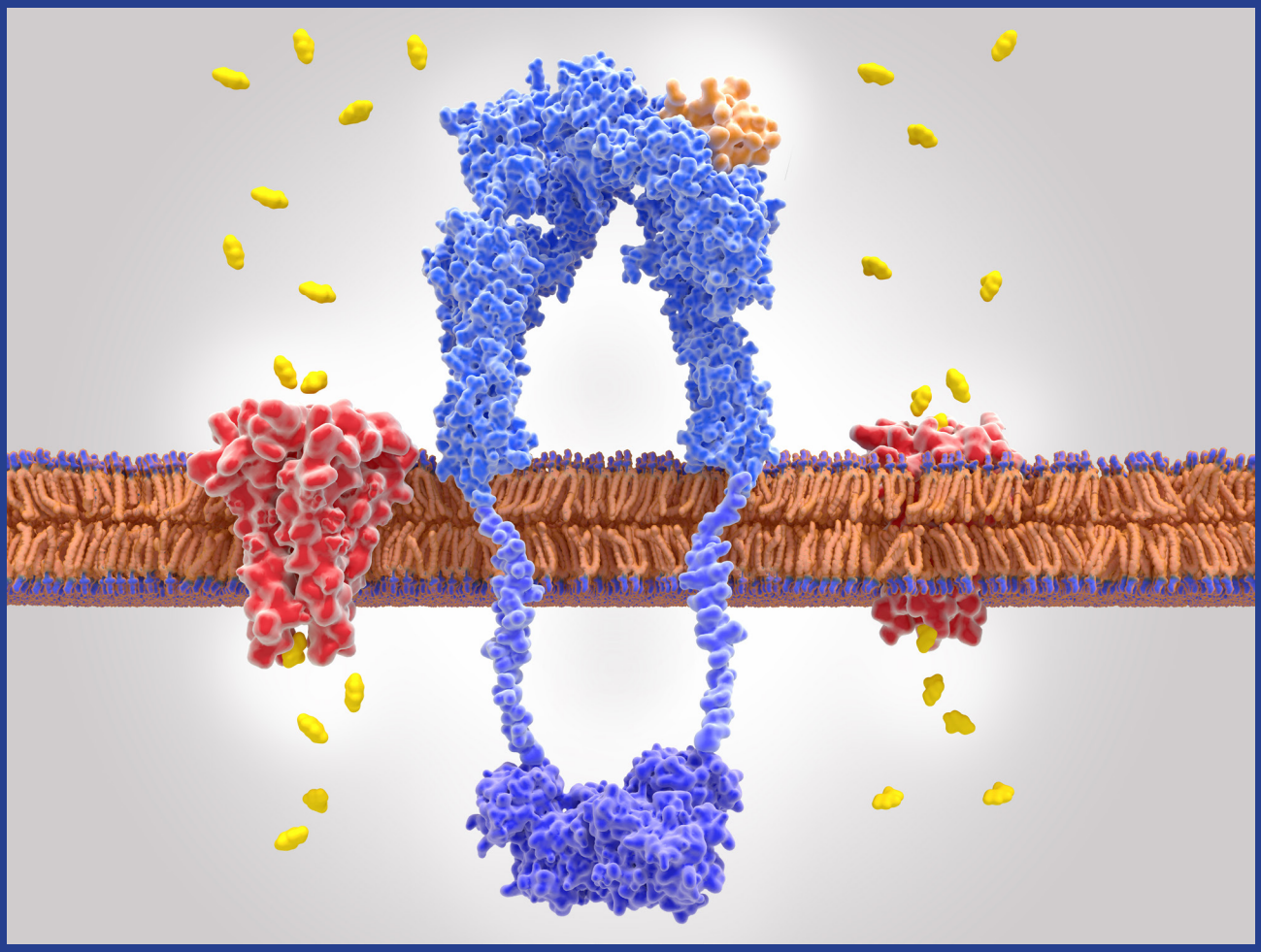
Fundamentals of BIOCHEMISTRY



Fundamentals of Biochemistry by Manjeet Kumari, PhD.

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The primary purpose of this book is to support equity and inclusiveness in education, so therefore, this book is dedicated to all students who want to learn biochemistry without breaking their bank!

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Table of Contents

Chapter #1 Chemical foundations of life

Introduction

Elements in various combinations comprise all matter, including living things. Some of the most abundant elements in living organisms include carbon, hydrogen, nitrogen, oxygen, sulfur, and phosphorus. These form the nucleic acids, proteins, carbohydrates, and lipids that are the fundamental components of living matter. Biologists must understand these important building blocks and the unique structures of the atoms that comprise molecules, allowing for cells, tissues, organ systems, and entire organisms to form.

All biological processes follow the laws of physics and chemistry, so in order to understand how biological systems work, it is important to understand the underlying physics and chemistry. For example, the flow of blood within the circulatory system follows the laws of physics that regulate the modes of fluid flow. The breakdown of the large, complex molecules of food into smaller molecules—and the conversion of these to release energy to be stored in adenosine triphosphate (ATP)—is a series of chemical reactions that follow chemical laws.

The properties of water and the formation of hydrogen bonds are key to understanding living processes. Recognizing the properties of acids and bases is important, for example, to our understanding of the digestive process. Therefore, the fundamentals of physics and chemistry are important for gaining insight into biological processes.

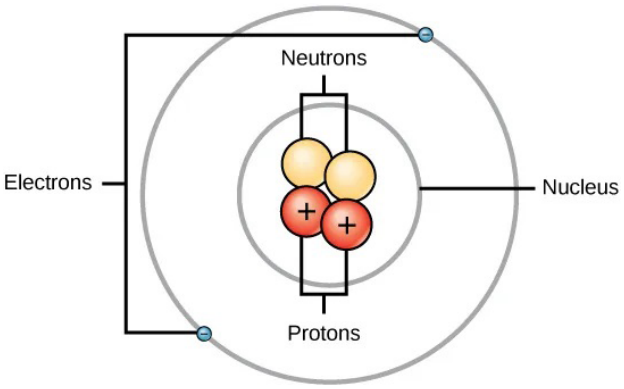

Fig 1.1 Elements, such as helium, depicted here, are made up of atoms. Atoms are made up of protons and neutrons located within the nucleus, with electrons in orbitals surrounding the nucleus.

Atoms, Isotopes, Ions, and Molecules: The building blocks

At its most fundamental level, life is made up of matter. Matter is any substance that occupies space and has mass. Elements are unique forms of matter with specific chemical and physical properties that cannot break down into smaller substances by ordinary chemical reactions. There are 118 elements, but only 98 occur naturally. The remaining elements are unstable and require scientists to synthesize them in laboratories.

Each element is designated by its chemical symbol, which is a single capital letter or, when the first letter is already "taken" by another element, a combination of two letters. Some elements follow the English term for the element, such as C for carbon and Ca for calcium. Other elements' chemical symbols derive from their Latin names. For example, the symbol for sodium is Na, referring to natrium, the Latin word for sodium.

The four elements common to all living organisms are oxygen (O), carbon (C), hydrogen (H), and nitrogen (N). In the nonliving world, elements are found in different proportions, and some elements common to living organisms are relatively rare on the earth as a whole. For example, the atmosphere is rich in nitrogen and oxygen but contains little carbon and hydrogen, while the earth's crust, although it contains oxygen and a small amount of hydrogen, has little nitrogen and carbon. In spite of their differences in abundance, all elements and the chemical reactions between them obey the same chemical and physical laws regardless of whether they are a part of the living or nonliving world.

The structure of the atom

To understand how elements come together, we must first discuss the element's smallest component or building block, the atom. An atom is the smallest unit of matter that retains all of the element's chemical properties. For example, one gold atom has all of the properties of gold in that it is a solid metal at room temperature. A gold coin is simply a very large number of gold atoms molded into the shape of a coin and contains small amounts of other elements known as impurities. We cannot break down gold atoms into anything smaller while still retaining the properties of gold.

An atom is composed of two regions: the nucleus, which is in the atom's center and contains protons and neutrons. The atom's outermost region holds its electrons in orbit around the nucleus, as Figure 1.1 illustrates. Atoms contain protons, electrons, and neutrons, among other subatomic particles. The only exception is hydrogen (H), which is made of one proton and one electron with no neutrons. Elements, such as helium, depicted here (Fig 1.1), are made up of atoms. Atoms are made up of protons and neutrons located within the nucleus, with electrons in orbitals surrounding the nucleus.

Protons and neutrons have approximately the same mass, about 1.67 × 10-24 grams. Scientists arbitrarily define this amount of mass as one atomic mass unit (amu) or one Dalton. Although similar in mass, protons and neutrons differ in their electric charge.

A proton is positively charged; whereas, a neutron is uncharged. Therefore, the number of neutrons in an atom contributes significantly to its mass, but not to its charge. Electrons are much smaller in mass than protons, weighing only 9.11 × 10-28 grams, or about 1/1800 of an atomic mass unit. Hence, they do not contribute much to an element's overall atomic mass. Therefore, when considering atomic mass, it is customary to ignore the mass of any electrons and calculate the atom's mass based on the number of protons and neutrons alone.

Although not significant contributors to mass, electrons do contribute greatly to the atom's charge, as each electron has a negative charge equal to the proton's positive charge. In uncharged, neutral atoms, the number of electrons orbiting the nucleus is equal to the number of protons inside the nucleus. In these atoms, the positive and negative charges cancel each other out, leading to an atom with no net charge. Accounting for the sizes of protons, neutrons, and electrons, most of the atom's volume—greater than 99 percent—is empty space. With all this empty space, one might ask why so-called solid objects do not just pass through one another. The reason they do not is that the electrons that surround

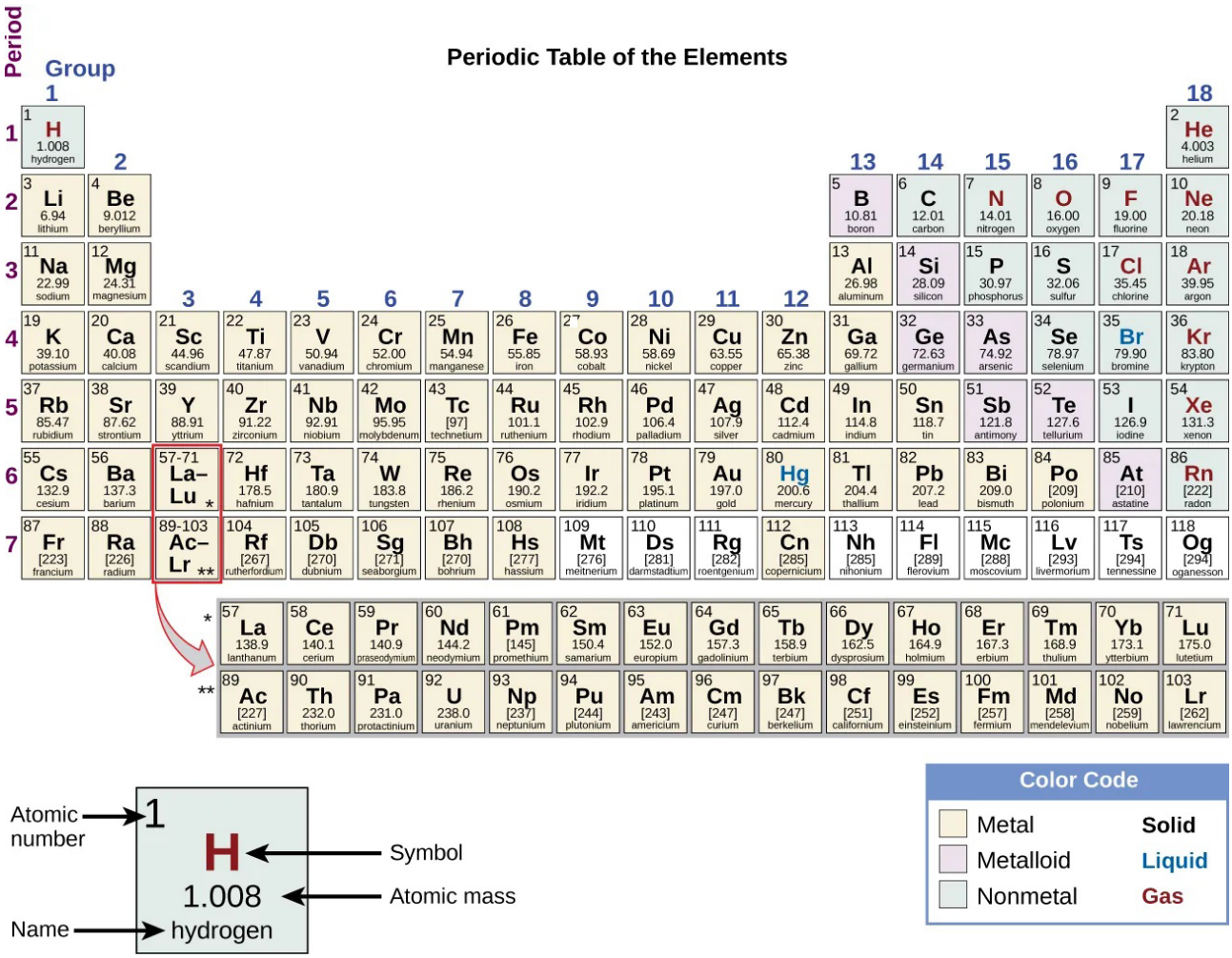

Fig 1.2 The periodic table shows each element's atomic mass and atomic number. The atomic number appears above the symbol for the element and the approximate atomic mass appears below it. Source: Biology 2e openstax.

all atoms are negatively charged and negative charges repel each other.

Atomic number and mass

Atoms of each element contain a characteristic number of protons and electrons. The number of protons determines an element's atomic number, which scientists use to distinguish one element from another. The number of neutrons is variable, resulting in isotopes, which are different forms of the same atom that vary only in the number of neutrons they possess. Together, the number of protons and neutrons determine an element's mass number.

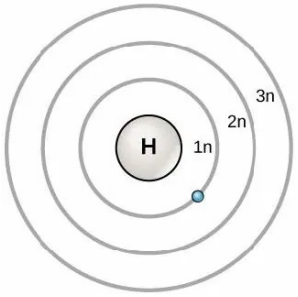

Fig 1.3 In 1913, Niels Bohrs developed the Bohr model in which electrons exist within principal shells. An electron normally exists in the lowest energy shell available, which is the one closest to the nucleus. Energy from a photon of light can bump it up to a higher energy shell, but this situation is unstable, and the electron quickly decays back to the ground state. In the process, it releases a photon of light. Source: Biology 2e Openstax

Note that we disregard the small contribution of mass from electrons in calculating the mass number. We can use this approximation of mass to easily calculate how many neutrons an element has by simply subtracting the number of protons from the mass number. Since an element's isotopes will have slightly different mass numbers, scientists also determine the atomic mass, which is the calculated mean of the mass number for its naturally occurring isotopes. Often, the resulting number contains a fraction For example, the atomic mass of chlorine (Cl) is 35.45 because chlorine is composed of several isotopes, some (the majority) with atomic mass 35 (17 protons and 18 neutrons)

Isotopes

Isotopes are different forms of an element that have the same number of protons but a different number of neutrons. Some elements—such as carbon, potassium, and uranium—have naturally occurring isotopes.

Carbon-12 contains six protons, six neutrons, and six electrons; therefore, it has a mass number of 12 (six protons and six neutrons). Carbon-14 contains six protons, eight neutrons, and six electrons; its atomic mass is 14 (six protons and eight neutrons). These two alternate forms of carbon are isotopes. Some isotopes may emit neutrons, protons, and electrons, and attain a more stable atomic configuration (lower level of potential energy); these are radioactive isotopes, or radioisotopes.

Radioactive decay (carbon-14 decaying to eventually become nitrogen-14) describes the energy loss that occurs when an unstable atom's nucleus releases radiation.

The Periodic Table: The periodic table organizes and displays different elements. Devised by Russian chemist Dmitri Mendeleev (1834–1907) in 1869, the table groups elements that, although unique, share certain chemical properties with other elements.

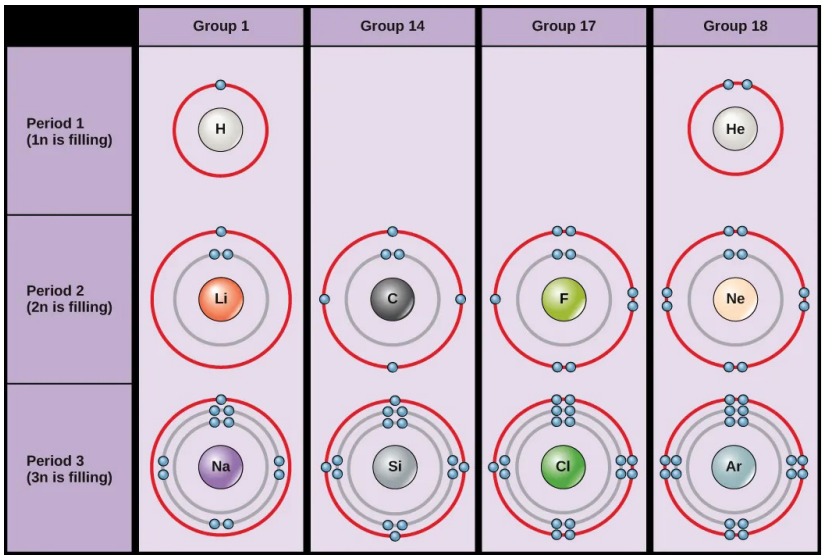

Fig 1.4 Bohr diagrams indicate how many electrons fill each principal shell. Group 18 elements (helium, neon, and argon) have a full outer, or valence, shell. A full valence shell is the most stable electron configuration. Elements in other groups have partially filled valence shells and gain or lose electrons to achieve a stable electron configuration. Source: Biology 2e

The properties of elements are responsible for their physical state at room temperature: they may be gases, solids, or liquids. Elements also have specific chemical reactivity, the ability to combine and to chemically bond with each other.

In the periodic table in Figure 1.2, the elements are organized and displayed according to their atomic number and are arranged in a series of rows and columns based on shared chemical and physical properties. In addition to providing the atomic number for each element, the periodic table also displays the element's atomic mass. Looking at carbon, for example, its symbol (C) and name appear, as well as its atomic number of six (in the upper left-hand corner) and its atomic mass of 12.11.

The periodic table groups elements according to chemical properties. Scientists base the differences in chemical reactivity between the elements on the number and spatial distri-

bution of an atom's electrons. Atoms that chemically react and bond to each other form molecules. Molecules are simply two or more atoms chemically bonded together. Logically, when two atoms chemically bond to form a molecule, their electrons, which form the outermost region of each atom, come together first as the atoms form a chemical bond.

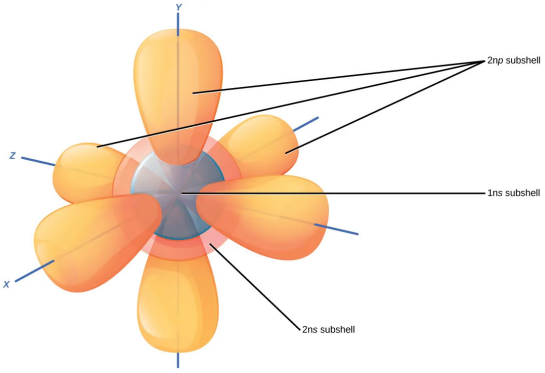

Fig 1.5 The s subshells are shaped like spheres. Both the 1n and 2n principal shells have an s orbital, but the size of the sphere is larger in the 2n orbital. Each sphere is a single orbital. Three dumbbell-shaped orbitals comprise p subshells. Principal shell 2n has a p subshell, but shell 1 does not.

Electron Shells and the Bohr Model

Note that there is a connection between the number of protons in an element, the atomic number that distinguishes one element from another, and the number of electrons it has. In all electrically neutral atoms, the number of electrons is the same as the number of protons. Thus, each element, at least when electrically neutral, has a characteristic number of electrons equal to its atomic number.

In 1913, Danish scientist Niels Bohr (1885–1962) developed an early model of the atom. The Bohr model shows the atom as a central nucleus containing protons and neutrons, with the electrons in circular orbitals at specific distances from the nucleus, as Figure 1.3 illustrates.

These orbits form electron shells or energy levels, which are a way of visualizing the number of electrons in the outermost shells. These energy levels are designated by a number and the symbol "n." For example, 1n represents the first energy level located closest to the nucleus.

Electrons fill orbitals in a consistent order: they first fill the orbitals closest to the nucleus, then they continue to fill orbitals of increasing energy further from the nucleus. If there are multiple orbitals of equal energy, they fill with one electron in each energy level before adding a second electron. The electrons of the outermost energy level determine the atom's energetic stability and its tendency to form chemical bonds with other atoms to form molecules.

Under standard conditions, atoms fill the inner shells first, often resulting in a variable number of electrons in the outermost shell. The innermost shell has a maximum of two electrons but the next two electron shells can each have a maximum of eight electrons. This is known as the octet rule, which states, with the exception of the innermost shell, that atoms are more stable energetically when they have eight electrons in their valence shell, the outermost electron shell. Figure 1.4 shows examples of some neutral atoms and their electron configurations. Notice that in Figure 1.4, helium has a complete outer electron shell, with two electrons filling its first and only shell. Similarly, neon has a complete outer 2n shell containing eight electrons. In contrast, chlorine and sodium have seven and one in their outer shells, respectively, but theoretically they would be more energetically stable if they followed the octet rule and had eight.

An atom may give, take, or share electrons with another atom to achieve a full valence shell, the most stable electron configuration. Looking at this figure, how many electrons do elements in group 1 need to lose in order to achieve a stable electron configuration? How many electrons do elements in groups 14 and 17 need to gain to achieve a stable configuration?

Understanding that the periodic table's organization is based on the total number of protons (and electrons) helps us know how electrons distribute themselves among the shells. The periodic table is arranged in columns and rows based on the number of electrons and their location. Examine more closely some of the elements in the table's far right column in Figure 1.4. The group 18 atoms helium (He), neon (Ne), and argon (Ar) all have filled outer electron shells, making it unnecessary for them to share electrons with other atoms to attain stability. They are highly stable as single atoms. Because they are non reactive, scientists coin them inert (or noble gases).

Compare this to the group 1 elements in the left-hand column. These elements, including hydrogen (H), lithium (Li), and sodium (Na), all have one electron in their outermost shells. That means that they can achieve a stable configuration and a filled outer shell by donating or sharing one electron with another atom or a molecule such as water. Hydrogen will donate or share its electron to achieve this configuration, while lithium and sodium will donate their electron to become stable. As a result of losing a negatively charged electron, they become positively charged ions.

Group 17 elements, including fluorine and chlorine, have seven electrons in their outmost shells, so they tend to fill this shell with an electron from other atoms or molecules, making them negatively charged ions. Group 14 elements, of which carbon is the most important to living systems, have four electrons in their outer shell allowing them to make several covalent bonds (discussed below) with other atoms.

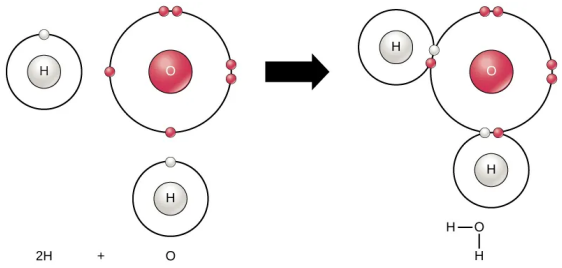

Fig 1.6 Two or more atoms may bond with each other to form a molecule. When two hydrogens and an oxygen share electrons via covalent bonds it forms a water molecule. Source: Biology 2e OpenStax

Thus, the periodic table's columns represent the potential shared state of these elements' outer electron shells that is responsible for their similar chemical characteristics.

Electron Orbitals: Although useful to explain the reactivity and chemical bonding of certain elements, the Bohr model does not accurately reflect how electrons spatially distribute themselves around the nucleus. They do not circle the nucleus like the earth orbits the sun, but we find them in electron orbitals. These relatively complex shapes result from the fact that electrons behave not just like particles, but also like waves.

Mathematical equations from quantum mechanics, which scientists call wave functions, can predict within a certain level of probability where an electron might be at any given time. Scientists call the area where an electron is most likely to be found its orbital. Recall that the Bohr model depicts an atom's electron shell configuration. Within each electron shell are subshells, and each subshell has a specified number of orbitals containing electrons.

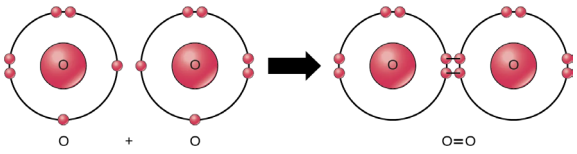

Fig 1.7 A double bond joins the oxygen atoms in an O_2 molecule.

While it is impossible to calculate exactly an electron's location, scientists know that it is most probably located within its orbital path. The letter s, p, d, and f designate the subshells. The s subshell is spherical in shape and has one orbital. Principal shell 1n has only a single s orbital, which can hold two electrons. Principal shell 2n has one s and one p subshell, and can hold a total of eight electrons.

The p subshell has three dumbbell-shaped orbitals, as Figure 1.5 illustrates. Subshells d and f have more complex shapes and contain five and seven orbitals, respectively. We do not show these in the illustration. Principal shell 3n has s, p, and d subshells and can hold 18 electrons. Principal shell 4n has s, p, d and f orbitals and can hold 32 electrons. Moving away from the nucleus, the number of electrons and orbitals in the energy levels increases. Progressing from one atom to the next in the periodic table, we can determine the electron structure by fitting an extra electron into the next available orbital.

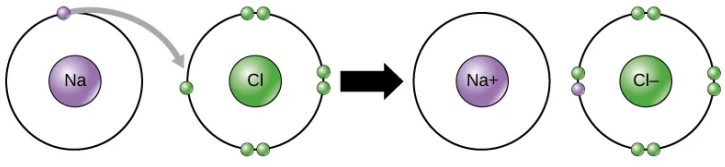

Fig 1.8 In the formation of an ionic compound, metals lose electrons and nonmetals gain electrons to achieve an octet. Source: Biology 2e OpenStax

The s subshells are shaped like spheres. Both the 1n and 2n principal shells have an s orbital, but the size of the sphere is larger in the 2n orbital. Each sphere is a single orbital. Three dumbbell-shaped orbitals comprise p subshells. Principal shell 2n has a p subshell, but shell 1 does not.

The closest orbital to the nucleus, the 1s orbital, can hold up to two electrons. This orbital is equivalent to the Bohr model's innermost electron shell. Scientists call it the 1s orbital because it is spherical around the nucleus. The 1s orbital is the closest orbital to the nu-

cleus, and it is always filled first, before any other orbital fills. Hydrogen has one electron; therefore, it occupies only one spot within the 1s orbital. We designate this as $1s^1$, where the superscripted 1 refers to the one electron within the 1s orbital.

Helium has two electrons; therefore, it can completely fill the 1s orbital with its two electrons. We designate this as $1s^2$, referring to the two electrons of helium in the 1s orbital. On the periodic table hydrogen and helium are the only two elements in the first row (period). This is because they only have electrons in their first shell, the 1s orbital. Hydrogen and helium are the only two elements that have the 1s and no other electron orbitals in the electrically neutral state.

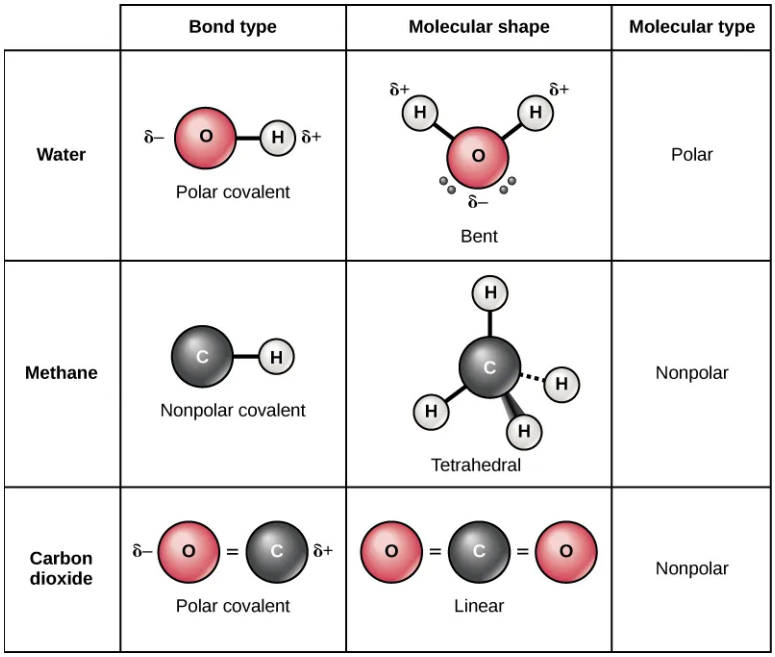

Fig 1.9 Whether a molecule is polar or nonpolar depends both on bond type and molecular shape. Both water and carbon dioxide have polar covalent bonds, but carbon dioxide is linear, so the partial charges on the molecule cancel each other out.

The second electron shell may contain eight electrons. This shell contains another spherical s orbital and three "dumbbell" shaped p orbitals, each of which can hold two electrons. After the 1s orbital fills, the second electron shell fills, first filling its 2s orbital and then its three p orbitals. When filling the p orbitals, each takes a single electron. Once each p orbital has an electron, it may add a second. Lithium (Li) contains three electrons that occupy the first and second shells. Two electrons fill the 1s orbital, and the third electron then fills the 2s orbital. Its electron configuration is $1s^2\,2s^1$.

Neon (Ne), alternatively, has a total of ten electrons: two are in its innermost 1s orbital and eight fill its second shell (two each in the 2s and three p orbitals). Thus it is an inert gas and energetically stable as a single atom that will rarely form a chemical bond with other atoms. Larger elements have additional orbitals, comprising the third electron shell. While the concepts of electron shells and orbitals are closely related, orbitals provide a more accurate depiction of an atom's electron configuration because the orbital model specifies the different shapes and special orientations of all the places that electrons may occupy.

Chemical Reactions and Molecules

All elements are most stable when their outermost shell is filled with electrons according to the octet rule. This is because it is energetically favorable for atoms to be in that configuration and it makes them stable.

However, since not all elements have enough electrons to fill their outermost shells, atoms form chemical bonds with other atoms thereby obtaining the electrons they need to attain a stable electron configuration. When two or more atoms chemically bond with each other, the resultant chemical structure is a molecule. The familiar water molecule, H_2O, consists of two hydrogen atoms and one oxygen atom. These bond together to form water, as Figure 1.6 illustrates. Atoms can form molecules by donating, accepting, or sharing electrons to fill their outer shells.

Chemical reactions occur when two or more atoms bond together to form molecules or when bonded atoms break apart. Scientists call the substances used in the beginning of a chemical reaction reactants (usually on the left side of a chemical equation), and we call the substances at the end of the reaction products (usually on the right side of a chemical equation). We typically draw an arrow between the reactants and products to indicate the chemical reaction's direction. This direction is not always a "one-way street." To create the water molecule above, the chemical equation would be:

$$2H + O \rightarrow H_2O$$

An example of a simple chemical reaction is breaking down hydrogen peroxide molecules,

each of which consists of two hydrogen atoms bonded to two oxygen atoms (H_2O_2). The reactant hydrogen peroxide breaks down into water, containing one oxygen atom bound to two hydrogen atoms (H_2O), and oxygen, which consists of two bonded oxygen atoms (O2). In the equation below, the reaction includes two hydrogen peroxide molecules and two water molecules. This is an example of a balanced chemical equation, wherein each element's number of atoms is the same on each side of the equation.

According to the law of conservation of matter, the number of atoms before and after a chemical reaction should be equal, such that no atoms are, under normal circumstances, created or destroyed.

$2\ H_2O_2$(hydrogen peroxide) $\rightarrow 2\ H_2O$ (water) + O_2 (oxygen)

Even though all of the reactants and products of this reaction are molecules (each atom remains bonded to at least one other atom), in this reaction only hydrogen peroxide and water are representatives of compounds: they contain atoms of more than one type of element. Molecular oxygen, alternatively, as Figure 1.7 shows, consists of two doubly bonded oxygen atoms and is not classified as a compound but as a homonuclear molecule.

Some chemical reactions, such as the one above, can proceed in one direction until they expend all the reactants. The equations that describe these reactions contain a unidirectional arrow and are irreversible. Reversible reactions are those that can go in either direction. In reversible reactions, reactants turn into products, but when the product's concentration goes beyond a certain threshold (characteristic of the particular reaction), some of these products convert back into reactants. At this point, product and reactant designations reverse. This back and forth continues until a certain relative balance between reactants and products occurs—a state called equilibrium.

A chemical equation with a double headed arrow pointing towards both the reactants and products often denote these reversible reaction situations.

For example, in human blood, excess hydrogen ions (H^+) bind to bicarbonate ions (HCO3-) forming an equilibrium state with carbonic acid (H_2CO_3). If we added carbonic acid to this system, some of it would convert to bicarbonate and hydrogen ions.

$HCO_3^- + H^+ \leftrightarrow H_2CO_3$

However, biological reactions rarely obtain equilibrium because the concentrations of the reactants or products or both are constantly changing, often with one reaction's product a reactant for another. To return to the example of excess hydrogen ions in the blood, forming carbonic acid will be the reaction's major direction.

However, the carbonic acid can also leave the body as carbon dioxide gas (via exhalation) instead of converting back to bicarbonate ion, thus driving the reaction to the right by the law of mass action. These reactions are important for maintaining homeostasis in our blood.

$H_2CO_3^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow CO_2 + H_2O$

Ions and Ionic Bonds

Some atoms are more stable when they gain or lose an electron (or possibly two) and form ions. This fills their outermost electron shell and makes them energetically more stable. Because the number of electrons does not equal the number of protons, each ion has a net charge. Cations are positive ions that form by losing electrons. Negative ions form by gaining electrons, which we call anions. We designate anions by their elemental name and change the ending to "-ide", thus the anion of chlorine is chloride, and the anion of sulfur is sulfide.

Scientists refer to this movement of electrons from one element to another as electron transfer. As Figure 1.8 illustrates, sodium (Na) only has one electron in its outer electron

shell. It takes less energy for sodium to donate that one electron than it does to accept seven more electrons to fill the outer shell. If sodium loses an electron, it now has 11 protons, 11 neutrons, and only 10 electrons, leaving it with an overall charge of +1. We now refer to it as a sodium ion.

Chlorine (Cl) in its lowest energy state (called the ground state) has seven electrons in its outer shell. Again, it is more energy-efficient for chlorine to gain one electron than to lose seven. Therefore, it tends to gain an electron to create an ion with 17 protons, 17 neutrons, and 18 electrons, giving it a net negative (–1) charge. We now refer to it as a chloride ion. In this example, sodium will donate its one electron to empty its shell, and chlorine will accept that electron to fill its shell. Both ions now satisfy the octet rule and have complete outermost shells.

Because the number of electrons is no longer equal to the number of protons, each is now an ion and has a +1 (sodium cation) or –1 (chloride anion) charge. Note that these transactions can normally only take place simultaneously: in order for a sodium atom to lose an electron, it must be in the presence of a suitable Ionic bonds form between ions with opposite charges. For instance, positively charged sodium ions molecule with zero net charge.

Physiologists refer to certain salts as electrolytes (including sodium, potassium, and calcium), ions necessary for nerve impulse conduction, muscle contractions, and water balance. Many sports drinks and dietary supplements provide these ions to replace those lost from the body via sweating during exercise.

Covalent Bonds and Other Bonds and Interactions

Another way to satisfy the octet rule is by sharing electrons between atoms to form covalent bonds. These bonds are stronger and much more common than ionic bonds in the molecules of living organisms. We commonly find covalent bonds in carbon-based organic molecules, such as our DNA and proteins. We also find covalent bonds in inorganic molecules like H_2O, CO_2, and O_2. The bonds may share one, two, or three pairs of electrons, making single, double, and triple bonds, respectively. The more covalent bonds between two atoms, the stronger their connection. Thus, triple bonds are the strongest.

The strength of different levels of covalent bonding is one of the main reasons living organisms have a difficult time in acquiring nitrogen for use in constructing their molecules, even though molecular nitrogen, N_2, is the most abundant gas in the atmosphere. Molecular nitrogen consists of two nitrogen atoms triple bonded to each other and, as with all molecules, sharing these three pairs of electrons between the two nitrogen atoms allows for filling their outer electron shells, making the molecule more stable than the individual nitrogen atoms. This strong triple bond makes it difficult for living systems to break apart this nitrogen in order to use it as constituents of proteins and DNA.

Forming water molecules provides an example of covalent bonding. Covalent bonds bind the hydrogen and oxygen atoms that combine to form water molecules as Figure 1.6 shows. The electron from the hydrogen splits its time between the hydrogen atoms' incomplete outer shell and the oxygen atoms' incomplete outer shell.

To completely fill the oxygen's outer shell, which has six electrons but which would be more stable with eight, two electrons (one from each hydrogen atom) are needed: hence, the well-known formula H2O. The two elements share the electrons to fill the outer shell of each, making both elements more stable.

Polar Covalent Bonds

There are two types of covalent bonds: polar and nonpolar. In a polar covalent bond, Figure 1.9 shows atoms unequally share the electrons and are attracted more to one nucleus than the other. Because of the unequal electron distribution between the atoms of different elements, a slightly positive (δ^+) or slightly negative (δ^-) charge develops. This partial charge is an important property of water and accounts for many of its characteristics.

Water is a polar molecule, with the hydrogen atoms acquiring a partial positive charge and the oxygen a partial negative charge. This occurs because the oxygen atom's nucleus is more attractive to the hydrogen atoms' electrons than the hydrogen nucleus is to the oxygen's electrons. Thus, oxygen has a higher electronegativity than hydrogen and the shared electrons spend more time near the oxygen nucleus than the hydrogen atoms' nucleus, giving the oxygen and hydrogen atoms slightly negative and positive charges, respectively. Another way of stating this is that the probability of finding a shared electron near an oxygen nucleus is more likely than finding it near a hydrogen nucleus.

Either way, the atom's relative electronegativity contributes to developing partial charges whenever one element is significantly more electronegative than the other, and the charges that these polar bonds generate may then be used to form hydrogen bonds based on the attraction of opposite partial charges. (Hydrogen bonds, which we discuss in detail below, are weak bonds between slightly positively charged hydrogen atoms to slightly negatively charged atoms in other molecules.) Since macromolecules often have atoms within them that differ in electronegativity, polar bonds are often present in organic molecules.

Nonpolar Covalent Bonds

Nonpolar covalent bonds form between two atoms of the same element or between different elements that share electrons equally. For example, molecular oxygen (O_2) is nonpolar because the electrons distribute equally between the two oxygen atoms. Figure 1.9 also shows another example of a nonpolar covalent bond—methane (CH_4). Carbon has four electrons in its outermost shell and needs four more to fill it. It obtains these four from four hydrogen atoms, each atom providing one, making a stable outer shell of eight electrons.

Carbon and hydrogen do not have the same electronegativity but are similar; thus, nonpolar bonds form. The hydrogen atoms each need one electron for their outermost shell, which is filled when it contains two electrons. These elements share the electrons equally among the carbons and the hydrogen atoms, creating a nonpolar covalent molecule.

Resources

Chapter image: Hand holding erath in a flask. Adobe stock #33645024 Licensed

Chapter adapted from Biology2e Open Stax. https://openstax.org/books/biology-2e/pages/1-introduction. Access for free at https://openstax.org/books/biology-2e/pages/1-introduction

Chapter #2 Water and buffers

Water

Humans have spent time looking for evidence of water in different planets because water is the essential for life. We're literally drowning in it, as water is by far the most abundant component of every cell. To understand life, we begin the discussion with the basics of water, because everything that happens in cells, even reactions buried deep inside enzymes, away from water, is influenced by water's chemistry.

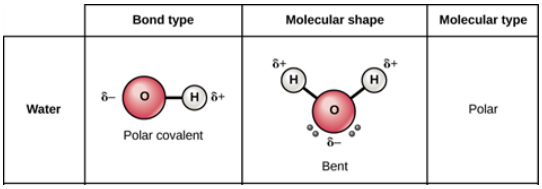

Figure 2.1 Water has polar covalent bond due to uneven sharing of electrons. Source: Biology 2e OpenStax.

The water molecule has wide 'V' shape (the HO-H angle is 104°) with uneven sharing of electrons between the oxygen and the hydrogen atoms (Figure 2.1).

Oxygen, with its higher electronegativity, holds electrons closer to itself than the hydrogens do. The hydrogens, as a result, are described as having a partial positive charge (typically designated as δ+) and the oxygen has a partial negative charge (written as δ-). Thus, water is a polar molecule because charges are distributed around it unevenly, not symmetrically.

Water as a solvent

Water is described as a solvent because of its ability to solvate (dissolve) many, but not all, molecules. Molecules that are ionic or polar dissolve readily in water, but non-polar substances dissolve poorly in water, if at all. Oil, for example, which is non-polar, separates from water when mixed with it. On the other hand, sodium chloride, which ionizes, and ethanol, which is polar, are able to form hydrogen bonds, so both dissolve in water. Ethanol's solubility in water is crucial for brewers, winemakers, and distillers – but for this property, there would be no wine, beer or spirits. As explained in an earlier section, we use the term hydrophilic to describe substances that interact well with water and dissolve in it and the term hydrophobic to refer to materials that are non-polar and do not dissolve in water. Table 2.1 illustrates some polar and non-polar substances. A third term, amphiphilic, refers to compounds that have both properties. Soaps, for example are amphiphilic, containing a long, non-polar aliphatic tail and a head that ionizes.

Hydrophilic vs Hydrophobic

Hydrophilic vs Hydrophobic Compounds

Hydrophobic	Hydrophilic
Nonpolar hydrocarbons (hexane)	Ionic compounds (NaCl)
Lipids (fats and cholesterol)	Polar organic compounds (alcohols, ketones or carbonyls)
	Weak acids (phosphates, amino acids)
	Sugars/carbohydrates

Table 2.1

Solubility

The solubility of materials in water is based in free energy changes, as measured by ΔG. Remember, from chemistry, that H is the enthalpy (heat at constant pressure) and S is entropy. Given this, $\Delta G = \Delta H - T\Delta S$, where T is the temperature in Kelvin. For a process to be favorable, the ΔG for it must be less than zero. From the equation, lowered ΔG values will be favored with decreases in enthalpy and/or increases in entropy. Let us first consider why non-polar materials do not dissolve in water. We could imagine a situation where the process of dissolving involves the "surrounding" of each molecule of the nonpolar solute in water, just like each sodium and each chloride ion gets surrounded by water molecules as salt dissolves.

Water organization

There is a significant difference, though between surrounding a non-polar molecule with water molecules and surrounding ions (or polar compounds) with water mol-

ecules. The difference is that since non-polar molecules don't really interact with water, the water behaves very differently than it does with ions or molecules that form hydrogen bonds. In fact, around each non-polar molecule, water gets very organized, aligning itself regularly. As any freshman chemistry student probably remembers, entropy is a measure of disorder, so when something becomes ordered, entropy decreases, meaning the ΔS is negative, so the $T\Delta S$ term in the equation is positive (negative of a negative). Since mixing a non-polar substance with water doesn't generally have any significant heat component, the ΔG is positive. This means, then, that dissolving a non-polar compound in water is not favorable and does not occur to any significant extent. Further, when the non-polar material associates with itself and not water, then the water molecules are free to mix, without being ordered, resulting in an increase of entropy. Entropy therefore drives the separation of non-polar substances from aqueous solutions.

Amphiphilic substances

Next, we consider mixing of an amphiphilic substance, such as a soap, with water (Figure 2.2). The sodium ions attached to the fatty acids in soap readily come off in aqueous solution, leaving behind a negatively charged molecule at one end and a non-polar region at the other end. The ionization of the soap causes in an increase in entropy - two particles instead of one. The non-polar portion of the negatively charged soap ion is problematic - if exposed to water, it will cause water to organize and result in a decrease of entropy and a positive ΔG.

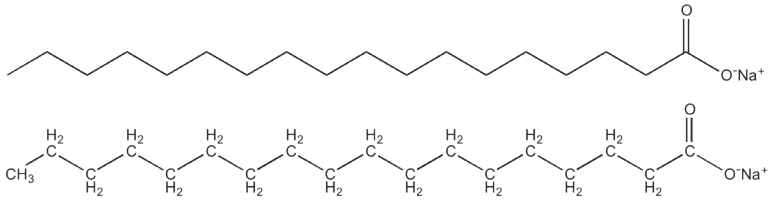

Fig 2.2 Structure of soap. Image by Aleia Kim

Since we know fatty acids dissolve in water, there must be something else at play. There is. Just like the non-polar molecules in the first example associated with each other and not water, so too do the non-polar portions of the soap ions associate with each other and exclude water. The result is that the soap ions arrange themselves as micelles with the non-polar portions on the interior of the structure away from water and the polar portions on the outside interacting with water. The interaction of the polar heads with water returns the water to its more disordered state (Fig 2.3).

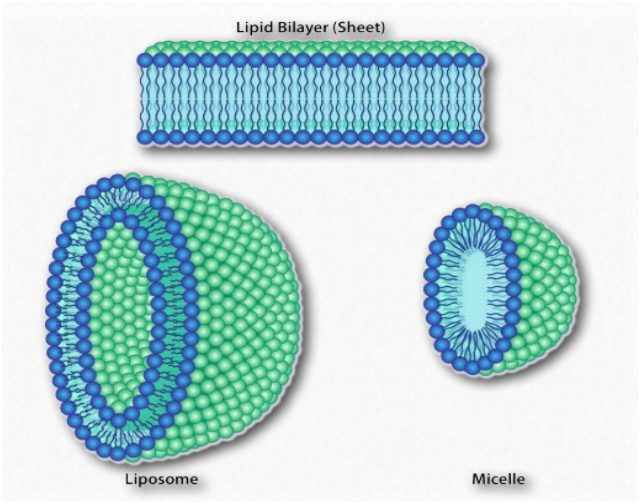

Fig 2.3 Structures formed by amphiphilic substances in water. Image by Aleia Kim

Yet another example is seen in the folding of globular proteins in the cytoplasm. Nonpolar amino acids are found in the interior portion of the protein (water excluded). Interaction of the non-polar amino acids turns out to be a driving force for the folding of proteins as they are being made in an aqueous solution.

Hydrogen bonds

The importance of hydrogen bonds in biochemistry (Figure 2.4) is hard to overstate.

Linus Pauling himself said, " . . . . I believe that as the methods of structural chemistry are further applied to physiological problems it will be found that the significance of the hydrogen bond for physiology is greater than that of any other single structural feature." In 2011, an IUPAC task group gave an evidence-based definition of hydrogen bonding that states, "The hydrogen bond is an attractive interaction between a hydrogen atom from a molecule or a molecular fragment X–H in which X is more electronegative than H, and an atom or a group of atoms in the same or a different molecule, in which there is evidence of bond formation."

Partial charges

The difference in electronegativity between hydrogen and the molecule to which it is covalently bound give rise to partial charges as described above. These tiny charges (δ+ and δ-) result in formation of hydrogen bonds, which occur when the partial positive charge of a hydrogen atom is attracted to the partial negative of another molecule. In water, that means the hydrogen of one water molecule is attracted to the oxygen of another (Figure 2.5).

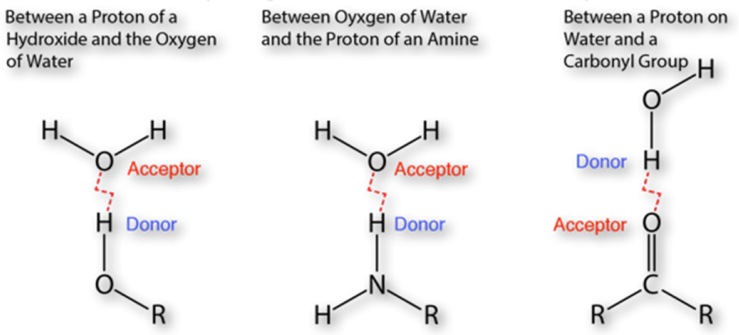

Fig 2.4 - Common hydrogen bonds in biochemistry

Since water is an asymmetrical molecule, it means also that the charges are asymmetrical. Such an uneven distribution is what makes a dipole. Dipolar molecules are important for interactions with other dipolar molecules and for dissolving ionic substances. Hydrogen bonds are not exclusive to water. In fact, they are important forces holding together macromolecules that include proteins and nucleic acids. Hydrogen bonds occur within and between macromolecules. The complementary pairing that occurs between bases in opposite strands of DNA, for example, is based on hydrogen bonds. Each hydrogen bond is relatively weak (compared to a covalent bond, for example - Table 2.2), but collectively they can be quite strong.

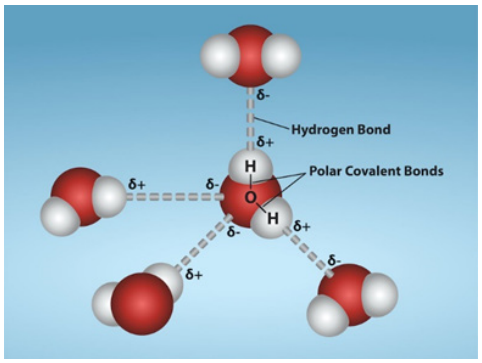

Fig. 2.5 The polarity of water. Credit: Rao, A., Fletcher, S., Ryan, K., Tag, A. and Hawkins, A. Department of Biology, Texas A&M University

Benefits of weak interactions

The weakness of hydrogen bond, however, is actually quite beneficial for cells, particularly as regards nucleic acids (Figure 2.6). The strands of DNA, for example, must be separated over short stretches in the processes of replication and the synthesis of RNA. Since only a few base pairs at a time need to be separated, the energy required to do this is small and the enzymes involved in the processes can readily take them apart, as needed.

Hydrogen bonds also play roles in binding of substrates to enzymes, catalysis, and protein-protein interaction, as well as other kinds of binding, such as protein-DNA, or antibody-antigen. As noted, hydrogen bonds are weaker than covalent bonds (Table 2.2) and their strength varies from very weak (1-2 kJ/mol) to fairly strong (29 kJ/mol). Hydrogen bonds only occur over relatively short distances (2.2 to 4.0 Å). The farther apart the hydrogen bond distance is, the weaker the bond is. The strength of the bond in kJ/mol represents the amount of heat that must be put into the system to break the bond - the larger the number, the greater the strength of the bond. Hydrogen bonds are readily broken using heat. The boiling of water, for example, requires breaking of H-bonds. When a biological structure, such as a protein or a DNA molecule, is stabilized by hydrogen bonds, breaking those bonds destabilizes the structure and can result in denaturation of the substance - loss of structure. It is partly for this reason that most proteins and all DNAs lose their native, or folded, structures when heated to boiling. For DNA molecules, denaturation results in complete separation of the strands from each other. For most proteins, this means loss of their characteristic three-dimensional structure and with it, loss of the function they performed. Though a few proteins can readily reassume their original structure when the solution they are in is cooled, most can't. This is one of the reasons that we cook our food. Proteins are essential for life, so denaturation of bacterial proteins results in death of any microorganisms contaminating the food.

Bond Energies

	Type of Bond	Bond Energy (kJ/mol)
Covalent Bonds	C—H	413
	O—H	460
Noncovalent Bonds	Hydrophobic Interaction	4-12
	Hydrogen Bond	20
	Ion-dipole Interaction	20

Table 2.2

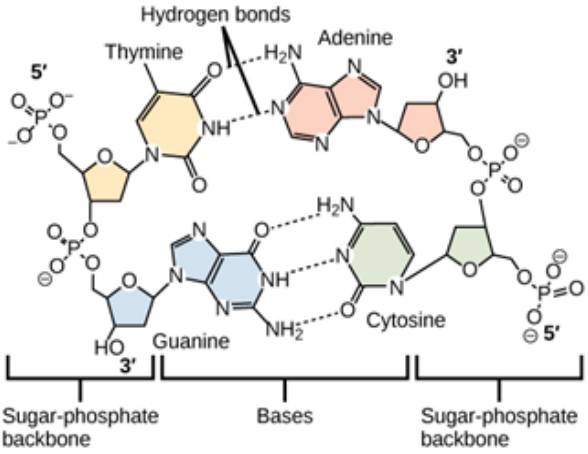

Fig 2.6 Hydrogen bonds in a base pair of DNA Source: Biology 2e OpenStax

Buffers

Water can ionize to a slight extent (10-7 M) to form H+ (proton) and OH- (hydroxide). We measure the proton concentration of a solution with pH, which is the negative log of the proton concentration. pH = -Log[H+] If the proton concentration, [H+]= 10-7 M, then the pH is 7 (Fig 2.7). We could just as easily measure the hydroxide concentration with the pOH by the parallel equation, pOH = -Log[OH-]

In pure water, dissociation of a proton simultaneously creates a hydroxide, so the pOH of pure water is 7, as well. This also means that pH + pOH = 14

Now, because protons and hydroxides can combine to form water, a large amount of one will cause there to be a small amount of the other. Why is this the case? In simple terms, if I dump 0.1 moles of H+ into a pure water solution, the high proton concentration will react with the relatively small amount of hydroxides to create water, thus reducing hydroxide concentration. Similarly, if I dump excess hydroxide (as NaOH, for example) into pure water, the proton concentration falls for the same reason.

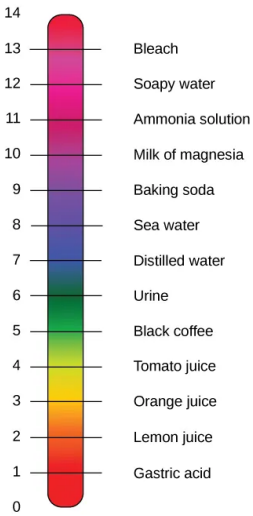

Fig 2.7 The pH scale measures hydrogen ions' (H+) concentration in a solution. (credit: modification of work by Edward Stevens). Source: Biology 2e OpenStax

Acids vs bases

Chemists use the term "acid" to refer to a substance which has protons that can dissociate (come off) when dissolved in water. They use the term "base" to refer to a substance that can absorb protons when dissolved in water. Both acids and bases come in strong and weak forms. (Examples of weak acids are shown in Table 2.3.). of Strong acids, such as HCl, dissociate completely in water. If we add 0.1 moles ($6.02x10^{22}$ molecules) of HCl to a solution to make a liter, it will have 0.1 moles of H+ and 0.1 moles of Cl- or $6.02x10^{22}$ molecules of each. There will be no remaining HCl when this happens. A strong base like NaOH also dissociates completely into Na^+ and OH^- .

Weak acids

Weak acids and bases differ from their strong counterparts. When you put one mole of acetic acid (HAc) into pure water, only a tiny percentage of the HAc molecules dissociate into H+ and Ac- (Fig 2.8). Clearly weak acids are very different from strong acids. Weak bases behave similarly, except that they accept protons, rather than donate them. Since we can view everything as a form of a weak acid, we will not use the term weak base here.

Weak Acid pKa Values

Name	Chemical Structure of Acid	Chemical Structure of Salt	pKa
Acetic Acid	CH_3COOH	CH_3COO^-	4.76
Formic Acid	$HCOOH$	$HCOO^-$	3.75
Lactic Acid	$CH_3CHOHCOOH$	$CH_3CH—HCOO^-$	3.86
Pyruvic Acid	$CH_3COCOOH$	$CH_3C—COO^-$	2.50
Oxalic Acid (1)	HOOC—COOH	HOOC—COO^-	1.23
Oxalic Acid (2)	HOOC—COO^-	$^-$OOC—COO^-	4.19
Carbonic Acid (1)	H_2CO_3	HCO_3^-	6.37
Carbonic Acid (2)	HCO_3^-	CO_3^{2-}	10.20
Malic Acid (1)	HOOC—CH_2—CHOH—COOH	HOOC—CH_2—CHOH—COO^-	3.40
Malic Acid (2)	HOOC—CH_2—CHOH—COO^-	$^-$OOC—CH_2—CHOH—COO^-	5.26
Malonic Acid (1)	HOOC—CH_2—COOH	HOOC—CH_2—COO^-	2.83
Malonic Acid (2)	HOOC—CH_2—COO^-	$^-$OOC—CH_2—COO^-	5.69
Phosphoric Acid (1)	H_3PO_4	$H_2PO_4^-$	2.14
Phosphoric Acid (2)	$H_2PO_4^-$	HPO_4^{2-}	7.20
Phosphoric Acid (3)	HPO_4^{2-}	PO_4^{3-}	12.40
Succinic Acid (1)	HOOC—CH_2—CH_2—COOH	HOOC—CH_2—CH_2—COO^-	4.21
Succinic Acid (2)	HOOC—CH_2—CH_2—COO^-	$^-$OOC—CH_2—CH_2—COO^-	5.63

Table 2.3

Clearing Confusion

Students are often puzzled and expect that [H+] = [A-] because the dissociation equation shows one of each from HA. This is, in fact, true ONLY when HA is allowed to dissociate in pure water. Usually the HA is placed into solution that has protons and hydroxides to affect things. Those protons and /or hydroxides change the H+ and A concentration unequally, since A- can absorb some of the protons and/or HA can release H+ when influenced by the OH- in the solution. Therefore, one must calculate the proton concentration from the pH using the Henderson Hasselbalch equation.

$$pH = pKa + \log([Ac^-]/[HAc])$$

You may wonder why we care about weak acids. You may never have thought much of weak acids when you were in General Chemistry. Your instructor described them as buffers and you probably dutifully memorized the fact that "buffers are substances that resist change in pH" without really learning what this meant. Buffers are much too important to be thought of in this way.

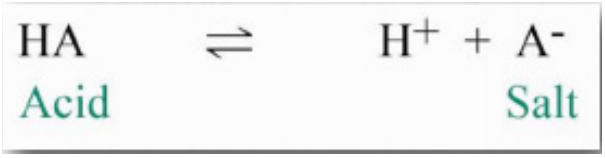

Fig 2.8 Dissociation of weak acid

UPS: Weak acids are critical for life because their affinity for protons causes them to behave like a UPS. We're not referring to the UPS that is the United Parcel Service®, but instead, to the encased battery backup systems for computers called Uninterruptible Power Supplies that kick on to keep a computer running during a power failure. The battery in a laptop computer is a UPS, for example. We can think of weak acids as Uninterruptible Proton Suppliers within certain pH ranges, providing (or absorbing) protons as needed. Weak acids thus help to keep the H^+ concentration (and thus the pH) of the solution they are in relatively constant. Consider the bicarbonate/carbonic acid system. Figure 2.9 shows what happens when H2CO3 dissociates. Adding hydroxide ions (by adding a strong base like NaOH) to the solution causes the H+ ions to react with OH- ions to make water. Consequently, the concentration of H+ ions would go down and the pH would go up. However, in contrast to the situation with a solution of pure water, there is a backup source of H+ available in the form of H2CO3. Here is where the UPS function kicks in. As protons are taken away by the added hydroxyl ions (making water), they are partly replaced by protons from the H2CO3. This is why a weak acid is a buffer. It resists changes in pH by releasing protons to compensate for those "used up" in reacting with the hydroxyl ions.

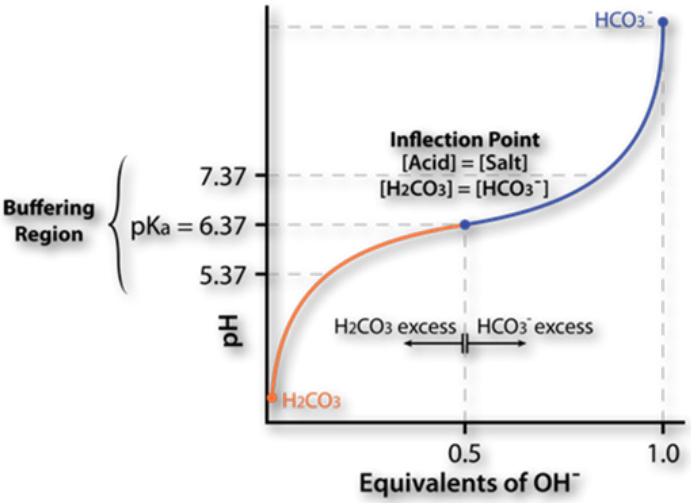

Fig 2.9 Titration curve for carbonic acid

Henderson-Hasselbalch

Henderson-Hasselbalch It is useful to be able to predict the response of the H2CO3 system to changes in H+ concentration. The Henderson-Hasselbalch equation defines the relationship between pH and the ratio of HCO_3^- and H_2CO_3. It is

$$pH = pKa + \log([HCO_3^-]/[H_2CO_3])$$

This simple equation defines the relationship between the pH of a solution and the ratio of HCO_3^- and H_2CO_3 in it. The new term, called the pKa, is defined as

$$pKa = -\text{Log } Ka,$$

just as

$$pH = -\text{Log } [H^+].$$

The Ka is the acid dissociation constant and is a measure of the strength of an acid. For a general acid, HA, which dissociates as

$$HA = H^+ + A^-,$$

$$Ka = [H^+][A^-]/[HA]$$

Thus, the stronger the acid, the more protons that will dissociate from it when added to water and the larger the value its Ka will have. Large values of Ka translate to lower values of pKa. As a result, the lower the pKa value is for a given acid, the stronger the weak acid is.

Constant pKa

Please note that pKa is a constant for a given acid. The pKa for carbonic acid is 6.37. By comparison, the pKa for formic acid is 3.75. Formic acid is therefore a stronger acid than acetic acid. A stronger acid will have more protons dissociated at a given pH than a weaker acid. Now, how does this translate into stabilizing pH? Figure 2.9 shows a titration curve. In this curve, the titration begins with the conditions at the lower left (very low pH). At this pH, the H_2CO_3 form predominates, but as more and more OH^- is added (moving to the right), the pH goes up, the amount of HCO_3^- goes up and (correspondingly), the amount of H_2CO_3 goes down. Notice that the curve "flattens" near the pKa (6.37).

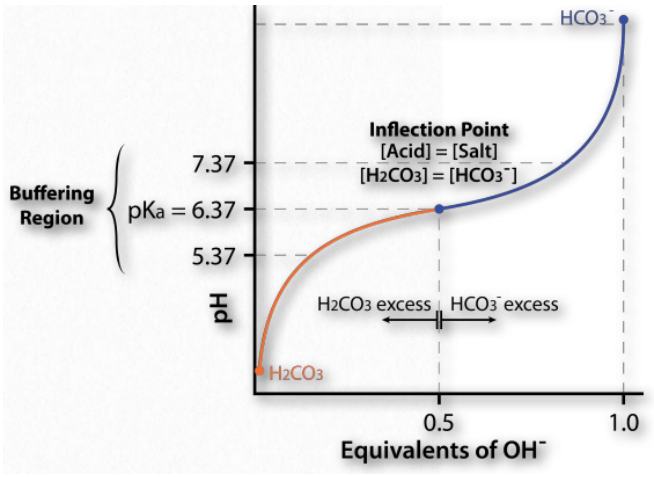

Figure 2.9 Titration curve for carbonic acid
Image by Aleia Kim

Buffering region

Flattening of the curve tells us is that the pH is not changing much (not going up as fast) as it did earlier when the same amount of hydroxide was added. The system is resisting a change in pH (not stopping the change but slowing it) in the region of about one pH unit above and one pH unit below the pKa. Thus, the buffering region of the carbonic acid/ bicarbonate buffer is from about 5.37 to 7.37. It is maximally strong at a pH of 6.37.

Now it starts to become apparent how the buffer works. HA can donate protons when extras are needed (such as when OH^- is added to the solution by the addition of NaOH). Similarly, A- can accept protons when extra H^+ are added to the solution (adding HCl, for example). The maximum ability to donate or accept protons comes when $[A^-] = [HA]$ This is consistent with the Henderson Hasselbalch equation and the titration curve.

When $[A^-] = [HA]$, pH = 6.37 + Log(1). Since Log(1) = 0, pH = 6.37 = pKa for carbonic acid. Thus for any buffer, the buffer will have maximum strength and display flattening of its titration curve when $[A^-] = [HA]$ and when pH = pKa. If a buffer has more than one pKa, then each pKa region will display the behavior.

Buffered vs non-buffered

To understand how well a buffer protects against changes in pH, consider the effect of adding .01 moles of HCl to 1.0 liter of pure water (no volume change) at pH 7, compared to adding it to 1.0 liter of a 1M acetate buffer at pH 4.76. Since HCl completely dissociates, in 0.01M (10^{-2} M) HCl you will have 0.01M H^+. For the pure water, the pH drops from 7.0 down to 2.0 (pH = -log(0.01M)). By contrast, the acetate buffer's pH after adding the same amount of HCl is 4.74. Thus, the pure water solution sees its pH fall from 7 to 2 (5 pH units), whereas the buffered solution saw its pH drop from 4.76 to 4.74 (0.02 pH units). Clearly, the buffer minimizes the impact of the added protons compared to the pure water.

Buffer capacity

It is important to note that buffers have capacities limited by their concentration. Let's imagine that in the previous paragraph, we had added the 0.01 moles HCl to an acetate buffer that had a concentration of 0.01M and equal amounts of Ac- and HAc. When we try to do the math in parallel to the previous calculation, we see that there are 0.01M protons, but only 0.005M A^- to absorb them. We could imagine that 0.005M of the protons would be

absorbed, but that would still leave 0.005M of protons unbuffered. Thus, the pH of this solution would be approximately pH = -log(0.005M) = 2.30 Exceeding buffer capacity dropped the pH significantly compared to adding the same amount of protons to a 1M acetate buffer. Consequently, when considering buffers, it is important to recognize that their concentration sets their limits. Another limit is the pH range in which one hopes to control proton concentration.

Multiple ionizable groups

Now, what happens if a molecule has two (or more) ionizable groups? It turns out, not surprisingly, that each group will have its own pKa and, therefore, will have multiple regions of buffering.

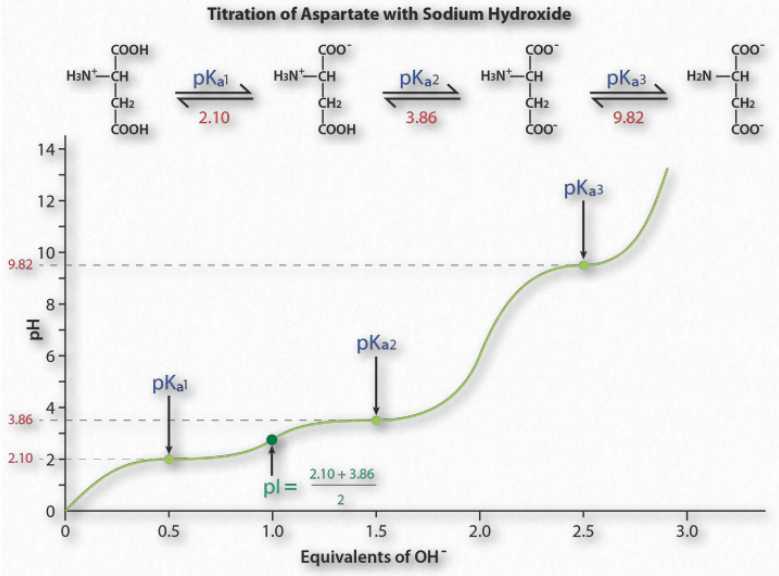

Fig 2.10 Titration of acidic amino acid

Figure 2.10 shows the titration curve for the amino acid aspartic acid. Note that instead of a single flattening of the curve, as was seen for acetic acid, aspartic acid's titration curve displays three such regions. These are individual buffering regions, each centered on the respective pKa values for the carboxyl group and the amine group. Aspartic acid has four possible charges: +1 (α-carboxyl group, α-amino group, and R group carboxyl each has a proton), 0 (α-carboxyl group missing proton, α- amino group has a proton, R-group carboxyl has a proton), -1 (α-carboxyl group and R-group carboxyl each lack a proton, α-amino group retains a proton), -2 (α-carboxyl, R-group carboxyl, and α-amino groups all lack extra proton).

Prediction: How does one predict the charge for an amino acid at a given pH? A good rule of thumb for estimating charge is that if the pH is more than one unit below the pKa for a group (carboxyl or amino), the proton is on. If the pH is more than one unit above the pKa for the group, the proton is off. If the pH is NOT more than one or less than one pH unit from the pKa, this simple assumption will not work. Further, it is important to recognize that these rules of thumb are estimates only. The pI (pH at which the charge of a molecule is zero) is an exact value calculated as the average of the two pKa values on either side of the zero region. It is calculated at the average of the two pKa values around the point where the charge of the molecule is zero. For aspartic acid, this corresponds to pKa1 and pKa2.

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Chapter #3 Amino acids

Introduction

All of the proteins on the face of the earth are made up of the same 20 amino acids. Linked together in long chains called polypeptides, amino acids are the building blocks for the vast assortment of proteins found in all living cells. All amino acids have the same basic structure, which is shown in Figure 3.1. At the "center" of each amino acid is a carbon called the α carbon and attached to it are four groups - a hydrogen, an α- carboxyl group, an α-amine group, and an R-group, sometimes referred to as a side chain. The α carbon, carboxyl, and amino groups are common to all amino acids, so the R-group is the only unique feature in each amino acid. (A minor exception to this structure is that of proline, in which the end of the R-group is attached to the α-amine.) With the exception of glycine, which has an R-group consisting of a hydrogen atom, all of the amino acids in proteins have four different groups attached to them and consequently can exist in two mirror image forms, L and D. With only very minor exceptions, every amino acid found in cells and in proteins is in the L configuration.

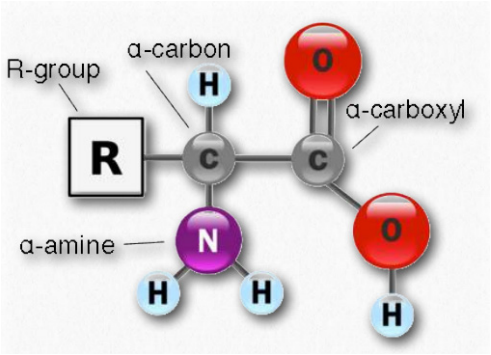


Figure 3.1 General amino acid structure

There are 22 amino acids that are found in proteins and of these, only 20 are specified by the universal genetic code. The others, selenocysteine and pyrrolysine use tRNAs that are able to base pair with stop codons in the mRNA during translation. When this happens, these unusual amino acids can be incorporated into proteins. Enzymes containing selenocysteine, for example, include glutathione peroxidases, tetraiodothyronine 5' deiodinases, thioredoxin reductases, formate dehydrogenases, glycine reductases, and selenophosphate synthetase. Pyrrolysine-containing proteins are much rarer and are mostly confined to archaea.

It is one of the more striking generalizations of biochemistry ...that the twenty amino acids and the four bases, are, with minor reservations, the same throughout Nature."

Francis Crick

Essential and non-essential amino acids

Nutritionists divide amino acids into two groups - essential amino acids (must be in the diet because cells can't synthesize them) and non-essential amino acids (can be made by cells). This classification of amino acids has little to do with the structure of amino acids. Essential amino acids vary considerable from one organism to another and even differ in humans, depending on whether they are adults or children. Table 2.1 shows essential and non-essential amino acids in humans.

Essential	Non-Essential
Histidine	Alanine
Isoleucine	Arginine
Leucine	Asparagine
Lysine	Aspartic acid
Methionine	Cysteine
Phenylalanine	Glutamic acid
Threonine	Glutamine
Tryptophan	Glycine
Valine	Proline
	Selenocysteine
	Serine
	Tyrosine

Table 3.1

Some amino acids that are normally nonessential, may need to be obtained from the diet in certain cases. Individuals who do not synthesize sufficient amounts of arginine, cysteine, glutamine, proline, selenocysteine, serine, and tyrosine, due to illness, for example, may need dietary supplements containing these amino acids.

Non-protein amino acids

There are also α-amino acids found in cells that are not incorporated into proteins. Common ones include ornithine and citrulline. Both of these compounds are intermediates in the urea cycle. Ornithine is a metabolic precursor of arginine and citrulline can be produced by the breakdown of arginine. The latter reaction produces nitric oxide, an important signaling molecule. Citrulline is the metabolic byproduct. It is sometimes used as a dietary supplement to reduce muscle fatigue.

R-group chemistry

We separate the amino acids into categories based on the chemistry of their R-groups. If you compare groupings of amino acids in different textbooks, you will see different names for the categories and (sometimes) the same amino acid being categorized differently by different authors. Indeed, we categorize tyrosine both as an aromatic amino acid and as a hydroxyl amino acid. It is useful to classify amino acids based on their R-groups, because it is these side chains that give each amino acid its characteristic properties. Thus, amino acids with (chemically) similar side groups can be expected to function in similar ways, for example, during protein folding.

Non-Polar	Carboxyl	Amine	Aromatic	Hydroxyl	Other
Alanine	Aspartic Acid	Arginine	Phenylalanine	Serine	Asparagine
Glycine	Glutamic Acid	Histidine	Tryptophan	Threonine	Cysteine
Isoleucine		Lysine	Tyrosine	Tyrosine	Glutamine
Leucine					Selenocysteine
Methionine					Pyrrolysine
Proline					
Valine					

Table 3.2 Amino acid categories (based on R-group properties)

We separate the amino acids into categories based on the chemistry of their R-groups. If you compare groupings of amino acids in different textbooks, you will see different names for the categories and (sometimes) the same amino acid being categorized differently by different authors. Indeed, we categorize tyrosine both as an aromatic amino acid and as a hydroxyl amino acid. It is useful to classify amino acids based on their R-groups, because it is these side chains that give each amino acid its characteristic properties. Thus, amino acids with (chemically) similar side groups can be expected to function in similar ways, for example, during protein folding.

Non-polar amino acids

Alanine (Ala/A) is one of the most abundant amino acids found in proteins, ranking second only to leucine in occurrence. A D-form of the amino acid is also found in bacterial cell walls. Alanine is non-essential, being readily synthesized from pyruvate. It is coded for by GCU, GCC, GCA, and GCG.

Glycine (Gly/G) is the amino acid with the shortest side chain, having an R-group consistent only of a single hydrogen. As a result, glycine is the only amino acid that is not chiral. Its small side chain allows it to readily fit into both hydrophobic and hydrophilic environments. Glycine is specified in the genetic code by GGU, GGC, GGA, and GGG. It is nonessential to humans.

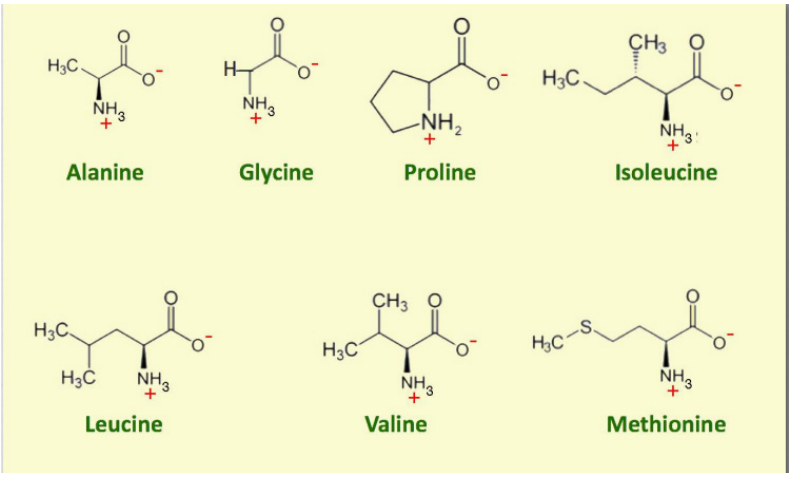


Fig 3.2 Non polar amino acids

Isoleucine (Ile/I) is an essential amino acid encoded by AUU, AUC, and AUA. It has a hydrophobic side chain and is also chiral in its side chain.

Leucine (Leu/L) is a branched-chain amino acid that is hydrophobic and essential. Leucine is the only dietary amino acid reported to directly stimulate protein synthesis in muscle, but caution is in order, as 1) there are conflicting studies and 2) leucine toxicity is dangerous, resulting in "the four D's": diarrhea, dermatitis, dementia and death . Leucine is encoded by six codons: UUA,UUG, CUU, CUC, CUA, CUG.

Methionine (Met/M) is an essential amino acid that is one of two sulfurcontaining amino acids - cysteine is the other. Methionine is non-polar and encoded solely by the AUG codon. It is the "initiator" amino acid in protein synthesis, being the first one incorporated

Amino acid	Short	Abbrev.	Side chain	Hydrophobic	pKa	Polar	pH	Small	Tiny	Aromatic or Aliphatic	van der Waals volume
Alanine	A	Ala	$-CH_3$	X	-	-	-	X	X	-	67
Cysteine	C	Cys	$-CH_2SH$	-	8.18	-	acidic	X	X	-	86
Aspartic acid	D	Asp	$-CH_2COOH$	-	3.90	X	acidic	X	-	-	91
Glutamic acid	E	Glu	$-CH_2CH_2COOH$	-	4.07	X	acidic	-	-	-	109
Phenylalanine	F	Phe	$-CH_2C_6H_5$	X	-	-	-	-	-	Aromatic	135
Glycine	G	Gly	-H	X	-	-	-	X	X	-	48
Histidine	H	His	$-CH_2-C_3H_3N_2$	-	6.04	X	weak basic	-	-	Aromatic	118
Isoleucine	I	Ile	$-CH(CH_3)CH_2CH_3$	X	-	-	-	-	-	Aliphatic	124
Lysine	K	Lys	$-(CH_2)_4NH_2$	-	10.54	X	basic	-	-	-	135
Leucine	L	Leu	$-CH_2CH(CH_3)_2$	X	-	-	-	-	-	Aliphatic	124
Methionine	M	Met	$-CH_2CH_2SCH_3$	X	-	-	-	-	-	-	124
Asparagine	N	Asn	$-CH_2CONH_2$	-	-	X	-	X	-	-	96
Pyrrolysine	O	Pyl	$-(CH_2)_4NHCOC_4H_5NCH_3$	-	-	X	weak basic	-	-	-	
Proline	P	Pro	$-CH_2CH_2CH_2-$	X	-	-	-	X	-	-	90
Glutamine	Q	Gln	$-CH_2CH_2CONH_2$	-	-	X	weak basic	-	-	-	114
Arginine	R	Arg	$-(CH_2)_3NH-C(NH)NH_2$	-	12.48	X	strongly basic	-	-	-	148
Serine	S	Ser	$-CH_2OH$	-	5.68	X	weak acidic	X	X	-	73
Threonine	T	Thr	$-CH(OH)CH_3$	-	5.53	X	weak acidic	X	-	-	93
Selenocysteine	U	Sec	$-CH_2SeH$	-	5.73	-	acidic	X	X	-	
Valine	V	Val	$-CH(CH_3)_2$	X	-	-	-	X	-	Aliphatic	105
Tryptophan	W	Trp	$-CH_2C_8H_6N$	-	5.885	X	weak basic	-	-	Aromatic	163
Tyrosine	Y	Tyr	$-CH_2-C_6H_4OH$	-	10.46	X	weak acidic	-	-	Aromatic	141

Table 3.3 - Properties of amino acid side chaingen bonds in biochemistry

into protein chains. In prokaryotic cells, the first methionine in a protein is formylated.

Proline (Pro/P) is the only amino acid found in proteins with an R-group that joins with its own α-amino group, making a secondary amine and a ring. Proline is a non-essential amino acid and is coded by CCU, CCC, CCA, and CCG. It is the least flexible of the protein amino acids and thus gives conformational rigidity when present in a protein. Proline's presence in a protein affects its secondary structure. It is a disrupter of α-helices and β-strands. Proline is often hydroxylated in collagen (the reaction requires Vitamin C - ascorbate) and this has the effect of increasing the protein's conformational stability. Proline hydroxylation of hypoxia-inducible factor (HIF) serves as a sensor of oxygen levels and targets HIF for destruction when oxygen is plentiful.

Valine (Val/V) is an essential, non-polar amino acid synthesized in plants. It is noteworthy in hemoglobin, for when it replaces glutamic acid at position number six, it causes hemoglobin to aggregate abnormally under low oxygen conditions, resulting in sickle cell disease. Valine is coded in the genetic code by GUU, GUC, GUA, and GUG.

Carboxyl Amino Acids

Aspartic acid (Asp/D) is a non-essential amino acid with a carboxyl group in its Rgroup. (Fig 3.3). It is readily produced by transamination of oxaloacetate. With a pKa of 3.9, aspartic acid's side chain is negatively charged at physiological pH. Aspartic acid is specified in the genetic code by the codons GAU and GAC.

Glutamic acid (Glu/E), which is coded by GAA and GAG, is a non-essential amino acid readily made by transamination of α- ketoglutarate. It is a neurotransmitter and has an R-group with a carboxyl group that readily ionizes (pKa = 4.1) at physiological pH.

Amine amino acids

Arginine (Arg/R) is an amino acid that is, in some cases, essential, but non-essential in others (Fig. 3.4). Premature infants cannot synthesize arginine. In addition, surgical trauma, sepsis, and burns increase demand for arginine. Most people, however, do not need arginine supplements. Arginine's side chain contains a complex guanidinium group with a pKa of over 12, making it positively charged at cellular pH. It is coded for by six codons - CGU, CGC, CGA, CGG, AGA, and AGG.

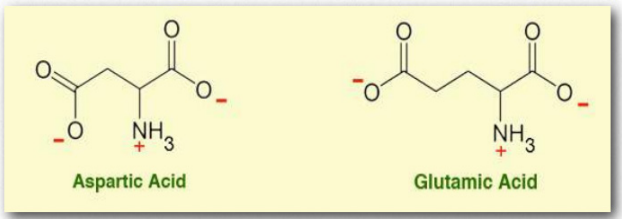


Fig 3.3 Carboxyl amino acids

Histidine (His/H) is the only one of the proteinaceous amino acids to contain an imidazole functional group. It is an essential amino acid in humans and other mammals. With a side chain pKa of 6, it can easily have its charge changed by a slight change in pH. Protonation of the ring results in two NH structures which can be drawn as two equally important resonant structures.

Fig 3.4 Amine amino acids

Lysine (Lys/K) is an essential amino acid encoded by AAA and AAG. It has an Rgroup that can readily ionize with a charge of +1 at physiological pH and can be posttranslationally modified to form acetyllysine, hydroxylysine, and methyllysine. It can also be ubiquitinated, sumoylated, neddylated, biotinylated, carboxylated, and pupylated, and. O-Glycosylation of hydroxylysine is used to flag proteins for export from the cell. Lysine is often added to animal feed because it is a limiting amino acid and is necessary for optimizing growth of pigs and chickens.

Aromatic amino acids

Phenylalanine (Phe/ F) is a non-polar, essential amino acid coded by UUU and UUC. It is a metabolic precursor of tyrosine. Inability to metabolize phenylalanine arises from the genetic disorder known as phenylketonuria. Phenylalanine is a component of the aspartame artificial sweetener. (Fig.3.5)

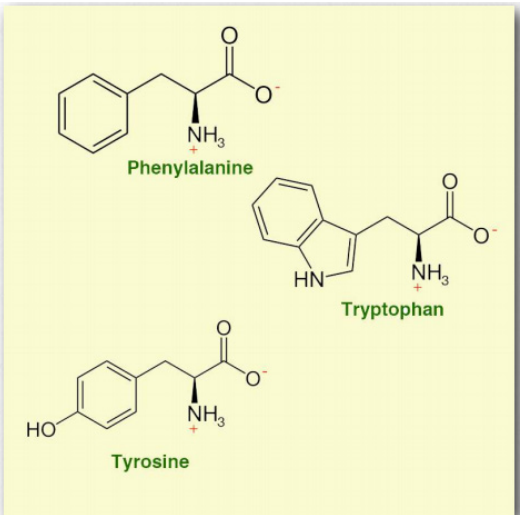


Fig 3.5 Aromatic amino acids

Tryptophan (Trp/W) is an essential amino acid containing an indole functional group. It is a metabolic precursor of serotonin, niacin, and (in plants) the auxin phytohormone. Though reputed to serve as a sleep aid, there are no clear research results indicating this.

Tyrosine (Tyr/Y) is a non-essential amino acid coded by UAC and UAU. It is a target for phosphorylation in proteins by tyrosine protein kinases and plays a role in signaling processes. In dopaminergic cells of the brain, tyrosine hydroxylase converts tyrosine to l-dopa, an immediate precursor of dopamine. Dopamine, in turn, is a precursor of norepinephrine and epinephrine. Tyrosine is also a precursor of thyroid hormones and melanin.

Hydroxyl amino acids

Serine (Ser/S) is one of three amino acids having an R-group with a hydroxyl in it (threonine and tyrosine are the others). It is coded by UCU, UCC, UCA, UGC, AGU, and AGC. Being able to hydrogen bond with water, it is classified as a polar amino acid (Fig 3.6). It

is not essential for humans. Serine is precursor of many important cellular compounds, including purines, pyrimidines, sphingolipids, folate, and of the amino acids glycine, cysteine, and tryptophan. The hydroxyl group of serine in proteins is a target for phosphorylation by certain protein kinases. Serine is also a part of the catalytic triad of serine proteases.

Threonine (Thr/T) is a polar amino acid that is essential. It is one of three amino acids bearing a hydroxyl group (serine and tyrosine are the others) and, as such, is a target for phosphorylation in proteins. It is also a target for Oglycosylation of proteins. Threonine proteases use the hydroxyl group of the amino acid in their catalysis and it is a precursor in one biosynthetic pathway for making glycine. In some applications, it is used as a pro-drug to increase brain glycine levels. Threonine is encoded in the genetic code by ACU, ACC, ACA, and ACG.

Tyrosine (Tyr/Y) is a non-essential amino acid coded by UAC and UAU. It is a target for phosphorylation in proteins by tyrosine protein kinases and plays a role in signaling processes. In dopaminergic cells of the brain, tyrosine hydroxylaseconverts tyrosine to ldopa, an immediate precursorof dopamine. Dopamine,in turn, is a precursor of norepinephrine and epinephrine. Tyrosine is also a precursor of thyroid hormones and melanin.

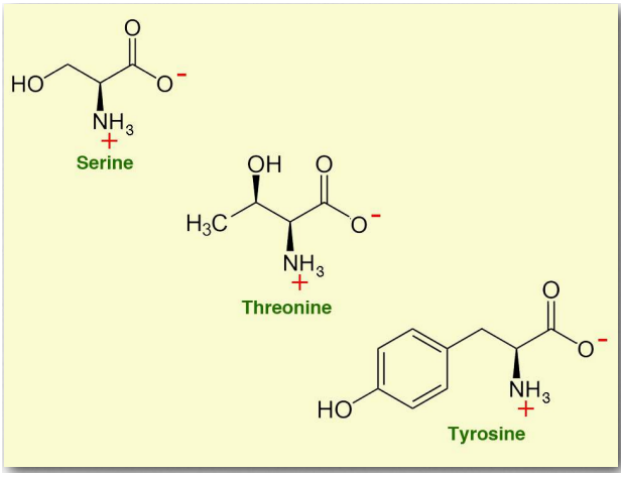


Fig 3.6 Hydroxyl amino acids

Other amino acids

Asparagine (Asn/N) is a non-essential amino acid coded by AAU and AAC. Its carboxyamide in the R-group gives it polarity. Asparagine is implicated in formation of acrylamide in foods cooked at high temperatures (deep frying) when it reacts with carbonyl groups. Asparagine can be made in the body from aspartate by an amidation reaction with an amine from glutamine. Breakdown of asparagine produces malate, which can be oxidized in the citric acid cycle. (Fig 3.7)

Cysteine (Cys/C) is the only amino acid with a sulfhydryl group in its side chain. It is nonessential for most humans, but may be essential in infants, the elderly and individuals who suffer from certain metabolic diseases. Cysteine's sulfhydryl group is readily oxidized to a disulfide when reacted with another one. In addition to being found in proteins, cysteine is also a component of the tripeptide, glutathione. Cysteine is specified by the codons UGU and UGC.

Glutamine (Gln/Q) is an amino acid that is not normally essential in humans, but may be in individuals undergoing intensive athletic training or with gastrointestinal disorders. It has a carboxyamide side chain which does not normally ionize under physiological pHs, but which gives polarity to the side chain. Glutamine is coded for by CAA and CAG and is readily made by amidation of glutamate. Glutamine is the most abundant amino acid in circulating blood and is one of only a few amino acids that can cross the blood-brain barrier.

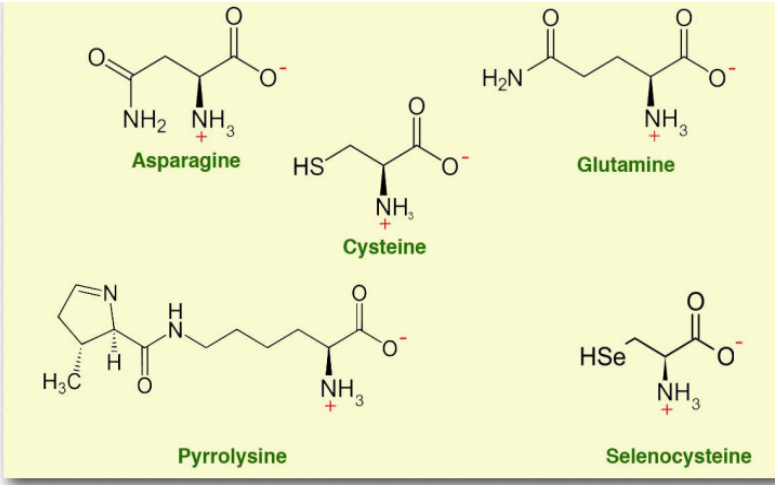


Fig 3.7 Other amino acids

Selenocysteine (Sec/U) is a component of selenoproteins found in all kingdoms of life. It is a component in several enzymes, including glutathione peroxidases and thioredoxin

reductases. Selenocysteine is incorporated into proteins in an unusual scheme involving the stop codon UGA. Cells grown in the absence of selenium terminate protein synthesis at UGAs. However, when selenium is present, certain mRNAs which contain a selenocysteine insertion sequence (SECIS), insert selenocysteine when UGA is encountered. The SECIS element has characteristic nucleotide sequences and secondary structure base-pairing patterns. Twenty five human proteins contain selenocysteine.

Pyrrolysine (Pyl/O) is a twenty second amino acid, but is rarely found in proteins. Like selenocysteine, it is not coded for in the genetic code and must be incorporated by unusual means. This occurs at UAG stop codons. Pyrrolysine is found in methanogenic archaean organisms and at least one methane-producing bacterium. Pyrrolysine is a component of methane-producing enzymes.

Ionizing groups

pKa values for amino acid side chains are very dependent upon the chemical environment in which they are present (Table 3.3). For example, the R-group carboxyl found in aspartic acid has a pKa value of 3.9 when free in solution, but can be as high as 14 when in certain environments inside of proteins, though that is unusual and extreme. Each amino acid has at least one ionizable amine group (α- amine) and one ionizable carboxyl group (α- carboxyl). When these are bound in a peptide bond, they no longer ionize. Some, but not all amino acids have R-groups that can ionize. The charge of a protein then arises from the charges of the α-amine group, the α- carboxyl group. and the sum of the charges of the ionized R-groups. Titration/ionization of aspartic acid is depicted in Figure 3.8. Ionization (or deionization) within a protein's structure can have significant effect on the overall conformation of the protein and, since structure is related to function, a major impact on the activity of a protein (Fig 3.8).

Table 3.3 Amino acid properties Wikipedia

Amino acid	Short	Abbrev.	Avg. mass (Da)	pI	pK_1 (α-COOH)	pK_2 (α-$^+NH_3$)
Alanine	A	Ala	89.09404	6.01	2.35	9.87
Cysteine	C	Cys	121.15404	5.05	1.92	10.70
Aspartic acid	D	Asp	133.10384	2.85	1.99	9.90
Glutamic acid	E	Glu	147.13074	3.15	2.10	9.47
Phenylalanine	F	Phe	165.19184	5.49	2.20	9.31
Glycine	G	Gly	75.06714	6.06	2.35	9.78
Histidine	H	His	155.15634	7.60	1.80	9.33
Isoleucine	I	Ile	131.17464	6.05	2.32	9.76
Lysine	K	Lys	146.18934	9.60	2.16	9.06
Leucine	L	Leu	131.17464	6.01	2.33	9.74
Methionine	M	Met	149.20784	5.74	2.13	9.28
Asparagine	N	Asn	132.11904	5.41	2.14	8.72
Pyrrolysine	O	Pyl	255.31			
Proline	P	Pro	115.13194	6.30	1.95	10.64
Glutamine	Q	Gln	146.14594	5.65	2.17	9.13
Arginine	R	Arg	174.20274	10.76	1.82	8.99
Serine	S	Ser	105.09344	5.68	2.19	9.21
Threonine	T	Thr	119.12034	5.60	2.09	9.10
Selenocysteine	U	Sec	168.053	5.47		
Valine	V	Val	117.14784	6.00	2.39	9.74
Tryptophan	W	Trp	204.22844	5.89	2.46	9.41
Tyrosine	Y	Tyr	181.19124	5.64	2.20	9.21

Most proteins have relatively narrow ranges of optimal activity that typically correspond to the environments in which they are found. It is worth noting that formation of peptide bonds between amino acids removes ionizable hydrogens from both the α- amine and α-carboxyl groups of amino acids. Thus, ionization/ deionization in a protein arises only from 1) the amino terminus; 2) carboxyl terminus; 3) R-groups; or 4) other functional groups (such as sulfates or phosphates) added to

amino acids post-translationally.

Carnitine

Not all amino acids in a cell are found in proteins. The most common examples include ornithine (arginine metabolism), citrulline (urea cycle), and carnitine (Figure 3.9). When fatty acids destined for oxidation are moved into the mitochondrion for that purpose, they travel across the inner membrane attached to carnitine. Of the two stereoisomeric forms, the L form is the active one. The molecule is synthesized in the liver from lysine and methionine.

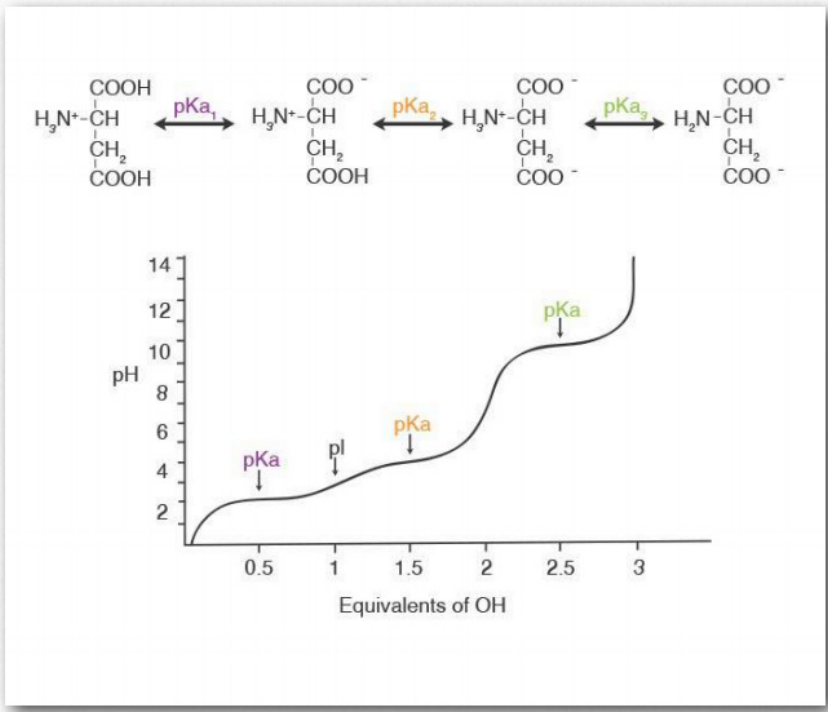


Fig 3.8 Titration curve for aspartic acid Image by Penelope Irving

From exogenous sources, fatty acids must be activated upon entry into the cytoplasm by being joined to coenzyme A. The CoA portion of the molecule is replaced by carnitine in the intermembrane space of the mitochondrion in a reaction catalyzed by carnitine acyltransferase I. The resulting acylcarnitine molecule is transferred across the inner mitochondrial membrane by the carnitin-eacylcarnitine translocase and then in the matrix of the mitochondrion, carnitine acyltransferase II replaces the carnitine with coenzyme A. We will discuss this in fat metabolism section.

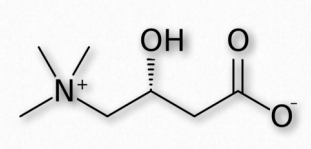
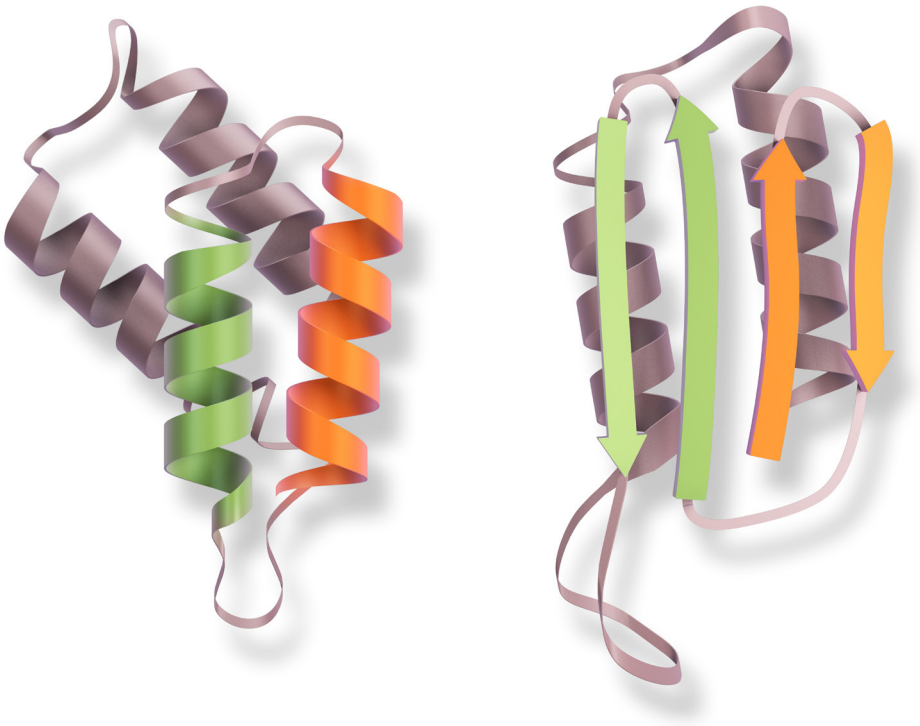


Fig 3.9 L-Carnitine

Resources:

Chapter page: Molecule of Glycine. A 3D model of a glycine amino acid in form of a schematic molecular structure freely levitating among of other organic substances. 3D-rendering graphics. Adobe stock #415629100 licensed.

Chapter #4 Proteins



Introduction

Proteins are the workhorses of the cell. Virtually everything that goes on inside of cells happens as a result of the actions of proteins. Among other things, protein enzymes catalyze the vast majority of cellular reactions, mediate signaling, give structure both to cells and to multicellular organisms, and exert control over the expression of genes. Life, as we know it, would not exist if there were no proteins. The versatility of proteins arises because of their varied structures.

Proteins are made by linking together amino acids, with each protein having a characteristic and unique amino acid sequence. To get a sense for the diversity of proteins that can be made using 20 different amino acids, consider that the number of different combinations possible with 20 amino acids is 20n, where n=the number of amino acids in the chain. It becomes apparent that even a dipeptide made of just two amino acids joined together gives us 202 = 400 different combinations. If we do the calculation for a short peptide of 10 amino acids, we arrive at an enormous 10,240,000,000,000 combinations. Most proteins are much larger than this, making the possible number of proteins with unique amino acid sequences unimaginably huge.

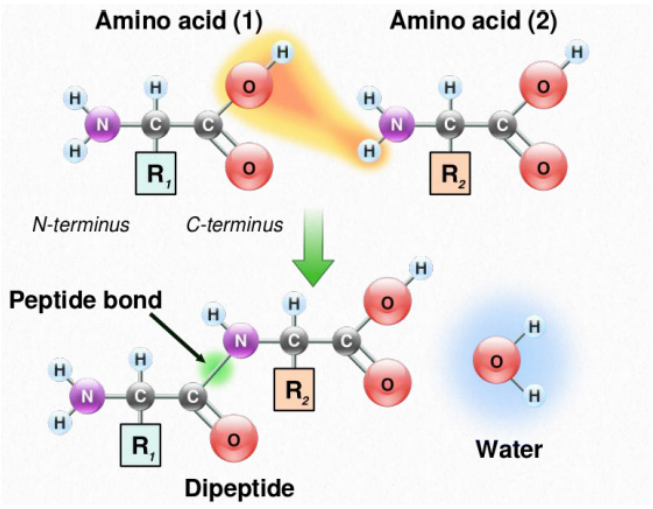


Figure 4.1 Formation of a peptide bond

Although amino acids serve other functions in cells, their most important role is as constituents of proteins. Proteins, as we noted, are polymers of amino acids.

Amino acids are linked to each other by **peptide bonds,** in which the carboxyl group of one amino acid is joined to the amino group of the next, with the loss of a molecule of water. Additional amino acids are added in the same way, by formation of peptide bonds between the free carboxyl on the end of the growing chain and the amino group of the next amino acid in the sequence. A chain made up of just a few amino acids linked together is called an oligopeptide (oligo=few) while a typical protein, which is made up of many amino acids is called a polypeptide (poly=many). The end of the peptide that has a free amino group is called the N-terminus (for NH_2), while the end with the free carboxyl is termed the C-terminus (for carboxyl).

As we've noted before, function is dependent on structure, and the string of amino acids must fold into a specific 3-D shape, or conformation, in order to make a functional protein.

Levels of Structure

The significance of the unique sequence, or order, of amino acids, known as the protein's primary structure, is that it dictates the 3-D conformation the folded protein will have. This conformation, in turn, will determine the function of the protein. We shall examine protein structure at four distinct levels (Figure 4.2) - 1) how sequence of the amino acids in a protein (primary structure) gives identity and characteristics to a protein (Figure 4.3); 2) how local interactions between one part of the polypeptide backbone and another affect protein shape (secondary structure); 3) how the polypeptide chain of a protein can fold to allow amino acids to interact with each other that are not close in primary structure (tertiary structure); and 4) how different polypeptide chains interact with each other within a multi-subunit protein (quaternary structure).

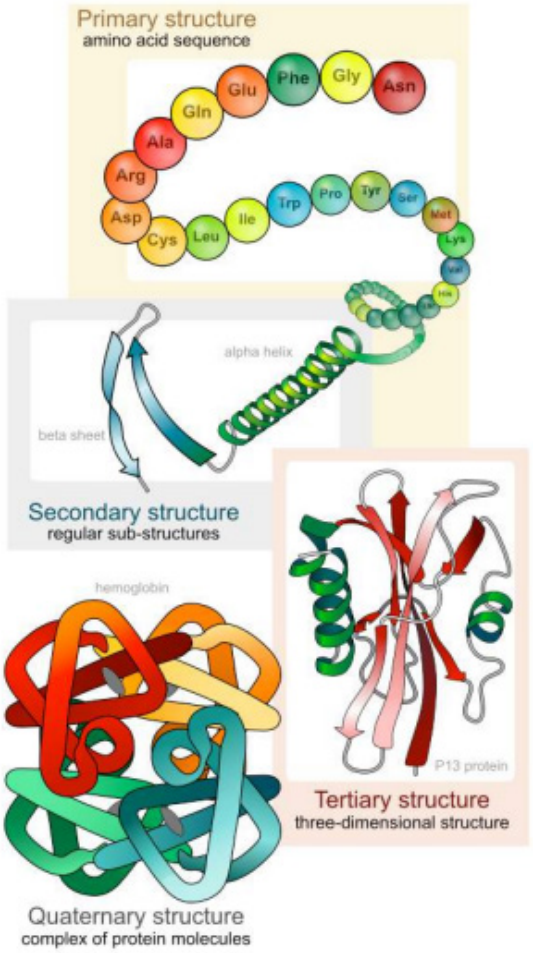


Fig 4.2 Four levels of protein structure

At this point, we should provide a couple of definitions. We use the term polypeptide to refer to a single polymer of amino acids. It may or may not have folded into its final, functional form. The term protein is sometimes used interchangeably with polypeptide, as in "protein synthesis". It is generally used, however, to refer to a folded, functional molecule that may have one or more subunits (made up of individual polypeptides). Thus, when we use the term protein, we are usually referring to a functional, folded polypeptide or peptides. Structure is essential for function. If you alter the structure, you alter the function - usually, but not always, this means you lose all function. For many proteins, it is not difficult to alter the structure.

Proteins are flexible, not rigidly fixed in structure. As we shall see, it is the flexibility of proteins that allows them to be amazing catalysts and allows them to adapt to, respond to, and pass on signals upon binding of other molecules or proteins. However, proteins are not infinitely flexible. There are constraints on the conformations that proteins can adopt and these constraints govern the conformations that proteins display.

Schematically, in Figure 4.3, we can see how sequential R-groups of a protein are arranged in an alternating orientation on either side of the polypeptide chain. Organization of R-groups in this fashion is not random. Steric hindrance can occur when consecutive R-groups are oriented on the same side of a peptide backbone (Figure 4.4)

Primary Structure

Primary structure is the ultimate determinant of the overall conformation of a protein. The primary structure of any protein arrived at its current state as a result of mutation and selection over evolutionary time. Primary structure of proteins is mandated by the sequence of DNA coding for it in the genome. Regions of DNA specifying proteins are known as coding regions (or genes).

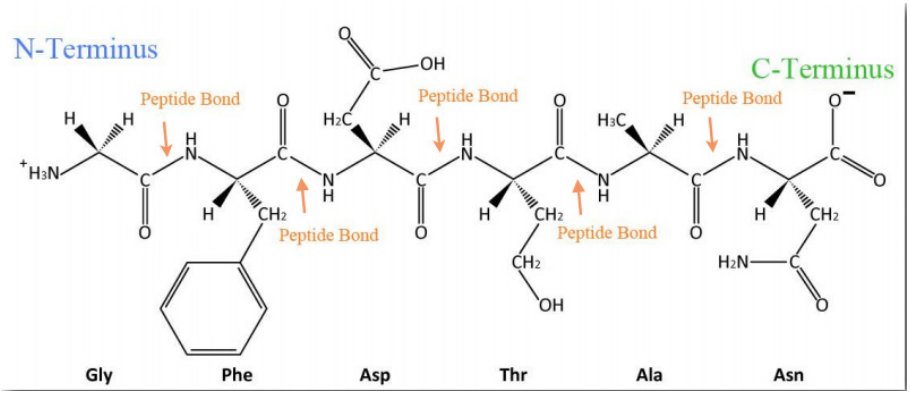


Fig 4.3 Sequence of a simple polypeptide Wikipedia

The base sequences of these regions directly specify the sequence of amino acids in proteins, with a one-to-one correspondence between the codons (groups of three consecutive bases) in the DNA and the amino acids in the encoded protein. The sequence of codons in DNA, copied into messenger RNA, specifies a sequence of amino acids in a protein.

The order in which the amino acids are joined together in protein synthesis starts defining a set of interactions between amino acids even as the synthesis is occurring. That is, a polypeptide can fold even as it is being made. The order of the R-group structures and resulting interactions are very important because early interactions affect later interactions. This is because interactions start establishing structures - secondary and tertiary. If a helical

structure (secondary structure), for example, starts to form, the possibilities for interaction of a particular amino acid Rgroup may be different than if the helix had not formed (Figure 4.4). R-group interactions can also cause bends in a polypeptide sequence (tertiary structure) and these bends can create (in some cases) opportunities for interactions that wouldn't have been possible without the bend or prevent (in other cases) similar interaction possibilities.

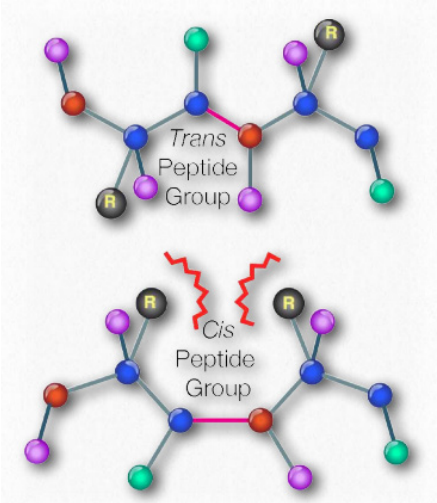


Fig 4.4 Cis vs trans orientation of R-groups around peptide bond Image by Aleia Kim

Secondary Structure

As protein synthesis progresses, interactions between amino acids close to each other begin to occur, giving rise to local patterns called secondary structure. These secondary structures include the well known α- helix and β-strands. Both were predicted by Linus Pauling, Robert Corey, and Herman Branson in 1951. Each structure has unique features.

α-helix

The α-helix (Fig 4.5) has a coiled structure, with 3.6 amino acids per turn of the helix (5 helical turns = 18 amino acids). Helices are predominantly right handed - only in rare cases, such as in sequences with many glycines can left handed α- helices form. In the α-helix, hydrogen bonds form between C=O groups and N-H groups in the polypeptide backbone that are four amino acids distant. These hydrogen bonds are the primary forces stabilizing the α-helix.

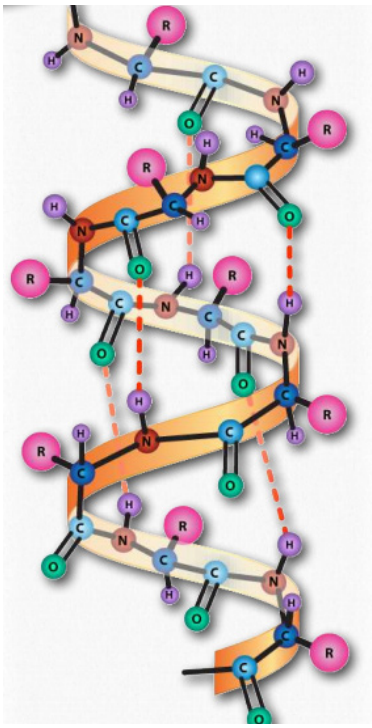


Fig 4.5 Cis vs trans orientation of R-groups around peptide bond Image by Aleia Kim

We use the terms rise, repeat, and pitch to describe the parameters of any helix. The repeat is the number of residues in a helix before it begins to repeat itself. For an α-helix, the repeat is 3.6 amino acids per turn of the helix. The rise is the distance the helix elevates with addition of each residue. For an α-helix, this is 0.15 nm per amino acid. The pitch is the distance between complete turns of the helix. For an α-helix, this is 0.54 nm. The stability of an α-helix is enhanced by the presence of the amino acid aspartate.

β strand/sheet

A helix is, of course, a three-dimensional object. A flattened form of helix in two dimensions is a common description for a β- strand. Rather than coils, β-strands have bends and these are sometimes referred to as pleats, like the pleats in a curtain. β-strands can be organized to form elaborately organized structures, such as sheets, barrels, and other arrangements.

Higher order β-strand structures are sometimes called supersecondary structures), since they involve interactions between amino acids not close in primary sequence. These structures, too, are stabilized by hydrogen bonds between carbonyl oxygen atoms and hydrogens of amine groups in the polypeptide backbone (Fig 4.6). In a higher order structure, strands can be arranged parallel (amino to carboxyl orientations the same) or anti-parallel (amino to carboxyl orientations opposite of each other (in Figure 4.6, the direction of the strand is shown by the arrowhead in the ribbon diagrams).

Turns

Turns (sometimes called reverse turns) are a type of secondary structure that, as the name suggests, causes a turn in the structure of a polypeptide chain. Turns give rise to tertiary structure ultimately, causing interruptions in the secondary structures (α- helices and β-strands) and often serve as connecting regions between two regions of secondary structure in a protein. Proline and glycine play common roles in turns, providing less flexibility (starting the turn) and greater flexibility (facilitating the turn), respectively.

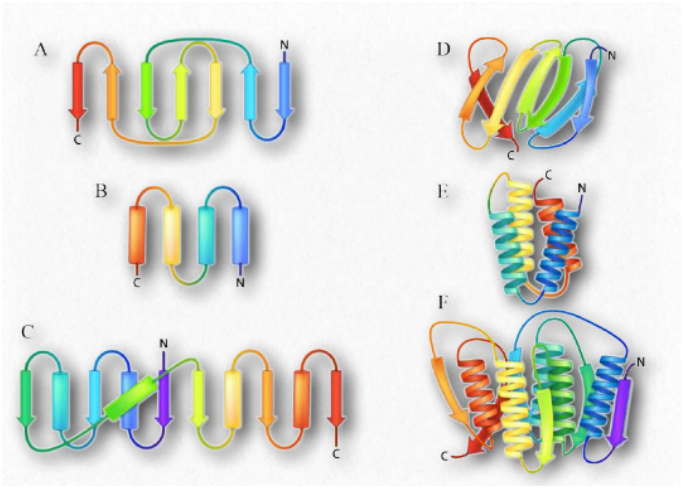


Fig 4.6 Ribbon depictions of supersecondary β-sheets (A-D) and α-helix arrangements (E-F) Image by Aleia Kim

Ramachandran plots

In 1963, G.N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan described a novel way to describe protein structure. If one considers the backbone of a polypeptide chain, it consists of a repeating set of three bonds. Sequentially (in the amino to carboxyl direction) they are 1) a rotatable bond (ψ) between α-carbon and α-carboxyl preceding the peptide bond, 2) a non-rotatable peptide bond (ω) between the α-carboxyl and α-amine groups), and 3) a rotable bond (φ) between the α-amine and α-carbon following the peptide bond . Note in Figure 4.7 that the amino to carboxyl direction is right to left.

The presence of the carbonyl oxygen on the α-carboxyl group allows the peptide bond to exist as a resonant structure, meaning that it behaves some of the time as a double bond. Double bonds cannot, of course, rotate, but the bonds on either side of it have some freedom of rotation. The φ and ψ angles are restricted to certain values, because some angles will result in steric hindrance. In addition, each type of secondary structure has a characteristic range of values for φ and ψ.

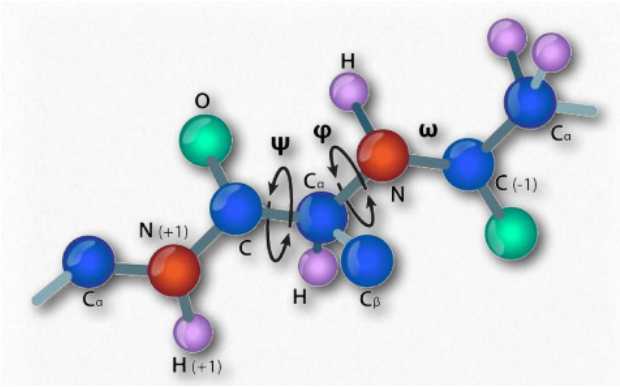
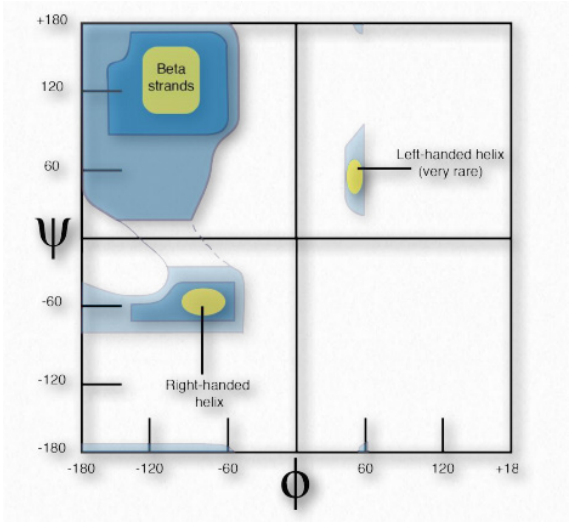


Fig 4.7 ω, ψ, and φ rotational angles in a peptide Image by Aleia Kim

Ramachandran and colleagues made theoretical calculations of the energetic stability of all possible angles from 0° to 360° for each of the φ and ψ angles and plotted the results on a Ramachandran Plot (also called a φ-ψ plot), delineating regions of angles that were theoretically the most stable (Figure 4.8). Three primary regions of stability were identified, corresponding to φ-ψ angles of β-strands (top left), right handed α- helices (bottom left), and lefthanded α-helices (upper right). The plots of predicted stability are remarkably accurate when compared to φ-ψ angles of actual proteins.

Interactive link: You can visit following link to rotate Phi and Psi angles to find angle combinations where there are no clashes of R groups. https://proteopedia.org/wiki/index.php/Tutorial:Ramachandran_principle_and_phi_psi_angles



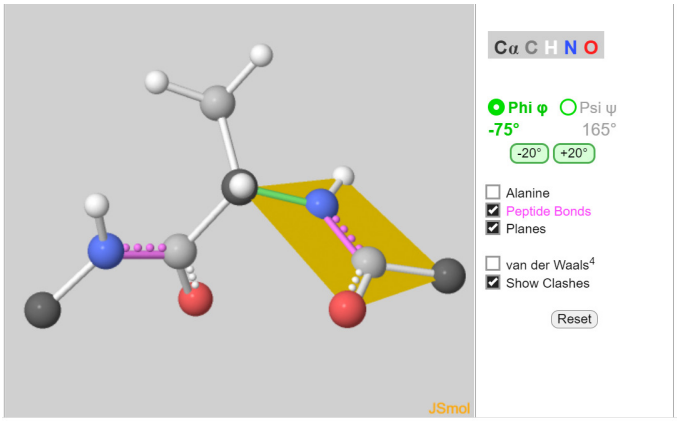


Fig 4.8 Theoretical Ramachandran plot Image by Penelope Irving

Secondary structure prediction

By comparing primary structure (amino acid sequences) to known 3D protein structures, one can tally each time an amino acid is found in an α-helix, β-strand/sheet, or a turn. Computer analysis of thousands of these sequences allows one to assign a likelihood of any given amino acid appearing in each of these structures (Table 4.1). Using these tendencies, one can, with up to 80% accuracy, predict regions of secondary structure in a protein based solely on amino acid sequence.

Amino Acid	α helix	Reverse turn	β sheet
Ala	1.41	0.82	0.72
Arg	1.21	0.90	0.84
Asn	0.76	1.34	0.48
Asp	0.99	1.24	0.39
Cys	0.66	0.54	1.40
Gln	1.27	0.84	0.98
Glu	1.59	1.01	0.52
Gly	0.43	1.77	0.58
His	1.05	0.81	0.80
Ile	1.09	0.47	1.67
Leu	1.34	0.57	1.22
Lys	1.23	1.07	0.69
Met	1.30	0.52	1.14
Phe	1.16	0.59	1.33
Pro	0.34	1.32	0.31
Ser	0.57	1.22	0.96
Thr	0.76	0.96	1.17
Trp	1.02	0.65	1.35
Tyr	0.74	0.76	1.45
Val	0.90	0.41	1.87

Table 4.1 Relative tendencies of each amino acid to be in a secondary structure. Higher values indicate greater tendency
Image by Penelope Irving

Hydrophobicity

The chemistry of amino acid Rgroups affects the structures they are most commonly found in. Subsets of their chemical properties can give clues to structure and, sometimes, cellular location. A prime example is the hydrophobicity (wateravoiding tendencies) of some R-groups (Table 4.2). Given the aqueous environment of the cell, such R-groups are not likely to be on the outside surface of a folded protein.

However, this rule does not hold for regions of protein that may be embedded within the lipid bilayers of cellular/ organelle membranes. This is because the region of such proteins that form the transmembrane domains are are buried in the hydrophobic environment in the middle of the lipid bilayer.

Supersecondary structure

Another element of protein structure is harder to categorize because it incorporates elements of secondary and tertiary structure. Dubbed supersecondary structure (or structural motifs), these structures contain multiple nearby secondary structure components arranged in a specific way and that appear in multiple proteins. Since there are many ways of making secondary structures from different primary structures, so too can similar motifs arise from different primary sequences. An example of a structural motif is shown in Figure 4.9

Amino Acid Hydropathy Scores

Amino Acid	One Letter Code	Hydropathy Score
Isoleucine	I	4.5
Valine	V	4.2
Leucine	L	3.8
Phenylalanine	F	2.8
Cysteine	C	2.5
Methionine	M	1.9
Alanine	A	1.8
Glycine	G	-0.4
Threonine	T	-0.7
Tryptophan	W	-0.9
Serine	S	-0.8
Tyrosine	Y	-1.3
Proline	P	-1.6
Histidine	H	-3.2
Glutamic acid	E	-3.5
Glutamine	Q	-3.5
Aspartic acid	D	-3.5
Asparagine	N	-3.5
Lysine	K	-3.9
Arginine	R	-4.5

Table 4.2

Tertiary structure

Proteins are distinguished from each other by the sequence of amino acids comprising them. The sequence of amino acids of a protein determines protein shape, since the chemical properties of each amino acid are forces that give rise to intermolecular interactions to begin to create secondary structures, such as α-helices and β-strands. The sequence also defines turns and random coils that play important roles in the process of protein folding.

Since shape is essential for protein function, the sequence of amino acids gives rise to all of the properties a protein has. As protein synthesis proceeds, individual components of secondary structure start to interact with each other, giving rise to folds that bring amino acids close together that are not near each other in primary structure. At the tertiary level of structure, interactions among the R-groups of the amino acids in the

Fig 4.9 Ribbon depiction of a β-hairpin. Shown are two β strands in turquoise interacting with each other.

protein, as well as between the polypeptide backbone and amino acid side groups play a role in folding (Fig 4.10).

Globular proteins

Folding gives rise to distinct 3-D shapes in proteins that are non-fibrous. These proteins are called globular. A globular protein is stabilized by the same forces that drive its formation. These include ionic interactions, hydrogen bonding, hydrophobic forces, ionic bonds, disulfide bonds and metallic bonds. Treatments such as heat, pH changes, detergents, urea and mercaptoethanol overpower the stabilizing forces and cause a protein to unfold, losing its structure and (usually) its function (Figure 4.11). The ability of heat and detergents to denature proteins is why we cook our food and wash our hands before eating - such treatments denature the proteins in the microorganisms on our hands. Organisms that live in environments of high temperature (over 50°C) have proteins with changes in stabilizing forces - additional hydrogen bonds, additional salt bridges (ionic interactions), and compactness may all play roles in keeping these proteins from unfolding.

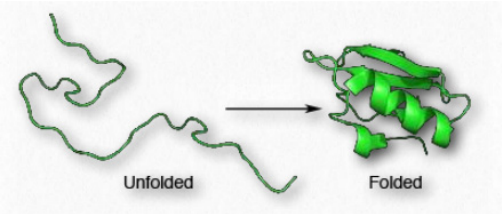


Fig 4.10 Ribbon depiction of a β-hairpin. Shown are two β strands in turquoise interacting with each other.

Protein stabilizing forces

Before considering the folding process, let us consider some of the forces that help to stabilize proteins.

Hydrogen bonds

Hydrogen bonds arise as a result of partially charged hydrogens found in covalent bonds. This occurs when the atom the hydrogen is bonded to has a greater electronegativity than hydrogen itself does, resulting in hydrogen having a partial positive charge because it is not able to hold electrons close to itself. Hydrogen partially charged in this way is attracted to atoms, such as oxygen and nitrogen that have partial negative charges, due to having greater electronegativities and thus holding electrons closer to themselves. The partially positively charged hydrogens are called donors, whereas the partially negative atoms they are attracted to are called acceptors. Hydrogen bonds are important forces in biopolymers that include DNA, proteins, and cellulose. All of these polymers lose their native structures upon boiling. Hydrogen bonds between amino acids that are close to each other in primary structure can give rise to regular repeating structures, such as helices or pleats, in proteins (secondary structure)

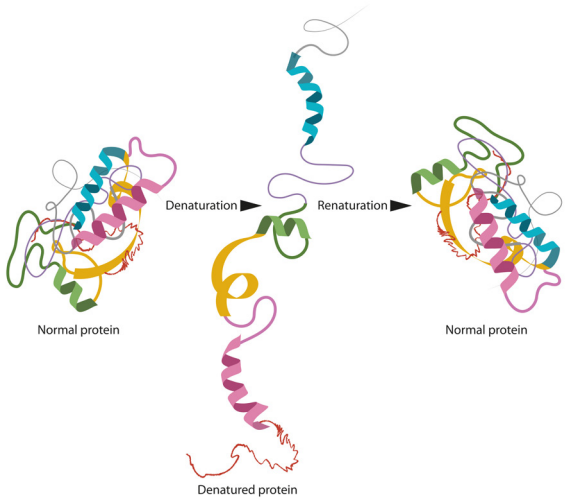


Fig 4.11 Proteins can be denatured and renatured

Ionic interactions

Ionic interactions are important forces stabilizing protein structure that arise from ionization of R-groups in the amino acids comprising a protein. These include the carboxyl amino acids, the amine amino acids as well as the sulfhydryl of cysteine and sometimes the hydroxyl of tyrosine.

Hydrophobic forces

Hydrophobic forces stabilize protein structure as a result of interactions that favor the exclusion of water. Non-polar amino acids (commonly found in the interior of proteins) favor associating with each other and this has the effect of excluding water. The excluded water has a higher entropy than water interacting with the hydrophobic side chains. This is because water aligns itself very regularly and in a distinct pattern when interacting with hydrophobic molecules.

When water is prevented from having these kinds of interactions, it is much more disordered that it would be if it could associate with the hydrophobic regions. It is partly for this reason that hydrophobic amino acids are found in protein interiors - so they can exclude water and increase entropy.

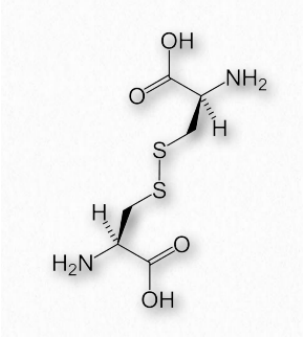


Fig 4.12 Disulfide bond

Disulfide bonds

Disulfide bonds, which are made when two sulfhydryl side-chains of cysteine are brought into close proximity, covalently join together different protein regions and can give great strength to the overall structure (Figures 4.12 & 4.13). These joined residues of cysteine are sometimes referred to as cystine. Disulfide bonds are the strongest of the forces stabilizing protein structure.

van der Waals forces

Van der Waals forces is a term used to describe various weak interactions, including those caused by attraction between a polar molecule and a transient dipole, or between two temporary dipoles. van der Waals forces are dynamic because of the fluctuating nature of the attraction, and are generally weak in comparison to covalent bonds, but can, over very short distances, be significant.

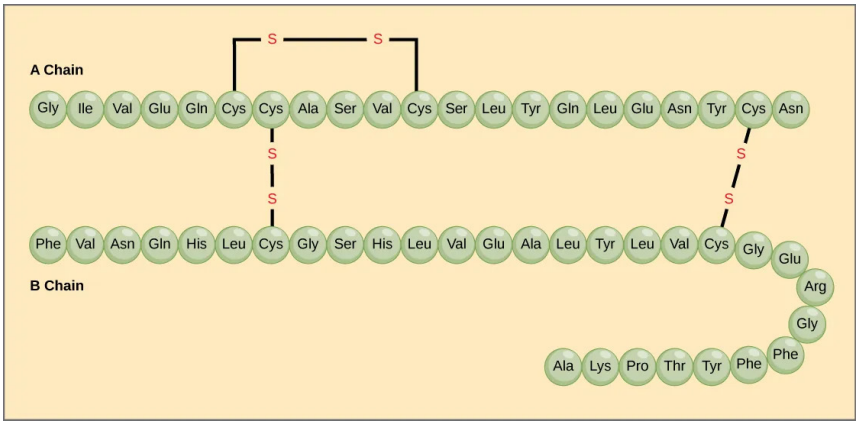


Fig 4.13 Bovine serum insulin is a protein hormone comprised of two peptide chains labelled as A and B. The three-letter abbreviations represent the amino acids' names in the order they are present indicate primary structure. The amino acid cysteine (cys) has a sulfhydryl (SH) group as a side chain. Two sulfhydryl groups can react in the presence of oxygen to form a disulfide (S-S) bond. Two disulfide bonds connect the A and B chains together, and a third helps the A chain fold into the correct shape. Note that all disulfide bonds are the same length, but have been drawn different sizes for clarity. Source Open Stax: Biology 2e

Quaternary structure

A fourth level of protein structure is that of quaternary structure. It refers to structures that arise as a result of interactions between multiple polypeptides. The units can be identical multiple copies or can be different polypeptide chains. Adult hemoglobin is a good example of a protein with quaternary structure, being composed of two identical chains called α and two identical chains called β. Alhough the α-chains are very similar to the β-chains, they are not identical. Both of the α- and the β-chains are also related to the single polypeptide chain in the related protein called myoglobin. Both myoglobin and hemoglobin have similarity in binding oxygen, but their behavior towards the molecule differ significantly. Notably, hemoglobin's multiple subunits (with quaternary structure) compared to myoglobin's single subunit (with no quaternary structure) give rise to these differences .

Protein misfolding

The proper folding of proteins is essential to their function. Misfolding of proteins (also called proteopathy) might have consequences

In some cases, this might simply result in an inactive protein. Protein misfolding also plays a role in numerous diseases, such as Mad Cow Disease, Alzheimers, Parkinson's Disease, and CreutzfeldJakob disease. Many, but not all, misfolding diseases affect brain tissue.

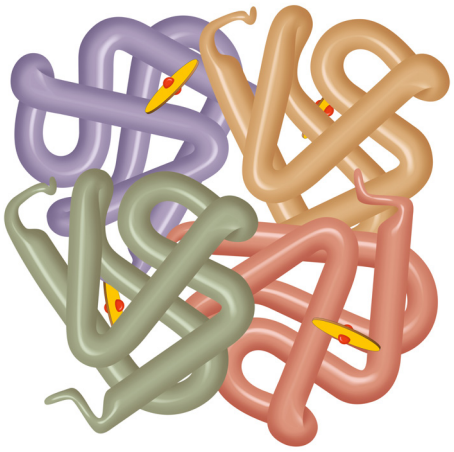
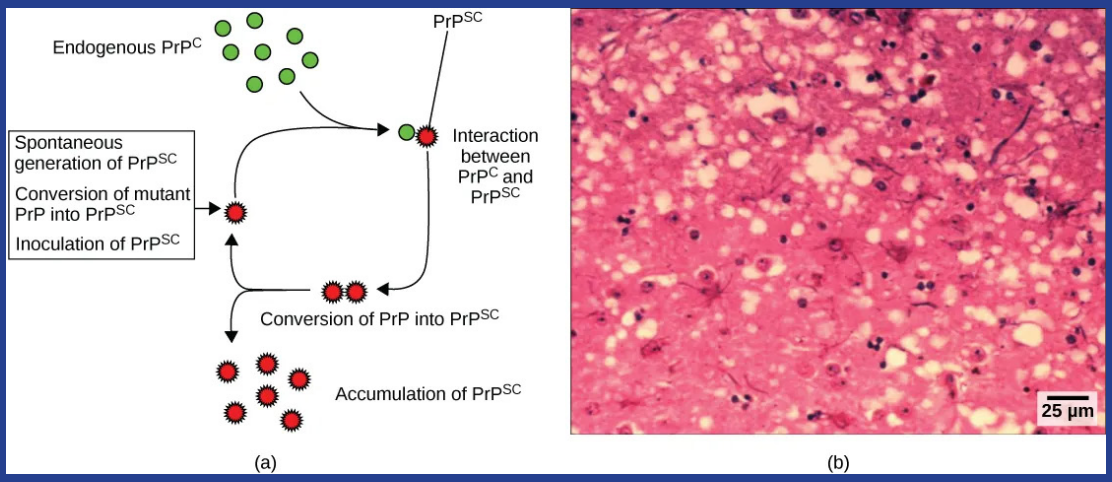


Fig 4.14 An artist's rendering of Quaternary structure of hemoglobin. Red dots reperesent iron.

Insoluble deposits

Misfolded proteins will commonly form aggregates called amyloids that are harmful to tissues containing them because they change from being soluble to insoluble in water and form deposits. The process by which misfolding occurs is not completely clear, but in many cases, it has been demonstrated that a "seed" protein which is misfolded can induce the same misfolding in other copies of the same protein. These seed proteins are known as prions and they act as infectious agents, resulting in the spread of disease. The list of human diseases linked to protein misfolding is long and continues to grow.

BSE was initially thought to only affect cattle. Cattle dying of the disease were shown to have developed lesions or "holes" in the brain, causing the brain tissue to resemble a sponge, hence the name Bovine Spongiform Encephalopathy. Later on in the outbreak, however, it was shown that a similar encephalopathy in humans, known as variant Creutzfeldt-Jakob disease (CJD), could be acquired from eating beef from animals infected with BSE. Indentification of BSE aka Mad cow disease in Alberta sparked bans by various countries on the importation of Alberta beef, causing considerable economic damage to the Candian beef industry. The cause of spongiform encephalopathies, such as kuru and BSE, is an infectious structural variant of a normal cellular protein called PrP (prion protein). It is this variant that constitutes the prion particle. PrPSc is the name given to a misfolded form of the same protein, that is associated with the development of disease symptoms. A third form of PrP, called PrPres is not infectious. The 'res' of PrPres indicates it is protease resistant. It is worth noting that all three forms of PrP have the same amino acid sequence and differ from each other only in the ways in which the polypeptide chains are folded.



BOX 4.1: Mad Cow Disease in humans. (a) Endogenous normal prion protein (PrPc) is converted into the disease-causing form (PrPsc) when it encounters this variant form of the protein. PrPsc may arise spontaneously in brain tissue, especially if a mutant form of the protein is present, or it may occur via the spread of misfolded prions consumed in food into brain tissue. (b) This prion-infected brain tissue, visualized using light microscopy, shows the vacuoles that give it a spongy texture, typical of transmissible spongiform encephalopathies. (credit b: modification of work by Dr. Al Jenny, USDA APHIS; scale-bar data from Matt Russell) Adapted from OpenStax: Biology 2e

Resources:

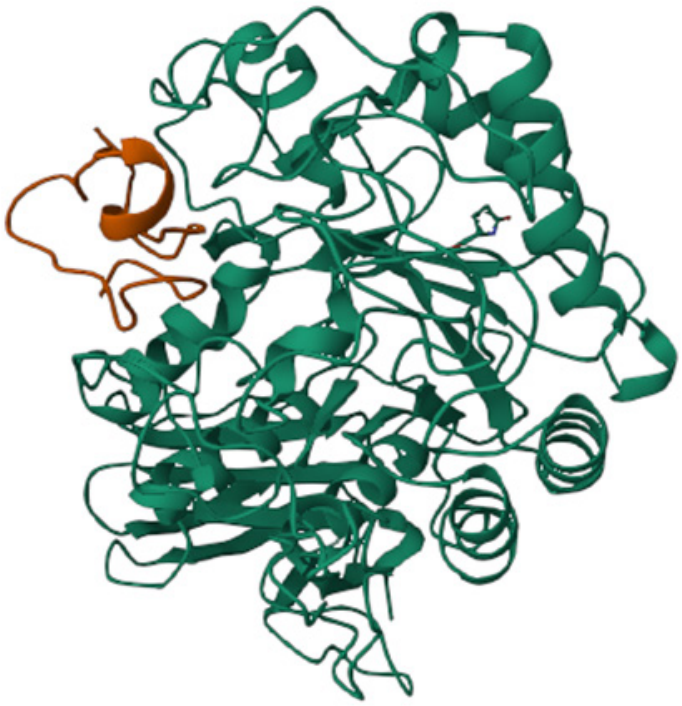
Chapter image: 3D Illustration Rendering. Prions, Prion protein mutation before and after. Biotechnology concept of Mad Cow, Illness of medical structure, bioinformatics medical design. Adobe stock # 332734875. Licensed.

Fig 4.11 Structure of normal and disassembled protein. Adobe stock # 361751370

Fig 4.14 Structure of human hemoglobin molecule. Adobe stock #486304832 licensed.

Chapter #5 Protein function

Enzyme: Catalysis



Introduction

Proteins are one of the most abundant organic molecules in living systems and have the most diverse range of functions of all macromolecules. Proteins may be structural, regulatory, contractile, or protective. They may serve in transport, storage, or membranes; or they may be toxins or enzymes. Each cell in a living system may contain thousands of proteins, each with a unique function. Their structures, like their functions, vary greatly. They are all, however, amino acid polymers arranged in a linear sequence.

We will discuss enzymes in this chapter. However, before discussing enzymes, it is appropriate to pause and discuss an important concept relating to chemical/biochemical reactions. That concept is equilibrium and it is very often misunderstood. The "equi" part of the word relates to equal, as one might expect, but it does not relate to absolute concentrations. What happens when a biochemical reaction is at equilibrium is that the concentrations of reactants and products do not change over time. This does not mean that the reactions have stopped. Remember that reactions are reversible, so there is a forward reaction and a reverse reaction: if you had 8 molecules of A, and 4 of B at the beginning, and 2 molecules of A were converted to B, while 2 molecules of B were simultaneously converted back to A, the number of molecules of A and B remain unchanged, i.e., the reaction is at equilibrium. However, you will notice that this does not mean that there are equal numbers of A and B molecules.

Activation energy

Figure 5.1 schematically depicts the energy changes that occur during the progression of a simple reaction. In order for the reaction to proceed, an activation energy must be overcome in order for the reaction to occur. In Figure 5.2, the activation energy for a catalyzed reaction is overlaid. As you can see, the reactants start at the same energy level for both catalyzed and uncatalyzed reactions and that the products end at the same energy for both as well. The catalyzed reaction, however, has a lower energy of activation (dotted line) than the uncatalyzed reaction. This is the secret to catalysis - overall ΔG for a reaction does NOT change with catalysis, but the activation energy is lowered.

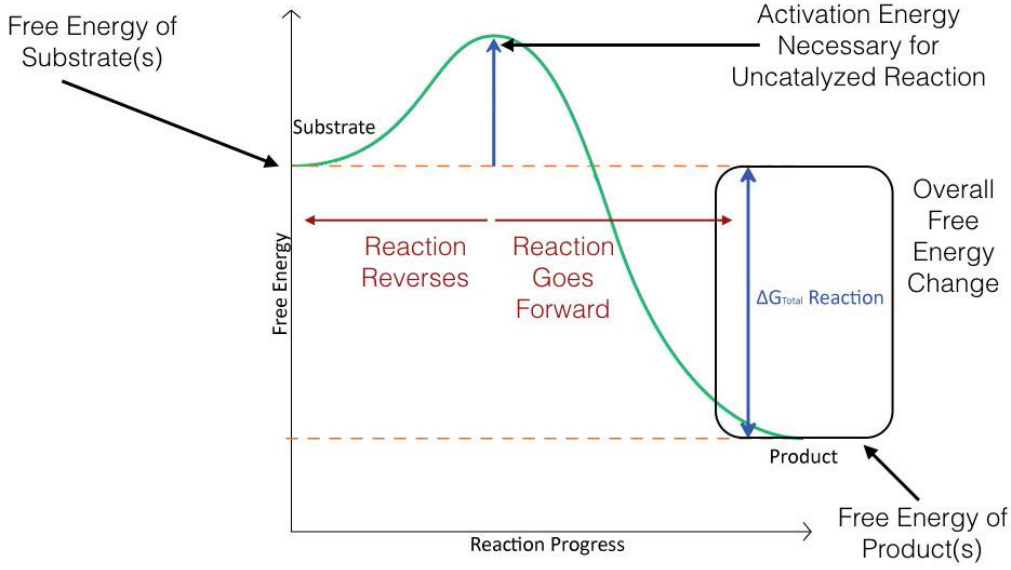


Fig 5.1 Energy changes during the course of an uncatalyzed reaction. Image by Aleia Kim

Reversibility

The extent to which reactions will proceed forward is a function of the size of the energy difference between the product and reactant states. The lower the energy of the products compared to the reactants, the larger the percentage of molecules that will be present as products at equilibrium. It is worth noting that since an enzyme lowers the activation energy for a reaction that it can speed the reversal of a reaction just as it speeds a reaction in the forward direction. At equilibrium, of course, no change in concentration of reactants and products occurs. Thus, enzymes speed the time required to reach equilibrium, but do not affect the balance of products and reactants at

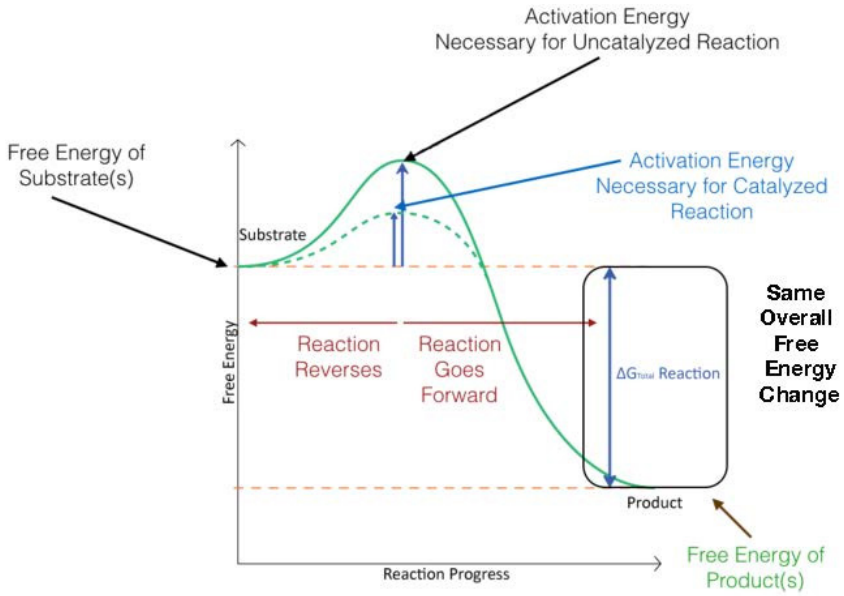


Table 5.2 Energy changes during the course of an uncatalyzed reaction (solid green line) and a catalyzed reaction (dotted green line). Image by Aleia Kim

equilibrium.

Exceptions

The reversibility of enzymatic reactions is an important consideration for equilibrium, the measurement of enzyme kinetics, for Gibbs free energy, for metabolic pathways, and for physiology. There are some minor exceptions to the reversibility of reactions, though. They are related to the disappearance of a substrate or product of a reaction. Consider the first reaction below which is catalyzed by the enzyme carbonic anhydrase:

$$CO_2+H_2O \rightleftharpoons H_2CO \rightleftharpoons HCO^-_3+H^+$$

In the forward direction, carbonic acid is produced from water and carbon dioxide. It can either remain intact in the solution or ionize to produce bicarbonate ion and a proton. In the reverse direction, water and carbon dioxide are produced. Carbon dioxide, of course, is a gas and can leave the solution and escape.

When reaction molecules are removed, as they would be if carbon dioxide escaped, the reaction is pulled in the direction of the molecule being lost and reversal cannot occur unless the missing molecule is replaced. In the second reaction occurring on the right, carbonic acid (H_2CO_3) is "removed" by ionization. This too would limit the reaction going back to carbon dioxide in water. This last type of "removal" is what occurs in metabolic pathways. In this case, the product of one reaction (carbonic acid) is the substrate for the next (formation of bicarbonate and a proton).

In the metabolic pathway of glycolysis, ten reactions are connected in this manner and reversing the process is much more complicated than if just one reaction was being considered.

General mechanisms of action

As noted above, enzymatically catalyzed reactions are orders of magnitude faster than uncatalyzed and chemical-catalyzed reactions. The secret of their success lies in a fundamental difference in their mechanisms of action.

Every chemistry student has been taught that a catalyst speeds a reaction without being consumed by it. In other words, the catalyst ends up after a reaction just the way it started so it can catalyze other reactions, as well. Enzymes share this property, but in the middle, during the catalytic action, an enzyme is transiently changed. In fact, it is the ability of an enzyme to change that leads to its incredible efficiency as a catalyst.

Changes

These changes may be subtle electronic ones, more significant covalent modifications, or structural changes arising from the flexibility inherent in enzymes, but not present in chemical catalysts. Flexibility allows movement and movement facilitates alteration of electronic environments necessary for catalysis. Enzymes are, thus, much more efficient than rigid

chemical catalysts as a result of their abilities to facilitate the changes necessary to optimize the catalytic process.

Substrate binding

Another important difference between the mechanism of action of an enzyme and a chemical catalyst is that an enzyme has binding sites that not only 'grab' the substrate (molecule involved in the reaction being catalyzed), but also place it in a position to be electronically induced to react, either within itself or with another substrate. The enzyme itself may play a role in the electronic induction or the induction may occur as a result of substrates being placed in very close proximity to each other. Chemical catalysts have no such ability to bind substrates and are dependent upon them colliding in the right orientation at or near their surfaces.

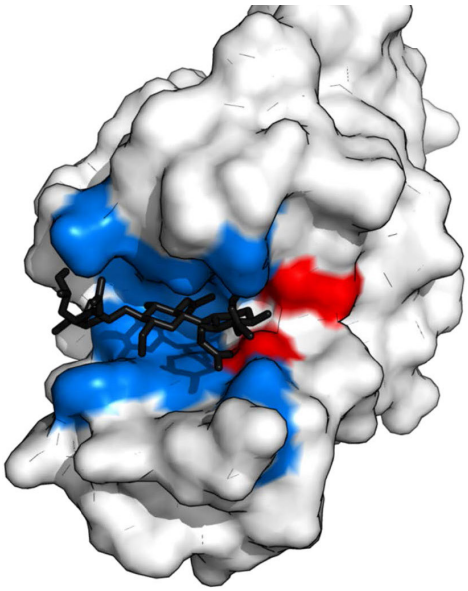


Fig 5.3 Lysozyme with substrate binding site (blue), active site (red) and bound substrate (black). Wikipedia

Active site

Reactions in an enzyme are catalyzed at a specific location within it known as the 'active site'. Substrates bind at the active site and are oriented to provide access for the relevant portion of the molecule to the electronic environment of the enzyme where catalysis occurs.

Enzyme flexibility

A difference between an enzyme and a chemical catalyst is that an enzyme is flexible. Its slight changes in shape (often arising from the binding of the substrate itself) help to optimally position substrates for reaction after they bind. (Fig 5.3)

Induced fit

These changes in shape are explained, in part, by Koshland's Induced Fit Model of Catalysis (Figure 5.4), which illustrates that not only do enzymes change substrates, but that substrates also transiently change enzyme structure. At the end of the catalysis, the enzyme is returned to its original state. Koshland's model is in contrast to the Fischer Lock and Key model, which says simply that an enzyme has a fixed shape that is perfectly matched for binding its substrate(s). Enzyme flexibility also is important for control of enzyme activity. Enzymes alternate between the T (tight) state, which is a lower activity state and the R (relaxed) state, which has greater activity.

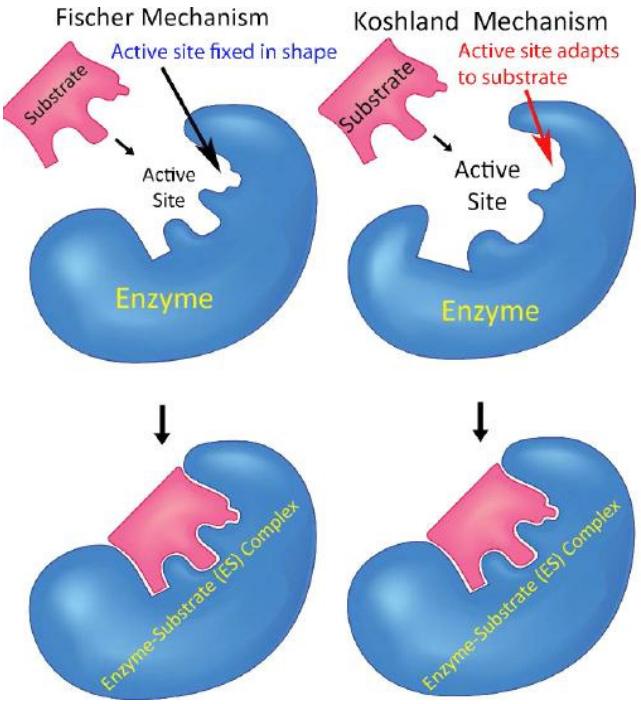


Fig 5.4 Fischer's lock and key model (left) Vs. Koshland's induced fit model (right). Image by Aleia Kim

The Koshland Induced Fit model of catalysis postulates that enzymes are flexible and change shape on binding substrate. Changes in shape help to 1) aid binding of additional substrates in reactions involving more than one substrate and/or 2) facilitate formation of an electronic environment in the enzyme that favors catalysis. This model is in contrast to the Fischer Lock and Key Model of catalysis which considers enzymes as having pre-formed substrate binding sites.

Ordered binding

The Koshland model is consistent with multi-substrate binding enzymes that exhibit ordered binding of substrates. For these systems, binding of

the first substrate induces structural changes in the enzyme necessary for binding the second substrate.

There is considerable experimental evidence supporting the Koshland model. Hexokinase, for example, is one of many enzymes known to undergo significant structural alteration after binding of substrate. In this case, the two substrates are brought into very close proximity by the induced fit and catalysis is made possible as a result.

Reaction types

Enzymes that catalyze reactions involving more than one substrate, such as

$A+B \rightleftharpoons C+D$

can act in two different ways. Enzymatic reactions can be of several types, as shown in Figure 4.7. In one mechanism, called sequential reactions, at some point in the reaction, both substrates will be bound to the enzyme. There are, in turn, two different ways in which this can occur - random and ordered.

Single Substrate - Single Product : $A \rightleftharpoons B$

Single Substrate - Multiple Products : $A \rightleftharpoons B + C$

Multiple Substrates - Single Products : $A + B \rightleftharpoons C$

Multiple Substrates - Multiple Products : $A + B \rightleftharpoons C + D$

Consider lactate dehydrogenase, which catalyzes the reaction below:

$NADH+Pyruvate \rightarrow Lactate+NAD^+$

This enzyme requires that NADH must bind prior to the binding of pyruvate. As noted earlier, this is consistent with an induced fit model of catalysis. In this case, binding of the NADH changes the enzyme shape/environment so that pyruvate can bind and without binding of NADH, the substrate cannot access the pyruvate binding site. This type of multiple substrate reaction is called *sequential ordered binding*, because the binding of substrates must occur in the right order for the reaction to proceed.

Random binding

A second mechanism of binding/catalysis is exhibited by creatine kinase which catalyzes the following reaction:

$Creatine+ATP \rightarrow Creatine\ phosphate+ADP$

For this enzyme, substrates can bind to it in any order. Creatine kinase displays sequential random binding. It is worth mentioning that random binding is not inconsistent with Koshland's induced fit model. Rather, random binding simply means that the enzyme's induced fit doesn't affect substrate binding sites and involves other parts of the enzyme. In summary, sequential binding can occur as ordered binding or as random binding.

Double displacement reactions

Not all enzymes that catalyze multi-substrate reactions, though, bind A and B by the sequential mechanisms above. This other category of enzyme includes those that exhibit what are called "ping-pong" (or double displacement) mechanisms. In these enzymes, the

enzyme functions as both a catalyst and a carrier of a group between individually bound substrates. Examples of this type of enzyme include the class of enzymes known as transaminases. A general form of the reactions catalyzed by these enzymes is shown in Figure 5.5

In reversible transaminase reactions, an oxygen and an amine are swapping between the molecules. It can be represented as follows (where N is the amine and O is the oxygen).

A=O + C=N ⇌ B-N + D=O

This reaction occurs not as one transfer reaction swapping the N and the O, but rather as a set of two half-reactions. In this case, the enzyme is a both donor and a carrier of the group being swapped. The first half-reaction goes as follows

A=O + Enz-N⇌B-N + Enz=O

Next a second half-reaction goes as

C-N + Enz=O ⇌ D=O + Enz=N

The sum of these half-reactions then is

A=O + C=N⇌B-N + D=O

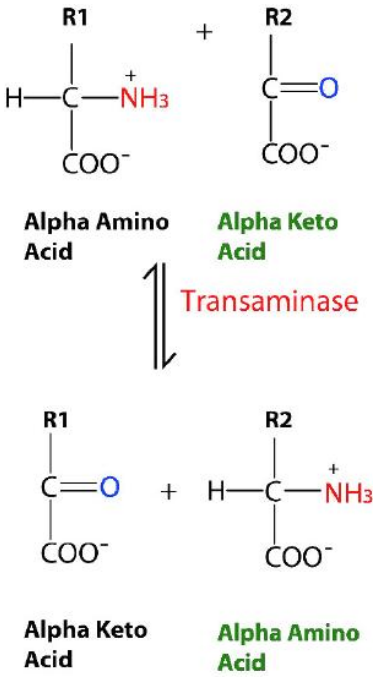


Fig 5.5 Double displacement reaction mechanism of a transaminase

Note that at no time did the enzyme bind both A and C simultaneously. It is also important to recognize that the enzyme existed in two states - Enz=O and Enz-N. The shuffling of the enzyme between these two states is what gives rise to the ping-pong name of this

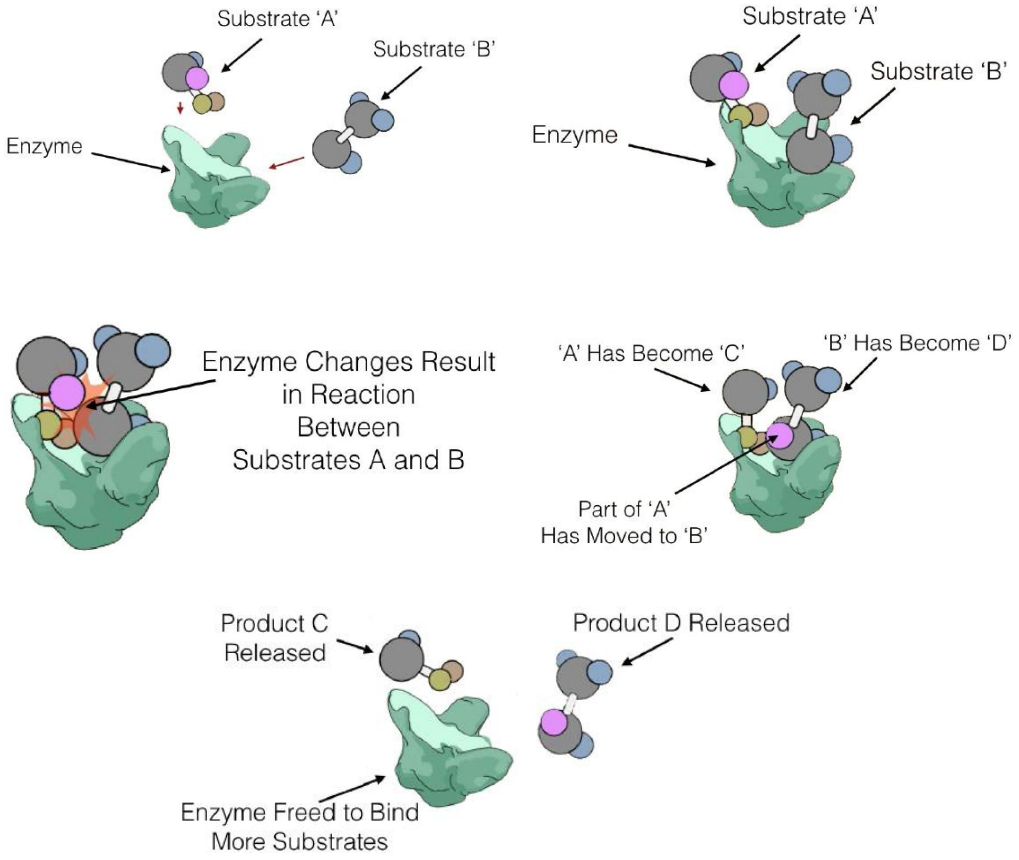


Fig 5.6 From left to right

1) Binding of substrates (A+B) to free enzyme

2) ES complex formation

3) ES* complex - The reaction occurs

4) EP complex - the reaction is complete.

5) E + P - the products are released

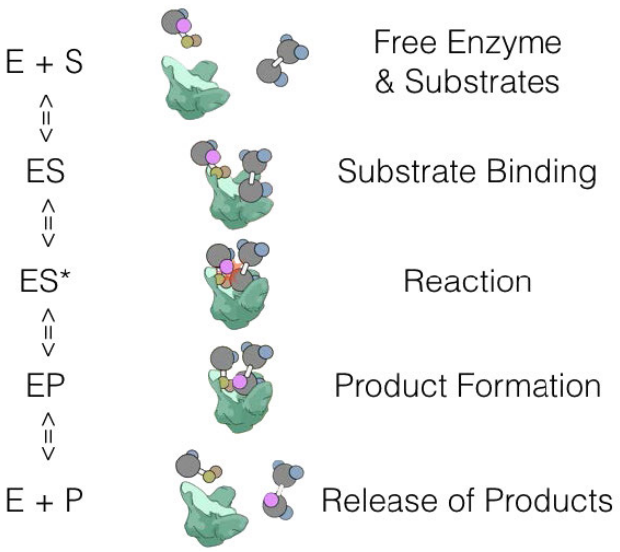


Fig 5.8 Summary of enzyme substrate reactions

mechanism - it literally goes back and forth like a ping-pong ball in a table tennis match.

Enzyme kinetics

To understand how an enzyme enhances the rate of a reaction, we must understand enzyme kinetics. We present a model here proposed by Leonor Michaelis and Maud Menten. In order to understand the model, it is necessary to understand a few parameters.

First, we describe a reaction in simple terms proceeding as follows

$$E + S \rightarrow ES \rightarrow E + P$$

where E is enzyme, S is substrate, and P is product. In this scheme, ES is the Enzyme-Substrate complex, which is simply the enzyme bound to its substrate.

We could define the ES state a bit further with

$$E + S \rightarrow ES \rightarrow ES^* \rightarrow EP \rightarrow E + P$$

where ES* is the activated state and EP is the enzyme-product complex before release of the product.

The first consideration we have is velocity. The velocity of a reaction is the rate of creation of product over time, measured as the concentration of product per time. The time is a critical consideration when measuring velocity. In a closed system (in which an enzyme operates), all reactions will advance towards equilibrium. Enzymatically catalyzed reactions are no different in the end result from non-enzymatic reactions, except that they get to equilibrium faster.

Equilibrium

At equilibrium, the ratio of product to reactant does not change. That is a property of equilibrium. Since the system is closed, the concentration of product over time will not change. The velocity will thus be zero under these conditions and we will have learned nothing about the reaction if we wait too long to study it.

Velocity

Consequently, in Michaelis-Menten kinetics, velocity is measured as initial velocity (V0). This is accomplished by measuring the rate of formation of product early in the reaction before equilibrium is established and under these conditions, there is very little if any of the reverse reaction occurring.

The other two assumptions are related. First, we use conditions where there is much more substrate than enzyme. This makes sense. If the substrate is not in great excess, then the enzyme's conversion of substrate to product will occur much faster than the enzyme can bind substrate.

Waiting for substrate

Thus, the enzyme would "wait" for substrate to bind if there were not sufficient amounts

of it to bind to the enzyme in a timely fashion (when substrate concentration is low). This would not give an accurate measure of velocity, since the enzyme would be inactive a good deal of the time. Because of this, we assume saturation of the enzyme with substrate will give a maximal velocity of the reaction.

Steady state

Last, the high concentration of substrate combined with measuring initial conditions results in studying reactions that are under so-called steady state conditions (Figure 5.9). When steady state occurs, the concentration of the ES complex over time is not significantly changing during the period of analysis (Fig 5.10).

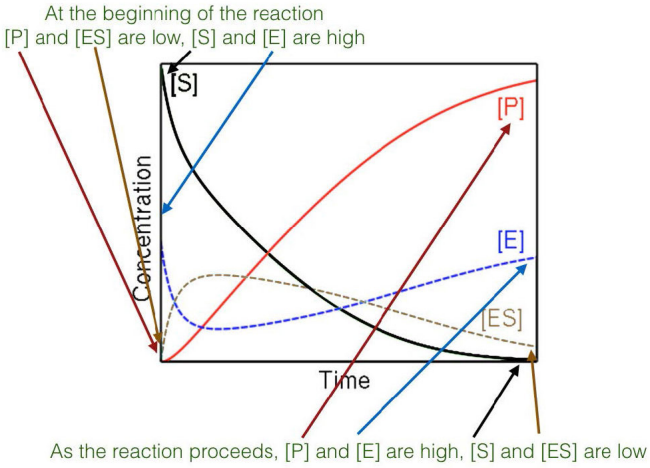


Fig 5.9 Concentration of product (P), substrate (S), enzyme (E), and enzyme-substrate complex (ES) versus time for an enzymatic reaction

Reiterating, the three assumptions for Michaelis-Menten kinetics are

- Measurement of initial velocity of a reaction
- Substrate in great excess compared to enzyme
- Reaction conditions occurring under steady state

Experimental considerations

Now we turn our attention to how studies of the kinetic properties of an enzyme are conducted. To perform an analysis, one would do the following experiment - 20 different tubes would be set up with enzyme buffer (to keep the enzyme stable), the same amount of enzyme, and then a different amount of substrate in each tube, ranging from tiny amounts in the first tubes to very large amounts in the last tubes. The reaction would be allowed to proceed for a fixed, short amount of time and then the reaction would be stopped and the amount of product contained in each tube would be determined.

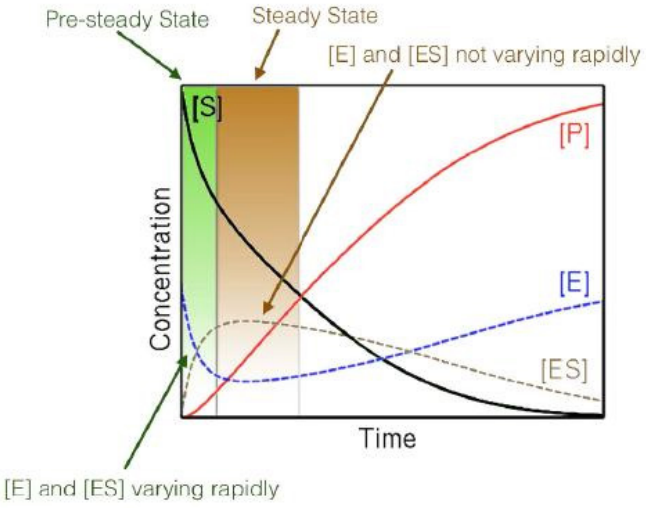


Fig 5.10 Steady state versus non-steady state conditions

The initial velocity (V0) of the reaction then would be the concentration of product found in each tube divided by the time that the reaction was allowed to run. Data from the experiment would be plotted on a graph using initial velocity (V0) on the Y-axis and the concentration of substrate on the X-axis, each tube, of course having a unique reaction velocity corresponding to a unique substrate concentration.

For an enzyme following Michaelis-Menten kinetics, a curve like that shown in Figure 5.11 or 5.12 would result. At low concentration of substrate, it is limiting and the enzyme converts it into product as soon as it can bind it. Consequently, at low concentrations of substrate, the rate of increase of [P] is almost linear with [S].

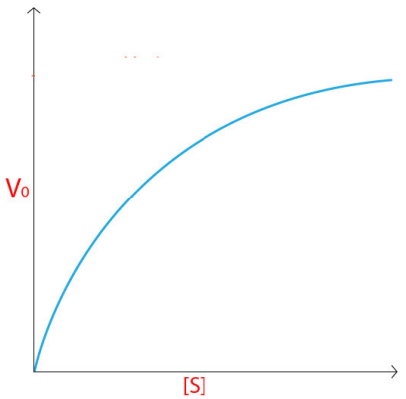


Fig 5.11 Kinetics of an enzyme. Image by Aleia Kim

Non-linear increase:

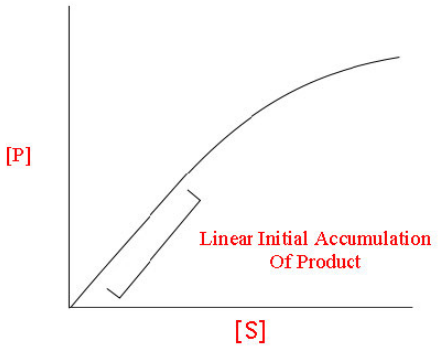


Fig 5.12 Linear relationship between [P] and [S] at low [S]

As the substrate concentration increases, however, the velocity of the reaction in tubes with higher substrate concentration ceases to increase linearly and instead begins to flatten out, indicating that as the substrate concentration gets higher and higher, the enzyme has a harder time keeping up to convert the substrate to product.

Saturation

Not surprisingly, when the enzyme becomes completely saturated with substrate, it will not have to wait for substrate to diffuse to it and will therefore be operating at maximum velocity.

For an enzyme following Michaelis-Menten kinetics will have its velocity (v) at any given substrate concentration given by the following equation:

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

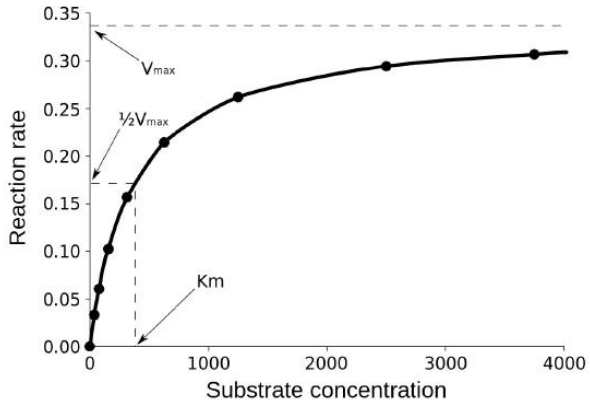


Fig 5.13 Vmax, Vmax/2, and Km

Vmax

Two terms in the equation above require explanation. The first is Vmax. It refers to the maximum velocity of an enzymatic reaction. Maximum velocity for a reaction occurs when an enzyme is saturated with substrate. Saturation is important because it means (per the assumption above) that none of the enzyme molecules are "waiting" for substrate after a product is released. Saturation ensures that another substrate is always instantly available. The unit of Vmax is concentration of product per time = [P]/time.

On a plot of initial velocity versus substrate concentration (V0 vs. [S]), Vmax is the value on the Y axis that the curve asymptotically approaches (dotted line in Figure 5.13). It should be noted that the value of Vmax depends on the amount of enzyme used in a reaction. If you double the amount of enzyme used, you will double the Vmax. If one wanted to compare the velocities of two different enzymes, it would be necessary to use the same amounts of enzyme in the reaction each one catalyzes.

Km

The second term is Km (also known as Ks). Referred to as the Michaelis constant, Km is the substrate concentration that causes the enzyme to work at half of maximum velocity (Vmax/2). What it measures, in simple terms, is the affinity an enzyme has for its substrate. The value of Km is inversely related to the affinity of the enzyme for its substrate. Enzymes with a high Km value will have a lower affinity for their substrate (will take more substrate to get to Vmax/2) whereas those with a low Km will have high affinity and take less substrate to get to Vmax/2. The unit of Km is concentration.

Affinities of enzymes for substrates vary considerably, so knowing Km helps us to understand how well an enzyme is suited to the substrate being used. Measurement of Km depends on the measurement of Vmax.

Common mistake

A common mistake students make in describing Vmax is saying that Km = Vmax /2. This

is, of course, not true. Km is a substrate concentration and is the amount of substrate it takes for an enzyme to reach Vmax /2. On the other hand Vmax /2 is a velocity and a velocity certainly cannot equal a concentration.

Kcat

It is desirable to have a measure of velocity that is independent of enzyme concentration. Remember that Vmax depended on the amount of enzyme used. For this, we use the Kcat, also known as the turnover number. Kcat is a number that requires one to first determine Vmax for an enzyme and then divide the Vmax by the concentration of enzyme used to determine Vmax. Thus,

Kcat = Vmax /[Enzyme]

Since Vmax has units of concentration per time and [Enzyme] has units of concentration, the units on Kcat are time-1. While that might seem unintuitive, it means that the value of Kcat is the number of molecules of product made by each molecule of enzyme in the time given. So, a Kcat value of 1000/sec means each enzyme molecule in the reaction at Vmax is producing 1000 molecules of product per second. Note that since Kcat is a calculated value, it cannot be read from a V vs [S] graph as Vmax and Km can.

Enzyme	Turnover Number (per second)
Carbonic anhydrase	600,000
3-Ketoesteroid isomerase	280,000
Acetylcholinesterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymostrypsin	100
DNA Polymerase I	15
Tryptophan synthetase	2
Lysozyme	0.5

Fig 5.14 Kcat (turnover number) values for several enzymes. Image by Aleia Kim

Amazing Kcat values

A Kcat value of 1000 molecules of product per enzyme per second might seem like a high value, but there are enzymes known (carbonic anhydrase, for example) that have a Kcat value of over 600,000/second (Figure 5.14). This astonishing value illustrates clearly why enzymes seem almost magical in their action. In contrast to Vmax, which varies with the amount of enzyme used, Kcat is a constant for an enzyme under given conditions.

Enzyme	K_{cat} / K_M ($s^{-1}M^{-1}$)
Acetylcholinesterase	1.6×10^8
Carbonic anhydrase	8.3×10^7
Catalase	4.0×10^7
Crotonase	2.8×10^8
Fumarase	1.6×10^8
Triose phosphate isomerase	2.4×10^8
β-Lactamase	1.0×10^8
Superoxide dismutase	7.0×10^9

Fig 5.15 Kcat/Km values for perfect enzymes. Image by Aleia Kim

As seen earlier, enzymes that follow Michaelis-Menten kinetics produce hyperbolic plots of Velocity (V0) versus Substrate Concentration [S]. Not all enzymes, though, follow Michaelis-Menten kinetics. Many enzymes have multiple protein subunits and these sometimes interact differently upon binding of a substrate or an external molecule.

Perfect enzymes

Now, if we think about what an ideal enzyme might be, it would be one that has a very high velocity and a very high affinity for its substrate. That is, it wouldn't take much substrate to get to Vmax/2 and the Kcat would be very high. Such enzymes would have values of Kcat / Km that are maximum. Interestingly, there are several enzymes that have this property and their maximal Kcat / Km values are all approximately the same. Such enzymes are referred to as being "perfect" because they have reached the maximum possible value.

Diffusion limitation

Why should there be a maximum possible value of Kcat / Km? The answer is that movement of substrate to the enzyme becomes the limiting factor for perfect enzymes. Movement of substrate by diffusion in water has a fixed rate at any temperature and

that limitation ultimately determines the maximum speed an enzyme can catalyze at. In a macroscopic world analogy, factories can't make products faster than suppliers can deliver materials. It is safe to say for a perfect enzyme that the only speed limit it has is the rate of substrate diffusion in water.

Given the "magic" of enzymes alluded to earlier, it might seem that all enzymes should have evolved to be "perfect." There are very good reasons why most of them have not.

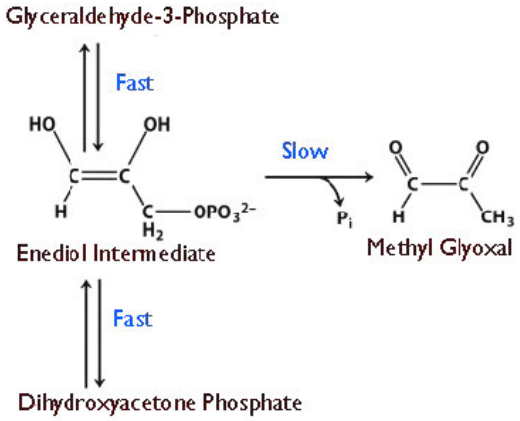


Fig 5.16 A high speed reaction avoids production of methyl glyoxal. Image by Aleia Kim

Speed

Speed is a dangerous thing. The faster a reaction proceeds in catalysis by an enzyme, the harder it is to control. As we all know from learning to drive, speeding causes accidents. Just as drivers need to have speed limits for operating automobiles, so too must cells exert some control on the 'throttle' of their enzymes. In view of this, one might wonder then why any cells have evolved any enzymes to perfection. There is no single answer to the question, but a common one is illustrated by triose phosphate isomerase, which catalyzes a reaction in glycolysis shown in Figure 5.16.

Lineweaver-Burk plots

The study of enzyme kinetics is typically the most math intensive component of biochemistry and one of the most daunting aspects of the subject for many students. Although attempts are made to simplify the mathematical considerations, sometimes they only serve to confuse or frustrate students. Such is the case with modified enzyme plots, such as Lineweaver-Burk (Figure 5.17).

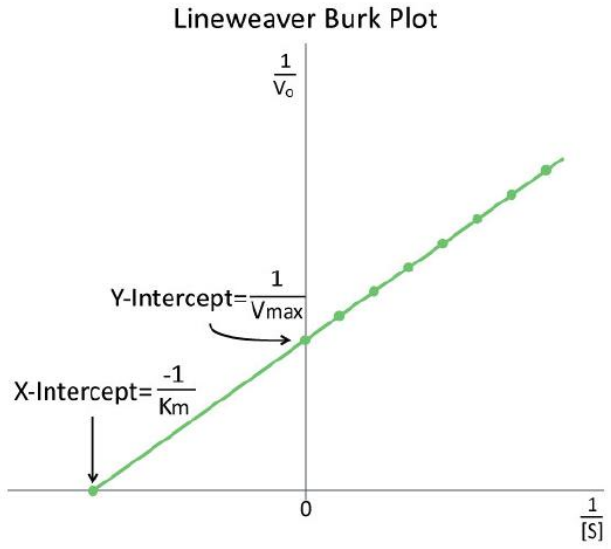


Fig 5.17 A Lineweaver-Burk plot of 1/V0 vs 1/[S]. Image by Aleia Kim

Indeed, when presented by professors as simply another thing to memorize, who can blame students? In reality, both of these plots are aimed at simplifying the determination of parameters, such as Km and Vmax

In making either of these modified plots, it is important to recognize that the same data is used as in making a V0 vs. [S] plot. The data are simply manipulated to make the plotting easier.

Double reciprocal

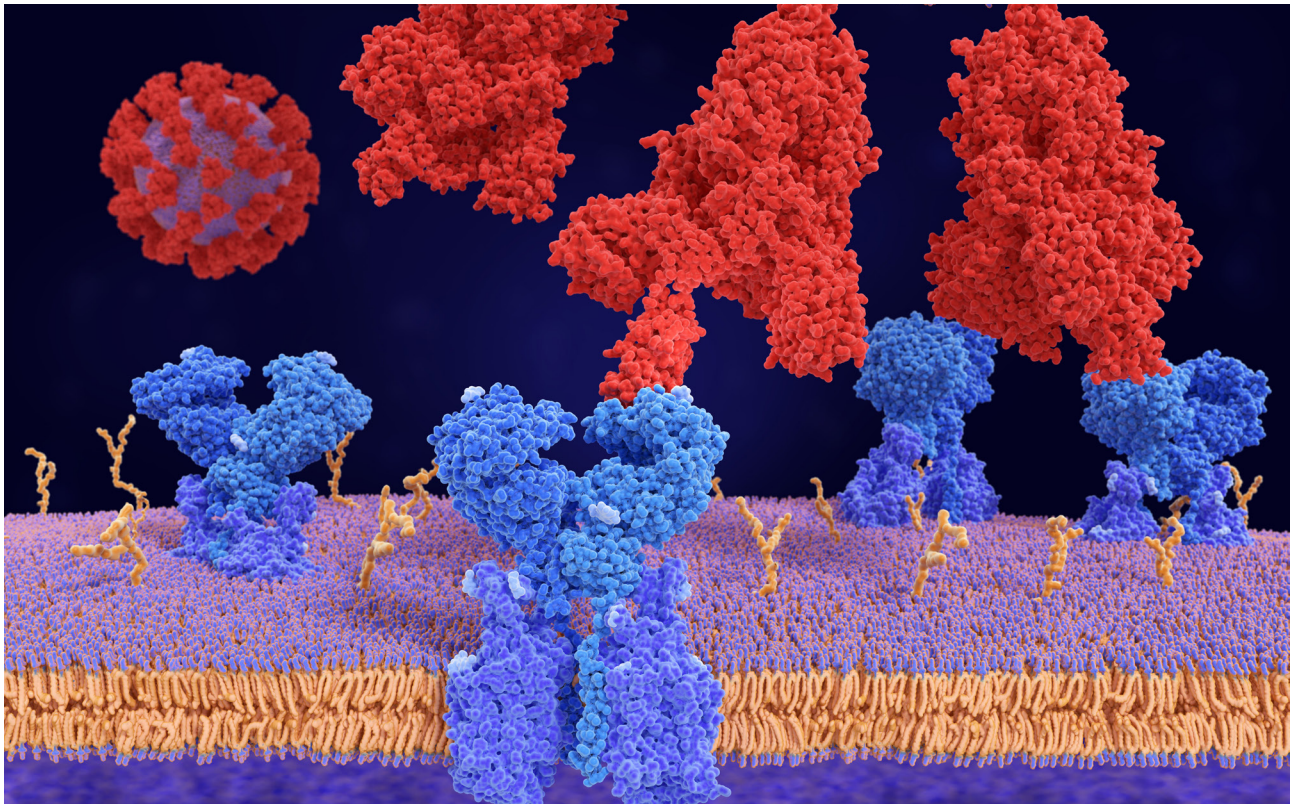
For a LineWeaver-Burk plot, the manipulation is using the reciprocal of the values of both the velocity and the substrate concentration. The inverted values are then plotted on a graph as 1/V0 vs. 1/[S]. Because of these inversions, Lineweaver-Burk plots are commonly referred to as 'double-reciprocal' plots. As can be seen in Figure 5.17, the value of Km on a Lineweaver Burk plot is easily determined as the negative reciprocal of the x-intercept , whereas the Vmax is the inverse of the y-intercept. Other related manipulation of kinetic data include Eadie-Hofstee diagrams, which plots V0 vs V0/[S] and gives Vmax as the Y-axis intercept with the slope of the line being -Km.

Resources:

Chapter page: α-Amlyase protein (green) in complex with amylase inhibitor (orange). Iamge from RCSB PDB (rcsb.org) of PBD ID 1CLV. (Pereira, P.J., Lozanov, V., Patthy, A., Huber, R., Bode, W., Pongor, S., Strobl, S.(1999) Structure 7: 1079-1088. https://doi.org/10.1016/s0969-2126(99)80175-0

Chapter #6 Protein function

Control of enzyme activity



Regulation of enzyme activity

Apart from their ability to greatly speed the rates of chemical reactions in cells, enzymes have another property that makes them valuable. This property is that their activity can be regulated, allowing them to be activated and inactivated, as necessary. This is tremendously important in maintaining homeostasis, permitting cells to respond in controlled ways to changes in both internal and external conditions.

Inhibition of specific enzymes by drugs can also be medically useful. Understanding the mechanisms that control enzyme activity is, therefore, of considerable importance.

Inhibition

We will first discuss four types of enzyme inhibition – competitive, non-competitive, uncompetitive, and suicide inhibition. Of these, the first three types are reversible. The last one, suicide inhibition, is not.

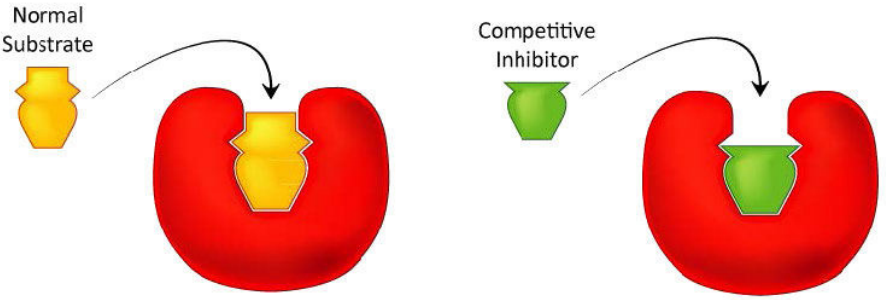


Fig 6.1 Competitive inhibitors resemble the normal substrate and compete for binding at the active site. Image by Aleia Kim

Competitive inhibition

Probably the easiest type of enzyme inhibition to understand is competitive inhibition and it is the one most commonly exploited pharmaceutically. Molecules that are competitive inhibitors of enzymes resemble one of the normal substrates of an enzyme (Fig 6.1). An example is methotrexate, which resembles the folate substrate of the enzyme dihydrofolate reductase (DHFR). This enzyme normally catalyzes the reduction of folate, an important reaction in the metabolism of nucleotides.

Inhibitor binding

When the drug methotrexate is present, some of the DHFR enzyme binds to it, instead of to folate, and during the time methotrexate is bound, the enzyme is inactive and unable to bind folate. Thus, the enzyme is inhibited. Notably, the binding site on DHFR for methotrexate is the active site, the same place that folate would normally bind. As a result, methotrexate 'competes' with folate for binding to the enzyme. The more methotrexate there is, the more effectively it competes with folate for the enzyme's active site. Conversely, the more folate there is, the less of an effect methotrexate has on the enzyme because folate outcompetes it (Fig 6.2).

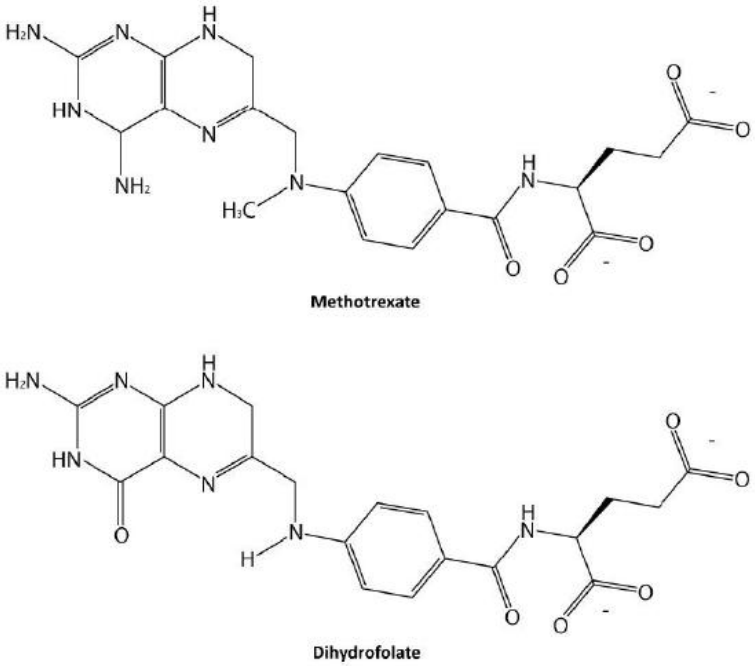


Fig 6.2 Methotrexate and dihydrofolate. Image by Ben Carson

No effect on Vmax

How do we study competitive inhibition?
It is typically done as follows. First, one performs a set of V0 vs. [S] reactions without

inhibitor (20 or so tubes, with buffer and constant amounts of enzyme, varying amounts of substrate, equal reaction times). V0 vs. [S] is plotted (Figure 6.3; red line), as well as 1/V0 vs. 1/[S] (Fig 6.4 green line). Next, a second set of reactions is performed in the same manner as before, except that a fixed amount of the methotrexate inhibitor is added to each tube. At low concentrations of substrate, the methotrexate competes for the enzyme effectively, but at high concentrations of substrate, the inhibitor will have a much reduced effect, since the substrate outcompetes it, due to its higher concentration (remember that the inhibitor is at fixed concentration).

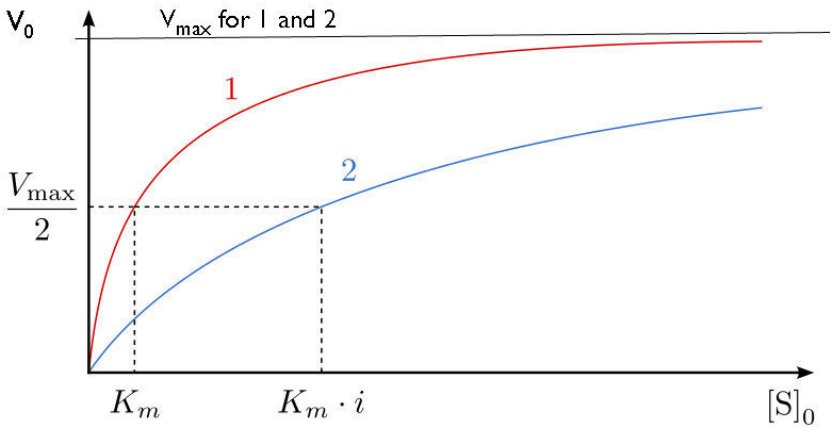


Fig 6.3 V0 vs [S] Plots for uninhibited reactions (red) and competitively inhibited reactions (blue). Both ultimately have same Vmax

Increased Km

In competitively inhibited reactions, the apparent Km of the enzyme for the substrate increases ($-1/Km$ gets closer to zero - red line in Figure 6.3) when the inhibitor is present compared to when the inhibitor is absent, thus illustrating the better competition of the inhibitor at lower substrate concentrations. It may not be obvious why we call the changed Km the apparent Km of the enzyme. The reason is that the inhibitor doesn't actually change the enzyme's affinity for the folate substrate. It only appears to do so. This is because of the way that competitive inhibition works. When the competitive inhibitor binds the enzyme, it is effectively 'taken out of action.' Inactive enzymes have NO affinity for substrate and no activity either. We can't measure Km for an inactive enzyme.

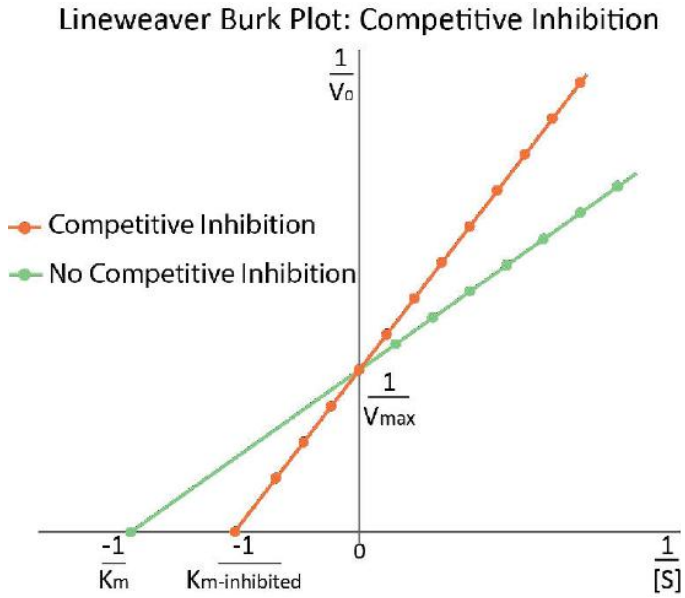


Fig 6.4 Lineweaver-Burk plots - uninhibited reactions (green). Competitively inhibited reactions (orange). Lines cross on Y-axis at 1/Vmax Since Vmax is the same for both reactions. Image by Aleia Kim

The enzyme molecules that are not bound by methotrexate can, in fact, bind folate and are active. Methotrexate has no effect on them and their Km values are unchanged. Why then, does Km appear higher in the presence of a competitive inhibitor? The reason is that the competitive inhibitor is having a greater effect of reducing the amount of active enzyme at lower concentrations of substrate than it does at higher concentrations of substrate. When the amount of enzyme is reduced, one must have more substrate to supply the reduced amount of enzyme sufficiently to get to Vmax/2.

It is worth noting that in competitive inhibition, the percentage of inactive enzyme changes drastically over the range of [S] values used. To start, at low [S] values, the greatest percentage of the enzyme is inhibited. At high [S], no significant percentage of enzyme is inhibited. This is not always the case, as we shall see in non-competitive inhibition.

Non-competitive inhibition

A second type of inhibition employs inhibitors that do not resemble the substrate and bind not to the active site, but rather to a separate site on the enzyme (Figure 6.5). The effect of binding a non-competitive inhibitor is significantly different from binding a competitive inhibitor because there is no competition. In the case of competitive inhibition,

the effect of the inhibitor could be reduced and eventually overwhelmed with increasing amounts of substrate. This was because increasing substrate made increasing percentages of the enzyme active. With non-competitive inhibition, increasing the amount of substrate has no effect on the percentage of enzyme that is active. Indeed, in non-competitive inhibition, the percentage of enzyme inhibited remains the same through all ranges of [S].

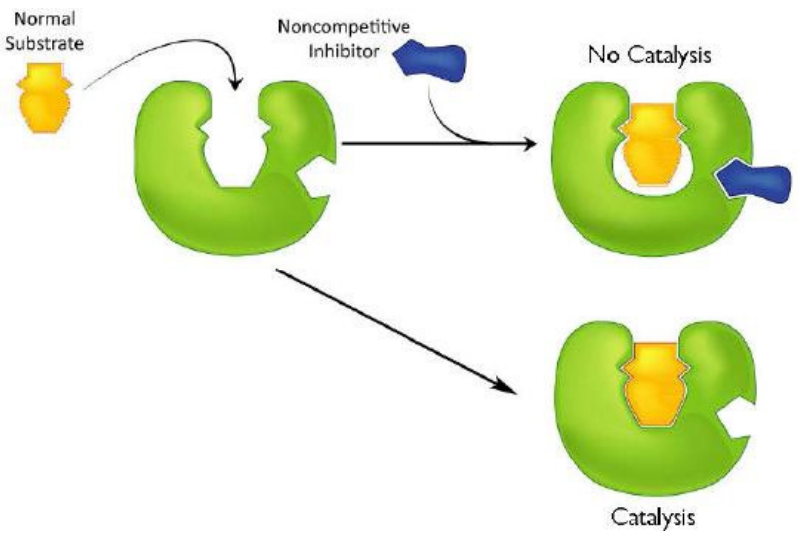


Fig 6.5 Non-competitive inhibition - inhibitor does not resemble the substrate and binds to a site other than the active site. Image by Aleia Kim

This means, then, that non-competitive inhibition effectively reduces the amount of enzyme by the same fixed amount in a typical experiment at every substrate concentration used. The effect of this inhibition is shown in Figure 6.6 & 6.7. As you can see, Vmax is reduced in non-competitive inhibition compared to uninhibited reactions.

This makes sense if we remember that Vmax is dependent on the amount of enzyme present. Reducing the amount of enzyme present reduces Vmax. In competitive inhibition, this doesn't occur detectably, because at high substrate concentrations, there is essentially 100% of the enzyme active and the Vmax appears not to change. Additionally, Km for non-competitively inhibited reactions does not change from that of uninhibited reactions. This is because, as noted previously, one can only measure the Km of active enzymes and Km is a constant for a given enzyme.

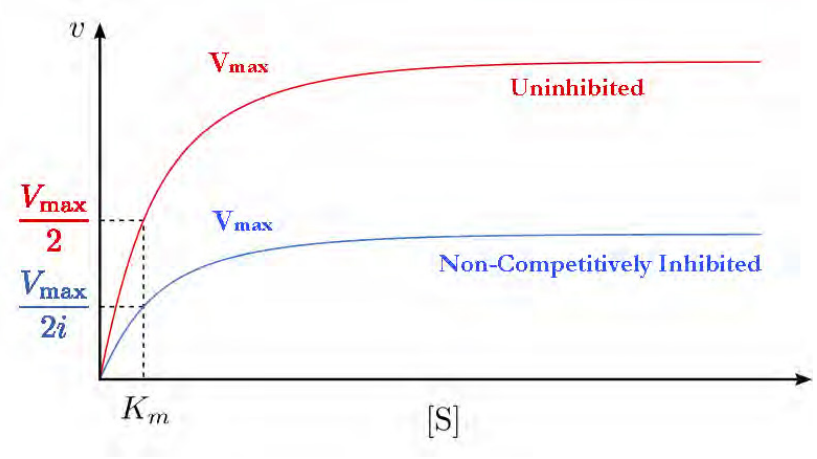


Fig 6.6 V0 vs [S] plots of uninhibited reactions (red) and non-competitively inhibited reactions (blue). Vmax is reduced, but Km values are unchanged in non-competitively inhibited reactions

Uncompetitive inhibition

A third type of enzymatic inhibition is that of uncompetitive inhibition, which has the odd property of a reduced Vmax as well as a reduced Km. The explanation for these seemingly odd results is rooted in the fact that the uncompetitive inhibitor binds only to the enzyme-substrate (ES) complex (Figure 6.8). The inhibitor-bound complex forms mostly under concentrations of high substrate and the ES-I complex cannot release product while the inhibitor is bound, thus explaining the reduced Vmax.

The reduced Km is a bit harder to conceptualize. The reason is that the inhibitor-bound complex effectively reduces the concentration of the ES complex. By Le Chatelier's Principle, a shift occurs to form additional ES complex, resulting in less free enzyme and more enzyme in the forms ES and ESI (ES with inhibitor). Decreases in free enzyme correspond to an enzyme with greater affinity for its substrate. Thus, paradoxically, uncompetitive inhibition both decreases Vmax and increases an enzyme's affinity for its substrate (Km-Figures 6.8 & 6.9).

Suicide inhibition

In contrast to the first three types of inhibition, which involve reversible binding of the inhibitor to the enzyme, suicide inhibition is irreversible, because the inhibitor becomes covalently bound to the enzyme during the inhibition. Suicide inhibition rather closely re-

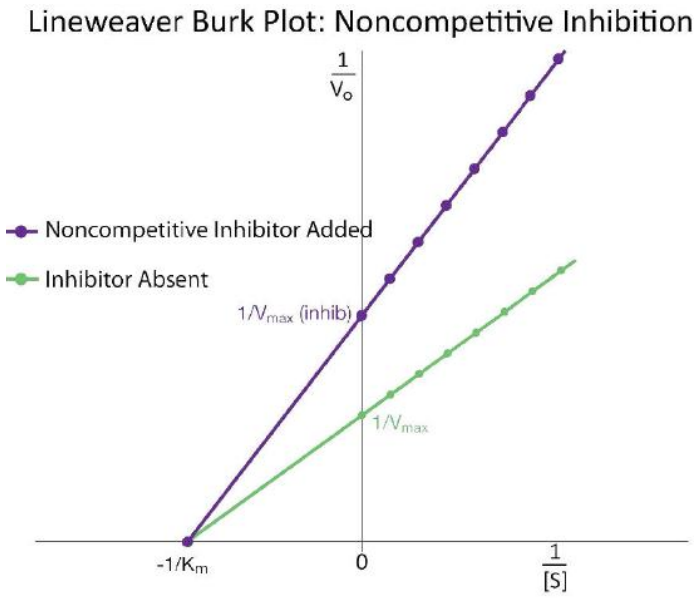


Fig 6.7 Lineweaver Burk plots of uninhibited reactions (green) and non-competitively inhibited reactions (purple). Image by Aleia Kim

sembles competitive inhibition because the inhibitor generally resembles the substrate and binds to the active site of the enzyme. The primary difference is that the suicide inhibitor is chemically reactive in the active site and makes a bond with it that precludes its removal. Such a mechanism is that employed by penicillin (Figure 6.11), which covalently links to the bacterial enzyme, DD transpeptidase and stops it from functioning. Since the normal function of the enzyme is to make a bond necessary for the peptidoglycan complex of the bacterial cell wall, the cell wall cannot properly form and bacteria cannot reproduce.

Control of enzymes

It is appropriate to talk at this point about mechanisms cells use to control enzymes. There are four general methods that are employed:

1) allosterism, 2) covalent modification, 3) access to substrate, and 4) control of enzyme synthesis/breakdown. Some enzymes are controlled by more than one of these methods.

Allosterism

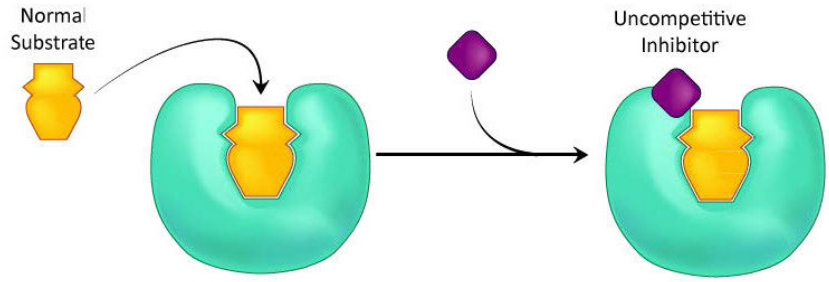


Fig 6.8 Uncompetitive inhibitor

The term allosterism refers to the fact that the activity of certain enzymes can be affected by the binding of small molecules. Molecules causing allosteric effects come in two classifications. Ones that are substrates for the enzymes they affect are called homotropic effectors and those that are not substrates are called heterotropic effectors.

Allosteric inhibition

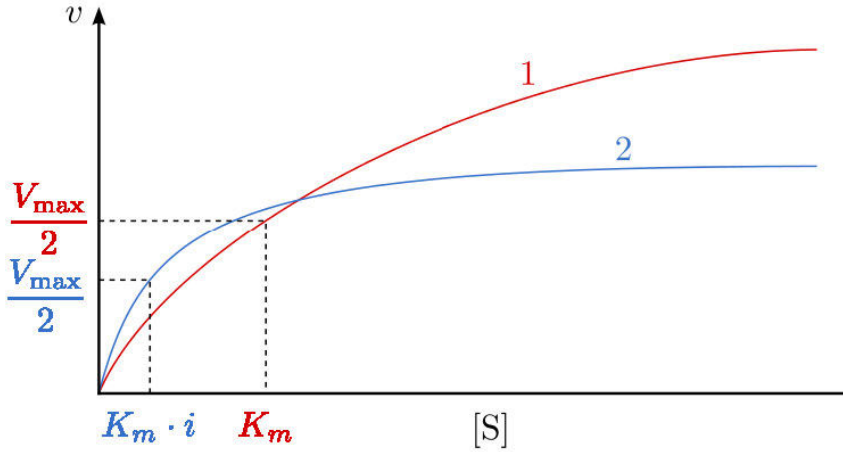


Fig 6.9 V0 vs [S] plot for uncompetitive inhibition (blue) and uninhibited reactions (red)

Allosterically, regulation of these enzymes works by inducing different physical states (shapes, as it were) that affect their ability to bind to substrate. When an enzyme is inhibited by binding an effector, it is converted to the T-state (T=tight), it has a reduced affinity for substrate and it is through this means that the reaction is slowed.

Allosteric activation

On the other hand, when an enzyme is activated by effector binding, it converts to the R-state (R=relaxed) and binds substrate much more readily. When no effector is present, the enzyme may be in a mixture of T- and R-states.

Feedback inhibition

An interesting kind of allosteric control is exhibited by HMG-CoA reductase, which catalyzes an important reaction in the pathway leading to the synthesis of cholesterol. Binding of cholesterol to the enzyme reduces the enzyme's activity significantly. Cholesterol is not a substrate for the enzyme, so it is therefore a heterotropic effector.

Notably, though, cholesterol is the end-product of the pathway that HMG-CoA reductase catalyzes a reaction in. When enzymes are inhibited by an end-product of the pathway in which they participate, they are said to exhibit feedback inhibition.

Feedback inhibition always operates by allosterism and further, provides important and efficient control of an entire pathway. By inhibiting an early enzyme in a pathway, the flow of materials (and ATP hydrolysis required for their processing) for the entire pathway is stopped or reduced, assuming there are not alternate supply methods.

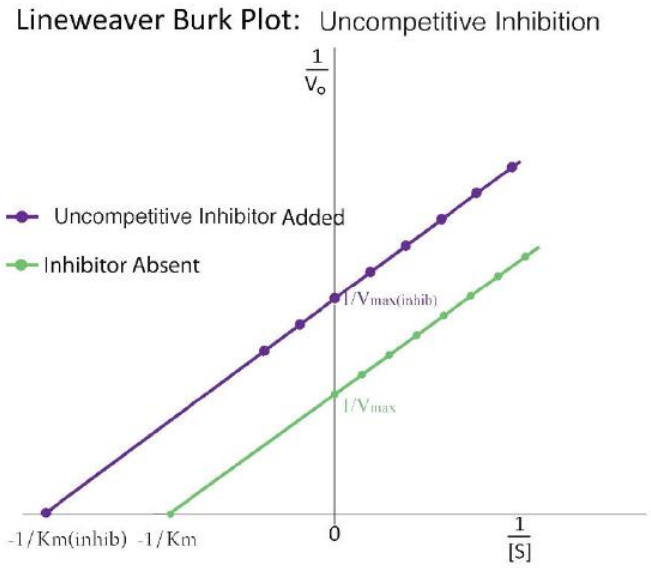


Fig 6.10 Uncompetitive inhibition (purple) and uninhibited reactions (green). Image by Aleia Kim

Pathway control

In the cholesterol biosynthesis pathway, stopping this one enzyme has the effect of shutting off (or at least slowing down) the entire pathway. This is significant because after catalysis by HMG-CoA

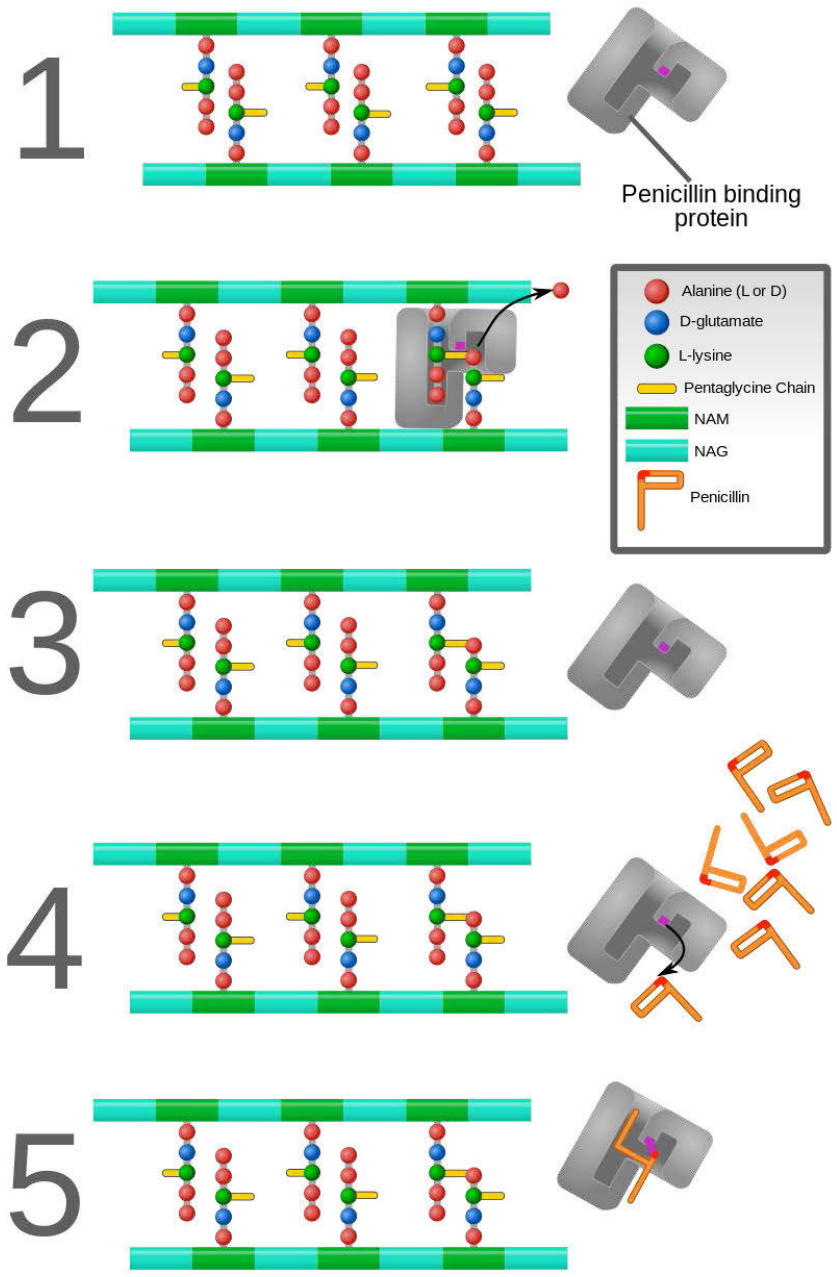


Fig 6.11 Action of penicillin. DD-transpeptidase builds peptidoglycan layer of bacterial cell wall (1-3). Binding of penicillin by DD-transpeptidase stops peptidoglycan synthesis (4-5). Wikipedia

reductase, there are over 20 further reactions necessary to make cholesterol, many of them requiring ATP energy. Shutting down one reactions stops all of them.

ATCase

Another interesting example of allosteric control and feedback inhibition is associated with the enzyme Aspartate Transcarbamoylase (ATCase). This enzyme, which catalyzes a step in the synthesis of pyrimidine nucleotides, has 12 subunits. These include six identical catalytic subunits and six identical regulatory subunits (Fig 6.12). The catalytic subunits bind to substrate and catalyze a reaction. The regulatory subunits bind to either ATP or CTP. If they bind to ATP, the enzyme subunits arrange themselves in the R-state.

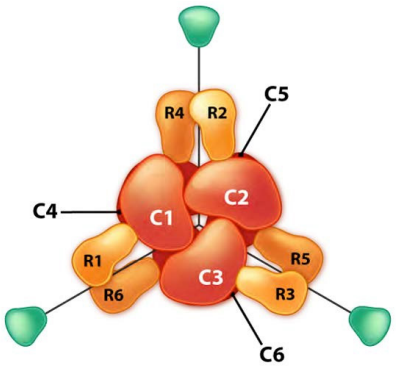


Fig. 6.12 Schematic structure of ATCase. Regulatory Units = R, Catalytic Units = C. Image by Aleia Kim

R-state : The R-state of ATCase allows the substrate to have easier access to the six active sites and the reaction occurs more rapidly. For the same amount of substrate, an enzyme in the R-state will have a higher velocity than the same enzyme that is not in the R-state. By contrast, if the enzyme binds to CTP on one of its regulatory subunits, the subunits will arrange in the T-state and in this form, the substrate will not have easy access to the active sites, resulting in a slower velocity for the same concentration of substrate compared to the R-state. ATCase is interesting in that it can also flip into the R-state when one of the substrates (aspartate) binds to an active site within one of the catalytic subunits.

Aspartate has the effect of activating the catalytic action of the enzyme by favoring the R-state. Thus, aspartate, which is a substrate of the enzyme is a homotropic effector and ATP and CTP, which are not substrates of the enzyme are heterotropic effectors of ATCase (Fig 6.13).

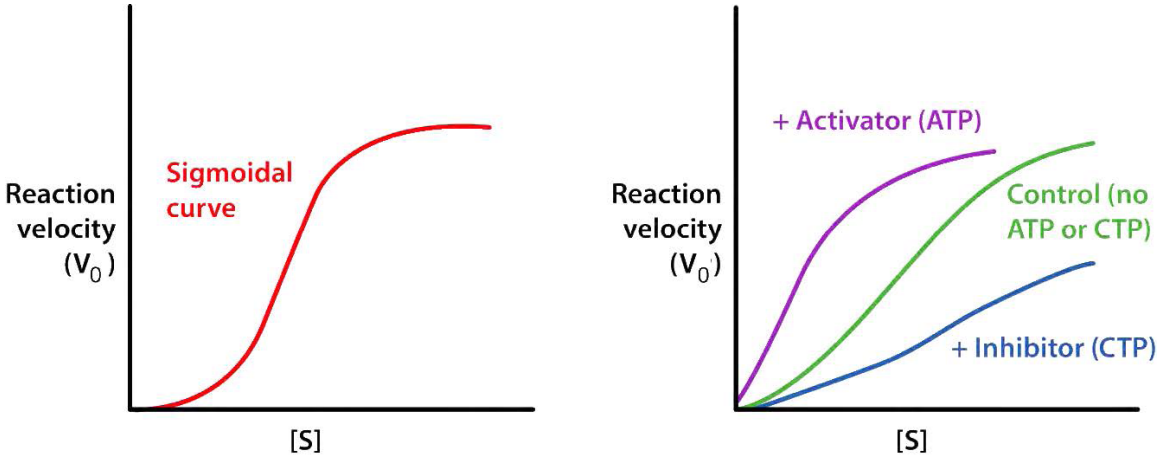


Fig. 6.13 Plots of V0 vs. [S] for ATCase. Left - Allosteric effect of aspartate. Right - Allosteric effects of ATP (activator) and CTP (inhibitor). Image by Pehr Jacobson

There are various models commonly used to explain how allosterism regulates multi-subunit enzyme activity. All models describe a Tense (T) state that is less catalytically active and a Relaxed (R) state that is more catalytically active. The models differ in how the states change.These models are

1) sequential model (also known as KNF),

2) Monod-Wyman-Changeux (MWC) model (also known as the concerted model)

3) Morpheein model.

We will look at the sequential model (6.14) where binding of an allosteric effector by one

subunit causes it to change from T to R state (or vice versa) and that change makes it easier for adjacent subunits to similarly change state. With this model , there is a cause/effect relationship between binding of an effector by one subunit and change of state by an adjacent subunit.

Please note that hemoglobin is not an enzyme but we use this example to learn how R and T states are altered by binding to Oxygen. Binding of one oxygen by one unit of the complex may induce that unit to flip to the R-state and, through interactions with other subunits, cause them to favor adopting the R configuration before they bind to oxygen. In this way, binding of one subunit favors binding of others and cooperativity can be explained by the change in binding affinity as oxygen concentration changes.

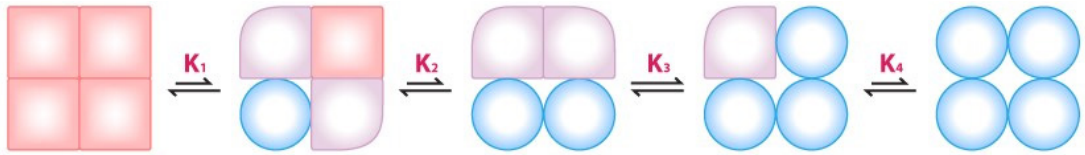


Fig 6.14 Sequential model of allosteric regulation. Round = R-State. Square = T-state.. Image by Aleia Kim

Covalent control of enzymes

Some enzymes are synthesized in a completely inactive form and their activation requires covalent bonds in them to be cleaved. Such inactive forms of enzymes are called zymogens. Examples include the proteins involved in blood clotting and proteolytic enzymes of the digestive system, such as trypsin, chymotrypsin, pepsin, and others.

Synthesizing some enzymes in an inactive form makes very good sense when an enzyme's activity might be harmful to the tissue where it is being made. For example, the painful condition known as pancreatitis arises when digestive enzymes made in the pancreas are activated too soon and end up attacking the pancreas.

Resources:

Chapter page Binding of the coronavirus spike proteins to ACE2 receptors mediates the virus penetration into human cells Adobe stock # 361166267 Licensed


7

Chapter #7 Carbohydrates

Introduction

Carbohydrates are a third major group of biomolecules. This diverse group is commonly described as sugars, or sac-charides, from the Greek word for sugar. The simplest carbohy- drates are called monosaccha- rides, or simple sugars. An example is glucose. Monosaccharides can be joined to make larger molecules. Disaccharides contain two monosaccharides. Sucrose is a disaccharide, containing both fructose and glucose. Polsaccharides are chains of many sugar subunits. Examples include glycogen and cellulose, both of which are polymers of glucose (but with different configurations). Carbohydrates are literally "hydrates of carbon." This designation derives from the generalized-formula of simple monosaccharides, which can be written in the form of $C_X(H_2O)_X$, where x is a digit typically between 3 and 8.

Not all sugars have this formula, however. Deoxyribose, the sugar found in every nucleotide in a DNA molecule lacks one oxygen and thus has the formula $C_5H_{10}O_4$.Carbohydrates are important in cells as energy sources (glucose, glycogen, amylose), asmarkers of cellular identity (oligosaccharides on the surface of cells of multicellular organisms), as structural components (cellulose in plants), and as constituents of nucleotides(ribose in RNA, deoxyribose in DNA). The building blocks of carbohydrates are simple sugars and it is here we begin our description.

Monosaccharides

The most common monosaccharides include glucose, fructose, galactose, ribose, and mannose. Of these sugars, all but one (fructose)exists as an aldehyde. Fructose and other less well known sugars are ketones. Figure 7.1 shows the structure of these sugars. Some discussion of nomenclature is appropriate.

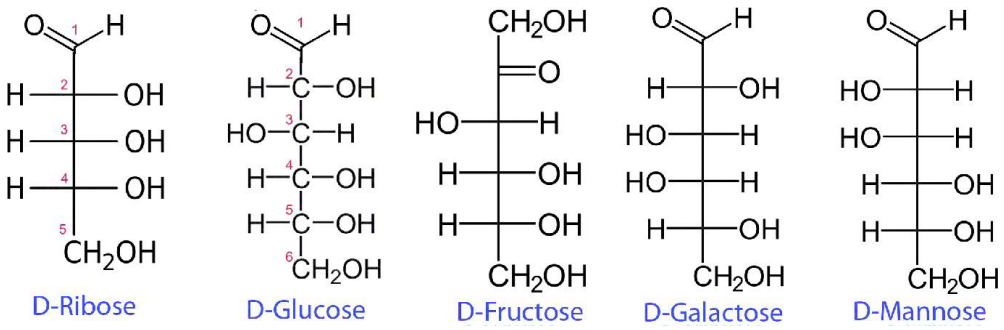


Fig 7.1 Common sugar structures

By convention, the letters 'ose' at the end of a biochemical name flags a molecule as a sugar.Thus, there are glucose, galactose, sucrose, and many other '-oses'.

Other descriptive nomenclature involves use of a prefix that tells how many carbons the sugar contains. For example, glucose, which contains six carbons, is described as a hexose. The following list shows the prefixes for numbers of carbons in a sugar:

•Tri- = 3, Tetr- = 4, Pent- = 5, Hex- =6, Hept- = 7, Oct- = 8

Other prefixes identify whether the sugar contains an aldehyde group (aldo-) or a ketone (keto-) group. Prefixes may be combined. Glucose, which is a 6-carbon sugar with an aldehyde group, can be described as an aldo hexose. The list that follows gives the common sugars and their descriptors.

•Ribose = aldo-pentose,

•Glucose = aldo-hexose

•Galactose = aldo-hexose

•Mannose = aldo-hexose

•Fructose = keto-hexose

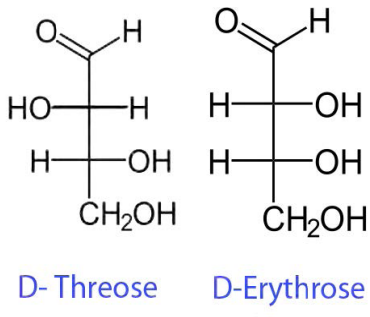


Fig 7.2 Diastereomers

Diastereomers

Sugars may have multiple asymmetric carbons and thus differ from each other in the configuration of hydroxyl groups on asymmetric carbons. Two sugars having the same chemical form (aldoses, for example) and the same number of carbons, but that differ only in the stereochemical orientations of their carbons are referred to as diastereomers (Figure 7.2). For example, glucose, galactose, and mannose all have the formula of $C_6H_{12}O_6$, but are chemically distinct from each other in the orientation of hydroxyl groups around the carbons within them (Fig 7.3).

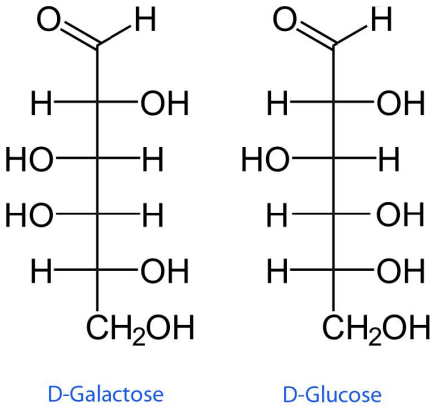


Fig 7.3 Epimers - D-Galactose and D-Glucose differ only in the configurationof carbon #4

Enantiomers and epimers

If two sugars are identical except for having one hydroxyl configured differently (such as glucose and galactose - Figure 7.3), they are diastereomers known as epimers. If the configuration of all of the hydroxyls of one sugar is exactly the opposite of their configuration in another sugar, the two sugars are mirror images of each other. Mirror images of sugars are known as enantiomers (Figure 7.4). Please note that due to the way sugars are named, L-glucose is the mirror image of D-glucose.

Sugars with five and six carbons can readily cyclize (Figure 7.5) and when they do, a new asymmetric carbon is created that didn't exist in the same sugars when they were in the straight chainform. This carbon has a special name - it is called the anomeric carbon and (like the other asymmetric carbons in sugars) it can have the hydroxyl in two different positions. These positions are referred to as α and β . Sugars, such as α-D-glucoseand β-D-glucose that differ only in the configuration of the anomeric carbon are referred to as anomers (Figure 7.6).

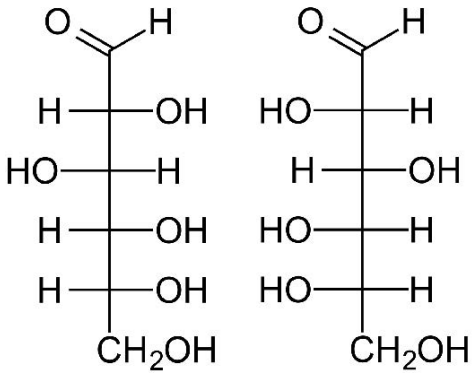


Fig 7.4 Enantiomers - D-Glucose (left) and L-Glucose (right) are mirror images

Sugars cyclizing to form rings with five atoms in them (Figure 7.5) are referred to as furanoses (named for furan) and those forming rings with six atoms, such as glucose in the same figure, are called pyranoses (named for pyran). The carbonyl carbon beomces the anomeric carbon in the ring by binding to the oxygen of a hydroxyl elsewhere in the chain. α- and β- forms of a given sugar can readily "flip" between each form in solution, so long as the anomeric hydroxyl is free. Most pentoses and hexoses can form both furanose and pyranose structures. Linking the anomeric hydroxyl to another group will create a glycoside and glycosides will remain locked in whichever α- or β- configuration they were in when the anomeric hydroxyl was altered.

Boat/chair conformations: Orbitals of carbon prefer to be in tetrahedral conformations and this means that the bonds between carbonsin a ring do not lie flat. Indeed, rings "pucker" to try to accommodate this tendency, giving rise to different 3D forms for any given sugar. Some of these form forms resemble boat structures, which others resemble chairs or envelopes (Figure 7.7). The stablest (and thus most abundant) of these forms have all of the hydoxyls in the equatorial positions, resulting in less steric hindrance.

Modified monosaccharides

Many modifications can occur on sugar residues. Common ones include oxidation,

reduction, phosphorylation, and substitution of an amine or an acetylamine for a hydroxyl. The ones that affect the anomeric hydroxyl group make glycosides, whereas modifications that don't affect the anomeric hydroxyl, (glucose-6-phosphate, for example),do not.

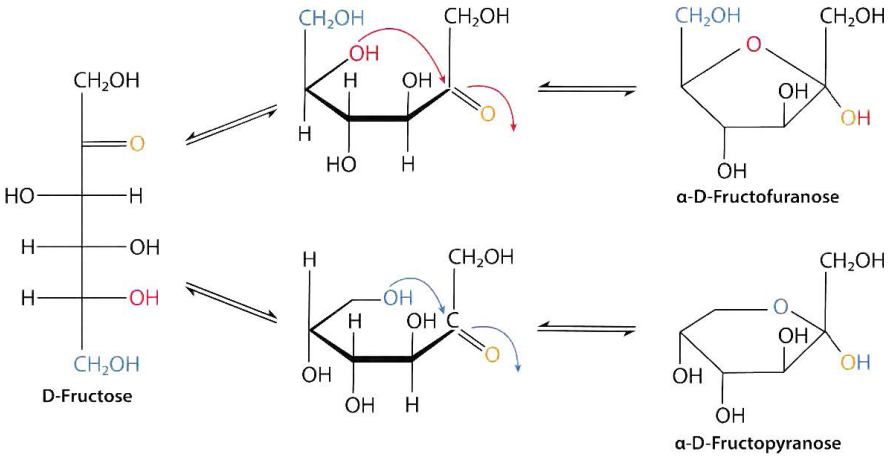


Fig 7.5 Conversion of D-fructose between furanose (top right), linear (left), and pyranose (bottom right) forms. Image by Pehr Jacobson

Oxidation/reduction

The last considerations for simple sugars relative to their structure are their chemical reactivity and modification. Sugars that are readily oxidized are called 'reducing sugars' because their oxidation causes other reacting molecules to be reduced. A test for reducing sugars is known as Benedict's test. In it, sugars are mixed and heated with an alkaline solution containing Cu^{++}. Reducing sugars will donate an electron to Cu^{++}, converting it to Cu^{+}, which will produce cuprous oxide Cu_2O, as an orange precipitate . Since Cu^{++} solution is blue, the change of color provides an easy visual indication of a reducing sugar. The aldehyde group of aldoses is very susceptible to oxidation, whereas ketoses are less so, but can easily be oxidized if, like fructose, they contain an α-hydroxyl and can tautomerize to an aldose.

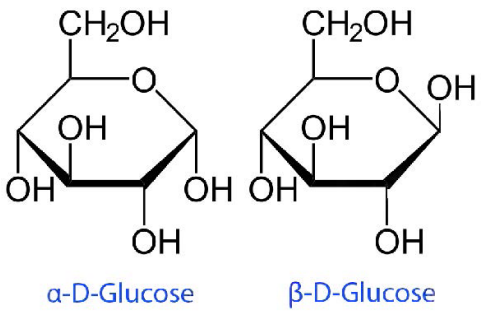


Fig 7.6 Anomers - α-DGlucose and β-D-Glucose differonly in the configuration of the anomeric carbon #1

Most monosaccharides are reducing sugars. This includes all of the common ones galactose, glucose, fructose, ribose, xylose, and mannose. Some disaccharides, such as lactose and maltose are reducing sugars since they have at least one anomeric carbon free, allowing that part of the sugar to linearize and yield an aldose. Sucrose, on the other hand has no anomeric carbons free - both are involved in a glycosidic linkage, so they cannot linearize and thus it is not a reducing sugar. Oxidation and reduction of sugars can occur in cells. As we will see, phosphorylation of sugars occurs routinely during metabolism.

Glucuronic acid

One oxidation product of glucose is glucuronic acid (Fig 7.8), a six carbon molecule where the CH_2OH on carbon six is oxidized to a carboxylic acid. Related oxidized sugars include galacturonic acid and mannuronic acid. Glucuronic acid is commonly conjugated to other molecules in the liver/bile by UDP-glucuronyltransferase enzymes to make the molecules more water soluble for excretion, since the carboxyl group of glucoronicacid ionizes readily at physiological pH. The reactions are usually done starting with glucuronic acid linked to UDP (UDPGlucuronic Acid). In addition, glucuronicacid is made from a UDP-glucose precur precursor. Glucuronic acid is a common constituent of glycosaminoglycans, proteoglycans, and glycoglycerolipids. Glucuronic acid is found in heparin, dermatan sulfate, chondroitin sulfate, hyaluronic acid, and keratan sulfate. Glucuronic acid is also a precursor of ascorbic acid (Vitamin C) in organisms that synthesize this compound.

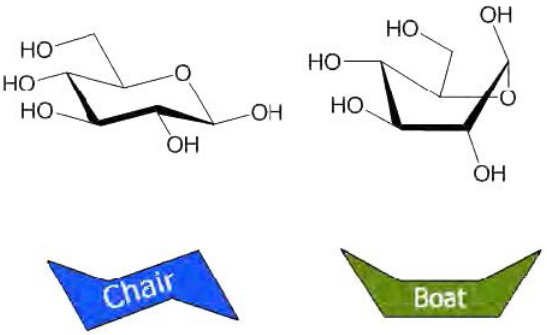


Fig 7.7 Anomers - α-DGlucose and β-D-Glucose differonly in the configuration of the anomeric carbon #1

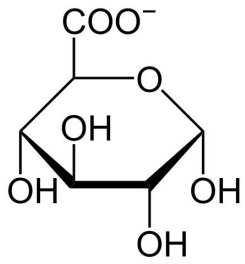


Fig 7.8 Glucuronic acid

Sugar alcohols

Reduction of aldoses or ketoses by hydrogenation produces the corresponding sugar alcohols. The compounds are widely used as thickeners of food or as artificial sweeteners, due to their ability to stimulate sweet receptors on the tongue. Common sugar alcohols (sugar progenitor in parentheses) include glycerol (glyceraldehyde), xylitol (xylose), sorbitol (Figure 7.9 - from glucose),galactitol (galactose), arabitol (arabinose), and ribitol (ribose).

Most of these compounds have a sweetness of between 0.4 and 1.0 times as sweet as sucrose, but provide considerably fewer calories per weight.

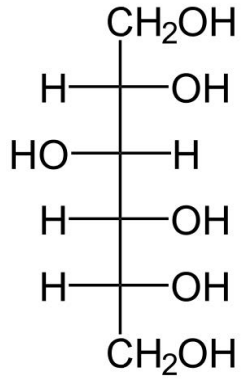


Fig 7.9 Sorbitol (also-called glucitol)

Xylitol is the sweetest of them with a sweetness equal to that of sucrose. Sugar alcohols are usedsometimes to mask the aftertaste of other artificial sweeteners. Many of them also produce a cooling sensation upon dissolving, due to that being an endothermic process for them, resulting in a pleasant mouth sensation. Last, they are poorly absorbed by intestines, and so have a low glycemic index.

Artificial sweeteners

Artificial sweeteners are compounds that stimulate taste receptors for sweetness, but are metabolized for energy inefficiently at best. Such compounds frequently are many times sweeter than table sugar (sucrose) on a weight/weight basis and are referred to as "intensely sweet." Most of the artificial sweeteners are not carbohydrates, but rather are able to stimulate the same sweet receptors that sugar does. Seven such compounds are approved for use in the U.S. - stevia, aspartame, sucralose, neotame, acesulfame potassium, saccharin. and advantame. The sugar alcohol known as sorbitol is also sometimes used as an artificial sweetener.

Disaccharides

Disaccharides (Figure 7.10) are made up of two monosaccharides. The most common ones include sucrose (glucose and fructose), lactose (galactose and glucose), and maltose (glucose and glucose). All of the common disaccharides contain at least one glycosidic bond. We name the disaccharides according to which carbons are linked to each other and the how the anomeric carbon of the glycosidic bond is configured. Lactose, for example, is described as β-Dgalactopyranosyl-(1→4)-D-glucose, or more succinctly as having an α-1,4 glycosidic bond.

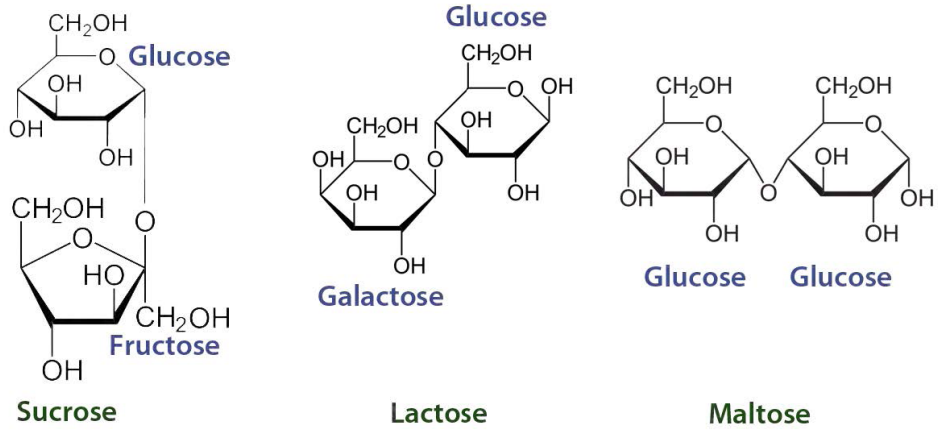


Fig 7.10 Common disaccharides - glycosidic bonds in rectangles

Oligosaccharides

As their name implies, oligosaccharides (Figure 7.11) are comprised of a few (typically 3 to 9) sugar residues. These often, but not always contain modified sugars. Unlikeall of the other saccharides, oligosaccharides are not typically found unattached to other cellular structures. Instead, oligosaccharides are found bound, for example, to sphingolipids(making cerebrosides or gangliosides) or proteins (making glycoproteins).

Oligosaccharides in membrane glycoproteins play important roles in cellular identity/recognition. The patterns of oligosaccharides displayed on the extracellular face of the plasma membrane acts as a sort of barcode that identifies specific cell types. The immune system recognizes these identity tags in the body. "Foreign" oligosaccharide structures trigger the immune system to attack them. While this provides a very good defense against invading cells of an organism, it also can pose significant problems when organs are transplanted from one individual into another, with rejection of donated organs, in some cases.

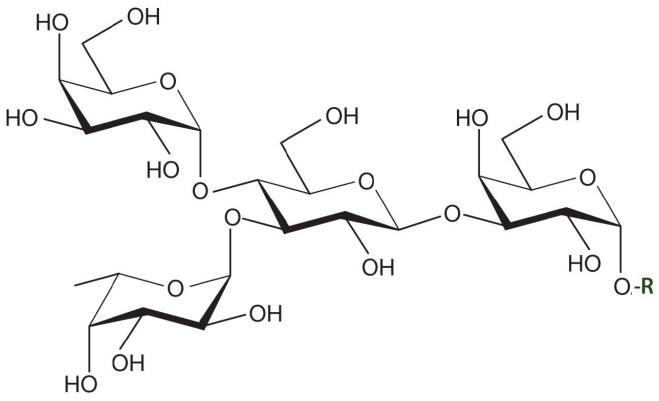


Fig 7.11 A Branched oligosaccharide attached to an R-Group

Organelle targeting

The oligosaccharides that are attached to proteins may also determine their cellular destinations. Improper glycosylation or errors in subsequent sugar modification patterns can result in the failure of proteins to reach the correct cellular compartment. For example, inclusion cell disease (also called I-cell disease) arises from a defective phosphotransferase in the Golgi apparatus. This enzyme normally catalyzes the addition of a phosphate to a mannose sugar attached to a protein destined for the lysosome. In the absence of a functioning enzyme, the unphosphorylated glycoprotein never makes it to the lysosome and is instead exported out of the cell where it accumulates in the blood and is excreted in the urine. Individuals with Icell disease suffer developmental delays, abnormal skeletal development, and restricted joint movement.

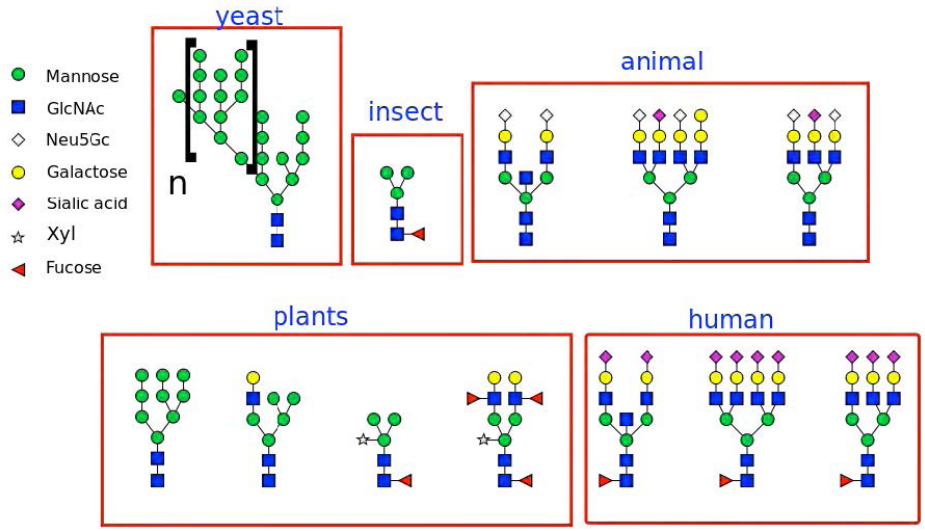


Fig7.12 N-linked glycosylation in various organisms. Wikipedia

Glycosylation

Sugars are commonly attached to proteins in a process called glycosylation. Typically the attachment is to a hydroxyl or other functional group. The majority of proteins synthesized in the endoplasmic reticulum are glycosylated.

Five classes of glycosylated products (called glycans if multiple carbohydrates are attached via glycosidic bonds) are known. They include:

N-linked glycans (Figure 2.165) - carbohydrate attached to N group of asparagine or arginine side chain

• O-linked glycans - carbohydrate attached to OH of serine, threonine, tyrosine, hydroxyproline,hydroxylysine, or lipids.

• Phosphoglycans - attachment to a phosphoserine

• Glypiation - linkage of a phosphatidyl inositol to link proteins to lipids via glycan linkages

• C-linked glycans - sugar attached to a carbon on a tryptphan side chain.

Glycosylation has several molecular/cellular functions. Some proteins require glycosylation to fold properly or to be stable. Glycosylated proteins on the plasma membrane serve as cellular identifiers. Blood types, for example, arise from differential glycosylation of a blood cell membrane protein. Glycosylation can also play an important role in cell-cell adhesion - important in the immune system.

Glycoproteins

Glycoproteins are a very diverse collection of saccharide containing proteins with many functions. Attachment of the saccharide to the protein is known as glycosylation. Secreted extracellular proteins and membrane proteins with exposed extracellular regions are often glycosylated. Saccharides attached to these may be short (oligosaccharides) or very large (polysaccharides). Glycoproteins play important roles in the immune system in antibodies and as components of the major histocompatibility complex (MHC). They are important for interactions between sperms and eggs, in connective tissues and are abundant in egg whites and blood plasma. Two glycoproteins (gp41 and gp120) are part of the HIV viral coat and are important in the infection process. Some hormones, such as erythropoietin, human chorionic gonadotropin, follicle-stimulating hormone and luteinizing hormone are also glycoproteins.

Glycation

Glycation is a chemical process (nonenzymatic) that occurs when a protein or lipid covalently binds to a sugar, such as glucose or fructose. Glycation differs from glycosylation in that the latter process is controlled by enzymes and results in specific attachment of specific sugars to biomolecules. Glycation, by contrast, is driven by two properties of monosaccharides 1) their chemistry and 2) their concentration. Glycations may be endogenous (occurring in an organism) or exogenous (occurring external to an organism). Exogenous glycation arises most commonly as a result of cooking of food and this results in attachment of sugars to lipids and/or proteins to form advanced glycation endproducts(AGEs).

Endogenous glycation, on the other hand, arises with a frequency that is proportional tothe concentration of free sugar in the body. These occur most frequently with fructose, galactose, and glucose in that decreasing order and are detected in the bloodstream. Both proteins and lipids can be glycated and the accumulation of endogenous advanced glycation endproducts (AGEs) is associated with Type 2 diabetes, as well as in increases in cardiovascular disease (damage to endothelium, cartilage, and fibrinogen), peripheral neuropathy (attack of myelin sheath), and deafness (loss of myelin sheath). One indicator of diabetes is increased glycation of hemoglobin in red blood cells, since circulating sugar concentration are high in the blood of diabetics. Hemoglobin glycation is measured in testing for blood glucose control in diabetic patients.

Polysaccharides

Long polymers of sugar residues are called polysaccharides and can be up to many thousands of units long. Polysaccharides are found free (not attached to other molecules) or bound to other cellular structures such as proteins. Some polysaccharides are homopolymers (containonly one kind of sugar), others are heteropolymers (glycosaminoglycans, hemicellulose). Polysaccharides function in energy storage (nutritional polysaccharides, such as glycogen, amylose, amylopectin, e.g.), structure enhancement (chitin, cellulose, e.g.), and lubrication (hyaluronic acid, e.g.). These individual categories of polysaccharides are discussed next.

Nutritional polysaccharides

This group of polysaccharides is used exclusively for storage of sugar residues. They are easily easily broken down by the organism making them, allowing for rapid release of sugar to meet rapidly changing energy needs.

Amylose

Amylose has the simplest structure of any of the nutritional polysaccharides, being made up solely of glucose polymers linked only by α-1,4 bonds (Figure 7.13). (Notethat the term 'starch' is actually a mixture ofamylose and amylopectin). Amylose is insoluble in water and is harder to digest than amylopectin (see below). The complexing of amylopectin with amylose facilitates its water solubility and its digestion. Amylose is produced in plants for energy storage and since plants don't have rapidly changing demands for glucose (no muscular contraction, for example), its compact structure and slow breakdown characteristics are consistent with plants' needs.

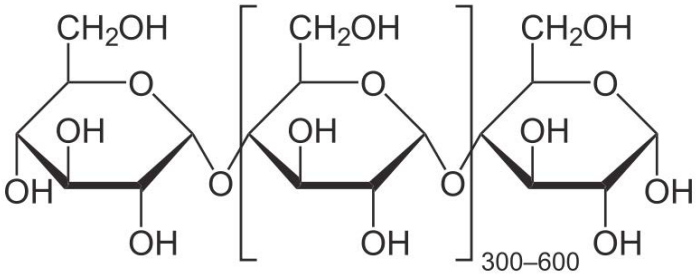


Fig 7.13 repeating units of amylosr

Amylopectin and glycogen

More complicated homopolymers of glucose are possessed by amylopectin in plants and glycogen (Figure 7.13) in animals. Both compounds contain long glucose chains withα-1,4 bonds like amylose, but unlike amylose, these long chains have branches of α-1,6 bonds. Amylopectin is the less-branched of the two, having such bonds about every 25-30 residues, whereas glycogen has branches about every 8-12 residues.

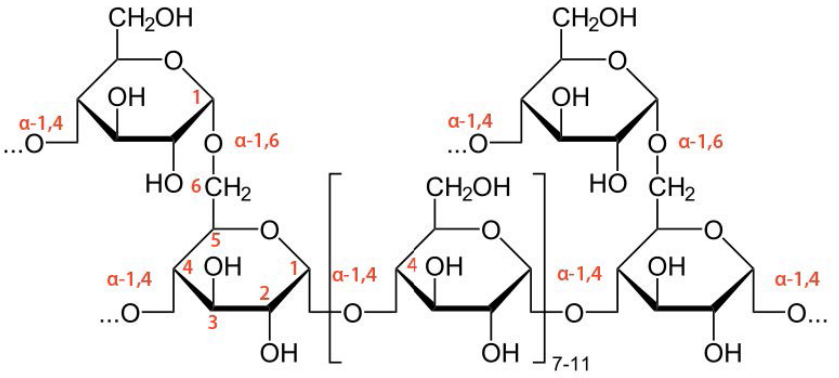


Fig 7.14 Structure of glycogen

Branching plays important roles in increasing water solubility and in providing more "ends" to the polymer. In animals, glycogen is broken down starting at the ends, so more ends means more glucose can be releasedq uickly. Again, plants, which have a lower need for quick release of glucose than animals get by with less branching and fewer ends.

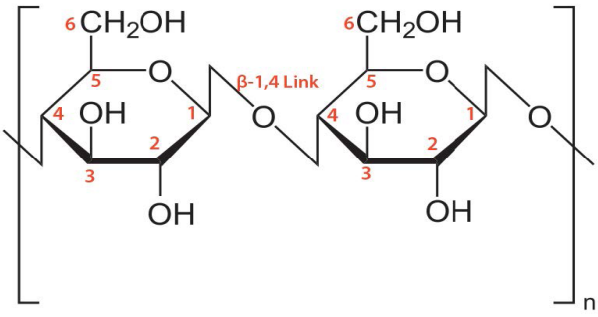


Fig 7.15 Cellulose with β-1,4 glycosidic links-between glucose sugars

Structural polysaccharides

An additional function of polysaccharides in cells relates to structure. Cellulose, which is a polymer of glucose with exclusive β-1,4 linkages between the units (Figure 7.15) is an important structural component of plants and fungi cells. Notably, most non-ruminant animals are unable to digest this polymer, as they lack the enzyme known as cellulase. Ruminants, such as cattle, however, contain in their rumen a bacterium that possesses this enzyme and allows them to obtain glucose energy from plants.

Another group of polysaccharides found in plant cell walls is the hemicelluloses. This class of molecules encompasses several branched heteropolymersof (mostly) D-pentose sugars alongwith a few hexoses and L-sugars as well.

Hemicelluloses are shorter than cellulose (500-3000 sugars versus 7000-15,000 sugars). Monomer sugars of polysaccharides besides glucose include xylose, mannose, galactose, rhamnose, and arabinose. Xylose is usually present in the greatest amount

Chitin

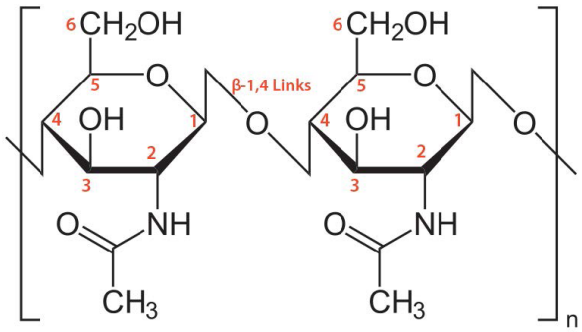


Fig 7.16 Chitin with β-1,4 links between N-acetylglucosamine sugars

Chitin (Figure 7.16) is another structural polysaccharide, being comprised of N-acetylglucosamine units joined by β-1,4 linkages. It is a primary component of the cell walls of fungi and is also prominent in the exoskeletons of arthropods and insects, as well as the beaks and internal shells of cephalopods. Chitin's structure was solved by Albert Hofmann in 1929. It is like cellulose except for the acetylamine group replacing the hydroxyl on position 2. This change allows hydrogen bonding to occur between adjacent polymers, thus providing greater strength.

Pectins

Another group of structural polysaccharides is the pectins (Figure 7.17). These com pounds are present in most primary plant cell walls and are abundant in non-woody parts of terrestrial plants. They are rich in galacturonic acid (α-1,4 links with no branches) and are used commercially as a gelling agent in jams/jellies, as well as a stabilizer in fruit juices and milk drinks. Pectin consumption may result in reduced blood cholesterol levels due to its tendency to 1) bind cholesterol and 2) to increase viscosity in the intestinal tract, thus reducing absorption of cholesterol from food. Pectins also trap carbohydrates in the digestive system and reduce their rate of absorption.

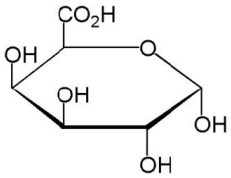


Fig 7.17 α-DGalacturonic acid - An important component of pectin polymers

Lectins

Lectins are not carbohydrates, but proteins that specifically bind to carbohydrate molecules found in animals and plants (where they are known as phytohemagglutinins) and are each highly specific for certain sugars. They function in cellular and molecular recognition, as well as cell adhesion. One lectin recognizes hydrolytic enzymes containing mannose-6-phosphate and targets them to be delivered to lysosomes. In the innate immune system, a mannose binding lectin helps defend against invading microbes.Other lectins have roles in inflammation and autoimmune disorders. Some viruses and bacteria use lectins to recognize and bind specific carbohydrate residues on the surface of target cells. Flu virus, for example, carries a lectin known as hemagglutinin (Fig 7.18) that binds to sialic acid and is essential for entrance of the virus into the target cell. After binding, the viral particle enters by endocytosis after the hemagglutinin has been cleaved by a protease. After replication of the virus inside of the cell, hemagglutinin and and a viral enzyme known as neuraminidase cluster in the cell membrane.

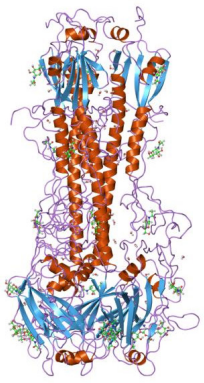


Fig 7.18 Hemagglutinin. http://www.ebi.ac.uk/

Viral RNA and associated viral proteins cluster near this membrane site and new viruses bud off in a portion of the cell's membrane after the hemagglutininsialic acid link to the infected cell is released by the neuraminidase cutting the bond between the sialic acid and the rest of the cell surface carbohydrate. Drugs, such as tamiflu, that interfere with neuraminidase work by preventing release of the viral particle. Unreleased particles will tend to aggregate and not function.

Some viral glycoproteins from hepatitis C virus may attach to lectins on the surface of liver cells in their infectious cycle. The bacterium *Helicobacter pylori* uses a cell surface lectin to bind oligosaccharides on epithelial cells lining the stomach. One lectin known as ricin is a very powerful toxin. It is produced in the endosperm of seeds of the castor oil plant and is of concern as a bioterrorism weapon as a result of its acute toxicity when inhaled or ingested.

Agar/agarose

A polysaccharide product that has numerous uses in laboratories is agar/agarose. Agarose is a polysaccharide polymer of D-galactose and 3,6-anhydro-Lgalactopyranose that is extracted from seaweed and has a repeating structure shown in Figure 7.19. Addition of agaropectin creates the material known as agar. Both substances make gel-like structures when boiled in water and then cooled. Agar is commonly used to make plates for culturing microorganisms and agarose is the common support of agarose gels used to separate DNA fragments in electrophoresis.

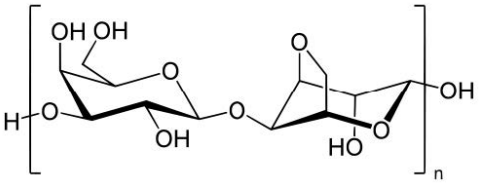


Fig 7.19 Repeating unit of agarose. 3,6-anhydro-L-galactopyranose

Glycosaminoglycans

Another variation on the polysaccharide theme is found in polymers known as the glycosaminoglycans. Previously known as mucopolysaccharides, glycosaminoglycans are polymers of unbranched repeating disaccharides. The repeating units of the disaccharide core of the molecules typically have an amino sugar (Nacetylglucosamine or Nacetylgalactosamine) and a uronic sugar (glucuronic acid or iduronic acid) or galactose. Glycosaminoglycans vary considerably in molecular mass, disaccharide structure, and sulfation. Glycosaminoglycans are organized in four groups - those found in connective tissue (linked to collagen) and they also act as lubricants for joints (hyaluronic acid in synovial fluid), asanti-clotting agents (heparin) and as components of mucus where they help to protect against infection.

Resources:

Chapter page: Delicious cupcakes on display with dark background Adobe stock #266867613. Licensed

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Chapter #8 Lipids

Introduction

Lipids are a diverse group of molecules that all share the characteristic that at least a portion of them is hydrophobic. Lipids play many roles in cells, including serving as energy storage (fats/oils), constituents of membranes (glycerophospholipids, sphingolipids, cholesterol), hormones (steroids), vitamins (fat soluble), oxygen/ electron carriers (heme), among others. For lipids that are very hydrophobic, such as fats/ oils, movement and storage in the aqueous environment of the body requires special structures. Other, amphipathic lipids, such as glycerophospholipids and sphingolipids spontaneously organize themselves into lipid bilayers when placed in water. Interestingly, major parts of many lipids can be derived from acetyl-CoA.

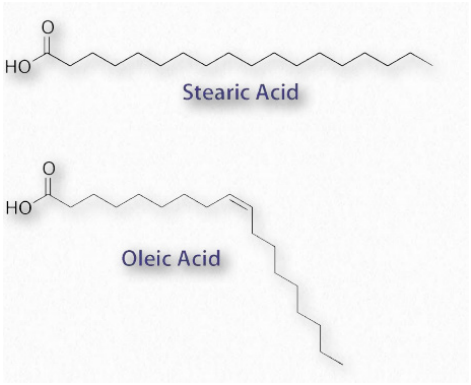

Fig 8.1 Saturated fatty acid (stearic acid) and unsaturated fatty acid (oleic acid)

Fatty acids

The most ubiquitous lipids in cells are the fatty acids. Found in fats, glycerophospholipids, sphingolipids and serving as as membrane anchors for proteins and other biomolecules, fatty acids are important for energy storage, membrane structure, and as precursors of most classes of lipids. Fatty acids, as can be seen from Figure 8.1 are characterized by a polar head group and a long hydrocarbon tail. Fatty acids with hydrocarbon tails that lack any double bonds are described as saturated, while those with one or more double bonds in their tails are known as unsaturated fatty acids. The effect of double bonds on the fatty acid tail is to introduce a kink, or bend, in the tail, as shown for oleic acid.

Common name	Chemical structure	
Caprylic acid	$CH_3(CH_2)_6COOH$	8
Capric acid	$CH_3(CH_2)_8COOH$	10
Lauric acid	$CH_3(CH_2)_{10}COOH$	12
Myristic acid	$CH_3(CH_2)_{12}COOH$	14
Palmitic acid	$CH_3(CH_2)_{14}COOH$	16
Stearic acid	$CH_3(CH_2)_{16}COOH$	18
Arachidic acid	$CH_3(CH_2)_{18}COOH$	20
Behenic acid	$CH_3(CH_2)_{20}COOH$	22
Lignoceric acid	$CH_3(CH_2)_{22}COOH$	24
Cerotic acid	$CH_3(CH_2)_{24}COOH$	26

Fig 8.2 Saturated fatty acids. Number of carbons in right column Wikipedia

Stearic acid, a saturated fatty acid, by contrast has a straight hydrocarbon tail. Figures 8.1-8.4 show the most common saturated and unsaturated fatty acids. Fatty acids with unsaturated tails have a lower melting temperature than those with saturated tails of the same length. Shorter tails also decrease melting temperature. These properties carry over to the fats/oils containing them.

Fatty acids with more than one double bond are called polyunsaturated. Plants are excellent sources of unsaturated and polyunsaturated fatty acids. The position of the double bond(s) in fatty acids has important considerations both for their synthesis and for their actions in the body. Biochemically, the double bonds found in fatty acids are predominantly in the cis configuration. So-called trans fats arise as a chemical by-product of partial hydrogenation of vegetable oil.

In humans, consumption of trans fat raises low density lipoprotein (LDL) levels and lowers high density lipoprotein (HDL) levels. Each is thought to contribute to the risk of developing coronary artery disease. The most common fatty acids in our body include palmitate, stearate, oleate, linolenate, linoleate, and arachidonate. Two notable shorter fatty acids are nonanoic (9 carbons) and decanoic acid (10 carbons), both of which appear to have anti-seizure effects. Decanoic acid directly inhibits excitatory neu-

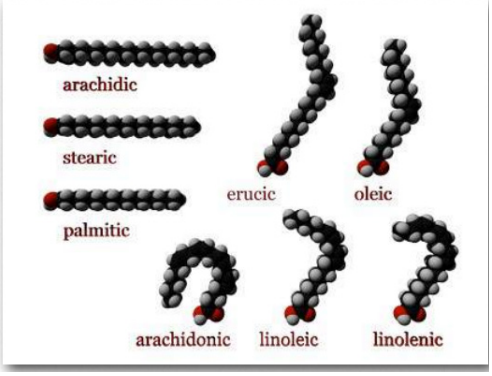

Fig 8.3 Fatty acid models. Carboxyl end labeled in red Wikipedia

Name	Double Bond Info	
Myristoleic acid	*cis*-Δ^9	14:1
Palmitoleic acid	*cis*-Δ^9	16:1
Sapienic acid	*cis*-Δ^6	16:1
Oleic acid	*cis*-Δ^9	18:1
Elaidic acid	*trans*-Δ^9	18:1
Vaccenic acid	*trans*-Δ^{11}	18:1
Linoleic acid	*cis,cis*-Δ^9,Δ^{12}	18:2
Linoelaidic acid	*trans,trans*-Δ^9,Δ^{12}	18:2
α-Linolenic acid	*cis,cis,cis*-$\Delta^9,\Delta^{12},\Delta^{15}$	18:3
Arachidonic acid	*cis,cis,cis,cis*-$\Delta^5\Delta^8,\Delta^{11},\Delta^{14}$	20:4
Eicosapentaenoic acid	*cis,cis,cis,cis,cis*-$\Delta^5,\Delta^8,\Delta^{11},\Delta^{14},\Delta^{17}$	20:5
Erucic acid	*cis*-Δ^{13}	22:1
Docosahexaenoic acid	*cis,cis,cis,cis,cis,cis*-$\Delta^4,\Delta^7,\Delta^{10},\Delta^{13},\Delta^{16},\Delta^{19}$	22:6

Fig 8.4 Unsaturated fatty acids. Right column Indicates number of carbons and double bonds Wikipedia

rot ransmission in the brain and may contribute to the anticonvulsant effect of the ketogenic diet.

Numbering

Figure 8.5 shows two different systems for locating double bonds in a fatty acid. The ω system counts carbons starting with the methyl end (shown in red) while the Δ system counts from the carboxyl end (shown in blue). For example, an ω-3 (omega 3) fatty acid would have a double bond at the third carbon from the methyl end. In the Δ system, a fatty acid that has a cis double bond at carbon 6, counting from the carboxyl end, would be written as cis-Δ6.

Fatty acids are described as essential fatty acids if they must be in the diet (can't be synthesized by the organism) and nonessential fatty acids if the organism can synthesize them. Humans and other animals lack the desaturase enzymes necessary to make double bonds at positions greater than Δ-9, so fatty acids with double bonds beyond this position must be obtained in the diet. Linoleic acid and linolenic acid, both fall in this category. Related unsaturated fatty acids can be made from these fatty acids, so the presence of linoleic and linolenic acids in the diet eliminates the need to have all unsaturated fatty acids in the diet. Both linoleic and linolenic acid contain 18 carbons, but linoleic acid is an ω-6 fatty acid, whereas linolenic acid is an ω-3 fatty acid. Notably, ω-6 fatty acids tend to be proinflammatory, whereas ω-3 fatty acids are lesser so.

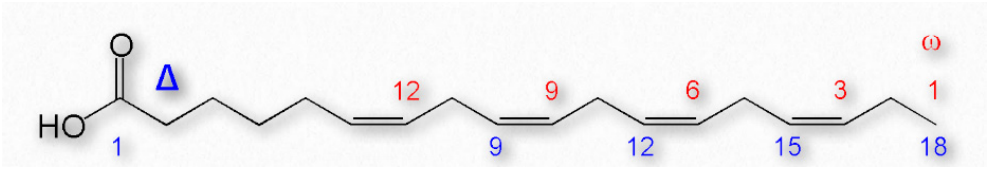

Fig 8.5 Δ and ω numbering systems for fatty acids Image by Pehr Jacobson

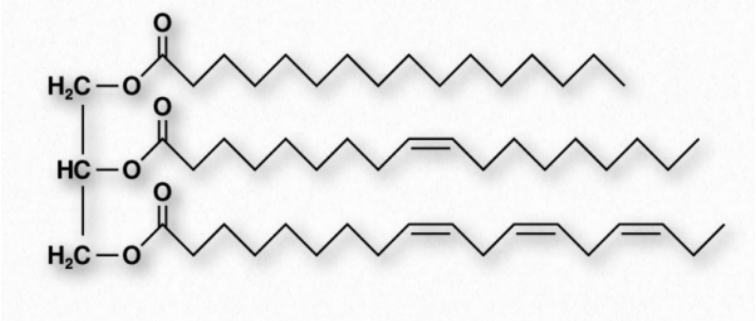

Fig 8.6 Structure of a fat/oil

Fats/oils

Fats and oils are the primary energy storage forms of animals and are also known as triacylglycerols and triglycerides, since they consist of a glycerol molecule linked via ester bonds to three fatty acids (Figure 8.6). Fats and oils have the same basic structure. We give the name fat to those compounds that are solid at room temperature and the name oil to those that are liquid at room temperature. Note that biological oils are not the same as petroleum oils. Increasing the number of unsaturated fatty acids (and the amount of unsaturation in a given fatty acid) in a fat decreases the melting temperature of it. Organisms like fish, which live in cool environments, have fats with more unsaturation and this is why fish oil contains polyunsaturated fatty acids.

Adipocytes

Fats are stored in the body in specialized cells known as adipocytes. Enzymes known as

lipases release fatty acids from fats by hydrolysis reactions (Figure 8.7). Triacylglycercol lipase is able to cleave the first two fatty acids from the fat. A second enzyme, monoacylglycerol lipase, cleaves the last fatty acid. Fats can be synthesized by replacing the phosphate on phosphatidic acid with a fatty acid.

Increasing the number of unsaturated fatty acids (and the amount of unsaturation in a given fatty acid) in a fat decreases the melting temperature of it. Organisms like fish, which live in cool environments, have fats with more unsaturation and this is why fish oil contains polyunsaturated fatty acids.

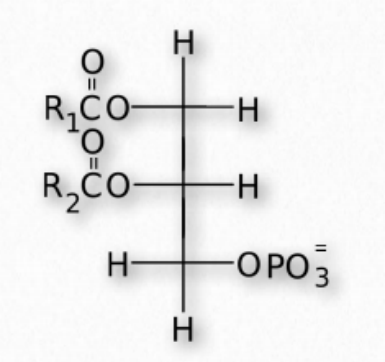

Fig 8.7 Structure of phosphatidic acid. R1 and R2 are alkyl groups of fatty acids.

Glycerophospholipids

Glycerophospholipids (phosphoglycerides) are important components of the lipid bilayer of cellular membranes. We will discuss cell membrane in the next chapter. Phosphoglycerides are structurally related to fats, as both are derived from phosphatidic acid (Figure 8.7). Phosphatidic acid is a simple glycerophospholipid that is usually converted into phosphatidyl compounds. These are made by esterifying various groups, such as ethanolamine, serine, choline, inositol, and others (Figure 8.8) to the phosphate of phosphatidic acid. All of these compounds form lipid bilayers in aqueous solution , due to their amphiphilic nature.

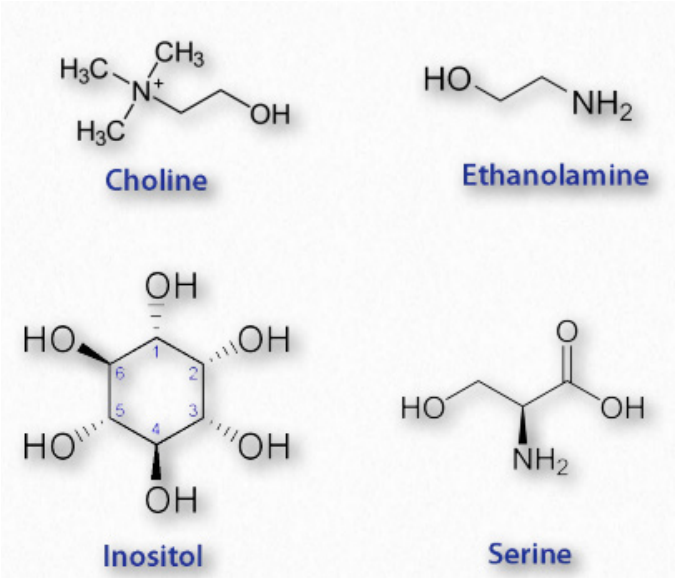

Fig 8.8 Four common components of phosphatides Wikipedia

Phosphatidylethanolamines

Since all glycerolipids can have a variety of fatty acids at positions 1 and 2 on the glycerol, they all are families of compounds. The phosphatidylethanolamines are found in all living cells and are one of the most common phosphatides, making up about 25% of them. They are common constituents of brain tissue and in the spinal cord, making up as much as 45% of the total phospholipids. Phosphatidylethanolamines are asymmetrically distributed across membranes, being preferentially located on the inner leaflet (closest to the cytoplasm) of the plasma membrane. Metabolically, phosphatidylethanloamines are precursors of phosphatidylcholines. Phosphatidylserines Phosphatidylserines are another group of phosphatidyl compounds that are preferentially distributed across the lipid bilayer of the plasma membrane. Like the phosphatidylethanolamines, phosphatidylserines are preferentially located on the inner leaflet of the plasma membrane. When apoptosis (cell suicide) occurs, the preferential distribution is lost and the phosphatidylserines appear on the outer leaflet where they serve as a signal to macrophages to bind and destroy the cell.

Phosphatidylcholines

Phosphatidylcholines (Figure 8.9) are another group of important membrane components. They tend to be found more commonly on the outer leaflet of the plasma membrane. Nutritionally, the compounds are readily obtained from eggs and soybeans. Phosphatidylcholines are moved across membranes by Phosphatidylcholine transfer protein (PCTP). This protein, which is sensitive to the levels of phosphatidylcholines, acts to stimulate the activity of a thioesterase (breaks thioester bonds, such as acyl-CoAs) and activates PAX3 transcription

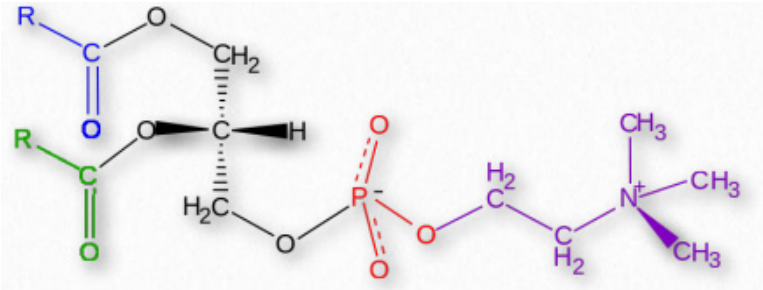

Fig 8.9 Phosphatidylcholine

factors.

Cardiolipins

Cardiolipins are an unusual set of glycerophospholipids in containing two diacylglycerol backbones joined in the middle by a diphosphoglycerol (Figure 8.10). It is an important membrane lipid, constituting about 20% of the inner mitochondrial membrane and is found in organisms from bacteria to humans. In both plants and animals, it is found almost totally in the inner mitochondrial membrane. The molecules appear to be required for both Complex IV and Complex III of the electron transport chain to maintain its structure. The ATP synthase enzyme (Complex V) of the oxidative phosphorylation system also binds four molecules of cardiolipin. It has been proposed that cardiolipin functions as a proton trap in the process of proton pumping by Complex IV. Cardiolipin also plays a role in apoptosis.

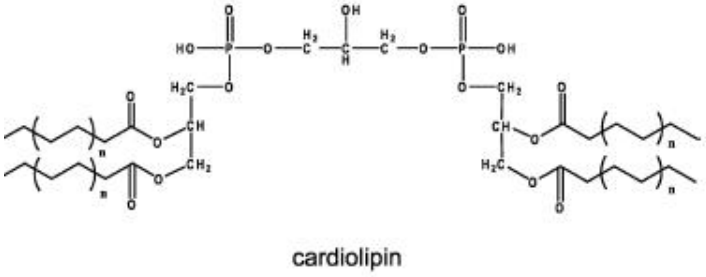

Fig 8.10 Cardiolipin-Rochellehx. Wikimedia Commons

The oxidation of cardiolipin by a cardiolipin-specific oxygenase causes cardiolipin to move from the inner mitochondrial membrane to the outer one, helping to form a permeable pore and facilitating the transport of cytochrome c out of the intermembrane space and into the cytoplasm - a step in the process of apoptosis.

Inositol

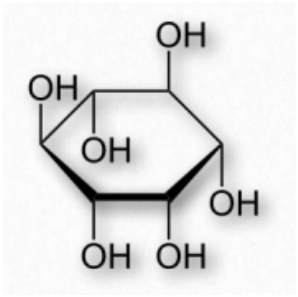

Fig 8.11 Inositol

Though technically not a lipid itself, inositol is found in many lipids. Inositol is a derivative of cyclohexane containing six hydroxyl groups - one on each carbon (Figure 8.11. It has nine different stereoisomers of which one, cis-1,2,3,5-trans-4,6- cyclohexanehexol (called myo-inositol) is the most common. It has a sweet taste (half that of sucrose).Numerous phosphorylated forms of the compound exist, from a single phosphate to six (one on each carbon). Phytic acid, for example, in plants, has six phosphates (Figure 8.12) that it uses to store phosphate. Inositol is produced from glucose and was once considered vitamin B8, but is made by the body in adequate amounts, so it is not now considered a vitamin. Phosphorylated forms of inositol are found in phosphoinositides, such as PIP2 and PIP3, both of which are important in signaling processes. Some of these include insulin signaling, fat catabolism, calcium regulation, and assembly of the cytoskeleton.

Phosphoinositides

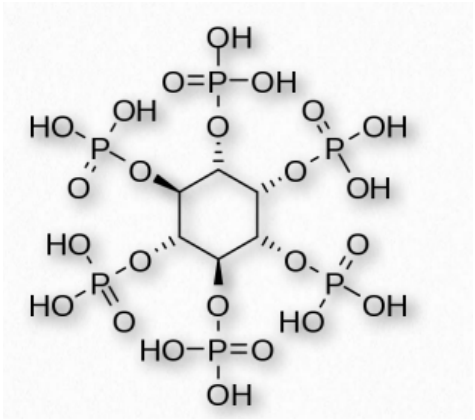

Fig 8.12 Phytic acid

Compounds based on phosphatidylinositol (PI) are often called phosphoinositides. These compounds have important roles in signaling and membrane trafficking. Hydroxyls on carbons 3,4, and 5 of the inositol ring are targets for phosphorylation by a variety of kinases. Seven different combinations are used. Steric hindrance inhibits phosphorylation of carbons 2 or 6. Naming of these phosphorylated compounds follows generally as PI(#P)P, PI(#P, #P)P, or PI(#P, #P, #P)P where #P refers to the number of the carbon where a phosphate is located. For example, PI(3)P refers to a phosphatidyl compound with a phosphate added to carbons 3 of the inositol ring, whereas PI(3,4,5)P is a phosphatidyl compound with a phosphate added to carbons 3,4,and 5.

Phosphatidylinositol-4,5- bisphosphate

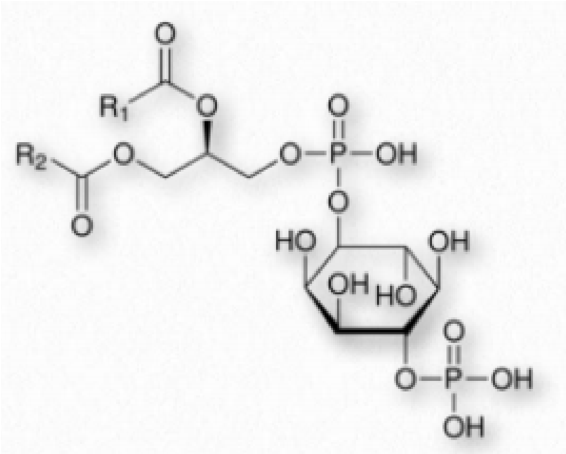

Fig 8.13 Phosphatidylinositol-4,5- phosphate

Phosphatidylinositol-4,5-bisphosphate (PIP2 - Figure 8.13) is a phospholipid of plasma membranes that functions in the phospholipase C signaling cascade. In this signaling pathway, hydrolysis catalyzed by phospholipase C releases inositol-1,4,5- trisphosphate (IP3) and diacylglycerol. Synthesis of PIP2 begins with phosphatidylinositol, which is phosphorylated at position 4 followed by phosphorylation at position 5 by specific kinases.

PIP2 can be phosphorylated to form the signaling molecule known as phosphatidylinositol (3,4,5)-trisphosphate (PIP3). Along with PIP3, PIP2 serves as a docking phospholipid for the recruitment of proteins that play roles in signaling cascades. Binding of PIP2 is also required by inwardly directed potassium channels.

Sphingolipids

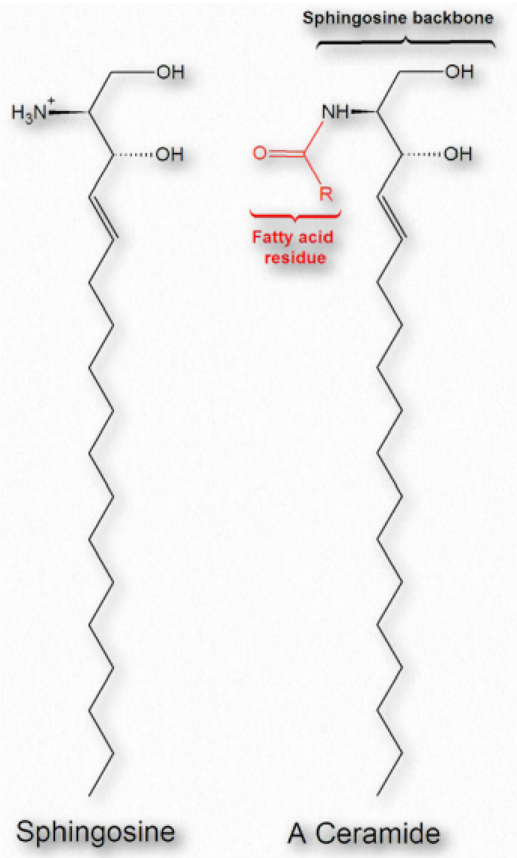

Fig 8.14 Sphingosine and a ceramide made from it Wikipedia

Fatty acids are also components of a broad class of molecules called sphingolipids. Sphingolipids are structurally similar to glycerophospholipids, though they are synthesized completely independently of them starting with palmitic acid and the amino acid serine. Sphingolipids are named for the amino alcohol known as sphingosine (Figure 8.14), though they are not directly synthesized from it. Figure 8.15 shows the generalized structure of sphingolipids.

If the R-group is a hydrogen, the molecule is called a ceramide. When the R-group is phosphoethanolamine the resulting molecule is sphingomyelin, an important component of the myelin sheath and lipid membranes. If a single, simple sugar is instead added, a cerebroside is created (Figure 8.16). Addition of a complex oligosaccharide creates a ganglioside.

Complex sphingolipids may play roles in cellular recognition and signaling. Sphingolipids are found most abundantly in plasma membrane and are almost completely absent from mitochondrial and endoplasmic reticulum membranes. In animals, dietary sphingolipids have been linked to reduced colon cancer, reductions in LDLs, and increases in HDLs. Like the glycerophospholipids, sphingolipids are amphiphilic. Most sphingolipids except sphingomyelin do not contain phosphate.

Other Lipids

Often lipids are broadly classified into storage and structure lipids, however, given the defination of lipids, there are mnay other compounds that fall under lipid. These include lipids that act as hormones, cofactors, pigments and signallng molecules.

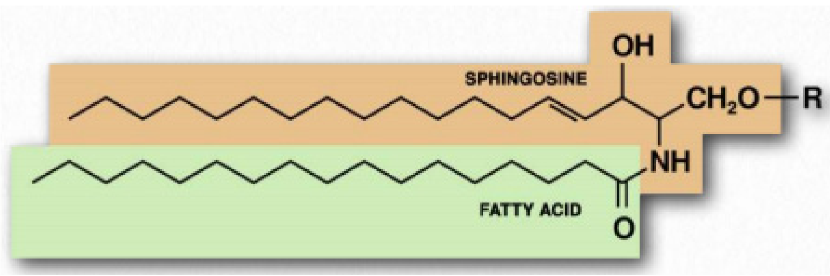

Fig 8.15 Wikipedia

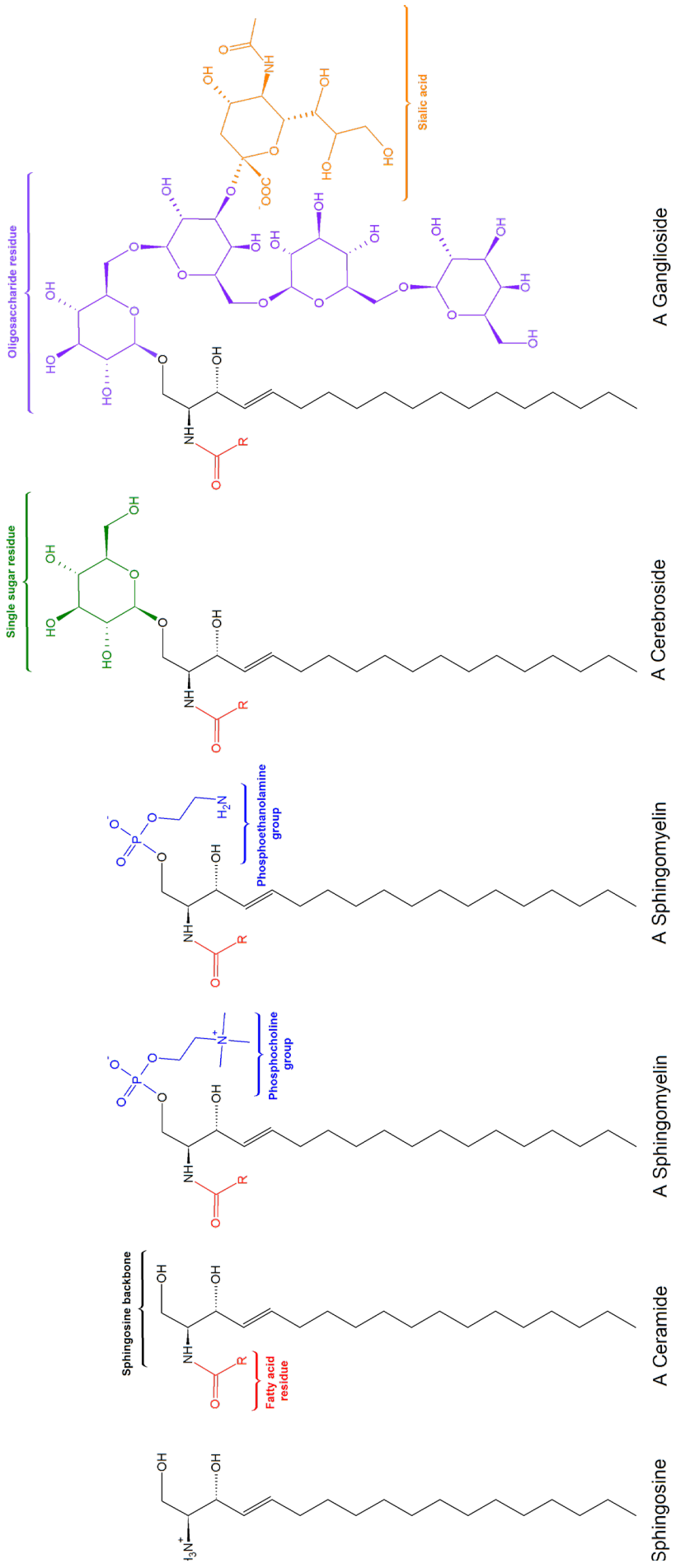

Fig 8.16 Categories of Sphingolipid. Colors represent various attachments to sphingosine chain. Wikipedia

Fatty acids made from omega-6 and omega-3 fatty acids include three important fatty acids containing 20 carbons. They include arachidonic acid (an ω-6 fatty acid with four double bonds (Δ-5,8,11,14) - Figure 8.16), eicosapentaenoic acid (an ω-3 fatty acid with five double bonds, and dihomo-γ-linolenic acid (an ω-6 fatty acid with three double bonds). The class of compounds known as eicosanoids is made by oxidation of these compounds. Subclasses include include prostaglandins, prostacyclins, thromboxanes, lipoxins, leukotrienes, and endocannabinoids. Eicosanoids play important roles affecting inflammation, immunity, mood, and behavior.

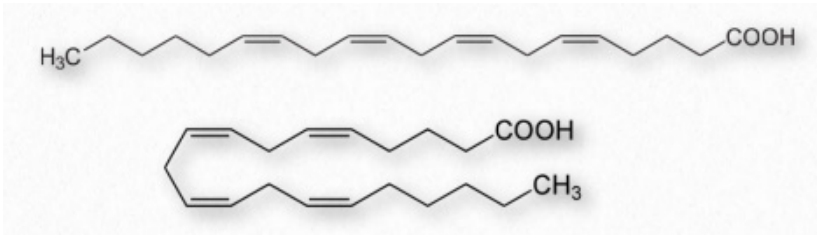

Fig 8.16 Arachidonic acid drawn as straight (top) and bent (bottom)

Prostaglandins

A collection of molecules acting like hormones, prostaglandins are derived from arachidonic acid and have many differing (even conflicting) physiological effects. These include constriction or dilation of vascular smooth muscle cells, induction of labor, regulation of inflammation, and action on the thermoregulatory center of the hypothalamus to induce fever, among others.

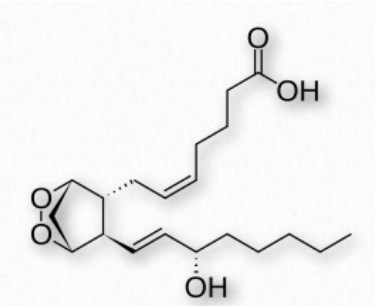

Fig 8.17 Prostaglandin PGH2

Prostaglandins are grouped with the thromboxanes (below) and prostacyclins (below), as prostanoids. The prostanoids, which all contain 20 carbons are a subclass of the eicosanoids. Prostaglandins are found in most tissues of higher organisms. They are autocrine or paracrine compounds produced from essential fatty acids. The primary precursor of the prostaglandins is the fatty acid known as arachidonic acid and the prostaglandin made from it is known as PGH2 (Figure 8.17), which, in turn is a precursor of other prostaglandins, as well as the prostacyclins and thromboxanes.

Thromboxanes

Thromboxanes play roles in clot formation and named for their role in thrombosis. They are potent vasoconstrictors and facilitate platelet aggregation. They are synthesized in platelets, as well. The anti-clotting effects of aspirin have their roots in the inhibition of synthesis of PGH2, which is the precursor of the thromboxanes. The most common thromboxanes are A2 (Figure 8.18) and B2.

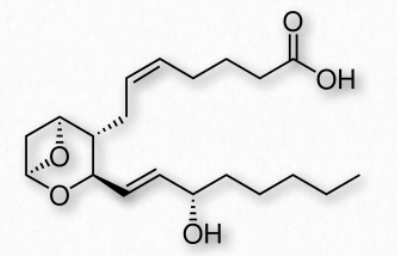

Fig 8.17 Prostaglandin PGH2

Prostacyclin

Prostacyclin (also known as prostaglandin I2 or PGI2 - Figure 8.18) counters the effects of thromboxanes, inhibiting platelet activation and acting as vasodilators. It is produced from PGH2 by action of the enzyme prostacyclin synthase.

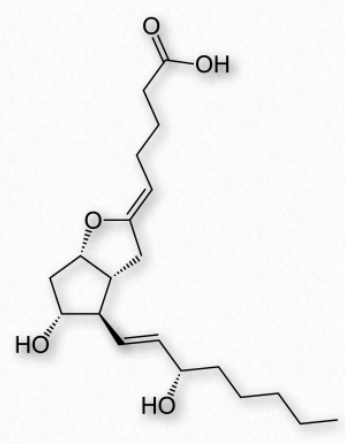

Fig 8.18 Prostacyclin

Leukotrienes

Another group of eicosanoid compounds are the leukotrienes (Figure 2.219). Like prostaglandins, leukotrienes are made from arachidonic acid. The enzyme catalyzing their formation is a dioxygenase known as arachidonate 5-lipoxygenase. Leukotrienes are involved in regulating immune responses. They are found in leukocytes and other immunocompetent cells, such as neutrophils, monocytes, mast cells, eosinophils, and basophils. Leukotrienes are associated with production of histamines and prostaglandins, which act as mediators of inflammation. Leukotrienes also trigger contractions in the smooth muscles of the bronchioles. When overproduced, they may pay a role in asthma and allergic reactions. Some treat-

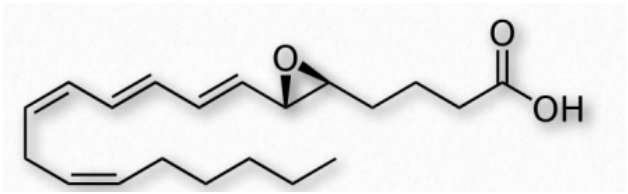

Fig 8.19 Leukotriene A4 (LTA4)

ments for asthma aim at inhibiting production or action of leukotrienes.

Cholesterol

Arguably, no single biomolecule has generated as much discussion and interest as has cholesterol (Figure 8.20). Certainly, from the perspective of the Nobel Prize committee, no small molecule even comes close, with 13 people having been awarded prizes for work on it. Evidence for cholesterol's importance comes from the study of brain tissue where it comprises 10-15% of the dry mass.

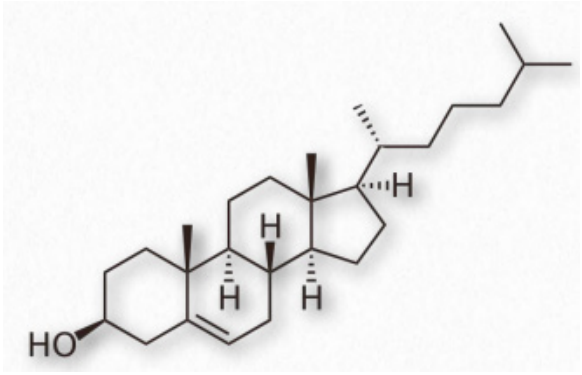

Fig 8.20 Structure of cholesterol

In animal membranes, cholesterol is important as an insulator for the transmission of signals in nerve tissue and it helps to manage fluidity of membranes over a wide range of temperatures. Cholesterol is made in many cells of the body, with the liver making the greatest amount. Cholesterol is only rarely found in prokaryotes (Mycoplasma, which requires it for growth, is an exception) and is found in only trace amounts in plants. Instead, plants synthesize similar compounds called phytosterols. On average, the body of a 150 pound adult male makes about 1 gram of cholesterol per day, with a total content of about 35 grams.

Cholesterol's (and other lipids') hydrophobicity requires special packaging into lipoprotein complexes (called chylomicrons, VLDLs, IDLs, LDLs, and HDLs) for movement in the lymph system and bloodstream. Though cholesterol can be made by cells, they also take it up from the blood supply by absorbing cholesterol-containing LDLs directly in a process called receptor-mediated endocytosis.

Oxidative damage to LDLs can lead to formation of atherosclerotic plaques and this is why cholesterol has gotten such a negative image in the public eye. The liver excretes cholesterol through the bile for elimination into the digestive system, but the compound is recycled there.

Cannabinoids

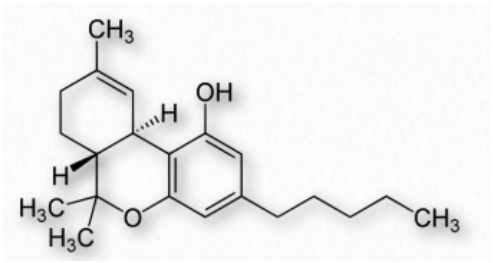

Fig 8.21 Tetrahydrocannabinol - Active ingredient in marijuana

Cannabinoids are a group of chemicals that bind to and have effects on brain receptors (cannabinoid receptors), repressing neurotransmitter release. Classes of these compounds include endocannabinoids (made in the body), phytocannabinoids (made in plants, such as marijuana), and synthetic cannabinoids (man-made).

Endocannabinoids are natural molecules derived from arachidonic acid. Cannabinoid receptors are very abundant, comprising the largest number of G-protein- 247 Figure 2.243 Tetrahydrocannabinol - Active ingredient in marijuana coupled receptors found in brain. The best known phytocannabinoid is Δ-9- tetrahydrocannabinol (THC), the primary psychoactive ingredient (of the 85 cannabinoids) of marijuana (Figure 8.21).

Anandamide

Anandamide (N-arachidonoylethanolamine) is an endocannabinoid neurotransmitter derived from arachidonic acid. It exerts its actions primarily through the CB1 and CB2 cannabinoid receptors, the same ones bound by the active ingredient in marijuana, Δ9-tetrahydrocannabinol. Anandamide has roles in stimulating eating/appetite and affecting motivation and pleasure.

It has been proposed to play a role in "runners high," an analgesic effect experienced from exertion, especially among runners. Anandamide appears to impair memory function in rats.

Anandamide has been found in chocolate and two compounds that mimic its effects (N-oleoylethanolamine and Nlinoleoylethanolamine) are present as well. The enzyme fatty acid amide hydrolase (FAAH) breaks down anandamide into free arachidonic acid and ethanolamine.

Resources and refrences:

Chapter page: Yellow fat Adobe stock #343269485 free-licensed.

Nelson, D. L., & Cox, M. M. (2017). Lehninger principles of biochemistry (7th ed.). W.H. Freeman.

Open Stax Biology 2e: Access for free at https://openstax.org/books/biology-2e

Fig 8.16 LHcheM, CC BY-SA 3.0 <https://creativecommons.org/licenses/by-sa/3.0>, via Wikimedia Commons

Chapter #9 Nucleic acids

Introduction

The nucleic acids, DNA and RNA, may be thought of as the information molecules of the cell. In this section, we will examine the structures of DNA and RNA, and how these structures are related to the functions these molecules perform.

We will begin with DNA, which is the hereditary information in every cell, that is copied and passed on from generation to generation. The race to elucidate the structure of DNA was one of the greatest stories of 20th century science. Discovered in 1869 by Friedrich Miescher, DNA was identified as the genetic material in experiments in the 1940s led by Oswald Avery, Colin MacLeod, and Maclyn McCarty. X-ray diffraction work of Rosalind Franklin and the observations of Erwin Chargaff were combined by James Watson and Francis Crick to form a model of DNA that we are familiar with today. Their famous paper, in the April 25, 1953 issue of Nature, opened the modern era of molecular biology. Arguably, that one-page paper has had more scientific impact per word than any other research article ever published. Today, every high school biology student is familiar with the double helical structure of DNA and knows that G pairs with C and A with T.

The double helix, made up of a pair of DNA strands, has at its core, bases joined by hydrogen bonds to form base pairs - adenine always paired with thymine, and guanine invariably paired with cytosine. Two hydrogen bonds are formed between adenine and thymine, but three hydrogen bonds hold together guanine and cytosine (Figure 9.1).

The complementary structure immediately suggested to Watson and Crick how DNA might be (and in fact, is) replicated and it further explains how information is DNA is transmitted to RNA for the synthesis of proteins. In addition to the hydrogen bonds between bases of each strand, the double helix is held together by hydrophobic interactions of the stacked, non-polar bases. Crucially, the sequence of the bases in DNA carry the information for making proteins. Read in groups of three, the sequence of the bases directly specifies the sequence of the amino acids in the encoded protein.

Structure

A DNA strand is a polymer of nucleoside monophosphates held together by phosphodiester bonds. Two such paired strands make up the DNA molecule, which is then twisted into a helix. In the most common B form, the DNA helix has a repeat of 10.5 base pairs per turn, with sugars and phosphate forming the covalent phosphodiester "backbone" of the molecule and the adenine, guanine, cytosine, and thymine bases oriented in the middle where they form the now familiar base pairs that look like the rungs of a ladder.

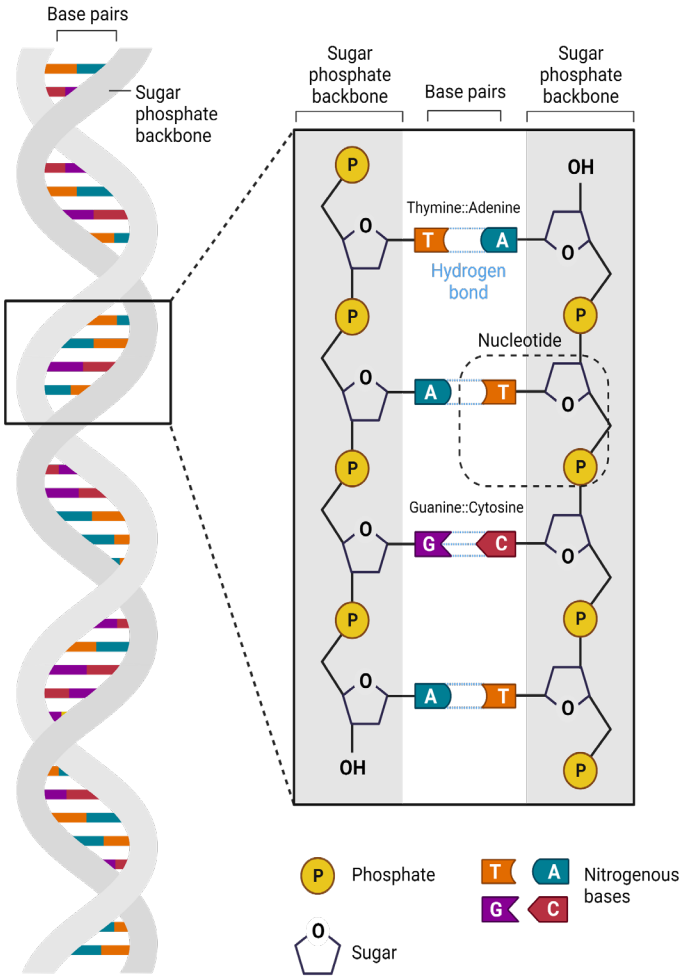

Fig 9.1 A DNA duplex with base pairs Adapted from Biorender by Manjeet Kumari

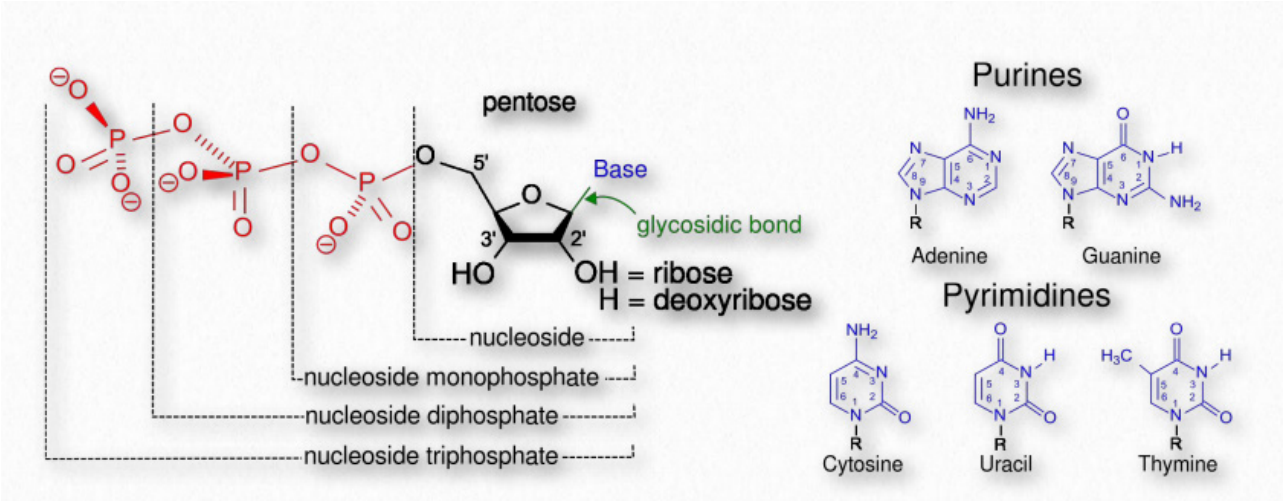

Fig 9.2 Nucleotides, nucleosides, and bases

Building blocks

The term nucleotide refers to the building blocks of both DNA (deoxyribonucleoside triphosphates, dNTPs) and RNA (ribonucleoside triphosphates, NTPs). In order to discuss this important group of molecules, it is necessary to define some terms.

Nucleotides (Fig 9.2) contain three primary structural components. These are a nitrogenous base, a pentose sugar, and at least one phosphate. Molecules that contain only a sugar and a nitrogenous base (no phosphate) are called nucleosides. The nitrogenous bases found in nucleic acids include adenine and guanine (called purines) and cytosine, uracil, or thymine (called pyrimidines). There are two sugars found in nucleotides - deoxyribose and ribose (Figure 9.2). By convention, the carbons on these sugars are labeled 1' to 5' (This is to distinguish the carbons on the sugars from those on the bases, which have their carbons simply labeled as 1, 2, 3, etc.). Deoxyribose differs from ribose at the 2' position, with ribose having an OH group, where deoxyribose has H.

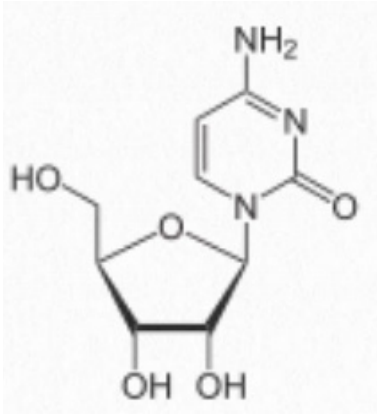

Fig 9.3 Cytidine

Nucleotides containing deoxyribose are called deoxyribonucleotides and are the forms found in DNA. Nucleotides containing ribose are called ribonucleotides and are found in RNA. Both DNA and RNA contain nucleotides with adenine, guanine, and cytosine, but with very minor exceptions, RNA contains uracil nucleotides, whereas DNA contains thymine nucleotides. When a base is attached to a sugar, the product, a nucleoside, gains a new name.

Uracil-containing = uridine (attached to ribose) / deoxyuridine (attached to deoxyribose)
Thymine-containing = ribothymidine (attached to ribose) / thymidine (attached to deoxyribose)
Cytosine-containing = cytidine (attached to ribose - Figure 9.3) / deoxycytidine (attached to deoxyribose)
Guanine-containing = guanosine (attached to ribose) / deoxyguanosine (attached to deoxyribose)
Adenine-containing = adenosine (attached to ribose) / deoxyadenosine (attached to deoxyribose)

Of these, deoxyuridine and ribothymidine are the least common. The addition of one or more phosphates to a nucleoside makes it a nucleotide. Nucleotides are often referred to as nucleoside phosphates, for this reason. The number of phosphates in the nucleotide is indicated by the appropriate prefixes (mono, di or tri).

Thus, cytidine, for example, refers to a nucleoside (no phosphate), but cytidine monophosphate refers to a nucleotide (with one phosphate). Addition of second and third phos-

phates to a nucleoside monophosphate requires energy, due to the repulsion of negatively charged phosphates and this chemical energy is the basis of the high energy triphosphate nucleotides (such as ATP) that fuel cells.

Deoxyribonucleotides

Individual deoxyribonucleotides are derived from corresponding ribonucleoside diphosphates via catalysis by the enzyme known as ribonucleotide reductase (RNR). The deoxyribonucleoside diphosphates are then converted to the corresponding triphosphates (dNTPs) by the addition of a phosphate group. Synthesis of nucleotides containing thymine is distinct from synthesis of all of the other nucleotides and will be discussed later.

Building DNA strands

Each DNA strand is built from dNTPs by the formation of a phosphodiester bond, catalyzed by DNA polymerase, between the 3'OH of one nucleotide and the 5' phosphate of the next. The result of this directional growth of the strand is that the one end of the strand has a free 5' phosphate and the other a free 3' hydroxyl group (Figure 2.130). These are designated as the 5' and 3' ends of the strand.

Hydrogen bonds

Hydrogen bonds between the base pairs hold a nucleic acid duplex together, with two hydrogen bonds per A-T pair (or per A-U pair in RNA) and three hydrogen bonds per G-C pair. The B-form of DNA has a prominent major groove and a minor groove tracing the path of the helix (Figure 9.5). Proteins, such as transcription factors bind in these grooves and access the hydrogen bonds of the base pairs to "read" the sequence therein.

Other forms of DNA besides the B-form are known (Figure 9.6). One of these, the A-form, was identified by Rosalind Franklin in the same issue of Nature as Watson and Crick's paper. Though the A-form structure is a relatively minor form of DNA and resembles the B-form, it turns out to be important in the duplex form of RNA and in RNA-DNA hybrids. Both the A form and the B-form of DNA have the helix oriented in what is termed the right-handed form.

Z-DNA

The A-form and the B-form stand in contrast to another form of DNA, known as the Z-form. ZDNA, as it is known, has the same base-pairing rules as the B and A forms, but instead has the helices twisted in the opposite direction, making a left-handed helix (Figure

Ribonucleotides as Energy Sources

Though ATP is the most common and best known cellular energy source, each of the four ribonucleotides plays important roles in providing energy. GTP, for example, is the energy source for protein synthesis (translation) as well as for a handful of metabolic reactions. A bond between UDP and glucose makes UDP-glucose, the building block for making glycogen. CDP is similarly linked to several different molecular building blocks important for glycerophospholipid synthesis (such as CDP-diacylglycerol).

The bulk of ATP made in cells is not from directly coupled biochemical metabolism, but rather by the combined processes of electron transport and oxidative phosphorylation in mitochondria and/or photophosphorylation that occurs in the chloroplasts of photosynthetic organisms. Triphosphate energy in ATP is transferred to the other nucleosides/nucleotides by action of enzymes called kinases. For example, nucleoside diphosphokinase (NDPK) catalyzes the following reaction

ATP+NDP <=>ADP+NTP

where 'N' of "NDP" and "NTP corresponds to any base. Other kinases can put single phosphates onto nucleosides or onto nucleoside monophosphates using energy from ATP.

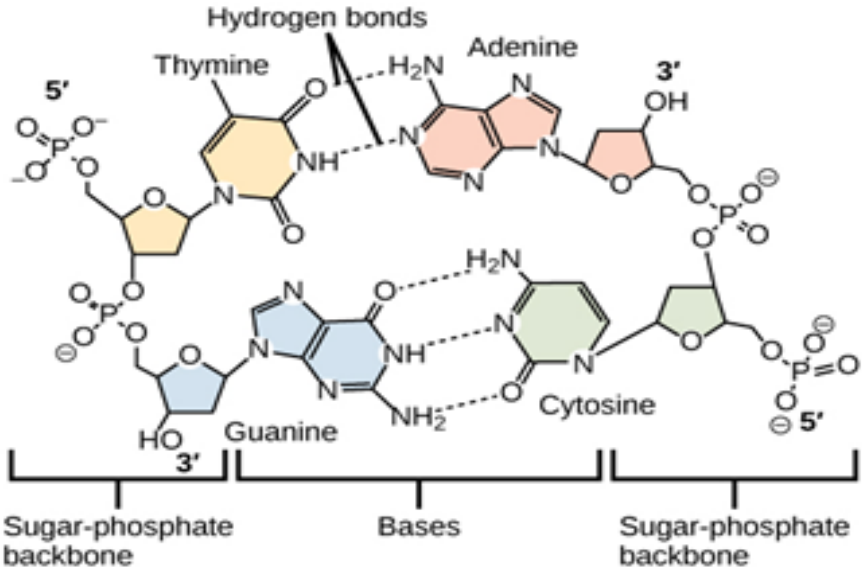

Fig 9.4 5'-3' Polarity of a DNA strand. Adapted from Openstax, Biology 2e

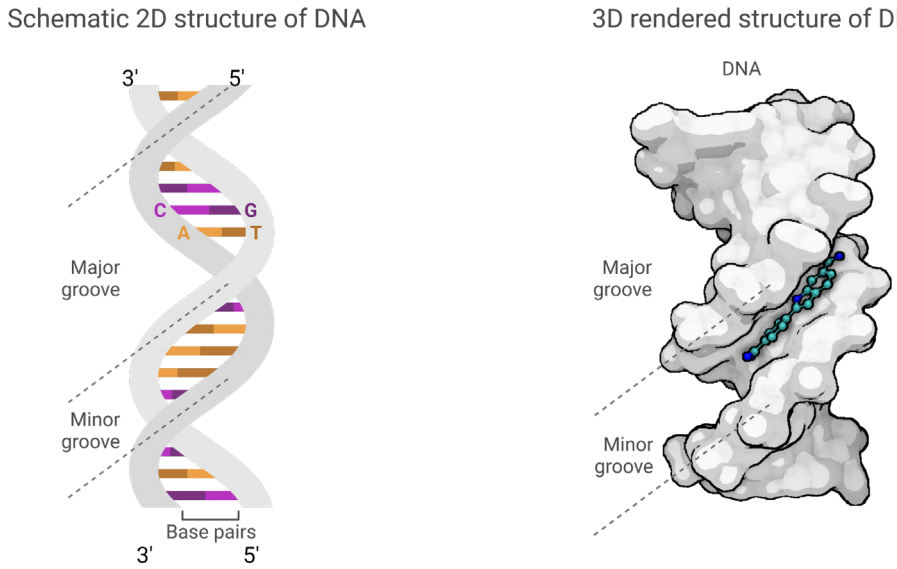

Fig 9.5 Major and minor grooves of DNA. The minor groove has been bound by a dye. Adapted from Biorender by Manjeet Kumari.

sion is discussed below.

Superhelicity

Short stretches of linear DNA duplexes exist in the B-form and have 10.5 base pairs per turn. Double helices of DNA in the cell can vary in the number of base pairs per turn they contain. There are several reasons for this. For example, during DNA replication, strands of DNA at the site of replication get unwound at the rate of 6000 rpm by an enzyme called helicase. The effect of such local unwinding at one place in a DNA has the effect increasing the winding ahead of it. Unrelieved, such 'tension' in a DNA duplex can result in structural obstacles to replication.

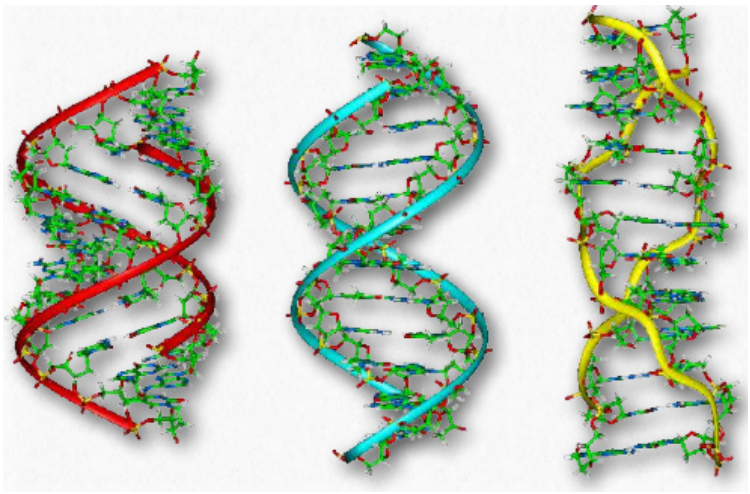

Fig 9.6 From left to right, the A-, B-, and Zforms of DNA

Such adjustments can occur in three ways. First, tension can provide the energy for 'flipping' DNA structure. Z-DNA can arise as a means of relieving the tension. Second, DNA can 'supercoil' to relieve the tension. Figures 9.7 shows negatively supercoiled, relaxed circular and positively supercoiled DNA interconverted by topoisomerase enzyme. In this method, the duplex crosses over itself repeatedly, much like a rubber band will coil up if one holds one section in place and twists another part of it. Third, enzymes called topoisomerases can act to relieve or, in some cases, increase the tension by adding or removing twists in the DNA. A "relaxed" DNA has 10.5 base pairs per turn. Each turn corresponds to one twist of the DNA. Using enzymes, it is possible to change the number of base pairs per turn. In either the case of increasing or decreasing the twists per turn, tension is introduced into the DNA structure.

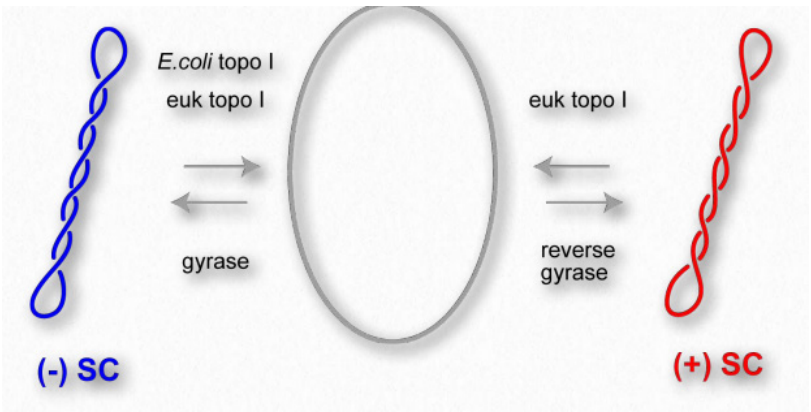

Fig 9.7 Supercoiled (SC) structures.

RNA

The structure of RNA is very similar to that of a single strand of DNA. Built of ribonucleotides, joined together by the same sort of phosphodiester bonds as in DNA, RNA uses uracil in place of thymine. In cells, RNA is assembled by RNA polymerases, which copy a DNA template in the much same way that DNA polymerases replicate a parental strand. During the synthesis of RNA, uracil is used across from an adenine in the DNA template. The building of messenger RNAs by copying a DNA template is a crucial step in the transfer of the information in DNA to a form that directs the synthesis of protein. Additionally, ribosomal and transfer RNAs serve important roles in "reading" the information in the mRNA codons and in polypeptide synthesis. RNAs are also known to play important roles in the regulation of gene expression.

RNA world

The discovery, in 1990, that RNAs could play a role in catalysis, a function once thought to be solely the domain of proteins, was followed by the discovery of many more so-called ribozymes- RNAs that functioned as enzymes. This suggested the answer to a long-standing chicken or egg puzzle - if DNA encodes proteins, but the replication of DNA requires proteins, how did a replicating system come into being? This problem could be solved if the first replicator was RNA, a molecule that can both encode information and carry out catalysis. This idea, called the "RNA world" hypothesis, suggests that DNA as genetic material and proteins as catalysts arose later, and eventually prevailed because of the advantages they offer. The lack of a 2'OH on deoxyribose makes DNA more stable than RNA. The double-stranded structure of DNA also provides an elegant way to easily replicate it. RNA catalysts, however, remain, as remnants of that early world. In fact, the formation of peptide bonds, essential for the synthesis of proteins, is catalyzed by RNA.

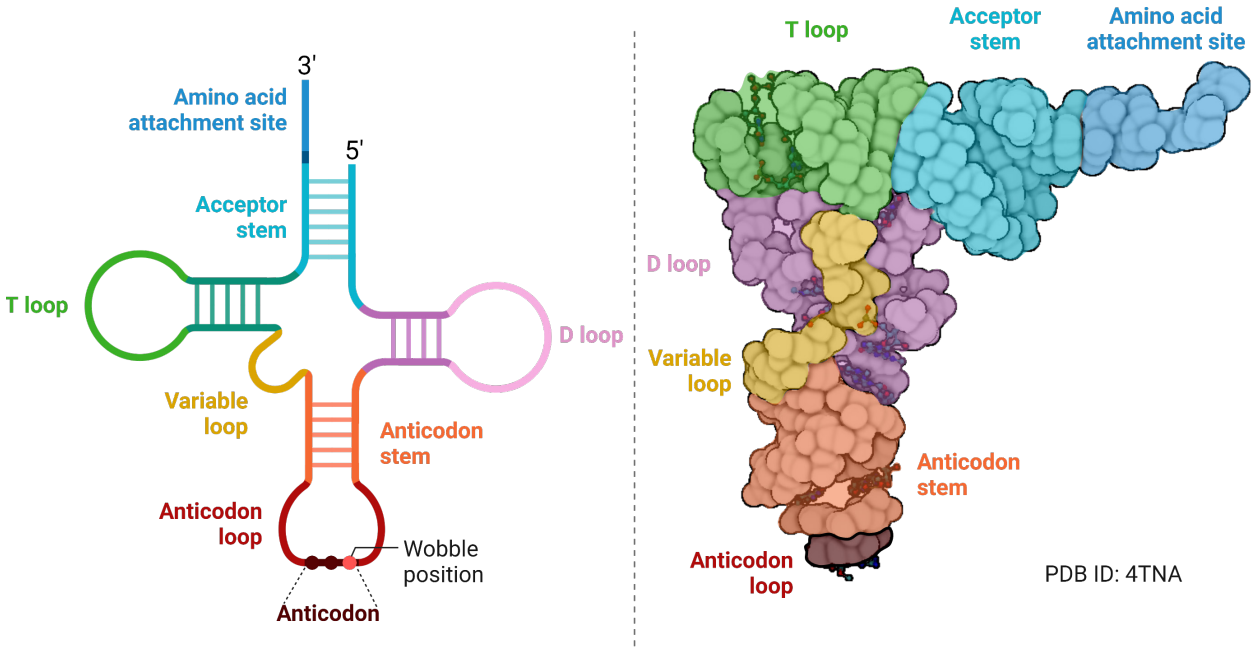

Fig 9.8 tRNA Images - 2D projection (left) and 3D projection (right). Adapted from Biorender by Manjeet Kumari

Secondary structure

With respect to structure, RNAs are more varied than their DNA cousin. Created by copying regions of DNA, cellular RNAs are synthesized as single strands, but they often

have self-complementary regions leading to "foldbacks" containing duplex regions. These are most easily visualized in the ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) (Figure 9.8), though other RNAs, including messenger RNAs (mRNAs), small nuclear RNAs (snRNAs), microRNAs (Figure 9.9), and small interfering RNAs (siRNAs) may each have double helical regions as well.

Fig 9.9 MicroRNA stem loops. Wikipedia

Base pairing

Base pairing in RNA is slightly different than DNA. This is due to the presence of the base uracil in RNA in place of thymine in DNA. Like thymine, uracil forms base pairs with adenine, but unlike thymine, uracil can, to a limited extent, also base pair with guanine, giving rise to many more possibilities for pairing within a single strand of RNA.

These additional base pairing possibilities mean that RNA has many ways it can fold upon itself that single-stranded DNA cannot. Folding, of course, is critical for protein function, and we now know that, like proteins, some RNAs in their folded form can catalyze reactions just like enzymes. Such RNAs are referred to as ribozymes. It is for this reason scientists think that RNA was the first genetic material, because it could not only carry information, but also catalyze reactions. Such a scheme might allow certain RNAs to make copies of themselves, which would, in turn, make more copies of themselves, providing a positive selection.

Stability

RNA is less chemically stable than DNA. The presence of the 2' hydroxyl on ribose makes RNA much more prone to hydrolysis than DNA, which has a hydrogen instead of a hydroxyl. Further, RNA has uracil instead of thymine. It turns out that cytosine is the least chemically stable base in nucleic acids. It can spontaneously deaminate and in turn is converted to a uracil. This reaction occurs in both DNA and RNA, but since DNA normally has thymine instead of uracil, the presence of uracil in DNA indicates that deamination of cytosine has occurred and that the uracil needs to be replaced with a cytosine. Such an event occurring in RNA would be essentially undetectable, since uracil is a normal component of RNA. Mutations in RNA have much fewer consequences than mutations in DNA because they are not passed between cells in division.

Catalysis

RNA structure, like protein structure, has importance, in some cases, for catalytic function. Like random coils in proteins that give rise to tertiary structure, single-stranded regions of RNA that link duplex regions give these molecules a tertiary structure, as well. Catalytic RNAs, called ribozymes, catalyze important cellular reactions, including the formation of peptide bonds in ribosomes. DNA, which is usually present in cells in strictly duplex forms (no tertiary structure, per se), is not known to be involved in catalysis.

RNA structures are important for reasons other than catalysis. The 3D arrangement of tRNAs is necessary for enzymes that attach amino acids to them to do so properly. Further, small RNAs called siRNAs found in the nucleus of cells appear to play roles in both gene regulation and in cellular defenses against viruses. The key to the mechanisms of these actions is the formation of short foldback RNA structures that are recognized by cellular proteins and then chopped into smaller units. One strand is copied and used to base pair with specific mRNAs to prevent the synthesis of proteins from them.

Denaturing nucleic acids

Like proteins, nucleic acids can be denatured. Forces holding duplexes together include hydrogen bonds between the bases of each strand that, like the hydrogen bonds in proteins, can be broken with heat or urea. (Another important stabilizing force for DNA arises from the stacking interactions between the bases in a strand.) Single strands absorb light at 260 nm more strongly than double strands. This is known as the hyperchromic effect (Figure 9.10) and is a consequence of the disruption of interactions among the stacked bases. The changes in absorbance allow one to easily follow the course of DNA denaturation. Denatured duplexes can readily renature when the temperature is lowered below the "melting temperature" or Tm, the temperature at which half of the DNA strands are in duplex form. Under such conditions, the two strands can re-form hydrogen bonds between the complementary sequences, returning the duplex to its original state. For DNA, strand separation and rehybridization are important for the technique known as the polymerase chain reaction (PCR). Strand separation of DNA duplexes is accomplished in the method by heating them to boiling. Hybridization is an important aspect of the method that requires single stranded primers to "find" matching sequences on the template DNA and form a duplex. Considerations for efficient hybridization (also called annealing) include temperature, salt concentration, strand concentration, and magnesium ion levels.

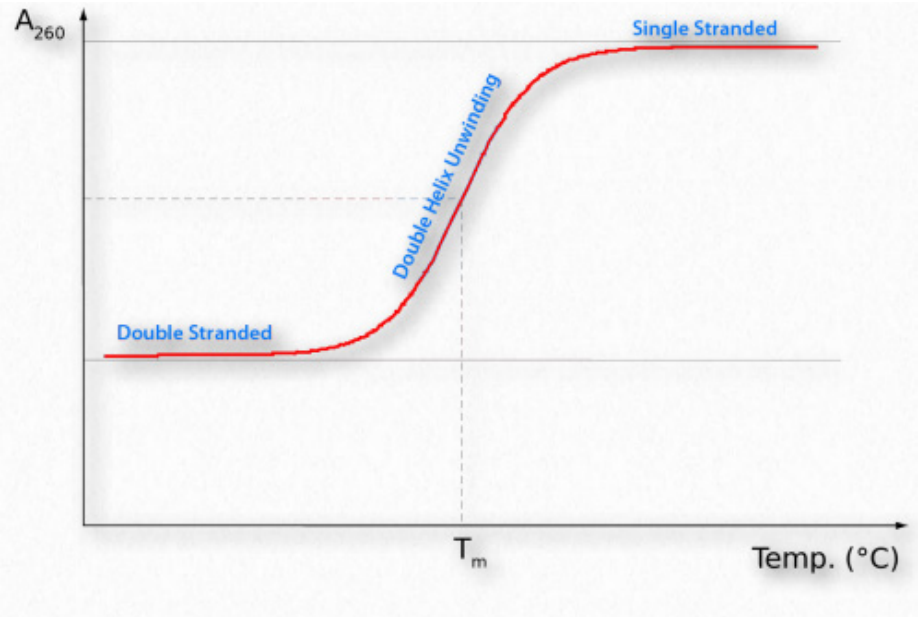

Fig 9.10 The hyperchromic effect Wikipedia

DNA packaging

DNA is easily the largest macromolecule in a cell. The single chromosome in small bacterial cells, for example, can have a molecular weight of over 1 billion Daltons. If one were to take all of the DNA of human chromosomes from a single cell and lay them end to end, they would be over 7 feet long. Such an enormous molecule demands careful packaging to fit within the confines of a nucleus (eukaryotes) or a tiny cell (bacteria). The chromatin system of eukaryotes is the best known, but bacteria, too, have a system for compacting DNA.

DNA in Bacteria

In bacteria, there is no nucleus for the DNA. Instead, DNA is contained in a structure called a nucleoid (Figure 9.11). It contains about 60% DNA with much of the remainder comprised of RNAs and transcription factors. Bacteria do not have histone proteins that DNA wrap around, but they do have proteins that help organize the DNA in the cell - mostly by making looping structures.

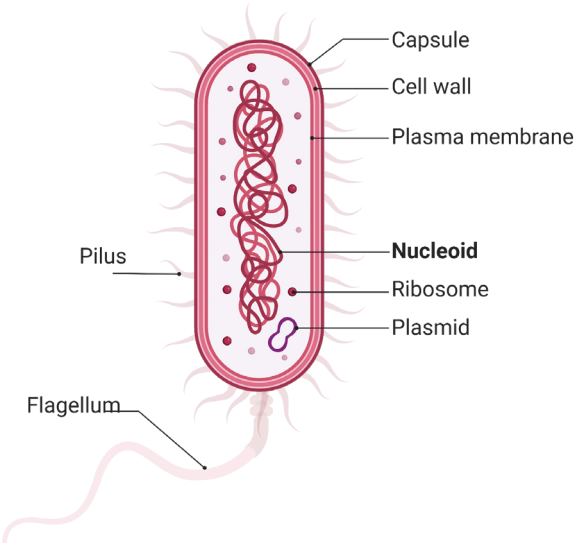

Fig 9.11 Nucloid in bacterium. Adapted from Biorender by Manjeet Kumari

These proteins are known as Nucleoid Associated Proteins and include ones named HU, H-NS, Fis, CbpA, and Dps. Of these, HU most resembles eukaryotic histone H2B and binds to DNA non-specifically. The proteins associate with the DNA and can also cluster, which may be the origin of the loops. It is likely these proteins play a role in helping to regulate transcription and respond to DNA damage. They may also be involved in recombination.

Eukaryotes

The method eukaryotes use for compacting DNA in the nucleus is considerably different, and with good reason - eukaryotic DNAs are typically much larger than prokaryotic DNAs, but must fit into a nucleus that is not much bigger than a prokaryotic cell. The strategy employed in eukaryotic cells is that of spooling - DNA is coiled around positively charged proteins called histones (Figure 9.12). These proteins, whose sequence is very similar in cells as diverse as yeast and humans, come in four types, dubbed H1, H2a, H2b, H3, and H4. A sixth type, referred to as H5 is actually an isoform of H1 and is rare. Two each of H2a, H2b, H3, and H4 are found in the core structure of what is called the fundamental unit of chromatin - the nucleosome (Figure 9.12 and 9.13).

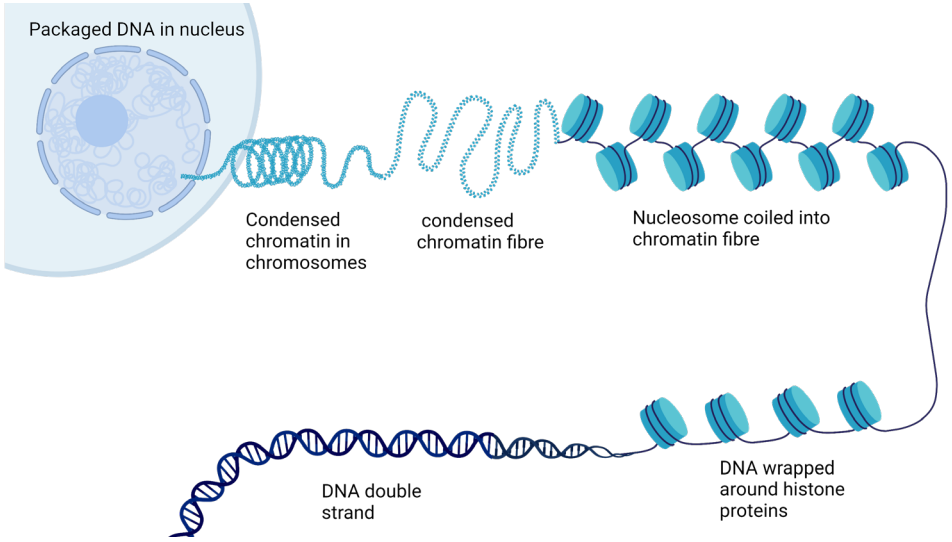

Fig 9.12 Packaging of DNA into a chromosome. Adapted from Biorender by Manjeet Kumari

Octamer

The core of 8 proteins is called an octamer. The stretch of DNA wrapped around the octamer totals about 147 base pairs and makes 1 2/3 turns around it. This complex is referred to as a core particle (Figure 9.13). A linker region of about 50-80 base pairs separate core particles. The term nucleosome then refers to a a core particle plus a linker region (Figure 9.13). Histone H1 sits near the junction of the incoming DNA and the histone core. It is often referred to as the linker histone. In the absence of H1, non-condensed nucleosomes resemble "beads on a string" when viewed in an electron microscope.

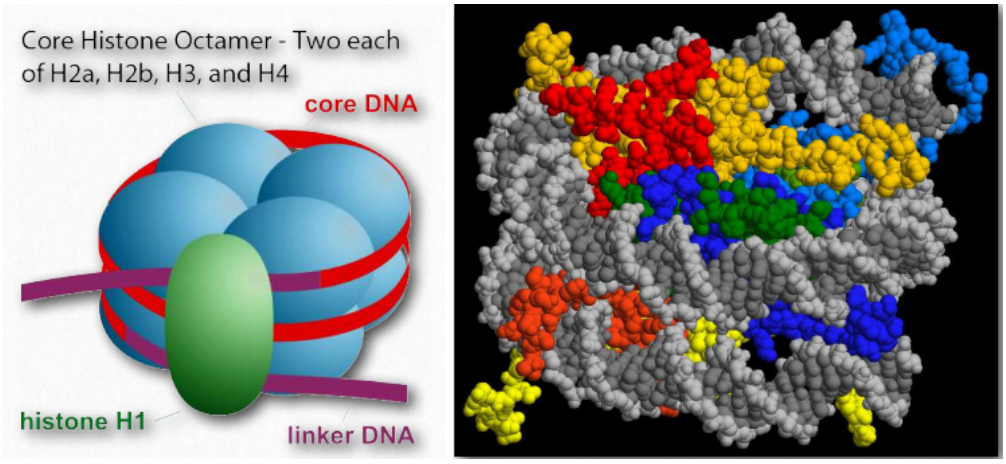

Fig 9.13 Left: Structure of nucleosome. Right: Core proteins (histones in color) with DNA (gray) wrapped around them. Wikipedia

For DNA, compression comes at different levels (Figure 9.12). The first level is at the nucleosomal level. Nucleosomes are stacked and coiled into higher order structures. 10 nm fibers are the simplest higher order structure (beads on a string) and they grow in complexity. 30 nm fibers consist of stacked nucleosomes and they are packed tightly. Higher level packing produces the metaphase chromosome found in meiosis and mitosis. The chromatin complex is a logistical concern for the processes of DNA replication and (particularly) gene expression where specific regions of

DNA must be transcribed. Altering chromatin structure is therefore an essential function for transcriptional activation in eukaryotes. One strategy involves adding acetyl groups to the positively charged lysine side chains to "loosen their grip" on the negatively charged DNA, thus allowing greater access of proteins involved in activating transcription to gain access to the DNA.

Resources:

Chapter page: A doctor with a stethoscope writes something against the background of an abstract image of DNA. Adobe stock #287290079 licensed

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10

Chapter #10 Membranes

Introduction

The plasma membrane, the cell membrane, has many functions, but the most basic one is to define the cell's borders and keep the cell functional. This protective membrane around cells contains many components, including cholesterol, proteins, glycolipids, glycerophospholipids, and sphingolipids. The last two of these will, when mixed vigorously with water, spontaneously form what is called a lipid bilayer (Figure 3.1), which serves as a protective boundary for the cell that is largely impermeable to the movement of most materials across it. With the notable exceptions of water, carbon dioxide, carbon monoxide, and oxygen, most polar/ionic require transport proteins to help them to efficiently navigate across the bilayer. The orderly movement of these compounds is critical for the cell to be able to 1) get food for energy; 2) export materials; 3) maintain osmotic balance; 4) create gradients for secondary transport; 5) provide electromotive force for nerve signaling; and 6) store energy in electrochemical gradients for ATP production (oxidative phosphorylation or photosynthesis). In some cases, energy is required to move the substances (active transport).

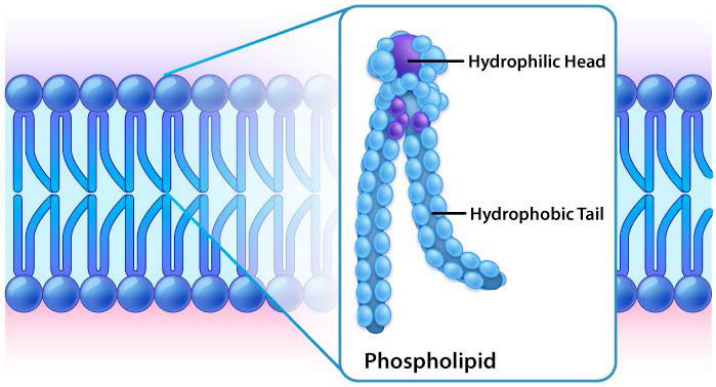


Fig 10.1 Lipid bilayer closeup Image by Aleia Kim

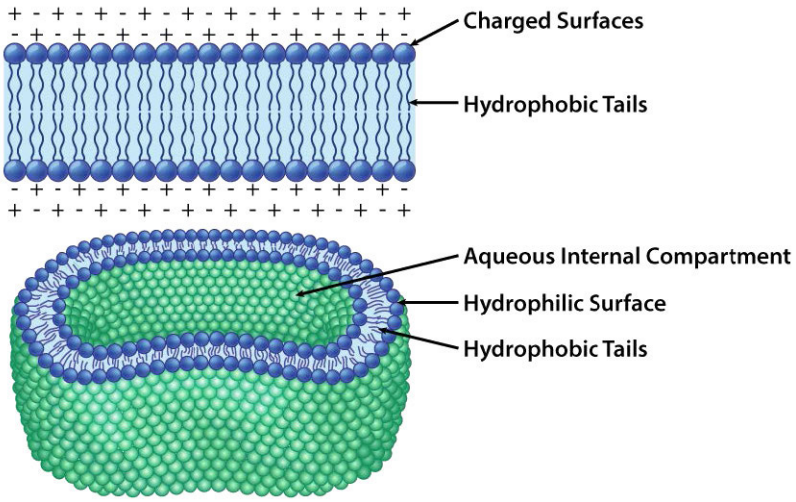


Fig 10.2 Organization of the lipid bilayer Image by Aleia Kim

Though some cells do not have cell walls (animal cells) and others do (bacteria, fungi, and plants), there is commonality among cells in that they all possess plasma membranes. There is also commonality in the components of the membranes, though the relative amount of constituents varies. Figures 10.1 and 10.2 illustrate the structure and environments of plasma membranes. All plasma membranes contain a significant amount of amphiphilic substances linked to fatty acids. These include the glycerophospholipids and the sphingolipids. The fatty acid(s) are labeled as hydrophobic tails in the figures.

Hydrophilic heads

The composition of the hydrophilic heads varies considerably. In glycerophospholipids, a phosphate is always present, of course, and it is often esterified to another substance to make a phosphatide (Figure 10.3). Common compounds linked to the phosphate (at the X position) include serine, ethanolamine, and choline (See lipid chapter). These vary in the their charges so in this way, the charge on the external or internal surface can be controlled.

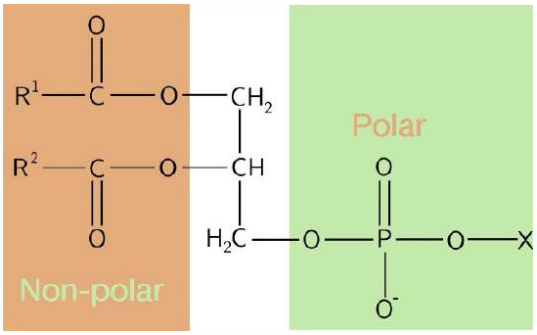


Fig 10.3 Schematic diagram of a phosphatide

In sphingolipids (Figure 10.4), the hydrophilic head can contain a phosphate linked to ethanolamine or choline and this describes the structure of sphingomyelin, an important component of neural membranes. Most sphingolipids lack the phosphate and

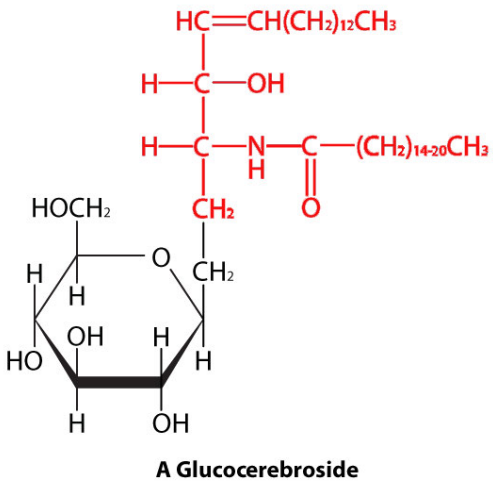


Fig 10.4 A sphingolipid. Polar head in black. Non-polar tail in red Image by Aleia Kim

have instead a hydrophilic head of a single sugar (cerebrosides) or a complex oligosaccharide (gangliosides).

In each case, the glycerophospholipid or sphingolipid has one end that is polar and one end that is non-polar. As we saw in the organization of amino acids with hydrophobic side chains occurring preferentially on the inside of a folded protein to exclude water, so too do the non-polar portions of these amphiphilic molecules arrange themselves so as to exclude water. Remember that the cytoplasm of a cell is mostly water and the exterior of the cell is usually bathed in an aqueous layer. It therefore makes perfect sense that the polar portions of the membrane molecules arrange themselves as they do - polar parts outside interacting with water and non-polar parts in the middle of the bilayer avoiding/excluding water (10.5).

Composition Bias

The plasma membrane has distinct biases of composition relative to its inside and the outside (Figure 10.5). First, glycosylation (of lipids and proteins) has the sugar groups located almost exclusively on the outside of the cell, away from the cytoplasm (Figure 10.6). Among the membrane lipids, sphingolipids are much more commonly glycosylated than glycerophospholipids. In addition, some of the glyerophospholipids are found preferentially on one side or the other (Figure 10.7). Phosphatidylserine and phosphatidylethanolamine are found preferentially within the inner leaflet of the plasma membrane, whereas phosphatidylcholine tends to be located on the outer leaflet. In the process of apoptosis, the phosphatidylserines appear on the outer leaflet where they serve as a signal to macrophages to bind and destroy the cell. Sphingolipids are found preferentially in the plasma membrane and are almost completely absent from mitochondrial and endoplasmic reticulum membranes (Figure 10.7).

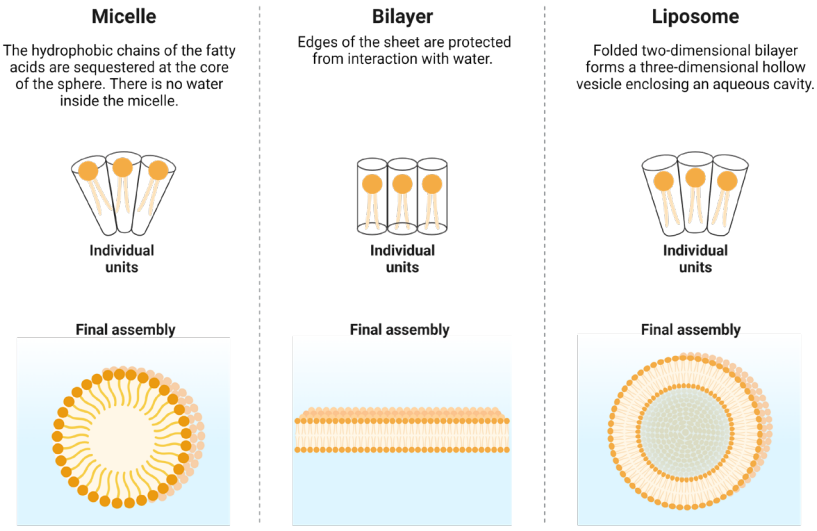


Fig 10.5 Types of amphipathic lipid aggregates. Adapted from Biorender by Manjeet Kumari

Bias of lipid composition also exists with respect to organelle membranes. The unusual diphosphodiglycerolipid known as cardiolipin, for example, is almost only found in mitochondrial membranes and like phosphatidylserine, its movement is an important step in apoptosis. In signaling, phosphatidylinositols play important roles providing second messengers upon being cleaved

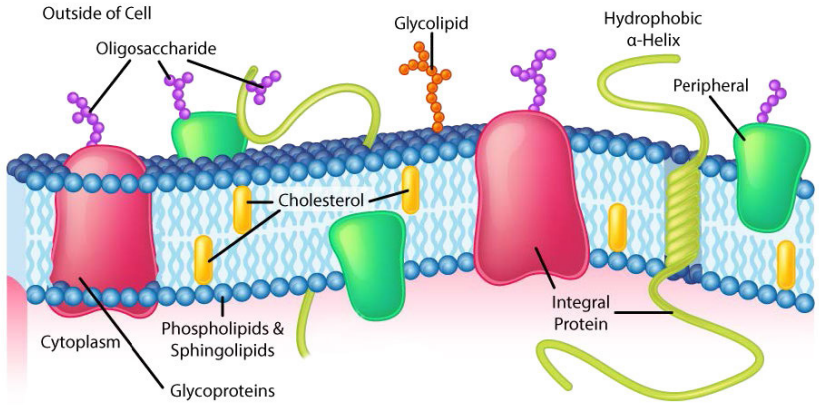


Fig 10.6 lipid bilayer Image by Aleia Kim

Lateral diffusion

Movement of lipids within each leaflet of the lipid bilayer occurs readily and rapidly due to membrane fluidity. This type of movement is called lateral diffusion and can be measured by

the technique called FRAP (Figure 10.8,). In this method, a laser strikes and stains a section of the lipid bilayer of a cell, leaving a spot as shown in B. Over time, the stain diffuses out ultimately across the entire lipid bilayer, much like a drop of ink will diffuse throughout when added to a glass of water. A measurement of the rate of diffusion gives an indication of the fluidity of a membrane.

Transverse Diffusion

While the movement in lateral diffusion occurs rapidly, movement of molecules from one leaflet over to the other leaflet occurs much more slowly. This type of molecular movement is called transverse diffusion and is almost nonexistent in the absence of enzyme action. Remember that there is a bias of distribution of molecules between leaflets of the membrane, which means that something must be moving them.

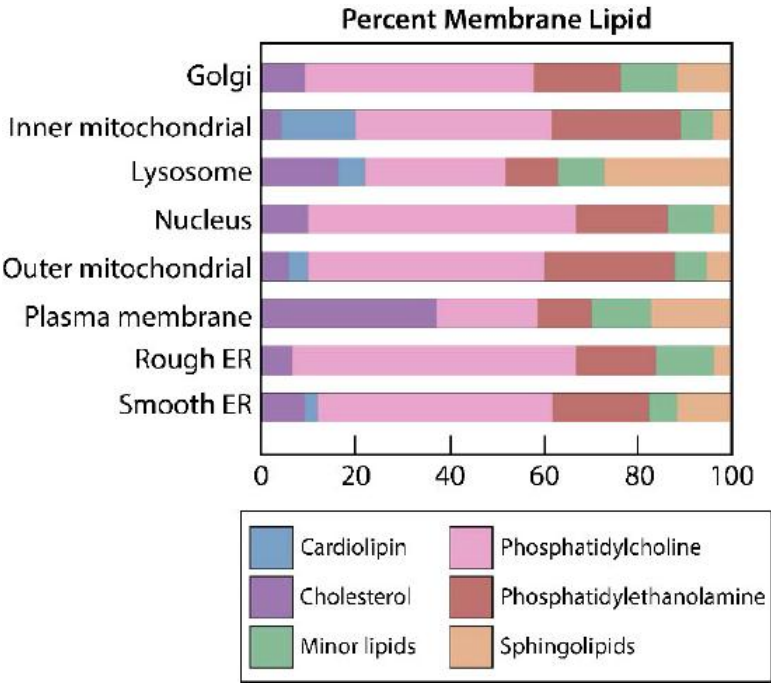


Fig 10.7 Distribution of lipids in organelle membranes Image by Pehr Jacobson

There are three enzymes that catalyze movement of compounds in transverse diffusion. Flippases move membrane glycerophospholipids/ sphingolipids from outer leaflet to inner leaflet (cytoplasmic side) of cell. Floppases move membrane lipids in the opposite direction. Scramblases move in either direction (Fig 10.9).

Cholestrol

Cholesterol's function in the lipid bilayer is complex. It influences membrane fluidity. Figure 10.10 shows the phase transition for a membrane as it is heated, moving from a more "frozen" character to that of a more "fluid" one as the temperature rises. The midpoint of this transition, referred to as the Tm, is influenced by the fatty acid composition of the lipid bilayer compounds. Longer and more saturated fatty acids will favor higher Tm values, whereas unsaturation and short fatty acids will favor lower Tm values. It is for this reason that fish, which live in cool environments, have a higher level of unsaturated fatty acids in them to use to make membrane lipids that will remain fluid at ocean temperatures. Interestingly, cholesterol does not change the Tm value, but instead widens the transition range between frozen and fluid forms of the membrane, allowing it to have a wider range of fluidity (Fig 10.11).

Lipid Rafts

Cholesterol is also abundantly found in membrane structures called lipid rafts. Lipid rafts are organized structures within the membrane typically containing signaling molecules and other integral membrane proteins. Lipid rafts affect membrane

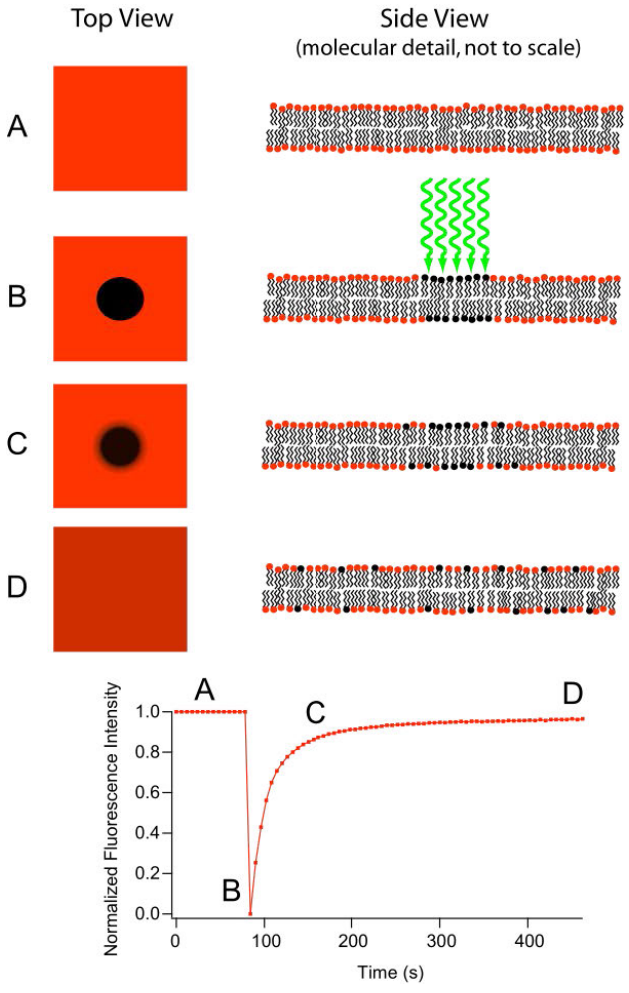


Fig 10.8 FRAP

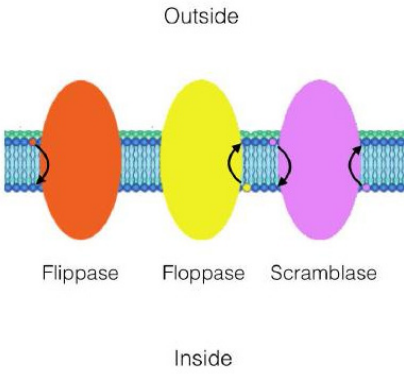


Fig10.9 Catalytic action of a flippase, a floppase, and a scramblase

fluidity, neurotransmission, and trafficking of receptors and membrane proteins. Distinguishing features of the rafts is that they are more ordered than the bilayers surrounding them, containing more saturated fatty acids (tighter packing and less disorganization, as a result) and up to 5 times as much cholesterol. They also are relatively rich in sphingolipids, with as much as 50% greater quantities of sphingomyelin than surrounding areas of the bilayer. Lipid rafts may provide concentrating platforms after individual protein receptors bind to ligands in signaling. After receptor activation takes place at a lipid raft, the signaling complex would provide protection from nonraft enzymes that could inactivate the signal.

Membrane proteins

Proteins in a lipid bilayer can vary in quantity enormously, depending on the membrane. Protein content by weight of various membranes typically ranges between 30 and 75% by weight. Some mitochondrial membranes can have up to 90% protein. Proteins linked to and associated with membranes come in several types.

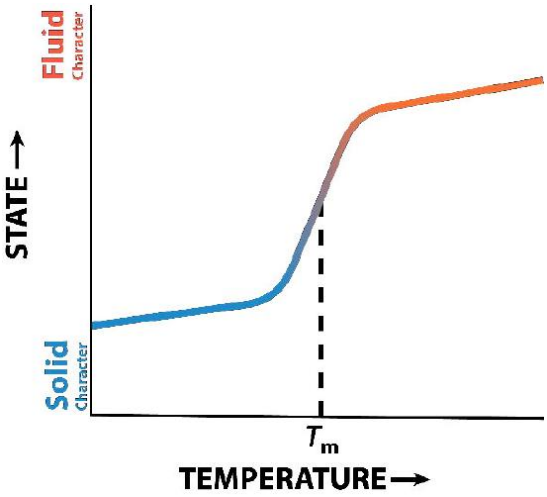


Fig 10.10 Membrane transition temperature Image by Aleia Kim

Transmembrane proteins

Transmembrane proteins are integral membrane proteins that completely span from one side of a biological membrane to the other and are firmly embedded in the membrane (Fig 10.12). Transmembrane proteins can function as docking sites for attachment (to the extracellular matrix, for example), as receptors in the cellular signaling system, or facilitate the specific transport of molecules into or out of the cell. Example of integrated/ transmembrane proteins include those involved in transport (e.g., Na+/K+ ATPase), ion channels (e.g., potassium channel of nerve cells) and signal transduction across the lipid bilayer (e.g., GProtein Coupled Receptors).

Peripheral membrane proteins (Fig 10.12) interact with part of the bilayer (usually does not involve hydrophobic interactions), but do not project through it. A good example is phospholipase A2, which cleaves fatty acids from glycerophospholipids in membranes. Associated membrane proteins typically do not have external hydrophobic regions, so they cannot embed in a portion of the lipid bilayer, but are found near them. Such association may arise as a result of interaction with other proteins or molecules in the lipid bilayer. A good example is ribonuclease.

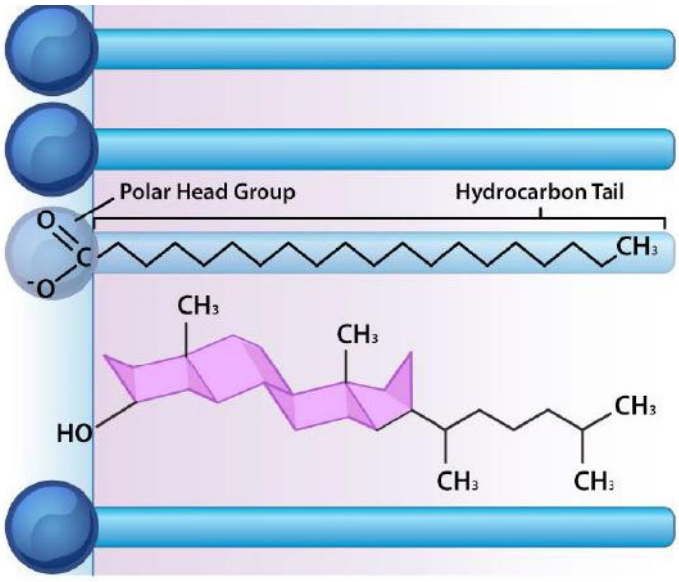


Fig 10.11 Cholesterol in the lipid bilayer Image by Aleia Kim

Anchored membrane proteins

Anchored membrane proteins are not themselves embedded in the lipid bilayer, but instead are attached to a molecule that is embedded in the membrane. The oncogene family of proteins known as ras are good examples.

Movement of materials across membranes

As noted earlier, it is essential for cells to be able to uptake nutrients. This function along with movement of ions and other substances is provided by proteins/protein complexes that are highly specific for the compounds they move.

Selective movement of ions by membrane proteins and the ions' extremely low permeability across the lipid bilayer are important for helping to maintain the osmotic balance of the cell and also for providing for the most important mechanism for it to make ATP - the process of oxidative phosphorylation.

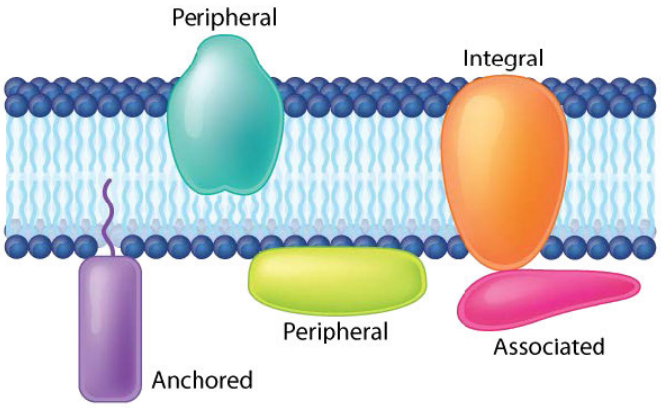


Fig 10.12 Membrane protein types Image by Aleia Kim

Terminology

A protein involved in moving only one molecule across a membrane is called a uniport (Figure 10.13). Proteins that move two molecules in the same direction across the membrane are called symports (also called synporters, synports, or symporters). If two molecules are moved in opposite directions across the bilayer, the protein is called an antiport. Proteins involved in moving ions are called ionophores.

If the action of a protein in moving ions across a membrane results in a net change in charge, the protein is described as electrogenic and if there is no change in charge the protein is described as electroneutral. When the driving force for movement through the membrane protein is simply diffusion, the process is called facilitated diffusion or passive transport and when the process requires other energy input, the process is called active transport.

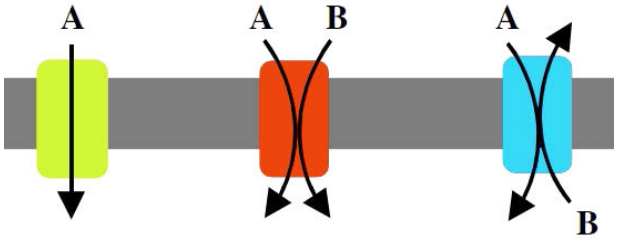


Fig 10.13 A uniport, a symport, and an antiport

Channels and transporters

With respect to movement of materials through membrane proteins, there is a difference between channels (sometimes called pores) and transporters. Channels largely provide openings with some specificity and molecules pass through them at close to the rate of diffusion. They usually involve movement of water or ions. Examples would be the sodium or potassium channels of nerve cells. Transporters have high specificity and transfer rates that are orders of magnitude slower. Transport proteins include the sodium-potassium pump, the sodium-calcium exchanger, amongst many others).

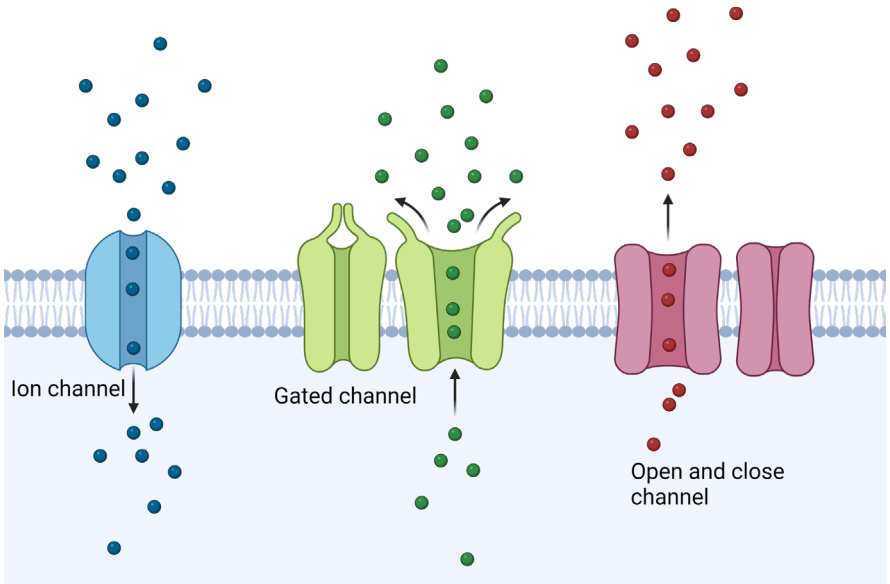


Fig 10.14 Ion transporters. Adapted from Biorender by Manjeet Kumari

Ion channels

Ion channels are pore-forming membrane proteins in the membranes of all cells that regulate movement of selected ions across a membrane (Figures 10.14). They help to establish the resting membrane potential and to affect action potentials and other electrical signals. They are very important in the process of nerve transmission. Ion channels control the flow of ions across secretory and epithelial cells, and consequently help to regulate cell volume by affecting osmotic pressure.

Ion channels are essential features of almost all cells, functioning as selective "tunnels" that restrict movement through them to ions with specific characteristics (typically size). The size of the opening is very narrow (usually one or two atoms wide) and is able to select even against ions that are too small.

Ion channels are controlled by mechanisms that include voltage, ligands, light, temperature, and mechanical deformation (stretch activated). Ligand-gated ion channels (LGICs) are transmembrane proteins which open to selectively allow ions such as Na+, K+, Ca++, or Cl− to pass through the membrane in response to the binding of a ligand messenger.

Sodium ion channels in the tongue for sugar receptors open in response to binding of sucrose, allowing sodium concentration in the nerve cell to increase and initiate a nerve signal to the brain. In this case, the default for the gate is to be closed and it opens in response to binding of a ligand (sucrose).

Transporter proteins

Not all facilitated transport occurs through ion channel proteins. Transporter proteins, as noted earlier facilitate movement of materials across a lipid bilayer, but are slower than ion

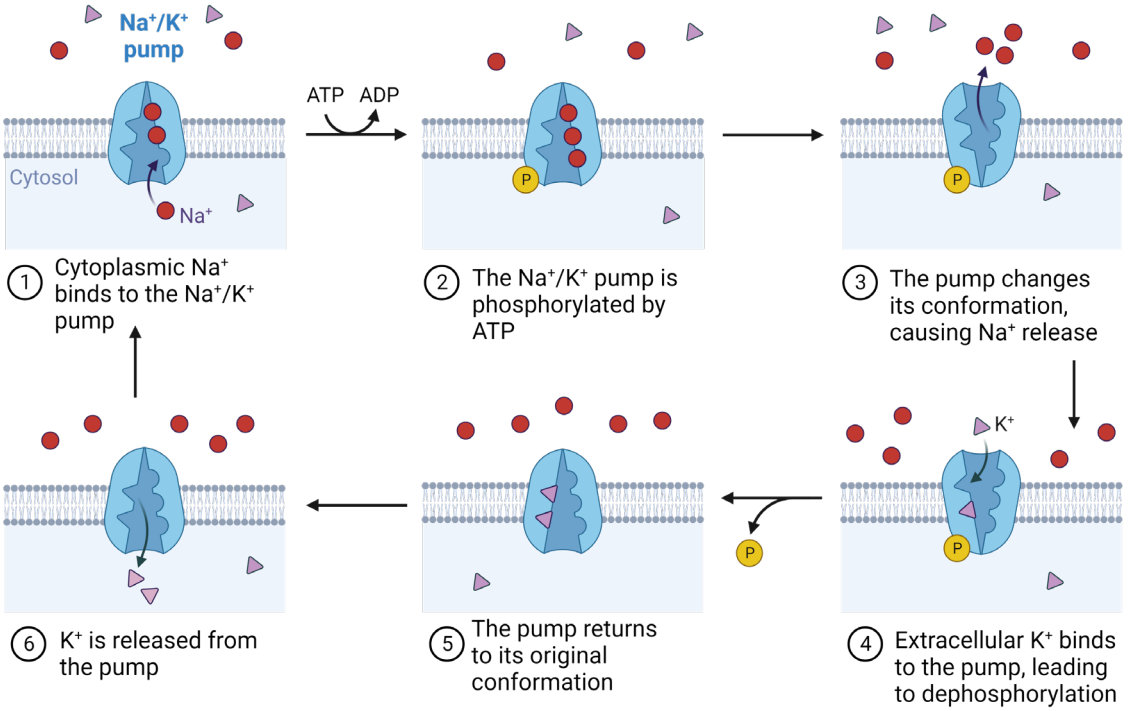


Fig 10.15 Sodium-Potassium pump. Adpated from Biorender by Manjeet Kumari

channels. Transporter proteins rely on a specific receptor site for proper recognition of the molecule to be moved.

Binding of the proper molecule causes a conformational change in the shape of the protein (an eversion) which results in a flipping of the open side of the protein to the other side of the lipid bilayer. In this way, the molecule is moved. Like ion channels, transporter proteins facilitate movement of materials in either direction, driven only by the concentration difference between one side and the other.

Many times, however, cells must move materials against a concentration gradient and when this occurs, another source of energy is required. This process is known as active transport. A good definition of active transport is that in active transport, at least one molecule is being moved against a concentration gradient. A common, but not exclusive, energy source is ATP (see Na+/K+ ATPase aka sodium-potassium pump, Fig 10.15), but other

energy sources are also employed. For example, the sodium-glucose transporter uses a sodium gradient as a force for actively transporting glucose into a cell. Thus, it is important to know that not all active transport uses ATP energy.

Na+/K+ ATPase

An important integral membrane transport protein is the Na+/K+ ATPase antiport (Figures 10.15), which moves three sodium ions out of the cell and two potassium ions into the cell with each cycle of action. In each case, the movement of ions is against the concentration gradient. Since three positive charges are moved out for each two positive charges moved in, the system is electrogenic.

The protein uses the energy of ATP to create ion gradients that are important both in maintaining cellular osmotic pressure and (in nerve cells) for creating the sodium and potassium gradients necessary for signal transmission. Failure of the system to function results in swelling of the cell due to movement of water into the cell through osmotic pressure. The transporter expends about one fifth of the ATP energy of animal cells. The cycle of action occurs as follows:

- Pump binds ATP followed by binding of 3 Na+ ions from cytoplasm of cell
- ATP hydrolysis results in phosphorylation of aspartate residue of pump. ADP is released
- Phosphorylated pump undergoes conformational change to expose Na+ ions to exterior of cell. Na+ ions are released.
- Pump binds 2 extracellular K+ ions.
- Pump dephosphorylates causing it to expose K+ ions to cytoplasm as pump returns to original shape.

Pump binds 3 Na+ ions, binds ATP and releases 2 K+ ions to restart process

The Na+/K+ ATPase is classified as a P-type ATPase. This category of pump is notable for having a phosphorylated aspartate intermediate and is present across the biological kingdoms - bacteria, archaeans, and eukaryotes. There are different types of ATPases. ATPases have roles in either the synthesis or hydrolysis of ATP.

1. F-ATPases (F1FO-ATPases) are present in mitochondria, chloroplasts and bacterial plasma membranes and are the prime ATP synthesizers for these systems. Each uses a proton gradient as its energy source for ATP production. Complex V of the mitochondrion is an F-type ATPase.

2. V-ATPases (V1VO-ATPases) are mostly found in vacuoles of eukaryotes . They utilize energy from ATP hydrolysis to transport solutes and protons into vacuoles and lysosomes, thus lowering their pH values.

The V-type and F-type ATPases are very similar in structure. The V-type uses ATP to pump protons into vacuoles and lysosomes, whereas F-types use proton gradients of the mitochondria and chloroplasts to make ATP. We will discuss their structure when we will study oxidative phophorylation.

Resources:

Chapter cover: Isolated liposome made of lipid bilayer 3d rendering. Adobe stock #471622041. Free-licensed

Fig 10.5 Adapted from "Types of Amphipathic Lipid Aggregates", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.

Fig 10.14 Adapted from "Transporters", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.

Fig 10.15 Adapted from "Sodium-Potassium Pump", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.

11

Chapter #11 Energy

Introduction

Living organisms are made up of cells, and cells contain a horde of biochemical components. Living cells, though, are not random collections of these molecules. They are extraordinarily organized or "ordered". By contrast, in the nonliving world, there is a universal tendency to increasing disorder. Maintaining and creating order in cells takes the input of energy. Without energy, life is not possible.

Oxidative Energy

The primary mechanism used by non-photosynthetic organisms to obtain energy is oxidation and carbon is the most commonly oxidized energy source. The energy released during the oxidative steps is "captured" in ATP and can be used later for energy coupling. The more reduced a carbon atom is, the more energy can be realized from its oxidation. Fatty acids are highly reduced, whereas carbohydrates are moderately so. Complete oxidation of both leads to carbon dioxide, which has the lowest energy state. Conversely, the more oxidized a carbon atom is, the more energy it takes to reduce it. In the series shown in Figure 11.1 the most reduced form of carbon is on the left. The energy of oxidation of each form is shown above it.

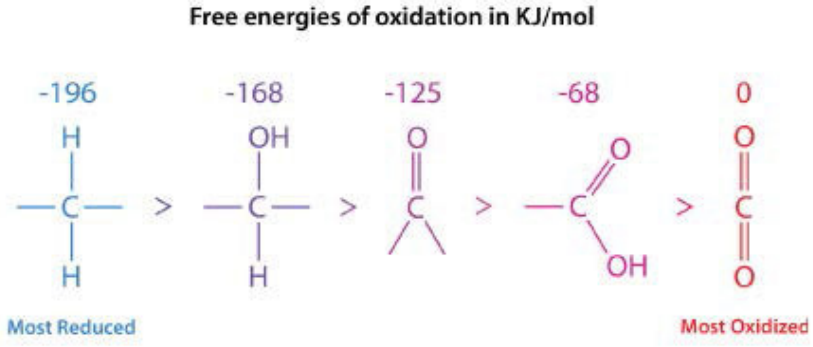


Fig 11.1 Five oxidation states of carbon

The reduction states of fatty acids and carbohydrates can be seen by their formulas.

Palmitic acid: $C_{16}H_{34}O_2$

Glucose: $C_6H_{12}O_6$

Palmitic acid only contains two oxygens per sixteen carbons, whereas glucose has six oxygen atoms per six carbons. Consequently, when palmitic acid is fully oxidized, it generates more ATP per carbon (128/16) than glucose (38/6). It is because of this that we

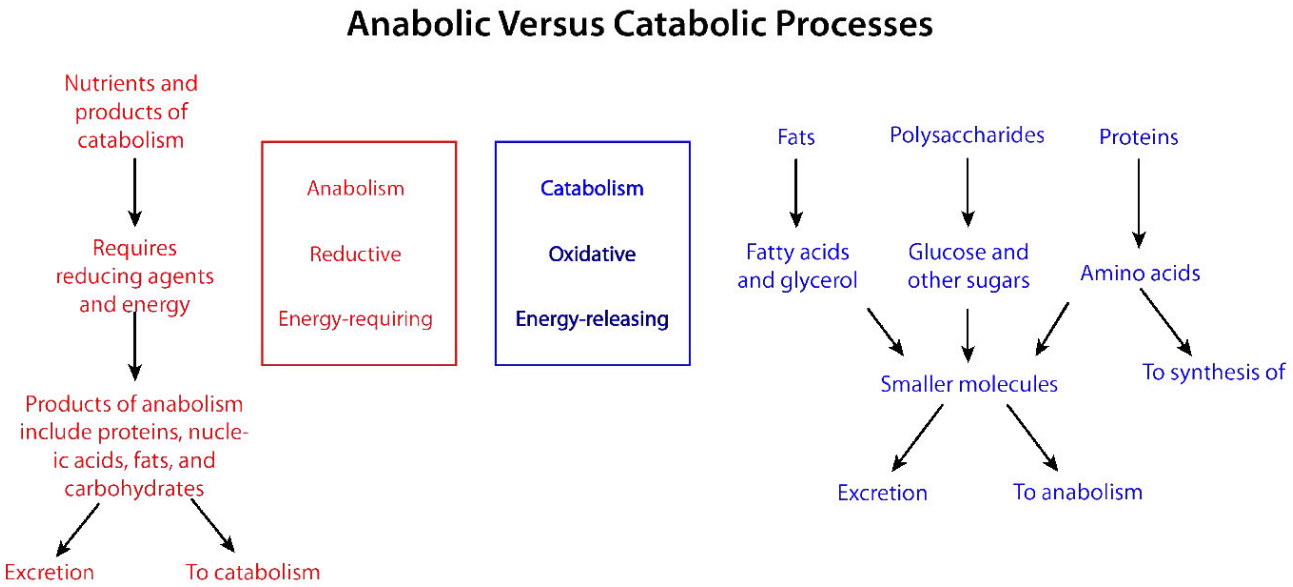


Fig 11.2 Synthesis and breakdown pathways in metabolism

use fat (contains fatty acids) as our primary energy storage material.

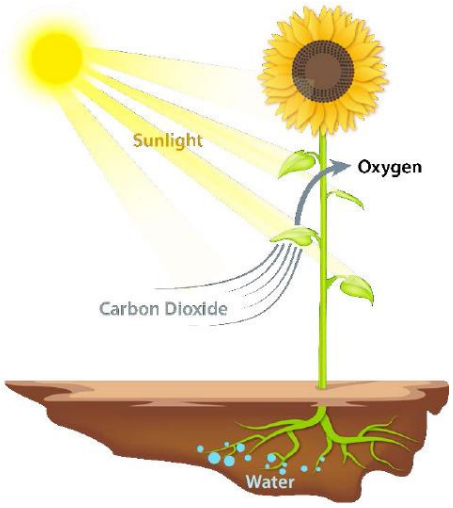


Fig 11.3 Movement of biological energy. Image by Aleia Kim

Oxidation vs. Reduction in Metabolism

Biochemical processes that break things down from larger to smaller are called catabolic processes. Catabolic processes are often oxidative in nature and energy releasing. Some, but not all, of that energy is captured as ATP. If not all of the energy is captured as ATP, what happens to the rest of it? The answer is simple. It is released as heat and it is for this reason we get hot when we exercise.

By contrast, synthesizing large molecules from smaller ones (for example, making proteins from amino acids) is referred to as anabolism. Anabolic processes are often reductive in nature (Figures 11.2) and require energy input. By themselves, they would not occur, as they are reversing oxidation and decreasing entropy (making many small things into a larger one). To overcome this energy barrier, cells must expend energy. For example, if one wishes to reduce CO_2 to carbohydrate, energy must be used to do so. Plants do this during the dark reactions of photosynthesis (

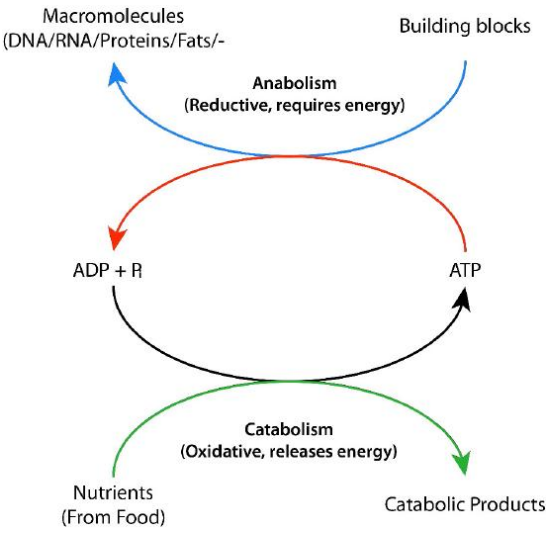


Fig 11.4 Cycling of biological energy via ADP and ATP. Image by Pehr Jacobson

The energy source for the reduction is ultimately the sun (Fig 11.3). The electrons for the reduction come from water, and the CO_2 is removed from the atmosphere and gets incorporated into a sugar.

Energy Coupling

The synthesis of the many molecules needed by cells needs the input of energy to occur. Cells overcome this energy obstacle by using ATP to "drive" the reaction (Figure 11.4) The energy needed to drive reactions is harvested in very controlled conditions in enzymes. This involves a process called 'coupling'. Coupled reactions rely on linking an energetically favorable reaction (i.e., one with a negative $\Delta G°'$) with the reaction requiring an energy input, which has a positive $\Delta G°'$. As long as the overall $\Delta G°'$ of the two reactions together is negative, the reaction can proceed. Hydrolysis of ATP is a very energetically favorable reaction that is commonly linked to many energy requiring reactions in cells. Without the hydrolysis of ATP (or GTP, in some cases), the reaction would not be feasible.

Entropy and energy

Most students who have had some chemistry know about the Second Law of Thermodynamics with respect to increasing disorder of a system. Cells are very organized or ordered structures, leading some to mistakenly conclude that life somehow violates the second law. In fact, that notion is incorrect. The second law doesn't say that entropy always increases, just that, left alone, it tends to do so, in an isolated system. Cells are not isolated systems, though, in that they obtain energy, either from the sun, if they are autotrophic, or food, if they are heterotrophic.

To counter the universal tendency towards disorder on a local scale requires energy. As an example, take a fresh deck of cards which is neatly aligned with Ace-King-Queen . . . . 4,3,2 for each suit. Throw the deck into the air, letting the cards scatter. When you pick them up, they will be more disordered than when they started. However, if you spend a few minutes (and expend a bit of energy), you can reorganize the same deck back to its previous, organized state. If entropy always increased everywhere, you could not do this. However, with the input of energy, you overcame the disorder. This illustrates an important concept: the

cost of fighting disorder is energy.

Biological energy

There are, of course, other reasons that organisms need energy. Muscular contraction, synthesis of molecules, neurotransmission, signaling, thermoregulation, and subcellular movements are examples. Where does this energy come from? The currencies of energy are generally high-energy phosphate-containing molecules. ATP is the best known and most abundant, but GTP is also an important energy source (energy source for protein synthesis). CTP is involved in synthesis of glycerophospholipids and UTP is used for synthesis of glycogen and other sugar compounds. In each of these cases, the energy is in the form of potential chemical energy stored in the multi-phosphate bonds. Hydrolyzing those bonds releases the energy in them.

Of the triphosphates, ATP is the primary energy source, acting to facilitate the synthesis of the others by action of the enzyme NDPK. ATP is made by three distinct types of phosphorylation – oxidative phosphorylation (in mitochondria), photophosphorylation (in chloroplasts of plants), and substrate level phosphorylation (in enzymatically catalyzed reactions).

Gibbs free energy in Biology

In order to understand how energy is captured, we must first understand Gibbs free energy and in doing so, we begin to see the role of energy in determining the directions chemical reactions take.

Gibbs free energy may be thought of as the energy available to do work in a thermodynamic system at constant temperature and pressure. Mathematically, the Gibbs free energy is given as:

$G=H–TS$

where H is the enthalpy, T is the temperature in Kelvin, and S is the entropy. At standard temperature and pressure, every system seeks to achieve a minimum of free energy. Thus, increasing entropy, S, will reduce Gibbs free energy. Similarly, if excess heat is available (reducing the enthalpy, H), the free energy can also be reduced.

Cells must work within the laws of thermodynamics, as noted, so all of their biochemical reactions, too, are ruled by these laws. Now we shall consider energy in the cell. The change in Gibbs free energy (ΔG) for a reaction is crucial, for it, and it alone, determines whether or not a reaction goes forward.

$\Delta G=\Delta H–T\Delta S$

There are three cases

$\Delta G < 0$: the reaction proceeds as written

$\Delta G = 0$: the reaction is at equilibrium

$\Delta G > 0$: the reaction runs in reverse

For a reaction

For a reaction

$aA \rightleftharpoons bB$

(where 'a' and 'b' are integers and A and B are molecules) at pH 7, ΔG can be determined by the following equation,

$\Delta G = \Delta G^{\circ\prime} + RT\ln([B]b/[A]a)$

For multiple substrate reactions, such as

$aA + cC \rightleftarrows bB + dD$

$\Delta G = \Delta G^{\circ\prime} + RT\ln\{([B]b[D]d)/([A]a[C]c)\}$

The $\Delta G^{\circ\prime}$ term is called the change in Standard Gibbs Free energy, which is the change in energy that occurs when all of the products and reactants are at standard conditions and the pH is 7.0. It is a constant for a given reaction.

In simple terms, we can collect all of the terms of the numerator together and call them {Products} and all of the terms of the denominator together and call them {Reactants},

$\Delta G = \Delta G^{\circ\prime} + RT\ln(\{Products\}\{Reactants\})$

For most biological systems, the temperature, T, is a constant for a given reaction. Since $\Delta G^{\circ\prime}$ is also a constant for a given reaction, the ΔG is changed almost exclusively as the ratio of {Products}/{Reactants} changes.

Importance of $\Delta G^{\circ\prime}$

If one starts out at standard conditions, where everything except protons is at 1M, the $RT\ln(\{Products\}/\{Reactants\})$ term is zero, so the $\Delta G^{\circ\prime}$ term equals the ΔG, and the $\Delta G^{\circ\prime}$ determines the direction the reaction will take (only under those conditions). This is why people say that a negative $\Delta G^{\circ\prime}$ indicates an energetically favorable reaction, whereas a positive $\Delta G^{\circ\prime}$ corresponds to an unfavorable one.

Increasing the ratio of {Products}/{Reactants} causes the value of the natural log (ln) term to become more positive (less negative), thus making the value of ΔG more positive. Conversely, as the ratio of {Products}/{Reactants} decreases, the value of the natural log term becomes less positive (more negative), thus making the value of ΔG more negative.

System response to stress

Intuitively, this makes sense and is consistent with Le Chatelier's Principle – a system responds to stress by acting to alleviate the stress. If we examine the ΔG for a reaction in a closed system, we see that it will always move to a value of zero (equilibrium), no matter whether it starts with a positive or negative value.

Another type of free energy available to cells is that generated by electrical potential. For example, mitochondria and chloroplasts partly use Coulombic energy (based on charge) from a proton gradient across their membranes to provide the necessary energy for the synthesis of ATP. Similar energies drive the transmission of nerve signals (sodium and potassium gradients) and the movement of some molecules in secondary active transport processes across membranes (e.g., H+ differential driving the movement of lactose).

From the Gibbs free energy change equation,

$\Delta G = \Delta H - T\Delta S$

it should be noted that an increase in entropy will help contribute to a decrease in ΔG. This happens, for example when a large molecule is being broken into smaller pieces or when the rearrangement of a molecule increases the disorder of molecules around it. The latter situation arises in the hydrophobic effect, which helps drive the folding of proteins.

Chemical and electrical potential

It is said that absence makes the heart grow fonder. We won't tackle that philosophical issue here, but we will say that separation provides potential energy that cells can and do harvest. The lipid bilayer of cell and (in eukaryotic cells) organelle membranes provide the necessary barrier for separation.

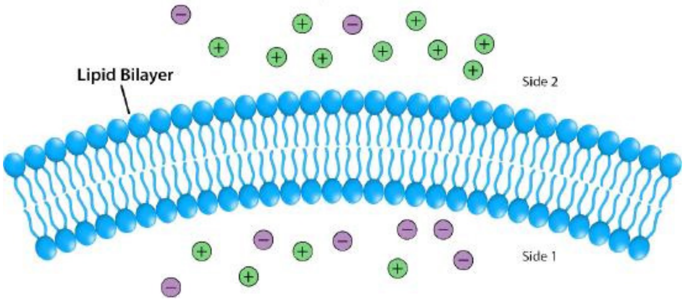


Fig 11.5 Differences in ion concentration across a membrane give rise to chemical and electrical gradients. Image by Pehr Jacobson

Impermeable to most ions and polar compounds, biological membranes are essential for processes that generate cellular energy. Consider Figure 11.4. A lipid bilayer separates two solutions with different concentrations of a solute. There is a greater concentration of negative ions in the bottom and a greater concentration of positive ions on the top.

Whenever there is a difference in concentration of molecules across a membrane, there is said to be a concentration gradient across it. A difference in concentration of ions across a membrane also creates a charge (or electrical) gradient. Because there is a difference in both the chemical concentration of the ions and in the charge on the two sides of the membrane, this is described as an electrochemical gradient.

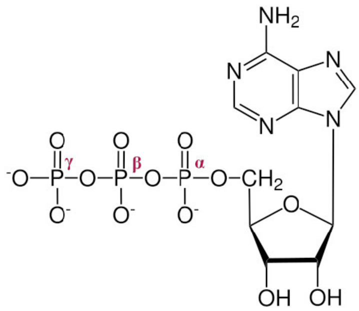


Fig 11.6 ATP showing α, β and γ phosphates

Such gradients function like batteries and contain potential energy. When the potential energy is harvested by cells, they can create ATP, transmit nerve signals, pump molecules across membranes, and more.

In discussing chemical potential, we must also consider reduction potential. Reduction potential measures the tendency of a chemical to be reduced by electrons. It is also designated by several other names/variables. These include redox potential, oxidation/reduction potential, ORP, pE, ε, E, and Eh. Reduction potential is measured in volts, or millivolts. A substance with a higher reduction potential will have a greater tendency to accept electrons and be reduced. If two substances are mixed in an aqueous solution, the one with the greater (more positive) reduction potential will tend to take electrons away, thus being reduced, from the one with the lower reduction potential, which becomes oxidized.

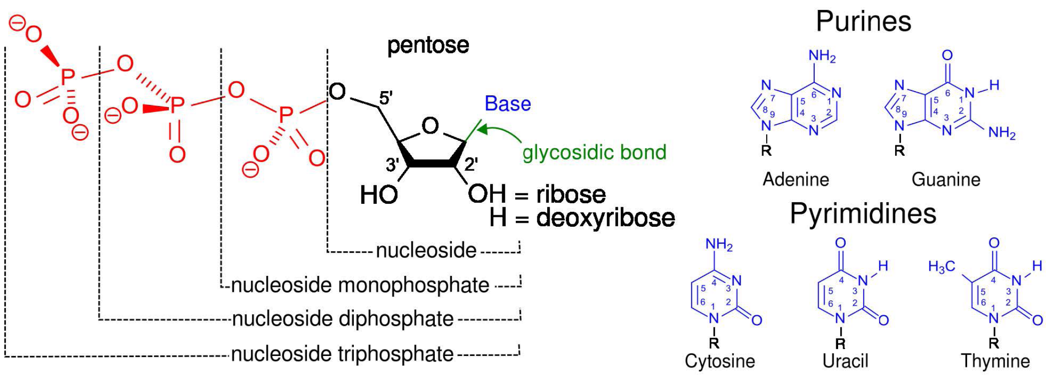


Fig 11.7 Nucleotides, nucleosides, and bases

Energy Storage in Triphosphates

Formation of triphosphates, like ATP, is essential to meeting the cell's energy needs for synthesis, motion, and signaling. In a given day, an average human body makes and breaks down more than its weight in triphosphates. This is especially remarkable considering that there is only about 250 g of the molecule present in the body at any given time. Energy in ATP is released by hydrolysis of a phosphate from the molecule. The three phosphates, starting with the one closest to the sugar are referred to as α, β, and γ (Figure 11.6) It is the γ phosphate that is cleaved in hydrolysis and the product is ADP. In a few reactions, the bond between the α and β is cleaved. When this happens, a pyrophosphate (β linked to γ) is released and AMP is produced. This latter reaction to produce AMP releases more energy ($\Delta G°'$ = -45.6 kJ/mol) than the first reaction which produces ADP

($\Delta G^{\circ\prime}$ = -30.5 kJ/mol).

Since triphosphates are the "currency" that meet immediate needs of the cell, it is important to understand how triphosphates are made. There are three phosphorylation mechanisms – 1) substrate level; 2) oxidative; and 3) photophosphorylation. We consider them here individually

Substrate level phosphorylation

The easiest type of phosphorylation to understand is that which occurs at the substrate level. This type of phosphorylation involves the direct synthesis of ATP from ADP and a high energy intermediate, typically a phosphate-containing molecule. Substrate level phosphorylation is a relatively minor contributor to the total synthesis of triphosphates by cells. An example substrate phosphorylation comes from glycolysis.

Phosphoenolpyruvate (PEP) + ADP $\rightleftharpoons$ Pyruvate + ATP

This reaction has a very negative $\Delta G^{\circ\prime}$ (-31.4 kJ/mol), indicating that the PEP contains more energy than ATP, thus tending to energetically favor ATP's synthesis. Other triphosphates can be made by substrate level phosphorylation, as well. For example, GTP can be synthesized by the following citric acid cycle reaction.

Succinyl-CoA + GDP + Pi $\rightleftharpoons$ Succinate + GTP + CoA-SH

Triphosphates can be interchanged readily in substrate level phosphorylations catalyzed by the enzyme Nucleoside Diphosphate Kinase (NDPK). A generalized form of the reactions catalyzed by this enzyme is as follows:

XTP + YDP $\rightleftharpoons$ XDP + YTP

where X = adenosine, cytidine, uridine, thymidine, or guanosine and Y can be any of these as well. Further, XTP and YDP can be any of the deoxynucleotides as well.

Last, an unusual way of synthesizing ATP by substrate level phosphorylation is via the reaction catalyzed by adenylate kinase

2 ADP $\rightleftharpoons$ ATP + AMP

This reaction is an important means of generating ATP when the cell doesn't have other sources of energy. Accumulation of AMP resulting from this reaction activates enzymes, such as phosphofructokinase, of glycolysis, which will catalyze reactions to give the cell additional, needed energy.

We will discuss oxidative phosphorylation and photophosphorylation in coming chapters.

Resources:

Chapter cover: Along the seawall in Stanley Park, downtown Vancouver, with Lions Gate Bridge in the background. Adobe stock #239929287. Free-licensed

12

Chapter #12 Cellular respiration

Glycolysis and pyruvate metabolism

Introduction

The term 'metabolism' (from the Greek, to change) is defined as sum total of all the chemical processes occurring within a living cell or organism that are necessary for the maintenance of life. In metabolism some substances are broken down to yield energy for vital processes (catabolism) while other substances, necessary for life, are synthesized (anabolism).
Studying the entire set of chemical processes necessary for life may seem intimidating, but in fact, there are two factors that make the study of metabolism much simpler than might be expected. The first is the unity of biochemical processes across species. Carl Sagan noted that "when you look more generally at life on Earth, you find that it is all the same kind of life. There are not many different kinds; there's only one kind. It uses about fifty fundamental biological building blocks, organic molecules." This means that what we learn in one organism will help us understand the biochemistry of all other organisms.

The second reassuring fact, in the words of Sir Frederick Gowland Hopkins, is that "in the study of the intermediate processes of metabolism we have to deal not with complex substances which elude ordinary chemical methods, but with the simple substances undergoing comprehensible reactions.

With that as our starting point lets look at Glycolysis as our first pathway.

Glycolysis

Carbohydrates, whether synthesized by photosynthetic organisms, stored in cells as glycogen, or ingested by heterotrophs, must be broken down to obtain energy for the cell's activities as well as to synthesize other molecules required by the cell. Starch and glycogen, polymers of glucose, are the main energy storage forms of carbohydrates in plants and animals, respectively. To use these sources of energy, cells must first break down the polymers to yield glucose. The glucose is then taken up by cells through transporters in cell membranes. The metabolism of glucose, as well as other six carbon sugars (hexoses) begins with the catabolic pathway called glycolysis. In this pathway, sugars are oxidized and broken down into pyruvate molecules. The corresponding anabolic pathway by which glucose is synthesized is termed gluconeogenesis. Neither glycolysis nor gluconeogenesis is a major oxidative/reductive process, with one step in each one involving loss/gain of electrons, but the product of glycolysis, pyruvate, can be completely oxidized to carbon dioxide. Indeed, without production of pyruvate from glucose in glycolysis, a major energy source for the cell would not be available.

Glucose is the most abundant hexose in nature and is traditionally used to illustrate the reactions of glycolysis, but fructose (in the form of fructose-6- phosphate) is also readily metabolized, while galactose can easily be converted into glucose for catabolism in the pathway as well. The end metabolic products of glycolysis are two molecules of ATP, two molecules of NADH and two molecules of pyruvate, which, in turn, can be oxidized further in the citric acid cycle.

Glycolysis begins with the six-carbon ring-shaped structure of a single glucose molecule and ends with two molecules of a three-carbon sugar called pyruvate. Glycolysis consists of two distinct phases. The first part of the glycolysis pathway traps the glucose molecule in the cell and uses energy to modify it so that the six-carbon sugar molecule can be split evenly into the two three-carbon molecules. The second part of glycolysis extracts energy from the molecules and stores it in the form of ATP and NADH—remember: this is the reduced form of NAD.

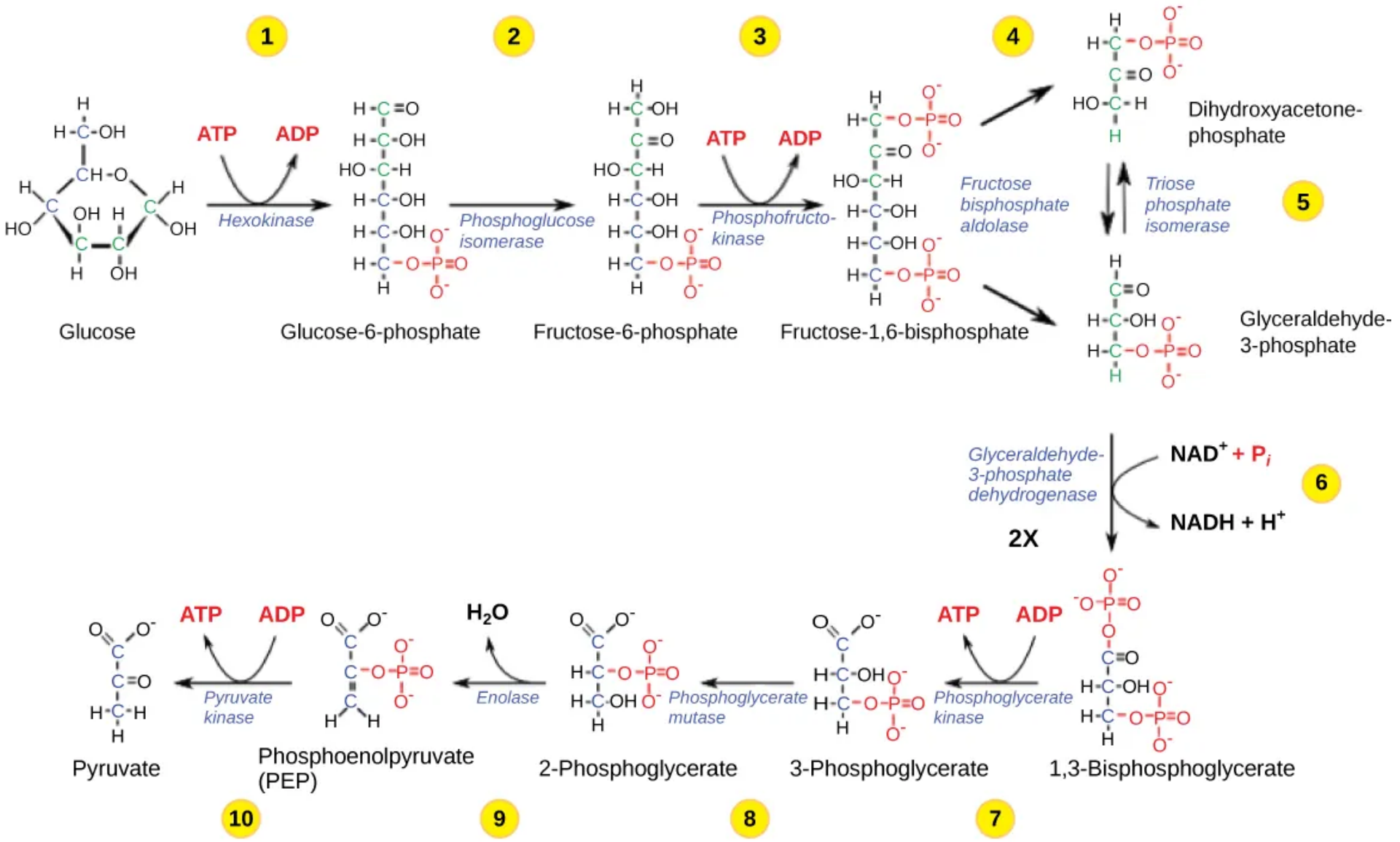


Fig 12.1 First and second half of Glycolysis. Adapted from OpenStax, Biology 2e. Access for free at https://openstax.org/books/biology-2e/pages/7-2-glycolysis

First Half of Glycolysis (Energy-Requiring Steps)

Reaction 1

Glucose gets a phosphate from ATP to make glucose-6-phosphate (G6P) in a reaction catalyzed by the enzyme hexokinase, a transferase enzyme.

Glucose + ATP ⇄ G6P + ADP + H+

Hexokinase is one of three regulated enzymes in glycolysis and is inhibited by one of the products of its action - G6P. Hexokinase has flexibility in its substrate binding and is able to phosphorylate a variety of hexoses, including fructose, mannose, and galactose.

Why phosphorylate glucose?

Phosphorylation of glucose serves two important purposes. First, the addition of a phosphate group to glucose effectively traps it in the cell, as G6P cannot diffuse across the lipid bilayer. Second, the reaction decreases the concentration of free glucose, favoring additional import of the molecule. G6P is a substrate for the pentose phosphate pathway and can also be converted to glucose-1-phosphate (G1P) for use in glycogen synthesis and galactose metabolism. (Fig 12.2)

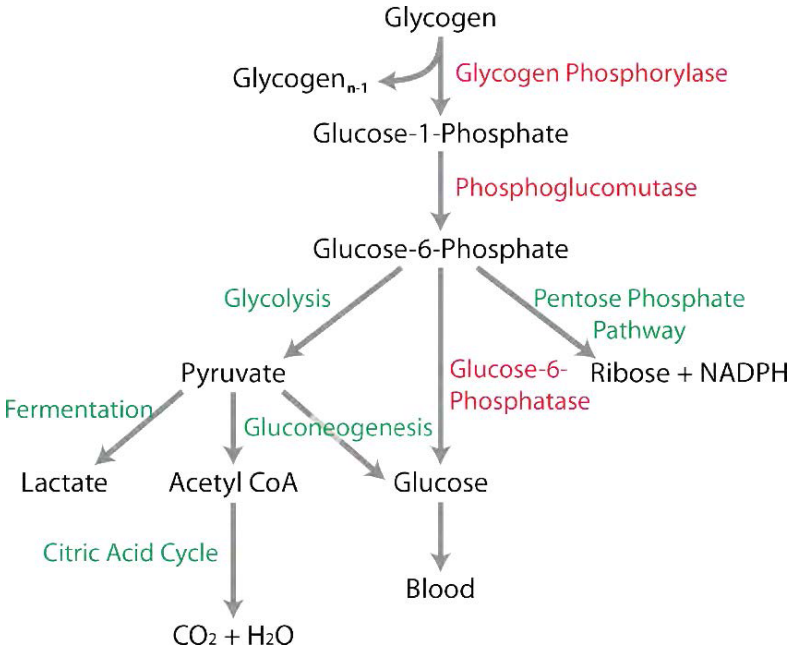


Fig 12.2 The centrality of glucose-6-phosphate. Image by Aleia Kim.

It is worth noting that the liver has an enzyme like hexokinase called glucokinase, which catalyzed by hexokinase has a much higher Km (lower affinity) for glucose. This is important, because the liver is a site of glucose synthesis (gluconeogenesis) where cellular concentrations of glucose can be relatively high. With a lower affinity glucose phosphorylating enzyme, glucose is not converted to G6P unless glucose concentrations get high, so the liver is able to release the glucose it makes into the bloodstream for the rest of the body to use.

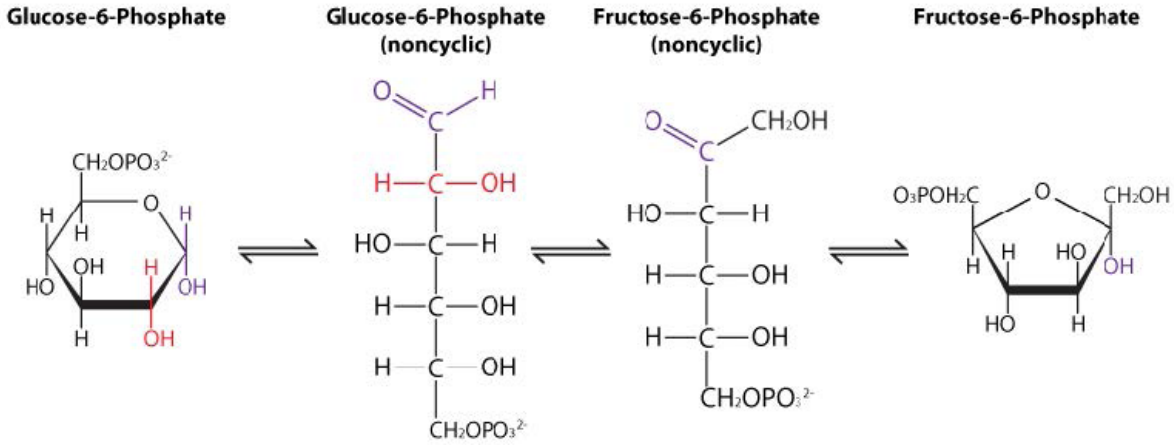


Fig 12.3 Mechanism of conversion of G6P to F6P in reaction

Reaction 2

Next, G6P is converted to fructose-6-phosphate (F6P), in a reaction catalyzed by the enzyme phosphoglucoisomerase.

G6P⇄ F6P

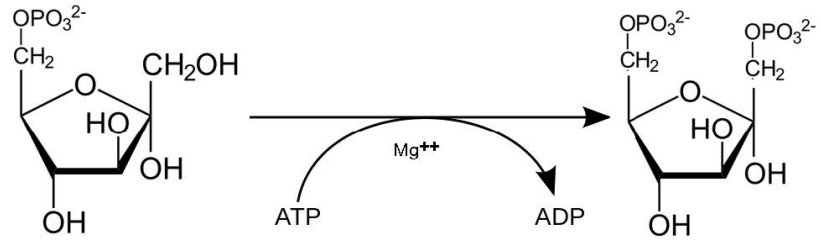


Fig 12.4 Reaction #3 - Conversion of F6P to F1,6BP by PFK Wikipedia

The reaction has a low ΔG°' , so it is readily favorable in either direction (Figure 12.3)- only slight changes in concentration of reactants.

Reaction 3

F6P+ATP⇄F1,6BP+ADP+H^+

The second input of energy occurs when F6P gets another phosphate from ATP in a reaction catalyzed by the enzyme phosphofructokinase-1 (PFK-1 - another transferase) to make fructose-1,6-bisphosphate (F1,6BP). PFK-1 is a very important enzyme regulating glycolysis, with several allosteric activators and inhibitors..

Like the hexokinase reaction the energy from ATP is needed to make the reaction energetically favorable. PFK-1 is the most important regulatory enzyme in the pathway and this reaction is the ratelimiting step. It is also one of three essentially irreversible reactions in glycolysis.

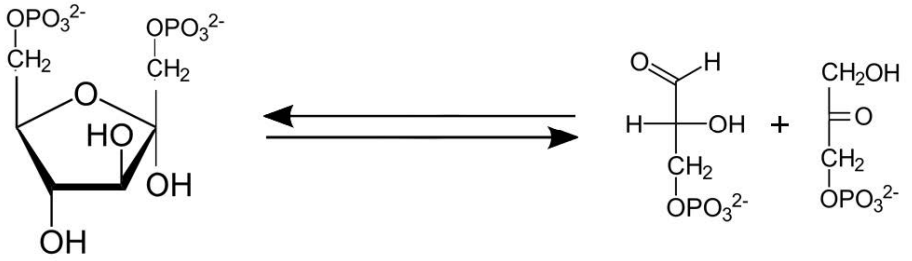


Fig 12.5 Reaction #4 - Breakdown of F1,6BP into GLYAL3P (left) and DHAP (right) by aldolase

A variant enzyme found in plants and some bacterial uses pyrophosphate rather than ATP as the energy source and due to the lower energy input from hydrolysis of the pyrophosphate, that reaction is reversible.

Reaction 4

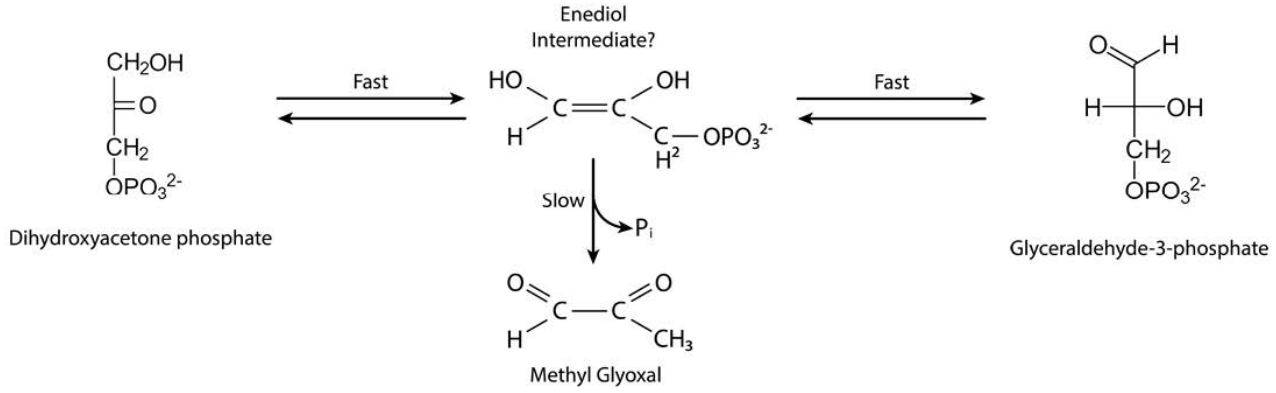


Fig 12.6 Reaction #5 - Triose phosphate isomerase with unstable, toxic intermediate (methyl glyoxal). Image by Ben Carson

F1,6BP ⇄ D-GLYAL3P + DHAP

With the glycolysis pump thus primed, the pathway proceeds to split the F1,6BP into two

3-carbon intermediates. This reaction catalyzed by the lyase known as aldolase is energetically a "hump" to overcome in the glycolysis direction ($\Delta G^{\circ\prime}$ = +24 kJ/mol) so to get over the energy hump, cells must increase the concentration the reactant (F1,6BP) and decrease the concentration of the products, which are D-glyceraldehyde- 3-phosphate (D-GLYAL3P) and dihydroxyacetone phosphate (DHAP).

Second half of Glycolysis (energy-generating steps)

Reaction 5

DHAP⇄D−GLYAL3P

In the next step, DHAP is converted to DGLYAL3P in a reaction catalyzed by the enzyme triosephosphate isomerase. At this point, the six carbon glucose molecule has been broken down to two units of three carbons each - D-GLYAL3P. From this point forward each reaction of glycolysis contains two of each molecule. Reaction #5 is fairly readily reversible in cells.

The enzyme is of note because it is one example of a "perfect enzyme." Enzymes in this category have very high ratios of Kcat/Km that approach a theoretical maximum limited only by the diffusion of substrate into the active site of the enzyme. The apparent reason for the enzyme evolving in this way is that the mechanism of the reaction produces an unstable, toxic intermediate (Figure 12.6). With the reaction proceeding as rapidly as it does, there is less chance of the intermediate escaping and causing damage in the cell.

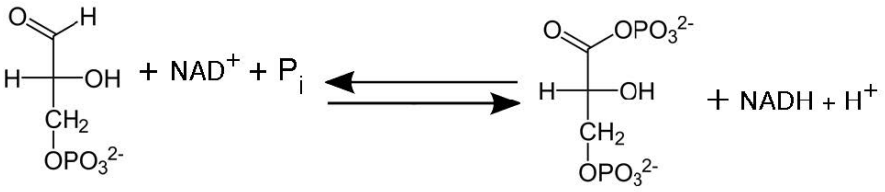


Fig 12.7 Reaction #6 - Oxidation of GLYAL3P, catalyzed by glyceraldehyde-3-phosphate dehydrogenase

Reaction 6

D-GLYAL3P + NAD+ + Pi ⇌ D-1,3BPG + NADH + H+

In this reaction, D-GLYAL3P is oxidized in the only oxidation step of glycolysis catalyzed by the enzyme glyceraldehyde-3- phosphate dehydrogenase, an oxidoreductase. The aldehyde in this reaction is oxidized, then linked to a phosphate to make an ester - D-1,3-bisphospho-glycerate (D- 1,3BPG). Electrons from the oxidation are donated to NAD+, creating NADH (Fig 12.7).

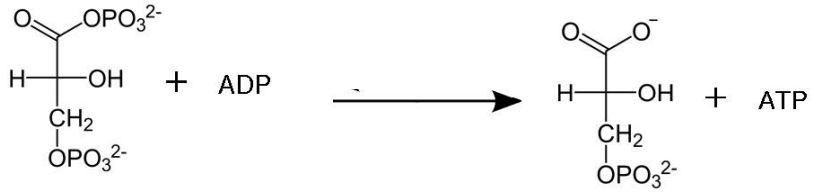


Fig 12.8 Reaction #7 - Substrate-level Phosphorylation by 1,3-BPG. Modified by Manjeet Kumari

NAD+ is a critical constituent in this reaction and is the reason that cells need a fermentation option at the end of the pathway.

Note here that ATP energy was not required to put the phosphate onto the oxidized D-GLYAL3P. The reason for this is because the energy provided by the oxidation reaction is sufficient for adding the phosphate.

Reaction 7

D−1,3BPG+ADP→3PG+ATP

The two phosphates in the tiny 1,3BPG molecule repel each other and give the molecule high potential energy. This energy is utilized by the enzyme phosphoglycerate kinase (another transferase) to phosphorylate ADP and make ATP, as well as the product, 3-phosphoglycerate (3-PG). This is an example of a substrate-level phosphorylation. Such mechanisms for making ATP require an intermediate with a high enough energy to phosphorylate ADP to make ATP (Fig 12.8).

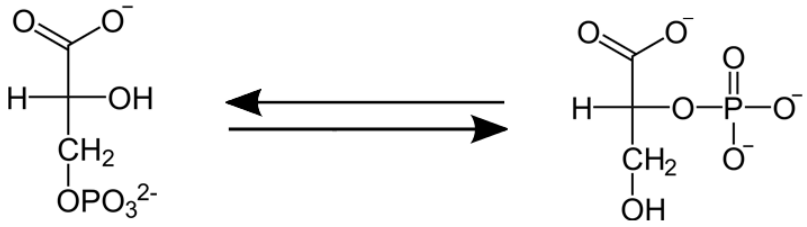


Fig 12.9 Reaction #8 - Conversion of 3-PG to 2-PG

Though there are a few substrate level phosphorylations in cells (including another one at the end of glycolysis), the vast major of ATP is made by oxidative phosphorylation in the mitochondria (in animals). In addition to oxidative phosphorylation, plants also make ATP by photophosphorylation in their chloroplasts. Since there are two 1,3 BPGs produced for every glucose, the two ATPs produced in this reaction replenish the two ATPs used to start the cycle and the net ATP count at this point of the pathway is zero.

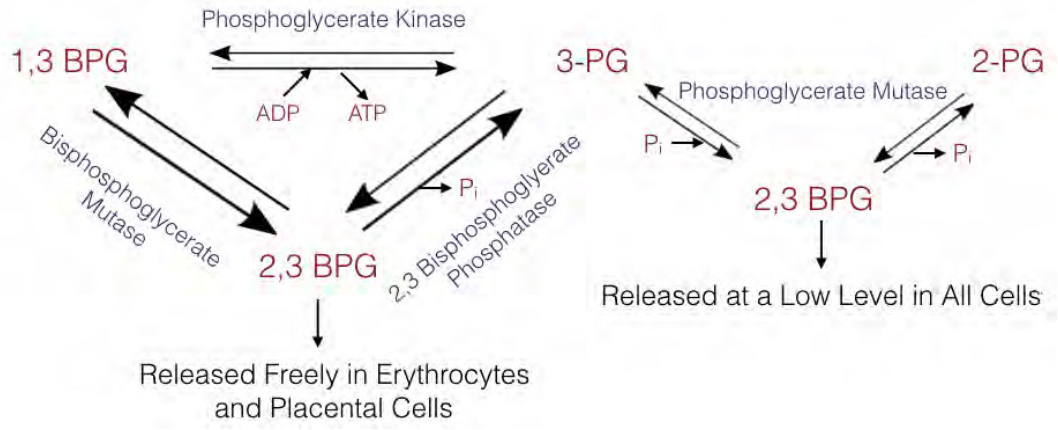


Fig 12.10 Two routes to formation of 2,3-BPG

Reaction 8

3−PG⇄2−PG

Conversion of the 3-PG intermediate to 2-PG (2- phosphoglycerate) occurs by an important mechanism. An intermediate in this readily reversible reaction (catalyzed by phosphoglycerate mutase - a mutase enzyme) is 2,3-BPG (Fig 12.9 and Fig 12.10). This intermediate, which is stable, is released with low frequency by the enzyme instead of being converted to 2-PG. 2,3BPG is important because it binds to hemoglobin and stimulates release of oxygen. The molecule can also be made from 1,3-BPG as a product of a reaction catalyzed by bisphophglycerate mutase . Cells which are metabolizing glucose rapidly release more 2,3-BPG and, as a result, get more oxygen, supporting their needs. Notably, cells which are metabolizing rapidly are using oxygen more rapidly and are more

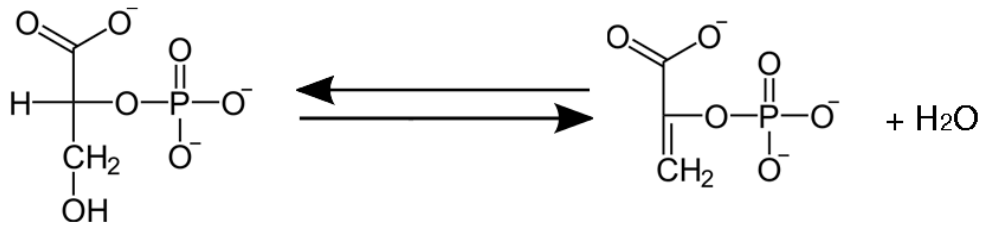


Fig 12.11 Reaction #9 - Enolase-catalyzed removal of water

likely to be deficient in it.

Reaction 9

2−PG⇄PEP+H2O

2-PG is converted by enolase (a lyase) to phosphoenolpyruvate (PEP) by removal of water, creating a very high energy intermediate (Fig 12.11). The reaction is readily reversible, but with PEP, the cell has one of its highest energy molecules and that is important for the next reaction.

Reaction 10

PEP+ADP+H^+→PYR+ATP

Conversion of PEP to pyruvate by pyruvate kinase is the second substrate level phosphorylation of glycolysis, creating ATP (Fig 12.12) This reaction is what some refer to as the "Big Bang" of glycolysis because there is almost enough energy in PEP to stimulate production of a second ATP (ΔG°' = 31.6 kJ/ mol), but it is not used. Consequently, this energy is lost as heat. If you wonder why you get hot when you exercise, the heat produced in the breakdown of glucose is a prime contributor and the pyruvate kinase reaction is a major source.

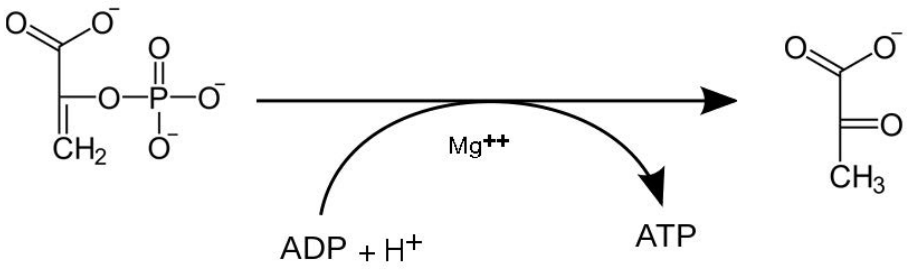


Fig 12.12 Reaction #10 - The big bang - PEP phosphorylate

Pyruvate kinase is the third and last enzyme of glycolysis that is regulated. The primary reason this is the case is to be able to prevent this reaction from occurring when cells are making PEP while going through gluconeogenesis. We will discuss gluconeogenesis elsewhere in this book.

Though glycolysis is a pathway focused on the metabolism of glucose and fructose, the fact that other sugars can be readily metabolized into glucose means that glycolysis can be used for extracting energy from them as well.

Pyruvate metabolism

As noted, pyruvate produced in glycolysis can be oxidized to acetyl-CoA, which is itself oxidized in the citric acid cycle to carbon dioxide. That is not the only metabolic fate of pyruvate, though. Here are metabolic fates of pyruvate:

1) Pyruvate is a oxidized to acetyl-CoA which enters citric acid cycle.

2) Pyruvate is a "starting" point for gluconeogenesis, being converted to oxaloacetate in the mitochondrion in the first step.

3) Pyruvate in animals can also be reduced to lactate by adding electrons from NADH. This reaction produces NAD+ and is critical for generating the latter molecule to keep the glycolysis going under conditions when there is no oxygen.

4) Pyruvate is a precursor of alanine which can be easily synthesized by transfer of a nitrogen from an amine donor, such as glutamic acid.

Pyruvate in animals can be reduced to lactate by adding electrons from NADH (Figure

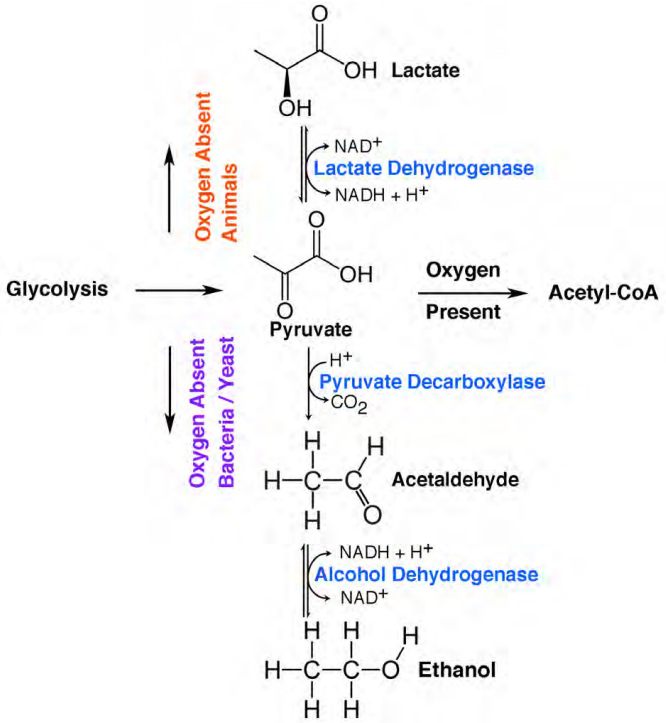


Fig 12.13 Pyruvate metabolism. When oxygen is absent, pyruvate is converted to lactate (animals) or ethanol (bacteria and yeast).

12.13). This reaction produces NAD+ and is critical for generating the latter molecule to keep the glyceraldehyde-3-phosphate dehydrogenase reaction of glycolysis (reaction #6) going under conditions when there is no oxygen (Fig 12.14).

This is because oxygen is necessary for the electron transport system (ETS) to operate and it performs the important function of converting NADH back to NAD+. When the ETS is running, NADH donates electrons to Complex I and is oxidized to NAD+ in the process, generating the intermediate needed for oxidizing GLYAL-3P. In the absence of oxygen,however, NADH cannot be converted to NAD+ by the ETS, so an alternative means of making NAD+ is necessary for keeping glycolysis running under low oxygen conditions (fermentation).

Bacteria and yeast generate NAD+ under oxygen deprived conditions by doing fermentation in a different way (Fig 12.15). They use NADH-requiring reactions that regenerate NAD+ while producing ethanol from pyruvate instead of making lactate. Thus, fermentation of pyruvate is essential to keep glycolysis operating when oxygen is limiting. It is also for these reasons that brewing of beer (using yeast) involves depletion of oxygen and muscles low in oxygen produce lactic acid (animals).

Pyruvate is a precursor of alanine which can be easily synthesized by transfer of a nitrogen from an amine donor, such as glutamic acid. Pyruvate can also be converted into oxaloacetate by carboxylation in the process of gluconeogenesis

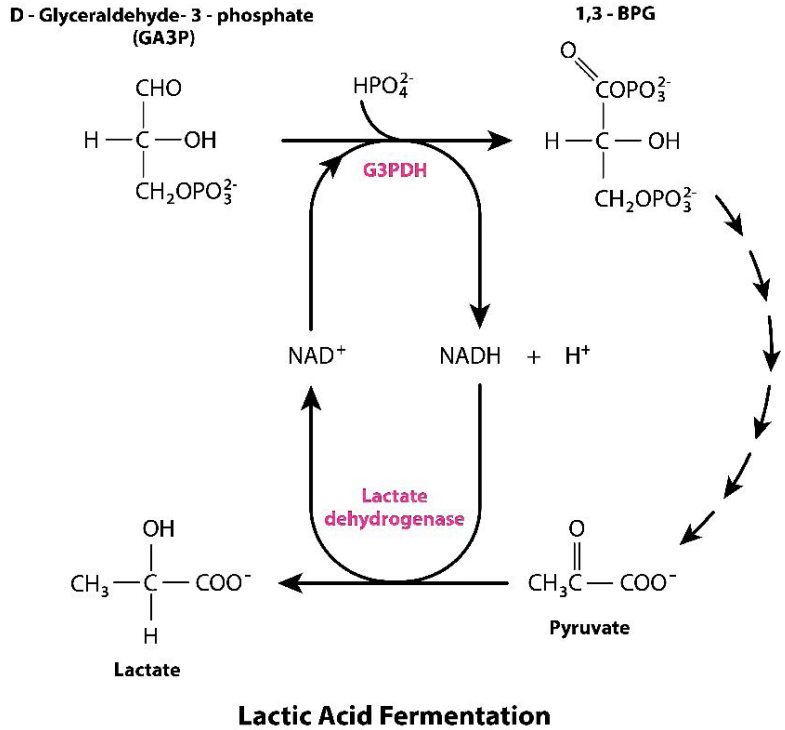


Fig 12.14. Image by Ben Carson

The enzymes involved in pyruvate metabolism include pyruvate dehydrogenase (makes acetyl-CoA), lactate dehydrogenase (makes lactate), transaminases (make alanine), pyruvate carboxylase (makes oxaloacetate), and pyruvate decarboxylase (a part of pyruvate dehydrogenase that makes acetaldehyde in bacteria and yeast).

Gluconeogenesis

The anabolic counterpart to glycolysis is gluconeogenesis (Figure 12.16), which occurs mostly in the cells of the liver and kidney and virtually no other cells in the body. In seven of the eleven reactions of gluconeogenesis (starting from pyruvate), the same enzymes are used as in glycolysis, but the reaction directions are reversed. Notably, the ΔG values of these reactions in the cell are typically near zero, meaning their direction can be readily controlled by changing substrate and product concentrations by small amounts.

The three regulated enzymes of glycolysis all catalyze reactions whose cellular ΔG val-

ues are not close to zero, making manipulation of reaction direction for their reactions non-trivial. Consequently, cells employ "work-around" reactions catalyzed by four different enzymes to favor gluconeogenesis, when appropriate.

Bypassing pyruvate kinase

Two of the enzymes (pyruvate carboxylase and PEP carboxykinase - PEPCK) catalyze reactions that bypass pyruvate kinase. F1,6BPase bypasses PFK-1 and G6Pase bypasses hexokinase. Notably, pyruvate carboxylase and G6Pase are found in the mitochondria and endoplasmic reticulum, respectively, whereas the other two are found in the cytoplasm along with all of the enzymes of glycolysis.

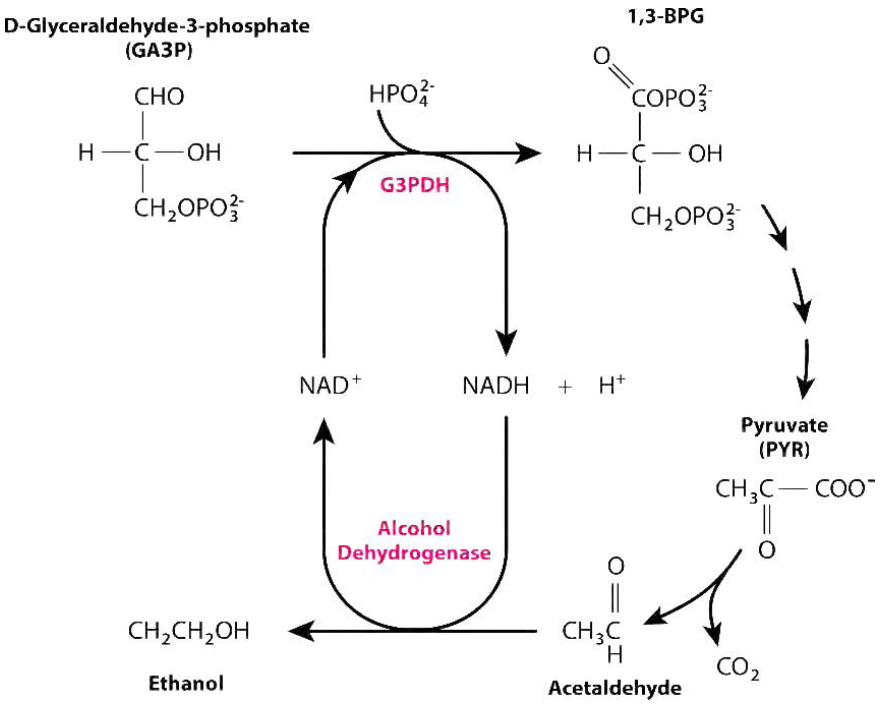


Fig 12.15. Formation of ethanol in microbial fermentation produces NAD+ for G3PDH Image by Ben Carson

Biotin: An important coenzyme used by pyruvate carboxylase is biotin. Biotin is commonly used by carboxylases to carry CO_2 to incorporate into the substrate. Also known as vitamin H, biotin is a water soluble B vitamin (B7) needed for many metabolic processes, including fatty acid synthesis, gluconeogenesis, and amino acid metabolism. Deficiency of the vitamin is rare, since it is readily produced by gut bacteria. There are many claims of advantages of taking biotin supplements, but there is no strong indication of benefits in most cases. Deficiencies are associated with inborn genetic errors, alcoholism, burn patients, and people who have had a gastrectomy. Some pregnant and lactating women may have reduced levels due to increased biotin catabolism.

Reciprocal regulation

All of the enzymes of glycolysis and nine of the eleven enzymes of gluconeogenesis are all in the cytoplasm, necessitating a coordinated means of controlling them. Cells generally need to minimize the extent to which paired anabolic and catabolic pathways are occurring simultaneously, lest they produce a futile cycle, resulting in wasted energy with no tangible product except heat. The mechanisms of controlling these pathways have opposite effects on catabolic and anabolic processes. This method of control is called reciprocal regulation.

Reciprocal regulation is a coordinated means of simultaneously controlling metabolic pathways that do opposite things. In reciprocal regulation, a single molecule (allosteric regulation) or a single covalent modification (phosphorylation/dephosphorylation, for example) has opposite effects on the different pathways (Fig 12.17).

Reciprocal allosteric effects

For example, in glycolysis, the enzyme known as phosphofructokinase (PFK-1) is allosterically activated by AMP and a molecule known as F2,6BP. The corresponding enzyme from gluconeogenesis catalyzing a reversal of the glycolysis reaction is known as F1,6BPase.F1,6BPase is inhibited by both AMP and F2,6BP. The advantage of reciprocalregulation schemes is that they are very efficient.

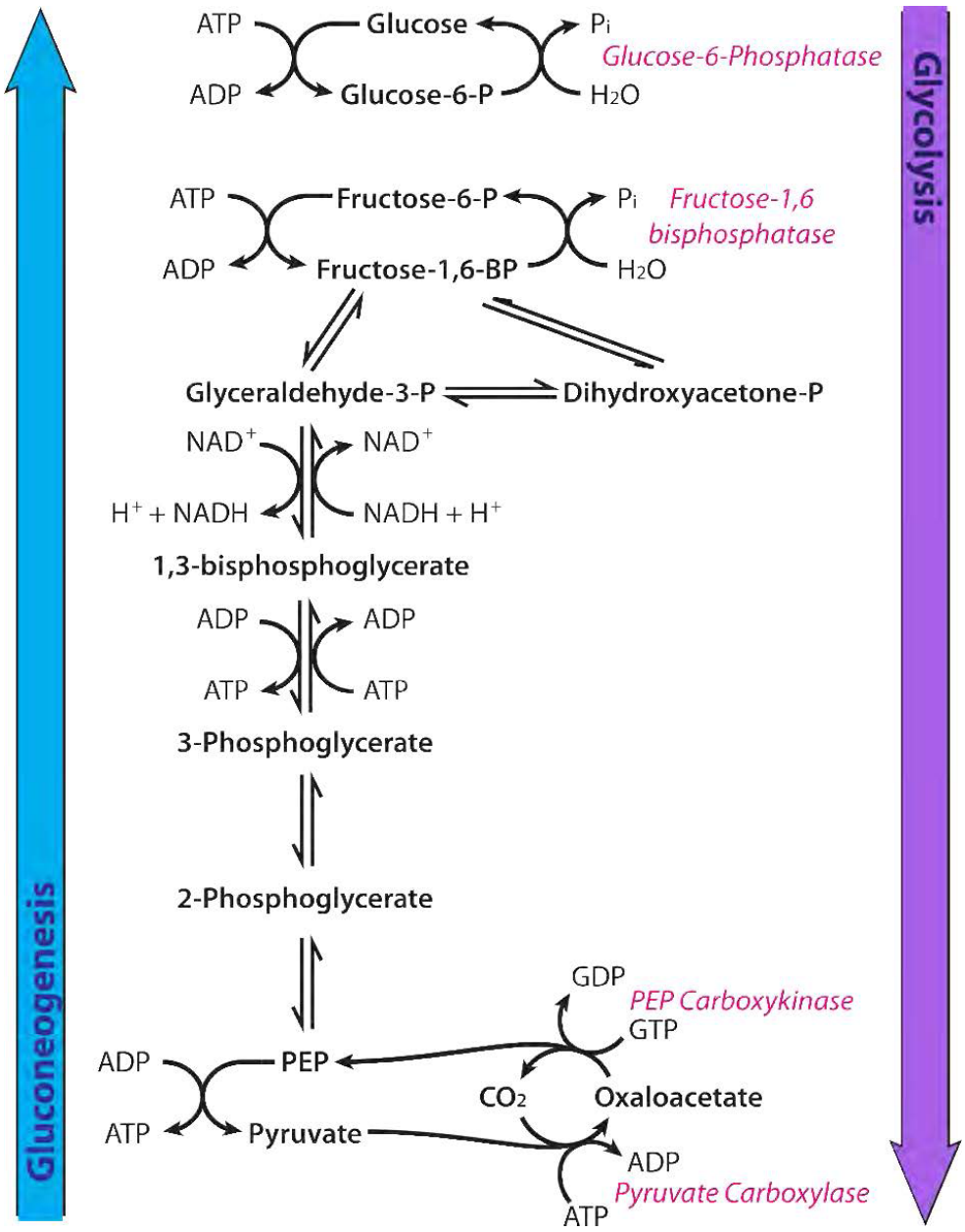


Fig 12.16. Gluconeogenesis and glycolysis. Only the enzymes differing in gluconeogenesis are shown. Image by Ben Carson

It doesn't take separate molecules or separate treatments to control two pathways simultaneously. Further, its simplicity ensures that when one pathway is turned on, the other is turned off.This is especially important with catabolic/anabolic regulation, because having both pathways going on simultaneously in a cell is not very productive, leading only to production of heat in a futile cycle. A simple futile cycle is shown on Figure 12.18.

If unregulated, the cyclic pathway in the figure (shown in black) will make ATP in creating pyruvate from PEP and will use ATP to make oxaloacetate from pyruvate. It will also use GTP to make PEP from oxaloacetate. Thus, each turn of the cycle will make one ATP, use one ATP and use one GTP for a net loss of energy. The process will start with pyruvate and end with pyruvate, so there is no net production of molecules.

Specific gluconeogenesis controls

Besides reciprocal regulation, other mechanisms help control gluconeogenesis. First, PEPCK is controlled largely at the level of synthesis. Overexpression of PEPCK (stimulated by glucagon, glucocorticoid hormones, and cAMP and inhibited by insulin) produces symp-

toms of diabetes. Glucose-6- phosphatase is present in low concentrations in many tissues, but is found most abundantly and importantly in the major gluconeogenic organs – the liver and kidney cortex.

Specific glycolysis controls

Control of glycolysis and gluconeogenesis is unusual for metabolic pathways, in that regulation occurs at multiple points. For glycolysis, this involves three enzymes:

1. Hexokinase (Glucose⇄ G6P)
2. Phosphofructokinase-1 (F6P⇄ F1,6BP)
3. Pyruvate kinase (PEP ⇄ Pyruvate).

Regulation of hexokinase: It is the simplest of these. The enzyme is unusual in being inhibited by its product, glucose-6-phosphate. This ensures when glycolysis is slowing down hexokinase is also slowing down to reduce feeding the pathway.

Allosteric Regulation of Glycolysis & Gluconeogenesis

Reciprocal Regulation

- AMP - Activates PFK-1, Inhibits F1,6BPase
- F2,6BP - Activates PFK-1, Inhibits F1,6BPase
- Citrate - Activates F1,6BPase, Inhibits PFK-1

Glycolysis Only

- ATP - Inhibits PFK-1 and Pyruvate Kinase
- Alanine - Inhibits Pyruvate Kinase

Gluconeogenesis Only

- ADP - Inhibits Pyruvate Carboxylase and PEPCK
- Acetyl-CoA - Activates Pyruvate Carboxylase

Regulation of Phosphofructokinase-1: PFK-1 has a complex regulation scheme Fig 20.19). First, it is reciprocally regulated (relative to F1,6BPase) by three molecules. F2,6BP activates PFK-1 and inhibits F1,6BPase. PFK-1 is also allosterically activated by AMP, whereas F1,6BPase is

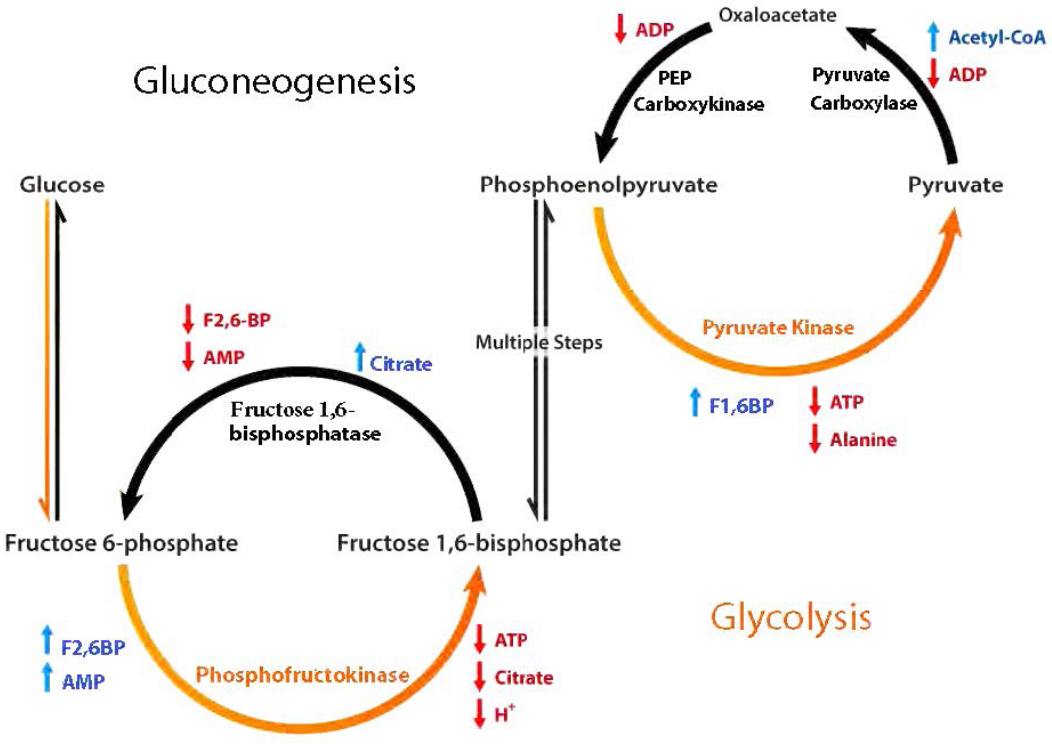


Fig 12.17 Regulation of glycolysis (orange path) and gluconeogenesis (black path)Image by Aleia Kim

inhibited. On the other hand, citrate inhibits PFK-1, but activates F1,6BPase. PFK-1 is also inhibite by ATP and is exquisitely sensitive to proton concentration, easily losing activity when the pH drops only slightly. Regulation of PFK-1 by F2,6BP is simple at the PFK-1 level, but more complicated at the level of synthesis of F2,6BP. Despite having a name sounding like a glycolysis/ gluconeogenesis intermediate (i.e. F1,6BP), F2,6BP is not an intermediate in either pathway. Instead, it is made from fructose-6-phosphate and ATP by the enzyme known as phosphofructokinase-2 (PFK- 2 - Figure 12.19).

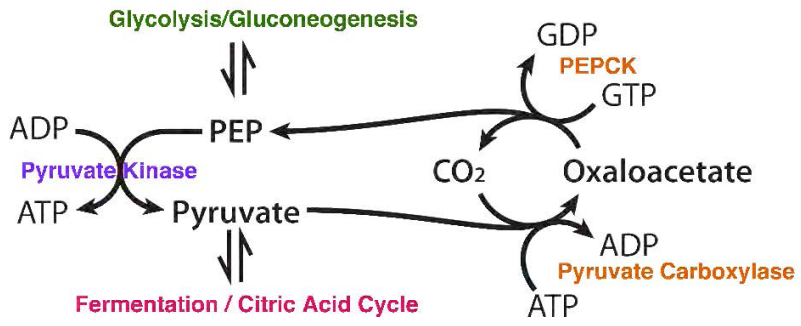


Fig 12.18. A simple futile cycle - follow the black lines. Image by Aleia Kim

Pyruvate kinase

It might also seem odd that pyruvate kinase, the last enzyme in the pathway, is regulated (Figure 12.1), but the reason is simple. Pyruvate kinase catalyzes the most energetically rich reaction of glycolysis. The reaction is favored so strongly in the forward direction that cells must do a'two-step' around it in the reverse direction when making glucose in the gluconeogenesis pathway.

In other words, it takes two enzymes, two reactions, and two triphosphates (ATP and GTP) to go from one pyruvate back to one PEP in gluconeogenesis. When cells are needing to make glucose, they can't be sidetracked by having the PEP they have made in gluconeogenesis be converted directly back to pyruvate by pyruvate kinase. Consequently, pyruvate kinase must be inhibited during gluconeogenesis or a futile cycle will occur and no glucose will be made.

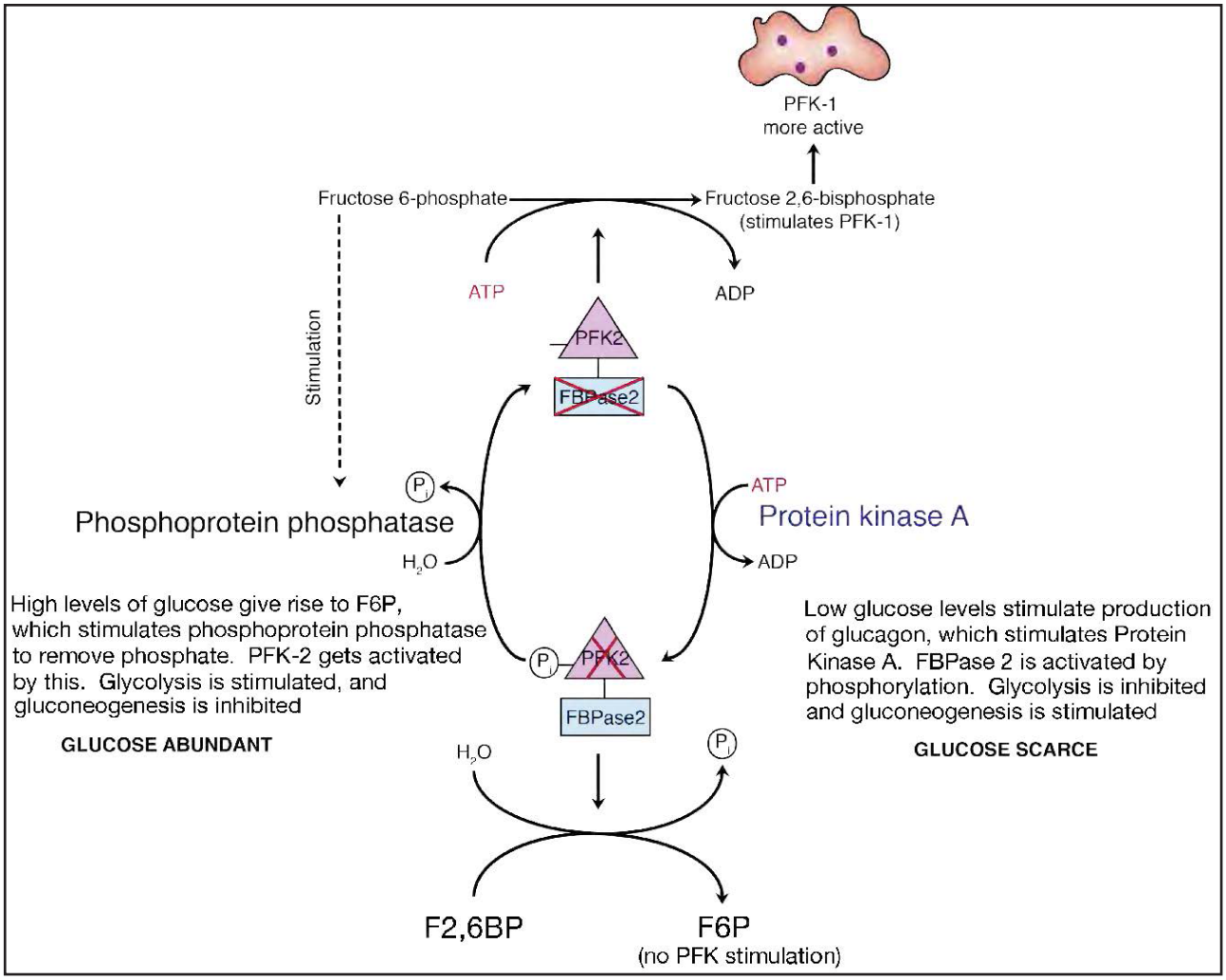


Fig 12.19. Regulation of Synthesis and Breakdown of F2,6BP. Image by Penelope Irving

Another interesting control mechanism called feedforward activation involves pyruvate kinase. Pyruvate kinase is activated allosterically by the glycolysis intermediate, F1,6BP. This molecule is a product of the PFK-1 reaction and a substrate for the aldolase reaction.

Cori cycle

With respect to energy, the liver and muscles act complementarily. The liver is the major organ in the body for the synthesis of glucose. Muscles are major users of glucose to make ATP. Actively exercising muscles use oxygen faster than the blood can deliver it. As a consequence, the muscles go anaerobic and produce lactate. This lactate is of no use to muscle cells, so they dump it into the blood. Lactate travels in the blood to the liver, which takes it up and reoxidizes it back to pyruvate, catalyzed by the enzyme lactate dehydrogenase (Figure 12.20).

Pyruvate in the liver is then converted to glucose by gluconeogenesis. The glucose thus made by the liver is dumped into the bloodstream where it is taken up by muscles and used for energy, completing the important intercellular pathway known as the Cori cycle.

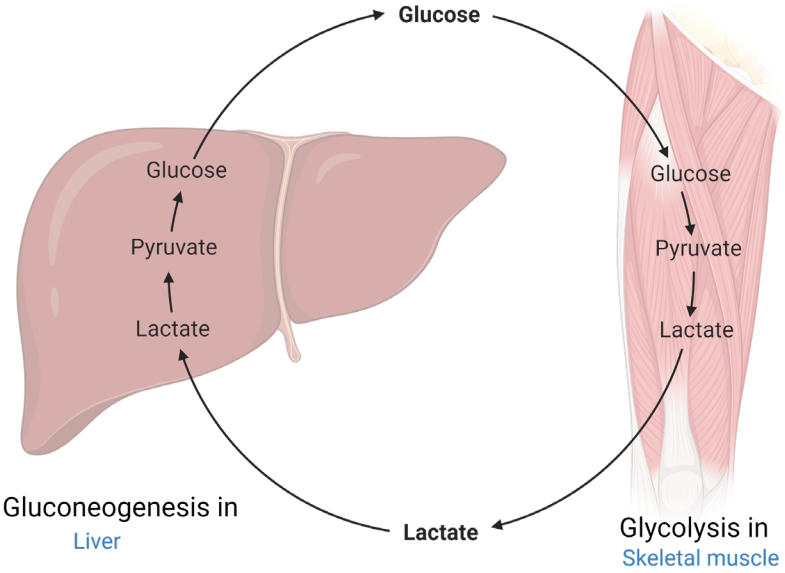


Figure 12.20 Cori Cycle. Adapted from Biorender by Manjeet Kumari

Resources:

Chapter page: Fit man running at the beach. Adobe stock #222896530 free-licensed.

https://openstax.org/books/biology-2e/pages/7-2-glycolysis

Fig 12.1 Adapted from OpenStax, Biology 2e

Fig 12:20 Adapted from "The cori cycle", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.

13

Chapter #13

Pyruvate oxidation and citric acid cycle

Introduction

The primary catabolic pathway in the body is the citric acid cycle because it is here that oxidation to carbon dioxide occurs for breakdown products of the cell's major building blocks - sugars, fatty acids, and amino acids. The pathway is cyclic and thus, doesn't really have a starting or ending point. All of the reactions occur in mitochondria, though one enzyme is embedded in the organelle's inner membrane. As needs change, cells may use a subset of the reactions of the cycle to produce a desired molecule rather than to run the entire cycle.

The molecule "feeding" the citric acid cycle is acetyl-CoA and it can be obtained from pyruvate (from glycolysis), from fatty acid β-oxidation, from ketone bodies, and from amino acid metabolism. Molecules from other pathways feeding into the citric acid cycle for catabolism make the citric acid cycle 'cataplerotic'. It is worth noting that acetyl-CoA has very different fates, depending on the cell's energy status/needs. The description below describes oxidation (catabolism) in citric acid cycle.

Pyruvate Oxidation (decarboxylation of pyruvate to form acetyl-CoA)

If oxygen is available, aerobic respiration will go forward. In eukaryotic cells, the pyruvate molecules produced at the end of glycolysis are transported into the mitochondria, which are the sites of cellular respiration. There, pyruvate is transformed into an acetyl group that will be picked up and activated by a carrier compound called coenzyme A (CoA). The resulting compound is called acetyl CoA. CoA is derived from vitamin B5, pantothenic acid. Acetyl CoA can be used in a variety of ways by the cell, but its major function is to deliver the acetyl group derived from pyruvate to citric acid cycle. Anabolically, acetyl-CoA is also very important for providing building blocks for synthesis of fatty acids, ketone bodies, amino acids and cholesterol.

The pyruvate dehydrogenase enzyme is a complex of multiple copies of three subunits that catalyze the decarboxylation of pyruvate to form acetyl-CoA. The reaction mechanism requires use of five coenzymes. Pyruvate dehydrogenase is an enormous complex in mammals with a size five times greater than ribosomes.

The three subunits are designated by E1, E2, and E3.

E1 Pyruvate decarboxylase

E2 Dihydrolipoamide acetyltransferase

E3 Dihydrolipoyl dehydrogenase.

Confusion arises with the name for E1. Some call it pyruvate dehydrogenase and others give it the name pyruvate decarboxylase. We will use pyruvate decarboxylase solely to refer to E1 and pyruvate dehydrogenase only to refer to the complex of E1, E2, and E3.

The catalytic actions of pyruvate dehydrogenase can be broken down into three steps, each taking place on one of the subunits. The steps, sequentially occurring on E1, E2, and E3, are 1) decarboxylation of pyruvate; 2) oxidation of the decarboxylated product; and 3) transfer of electrons to ultimately form NADH (Figure 13.1).

Catalysis

The catalytic process begins after binding of the pyruvate substrate with activation of the thiamine pyrophosphate coenzyme through formation of an ylide intermediate. The nucleophilic carbanion of the ylide attacks the electrophilic ketone carbon on the pyruvate, releasing carbon dioxide and creating an enol that loses a proton on the carbon to become

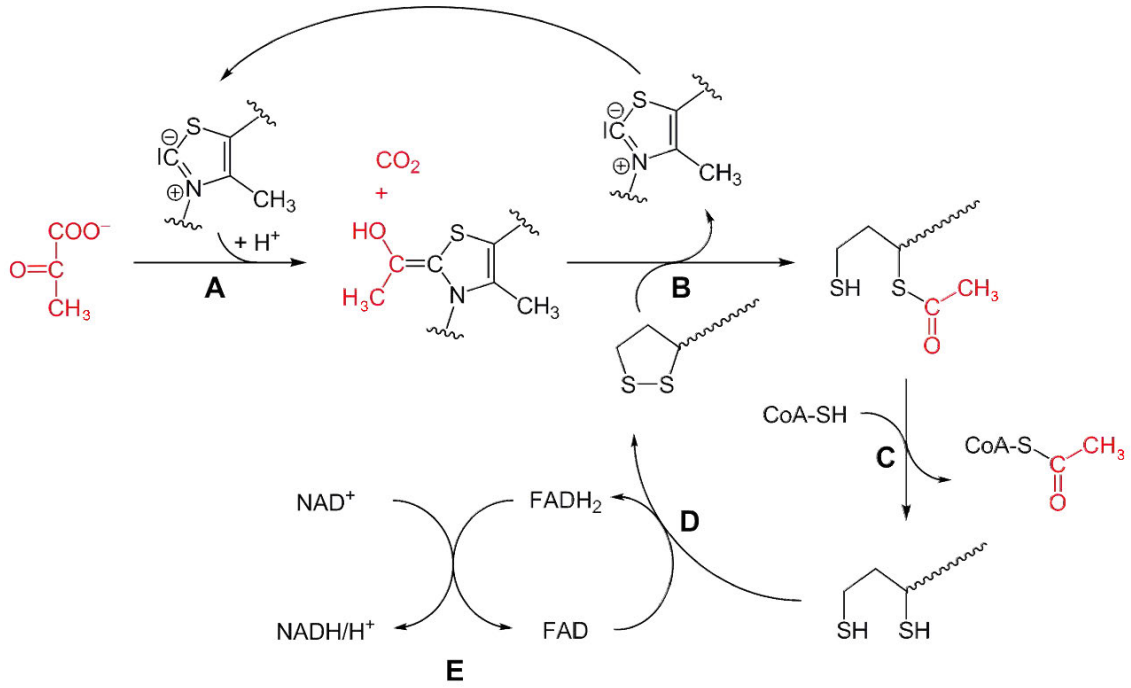


Fig 13.1 Mechanism of action of pyruvate decarboxylation and oxidation by pyruvate dehydrogenase.

a 1,3 dipole that includes the positively charged nitrogen of the thiamine. The reaction (step A in Figure 13.1) is a non-oxidative decarboxylation. Oxidation of the two carbon hydroxyethyl unit occurs in the transfer to the lipoamide.

Reductive acetylation

Reductive acetylation occurs next (Step B) as the 2-carbon hydroxyethyl unit is transferred to lipoamide on E2. (Lipoamide is the name for a molecule of lipoic acid covalently attached to a lysine side chain in the E2 subunit). In prokaryotes in the absence of oxygen, the hydroxyethyl group is not passed to lipoamide, but instead is released as free acetaldehyde , which can accept electrons from NADH (catalyzed by alcohol dehydrogenase) and become ethanol in the process of fermentation. In the presence of oxygen in almost all aerobic organisms, the process continues with transfer of the hydroxyethyl unit to E2.

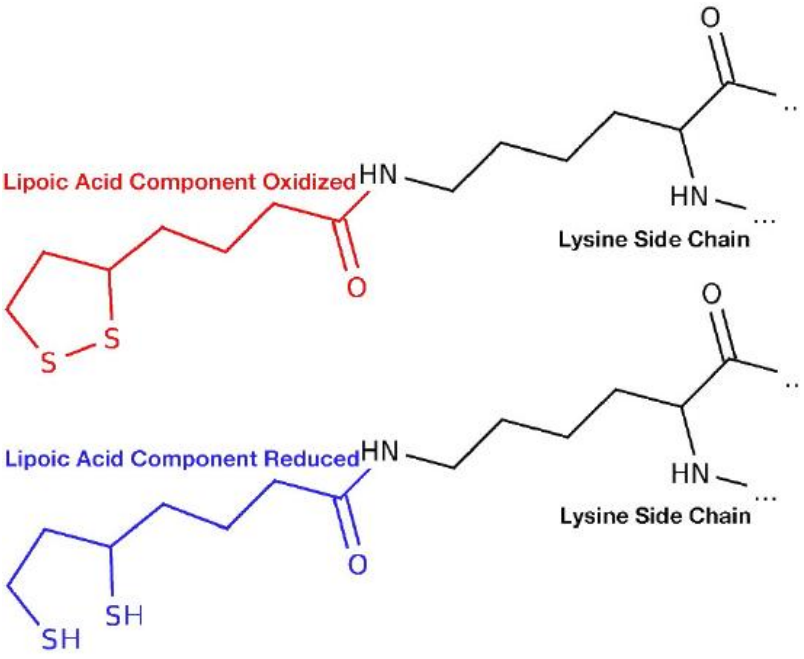


Fig 13.2 Lipoamide

Oxidation step

Transfer of the hydroxyethyl group from E1 to the lipoamide coenzyme in E2 is an oxidation, with transfer of electrons from the hydroxyethyl group to lipoamide's disulfide (reducing it) and formation on the lipoamide of an acetyl-thioester (oxidizing it, Figure 13.2).

The acetyl group is then transferred from lipoamide to coenzyme A in E2 (Step C in Figure 13.1), forming acetyl-CoA, which is released and leaving reduced sulfhydryls on the lipoamide. In order for the enzyme to return to its original state, the disulfide bond on lipoamide must be re-formed. This occurs with transfer of electrons from reduced lipoamide to an FAD covalently bound to E3 (Step D, Figure 13.1). This reduces FAD to $FADH_2$.

Formation of NADH

In the last step in the process, electrons from $FADH_2$ are transferred to external NAD^+, forming NADH (Step E) and completing the overall cycle. Then enzyme can then begin another catalytic round by binding to a pyruvate.

Regulation of pyruvate dehydrogenase

Covalent modification regulation of pyruvate dehydrogenase occurs as a result of phosphorylation by pyruvate dehydrogenase kinase (PDK) or dephosphorylation by pyruvate dehydrogenase phosphatase (PDP. - Figure 13.3).

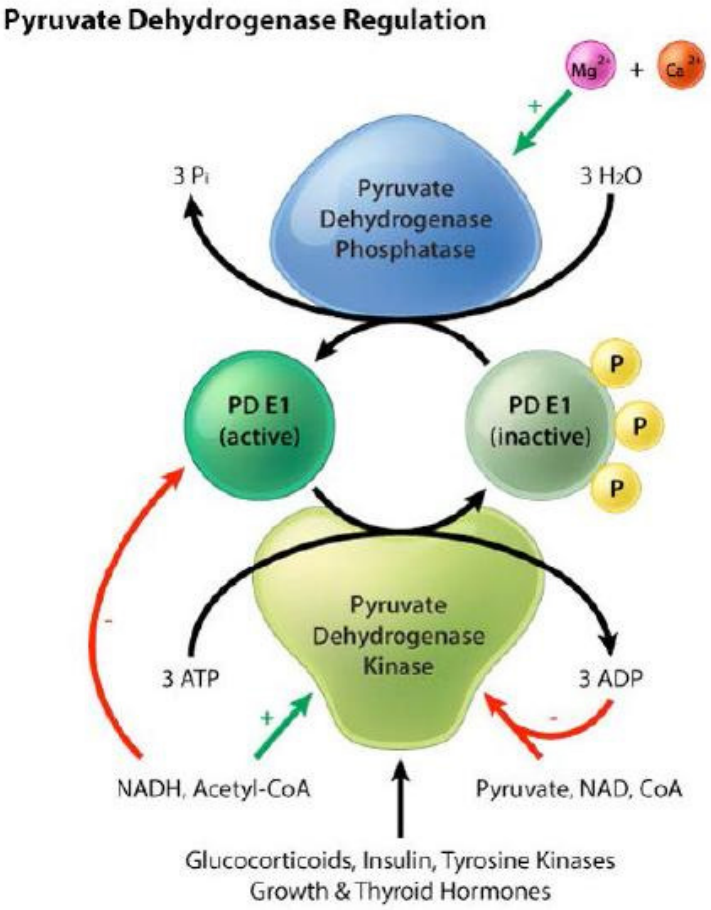


Fig 13.3 Regulation scheme for pyruvate dehydrogenase (PD). Image by Aleia Kim

PDK puts phosphate on any one of three serine residues on the E1 subunit, which causes pyruvate kinase to not be able to perform its first step of catalysis - the decarboxylation of pyruvate. PDP can remove those phosphates. PDK is allosterically activated in the mitochondrial matrix when NADH and acetyl-CoA concentrations rise.

Thus, the products of the pyruvate dehydrogenase reaction inhibit the production of more products by favoring its phosphorylation by PDK. Pyruvate, a substrate of pyruvate dehydrogenase, inhibits PDK, so increasing concentrations of substrate activate pyruvate dehydrogenase by reducing its phosphorylation by PDK. As concentrations of NADH and acetyl-CoA fall, PDP associates with pyruvate kinase and removes the phosphate on the serine on the E1 subunit. Insulin and calcium can also activate the PDP. This is very important in muscle tissue, since calcium is a signal for muscular contraction, which requires energy

Citric acid cycle reactions

Focusing on the pathway itself (Figure 13.4), the usual point to start discussion is addition of acetyl-CoA to oxaloacetate (OAA) to form citrate.

$$\text{Acetyl-CoA} + \text{Oxaloacetate} \rightarrow \text{Citrate} + \text{CoA-SH}$$

Acetyl-CoA for the pathway can come from a variety of sources. The reaction joining it to OAA is catalyzed by citrate synthase and the $\Delta G°'$ is fairly negative. This, in turn, helps to "pull" the malate dehydrogenase reaction preceding it in the cycle.

In the next reaction, citrate is isomerized to isocitrate by action of the enzyme called aconitase.

$$\text{Citrate} \rightleftarrows \text{Isocitrate}$$

Isocitrate is a branch point in plants and bacteria for the glyoxylate cycle. Oxidative decarboxylation of isocitrate by isocitrate dehydrogenase produces thefirst NADH and yields α-ketoglutarate.

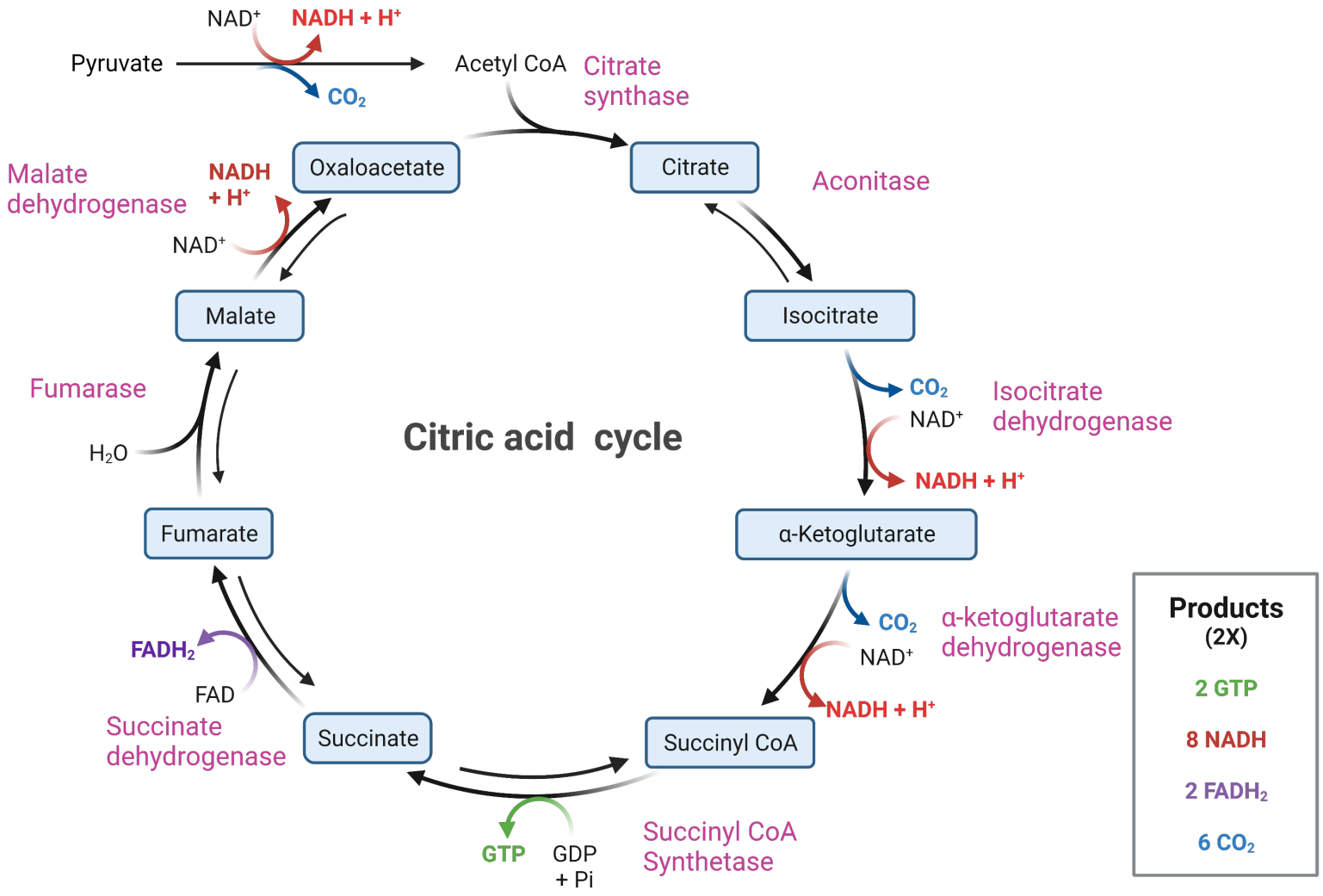


Fig 13.4
Citric acid cycle. Oxidation of pyruvate is included to show overall yield from two molecules of pyruvate. Adapted from Biorender by Manjeet Kumari

$$\text{Isocitrate} + NAD^+ \rightarrow \alpha\text{-ketoglutarate} + NADH + CO2$$

Decarboxylation of α-ketoglutarate produces succinyl-CoA and is catalyzed by α-ketoglutarate dehydrogenase.The enzyme α-ketoglutarate dehydrogenase is structurally very similar to pyruvate dehydrogenase and employs the same five coenzymes– NAD+, FAD, CoA-SH, thiamine pyrophosphate, and lipoamide.

$$\alpha\text{-ketoglutarate} + NAD^+ + \text{CoA-SH} \rightarrow \text{Succinyl-CoA} + NADH + CO_2$$

The remainder of the citric acid cycle involves conversion of the four carbon succinyl-CoA into oxaloacetate. Succinyl-CoA is converted to succinate in a reaction catalyzed by succinyl-CoA synthetase and a GTP is produced, as well – the only substrate level phosphorylation in the cycle.

$$\text{Succinyl-CoA} + GDP + Pi \rightleftarrows \text{Succinate} + GTP + \text{CoA-SH}$$

The energy for the synthesis of the GTP comes from hydrolysis of the high energy thioester bond between succinate and the CoA-SH. Evidence for the high energy of a thioester bond is also evident in the citrate synthase reaction, which is also very energetically favorable.

Oxidation of succinate occurs in the next step, catalyzed by succinate dehydrogenase.

$$\text{Succinate} + FAD \rightleftarrows \text{Fumarate} + FADH_2$$

This interesting enzyme both catalyzes this reaction and participates in the electron transport system, funneling electrons from the $FADH_2$ it gains in the reaction to coenzymeQ. The product of the reaction fumarate, gains a water across its trans double bond in the next reaction, catalyzed by fumarase to form malate.

$$\text{Fumarate} + H_2O \rightleftarrows \text{L-Malate}$$

Malate is important also for transporting electrons across membranes in the malate-aspartate shuttle and in ferrying carbon dioxide from mesophyll cells to bundle sheath cells in C4 plants

Conversion of malate to oxaloacetate by malate dehydrogenase is a rare biological oxidation that has a ΔG°' with a positive value (29.7 kJ/mol).

$$\text{L-Malate} + NAD^+ \rightleftarrows \text{Oxaloacetate} + NADH$$

The reaction is 'pulled' by the energetically favorable conversion of oxaloacetate to citrate in the citrate synthase reaction described above. It is worth noting that reversal of the citric acid cycle theoretically provides a mechanism for assimilating CO2. In fact, this reversal has been noted in both anaerobic and microaerobic bacteria, where it is called the Arnon-Buchanan cycle

Inhibitors and activators of citric acid cycle

High energy molecular indicators, such as ATP and NADH will tend to inhibit the cycle and low energy indicators (NAD^+, AMP, and ADP) will tend to activate the cycle. Pyruvate dehydrogenase, which catalyzes formation of acetyl-CoA for entry into the cycle is allosterically inhibited by its product (acetyl-CoA), as well as by NADH and ATP.

Enzymes

Regulated enzymes in the cycle include citrate synthase (inhibited by NADH, ATP, and succinyl-CoA), isocitrate dehydrogenase (inhibited by ATP, activated by ADP and NAD+), and α-ketoglutarate dehydrogenase (inhibited by NADH and succinyl-CoA and activated by AMP).

Anaplerotic/cataplerotic pathway

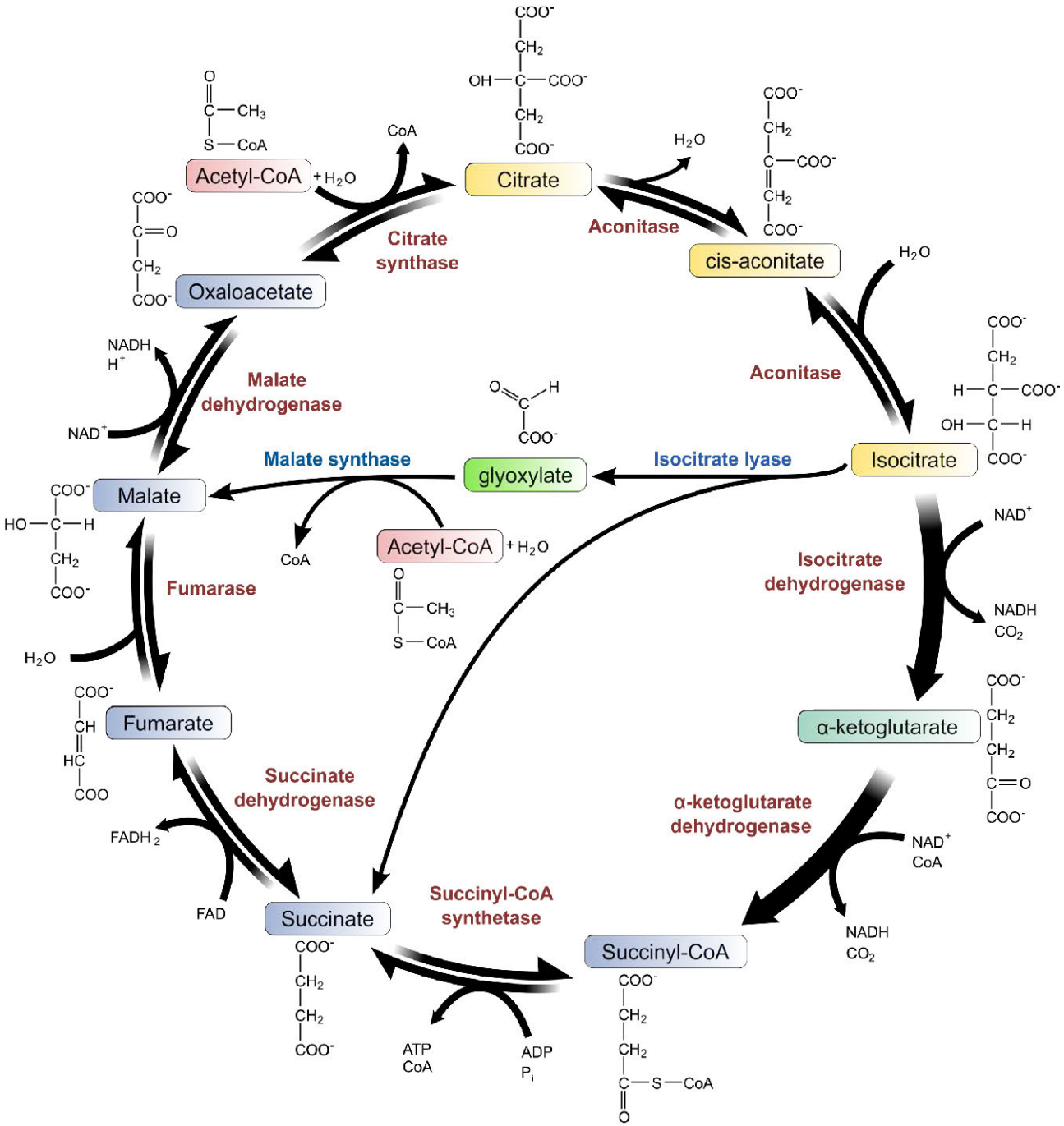


Fig 13.5 Reactions of the glyoxylate cycle. Wikipedia

The citric acid cycle is an important catabolic pathway oxidizing acetyl-CoA into CO_2 and generating ATP, but it is also an important source of molecules needed by cells and a mechanism for extracting energy from amino acids in protein breakdown and other breakdown products. This ability of the citric acid cycle to supply molecules as needed and to absorb metabolic byproducts gives great flexibility to cells. When citric acid cycle

intermediates are taken from the pathway to make other molecules, the term used to describe this is cataplerotic, whereas when molecules are added to the pathway, the process is described as anaplerotic. We will discuss some of these pathways later when we will discuss starvation and diabetes.

Glyoxylate cycle

A pathway related to the citric acid cycle found only in plants and bacteria is the glyoxylate cycle (Figures 13.4). The glyoxylate cycle, which bypasses the decarboxylation reactions while using most of the non-decarboxylation reactions of the citric acid cycle, does not operate in animals, because they lack two enzymes necessary for it – isocitrate lyase and malate synthase. The cycle occurs in specialized plant peroxisomes called glyoxysomes. Isocitrate lyase catalyzes the conversion of isocitrate into succinate and glyoxylate. Because of this, all six carbons of the citric acid cycle survive each turn of the cycle and do not end up as carbon dioxide.

Fig 13.6 A germinating seed

Succinate continues through the remaining reactions to produce oxaloacetate. Glyoxylate combines with another acetyl-CoA (one acetyl-CoA was used to start the cycle) to create malate (catalyzed by malate synthase). Malate can, in turn, be oxidized to oxaloacetate.

It is at this point that the glyoxylate pathway's contrast with the citric acid cycle is apparent. After one turn of the citric acid cycle, a single oxaloacetate is produced and it balances the single one used in the first reaction of the cycle. Thus, in the citric acid cycle, there is no net production of oxaloacetate in each turn of the cycle.

On the other hand, thanks to assimilation of carbons from two acetyl-CoA molecules, each turn of the glyoxylate cycle results in two oxaloacetates being produced, after starting with one. The extra oxaloacetate of the glyoxylate cycle can be used to make other molecules, including glucose in gluconeogenesis. This is particularly important for plant seed germination (Figure 13.5 and 13.6), since the seedling is not exposed to sunlight. With the glyoxylate cycle, seeds can make glucose from stored lipids.

Because animals do not run the glyoxylate cycle, they cannot produce glucose from acetyl-CoA in net amounts, but plants and bacteria can. As a result, plants and bacteria can turn acetyl-CoA from fat into glucose, while animals can't! Bypassing the oxidative decarboxylations (and substrate level phosphorylation) has energy costs, but, there are also benefits. Each turn of the glyoxylate cycle produces one $FADH_2$ and one NADH instead of the three NADHs, one $FADH_2$, and one GTP made in each turn of the citric acid cycle.

The citric acid cycle is a major catabolic pathway producing a considerable amount of energy for cells, whereas the glyoxylate cycle's main function is anabolic - to allow production of glucose from fatty acids in plants and bacteria. The two pathways are physically separated from each other (glyoxylate cycle in glyoxysomes / citric acid cycle in mitochondria).

Resources:

Chapter page: Citric acid on a dark stone background. Adobe stock #414084968 free-licensed.

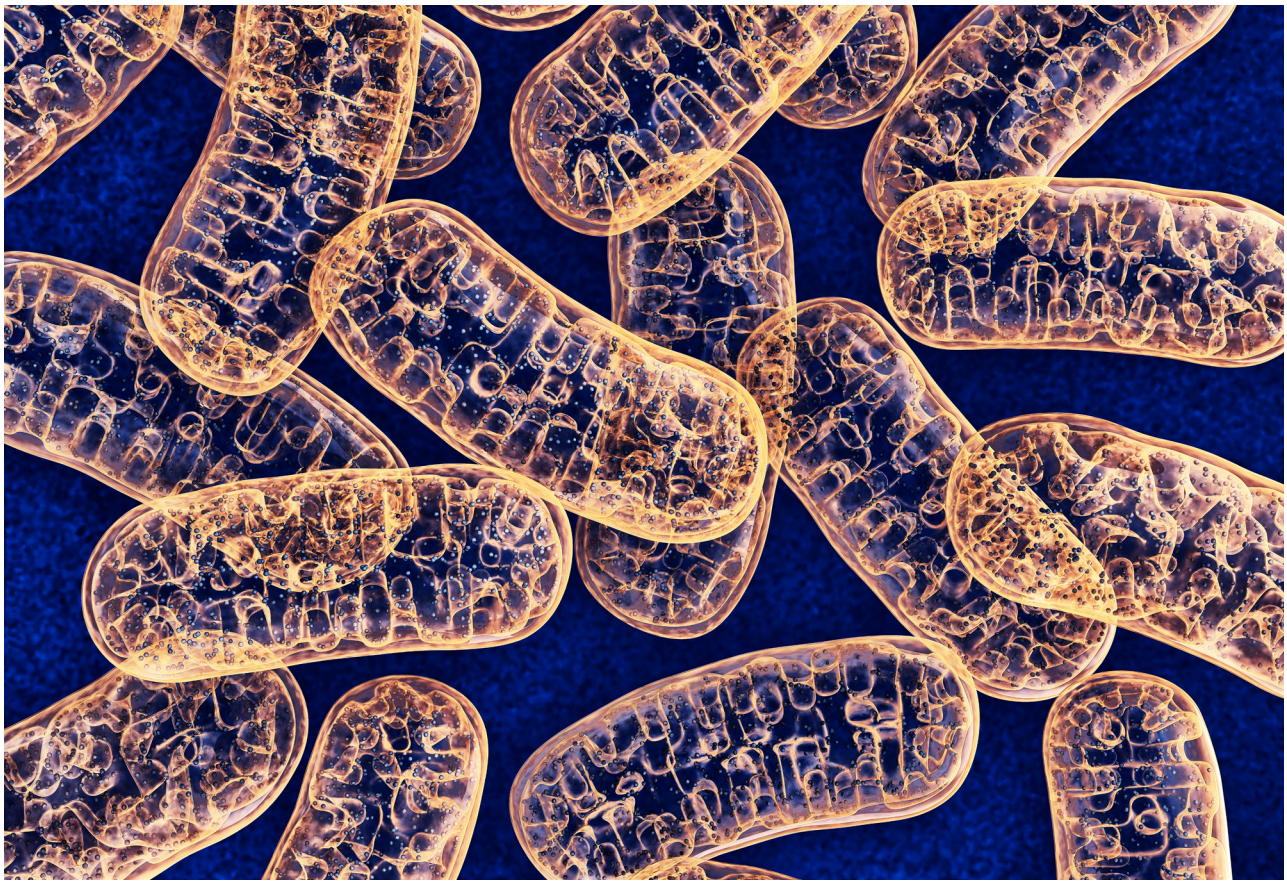
Fig 13.4 Adapted from "Citric acid cycle", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.

Figure 13.6 Close up Organic Sprouting beans on Cultivated soil. Adobe stock # 292792740 free-licensed.

14

Chapter #14 Cellular respiration

Electron transport chain and oxidative phosphorylation



Introduction

You have just read about following pathways in glucose catabolism—glycolysis and the citric acid cycle—that generate ATP. Most of the ATP generated during the aerobic catabolism of glucose, however, is not generated directly from these pathways. Instead, it is derived from a process that begins by moving electrons through a series of electron carriers (i.e. NADH and $FADH_2$)that undergo redox reactions. This process causes hydrogen ions to accumulate within the intermembranous space. Therefore, a concentration gradient forms in which hydrogen ions diffuse out of the intermembranous space into the mitochondrial matrix by passing through ATP synthase. The current of hydrogen ions powers the catalytic action of ATP synthase, which phosphorylates ADP, producing ATP. We will discuss electron tranport chain and oxidative phosphorylation in this chapter.

Electron transport chain

The electron transport system, located in the inner mitochondrial membrane (Fig. 14.1), transfers electrons donated by the reduced electron carriers NADH and $FADH_2$ (obtained from glycolysis, the citric acid cycle or fatty acid oxidation) through a series of electrons acceptors, to oxygen. As we shall see, movement of electrons through complexes of the electron transport system essentially "charges" a battery that is used to make ATP in oxidative phosphorylation. In this way, the oxidation of sugars and fatty acids is coupled to the synthesis of ATP, effectively extracting energy from food.

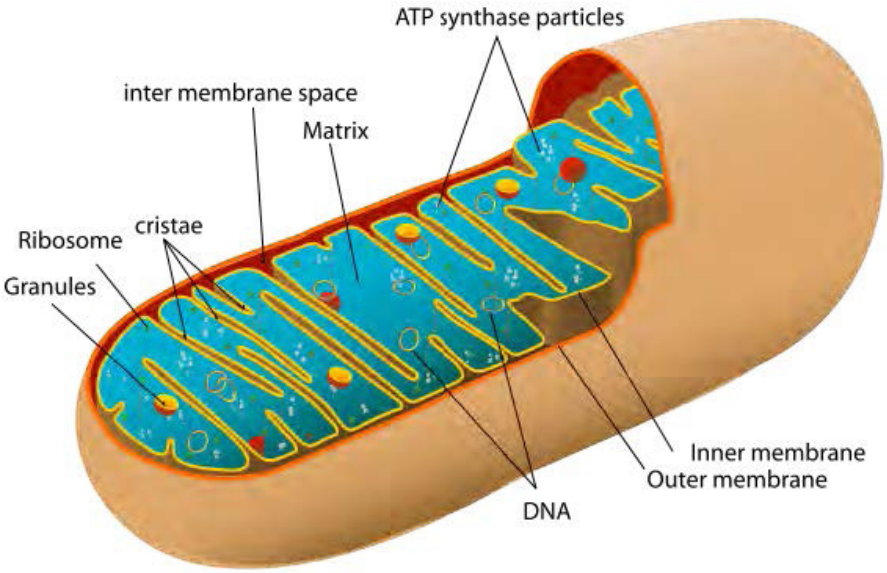


Fig 14.1 Mitochondria

Chemiosmotic model

Dr. Peter Mitchell introduced a radical proposal in 1961 to explain the mechanism by which mitochondria make ATP. It is known as the chemiosmotic hypothesis and has been shown over the years to be correct. Mitchell proposed that synthesis of ATP in mitochondria depends on an electrochemical gradient, across the mitochondrial inner membrane, that arises ultimately from the energy of reduced electron carriers, NADH and $FADH_2$.

Electron transport

Further, the proposal states that the gradient is created when NADH and $FADH_2$ transfer their electrons to an electron transport system (ETS) located in the inner mitochondrial membrane. Movement of electrons through a series of of electron carriers is coupled to the pumping of protons out of the mitochondrial matrix across the inner mitochondrial membrane into the space between the inner and outer membranes. The result is creation of a gradient of protons whose potential energy can be used to make ATP. Electrons combine with oxygen and protons at the end of the ETS to make water.

ATP synthase

In oxidative phosphorylation, ATP synthesis is accomplished as a result of protons re-entering the mitochondrial matrix via the transmembrane ATP synthase complex, which combines ADP with inorganic phosphate to make ATP. Central to the proper functioning of mitochondria through this process is the presence of an intact mitochondrial inner membrane impermeable to protons.

Tight coupling

When this is the case, tight coupling is said to exist between electron transport and the synthesis of ATP (called oxidative phosphorylation). Chemicals which permeabilize the inner mitochondrial membrane to protons cause uncoupling, that is, they allow the protons to leak back into the mitochondrial matrix, rather than through the ATP synthase, so that the movement of electrons through the ETS is no longer linked to the synthesis of ATP.

Mitochondria are called the power plants of the cell because most of a cell's ATP is produced there in the process of oxidative phosphorylation. The mechanism by which ATP is made in oxidative phosphorylation is one of the most interesting in all of biology.

ETC has following primary considerations. The first is electrical – electrons from reduced electron carriers, such as NADH and $FADH_2$, enter the electron transport system via Complex I and II, respectively. As seen in Figure 14.2 and Figure 14.3, electrons move from one complex to the next, not unlike the way they move through an electrical circuit. Such movement occurs a a result of a set of reduction-oxidation (redox) reactions with electrons moving from a more negative reduction potential to a more positive one. One can think of this occurring as a process where carriers "take" electrons away from complexes with lower reduction potential, much the way a bully takes lunch money from a smaller child. In this scheme, the biggest "bully" is oxygen in Complex IV. Electrons gained by a carrier cause it to be reduced, whereas the carrier giving up the electrons is oxidized.

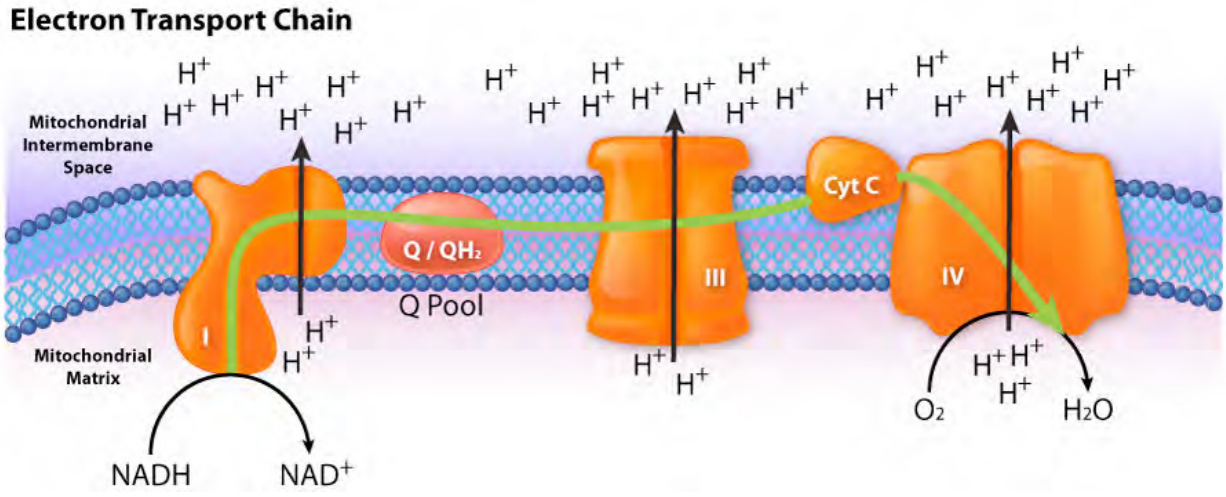


Fig 14.2 Flow of electrons from NADH into the electron transport system. Entry is through complex I

Entry of electrons to system

Movement of electrons through the chain begins either by 1) transfer from NADH to Complex I (Figure 14.2) or 2) movement of electrons through a covalently bound $FADH_2$ (Figure 14.3 and 14.4) in the membrane-bound succinate dehydrogenase (Complex II).

Both Complex I and II pass electrons to the inner membrane's coenzyme Q. In each case, coenzyme Q accepts electrons in pairs and passes them off to Complex III (CoQH2-cytochrome c reductase) singly. Coenzyme Q thus acts as a traffic cop, regulating the flow of

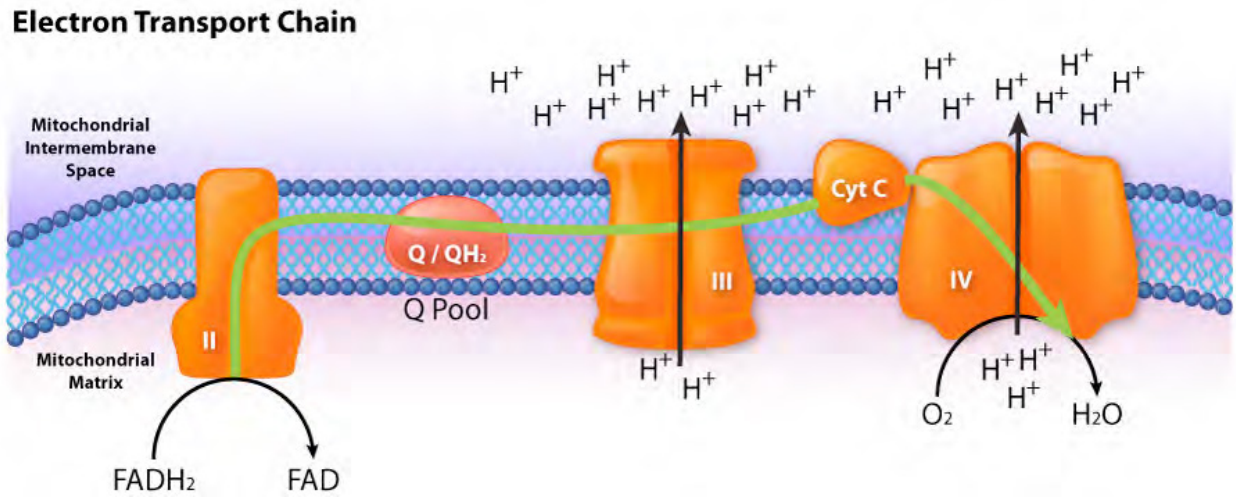


Fig 14.3 Flow of electrons from $FADH_2$ into the electron transport chain. Entry is through complex II.

electrons through the ETS.

Docking station

Complex III is a docking station or interchange for the incoming electron carrier (coenzyme Q) and the outgoing carrier (cytochrome c). Movement of electrons from Coenzyme Q to Complex III and then to cytochrome C occurs as a result of what is referred to as the Q-cycle.

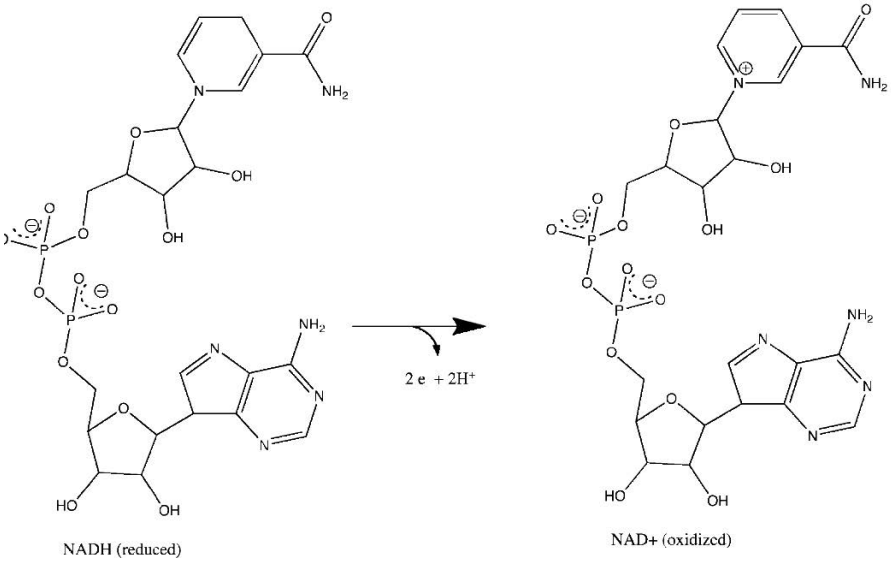


Fig 14.4.Loss of electrons by NADH to form NAD+. Relevant reactions occur in the top ring of the molecule.

Complex III acts to ferry electrons from CoQ to cytochrome c. Cytochrome c takes one electron from Complex III and passes it to Complex IV (cytochrome oxidase). Complex IV is the final protein recipient of the electrons. It passes them to molecular oxygen (O_2) to make two molecules of water. Making two water molecules requires four electrons, so Complex IV must accept, handle, and pass to molecular oxygen four separate electrons, causing the oxidation state of oxygen to be sequentially changed with addition of each electron.

Proton pumping

As electrons pass through complexes I, III, and IV, there is a release of a small amount of energy at each step, which is used to pump protons from the mitochondrial matrix (inside of mitochondrion) and deposit them in the intermembrane space (between the inner and outer membranes of the mitochondrion). The effect of this redistribution is to increase the electrical and chemical potential across the membrane.

Lets look at these components of ETC in a little more detail.

Complex I

Complex I (also called NADH:ubiquinone oxidoreductase or NADH dehydrogenase

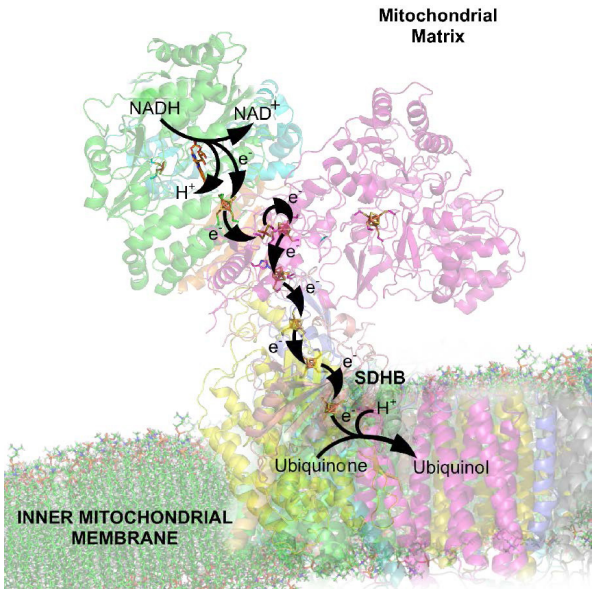


Fig14.5 Complex I embedded in the innermitochondrial membrane. The mitochondrial matrix at the top.

(ubiquinone)) is the electron acceptor from NADH in the electron transport chain and the largest complex found in it.

Complex I contains 44 individual polypeptide chains, numerous iron-sulfur centers, a molecule of flavin mononucleotide (FMN) and has an L shape with about 60 transmembrane domains. In the process of electron transport through it, four protons are pumped across the inner membrane into the intermembrane space and electrons move from NADH to coenzyme Q, converting it from ubiquinone (no electrons) to ubiquinol (gain of two electrons). An intermediate form, ubisemiquinone (gain of one electron), is found in the Q-cycle.

Electrons travel through the complex via seven primary iron sulfur centers. The best known inhibitor of the complex, rotenone, works by binding to the CoQ binding site. Other inhibitors include ADP-ribose (binds to the NADH site) and piericidin A (rotenone analog). The process of electron transfer through complex I is reversible and when this occurs, superoxide (a reactive oxygen species) may be readily generated.

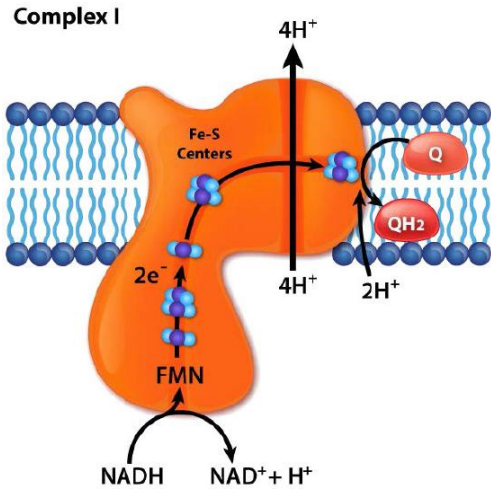


Fig 14.6 Complex I

Complex II

Complex II (also called succinate dehydrogenase or succinate-coenzyme Q reductase) is a membrane bound enzyme of the citric acid cycle that plays a role in the electron transport process, transferring electrons from its covalently bound $FADH_2$ to coenzyme Q (Figure 14.7). The process occurs with transfer of electrons from succinate to FAD to form FADH2 and fumarate. $FADH_2$, in turn, donates electrons to a relay system of iron-sulfur groups and they ultimately reduce ubiquinone (CoQ) along with two protons from the matrix to ubiquinol. The role of the heme group in the process is not clear. Inhibitors of the process include carboxin, malonate, malate, and oxaloacetate. The role of citric acid cycle intermediates as inhibitors is thought to be due to inhibition of the reversal of the transfer process which can produce superoxide.

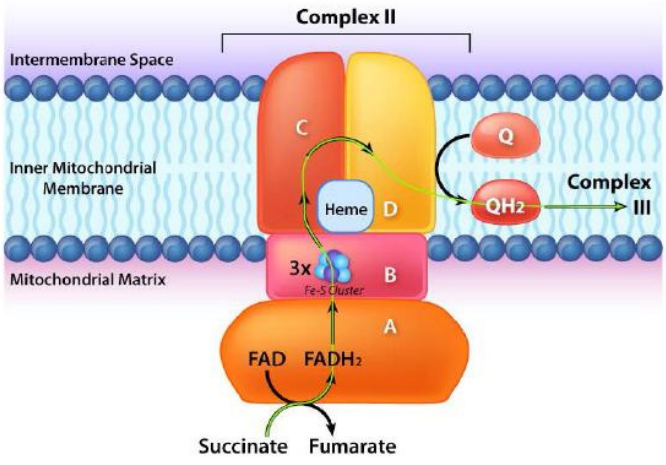


Fig 14.7 Complex II

Coenzyme Q

Coenzyme Q (Figure 14.8) is a 1,4 benzoquinone whose name is often given as Coenzyme Q10, CoQ, or Q10. The 10 in the name refers to the number of isoprenyl units it contains that anchor it to the mitochondrial inner membrane. CoQ is a vitamin-like lipid substance found in most eukaryotic cells as a component of the electron transport system. The requirement for CoQ increases with increasing energy needs of cells, so the highest concentrations of CoQ in the body are found in tissues that are the most metabolically active - heart, liver, and kidney.

Complex III

Complex III (also known as coenzyme Q : cytochrome c-oxidoreductase or the cytochrome bc1 complex - Figure 14.9) is the third elec-

tron accepting complex of the electron transport system. It is a transmembrane protein with multiple subunits present in the mitochondria of all aerobic eukaryotic organisms and and the cell membrane of almost all bacteria. The complex contains 11 subunits, a 2-iron ferredoxin, cytochromes b and c1 and belongs to the family of oxidoreductase enzymes.

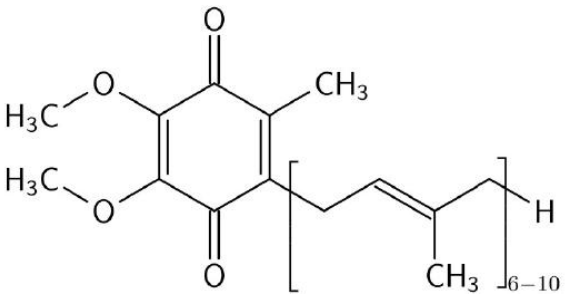


Fig 14.8 Coenzyme Q

It accepts electrons from coenzyme Q in electron transport and passes them off to cytochrome c. In this cycle, known as the Q cycle, electrons arrive from CoQ in pairs, but get passed to cytochrome c individually. In the overall process, two protons are consumed from the matrix and four protons are pumped into the intermembrane space. Movement of electrons through the complex can be inhibited by antimycin A, myxothiazol, and stigmatellin. Complex III is also implicated in creation of superoxide (a reactive oxygen species) when electrons from it leak out of the chain of transfer. The phenomenon is more pronounced when antimycin A is present.

Q-cycle

In the Q-cycle, electrons are passed from ubiquinol (QH2) to cytochrome c using Complex III as an intermediary docking station for the transfer. Two pair of electrons enter from QH2 and one pair is returned to another CoQ to re-make QH2. The other pair is donated singly to two different cytochrome c molecules. The Q-cycle happens in a two step process:

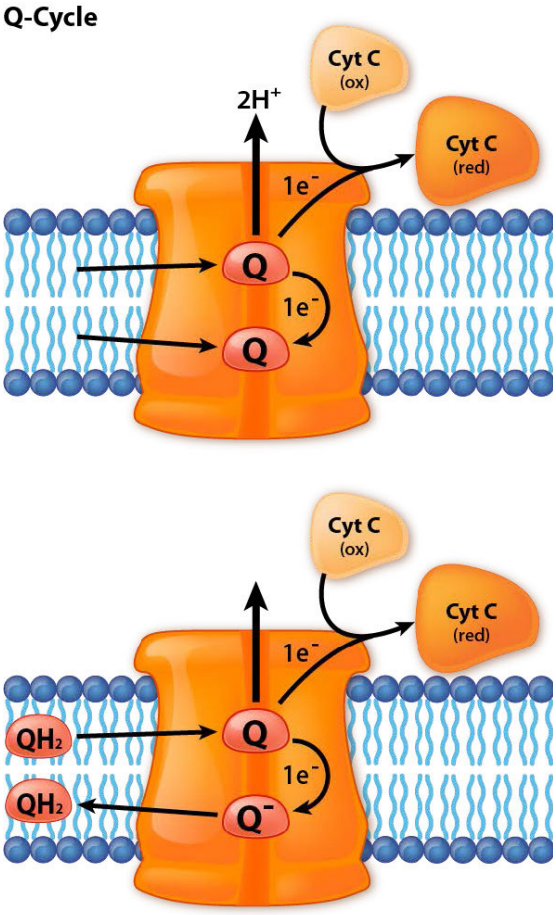


Fig 14.9 Q-cycle

Step one

First, a ubiquinol (CoQH2) and a ubiquinone (CoQ) dock at Complex III. Ubiquinol transfers two electrons to Complex III. One electron goes to a docked cytochrome c, reducing it and it exits (replaced by an oxidized cytochrome c). The other goes to the docked uniquinone to create the semi-reduced semiubiquinone (CoQ.-) and leaving behind a ubiquinone, which exits. This is the end of step 1.

Step two

The gap left behind by the ubiquinone (Q) that departed is replaced by another ubiquinol (QH2). It too donates two electrons to Complex III, which splits them. One goes to the newly docked oxidized cytochrome c, which is reduced and exits. The other goes to the ubisemiquinone. Two protons from the matrix combine with it to make another ubiquinol. It and the ubiquinone created by the electron donation exit Complex III and the process starts again. In the overall process, two protons are consumed from the matrix and four protons are pumped into the intermembrane space.

Cytochrome c

Cytochrome c (Figure 14.10) is a small (12,000 Daltons), highly conserved protein, from unicellular species to animals, that is loosely associated with the inner mitochondrial membrane where it functions in electron transport. It contains a heme group which is used to carry a single electron from Complex III to Complex IV. Cytochrome c also plays an important role in apoptosis in higher organisms. Damage to the mitochondrion that

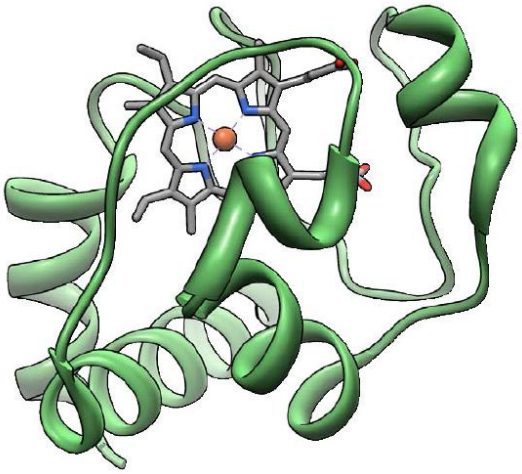


Fig 14.10 Cytochrome c with bound heme Group. Wikipedia.

results in release of cytochrome c can stimulate assembly of the apoptosome and activation of the caspase cascade that leads to programmed cell death.

Complex IV

Complex IV, also known as cytochrome c oxidase is a 14 subunit integral membrane protein at the end of the electron transport chain (Figure 14.11). It is responsible for accepting one electron each from four cytochrome c proteins and adding them to molecular oxygen (O2) along with four protons from the mitochondrial matrix to make two molecules of water. Four protons from the matrix are also pumped into the intermembrane space in the process. The complex has two molecules of heme, two cytochromes (a and a3), and two copper centers (called CuA ad CuB). Cytochrome c docks near the CuA and donates an electron to it. The reduced CuA passes the electron to cytochrome a, which turns it over to the a3-CuB center where the oxygen is reduced. The four electrons are thought to pass through the complex rapidly resulting in complete reduction of the oxygen-oxygen molecule without formation of a peroxide intermediate or superoxide, in contrast to previous predictions.

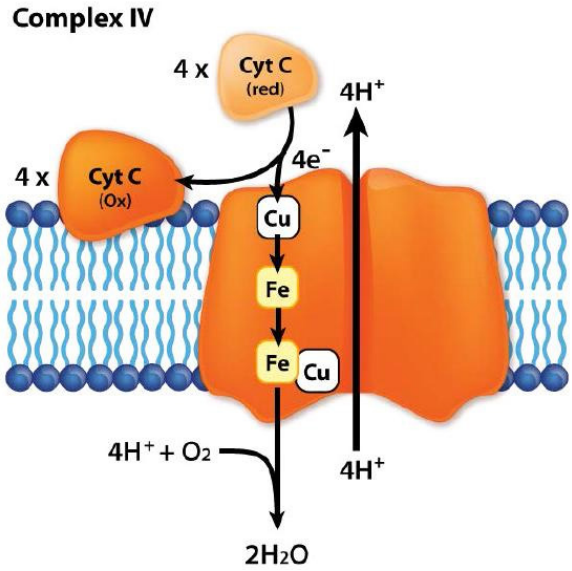


Fig 14.11 Movement of electrons and protons-through complex IV. Matrix is down. Image by Aleia Kim

Respirasome

There has been speculation for many years that a supercomplex of electron carriers (Figure 14.12)in the inner membrane of the mitochondrion may exist in cells with individual carriers making physical contact with each other. This would make for more efficient transfer reactions, minimize the production of reactive oxygen species and be similar to metabolons of metabolic pathway enzymes, for which there is some evidence. Now, evidence appears to be accumulating that complexes I, III, and IV form a supercomplex, which has been dubbed the respirasome1.

Oxidative phosphorylation

The process of oxidative phosphorylation uses the energy of the proton gradient established by the electron transport system as a means of phosphorylating ADP to make ATP (Figure 14.12). The establishment of the proton gradient is dependent upon electron transport. If electron transport stops or if the inner mitochondrial membrane's impermeability to protons is compromised, oxidative phosphorylation will not occur because without the proton gradient to drive the ATP synthase, there will be no synthesis of ATP.

ATP synthase

The protein complex harvesting energy from the proton gradient and using it to make ATP from ADP is an enzyme that has several names - Complex V, PTAS (Proton Translocating ATP Synthase), and ATP synthase (Figure14.12 and 14.13). Central to its function is the movement of protons through it (from the intermembrane space back into the matrix). Protons will only provide energy to make ATP if their concentration is greater in the intermembrane space than in the matrix and if ADP is available.

It is possible, in some cases, for the concentration of protons to be greater inside the matrix than outside of it. When this happens, the ATP synthase can run backwards, with protons moving from inside to out, accompanied by conversion of ATP to ADP + Pi. This is usually not a desirable circumstance and there are some controls to reduce its occurrence.

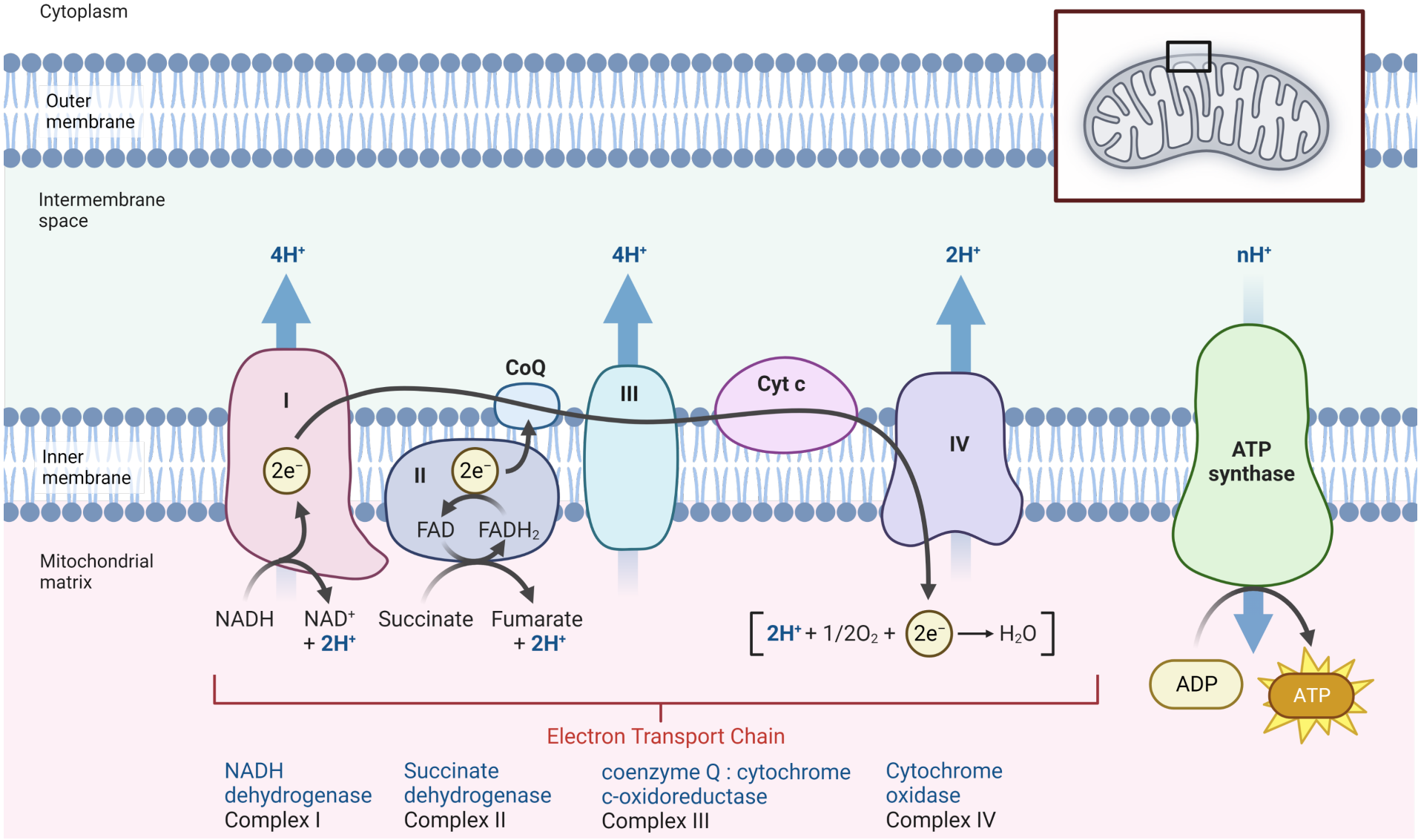


Figure 14.12 Electron transport chain and ATP synthase. Adapted from Biorender by Manjeet Kumari

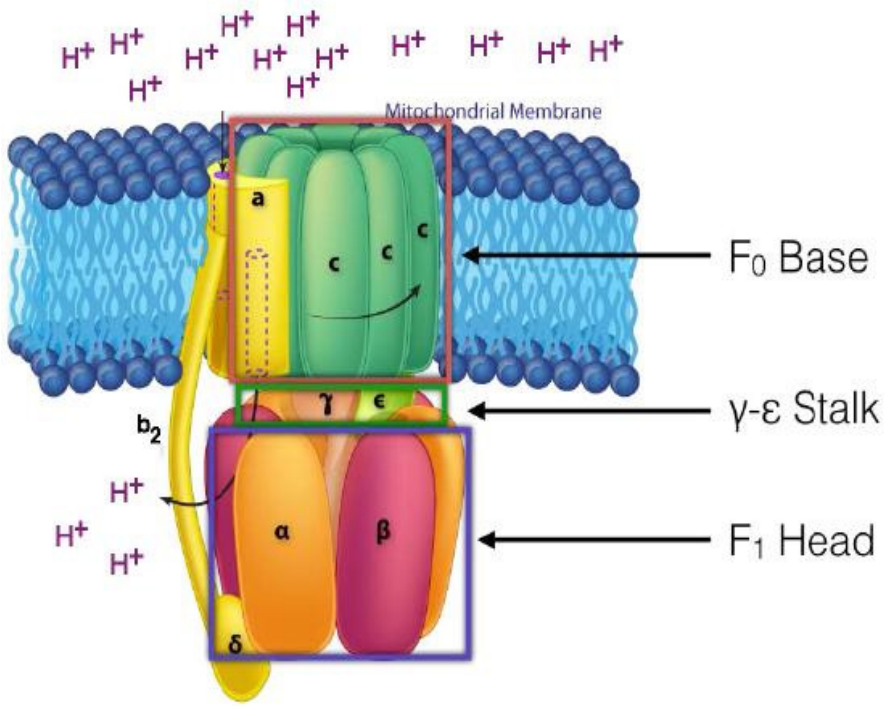


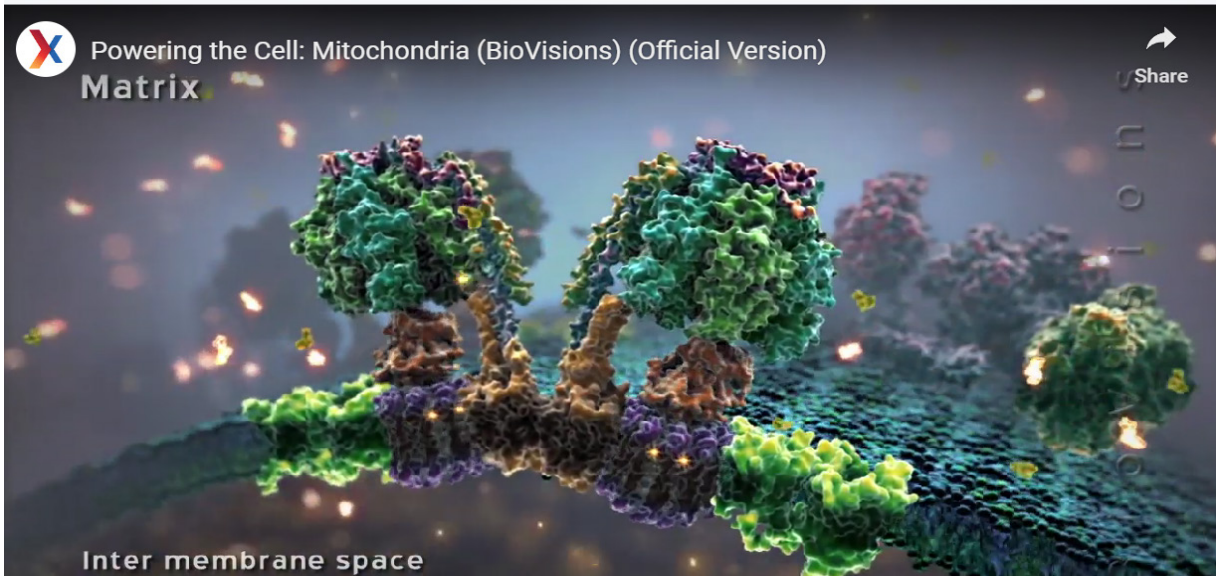
Fig 14.13 ATP synthase is multi-subunit membrane protein, which acts like a turbine at a hydroelectric dam. The movement of protons through the ATP Synthase c-ring causes it and the γ-ε stalk attached to it to turn. It is this action that is necessary for making ATP.

Normally, ATP concentration will be higher inside of the mitochondrion and ADP concentration be higher outside the mitochondrion. However, when the rate of ATP synthesis exceeds the rate of ATP usage, then ATP concentrations rise outside the mitochondrion and ADP concentrations fall everywhere.

This may happen, for example, during periods of rest. It has the overall effect of reducing transport and thus lowering the concentration of ADP inside the matrix. Reducing ADP concentration in the matrix reduces oxidative phosphorylation.

Another important consideration is that when ATP is made in oxidative phosphorylation, it is released into the mitochondrial matrix, but must be transported into the cytosol to meet the energy needs of the rest of the cell. This is accomplished by action of the adenine nucleotide translocase, an antiport that moves ATP out of the matrix in exchange for ADP moving into the matrix. This transport system is driven by the concentrations of ADP and ATP and ensures that levels of ADP are maintained within the mitochondrion, permitting continued ATP synthesis.

One last requirement for synthesis of ATP from ADP is that phosphate must also be imported into the matrix. This is accomplished by action of the phosphate translocase, which is a symport that moves phosphate into the mitochondrial matrix along with a proton.



Figure/Animation 14.14. "Video by LabXchange © The President and Fellows of Harvard College" Used with permission.

There is evidence that the two translocases and ATP synthase may exist in a complex, which has been dubbed the *ATP synthasome*. In summary, the electron transport system charges the battery for oxidative phosphorylation by pumping protons out of the mitochondrion. The intact inner membrane of the mitochondrion keeps the protons out, except for those that re-enter through ATP Synthase. The ATP Synthase allows protons to re-enter the mitochondrial matrix and harvests their energy to make ATP (Figure/animation 14.14). In

ATP Synthase, the spinning components, or rotor, are the membrane portion (c ring) of the F0 base and the γ-ε stalk, which is connected to it. The γ-ε stalk projects into the F1 head of the mushroom structure. The F1 head contains the catalytic ability to make ATP. The F1 head is hexameric in structure with paired α and β proteins arranged in a trimer of of dimers. ATP synthesis occurs within the β subunits.

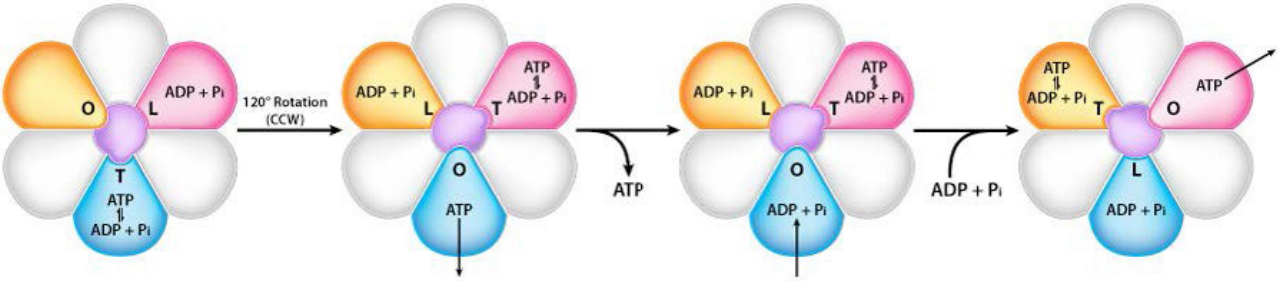


Fig 14.15. L, T and O structure of F1 Head of ATP synthase.

Rotation of γ unit

Turning of the γ shaft (caused by proton flow) inside the α-β trimer of the F1 head causes each set of β proteins to change structure slightly into three different forms called Loose, Tight, and Open (L,T,O - Figure 14.15). Each of these forms has a function. The Loose form binds ADP + Pi. The Tight form "squeezes" them together to form the ATP. The Open form releases the ATP into the mitochondrial matrix. Thus, as a result of the proton flow through the ATP synthase, from the intermembrane space into the matrix, ATP is made from ADP and Pi.

Respiratory Control

When a mitochondrion has an intact inner membrane and protons can only return to the matrix by passing through the ATP synthase, the processes of electron transport and oxidative phosphorylation are said to be tightly coupled.

Interdependence

In simple terms, tight coupling means that the processes of electron transport and oxidative phosphorylation are interdependent. Without electron transport going on in the cell, oxidative phosphorylation will soon stop.

The reverse is also true, because if oxidative phosphorylation stops, the proton gradient will not be dissipated as it is being built by the electron transport system and will grow larger and larger. The greater the gradient, the greater the energy needed to pump protons out of the mitochondrion. Eventually, if nothing relieves the gradient, it becomes too large and the energy of electron transport is insufficient to perform the pumping. When pumping stops, so too does electron transport.

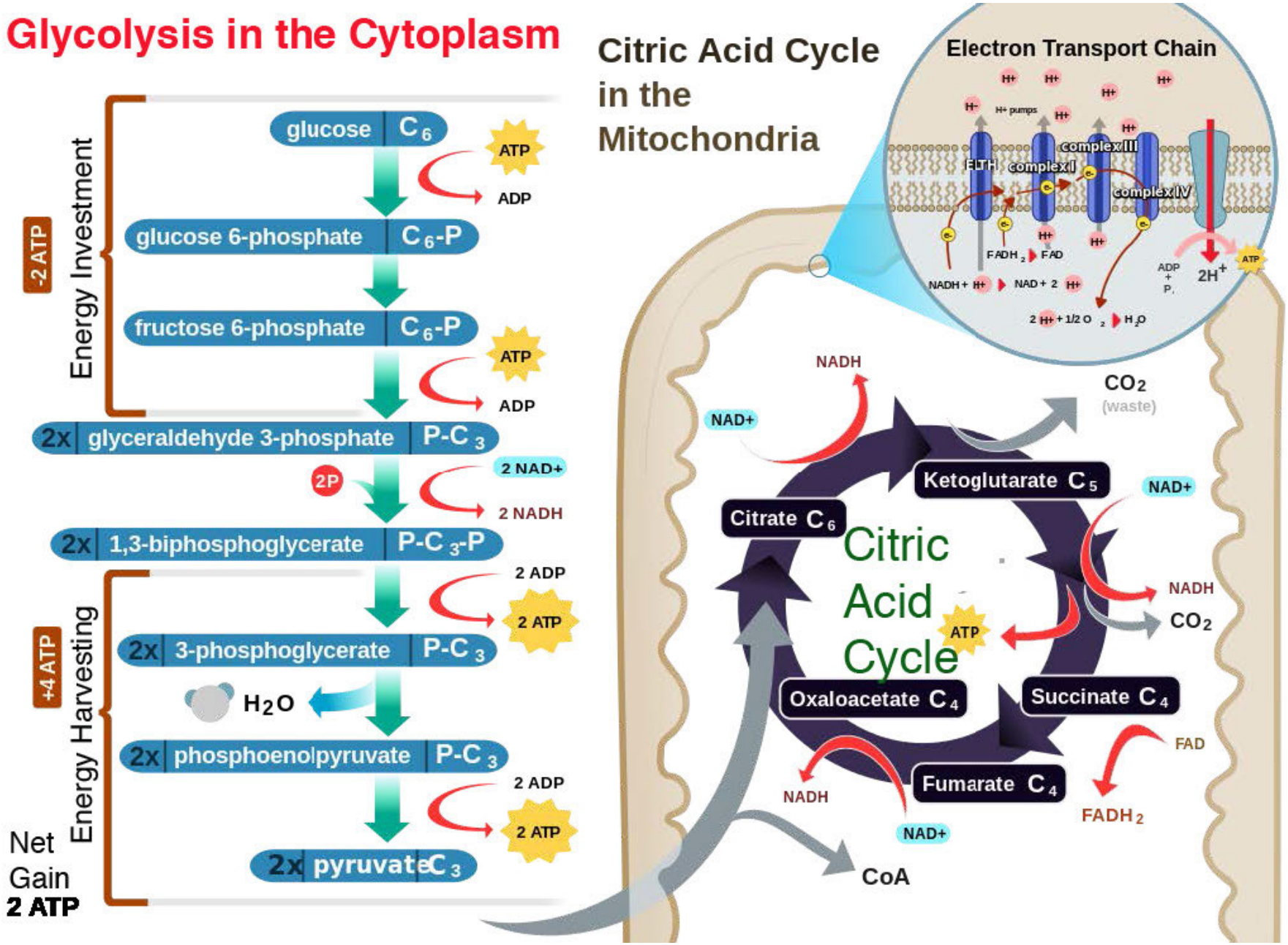
ADP dependence

Another relevant point is that ATP synthase is totally dependent upon a supply of ADP. In the absence of ADP, the ATP synthase stops functioning and when it stops, so too does movement of protons back into the mitochondrion. With this information, it is possible to understand the link between energy usage and metabolism. The root of this, as noted, is respiratory control.

At rest

To illustrate these links, let us first consider a person, initially at rest, who then suddenly

Fig 14.17. Respiration overview in eukaryotic cells. Wikipedia



jumps up and runs away. At first, the person's ATP levels are high and ADP levels are low (no exercise to burn ATP), so little oxidative phosphorylation is occurring and thus the proton gradient is high. Electron transport is moving slowly, if at all, so it is not using oxygen and the person's breathing is slow, as a result.

Exercise

When running starts, muscular contraction, which uses energy, causes ATP to be converted to ADP. Increasing ADP in muscle cells favors oxidative phosphorylation to attempt to make up for the ATP being burned. ATP synthase begins working and protons begin to come back into the mitochondrial matrix. The proton gradient decreases, so electron transport re-starts.

Electron transport needs an electron acceptor, so oxygen use increases and when oxygen use increases, the person starts breathing more heavily to supply it. When the person stops running, ATP concentrations get rebuilt by ATP synthase. Eventually, when ATP levels are completely restored, ADP levels fall and ATP synthase stops or slows considerably. With little or no proton movement, electron transport stops because the proton gradient is too large. When electron transport stops, oxygen use decreases and the rate of breathing slows down.

Importance of electron tranport

Electron transport is the way in which reduced electron carriers, NADH and $FADH_{2,}$ donate their electrons to the ETS, becoming oxidized to NAD^+ and FAD, respectively Oxidized carriers, such as NAD^+ and FAD are needed by catabolic pathways, like glycolysis, the citric acid cycle, and fatty acid oxidation. Anabolic pathways, such as fatty acid/fat synthesis and gluconeogenesis rely on reduced electron carriers, such as $FADH_2$, NADH, and the related carrier, NADPH.

Inhibitors

Electron tranport inhibitors

Common inhibitors of electron transport include rotenone and amytal, which stop movement of electrons past Complex I, malonate, malate, and oxaloacetate, which inhibit movement of electrons through Complex II, antimycin A which stops movement of electrons past Complex III, and cyanide, carbon monoxide, azide, and hydrogen sulfide, which inhibit electron movement through Complex IV. All of these compounds can stop electron transport directly (no movement of electrons) and oxidative phosphorylation indirectly (proton gradient will dissipate). While some of these compounds are not commonly known, almost everyone is aware of the hazards of carbon monoxide and cyanide, both of which can be lethal.

ATP synthase inhibitors

It is also possible to use an inhibitor of ATP synthase to stop oxidative phosphorylation directly (no ATP production) and electron transport indirectly (proton gradient not relieved so it becomes increasingly difficult to pump protons out of matrix). Oligomycin A is an inhibitor of ATP synthase.

A dangerous drug 2,4-DNP

Imagine a dam holding back water with a turbine generating electricity through which water must flow. When all water flows through the turbine, the maximum amount of electricity can be generated. If one pokes a hole in the dam, though, water will flow through the hole and less electricity will be created. The generation of electricity will thus

be uncoupled from the flow of water. If the hole is big enough, the water will all drain out through the hole and no electricity will be made.

Imagine, now, that the proton gradient is the equivalent of the water, the inner membrane is the equivalent of the dam and the ATP synthase is the turbine. When protons have an alternate route, little or no ATP will be made because protons will pass through the membrane's holes instead of spinning the turbine of ATP synthase.

It is important to recognize, though, that uncoupling by 2,4 DNP works differently from the electron transport inhibitors or the ATP synthase inhibitor. In those situations, stopping oxidative phosphorylation resulted in indirectly stopping electron transport, since the two processes were coupled and the inhibitors did not uncouple them. Similarly, stopping electron transport indirectly stopped oxidative phosphorylation for the same reason.

Such is not the case with 2,4 DNP. Stopping oxidative phosphorylation by destroying the proton gradient allows electron transport to continue unabated (it actually stimulates it), since the proton gradient cannot build no matter how much electron transport runs. Consequently, electron transport runs like crazy but oxidative phosphorylation stops. When that happens, NAD^+ and FAD levels rise, and catabolic pathways run unabated with abundant supplies of these electron acceptors. The reason such a scenario is dangerous is because the body is using all of its nutrient resources, but no ATP is being made. Lack of ATP leads to cellular (and organismal) death. In addition, the large amounts of heat generated can raise the temperature of the body to unsafe levels. This fact, and the associated increase in metabolic rate, led to DNP being used as a weight loss drug in the 1930s. Touted as an effortless way to lose weight without having to eat less or exercise more, it was hailed as a magic weight loss pill. It quickly became apparent, however, that this was very dangerous. Many people died from using this drug before laws were passed to ban the use of DNP as a weight loss aid.

Thermogenin

One of the byproducts of uncoupling electron transport is the production of heat. The faster metabolic pathways run, the more heat is generated as a byproduct. Since 2,4 DNP causes metabolism to speed up, a considerable amount of heat can be produced. Controlled uncoupling is actually used by the body in special tissues called brown fat. In this case, brown fat cells use the heat created to help thermoregulate the temperature of newborn children.

Permeabilization of the inner membrane is accomplished in brown fat by the synthesis of a protein called thermogenin (also known as uncoupling protein). Thermogenin binds to the inner membrane and allows protons to pass through it, thus bypassing the ATP synthase (like 2,4 DNP). this results in activation of catabolic pathways and the more catabolism occurs, the more heat is generated. Uncoupling by thermogenin, serves the important purpose of keeping newborn infants warm. But in adults, uncoupling merely wastes the energy that would have been harvested as ATP. In other words, it mimics starvation, even though there is plenty of food, because the energy is dissipated as heat.

Resources:

Chapter page: Mitochondria - microbiology 3d illustration Adobe stock #171126004 licensed.

Fig 14.14. https://www.labxchange.org/library/items/lb:HarvardX:1da15a56:video:1

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Fig 14:12 Adapted from "Electron transport chain", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.

15

Chapter #15

Photosynthesis and photophosphorylation

Introduction

The metabolic processes in all organisms—from bacteria to humans—require energy. To get this energy, many organisms access stored energy by eating, that is, by ingesting other organisms. But where does the stored energy in food originate? All of this energy can be traced back to photosynthesis. It is the only biological process that can capture energy that originates from sunlight and converts it into chemical compounds (carbohydrates) that every organism uses to power its metabolism. It is also a source of oxygen necessary for many living organisms. In brief, the energy of sunlight is "captured" to energize electrons, whose energy is then stored in the covalent bonds of sugar molecules. How long lasting and stable are those covalent bonds? The energy extracted today by the burning of coal and petroleum products represents sunlight energy captured and stored by photosynthesis 350 to 200 million years ago during the Carboniferous Period!

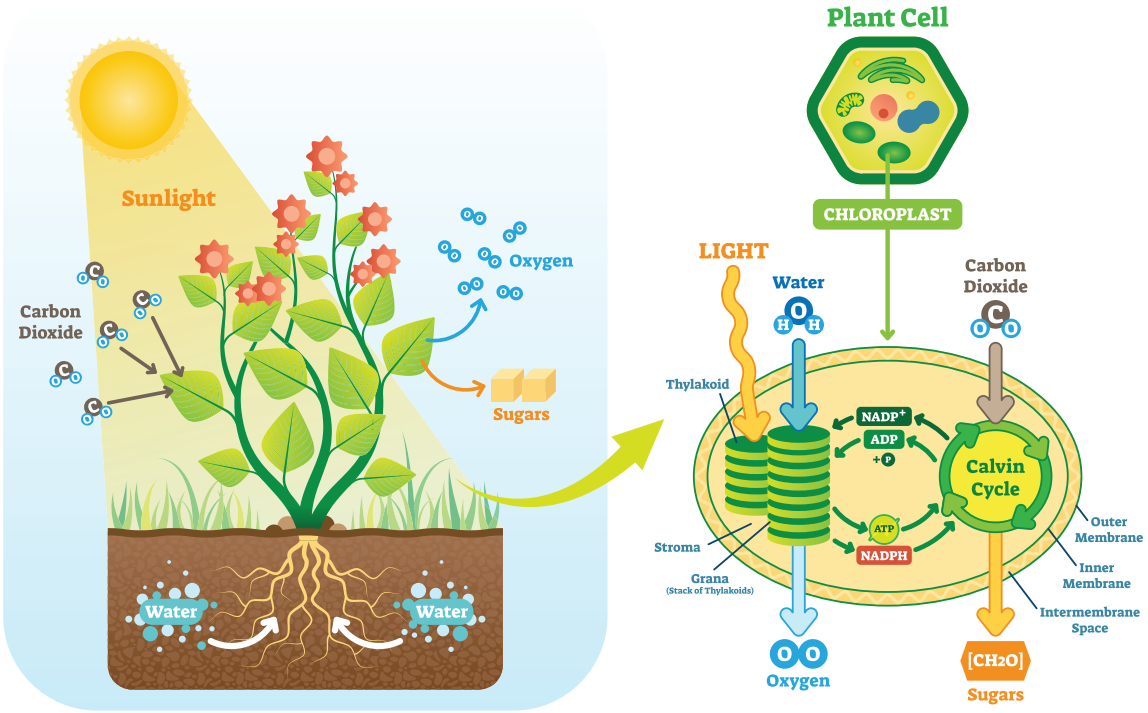

Fig 15.1 Photosynthesis takes place in two stages: light-dependent reactions and the Calvin cycle. Light-dependent reactions, which take place in the thylakoid.

Photosynthesis takes place in two sequential stages: the *light-dependent reactions* and the *light-independent reactions* (Figure 15.1). In the light-dependent reactions, energy from sunlight is absorbed by chlorophyll and that energy is converted into stored chemical energy. In the light-independent reactions, the chemical energy harvested during the light-dependent reactions drives the assembly of sugar molecules from carbon dioxide.

$$CO_2 + H_2O \rightarrow CH_2O + O_2$$

Therefore, although the light-independent reactions do not use light as a reactant, they require the products of the light-dependent reactions to function. In addition, however, several enzymes of the light-independent reactions are activated by light.

Before we move on to details of these reactions lets look at chloroplasts, orgnelles that are site of photosythesis.

Chloroplasts are found in almost all aboveground plant cells, but are primarily concentrated in leaves. The interior of a leaf, below the epidermis is made up of photosynthesis tissue called mesophyll, which can contain up to 800,000 chloroplasts per square milli-

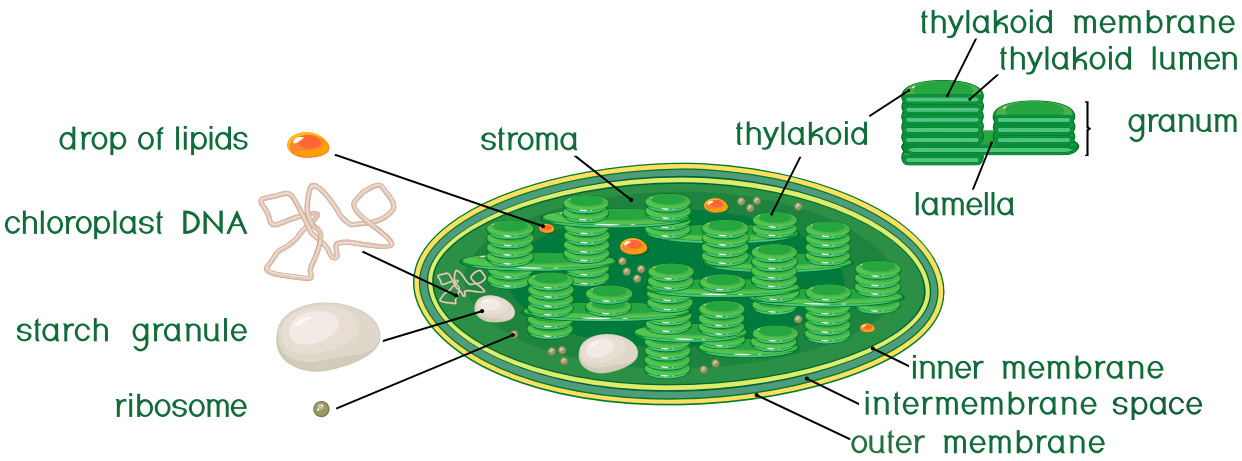

Fig 15.2 Structure of Cholorplast

meter.The chloroplast's membrane has a phospholipid inner membrane, a phospholipid outer membrane, and a region between them called the intermembrane space (Figure 15.2). Within the inner chloroplast membrane is the stroma, in which the chloroplast DNA and the enzymes of the Calvin cycle are located. Also within the stroma are stacked, flattened discs known as thylakoids which are defined by their thylakoid membranes. The space within the thylakoid membranes are termed the thylakoid spaces or thylakoid lumen. The protein complexes containing the light-absorbing pigments, known as photosystems, are located on the thylakoid membrane. Besides chlorophylls, carotenes and xanthophylls are also present, allowing for absorption of light energy over a wider range. The same pigments are used by green algae and land plants.

Brown algae and diatoms add fucoxanthin (a xanthophyll) and red algae add phycoerythrin to the mix. In plants and algae, the pigments are held in a very organized fashion complexes called antenna proteins that help funnel energy, through resonance energy transfer, to the reaction center chlorophylls. A system so organized is called a light harvesting complex. The electron transport complexes of photosynthesis are also located on the thylakoid membranes.

Light dependent reactions of photosynthesis

In chloroplasts, the light reactions of photosynthesis involving electron transfer occur in the thylakoid membranes (Figure 15.3). The chloroplasts are where the energy of light is captured, electrons are stripped from water, oxygen is liberated, electron transport occurs, NADPH is formed, and ATP is generated. The thylakoid membrane corresponds to the inner membrane of the mitochondrion for transport of electrons and proton pumping.

The thylakoid membrane does its magic using four major protein complexes. These include Photosystem II (PS II, or P680), Cytochrome b6f complex (Cyt b6f), Photosystem I (PS I, or P700), and ATP synthase. *Photophosphorylation* is process that uses these complexes to capture light energy, create a proton gradient from electron movement, capture light energy (again), and use proton gradient energy from the overall process to synthesize ATP.

Light harvesting

Harvesting the energy of light begins in PS II with the absorption of a photon of light at a reaction center. PS II performs this duty best with light at a wavelength of 680 nm and it readily loses an electron to excitation when this occurs, leaving PS II with a positive charge. This electron must be replaced. The ultimate replacement source of electrons is water, but water must lose four electrons and PS II can only accept one at a time.

Manganese centers (also known as Oxygen Evolving Complex)

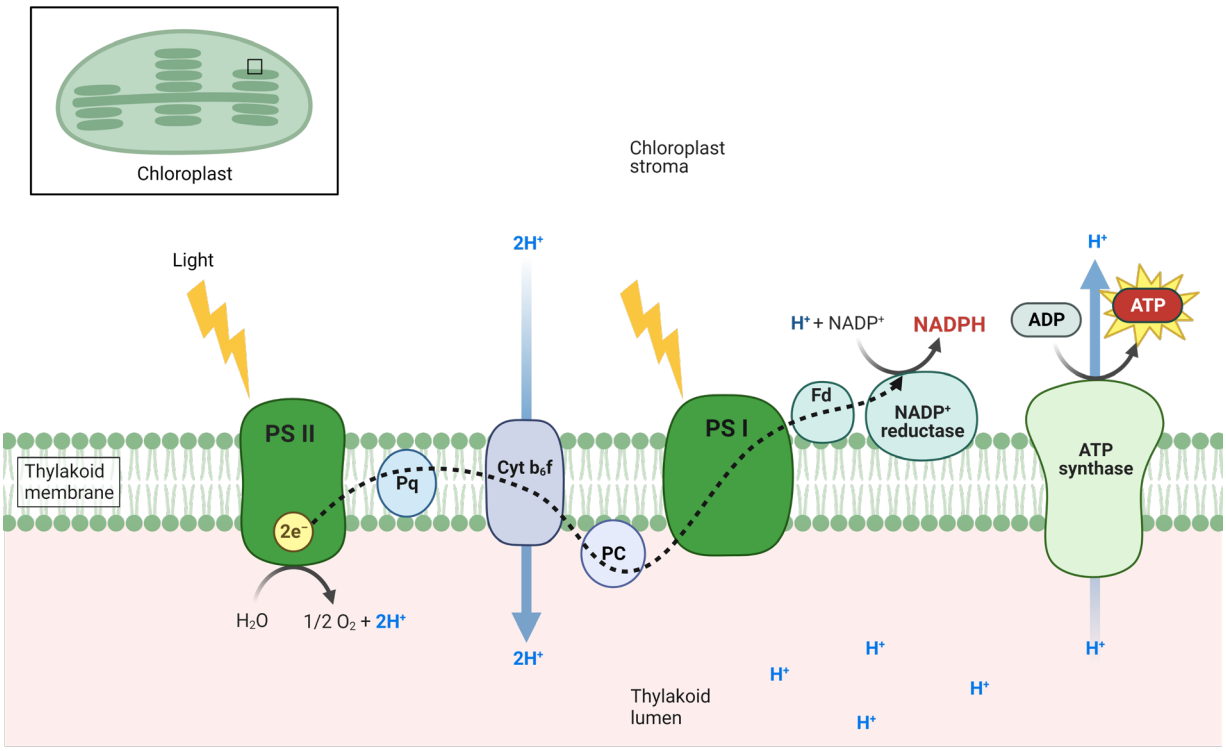

Fig 15.3 Complexes in the thylakoid membrane. Adapted from biorender by Manjeet Kumari

An in-

Oxygen Evolving Complex (OEC) contains four manganese centers that provide the immediate replacement electron that PSII requires. After four electrons have been donated by the OEC to PS II, the OEC extracts four electrons from two water molecules, liberating oxygen and dumping four protons into the thylakoid space, thus contributing to the proton gradient. The excited electron from PS II must be passed to another carrier very quickly, lest it decay back to its original state. It does this, giving its electron within picoseconds to pheophytin.

Pheophytin passes the electron on to protein-bound plastoquinones . The first is known as PQA. PQA hands the electron off to a second plastoquinone (PQB), which waits for a second electron and collects two protons to become PQH2, also known as plastoquinol (Figure 15.3). PQH2 passes these to the Cytochrome b6f complex (Cb6f) which uses passage of electrons through it to pump protons into the thylakoid space. ATP synthase makes ATP from the proton gradient created in this way. Cyt b6f drops the electron off at plastocyanin, which holds it until the next excitation process begins with absorption of another photon of light at 700 nm by PS I.

Absorption of light at PS I

With absorption of a photon of light by PS I, a process begins, that is similar to the process in PS II. PS I gains a positive charge as a result of the loss of an excited electron and pulls the electron in plastocyanin away from it. Meanwhile, the excited electron from PS I passes through an iron-sulfur protein, which gives the electron to ferredoxin (another iron sulfur protein). Ferredoxin then passes the electron off to the last protein in the system known as Ferredoxin:$NADP^+$ oxidoreductase, which gives the electron and a proton to $NADP^+$, creating NADPH.

Note that reduction of $NADP^+$ to NADPH requires two electrons and one proton, so the four electrons and two protons from oxidation of water will result in production of two molecules of NADPH. At this point, the light cycle is complete - water has been oxidized, ATP has been created, and NADPH has been made. The electrons have made their way from water to NADPH via carriers in the thylakoid membrane and their movement has released sufficient energy to make ATP. Energy for the entire process came from four photons of light.

The two photosystems performing all of this magic are protein complexes that are similar in structure and means of operation. They absorb photons with high efficiency so that whenever a pigment in the photosynthetic reaction center absorbs a photon, an electron from the pigment is excited and transferred to another molecule almost instantaneously. This reaction is called photo-induced charge separation and it is a unique means of transforming light energy into chemical forms.

Cyclic photophosphorylation

Besides the path described above for movement of electrons through PS I, plants have an alternative route that electrons can take. Instead of electrons going through ferredoxin to form NADPH, they instead take a backwards path through the the proton-pumping b6f complex. This system, called cyclic photophosphorylation (Figure 15.4) which generates more ATP and no NADPH, is similar to a system found in green sulfur bacteria. The ability of plants to switch between non-cyclic and cyclic photosystems allows them to make the proper ratio of ATP and NADPH they need for assimilation of carbon in the dark phase

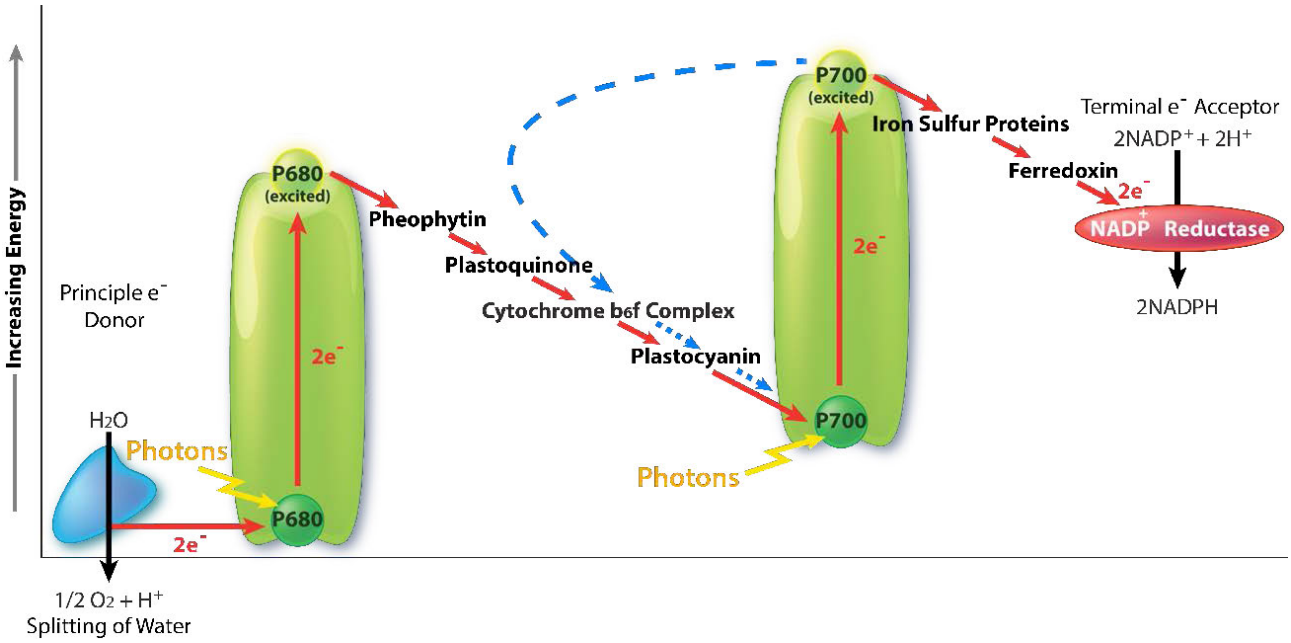

Fig 15.4 Movement of electrons through photosystems. Cyclic photophosphorylation shown by blue dashed line. Image by Aleia Kim and Pehr Jacobson

of photosynthesis. This ratio turns out to be 3 ATPs to 2 NADPHs.

Photosynthetic energy

The output of the photophosphorylation part of photosynthesis (O_2, NADPH, and ATP), of course, is not the end of the process of photosynthesis. For the growing plant, the NADPH and ATP are used to capture carbon dioxide from the atmosphere and convert it (ultimately) into glucose and other important carbon compounds. This, as noted previously, occurs in the Calvin Cycle in what is called the dark phase of the process. The oxygen liberated in the process is a necessary for respiration of all aerobic life forms on Earth. In-

Have you wondered how these complexes will look in the membrane?

You can search up proteins given in this book in the protein data bank and visualize their 3D interactive structure. Have fun!

Here is the link for PS II supercomplex from pea plant.

https://www.rcsb.org/structure/5xnl

deed, it is believed that essentially all of the oxygen in the atmosphere today is the result the splitting of water in photosynthesis over the many eons that the process has existed.

The Calvin Cycle

After the energy from the sun is converted into chemical energy and temporarily stored in ATP and NADPH molecules, the cell has the fuel needed to build carbohydrate molecules for long-term energy storage. The products of the light-dependent reactions, ATP and NADPH, have lifespans in the range of millionths of seconds, whereas the products of the light-independent reactions (carbohydrates and other forms of reduced carbon) can survive almost indefinitely as discussed earlier. The carbohydrate molecules made will have a backbone of carbon atoms. But where does the carbon come from? It comes from carbon dioxide—the gas that is a waste product of respiration in microbes, fungi, plants, and animals.

Reactions of the Calvin cycle take place in regions of the chloroplast known as the stroma, the fluid areas outside of the thylakoid membranes. The cycle can be broken into three stages:

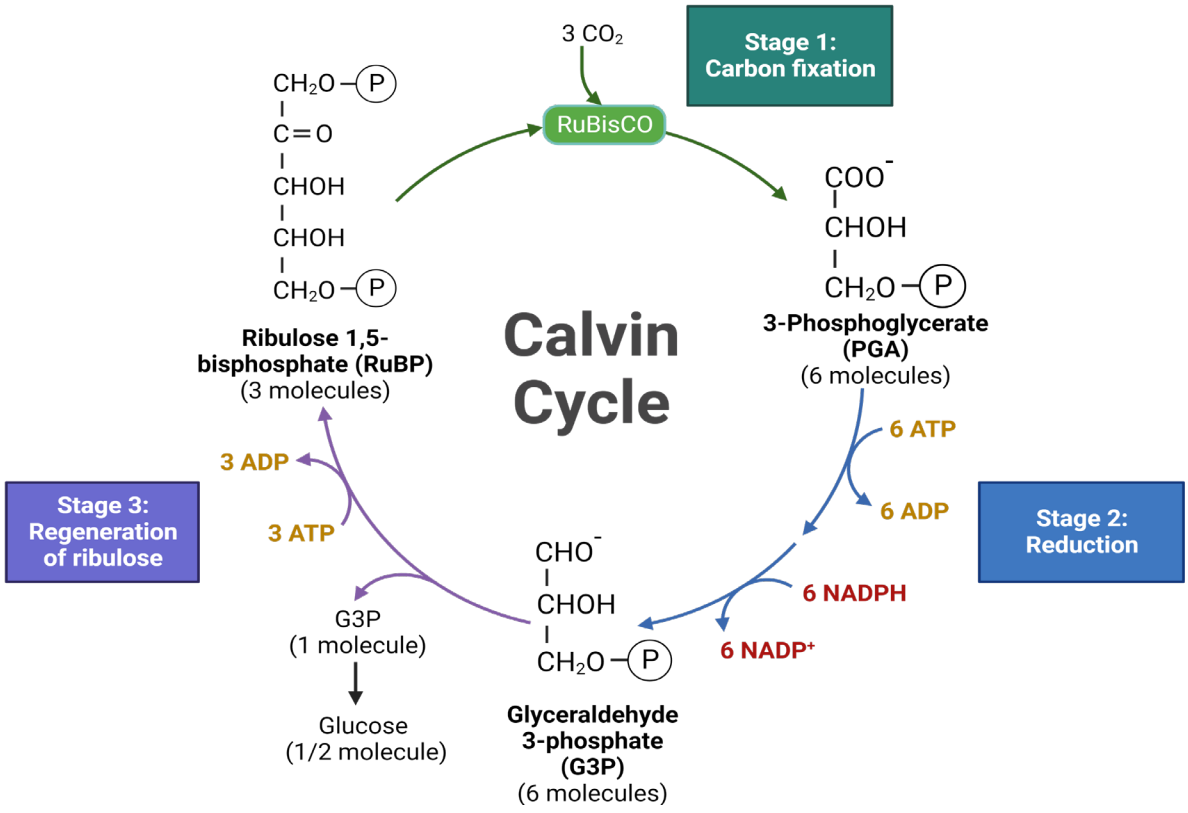

Fig 15.5 Calvin cycle. Adapted from Bioender by Manjeet Kumari.

1) CO_2 fixation
2) Reduction reactions
3) Regeneration of the starting material, ribulose 1,5 bisphosphate (Ru1,5BP).

Though reduction of carbon dioxide to glucose ultimately requires electrons from twelve molecules of NADPH (and 18 ATPs), it is confusing because one reduction occurs 12 times (1,3 BPG to GLYAL-3P) to input the overall reduction necessary to make one glucose (Figure 15.5). Another reason students find the pathway confusing is because the carbon dioxide molecules are absorbed one at a time into six different molecules of Ru1,5BP. At no point are the six carbons ever together in the same molecule to make a single glucose.

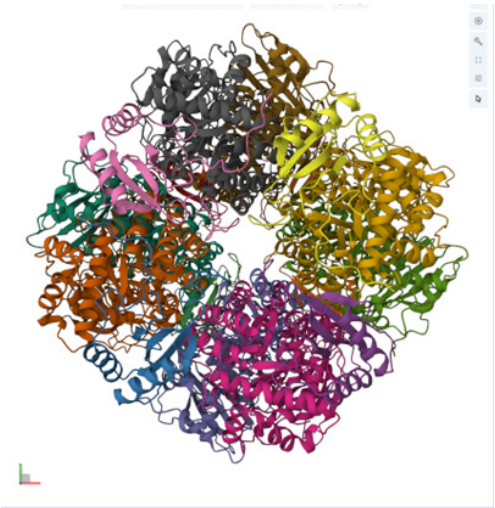

Fig 15.6 Rubisco. Click on the image. Interactive mode available when PDF viewed in interactive mode. https://www.rcsb.org/3d-sequence/1RCX?assemblyId=1

Instead, six molecules of Ru1,5BP (5Cx6=30 carbons) gain six more carbons via carbon dioxide and then split into 12 molecules of 3- phosphoglycerate (3Cx12=36 carbons). The gain of six carbons allows two, three carbon (3C) molecules to be produced in excess for each turn of the cycle. These two molecules molecules are then converted into glucose using the enzymes of gluconeogenesis. The other ten molecules of 3-PG are used to regenerate the six molecules of Ru1,5BP

Like the citric acid cycle, the Calvin cycle doesn't really have a starting or ending point, but we can think of the first reaction as the fixation of carbon dioxide to Ru1,5BP. This reaction is catalyzed by the enzyme known as ribulose-1,5 bisphosphate carboxylase (RUBISCO - Figure 15.6). The resulting six carbon intermediate is unstable and is rapidly converted to two molecules of 3- phosphoglycerate.

As noted, if one starts with 6 molecules of Ru1,5BP and makes 12 molecules of 3-PG, the extra 6 carbons that are a part of the cycle can be shunted off as two three-carbon molecules of glyceraldehyde-3-phosphate (GLYAL3P) to gluconeogenesis, leaving behind 10 molecules to be reconverted into 6 molecules of Ru1,5BP. This occurs in what is called the resynthesis phase. The resynthesis phase requires multiple steps, but only utilizes two enzymes unique to plants - sedoheptulose-1,7 bisphosphatase and phosphoribulokinase. RUBISCO is the third (and only other) enzyme of the pathway that is unique to plants. All of the other enzymes of the pathway are common to plants and animals and include some found in the pentose phosphate pathway and gluconeogenesis.

Photorespiration

In the Calvin cycle of photosynthesis, the enzyme ribulose-1,5-bisphosphate carboxylase (RUBISCO) catalyzes the addition of carbon dioxide to ribulose-1,5- bisphosphate (Ru1,5BP) to create two molecules of 3-phosphoglycerate. Molecular oxygen (O_2), however, competes with CO_2 for this enzyme, so about 25% of the time, the molecule that gets added is not CO_2, but rather O_2. When this happens, the following reaction occurs

$$Ru1,5BP + O_2 \rightleftarrows Phosphoglycolate + 3\text{-}phosphoglycerate + 2H^+$$

This is the first step in the process known as photorespiration. The process of photorespiration is inefficient relative to the carboxylation of Ru1,5BP. Phosphoglycolate is converted to glyoxylate in the glyoxysome and then transamination of that yields glycine. Two glycines can combine in a complicated coupled set of reactions in the mitochondrion shown next.

$$2\ Glycine + NAD^+ + H_2O \rightleftarrows Serine + CO_2 + NH_3 + NADH + H^+$$

Deamination and reduction of serine yields pyruvate, which can be then be converted back to 3-phosphoglycerate. The end point of oxygenation of Ru1,5BP is the same as the carboxylation of Ru1,5BP reactions, but there are significant energy costs associated with it, making the process less efficient.

C_4 plants

The Calvin cycle is the means by which plants assimilate carbon dioxide from the atmosphere, ultimately into glucose. Plants use two general strategies for doing so. The first is employed by plants called C_3 plants (most plants) and it simply involves the pathway described above. They are called C_3 plants because the first stable intermediate after absorbing carbon dioxide contains three carbons - 3-phosphoglycerate. Another class of plants, called C_4 plants employ a novel strategy for concentrating the CO2 prior to assimilation. C_4 plants (e.g. maize) are generally found in hot, dry environments where conditions would otherwise

favor the wasteful photorespiration reactions of RUBISCO and loss of water.

In C_4 plants, carbon dioxide is captured in special mesophyll cells first by phosphoenolpyruvate (PEP) to make oxaloacetate (contains four carbons and gives the C_4 plants their name). The oxaloacetate is converted to malate and transported into bundle sheath cells where the carbon dioxide is released and captured by Ru1,5BP, as in C_3 plants. The Calvin cycle proceeds from there. The advantage of the C_4 plant scheme is that it allows concentration of carbon dioxide while minimizing loss of water and photorespiration.

CAM plants

Crassulacean acid pathway (CAM) is present in plants that must conserve every drop of water by keeping stomata closed during the hot and dry day. Cacti, for instance, keep their stomata closed during the day but open them in the night. These plants store CO_2 in the form of malate which is sequesterd in the vacoule during the night. When sun rises, malate is tranported out of vacoule, carbon dioxide is released and enters Calvin cycle. CAM plants show temporal separation, whereas C_4 plants allow spatial separation to fix CO_2.

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https://openstax.org/books/biology-2e/pages/1-introduction

Fig 15.1 Photosynthesis biological vector illustration diagram with plan cell scheme. Adobe stock 199928897 Licensed by Manjeet Kumari.

Fig 15.2 Chloroplast structure with titles. Adobe stock #185026158. Licensed by Manjeet Kumari.

Fig 15:3 Adapted from "Light Dependent Reactions of Photosynthesis", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.

Fig 15:5 Adapted from "Calvin Cycle", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.

Fig 15:6 Image from the RCSB PDB (RCSB.org) of PDB ID 1RCX (Taylor, T.C., Andersson, I. (1997) J Mol Biol 265: 432-444. https://doi.org/10.1006/jmbi.1996.0738

16

Chapter #16

Fatty acid metabolism

Introduction

With more than 13% of the world population currently living with obesity, there is a tremendous amount of interest in the metabolism of fat and fatty acids. Fat is the most important energy storage form of animals, storing considerably more energy per carbon than carbohydrates, but its insolubility in water requires the body to package it specially for transport. Surprisingly, fat/fatty acid metabolism is not nearly as tightly regulated as that of carbohydrates. Neither are the metabolic pathways of breakdown and synthesis particularly complicated, either.

Lipid movement in body

Before we discuss the breakdown and synthesis of fat, let us first discuss movement of lipid in the body. *Lipoprotein complexes* are combinations of apolipoproteins and lipids bound to them that solubilize fats and other non-polar molecules, such as cholesterol, so they can travel in the bloodstream between various tissues of the body. The apolipoproteins provide the emulsification necessary for this. Lipoprotein complexes are formed in tiny "balls" with the water soluble apolipoproteins on the outside and non-polar lipids, such as fats, cholesteryl esters, and fat soluble vitamins on the inside.

They are categorized by their densities. These include (from highest density to the lowest) high density lipoproteins (HDLs), Low Density Lipoproteins (LDLs), Intermediate Density Lipoproteins (IDLs), Very Low Density Lipoproteins (VLDLs) and the chylomicrons. These particles are synthesized in the liver and small intestines.

Each lipoprotein complex contain a characteristic set of apolipoproteins, as shown in Figure16.1. ApoC-II and ApoC-III are notable for their presence in all the lipoprotein complexes and the roles they play in activating (ApoC-II) or inactivating (ApoC-III) lipoprotein lipase. Lipoprotein lipase is a cellular enzyme that catalyzes the breakdown of fat from the complexes. ApoE is useful for helping the predict the likelihood of the occurrence of Alzheimer's disease in a patient.

The movement of fats in the body is important because they are not stored in all cells. Only specialized cells called adipocytes store fat.There are three pathways:

1) Exogenous pathway
2) Endogenous pathway
3) Reverse transport pathway

Name	Lipoprotein Complex(es)	Function
ApoA-I	HDL	Promotes Fat Movement to Liver
ApoA-II	HDL	Inhibit LCAT
ApoA-IV	Chylomicrons / HDL	Activate LCAT
AboB-48	Chylomicrons	Cholesterol Transport
AboB-100	VLDL / LDL	Bind LDL Receptor
ApoC-I	VLDL / LDL	Unknown
ApoC-II	All	Activate Lipoprotein Lipase
ApoC-III	All	Inhibit Lipoprotein Lipase
ApoD	HDL	Unknown
ApoE	VLDL / Chylomicrons / HDL	Clearance of Chylomicrons Remnants and VLDLs

Fig 16.1 Apolipoproteins

Exogenous pathway

Dietary fat entering the body from the intestinal system must be transported, as appropriate, to places needing it or storing it. This is the function of the exogenous pathway of lipid movement in the body. All dietary lipids (fats, cholesterol, fat soluble vitamins, and

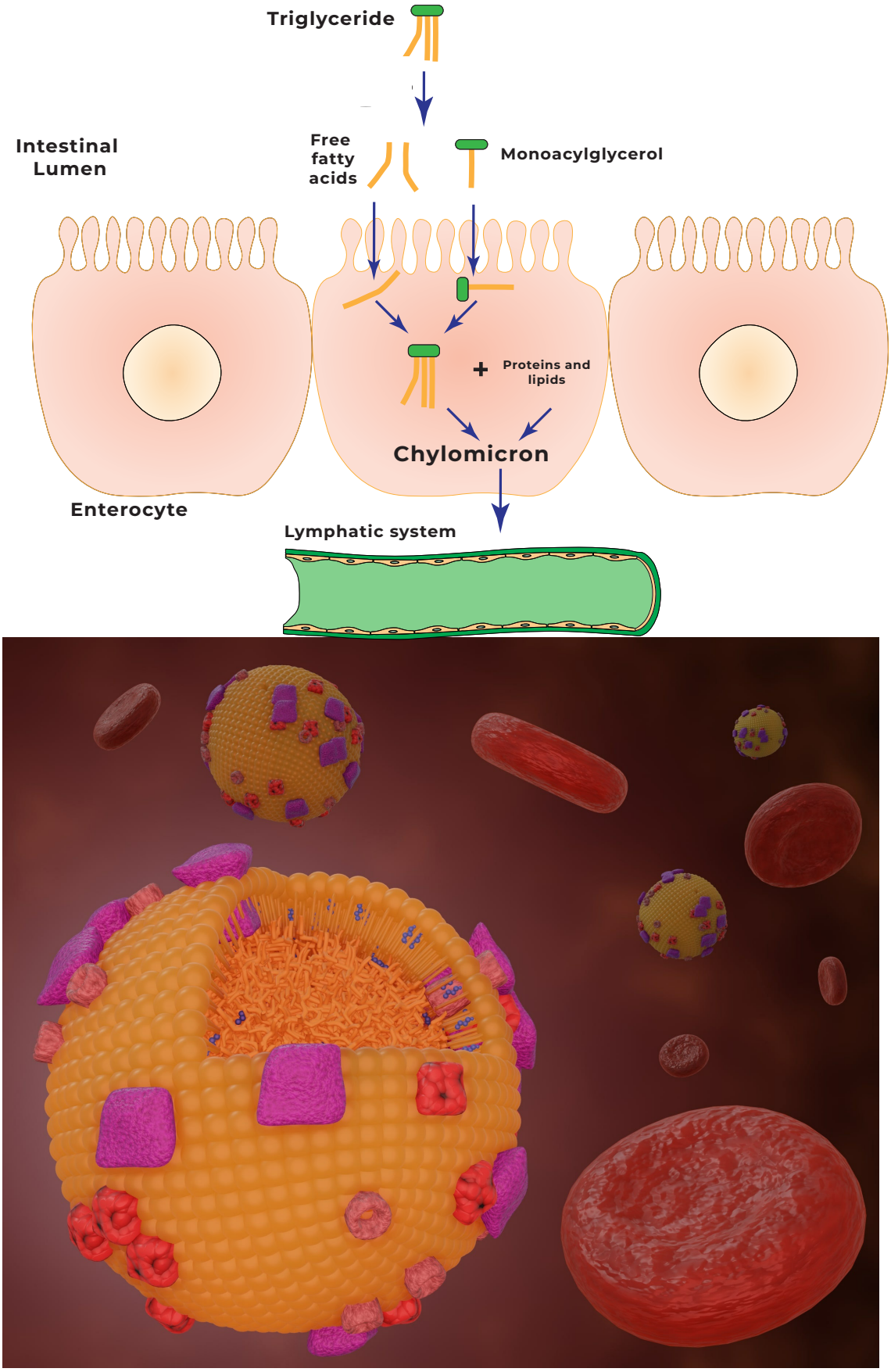


Fig 16.2
Top: Chylomicron formation, Bottom: Chylomicron schematic rendering. Colored structures on surface represent apoproteins on phopholipid layer. Blue dots represent cholestrol. Core represent triglycerides.

other lipids) are moved by it. In the case of dietary fat, it begins its journey after ingestion first by being solubilized by bile acids in the intestinal tract. After passing through the stomach, pancreatic lipases clip two fatty acids from the fat, leaving a monoacyl glycerol. The fatty acids and monoacyl glycerol are absorbed by intestinal cells (enterocytes) and reassembled back into a fat, and then this is mixed with phospholipids, cholesterol esters, and apolipoprotein B-48 and processed to form chylomicrons (Figures 16.2) in the Golgi apparatus and smooth endoplasmic reticulum.

These are exocytosed from the cell into lymph capillaries called lacteals. The chylomicrons pass through the lacteals and enter the bloodstream via subclavian vein. Within the bloodstream, lipoprotein lipase breaks down the fats causing the chylomicron to shrink and become what is known as a chylomicron remnant. It retains its cholesterol and other lipid molecules.

The chylomicron remnants travel to the liver where they are absorbed. This is accomplished by receptors in the liver that recognize and bind to the ApoE of the chylomicrons. The bound complexes are then internalized by endocytosis, degraded in the lysosomes, and the cholesterol is disbursed in liver cells.

Endogenous pathway

The liver plays a central role in managing the body's needs for lipids. When lipids are needed by the body or when the capacity of the liver to contain more lipids than is supplied by the diet, the liver packages up fats and cholesterol esters into Very Low Density Lipoprotein (VLDL) complexes and exports them via the endogenous pathway. VLDL complexes contain ApoB-100, ApoC-I, ApoC-II, ApoC-III, and ApoE apolipoproteins. VLDLs enter the blood and travel to muscles and adipose tissue where lipoprotein lipase is activated by ApoC-II.

In the muscle cells, the released fatty acids are taken up and oxidized. By contrast, in the adipoctyes, the fatty acids are taken up and reassembled back into triacylglycerides (fats) and stored in fat droplets. Removal of fat from the VLDLs causes them to shrink, first to Intermediate Density Lipoprotein (IDL) complexes (also called VLDL remnants) and then to Low Density Lipoprotein (LDL) complexes.

Reverse transport pathway

Another important consideration of the movement of lipids in the body is the reverse transport pathway. It is also called the reverse cholesterol transport pathway, since cholesterol is the primary molecule involved. This pathway involves the last class of lipoprotein complexes known as the High Density Lipoproteins (HDLs). In contrast to the LDLs, which are commonly referred to as "bad cholesterol", the HDLs are known as "good cholesterol." HDLs are synthesized in the liver and small intestine. They contain little or no lipid when made (called depleted HDLs), but serve the role of "scavenger" for cholesterol in the blood and from remnants of other (damaged) lipoprotein complexes in the blood. It returns its load of cholesterol back to the liver or, alternatively, to LDL molecules for endocytosis. HDLs have the effect of lowering levels of cholesterol and it is for that reason they are described as "good cholesterol."

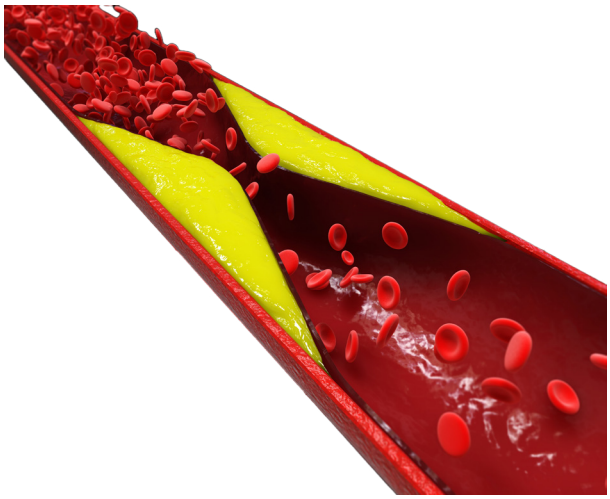


Fig 16.3 Atherosclerosis with plaque in blood vessel

Good cholesterol / bad cholesterol

It is commonly accepted that "high cholesterol" levels are not healthy. This is due, at

least indirectly, to the primary carriers of cholesterol, the LDLs. A primary function of the LDLs is to deliver cholesterol and other lipids directly into cells by receptor mediated endocytosis. High levels of LDLs, though, are correlated with formation of atherosclerotic plaques (Figure 16.3) and incidence of atherosclerosis, leading to the description of them as "bad cholesterol." This is because when LDL levels are very high, plaque formation begins. It is thought that reactive oxygen species (higher in the blood of smokers) causes partial oxidation of fatty acid groups in the LDLs. When levels are high, they tend to accumulate in the extracellular matrix of the epithelial cells on the inside of the arteries. Macrophages of the immune system take up the damaged LDLs (including the cholesterol).

Since macrophages can't control the amount of cholesterol they take up, cholesterol begins to accumulate in them and they take on appearance that leads to their being described as "foam cells." With too much cholesterol, the foam cells, however, are doomed to die by the process of programmed cell death (apoptosis). Accumulation of these, along with scar tissue from inflammation result in formation of a plaque. Plaques can grow and block the flow of blood or pieces of them can break loose and plug smaller openings in the blood supply, ultimately leading to heart attack or stroke.

On the other hand, high levels of HDL are inversely correlated with atherosclerosis and arterial disease. Depleted HDLs are able to remove cholesterol from foam cells. This occurs as a result of contact between the ApoA-I protein of the HDL and a transport protein on the foam cell (ABC-G1). Another transport protein in the foam cell, ABCA-1 transports extra cholesterol from inside the cell to the plasma membrane where it is taken up into the HDL and returned to the liver or to LDLs by the reverse transport cholesterol pathway.

Breakdown of fat

Breakdown of fat in adipocytes requires catalytic action of three enzymes. The first of these is controlled by binding of hormones to the cell membrane (Figure 16.4). It is the only regulated enzyme of fat breakdown and is known as hormone sensitive triacylglycerol lipase (HSTL). It removes the first fatty acid from the fat. Diacylglyceride lipase removes the second one and monoacylglyceride lipase removes the third. As noted, only the first one is regulated and it appears to be the rate limiting reaction when active.

Epinephrine activation

As shown in Figure 16.4, activation of HSTL is accomplished by epinephrine stimulation process and that it overlaps with the same activation that stimulates glycogen breakdown and gluconeogenesis.

This coordination is very important. Each of the pathways stimulated by the epinephrine signaling system aims to provide the body with more materials to catabolize for energy - sugars and fatty acids. The HSTL is inhibited by dephospohrylation and this is stimulated by binding of insulin to its cell membrane receptor.

Perilipin

A protein playing an important roles in regulation of fat breakdown is perilipin. Perilipin associates with fat droplets and helps regulate action of HSTL, the enzyme catalyzing the first reaction in fat catabolism. When perilipin is not phosphorylated, it coats the fat droplet and prevents HSTL from getting access to it. Activation of protein kinase A in the epinephrine cascade, however, results in phosphorylation of both perilipin and HSTL. When this occurs, perilipin loosens its tight binding to the fat droplet, allowing digestion of the fat to begin by HSTL.

Perilipin expression is high in obese organisms and some mutational variants have been associated with obesity in women. Another mutation reduces perilipin expression and is associated with greater lipolysis (fat breakdown) in women. Mice lacking perilipin eat more

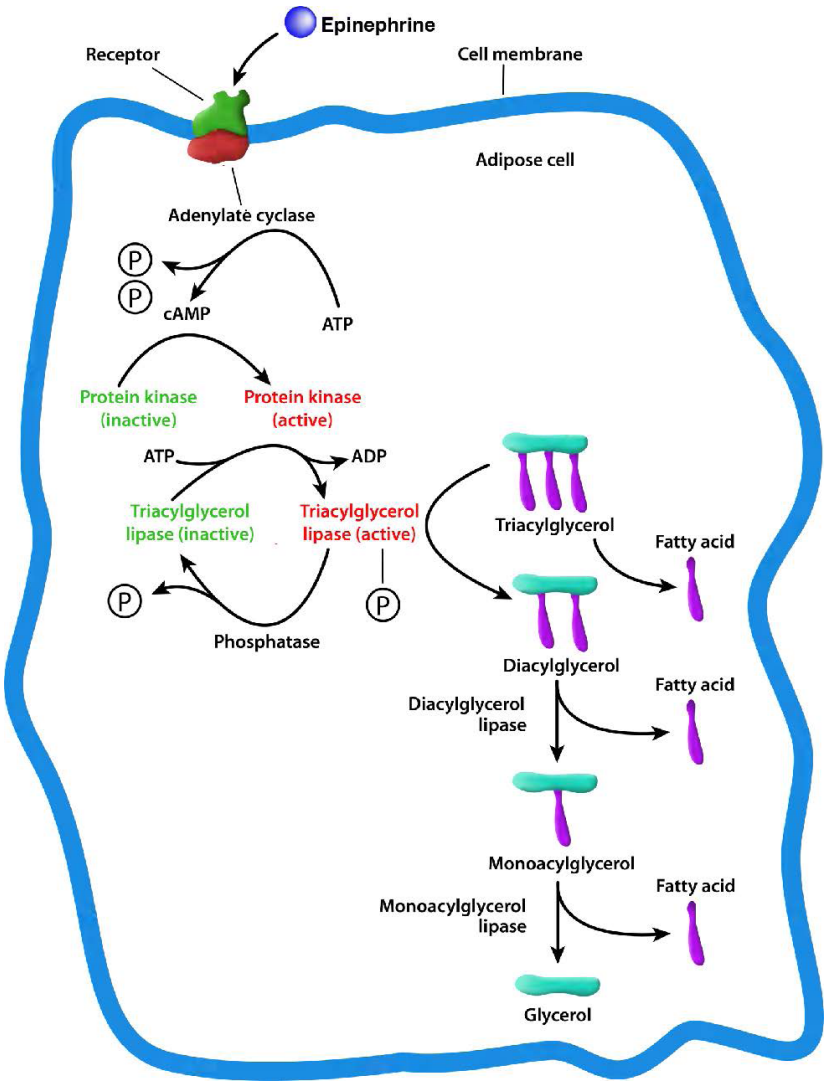


Fig 16.4 Breakdown of fat in adipocytes. Image by Pehr Jacobson

food than wild-type mice, but gain 1/3 less weight when on the same diet.

Fatty acids released from adipocytes travel in the bloodstream bound to serum albumin. Arriving at target cells, fatty acids are taken up by membrane-associated fatty acid binding proteins, which help control cellular fatty acid uptake by transport proteins

Upon arrival inside of target cells, fatty acids are oxidized in a process that chops off two carbons at a time to make acetyl-CoA, which is subsequently oxidized in the citric acid cycle. Depending on the size of the fatty acid, this process (called β-oxidation) will begin in either the mitochondrion or the peroxisomes.

Oxidation in mitochondria

To be oxidized in the mitochondrion, fatty acids must first be attached to coenzyme A (CoA-SH or CoA) and transported through the cytoplasm and the outer mitochondrial membrane. In the mitochondrion's intermembrane space, the CoA on the fatty acid is replaced by a carnitine (Figure 16.5) in order to be moved into the matrix. After this is done, the fatty acid linked to carnitine is transported into the mitochondrial matrix and in the matrix the carnitine is replaced again by coenzyme A. It is in the mitochondrial matrix where the oxidation occurs. The fatty acid linked to CoA (called an acyl-CoA) is the substrate for fatty acid oxidation. Steps

The process of fatty acid oxidation (Fig 16.6) is fairly simple. The reactions all occur

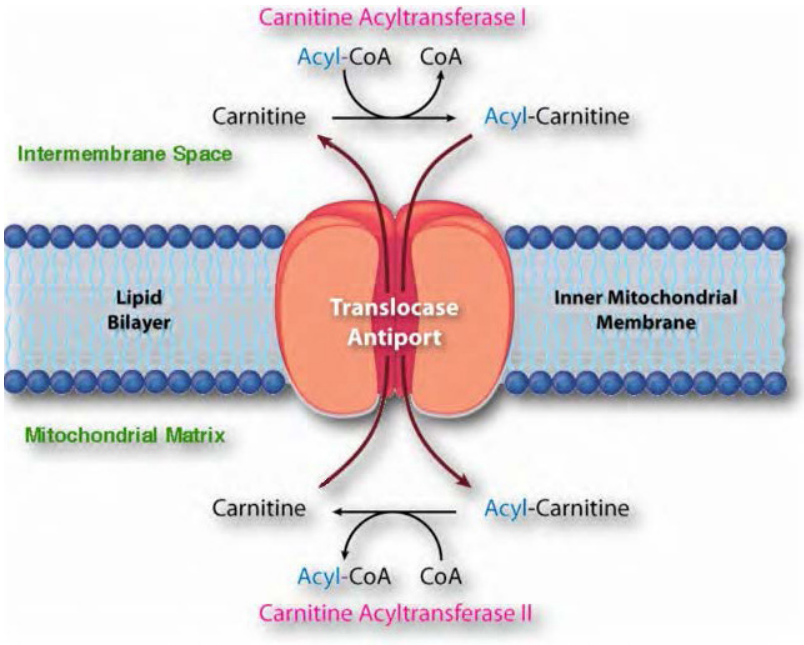


Fig 16.5 Transport of fatty acid (acyl group) across mitochondrial inner membrane

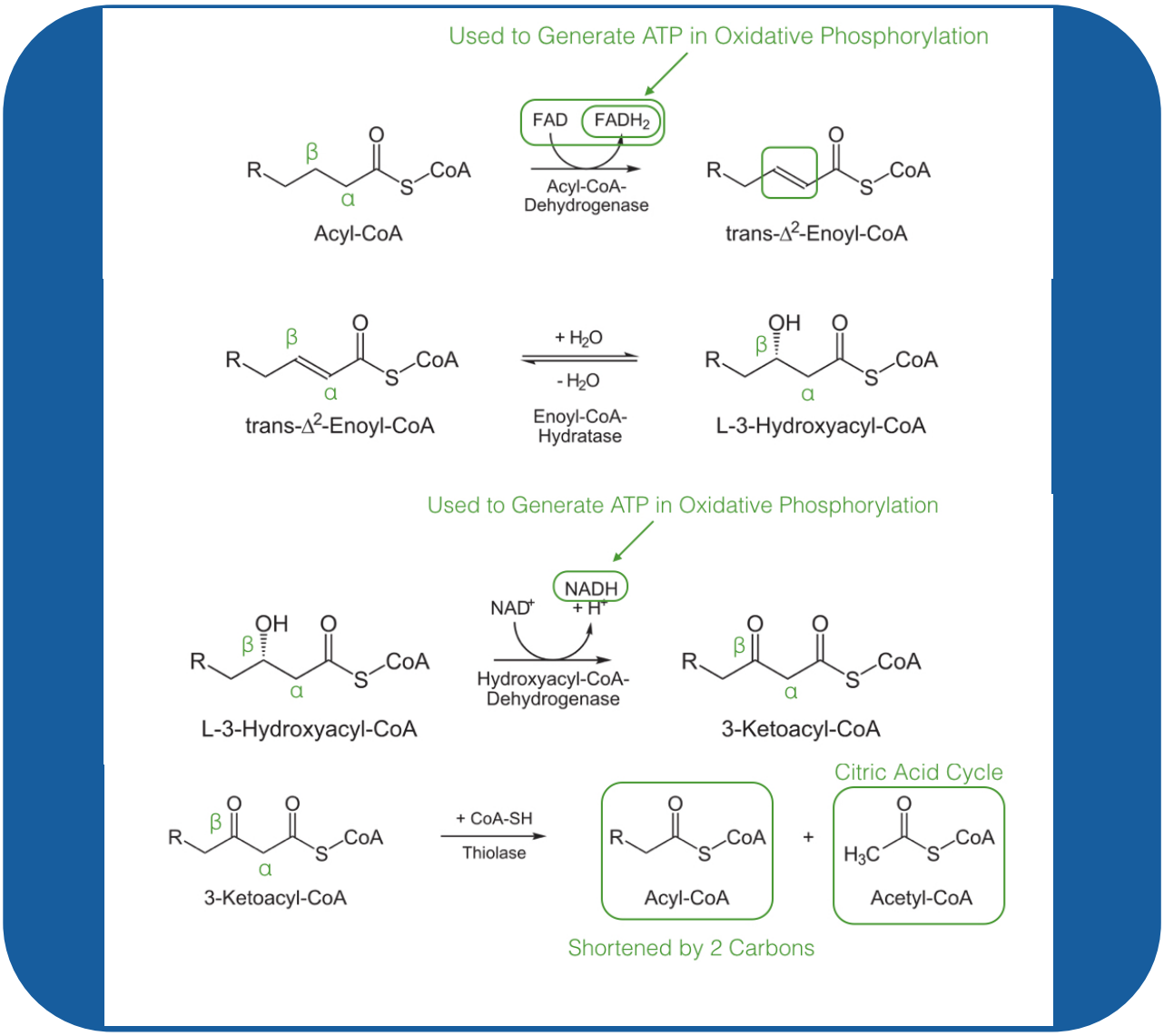
between carbons 2 and 3 (with #1 being the one linked to the CoA) and sequentially include the following steps:

1) Dehydrogenation to create FADH2 and a fatty acyl group with a double bond between carbons 2 and 3 in the trans configuration;

2) Hydration across the double bond to put a hydroxyl group on carbon 3 in the L configuration;

3) Oxidation of the hydroxyl group to make a ketone; and

4) Thiolytic cleavage to release acetyl-CoA and a fatty acid two carbons shorter than the starting one.



Enzymes of β-oxidation

Two of the enzymes of β-oxidation are notable. The first is acyl-CoA dehydrogenase and second is thiolase. Acyl-CoA dehydrogenase catalyzes the dehydrogenation in the first reaction and yields $FADH_2$. The enzyme comes in three different forms – ones specific for long, medium, or short chain length fatty acids. The first of these is sequestered in the peroxisomes of animals whereas the ones that work on medium and shorter chain fatty acids are found in the mitochondria. Action of all three enzymes is typically needed to oxidize a fatty acid. Plants and yeast perform β-oxidation exclusively in peroxisomes.

The most interesting of the acyl-CoA dehydrogenases is the one that works on medium length fatty acids. This one, which is the one most commonly deficient in animals, has been associated with sudden infant death syndrome. Reactions two and three in β-oxidation are catalyzed by enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase, respectively. The latter reaction yields an NADH.

Thiolase

The second notable enzyme of β-oxidation is thiolase because this enzyme not only catalyzes the formation of acetyl-CoAs in β-oxidation, but also the joining of two acetyl-CoAs (essentially the reversal of the last step of β-oxidation) to form acetoacetyl-CoA – essential for the pathways of ketone body synthesis and cholesterol biosynthesis.

Regulation of fatty acid oxidation

Breakdown of fatty acids is controlled at different levels. The first is by control of the availability of fatty acids from the breakdown of fat. As noted above, this process is by regulating the activity of hormone-sensitive triacylglycerol lipase (HSTL) activity by epinephrine (stimulates) and insulin (inhibits).

A second level of control of fatty acid availability is by regulation of carnitine acyl transferase. This enzyme controls the swapping of CoA on an acyl-CoA molecule for carnitine, a necessary step for the fatty acid to be imported into the mitochondrion for oxidation.

The enzyme is inhibited by malonyl-CoA, an intermediate in fatty acid synthesis. Thus, when fatty acids are being synthesized, import of them into the mitochondrion for oxidation is inhibited. Lastly, the last enzyme in the β-oxidation cycle, thiolase, is inhibited by acetyl-CoA.

Fatty acid synthesis

Synthesis of fatty acids occurs in the cytoplasm and endoplasmic reticulum of the cell and is chemically similar to the reverse of the β-oxidation process.. The first of these occur in preparing substrates for the reactions that grow the fatty acid. Fatty acid synthesis occurs in the cytoplasm of eukaryotic cells. Transport of acetyl-CoA from the mitochondrial matrix occurs when it begins to build up. This happens when the citric acid cycle slows or stops from lack of exercise.

Two molecules can play roles in moving acetyl-CoA to the cytoplasm – citrate and acetylcarnitine. Joining of oxaloacetate with acetyl-CoA in the mitochondrion creates citrate which gets transported across the membrane, followed by action of citrate lyase in the cytoplasm of the cell to release acetyl-CoA and oxaloacetate. Additionally, when free acetyl-CoA accumulates in the mitochondrion, it may combine with carnitine and be transported out to the cytoplasm.

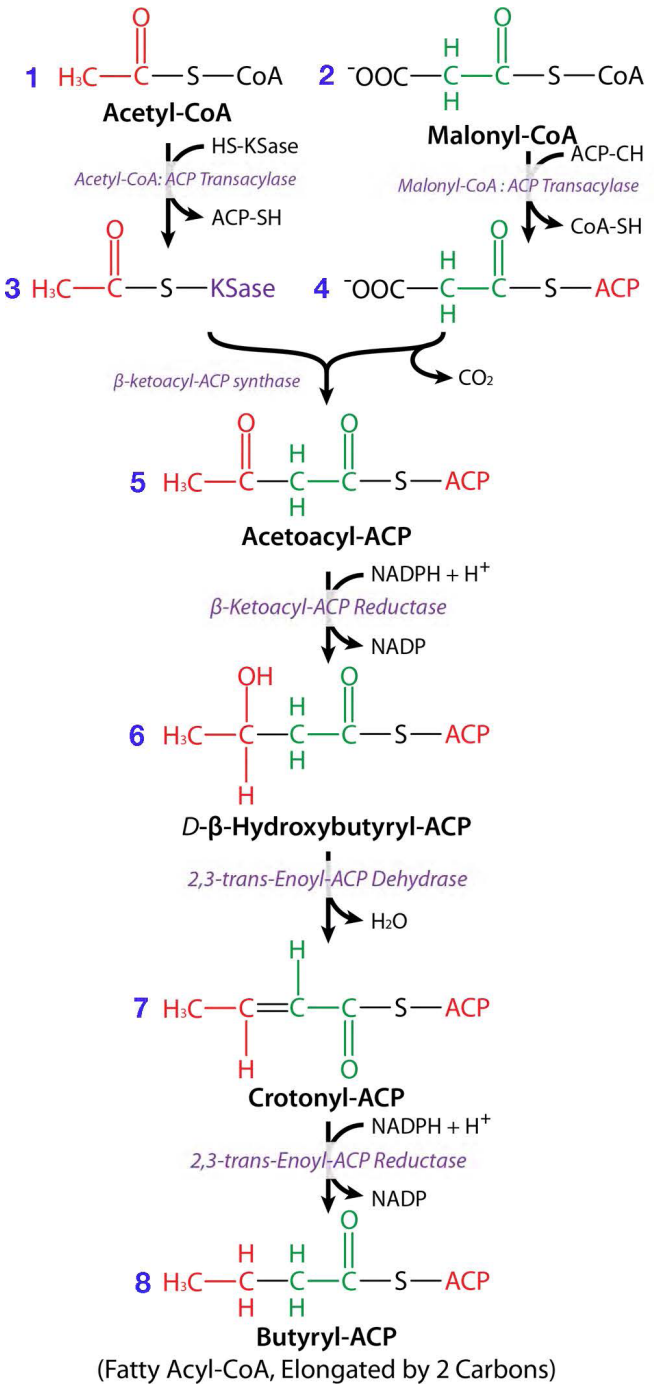


Fig 16.7 One round of fatty acid synthesis. Image by Aleia Kim

Fatty acid synthase

In animals, six different catalytic activities necessary to fully make palmitoyl-CoA are contained in a single complex called Fatty Acid Synthase.

These include:

1)Transacylases (MAT) for swapping CoA-SH with ACP-SH on acetyl-CoA and malonyl-CoA;

2) A synthase (KS) to catalyze addition of the two carbon unit from the three carbon malonyl-ACP in the first step of the elongation process;

3) A reductase (KR) to reduce the ketone;

4) A dehydrase (DH) to catalyze removal of water;

5) A reductase (ER) to reduce the trans double bond and

6) A thioesterase (TE) to cleave the finished palmitoyl -CoA into palmitic acid and CoA-SH.

In the middle of the complex is a site for binding the ACP portion of the growing fatty acid chain to hold it as the other part of the fatty acid is rotated into positions around the enzyme complex for each catalysis. In bacteria, these six activities are found on separate enzymes and are not part of a complex.

Cytoplasmic reactions

The process of making a fatty acid in the cytoplasm starts with two acetyl-CoA molecules. One is converted to malonyl-CoA by adding a carboxyl group. This reaction is catalyzed by the enzyme acetyl-CoA carboxylase (ACC), the only regulated enzyme of fatty acid synthesis and the only one separate from the fatty acid synthase. Next, both acetyl-CoA and malonyl-CoA have their CoA portions replaced by a carrier protein known as ACP (acyl-carrier protein) to form acetyl-ACP (catalyzed by acetyl-CoA : ACP transacylase - MAT in Figure 16.8) and malonyl-ACP (catalyzed by malonyl-CoA : ACP transacylase - MAT in Figure 16.8). Joining of a fatty acyl-ACP (in this case, acetyl-ACP) with malonyl-ACP splits out the carboxyl group from malonyl-ACP that was added to it and creates the acetoacyl-ACP intermediate (catalyzed by β-ketoacyl-ACP synthase - KS on Figure 16.8) .

From this point forward, the chemical reactions resemble those of β-oxidation reversed. First, the ketone is reduced to a hydroxyl using NADPH (catalyzed by β-ketoacyl-ACP reductase - KR). In contrast to the hydroxylated intermediate of β-oxidation, the intermediate here (D-β- hydroxybutyryl-ACP) is in the D-configuration.

Dehydration

Next, water is removed from carbons 2 and 3 of the hydroxyl intermediate in a reaction

catalyzed by 2,3-trans-enoyl-ACP dehydrase - DH on Figure 16.8 This yields a trans doubled-bonded molecule. Last, the double bond is hydrogenated to yield a saturated intermediate by 2,3-trans-enoyl-ACP reductase - ER on Figure 16.8. This completes the first cycle of synthesis.

Additional cycles involve addition of more two-carbon units from malonyl-ACP to the growing chain until ultimately an intermediate with 16 carbons is produced (palmitoyl-ACP). At this point, a thioesterase cleaves the ACP from the palmitoyl-ACP to yield palmitic acid and the cytoplasmic synthesis ceases. Fatty acids are synthesized in the saturated form and desaturation occurs later in the endoplasmic reticulum. R eactions to elongate the fatty acid (with elongases) may also occur to make unsaturated fatty acids of varying lengths. Desaturases are named according to the location of the double bonds they introduce in fatty acids. The delta (Δ) system number the carbon at the carboxyl end as number 1 and the omega (ω) number system numbers the carbon at the methyl end as number 1. Humans have desaturases named as Δ5, Δ6, and Δ9. A Δ9 desaturase, for example, could convert stearic acid into oleic acid, because stearic acid is a saturated 18 carbon fatty acid and oleic acid is an 18 carbon fatty acid with only one double bond - at position Δ9.

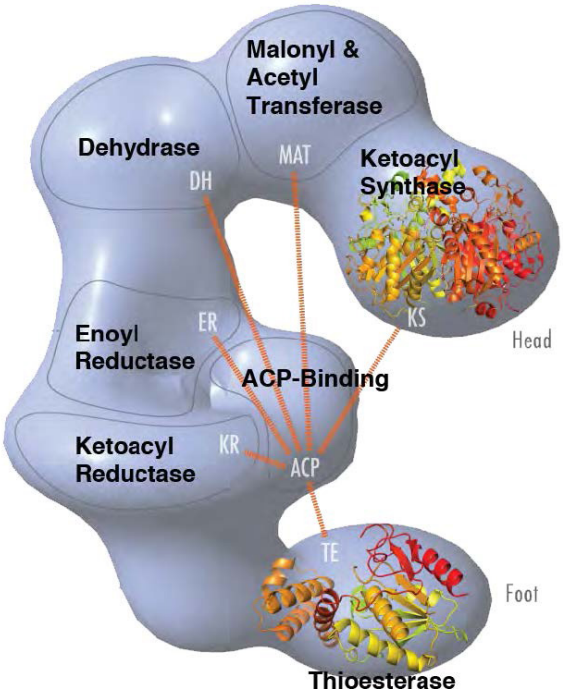


Fig 16.8 Fatty acid synthase complex and 3D model movie of FAS complex.

Polyunsaturated fatty acids require the action of multiple enzymes and (in some cases) the action of elongases. Arachidonic acid, for example, is a 20 carbon fatty acid with four double bonds and its synthesis requires both an elongase (to increase the length of the fatty acid from 16 to 20) and multiple desaturases - one for each desaturated double bond..

Animals are limited in the fatty acids they can make, due to an inability of their desaturases to catalyze reactions beyond carbons Δ9. Thus, humans can make oleic acid, but cannot synthesize linoleic acid (Δ9,12) or linolenic acid (Δ9,12,15). Consequently, these two must be provided in the diet and are referred to as essential fatty acids.

Resources:

Chapter page: Frech fries prepared in hot oil Adobe stock #265509234 Free-licensed.

Kumari, M., and Kozyrskyj, A. L. (2017) Gut microbial metabolism defines host metabolism: an emerging perspective in obesity and allergic inflammation. Obesity Reviews, 18: 18– 31. doi: 10.1111/obr.12484.

Fig 16.2 . Top:Chylomicron formation Adobe stock 430688136 Free-Licensed by Manjeet Kumari. Bottom: Chylomicron 554760608 Licensed by Manjeet Kumari

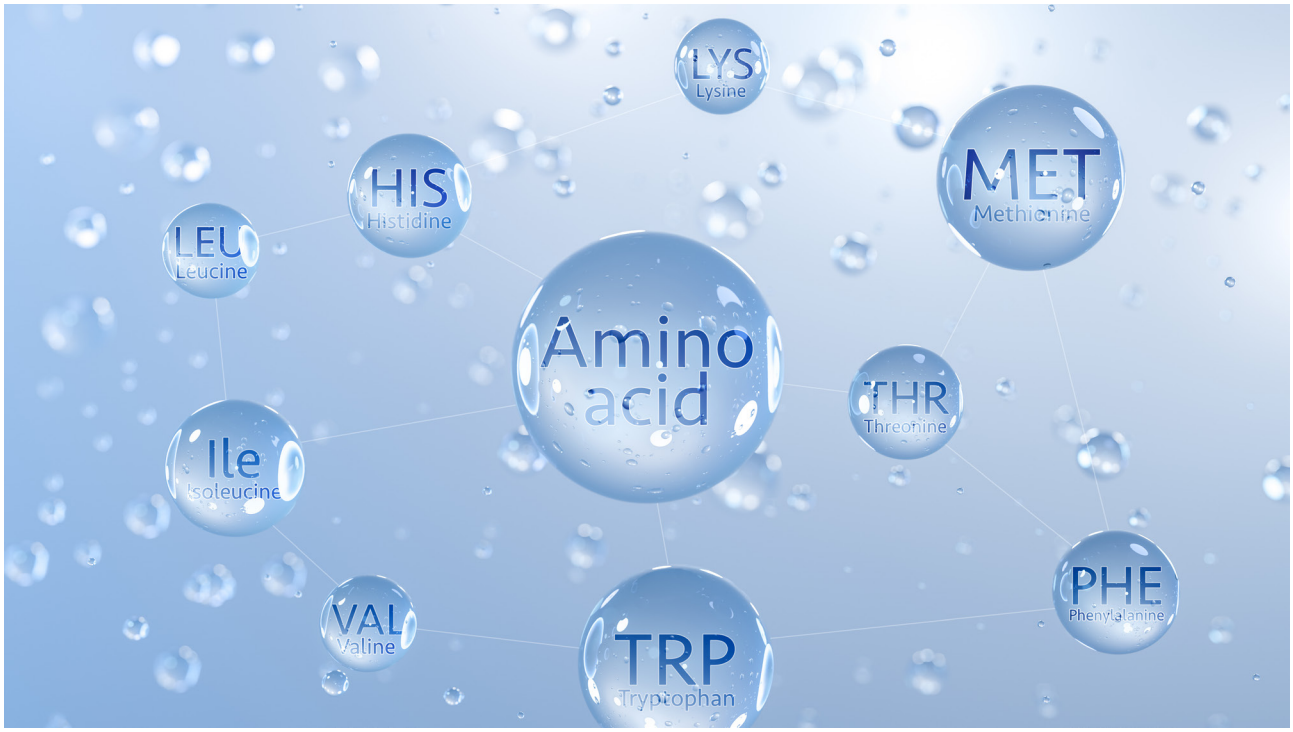
Fig 16.3 Atherosclerosis with plaque in vessel. Adobe stock #394303002. Free-Licensed by Manjeet Kumari.

Fig 16:8 Image from the RCSB PDB (RCSB.org) of PDB ID 2CF2 (Maier, T., Jenni, S., Ban, N.(2006) Science 311: 1258. https://doi.org/10.1126/science.1123248

17

Chapter #17

Amino acid and nucleotide metabolism



Introduction

In contrast to some of the metabolic pathways described to this point, amino acid metabolism is not a single pathway. The 20 universal amino acids (Fig 17.1) have some parts of their metabolism that overlap with each other, but others are very different from the rest.

Essential	Non-Essential
Histidine	Alanine
Isoleucine	Arginine
Leucine	Asparagine
Lysine	Aspartic acid
Methionine	Cysteine
Phenylalanine	Glutamic acid
Threonine	Glutamine
Tryptophan	Glycine
Valine	Proline
	Selenocysteine
	Serine
	Tyrosine

Fig 17.1 Essential and non-essential amino acids

Before beginning discussion of the pathways, it is worthwhile to discuss a reaction common to the metabolism of most of the amino acids and other nitrogen-containing compounds and that is transamination. In cells, nitrogen is a nutrient that moves from one molecule to another in a sort of hand-off process. A common transamination reaction is shown in Figure 17.2. This is essentially removing amino group by emzymes called transaminases or aminotransferases.

Glutamate and glutamine play central roles in transamination, each containing one more amine group than α-ketoglutarate and glutamate, respectively. Transamination reactions occur by a ping-pong mechanism and involve swaps of amines and oxygens in Schiff base reactions. Two amino acids, glutamine and asparagine are the products of gaining an amine in their respective R-groups in reactions involving ammonium ion.

Amino acids are divided according to the pathways involved in their degradation. There are three general categories. Ones that yield intermediates in the glycolysis pathway are called glucogenic and those that yield intermediates of acetyl-CoA or acetoacetate are called ketogenic. Those that involve both are called glucogenic and ketogenic.

Amino acids largely produce breakdown products related to intermediates of the citric acid cycle or glycolysis, but this isn't the complete picture. Some amino acids, like tryptophan, phenylalanine, and tyrosine yield hormones or neurotransmitters on further metabolism. Others like cysteine and methionine must dispose of their sulfur and all of the amino acids must rid themselves of nitrogen, which can happen via the urea cycle.

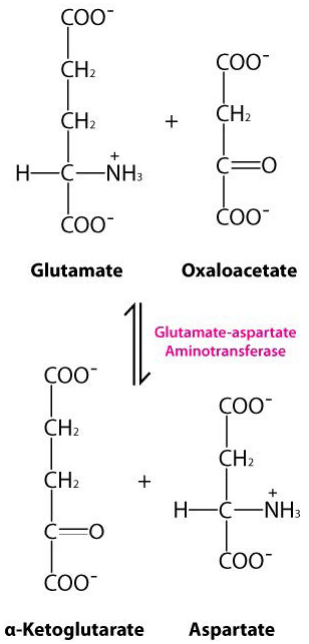


Fig 17.2 Example of a transaminase (aminotransferase) reaction.

Urea cycle

The urea cycle holds the distinction of being the first metabolic cycle discovered - in 1932, five years before the citric acid cycle. It is an important metabolic pathway for balancing nitrogen in the bodies of animals and it takes place primarily in the liver and kidney.

Organisms, like humans, that excrete urea are called ureotelic. Those that excrete uric acid (birds, for example) are called uricotelic and those that excrete ammonia (fish) are ammonotelic. Ammonia, of course, is generated by metabolism of amines and is toxic, so managing levels of it is critical for any organism. Excretion of ammonia by fish is one reason that an aquarium periodically requires cleaning and replacement of water.

Liver failure can lead to accumulation of nitrogenous waste and exacerbates the problem. As shown in Figure 17.3, the cycle contains five reactions, with each turn of the cycle producing a molecule of urea. Of the five reactions, three occur in the cytoplasm and two take place in the mitochondrion.

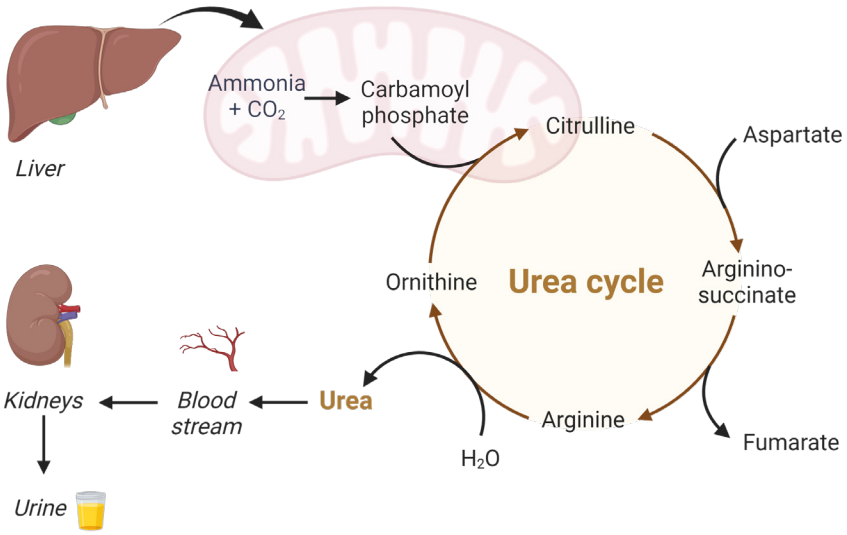


Fig 17.3
Urea Cycle

Ornithine synthesis

Though the cycle doesn't really have a starting point, a common place to begin discussion is with the molecule of ornithine.

Ornithine is found in the cytoplasm and is transported into the mitochondrion by the ornithine-citrulline antiport of the inner mitochonrial membrane. In the matrix of the mitochondrion, two reactions occur relevant to the cycle. The first is formation of carbamoyl phosphate from bicarbonate, ammonia, and ATP catalyzed by carbamoyl phosphate synthetase I. Carbamoyl phosphate then combines with ornithine in a reaction catalyzed by ornithine transcarbamoylase to make citrulline.

The citrulline is transported out to the cytoplasm by the ornithine-citrulline antiport mentioned above. In the cytoplasm, citrulline combines with L-aspartate using energy of ATP to make citrullyl-AMP (an intermediate) followed by argininosuccinate. The reaction is catalyzed by argininosuccinate synthase. Next, fumarate is split from argininosuccinate by argininosuccinate lyase to form arginine. Water is used by arginase to cleave arginine into urea and ornithine, completing the cycle.

Urea is less toxic than ammonia and is released in the urine. Some organisms make uric acid for the same reason.

It is worth noting that aspartic acid, ammonia, and bicarbonate enter the cycle and fumarate and urea are produced by it. Points to take away include 1) ammonia is converted to urea using bicarbonate and the amine from aspartate 2)aspartate is converted to fumarate which releases more energy than if aspartate were converted to oxaloacetate, since conversion of fumarate to malate to oxaloacetate in the citric acid cycle generates an NADH, but direct conversion of aspartate to oxaloacetate does not; and 3) glutamate and aspartate are acting as shuttles to funnel ammonia into the cycle.

Urea cycle regulation

The urea cycle is controlled both allosterically and by substrate concentration. The cycle requires N-acetylglutamate (NAG) for allosteric activation of carbamoyl phosphate synthetase I. The enzyme that catalyzes synthesis of NAG, NAG synthetase, is activated by arginine and glutamate. Thus, an indicator of high amine levels, arginine, and an important shuttler of amine groups, glutamate, stimulates the enzyme that activates the cycle.

The reaction catalyzed by NAG synthetase is

Acetyl-CoA + L-glutamate →CoA-SH + N-acetyl-L-glutamate

At the substrate level, all of the other enzymes of the urea cycle are controlled by the concentrations of substrates they act upon. Only at high concentrations are the enzymes fully utilized.

Complete deficiency of any urea cycle enzyme is fatal at birth, but mutations resulting

in reduced expression of enzymes can have mixed effects. Since the enzymes are usually not limiting for these reactions, increasing substrate can often overcome reduced enzyme amounts to a point by simply fully activating enzymes present in reduced quantities.

Ammonia accumulation

However, if the deficiencies are sufficient, ammonium can accumulate and this can be quite problematic, especially in the brain, where mental deficiencies or lethargy can result. Reduction of ammonium concentration relies on the glutamate dehydrogenase reaction (named for the reverse reaction).

α-ketoglutarate + NH3 + NAD(P)H ⇄ Glutamate + NADP+

Additional ammonia can be taken up by glutamate in the glutamine synthetase reaction.

Glutamate + NH3 + ATP → Glutamine + ADP + Pi

The result of these reactions is that α-ketoglutarate and glutamate concentrations will be reduced and the concentration of glutamine will increase. For the brain, this is a yin/yang situation. Removal of ammonia is good, but reduction of α-ketoglutarate concentration means less energy can be generated by the citric acid cycle. Further, glutamate is, itself, an important neurotransmitter and a precursor of another neurotransmitter - γ-aminobutyric acid (GABA).

.So far we have seen that nitrogen, if not reused for synthesis of other nitrogenous compounds, is excreted as urea (Fig. 17.3). The left over carbon skeleton of amino acid is sent as intermediates of citric acid cycle, glycolytic pathway or acetyl-CoA (Fig. 17.4)

Amino acid anbolism

It is also important to recognize that organisms differ considerably in the amino acids that they can synthesize. Humans, for example, cannot make 9 of the 20 amino acids needed to make proteins, and the number of these that can be synthesized in needed amounts varies between adults and children.

Amino acids that cannot be made by an organism must be in the diet and are called essential amino acids. Non-essential amino acids are those an organism can make in sufficient quantities. Though amino acids do not have a common pathway of metabolism, they are often organized in "families" of amino acids (Table 17.1) with overlapping metabolic reactions common to members of each group. On the next page you will find amino acids biosynthetic pathways classified based on precursor molecule.. The details of these pathways are not discussed here.

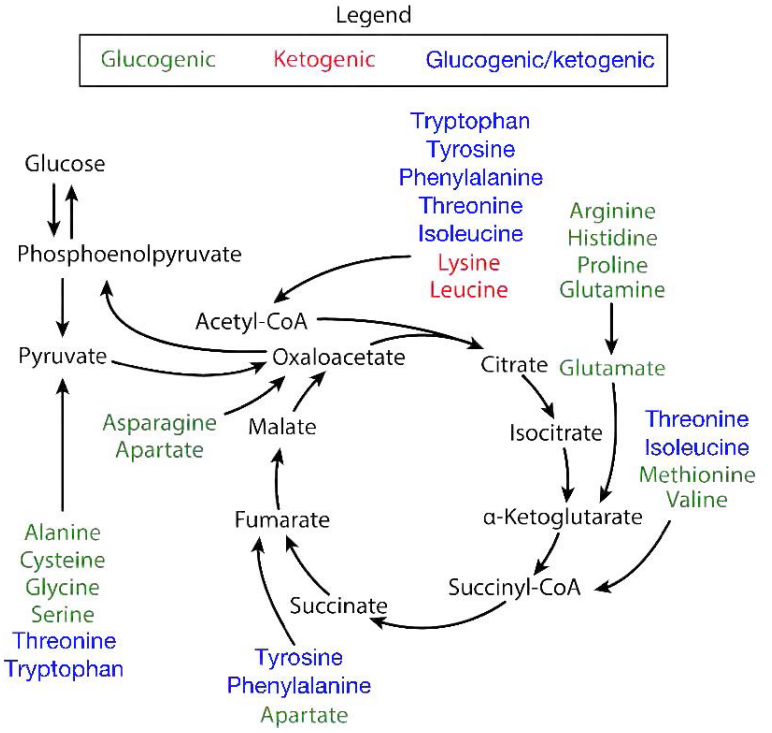


Fig 17.4 Breakdown pathways for amino acids. Some yield more than one intermediate

Table 17.1 Common biosynthetic pathways for amino acid synthesis. Table by Manjeet Kumari

Family	Amino acids
α-Ketoglutarate (as precursor) family	This family of amino acids arises from α-ketoglutarate of the citric acid cycle. It includes the amino acids • glutamic acid, • glutamine, • proline, • arginine. It is also called the glutamate family,
3-phosphoglycerate (as precursor) family	• Cysteine • Glycine • Serine
Oxaloacetate (as precursor) family	• Asparagine • Aspartate • Methionine • Threonine • Lysine
PEP and erythrose-4-phosphate (as precursor) family	All three aromatic amino acids are also important sources of hormones, neurotransmitters, and even the skin pigment melanin • Tryptophan, • Phenylalanine • Tyrosine
Ribose-5-phosphate (as precursor) family	• Histidine. It is also called histidine family.
Pyruvate (as precursor) family	• Alanine • Valine • Leucine • isoleucine

Nucleotide metabolism

Nucleotides are most often thought of as the building blocks of the nucleic acids, DNA and RNA. While this, is, of course, a vital function, nucleotides also play other important roles in cells. Ribonucleoside triphosphates like ATP, CTP, GTP and UTP are necessary, not just for the synthesis of RNA, but as part of activated intermediates like UDP-glucose in biosynthetic pathways. ATP is also the universal "energy currency" of cells, and coupling of energetically unfavorable reactions with the hydrolysis of ATP makes possible the many reactions in our cells that require an input of energy. Adenine nucleotides serve as components of $NAD(P)^+$ and FAD. Nucleotides can also serve as allosteric and metabolic regulators. The synthesis and breakdown pathways for nucleotides and the molecules derived from them are thus, of vital importance to cells. We will not discuss the catabolic and anabolic pathways of nucleotide in detail here as it is beyond scope of our second year, one semester biochem course. Some key points are covered here because regulation of nucleotide synthesis, especially for deoxyribonucleotides, is important to ensure that the four nucleotides are made in the right proportions, as imbalances in nucleotide concentrations can lead to increases in mutation rates.

Pathways of nucleotide metabolism are organized in two major groups and one minor one. These include, respectively, metabolism of 1) purines; 2) pyrimidines; and 3) deoxyribonucleotides.

Each group can be further subdivided into pathways that make nucleotides from simple precursors (*de novo* pathways) and others that use pieces of nucleotides to reassemble full ones (salvage pathways). Notably, de novo synthesis pathways for all of the nucleotides begin with synthesis of ribonucleotides. Deoxyribonucleotides are made from the ribonucleotides. (Fig 17.5 and 17.6)

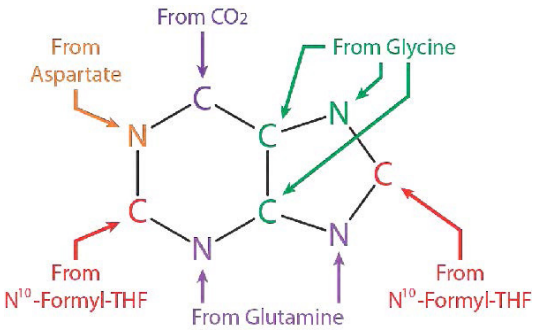


Fig 17.5 Origin of atoms in purines. Image by Aleia Kim

Besides salvage and being built into nucleic acids, nucleotides can be broken down into simpler component molecules. Some of these molecules, such as uric acid, can have significant impact on organisms

Breakdown of purine nucleotides starts with nucleoside monophosphates, which can be produced by breakdown of an RNA, for example, by a nuclease.

Metabolism of AMP and GMP converge at xanthine. First, AMP is dephosphorylated by nucleotidase to create adenosine, which is then deaminated by adenosine deaminase to yield inosine. Alternatively, AMP can be deaminated by AMP deaminase to yield IMP.

IMP is also an intermediate in the synthesis pathway for purine anabolism. Dephosphorylation of IMP (also by nucleotidase) yields inosine. Inosine has ribose stripped from it by action of purine nucleotide phosphorylase to release hypoxanthine. Hypoxanthine is oxidized to xanthine in a hydrogen peroxide-generating reaction catalyzed by xanthine oxidase.

Catabolism of GMP proceeds independently, though similarly. First, phosphate is removed by nucleotidase to yield guanosine. Guanosine is stripped of ribose to yield free guanine base, which is deaminated by guanine deaminase (also called guanase) to produce xanthine. Xanthine is catabolised as shown in Figure 17.7

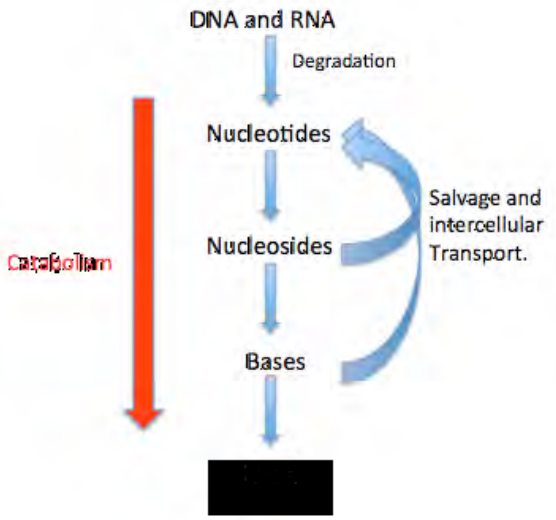


Fig 17.6 Catabolism and salvage of nucleotides from DNA and RNA leading to Urea formation. Wikipedia

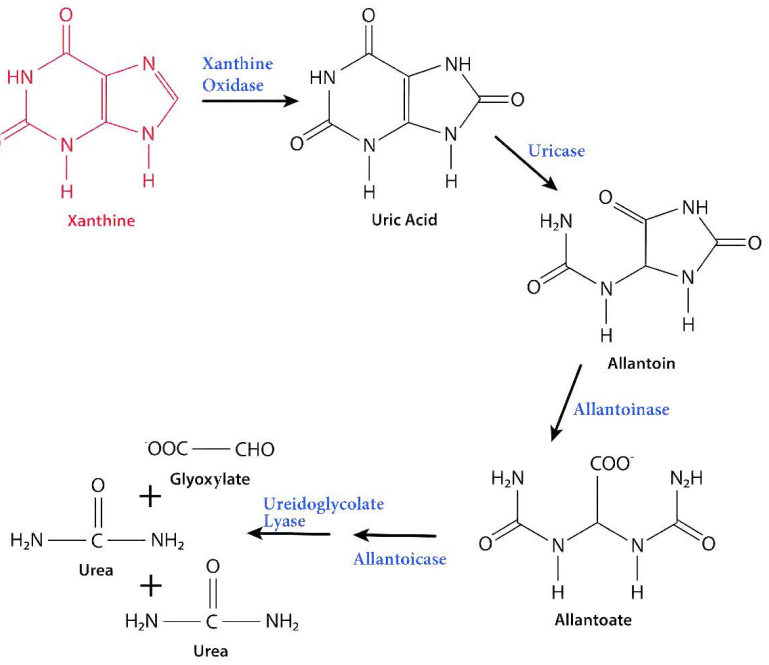


Fig 17.7 Purine catabolism. Image by Pehr Jacobson

Resources:

Chapter page: Essential amino acid symbols located on drops with structural links, 3d rendering of dietary supplements Adobe stock #551173684 licensed.

Fig 17.3 Adapted from "Urea cycle", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.

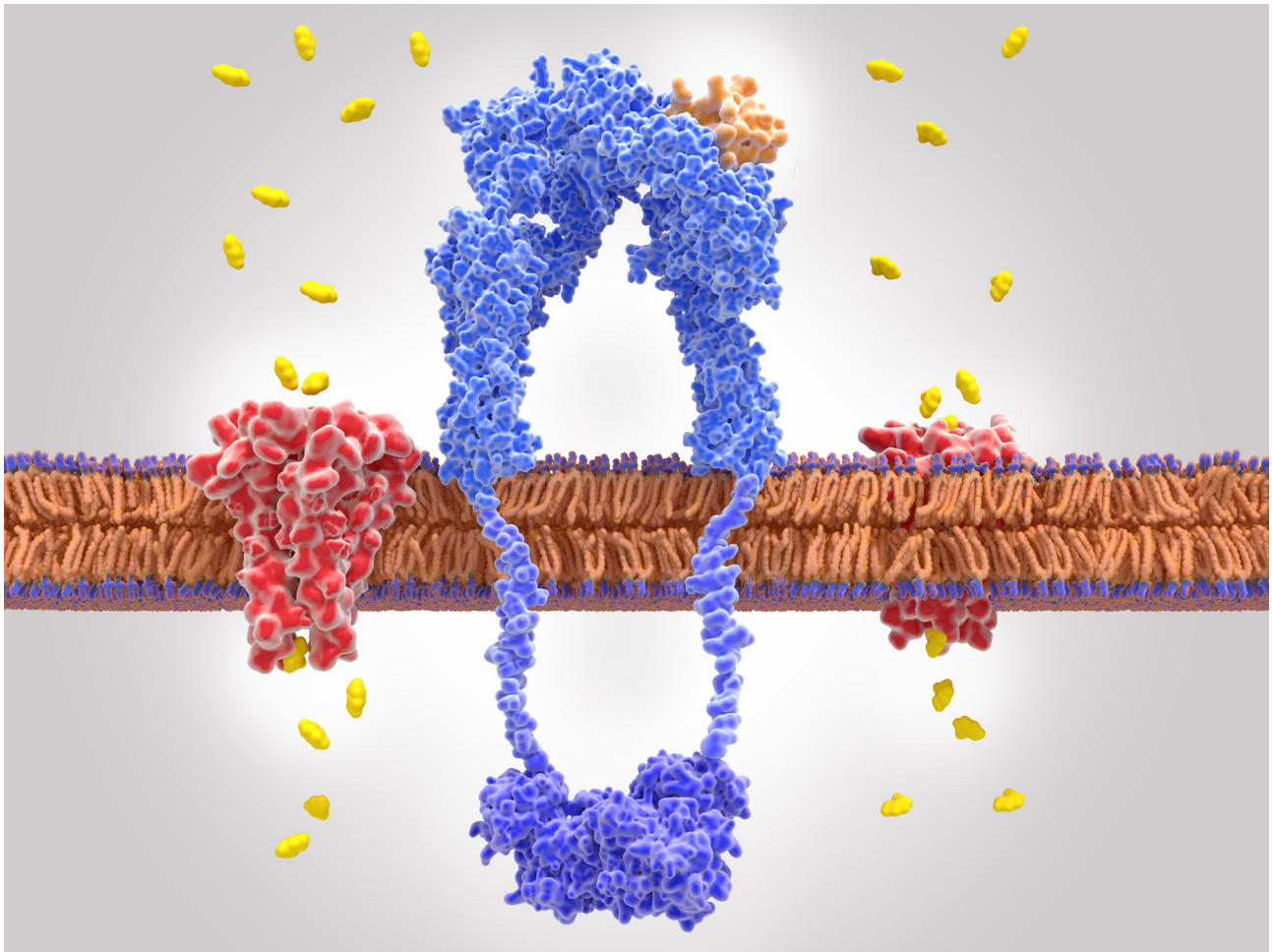
To learn more about ribonucleotide synthesis check following link. Used by permission. Video from HarvardX, available on LabXchange.org

https://www.labxchange.org/library/items/lb:HarvardX:601df683:video:1

18

Chapter #18

Feed-Fast



Introduction

In this last chapter we will discuss how cells manage different metabolic pathwys when there is plenty of sugar (fed) and when there is none (starvation)! Uncontrolled diabetes is a metabolic disorder that mimics starvation state.

Glucose

Animals store glucose primarily in liver and muscle in the form of a compound related to amylopectin known as glycogen. Remember that amylopectin is present in plants. The structural differences between glycogen and amylopectin are solely due to the frequency of the α-1,6 branches of glucoses. In glycogen they occur about every 10 residues instead of every 30-50, as in amylopectin (Figure 18.1).

Glycogen provides an additional source of glucose besides that produced via gluconeogenesis. Because glycogen contains so many glucoses, it acts like a battery backup for the body, providing a quick source of glucose when needed and providing a place to store excess glucose when glucose concentrations in the blood rise. Recall that excess glucose can cause glycation of proteins besides other issues for the cell.

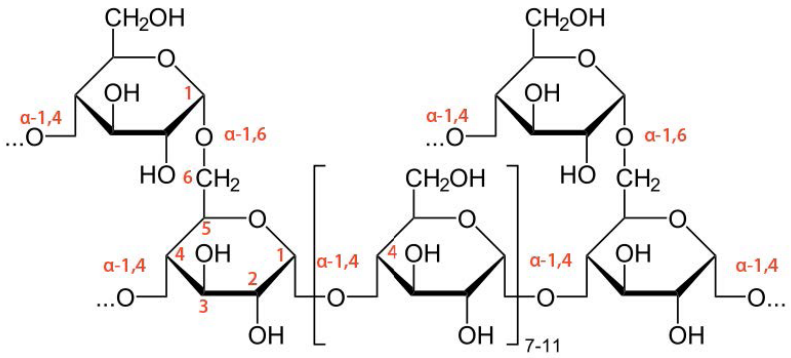


Fig 18.1 Glycogen Structure - α-1,4 links with α-1,6 branches every 7-10 residues.

In fact, high level of glucose activates secretion of insulin (a hormone produced by the beta cells of the pancreas) which functions to lower blood glucose levels. The chapter image on the left page (and figure 18.2) shows when insulin binds to insulin receptors on the surface of cell, a cascade of reaction leads to fusion of GLUT protein containing vesicle with plasma membrane of cell. This leads to transport of glucose in the cell thus lowering the concentration of glucose in the blood. This glucose is immediately phosphorylated (G6P) to enter glycolysis if there is need for energy, otherwise it can be stored into glycogen.

Just as in gluconeogenesis, the cell has a separate mechanism for glycogen breakdown that is distinct from glycogen synthesis

Glycogen breakdown (Glycogenolysis)

Breakdown of glycogen involves:

1) release of glucose-1-phosphate (G1P),

2) rearranging the remaining glycogen (as necessary) to permit continued breakdown, and

3) conversion of G1P to G6P for further metabolism. This G6P can be a) used in glycolysis, b) converted to glucose by gluconeogenesis, or c) oxidized in the pentose phosphate pathway.

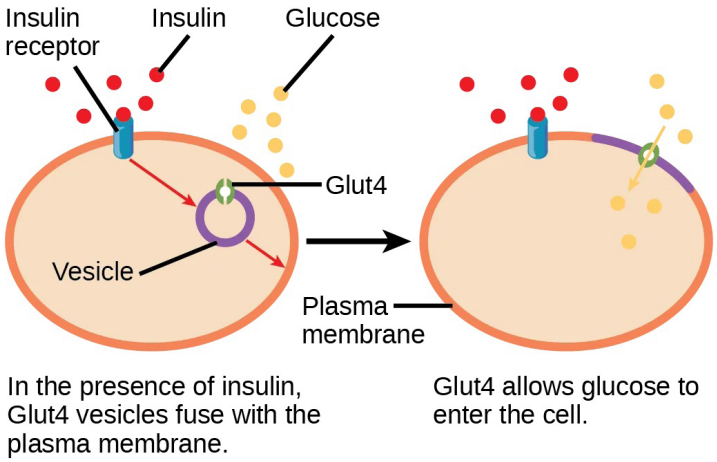


Fig 18.2 Insulin dependent uptake of glucose. Source: Opne Stax Biology 2e.

Glycogen phosphorylase (sometimes simply called phosphorylase) catalyzes breakdown of glycogen into glucose-1- Phosphate (G1P - Figure 18.3). The reaction that produces

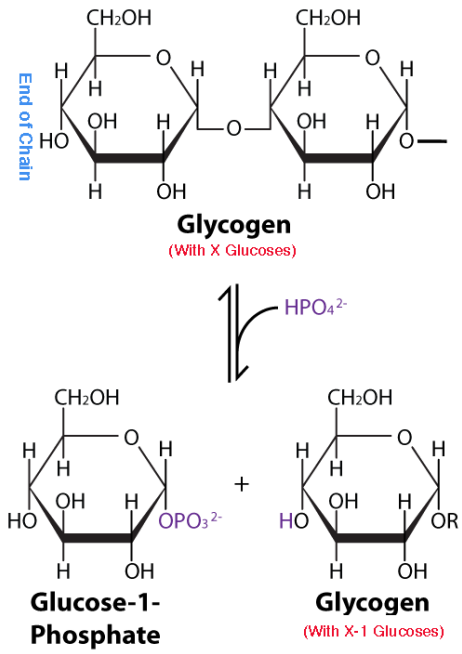


Fig 18.3 Breaking of α-1,4 bonds of glycogen by glycogen phosphorylase
Image by Aleia Kim

G1P from glycogen is a phosphorolysis, not a hydrolysis reaction. The distinction is that hydrolysis reactions use water to cleave bigger molecules into smaller ones, but phosphorolysis reactions use phosphate instead for the same purpose. Note that the phosphate is just that - it does NOT come from ATP. Since ATP is not used to put phosphate on G1P, the reaction saves the cell energy.

G1P can be converted to G6P by action of an enzyme called phosphoglucomutase. This reaction is readily reversible, allowing G6P and G1P to be interconverted as the concentration of one or the other increases.

Another hormone of interest here is glucagon. It is produced by alpha cells of pancreas. When blood glucose falls below the normal level, binding of glucagon to appropriate cell receptors stimulates a phosphorylation cascade which simultaneously activates breakdown of glycogen by glycogen phosphorylase and inhibits synthesis of glycogen by glycogen synthase.

Glycogen synthesis (Glycogenesis)

The anabolic pathway opposing glycogen breakdown is that of glycogen synthesis. Just as cells reciprocally regulate glycolysis and gluconeogenesis to prevent a futile cycle between these pathways, so too do cells use reciprocal schemes to regulate glycogen breakdown (glycogenolysis) and synthesis (glycogenesis).

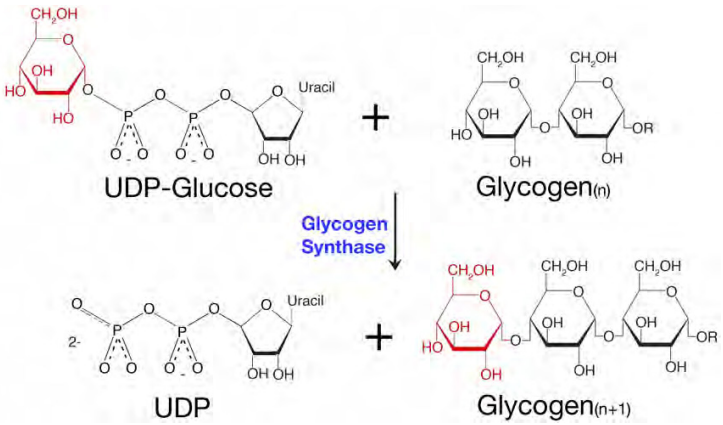


Fig 18.4 Catalytic activity of glycogen synthase.
Image by Penelope Irving

Synthesis of glycogen starts with G1P, which is converted to an 'activated' intermediate, UDP-glucose. This activated intermediate is what 'adds' the glucose to the growing glycogen chain in a reaction catalyzed by the enzyme known as glycogen synthase (Figure 18.4). Once the glucose is added to glycogen, the glycogen molecule may need to have branches inserted in it by the enzyme known as branching enzyme.

Pentose phosphate pathway

The pentose phosphate pathway (PPP also called the hexose monophosphate shunt) is an oxidative pathway involving sugars that is sometimes described as a parallel pathway to glycolysis. It is, in fact, a pathway with multiple inputs and outputs (Figure 18.5). PPP is also a major source of NADPH for biosynthetic reactions and can provide ribose-5-phosphate for nucleotide synthesis.

Though when drawn out, the pathway's "starting point" is often shown as glucose-6-phosphate (G6P), in fact there are multiple entry points including other glycolysis intermediates, such as fructose-6-phosphate (F6P) and glyceraldehyde-3-phosphate (GLYAL-3-P), as well as less common sugar compounds with 4,5, and 7 carbons.

The multiple entry points and multiple outputs gives the cell tremendous flexibility to meet its needs by allowing it to use a variety of materials to make any of these products

In the reversible reactions of the pentose phosphate pathway, one can see how glycolysis intermediates can easily be rearranged and made into other sugars. Thus, GLYAL-3-Pand F6P can be readily made into Ribose-5-phosphate for nucleotide synthesis. Involvement of F6P in the pathway permits cells to continue making nucleotides (by making R-5-P) or tryptophan (by making E-4-P) even if the oxidative reactions of PPP are inhibited.

Fat

Fatty acid oxidation also known as beta oxidation produce acetyl CoA, NADH and $FADH_2$. Acetyl-CoA is one of the most "connected"metabolites in biochemistry, appearing in fatty acid oxidation/synthesis, pyruvate oxidation, the citric acid cycle, amino acid anabolism/catabolism, ketone body metabolism, steroid/bile acid synthesis, and (by extension from fatty acid metabolism) prostaglandin synthesis.

Ketone bodies (acetoacetic acid, acetone, and β-hydroxybutyrate) are of special interest in this chapter because these are made when the blood levels of glucose fall very low (Figure 18.6).

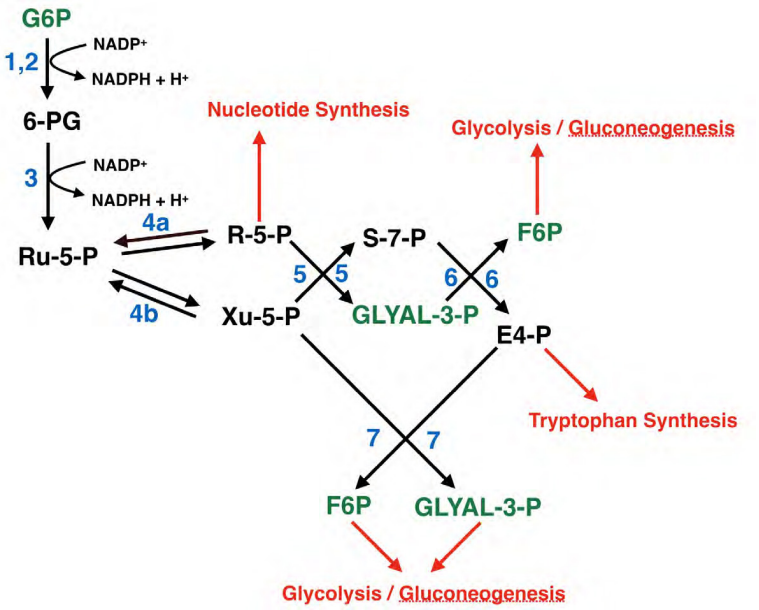


Fig 18.5 The Pentose Phosphate Pathway Enzymes
1 = G6P dehydrogenase,
2 = 6-Phosphogluconolactonase,
3 = 6-PG dehydrogenase,
4a = Ribose 5-phosphate isomerase
4b = Ribulose 5-phosphate 3-epimerase,
5,7 =Transketolase
6 = Transaldolase

Normally, glucose is the body's primary energy source. It comes from the diet, from the breakdown of storage carbohydrates, such as glycogen, or from glucose synthesis (gluconeogenesis). Since the primary stores of glycogen are in muscles and liver and since gluconeogenesis occurs only in liver, kidney, and gametes, when the supply of glucose is depleted (glycogen provide only one day supply), the liver must supply an alternate energy source in the form of ketone bodies. Most cells of the body can use ketone bodies as energy sources.

Biosynthetic pathway that make ketone bodies is called ketogenesis. Ketone bodies can be made in liver from the breakdown of fatty acids in the mitochondria. Recall from Chapter 16 that beta-oxidation of fatty acids releases acetyl CoA. Under conditions of prolonged fasting/diabetes, TCA cycle intermediates are depleted because oxaloacetate is channeled away to make glucose via gluconeogenesis. Thus, acetyl CoA derived from beta-oxidation is used for ketogenesis. Three key enzymes of ketogenesis are 1) Thiolase, 2) 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) Synthase and 3) HMG-CoA Lyase. The first enzyme, thiolase, converts two acetyl CoA molecules to acetoacetylCoA. Acetoacetyl-CoA is then combined with another acetyl-CoA resulting in formation of HMG-CoA. This step is catalysed by second enzyme HMGCoA synthase. (Fig 18.7 blue). The third enzyme HMG-CoA lyase then strips an acetyl-CoA from HMG-CoA to produce acetoacetate (a ketone body). This acetoacetate can have two fates: a) it can undergo non enzymatic decarboxylation to forms another ketone body called acetone, and b) forms β-hydroxybutyrate, the most abundant ketone body in blood.

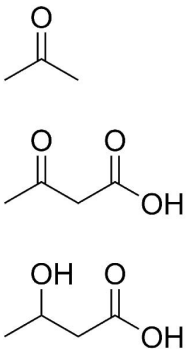


Fig 18.6 Three ketone bodies - acetone (top), acetoacetic acid (middle), and β-hydroxybutyrate (bottom)

When body is producing ketone bodies during prolonged starvation, this stage is known as ketosis. While ketogenesis is the biochemical pathways to produce ketone bodies, ketolysis is the pathway for cells to use ketone bodies for energy. Ketogenesis in the liver and ketolysis in non-liver cells (extra hepatic tissue) is critical for survival, particularly for brain. In fact, β-hydroxybutyrate travels readily in the blood and crosses the blood-brain barrier to fuel brain cells. Ketone bodies are then converted to acetyl-CoA by reversing the pathway that makes them(Figure 18.7). This Acetyl CoA, of course, can be used for ATP synthesis via the citric acid cycle. People who are very hypoglycemic(including some diabetics) will produce ketone bodies and these are often first detected by the smell of acetone on their breath. Acetone is a dead metabolic molecule which is not converted back to acetyl-CoA but rather excreted in urine or exhaled out in breath.

It is important to emphasize here that use of ketone bodies for energy in limited to extra hepatic tissue only to prevent futile cycle in liver. This is achieved by regulation of enzymes of ketolysis. Ketolysis as mentioned above is reversal of ketogenesis where β-hydroxybutyrate in converted to acetoacetate, then to acetoacetyl CoA and finally to AcetylCoA.

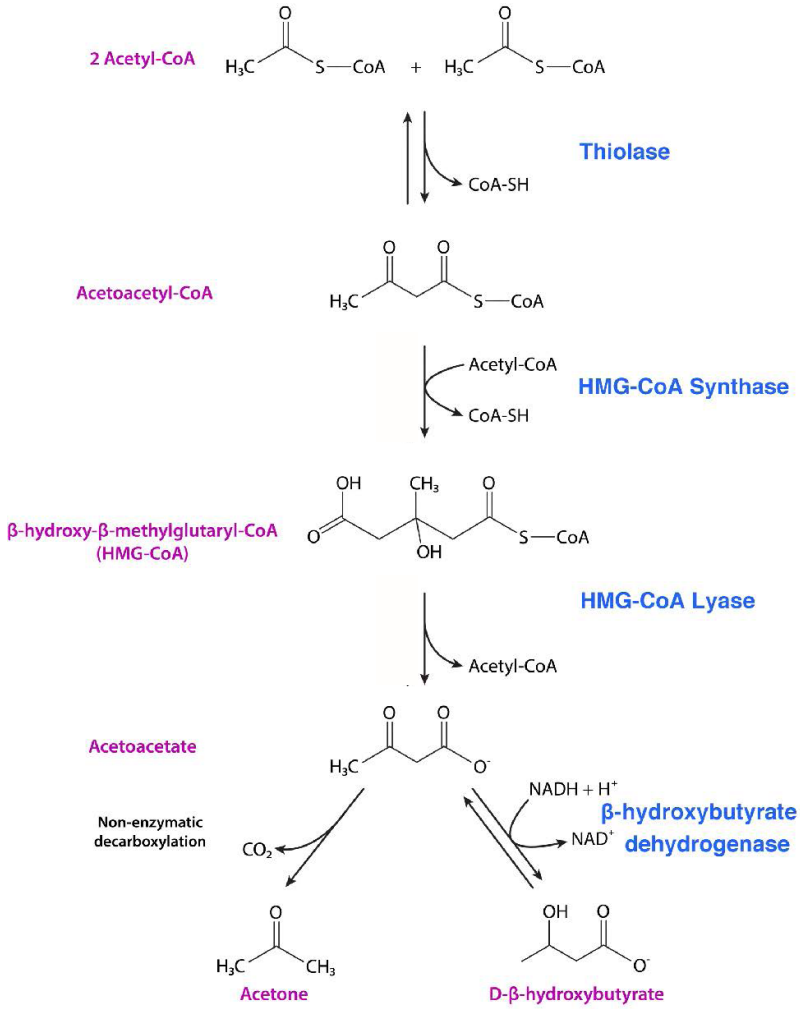


Table 18.7 Ketone body metabolism. Image by Pehr Jacobson.

However, conversion of acetoacetate to acetoacetyl CoA needs need another enzyme called succinyl-CoA:3-ketoacid coenzyme A transferase (SCOT). Interestingly, mRNA levels of SCOT are not detectable in the liver but present in all extra-hepatic tissues.

It is certainly puzzling that why brain depends on glucose instead of fat as a source of energy and why fat needs to be converted to ketones as an alternate source of fuel?

Although fat generate more energy compare to glucose (BOX 18. 1), fat is not a primary source of energy for brain and must be converted to ketone bodies in mitochondria of liver cells when body enters starvation. This is because a) beta oxidation has higher oxygen requirement than glucose so there may be risk of hypoxia (low oxygen) in brain cells, b) there is less risk of oxidative stress due to superoxides produced during beta oxidation and, c) slower rate of beta oxidation of fatty acids in brain and hence ATP generation via beta oxidation is slower process than glucose oxidation.

Acidosis

The term acidosis refers to conditions in the body where the pH of arterial blood drops below 7.35. It is the opposite of the condition of alkalosis, where the pH of the arterial blood rises above 7.45. Normally, the pH of the blood stays in this narrow pH range. pH values of the blood lower than 6.8 or higher than 7.8 can cause irreversible damage and may be fatal. Acidosis may have roots in metabolism (metabolic acidosis) or in respiration (respiratory acidosis). Ketoacidosis is a condition when ketone bodies build up in blood and lowers the pH of blood. Acidosis can lead to cell damage.

There are several causes of acidosis. In metabolic acidosis, production of excess lactic acid or failure of the kidneys to excrete acid can cause blood pH to drop. Lactic acid is produced in the body when oxygen is limiting, so anything that interferes with oxygen delivery may create conditions favoring production of excess lactic acid. These may include restrictions in the movement of blood to target tissues, resulting in hypoxia (low oxygen conditions) or decreases in blood volume. Issues with blood movement can result from heart problems, low blood pressure, or hemorrhaging.

Strenuous exercise can also result in production of lactic acid due to the inability of the blood supply to deliver oxygen as fast as tissues require it (hypovolemic shock). At the end of the exercise, though, the oxygen supply via the blood system quickly catches up.

Respiratory acidosis arises from accumulation of carbon dioxide in the blood. Causes include hypoventilation, pulmonary problems, emphysema, asthma, and severe pneumonia.

Protein and nucleic acids

Proteins and nucleic acids as source of nutrient under starvation conditions is expensive because cell has to remove nitrogen before skeleton becomes available for oxidation. Remember that there are 20 amino acids and there different pathways of amino acid oxidation. Overall, carbon skeleton from amino acids feed into TCA cycle and nitrogen is dispensed via urea cycle as discussed in chapter 17.

Glucose-alanine cycle

The glucose alanine cycle (also known as the Cahill Cycle), has been described as the amine equivalent of the Cori cycle. The Cori cycle, of course, exports lactate from muscles (when oxygen is limiting) to the liver via the bloodstream. The liver, in turn, converts lactate to glucose, which it ships back to the muscles via the bloodstream. The Cori Cycle is an essential source of glucose energy for muscles during periods of exercise when oxygen is used faster than it can be delivered.

In the glucose-alanine cycle, cells are generating toxic amines and must export them. This is accomplished by transaminating pyruvate (the product of glycolysis) to produce the amino acid alanine. Recall transamination reactions from chapter 17.

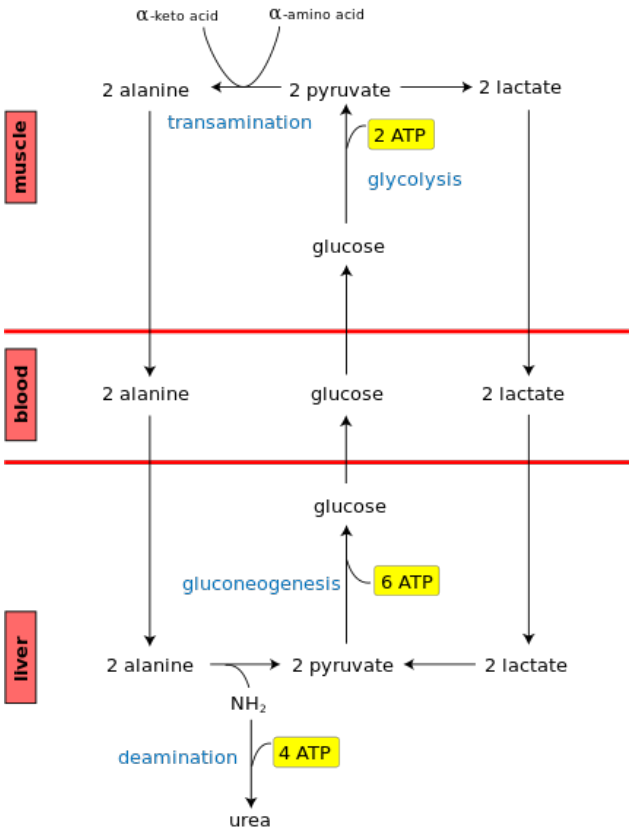


Fig 18.8 Glucose-alanine cycle along with Cori cycle. Wikipedia

BOX 18.1: Comparing yields of fat and glucose oxidations

Fat (Palmitic acid) oxidation:

A 16C fatty acid will have 7 beta-oxidations. Each oxidation step yields 1 NADH and 1 $FADH_2$.

1 NADH produces 2.5 ATP therefore, 7 NADH x 2.5 ATP= 17.5 ATP

1 $FADH_2$ produces 1.5 ATP therefore, 7 $FADH_2$ x 1.5=10.5 ATP

So, total 28 ATPs (17.5+10.5) are made by beta oxidation itself. Then, we also have 8 acetyl-CoAs (products of beta oxidation) which enter TCA cycle of mitochondria.

One acetyl-CoA produces 3 NADH, 1 $FADH_2$ and 1 ATP (GTP) in one turn of TCA cycle resulting in 10 ATPs. So, 8 acetyl-CoA x 10 ATP= 80 ATPs

If you combine ATP yield from beta-oxidation (20 ATPs) and acetyl-CoA via TCA cycle (80 ATPs), then total is 108 ATPs/one palmitic acid chain

Glucose oxidation

Glycolysis=2 ATP, 2 NADH=2 ATP + (2 x 2.5 ATP)= 7 ATPs

pyruvate oxidation=2 NADH= 2 x 2.5 ATPs = 5 ATPs

TCA cycle=6 NADH, 2 $FADH_2$, 2 GTP= (6 x 2.5 ATP)+ (2 x 1.5 ATP)+ 2 ATP=20 ATPs.

In total, 20+7+5= 32 ATPs/glucose molecule. Often yield is given in a range to account for how NADH moves from cytoplasm to mitochondria to give electrons to ETC.

For further learning: https://youtu.be/acA5iF1zrDI

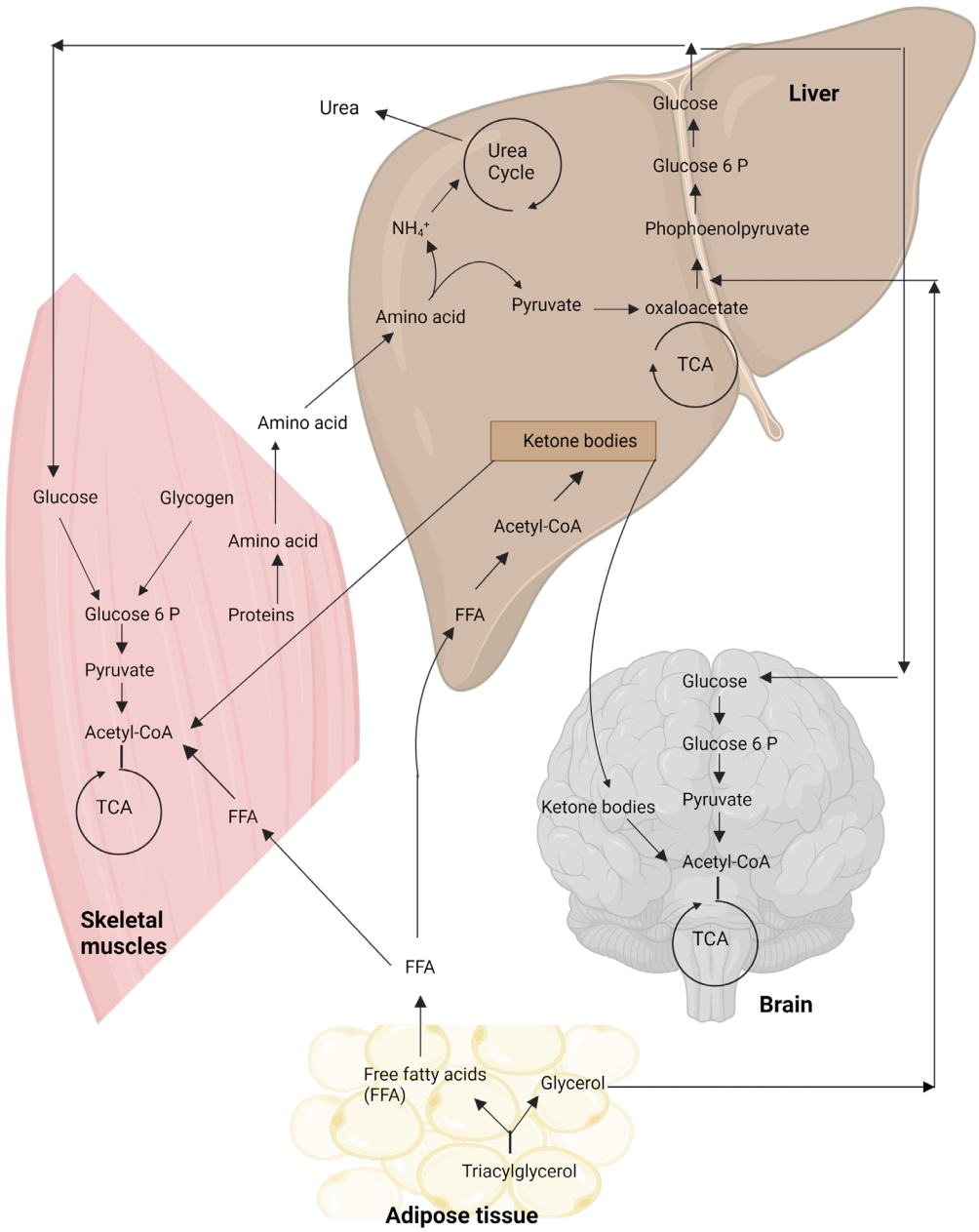


Table 18.9 Cross talk between pathways and organs under fasting/ starvation. Image by Manjeet Kumari. Created with Biorender.com

Pyruvate + Glutamate ⇄ Alanine + α-ketoglutarate

The glucose-alanine process requires the enzyme alanine aminotransferase, which is found in muscles, liver, and intestines. Alanine is exported in the process to the blood and picked up by the liver, which deaminates it to release the amine for synthesis of urea and excretion. The pyruvate left over after the transamination is a substrate for gluconeogenesis. Glucose produced in the liver is then exported to the blood for use by cells, thus completing the cycle (Figure 18.8).

Diabetes: Type-I Diabetes mimics starvation state because in the absence of insulin there in no glucose in the cell. If diabetes go untreated then body will activate gluconeogenesis and soon TCA cycle intermediates will deplete resulting in channeling acetyl-CoA to make ketone bodies. This leads to ketosis and acidosis. In addition, oxidation of amino acids for the source of energy will lead to high level to ammonia which causes comatose state and cerebral edema.

The cross talk among various pathways and organs on the next page shows how body manages fasting/starvation state.

We finish this book with gratitude to two Canadian scientists, Dr. Frederick Banting and Dr. John Macleod, whose discovery of insulin has saved millions of lives. If someone is diagnosed with type 1 diabetes today then it is no longer a death sentence.

Resources:

Chapter page: Binding of insulin to the insulin receptor leads to glucose uptake into the cell Adobe stock #277364367 licensed.

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Fig 18.8 Glucose-alanine cycle along with Cori cycle. https://en.wikipedia.org/wiki/Cahill_cycle

Fig 18.9 Created with BioRender.com (2023).

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