3. PRINCIPLES OF CHEMISTRY

...every day is bringing us to look more and more to chemistry to explain the physiology of our own bodies.

- Peter Mere Latham

Chemistry is the branch of knowledge that deals with the composition of substances, the relation of properties to composition, and interactions among substances. There is a certain overlap of chemistry with physics, because each of these substances are present in the physical world and subject to the world's physical laws. However, much of what distinguishes chemistry from physics is the attention given to electrons and their energies in the field of chemistry. It is the interactions among electrons from different elements that cause compound materials to form.

The reason to study chemistry is to understand many of the basic functions of life. General chemistry is the study of the interactions among different substances, especially those involving the electrons whizzing around the nuclei of those substances. Biochemistry relates more to configurations of molecules, and how they interact because of their shapes. Physical chemistry is the field that considers physical environmental effects, especially temperature, pressure, and interactions among mixtures of compounds on chemical activities. All of these are important in the understanding of intracellular processes and of the responses of the living entity (the biological unit) to its physical, chemical, and biological environs (Figure 3.0.1). In this

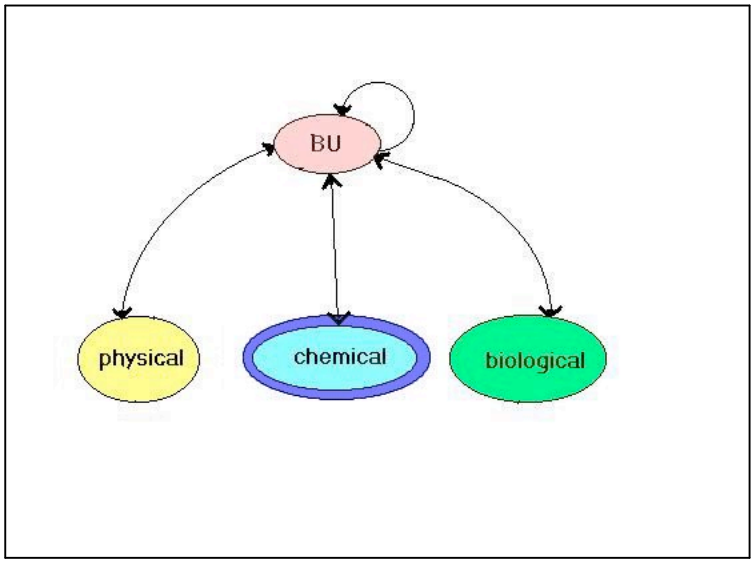


Figure 3.0.1. The biological unit (BU) is affected by interactions with its physical, chemical, and biological environment. Likewise, environmental elements are affected by the BU. Self-adjustment is also a possibility. The chemical environment determines many basic responses.

chapter, we consider some of the details of chemical processes important for life and important to understand how living systems can be manipulated. Such is the fundamental interest of a biological engineer.

Relevant chemical principles are:

1. *There is a periodicity of properties of elements, and these properties are related to numbers of electrons and their energy states in elemental atoms.* A classification of elements is thus possible, based upon similarities and differences. Biological substitution of one element for another is thus possible when the preferred element is scarce, as explained in Sections 3.1 and 3.2.

2. *Elements can combine to form compounds with different properties.* Compounds, especially those based on carbon, are the basis for most of the biochemical complexity of living things. See Sections 3.2 and 3.6.

3. *Molecular configurations determine usages.* Highly complex molecular structures may be formed when many atoms representing various elements are joined together. Particular configurations are created when locally unbalanced electrical charges either attract or repel other charges, either in the same compound or in neighboring compounds. Functions of many biochemicals depend on the ways in which they physically fit together with other compounds. See Section 3.7.

4. *Chemical reactions occur spontaneously when they yield energy to the environment.* These reactions are called exothermic, or heat generating, reactions. They are important because they are the sources for energy in biological systems. Certain compounds can act as chemical energy stores, and the energy can be made available for other uses. Especially important are those chemical reactions that require energy *from* the environment to proceed, as explained in Sections 3.4, 3.9, and 3.11.

5. *Reaction rates depend upon reactant concentrations, temperatures, and pressures.* In order for a chemical reaction to occur, there must be contact between the reactants, and the contact must be energetic enough to overcome atomic repulsion. Biochemical reactions are notable because compounds such as enzymes bring reactants in close proximity and increase local concentrations, thus promoting energetic contact. Temperature increases molecular kinetic energy and pressure increases concentration. See Sections 3.5 and 3.10.

6. *Intermediate reactions are most important to living things.* This includes intermediate energy and intermediate rates. Fast reactions, or

ones that deliver too much energy, or those with products that are too stable, cannot be readily controlled. Slow reactions use enzymes to speed them. A common means has not been developed to slow fast reactions. Biochemicals cannot be too basic or too acidic. Weak acids/bases can be buffered readily; strong acids and bases cannot, and so tend to extremes. See Sections 3.4, 3.7, 3.9, and 3.10.

3.1 Periodic Nature of Elements

To say…that a man is made up of certain chemical elements is a satisfactory description only for those who intend to use him as a fertilizer.
- Herbert J. Muller

When the elements are arranged by increasing mass, it soon becomes apparent that there is a periodic nature to elements with similar properties. This periodicity is even more readily apparent if the elements are arranged according to increasing number of electrons they possess (Figure 3.1.1). For instance, most elements react with other elements, but there are some like helium, neon, argon, krypton, xenon, and radon, with 2, 10, 18, 36, 54, and 86 electrons, that do not react. Each of these exhibits similar physical properties as well; each of these is a gas, for instance. The elements that directly follow the inert gases: lithium, sodium, potassium, rubidium, cesium, and francium, with 3, 11, 19, 37, 55, and 87 electrons, are all metals with shiny surfaces and are good conductors of heat and electricity. They are called the alkali metals because they all form solutions in water that counteract acids.

The elements that directly precede the inert gases: fluorine, chlorine, bromine, iodine, and astatine, with 9, 17, 35, 53, and 85 electrons, also resemble each other. They are nonmetals, poor heat and electric conductors, and form salts when reacted with the alkali metals. Because of this, these elements are called *halogens*, or salt-formers. The halogens form acidic solutions in water.

The elements that fall between the alkali metals and the halogens show a progressive gradation of properties between the two extremes. For instance, the elements magnesium (12 electrons), aluminum (13), silicon (14), phosphorus (15), and sulfur (16), which fall between sodium (11) and chlorine (17) show a decrease in metallic character and an increasing tendency to form less alkaline and more acidic solutions in water.

Those elements that lie midway between the alkali metals and the halogens are special to the life sciences. These elements: carbon (6), silicon (14), germanium (32), tin (50), and lead (82), include elements that have neither strong metallic nor strong alkali properties. The elements silicon and germanium are especially important in the electronics industry because, as semiconductors, they have controllable electrical conduction properties. Carbon is the basic element for all known life.

The periodic table of the elements is an arrangement of elements that accents the regular recurrence of similar physical and chemical properties of

the elements. It shows elements that are in the same family, or group, with similar properties, and it shows the different periods, with gradations of physical properties from the alkali metals to the halogens. While the periodic table does not explain all property variations and is more complicated than expected in certain transition regions, it is a good means to classify general chemical properties in an organized way.

Families (or groups) of elements are arranged vertically in the periodic chart. These are the elements with similar chemical and physical properties, although these properties weaken from top to bottom. Elements at the top of the chart are lighter and less dense; elements at the bottom have greater masses and are more dense. Elements arranged horizontally form a recurring

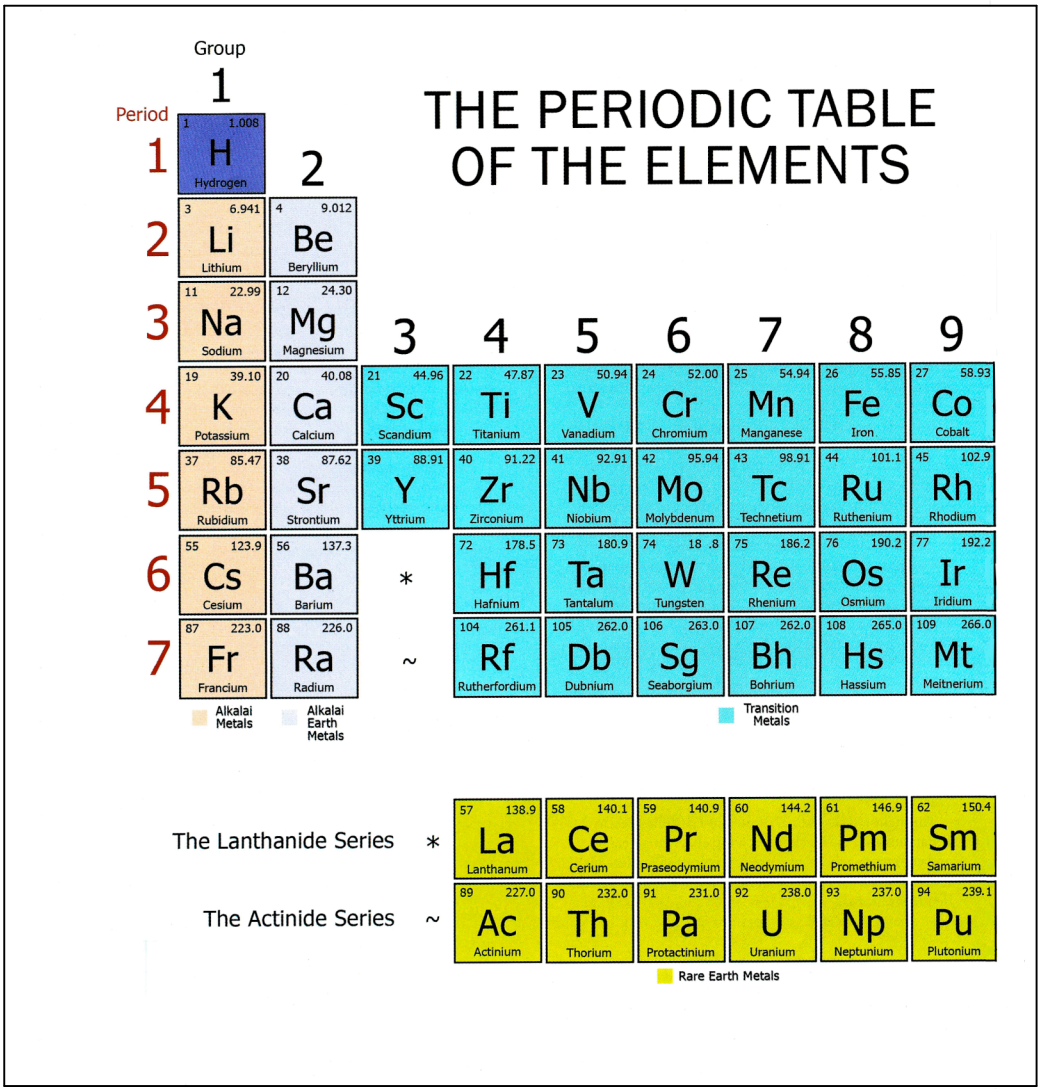


Figure 3.1.1. Periodic chart of the elements.

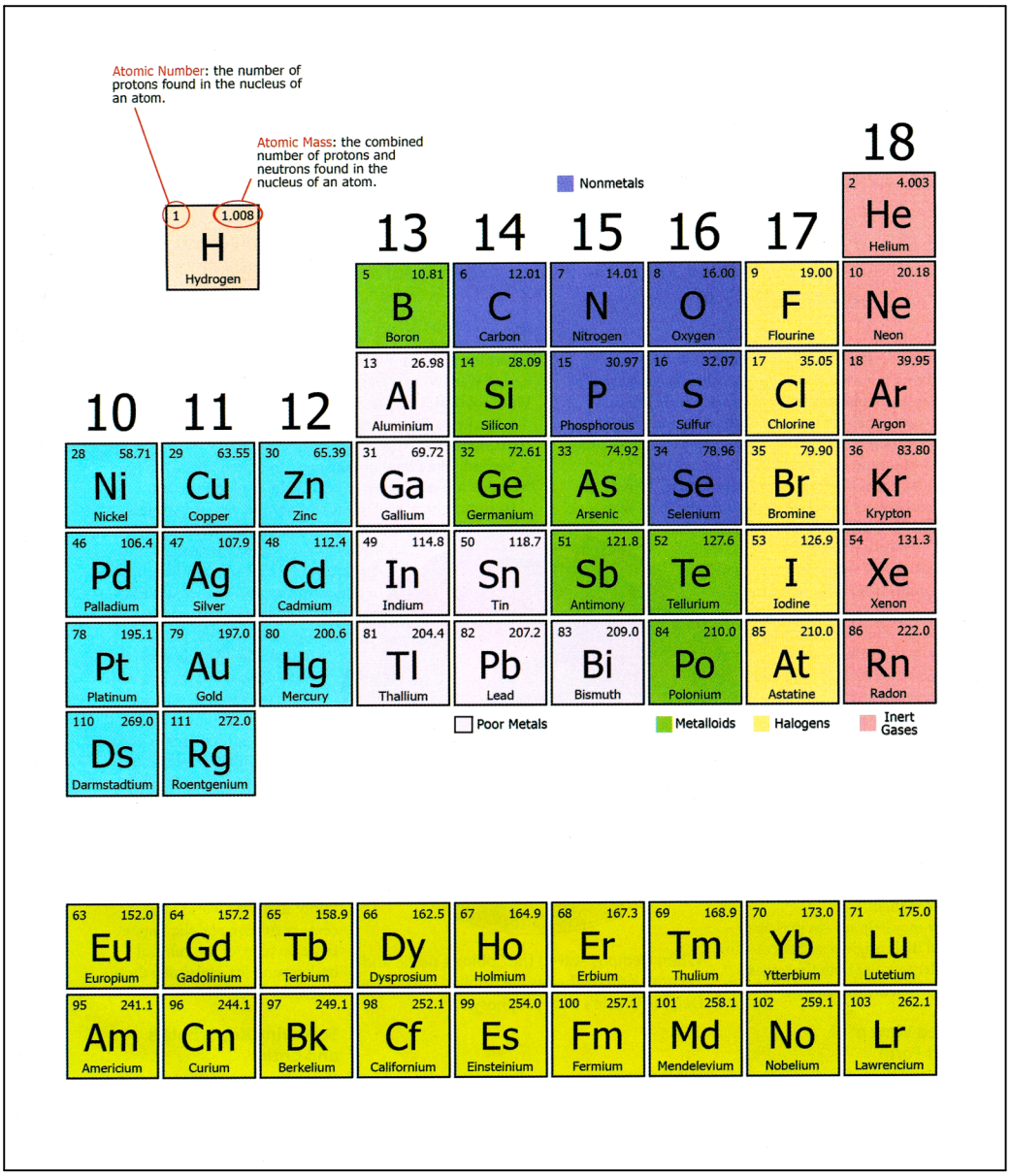


Figure 3.1.1 (cont.). Periodic chart of the elements.

pattern of transition from highly alkaline to highly acidic natures. Elements most chemically active are those at the right and left edges of the chart; those in the middle do not react with other elements very vigorously. The last group at the far right of the chart has elements that are completely inert chemically. They do not take part in chemical reactions, so they are neither necessary nor harmful for life. Elements heavier than atomic number 53 (Iodine) are

apparently not used in living things (see Section 6.3.1), and elements heavier than 92 are not found in nature.

It sometimes happens in living systems that, when the primary element is not immediately available, a substitution can be made with an element with similar properties. Thus, an element from the same group can sometimes be substituted for an element not readily available. For instance, strontium (38) can sometimes be found to substitute for calcium (20) in the bones when strontium is more available than calcium. Radioactive strontium isotopes are sometimes released in nuclear accidents so the incorporation of strontium into the bones is a cause for concern. The relative stability of lead compounds in the body is the result of its appearance in the same group as carbon and silicon. However, lead has toxic neurological effects that make it a dangerous element.

Lead incorporated into the body can have severe effects on the kidneys and the nervous system. Over time, an excess amount of lead leads to a decline of health and to neurological disruptions. It is thought that one of the contributing causes to the decline of the Roman Empire was the widespread use among the upper classes of lead water pipes. Over time, the ruling classes became less able to rule, less vigorous, and the civilization declined.

Arsenic (33) is dangerous because it can substitute for phosphorus (15), and phosphorus is nearly everywhere in living things.

Selenium is a micronutrient required by grazing animals. Sheep require 0.1 ppm (parts per million) in their total diet, with 0.3 ppm being better, and levels over 3 ppm being toxic. Forage plants do not require selenium at all, but because selenium is chemically similar to sulfur (being in the same chemical family), plant roots will absorb it from the soil and incorporate it into amino acids in place of sulfur. When selenium is present in the soil in large enough quantities, forage plants supply the selenium needs of the sheep grazing on them.

The periodic nature of chemical properties is explained by the distribution of energy levels of the electrons found outside the nucleus of each atom. Quantum mechanics successfully predicts the discrete energy levels of these electrons and the number of electrons that possess these levels of energy (Figure 3.1.2). For instance, only two electrons can have the lowest level of energy, eight electrons can have the next lowest level, and 18 electrons can have the third lowest energy level. The maximum number of electrons for these atoms is 2 (lowest energy level filled), 10 (2 at the lowest level plus 8 at the next level), and 28 (2 at the lowest level plus 8 at the second lowest level plus 18 at the third lowest level). If the number of electrons at each of these levels is equal to the maximum, then the elements exhibit inert properties; the inert gas helium has 2 electrons and neon has 10.

Thereafter, things become more complicated because there are many higher electron energy levels and each of these has sublevels. But the chemical properties of an element are mainly determined by the number of electrons at the highest energy level. There is a tendency for atoms to complete the maximum number of electrons at the highest energy level for

that element. Thus, if an atom has 2 electrons at its lowest energy level and 7 electrons at the next energy level, which is the highest energy level for that atom, then the atom tends to want to combine with another atom that has 1 electron at the highest energy level. The addition of that 1 electron completes the maximum number (8) of electrons at the second energy level for the first element (Figure 3.1.3). In this combination, both atoms are able to achieve the desired number (8) of electrons at the highest energy level for each atom. This combination involves fluorine (2 + 7 electrons) and lithium (2 + 1 electrons).

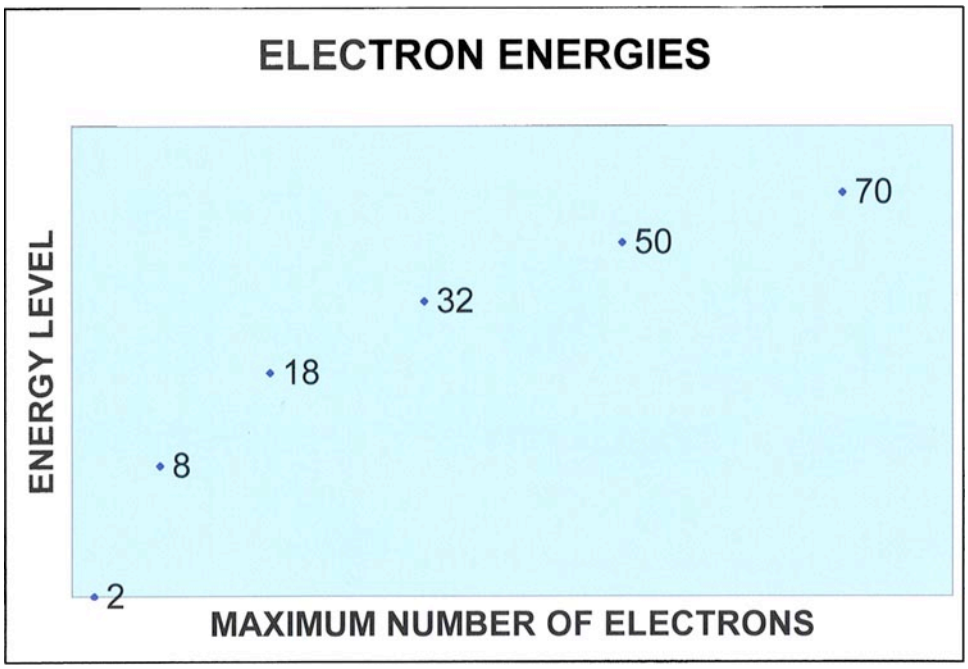


Figure 3.1.2. The maximum number of electrons at different energy levels is fixed. There are no allowable intermediate energy levels.

In a similar fashion, an atom of oxygen, with 6 highest energy level electrons combines readily with beryllium with 2 highest level electrons. Each atom then has 8 electrons in its highest energy level. The same result could be achieved among oxygen (2 + 6 electrons) and two lithium (2 + 1 electrons) atoms.

Similar chemical properties are found whenever an element requires the same number of electrons to complete its highest energy level. Thus, fluorine, chlorine, bromine, iodine, and astatine all lack 1 electron from completeness. Lithium, sodium, potassium, and rubidium each have 1 extra electron. Each of these families of elements exhibits many similar properties among the individual elements. They are not all alike, however, but they do share many properties.

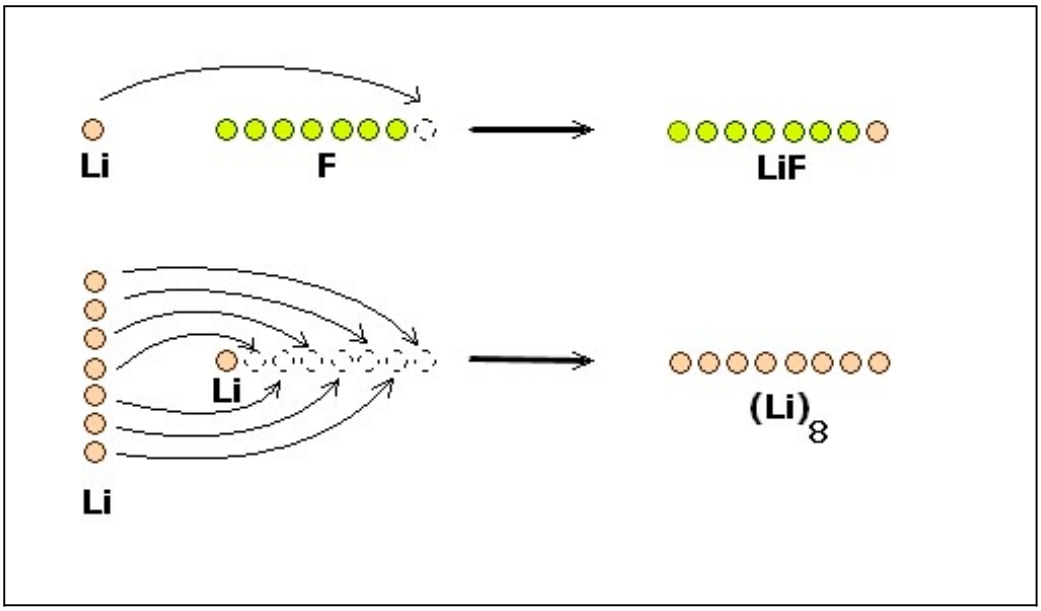


Figure 3.1.3. The addition of one electron at the highest energy level to seven electrons at the same level results in 8 electrons, a very stable configuration. Eight electrons can be satisfied by Lithium (Li = +1) and Fluorine (F = +7, or –1) together, or by 8 Li atoms.

The Mole

Atoms and molecules are extremely small and difficult to deal with on a one-to-one basis. Then, too, there are attractions, repulsions, or other interactions among these units that make it difficult to treat them as independent. Thus, there is a clear advantage to grouping atomic or molecular units into larger parcels.

Chemists have long agreed that the sizes of these parcels should have a mass value that scales from the molecular mass (number of protons and neutrons in the nuclei of the atoms making up the molecule). The mass scale used is left to the discretion of the individual chemist. Thus, mass units commonly used are pounds, grams, and kilograms; others may also be used.

A mole is the mass of a substance in grams that corresponds to its molecular mass, expressed in grams. Thus, a mole of water (H_2O) has a mass of 18 grams (2H = 2, 1O = 16, total molecular mass = 16 + 2 = 18). This mole may also be called a gram-mole for clarity.

A kilogram-mole is the mass of a substance in kilograms that equals the molecular mass (still expressed in grams) of the substance. A kilogram-mole is 1000 times as large as a gram-mole. A pound-mole is 454 times as large as a gram-mole.

The Mole, cont.

There is a definite number of atoms or molecules in a mole. This is called the Avagadro number and is equal to 6.0235×10^{23}. This many molecules or atoms are in a sample containing 1 mole of a substance. A sample with one kg-mole would contain 6.0235×10^{26} atoms or molecules.

Valence is the term given to the excess number of electrons that an element needs to complete its highest energy level. Thus, sodium has a valence of +1, whereas fluorine has a valence of –1. Fluorine could also be assigned the valence of +7, but this is a very unlikely state for fluorine. Sodium could also be given a valence of –7, but, again, it is unlikely that sodium would contribute 7 electrons to complete the highest energy level of another element. It is much more likely that it would complete its own highest energy level by donating its excess electron to another element with valence of –1.

For elements that appear in the middle of the Periodic Table, valence levels are +3, +4, or +5. These elements may appear to be able either to donate or accept electrons. Thus, phosphorus may have a valence of either –3 (most likely) or +5 (less likely). Carbon, with a valence of either +4 or –4 can act as either an electron donor or acceptor. This property makes carbon a very versatile element and we shall see later how this makes carbon special to life on Earth.

Applications and Predictions

1. There will be many biochemical variations that differ in one element substituting for another similar element.
2. Elements with higher numbers of electrons will usually have larger physical sizes than elements with smaller numbers of electrons. Larger sizes will usually mean that they are not as mobile.
3. Ionic charge of elements can be predicted by the location of an element on the Table of Elements. Elements on the left will tend to form positively charged ions and elements on the right will form negatively charged ions.
4. Elements with higher numbers of electrons, and protons in their nuclei, will be larger and less able to penetrate cell membranes.
5. Properties of a certain element can be inferred from its position in the Periodic Table of Elements.

3.2 Chemical Bonding

Slowly, slowly, slowly we oxidize!
Become old and rusty,
Fungoid and musty,
Diminish in size;
Reputation decreases and self-conceit ceases –
Cares fret and wear out facial lines incessantly,
Yet doctors grow old rather pleasantly!

- Isaac N. Himes

There are several ways that atomic elements bond together to form molecular compounds. The end result of most of these chemical reactions is that the atoms that participate end up with 8 electrons (an octet) in their uppermost energy levels. This electron octet appears to be a very stable atomic configuration.

3.2.1 Ionic Bonds

My name is Bond, Ionic Bond; Taken, not shared!

-Caren Thomas

The most stable compounds formed are those where electrons are transferred from an electron donor atom to an electron acceptor atom. Energy is required to remove an electron from a neutral atom to form a positive ion (called a *cation*). Energy is released from a neutral atom when it forms a negative ion (called an *anion*). If the electron affinity energy of one atom is greater than the energy required to strip electrons from another, then the reaction proceeds spontaneously, producing excess energy in the form of heat. If the energy required to strip one or more electrons is greater than the electron affinity energy, then the reaction will not proceed spontaneously.

The alkali metals have low energy requirements for electron removal. This is because removal of the one excess electron leaves a stable 8 electrons at the highest energy level. It is thus relatively easy to form cations from this group with a positive charge. An example of this is sodium, which forms a cation with charge of (+1) according to the following chemical equation:

$$Na + 5.1\ ev = Na^{+} + e^{-} \qquad (3.2.1)$$

where Na is the symbol for sodium (from the Latin name Natrium), Na^{+} is the symbol for a sodium ion with (+1) charge, and e^{-} is the symbol for an electron with a negative charge. The 5.1 ev term is an energy unit of 5.1 electron-volts (each ev is equivalent to 3.8×10^{-20} cal). Without the energy term on the left hand side of the equation, both sides would not be equivalent.

Metals with two electrons at the highest energy level (valence of 2) also have relatively low energy requirements to remove the two excess

electrons to form cations with (+2) charge. The halogens, with 7 excess electrons, have high energy requirements to remove all 7 electrons, but release relatively large amounts of energy when an electron is added to produce the stable octet. This forms ions with (–1) charge, as illustrated by chlorine:

$$Cl + e^- = Cl^- + 3.75 \text{ ev} \qquad (3.2.2)$$

where the symbol Cl denotes a chlorine atom, Cl^- denotes a chloride ion with a (–1) charge, and e^- denotes an electron with (–1)charge. Note that 3.75 ev of energy is liberated by this action.

Elements with 6 excess electrons (such as oxygen and sulfur) are similar to the halogens, but form anions with (–2) charge.

When an Na^+ and a Cl^- contact each other, they form NaCl, or common table salt, that releases enough energy that the entire process of stripping an electron from the sodium atom, adding an electron to the chlorine atom, and combining the two resulting ions into an NaCl molecule (a process that liberates energy) results in the liberation of considerable amounts of heat. This type of chemical bond is called an *ionic bond*.

In terms of effort and flow variables, electron affinity represents one effort variable, electron removal energy represents a second effort variable, and combination energy represents a third effort variable. The flow variable is liberated heat. If the sum of the three effort variables is positive, heat is liberated; if the sum is negative, then heat is absorbed. Resistance, or the ratio of effort to flow variables, is proportional to the spontaneity of the chemical reaction.

Ions are very important in biological processes because they represent the charge carriers in biological organisms. In the chemical soup inside biological entities, electrical current is carried by ions rather than by solitary electrons.

Redox Reactions and Electron Transfer

A biologically very important class of chemical reactions is called reduction-oxidation, or *redox* for short. In these reactions, electrons are transferred from one reactant to another:

$$A^+ + B \rightarrow A + B^+$$

Here an electron is transferred from an atom of B to a cation A^+. In the process, A^+ is said to be *reduced* and B is said to be *oxidized*.

Oxygen is one of the most common (although not the only) oxidizing agents. When oxygen is added to another atom, it forms an oxide, and the other atom is said to be oxidized:

$$4\,Fe + 3\,O_2 \rightarrow 2\,Fe_2\,O_3$$

Redox Reactions and Electron Transfer, cont.

Here, iron is oxidized to form iron oxide. Each iron cation in the molecule carries a charge of +3 and each oxygen anion in the molecule has a charge of –2.

Removing the oxygen means reduction. If the above chemical reaction were to be driven backwards, for instance by the addition of a lot of heat energy to the iron oxide, elemental iron would be formed and the iron would be said to be reduced.

Although oxygen was recognized as the first oxidizing agent, it is not the only such substance. Other common oxidizing agents include sulfur, phosphorus, and chlorine. The common property of each of these is that they lose electrons in the process. So, generally speaking, when a substance (such as the aforementioned iron) loses electrons, it is said to be oxidized. When the substance gains electrons it is said to be reduced:

$$\text{electrons} \xrightarrow{\text{lost}} \text{oxidation, positive charge increases}$$

$$\text{electrons} \xrightarrow{\text{gained}} \text{reduction, positive charge decreases}$$

An *oxidizing agent* is a substance, such as oxygen, that can oxidize something else. A *reducing agent* is the substance that can reduce something else. In every redox reaction, there is always at least one oxidizing agent and one reducing agent. The oxidizing agent is reduced and the reducing agent is oxidized. In the iron example,

$$4\,Fe + 3\,O_2 \rightarrow 2\,Fe_2O_3$$

the oxygen is the oxidizing agent for iron, and the iron is the reducing agent for the oxygen.

Hydrogen is a common reducing agent because it is an electron donor. When hydrogen is added to a molecule, an electron is transferred from the hydrogen atom to the molecule: the molecule is reduced and the hydrogen is oxidized (even without the presence of oxygen).

NAD (nicotinamide adenine dinucleotide) is a coenzyme common in respiratory chemical reactions. The oxidized form is NAD, and the reduced form is NADH. This illustrates that removal of a hydrogen atom is in itself an oxidative process.

Summarizing, then,

a substance that loses electrons is oxidized;

a substance that gains electrons is reduced.

3.2.2 Covalent Bonds

New functions come from new molecules. *-Bruce Yu*

The second type of chemical bonding is the *covalent bond*, which occurs whenever one atom is not strong enough to cause an electron to shift completely from one atom to another. In that case the two (or more) atoms share the electrons needed to complete their octets. Such covalent electron sharing occurs when two atoms of the same element combine, as with O_2, N_2, or H_2. Covalent bonding is especially important for the elements in the middle of the periodic table, those with 3, 4, or 5 electrons at their highest energy level.

Covalent bonds vary between the extremes of equal sharing of electrons to nearly complete donor-acceptor ionic bonding. Depending on the equality of sharing and the geometrical configuration of the molecule, a molecule may exhibit an unequal charge distribution from one side to the other. Such a molecule with a positive end and a negative end is called a *polar* molecule.

3.2.3 Electronegativity

To the ass, or the sow, their own offspring appear the fairest in creation. *-Latin proverb*

Electronegativity is the term used to describe the relative affinity of an atom to attract shared electrons. The higher the electronegativity of an element, the more tightly will electrons be bound to that type of atom. Many different energies contribute to the electronegativity, including energies associated with the nuclear charge, the size of the nucleus, and partial shielding of nuclear charge by other electrons.

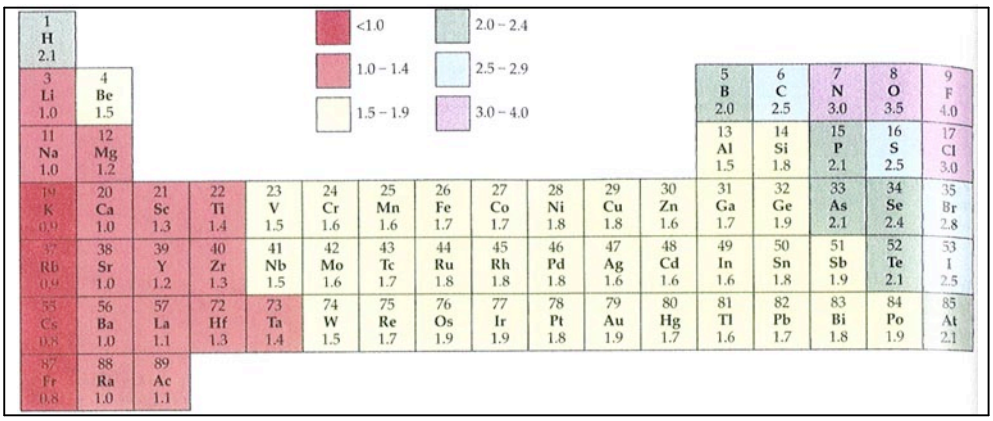


Figure 3.2.1. Table of electronegativities. Elements with much different electronegativities will tend to have high ionic affinity for one another. Elements with similar electronegativities will tend to share electrons.

In general, electronegativities increase from left to right in the periodic table of the elements (see Section 3.1), and decrease from top to bottom (Figure 3.2.1). Inert elements placed on the far right of the table have electronegativities of zero.

Elements with low electronegativities (but not zero) have relatively low electron affinities, which means that they can lose electrons readily. Elements with high electronegativities hold tightly to their electrons, and can readily attract electrons from other elements. Thus, two elements with very different electronegativities would be expected to form ionic bonds where electrons are donated by the low electronegative element to the element with high electronegativity. Furthermore, this combination would be expected to be very polar, with excess electrons located at the position occupied by the more electronegative atom.

Elements with nearly equal electronegativities would be expected to form covalent bonds, and share their electrons nearly equally. A molecule with this combination would be expected to be nonpolar.

3.2.4 *Water As a Polar Molecule*

Money is the best cosmetic. *-Gregory Nunn*

Water is a polar molecule (Figure 3.2.2). There is an angle of 104.5^{o} between the two hydrogen atoms with the oxygen atom at the vertex. The covalent bonds in the water molecule result in the oxygen atom being negative and the hydrogen atoms being relatively positive. If there were 180^{o} between the hydrogen atoms with the oxygen atom in between, then there would be a positive charge on each end of the water molecule, and the molecule would be *nonpolar*. Such is the structure of carbon dioxide, which is nonpolar. Water, however, does not have a 180^{o} angle between the hydrogen atoms and is polar.

Polarity is important in biological systems, because polar molecules are sometimes attracted to other polar molecules on the surface of cells, and helps to bind these together. When this happens, we call the surface *hydrophilic*, meaning that it attracts water and other polar substances.

When several water molecules are brought together, there is an attraction between the negative oxygen end of one molecule with the positive hydrogen end of another. Unlike charges attract each other. The result is that water tends to be structured even as a liquid. The hydrogen atoms arrange themselves as a tetrahedron around each oxygen atom. In a container with vast numbers of water molecules this structure continues in three dimensions.

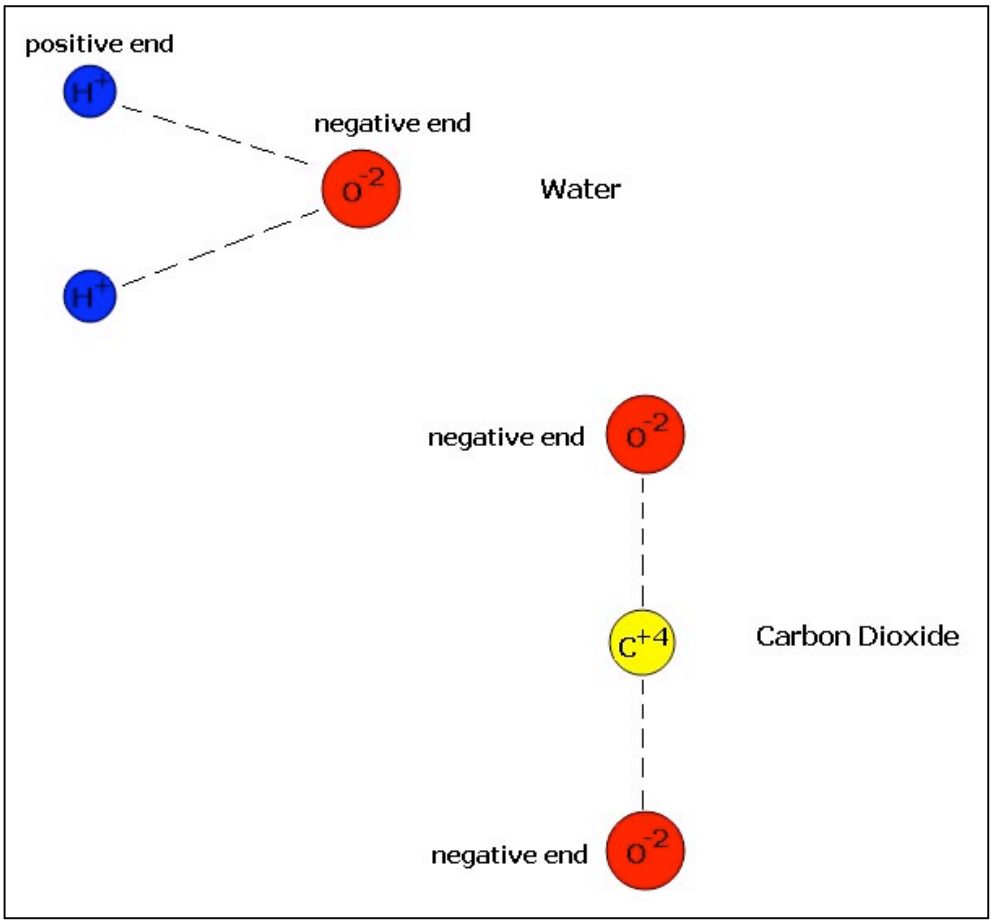


Figure 3.2.2. Water as a polar molecule. Carbon dioxide as a nonpolar molecule.

Ice is a crystal with this solidified structure (Figure 3.2.3). The arrangement of ice is such that it is honeycombed with hexagonal channels that gives ice its relatively low density. When ice melts, the structure becomes less orderly but is not completely destroyed. The arrangement of tetrahedra is more random and constantly changing. Nonetheless, some of the structure still remains.

It is fortunate for life that solid ice has a lower density than liquid water. Life can exist in warmer water beneath a frozen layer of colder ice that floats above because of its lower density. If ice were more dense, the water would be exposed to colder environments and would certainly all freeze, killing many of the organisms suspended in it.

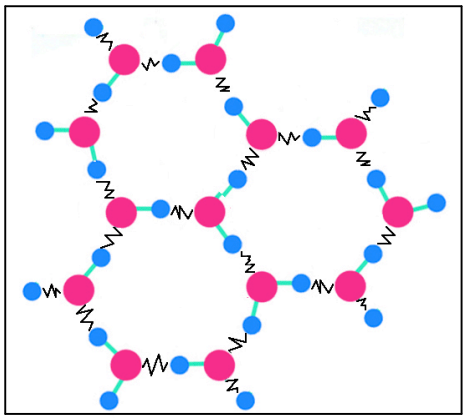


Figure 3.2.3. Two-dimensional representation of the structure of ice. Each oxygen atom is covalently bound to two hydrogen atoms and hydrogen bonded to two additional hydrogen atoms. The resulting structure is a tetrahedron not able to be shown completely in two dimensions. Ice has a hexagonal cellular structure that is relatively low density compared to water.

3.2.5 *Hydrogen Bonds*

A world that includes the Human Element, along with hydrogen, oxygen and the other elements, is a very different world indeed. Suddenly, chemistry is put to work solving human problems. Bonds are formed between aspirations and commitments. And the energy released from reactions fuels a boundless spirit that will make the planet a safer, cleaner, more comfortable place for generations to come.

-Dow

Positive hydrogen ions can act as bridges between two negative atoms, as they do between two oxygen atoms in water. This bridging is called a *hydrogen bond*, and is the result of polarities in molecules. Hydrogen bonding occurs in other molecules besides water, but the hydrogen bond is only found between two electronegative atoms. The result of hydrogen bonding, besides the structure already discussed for water, is a higher boiling point than would otherwise be expected. The structures of proteins, too, depend on hydrogen bonds that bridge between nitrogen and oxygen atoms.

Hydrogen bonding is what gives liquid water its structure. When a solute is introduced into liquid water, considerable energy is required to tear water molecules apart from their neighbors in order to make room for solute particles. Hydrogen bonds can be broken between water molecules and established between a water oxygen atom and a portion of a solute molecule. In the case of ammonia (NH_3), a hydrogen bond is established between the N of NH_3 and the O of H_2O. A hydrogen bond between the O of C_2H_5OH (ethanol) and the O of H_2O, and a hydrogen bond between the O of $C_{12}H_{22}O_{11}$

(sucrose) and the O of H_2O allow ethanol and sucrose to dissolve readily in water.

Many solutes, such as the ethanol and sucrose mentioned above, exist in solution as whole molecules. They are assisted in becoming dissolved solutes by the hydrogen bonds that form between them and water molecules. Otherwise, they would not liberate enough energy to break apart the structure of liquid water; they would not dissolve; and they would remain separate from the water.

Other solutes, called *electrolytes*, consist of molecules that dissociate to form charged ions in aqueous solution (they do not dissociate unless water is present). Thus, HCl is found in water as H^+ and Cl^- ions; NaCl is found in water as Na^+ and Cl^- ions. The polar water molecule becomes attracted to these ions, the negative oxygen end to the H^+ ions (to form a H_3O^+ complex) and the positive hydrogen end is attracted to the Cl^- ions.

Water is thus attached to each ion because of the electropolar nature of the water molecule and the charged ion in solution. Water is always associated with any dissolved species and will affect its properties. There may be an amelioration of the effects of ionic charge on its surroundings because of the shielding effect of the water molecules surrounding the ion. There is always a size enhancement to any dissolved particle because the surrounding water clings to each dissolved particle. For example, a bare hydrogen ion is nothing but a proton of essentially zero size. The H_3O^+ ion, which is the form that H^+ ions actually take, has a volume about 10^{15} times bigger than H^+, and is comparable in size to other ions.

Because solutes disrupt the normal pattern of water structure, solutions have freezing temperatures lower than that of pure water (Feeney, 1974) and solutions have boiling temperatures higher than that of pure water. Differences in freezing and boiling points are determined almost solely by the number of solute particles present and not by their properties. Totally dissociated compounds, such as table salt (NaCl), will have twice as much freezing point depression and boiling point elevation as will nondissociated compounds such as sucrose. Incompletely dissociated compounds will have intermediate effects.

3.2.6 *Van der Waals Forces*

Every artist was first an amateur. *-Ralph Waldo Emerson*

Even nonpolar molecules form attractive forces among themselves. Due to the nonstationary nature of electrons moving about the nuclei of the atoms making up the molecule, polarities may be temporarily formed in what over a long time may be considered to be a nonpolar molecule. When this happens, it influences electron positioning in neighboring molecules. Electrons repel electrons; protons attract electrons. Attraction between

molecules occurs when polarities among neighboring molecules become more or less synchronized. These forces are called *van der Waals forces*.

Van der Waals forces are present in all molecules. They are relatively weak attractive forces, certainly weaker than covalent bonds, which, in turn, are weaker than ionic bonds. Higher temperatures, indicating more molecular kinetic energy, weakens the relative strength of van der Waals forces, but higher pressures, pushing molecules closer together, increases their relative strength.

Geckos' Feet

Geckos are lizard-like creatures that can scamper up walls and hang from ceilings. Whether the surface is made from wood, metal, or glass, the gecko can climb it. What enables the gecko to do this? Is it glue, suction, or a thin film of water? None of these, as it turns out.

The gecko has feet with millions of microscopic hairs sprouting from the bottom. The hairs, called retae, split into as many as 1000 hairlets, each capped with a triangular bacterium-sized pad called a spatula (the shape is reminiscent of a hamburger flipper). These spatulae contact the climbing surface and induce van der Waals forces. Each spatula is capable of supporting the weight of an ant. Together, the spatulae could theoretically hoist a 130 kg man.

Although van der Waals forces are very weak, enough of them together serve the gecko very well. And now that the secret of the gecko's climbing is known, new glues can be made with artificial spatulae (Autumn et al, 2002).

3.2.7 *Metallic Bonding*

Every absurdity has a champion to defend it. *-Goldsmith*

A last type of bonding is *metallic bonding* in which the atomic electrons are so weakly held to the atomic nuclei that they essentially form an electron cloud shared by all nuclei. The attraction of the positive nuclei for the shared electrons is the force that holds the metal crystal lattice together. Metallic bonds are not important within living creatures.

Example 3.2.1 Wastewater Bioreactor Troubleshooting

Municipal wastewater can be treated biologically in a bioreactor, consisting of a basin containing wastewater and activated sludge (solids with active microbes). In a batch mode, 1) the reactor is filled with wastewater, 2) the mixture of wastewater and sludge is aerated, 3) the contents are allowed to settle, 4) effluent is decanted and some sludge is removed, and 5) the system idles until the next cycle. Sometimes there are problems with anoxic conditions, odor, low capacity, and poor effluent quality. What are possible causes and solutions for these problems?

Solution: All of these problems are related, and solving them can be counterintuitive. One possible solution would be to clean the bioreactor and begin again, but this may be very expensive and not entirely satisfactory.

Anoxic conditions are caused by a large demand for oxygen in the wastewater. Demand can be caused either by biological substances (BOD, or biological oxygen demand), or by chemicals (COD, or chemical oxygen demand). Adding more wastewater will not dilute BOD or COD, and may make matters worse. The solution to this problem is to lengthen the step 2 of the cycle.

Anoxic conditions favor sulfur instead of oxygen as an oxidizing agent. Hydrogen sulfide (H_2S) is formed that has a rotten egg odor. Raising pH reduces the concentration of H_2S compared to other sulfide species, thereby reducing odor.

Temperature has a profound effect on bioreactor performance. The rate at which gases, such as oxygen, can be dissolved decreases rapidly as temperature increases; chemical reaction rates increase exponentially with temperature; thermal agitation increases water turbidity as temperature increases. Because of these, anoxic conditions are more likely to occur at higher temperatures.

The solutions to these problems are, consequently, to improve aeration, monitor pH, and cool the bioreactor (Marx et al, 2003).

Example 3.2.2 Natural Halocarbons as Bioactive Compounds

In the search for useful biochemicals naturally produced by plants and animals, the class of halocarbons is still in the process of being explored for exploitation. These compounds, combining one or more halogens with carbon, are usually quite stable and have interesting uses. Some of these compounds are already in use and are synthesized industrially. Others are still being found and uses are being determined. Some of these compounds are (Gribble, 2004):

compound	produced by	use
brominated dioxin	sponges, corals, seaweeds	prevent barnacle growth
panacene	sea hare	taste discourages being eaten
methyl bromide	cabbage, broccoli, turnips, canola, evergreen trees, potatoes	natural pesticide
fluoroacetic acid	Australian and African plants	poisonous to grazers
thyroxine	many vertebrates	metabolic regulator
chloroform	termites	metabolic waste product
epibatidine	small frog	painkiller
vancomycin	?	antibiotic

Other compounds are finding uses as hormones, pheromones, toxins, bleaches, anti-cancer agents, anesthetics, and others. As more of these compounds become known, who knows what benefits might be derived from the effort?

Example 3.2.3 Detection of Skin Cancer by Classification of Raman Spectra

Malignant melanoma skin cancer is often lethal. Unfortunately, even experienced dermatologists misdiagnose this type of cancer 25-35% of the time.

Raman spectra are obtained by pointing a laser beam at a sample. Molecules in the sample become excited and scatter energy back to the probe. Frequency shifts in the spectra depend on the types of molecules present, and frequency shifts can be used as signatures identifying specific molecules.

Raman spectra can be used as inputs to neural networks for the purpose of diagnosing skin cancer types (Sigurdsson et al, 2004). Signals must be preprocessed to reduce variability and background from skin fluorescence. Although complicated, these steps improve the reliability of skin lesion classification.

Example 3.2.4 Nitric Oxide Messenger

Nitric oxide is a small gaseous molecule that is synthesized in many animals from barnacles to humans. It plays a role as a biological messenger with a broad range of physiological processes, including neurotransmission, blood clotting, blood pressure control, and immune system response.

Applications and Predictions

1. Compounds formed from ionic bonds will usually be extremely stable, and are rarely formed by living systems.
2. Polar molecules will be hydrophilic. Nonpolar molecules will be hydrophobic.
3. Water inside the cell will be structured, not free.
4. The expansion of freezing water will disrupt cellular structure and will kill the cells.
5. Compounds with hydrogen bonds will be firmer, slower to melt, and harder to boil than compounds without hydrogen bonds. The fats and oils deposited in the hands, where it is relatively cool, will have fewer hydrogen bonds (are said to be less saturated) than do fats and oils deposited in the center of the body.
6. Hydrated Na^+ will be much larger than hydrated K^+.
7. Organisms adapted to cold environments will use dissolved solutes as antifreeze compounds.
8. Water will be an excellent solvent for polar molecules or ions.
9. Polar compounds (like water) will not mix well with nonpolar compounds (like oil).
10. Chemical bonding controls the movement of energy from one compound to the next.
11. Energy can be stored by forming chemical bonds; energy can be released by breaking chemical bonds.
12. The strongest bonds in protein formation will be covalent bonds.
13. The most durable compounds found in living things will have the strongest chemical bonds; the compounds most likely to be manipulated will have the weakest bonds.
14. Elements with similar electronegativities will tend to form covalent bonds.

3.3 Chemical Equilibrium

The ultimate aim of the modern movement in biology is in fact to explain all biology in terms of physics and chemistry. *-Francis Crick*

A general chemical equation can be given by the form:

$$mA + nB = pC + qD + \text{energy} \qquad (3.3.1)$$

where m, n, p, and q are coefficients needed to balance elements in the compounds A, B, C, and D. The amount of energy may be either positive (*exothermic*, or heat producing) or negative (*endothermic*, or heat using). If all compounds are gases, then the *equilibrium constant* (K) for this equation equals the product of concentrations of the chemical products raised to appropriate powers divided by the product of concentrations of reactants raised to appropriate powers:

$$K = \frac{(\text{concentration of C})^p\ (\text{concentration of D})^q}{(\text{concentration of A})^m\ (\text{concentration of B})^n} \qquad (3.3.2)$$

where concentrations are normally given in moles per liter, and the exponent values are the same as the coefficients in the general equation (3.3.1). For instance, the oxidation of hydrogen to form water vapor,

$$2H_2 + O_2 = 2H_2O + 2.42 \times 10^5\ \text{N·m} \qquad (3.3.3)$$

has a chemical equilibrium given by:

$$K = \frac{(\text{concentration of water})^2}{(\text{concentration of hydrogen})^2\ (\text{concentration of oxygen})}$$

$$= 1.11 \times 10^{40} \qquad (3.3.4)$$

that defines the ratios of concentrations of products (H_2O) to reactants (H_2 and O_2). The large value of K means that almost no excess hydrogen and oxygen remain in the system; all are converted to water vapor as long as the original number of moles of hydrogen was twice that of oxygen.

If all reactants and products are in solution, then the equilibrium constant can assume several meanings. If the substance in solution is an acid or base that dissociates in solution, then the equilibrium constant becomes the *dissociation constant*. For example, some acetic acid molecules dissociate into hydrogen (H^+) and acetate ($C_2H_3O_2^-$) ions:

$$C_2H_4O_2 \leftrightarrow H^+ + C_2H_3O_2^- \qquad (3.3.5)$$

with a dissociation constant of 1.8×10^{-5} at 25°C:

$$K = \frac{(\text{concentration of } H^+)\,(\text{concentration of } C_2H_3O_2^-)}{(\text{concentration of } C_2H_4O_2)}$$

$$= 1.8 \times 10^{-5} \qquad (3.3.6)$$

Similar equilibrium constants have been found and tabulated for solubilities and precipitations.

Concentration Measures

Chemical concentrations are given in different units depending on the field of technology they come from. They can be converted from one to another, if need be, through suitable conversion equations given in Johnson (1999). Numerical values of equilibrium constants depend on the concentration units used, so the conventional concentrations are given in moles per liter. Other commonly used concentration units are:

M	Molarity	moles solute / liter solution
m	molality	moles solute / kilogram solvent
μ	mole fraction	moles solute / moles solution
N	normality	gram equivalents / liter solution
ψ	grams / liter	grams solute / liter solution
ppm	parts per million	grams solute / 10^6 grams solvent or liters solute / 10^6 liters solvent
v%	volume percent	liters solute / 100 liters solution
w%	weight percent	grams solute / 100 grams solution
X	mass fraction	grams solute / grams solution
ρ	density	kilograms solution / cubic meter solution

With such a large number of concentration terms used, one must be quite sure that units are specified and understood.

Applications and Predictions

1. Acetic acid will be a weak acid.
2. The heat energy that accompanies the formation of water from hydrogen and oxygen will be so large that it can damage living tissue unless dissipated.

3. A chemical reaction at equilibrium will remain at equilibrium until energy is added.
4. If not in chemical equilibrium, a reaction will either create energy or need energy to proceed.
5. Chemical reactions will be important in bioreactors; the stomach is one form of bioreactor.
6. The physical environment (temperature and pressure) will change the outcome of a reaction.

3.4 Acids and Bases

The greater the difficulty, the more glory in surmounting it.
-Epicurus

Acids and bases are very important in the biological world. An *acid* is a chemical that increases the concentration of hydrogen ions (H^+) when dissolved in pure water. In more general terms, acids are proton (equivalent to H^+) donors. A *base* is a substance that increases the concentration of hydroxide ions (OH^-) when dissolved in pure water. In more general terms, a base is a proton acceptor.

3.4.1 *Strong and Weak Acids and Bases*

Life is nothing but an electron looking for a place to rest.
-Albert Szent-Györgyi

Acids and bases may be either strong or weak. Strong acids and bases dissociate almost completely in dilute water solution into anions and cations:

$$HX + H_2O \rightarrow H^+ + X^- + H_2O \rightarrow H_3O^+ + X^- \qquad (3.4.1)$$

where HX is any typical acid containing hydrogen (H) and another element (X), and H_3O^+ is the hydronium ion composed of a H^+ cation and an associated water molecule. The hydronium ion is the common form of H^+ in water.

$$YOH \rightarrow Y^+ + OH^- \qquad (3.4.2)$$

where YOH is any typical base containing hydroxide (OH) with another element (Y). Hydroxide ions do not form complexes in association with water analogous to the hydronium ion. Weak acids and bases dissociate only partly in water, and range from those with almost no dissociation to those with nearly complete dissociation:

$$HX + H_2O \leftrightarrow H^+ + X^- + H_2O \rightarrow H_3O^+ + X^- \qquad (3.4.3)$$

Here, the double-ended arrow indicates that the dissociation proceeds both ways. Under certain conditions the dissociation into ions proceeds more completely, and under other conditions the undissociated form predominates. Most acids and bases are weak. Such weak acids or bases can be used as *buffers* against large changes in acidity of a solution.

One such buffer substance commonly employed in living organisms is carbonic acid ($H_2\ CO_3$), which is weakly dissociated into hydrogen ions and bicarbonate ions (neglecting the H_3O^+ complex actually formed):

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^- \qquad (3.4.4)$$

Depending on the acidity of the blood (which means the presence of H^+ ions from other sources), more H^+ ions or less H^+ ions may be released from carbonic acid. The more acid the blood, the more H^+ ions that are present, and the more the reaction will be driven back to the undissociated H_2CO_3 form. Thus, this chemical acts to oppose large changes in acidity levels, and internal optimal conditions are maintained.

3.4.2 *Salts*

Remember, if you're not part of the solution, you're part of the precipitate. *-Unknown*

Acids and bases react together to form salts:

$$HX + YOH \leftrightarrow H^+ + X^- + Y^+ + OH^- \leftrightarrow XY + H_2O \qquad (3.4.5)$$

where the chemical XY is a salt.

Some salts are readily soluble in water, and some are not (Kotz and Treichel, 1999). If they are insoluble, then they precipitate and have no further influence on the solution. If they are partly or completely soluble in water, then they often dissociate into ionic form, a process called *hydrolysis*. For instance,

$$NaCl \rightarrow Na^+ + Cl^- \qquad (3.4.6)$$

The product of anion concentration and cation concentration in a *saturated solution* is a form of equilibrium constant (equation 3.3.2), except that the solid form of the salt has no meaningful concentration term. The *solubility product constant*, K_{sp}, expresses the ability of a salt to dissolve and dissociate in water;

$$K_{sp} = (\text{anion concentration})\,(\text{cation concentration}) \qquad (3.4.7)$$

If NaCl is the only salt in solution, then the concentrations of Na^+ and Cl^- would be identical. However, in the complex solutions typical of biological systems, both Na^+ and Cl^-, as well as other ions, are contributed by other chemicals, and it is unlikely that the two concentrations would be the same.

If a salt is the product of a weak acid and a strong base, then, when the salt dissolves and dissociates in water, it will react with the water to form a strongly basic solution.

$$XY + H_2O \rightarrow X^+ + Y^- + H_2O \rightarrow HY + X^+ + OH^- \leftrightarrow H^+ + Y^- + X^+ + OH^- \qquad (3.4.8)$$

The acid HY weakly dissociates, and more OH^- ions are produced in the water than are H^+ ions. Thus, the solution becomes basic.

The opposite is true for a salt formed from a strong acid and a weak base. The salt in solution forms an acidic solution.

Sodium chloride, shown previously, is the salt product of a strong acid (HCl, hydrochloric acid) reacting with a strong base (NaOH, sodium hydroxide). Thus, NaCl in water solution will not significantly affect the acidity of the solution.

3.4.3 *pH*

Chemistry will liberate the laborer. *-Johann Most*

Water by itself tends to dissociate somewhat into ions:

$$2H_2O \leftrightarrow H_3O^+ + OH^- \qquad (3.4.9)$$

The equilibrium constant for this reaction is:

$$K = \frac{(\text{concentration of } H_3O^+)\,(\text{concentration of } OH^-)}{(\text{concentration of } H_2O)^2} \qquad (3.4.10)$$

Because the concentration of water is nearly constant at 55.5 M (molar, see Section 3.3), the equilibrium constant for water (K_w) is designated to be:

$$K_w = (\text{concentration of } H_3O^+)\,(\text{concentration of } OH^-)$$

$$= 1.0 \times 10^{-14} \qquad (3.4.11)$$

Pure water is neither acidic nor basic, being called *neutral.* In this case the concentrations of H_3O^+ and OH^- are equal. In other words, the concentration of H_3O^+ is 1.0×10^{-7} M at 25ºC.

The most common measurement of acidity is *pH*, given as the negative logarithm of the hydrogen ion concentration:

$$pH = -\log_{10}(\text{concentration of } H_3O^+) \qquad (3.4.12)$$

A pH of 7.0 is neutral; pH values less than this are acidic, and solutions with pH values greater than this are basic (Figure 3.4.1). Because the product of the H_3O^+ and OH^- concentrations is constant, when pH decreases, the concentration of H_3O^+ increases and the concentration of OH^- must decrease.

pH has a profound effect on biological systems. Solubility of most biochemicals is affected by pH, so the availability of certain ions in the environment and inside organisms is very sensitive to pH. The concentrations of some metal ions might be too low to sustain life at some pH values, or so high as to be toxic at other pH values. Activities of many enzymes are pH

sensitive, and thus metabolic reactions can change dramatically with slight shifts in pH. H^+ and OH^- can combine with different molecules and directly alter their functions. At both extremes, chemical burning can result.

Because of these effects, living things contain elaborate buffer systems to maintain constant pH levels. These buffer systems depend on the dissociations of weak acids and bases to perform their functions. The pH of normal human blood is usually taken to be 7.40 (slightly basic). Even a change in blood pH of 0.1, easily occurring in working muscles, causes a large shift in the availability of oxygen.

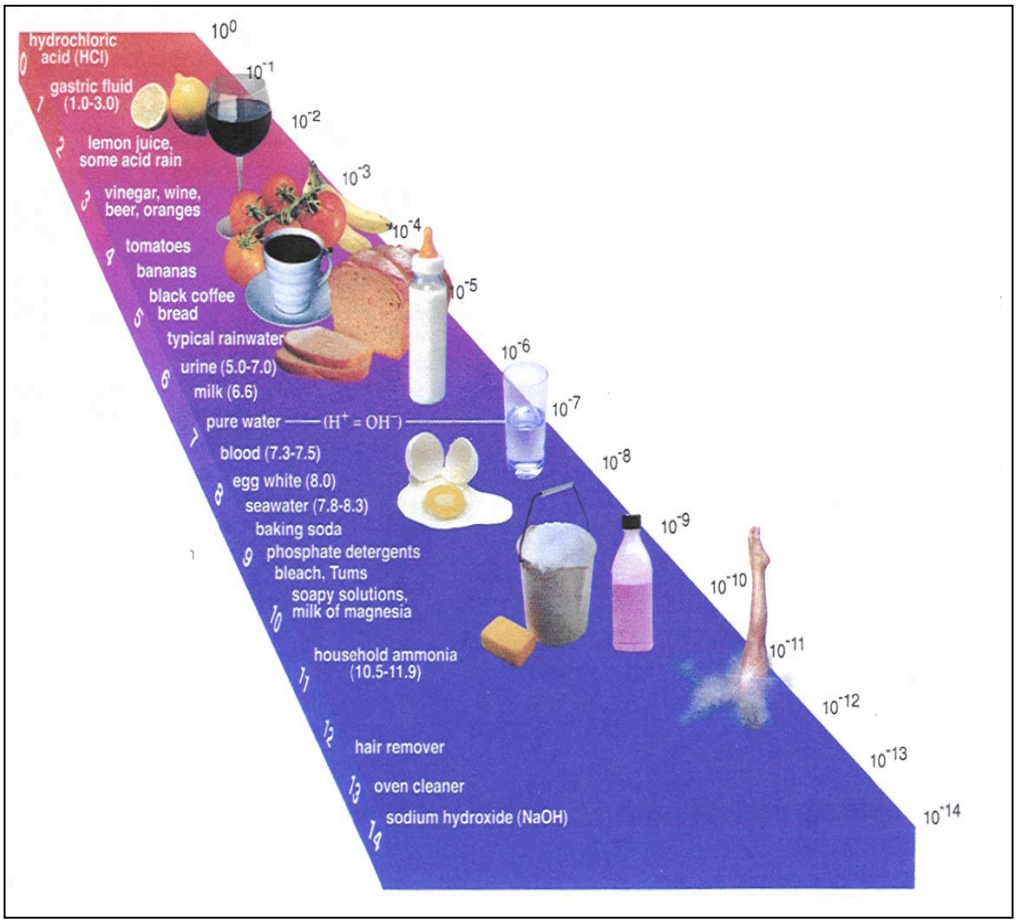


Figure 3.4.1. pH values of common substances (Starr, 2000).

Immediate buffering of the blood uses bicarbonate / carbonic acid, as mentioned earlier. Removal of carbon dioxide through the respiratory system can help restore pH balance in the short term. Over a longer time, kidney selective excretion of different ions is meant to restore blood pH levels to normal.

A common means to inhibit microbial growth is to preserve foods with low pH liquids. Many canned fruits have pH low enough that microbial

spoilage is extremely unusual. Additional organic acids, such as ascorbic acid, are routinely added to assist preservation and prevent enzymatic oxidation.

Applications and Predictions

1. One means to remove a toxic chemical from the environment is to precipitate it.
2. There will be a high concentration of bicarbonate in the blood.
3. Strong acids and bases can be used as disinfectants
4. Most organisms will have pH values close to neutral.
5. Some substances will rise to toxic levels at abnormal pH values.
6. Because pH is a logarithmic scale, the acidity of the stomach (as low as pH 1.0) is millions of times more acidic than cellular fluids (typically near pH 7.0).
7. Strong acids will be neutralized by strong bases.

3.5 Reaction Rates

A drug is a substance which when injected into a guinea pig produces a scientific paper. *-Unknown*

3.5.1 *Collision Theory*

Man fools himself. He prays for a long life, and he fears an old age.
-Chinese proverb

The *chemical collision theory* has been able to explain many of the observations related to chemical kinetics. The assumption made for this theory is that particles must physically collide for a chemical reaction to occur. In addition, these collisions must be effective; without sufficient force, the electrons surrounding the nuclei of the atoms involved would just repel each other and the atoms would not combine. Thus, the rate of any step in a reaction is directly proportional to: 1) the number of collisions per unit time and 2) the fraction of these collisions that are effective.

The number of collisions for gases at Standard Temperature and Pressure (STP: 0^{o}C and 1 atm) has been calculated to be more than 10^{30} per second. If all these collisions were effective, then reaction rates would be extremely fast. However, this is not true because only a small fraction of collisions are effective. The extra amount of energy required in a collision to overcome interatomic repulsive forces and produce a chemical reaction is known as the *energy of activation*. Its magnitude depends on properties of the reactants.

3.5.2 Intermediate Reactions

Our biomedical engineering world is like that. We have the mundane, the ordinary, and the commonplace. We have hard work, unsolvable problems, and so many distractions. But we also have sublime moments when we realize that we have made progress, that what we are doing is important, and what we are doing is good. *-Raj Tonnash*

It is common to express a chemical reaction in its simplest possible terms. Thus,

$$A + B \rightarrow AB \qquad (3.5.1)$$

is the net chemical balance normally cited. However, there may be several intermediate steps:

$$A + B \rightarrow C \qquad (3.5.2a)$$
$$C + B \rightarrow D \qquad (3.5.2b)$$
$$D + A \rightarrow AB \qquad (3.5.2c)$$

where C and D are intermediate products that may be so short-lived as to be unobservable under normal circumstances. Each step (a), (b), and (c) has its own reaction rate and its own activation energy. Thus the reaction rate for (3.5.1) depends on the slowest reaction rate (a), (b), or (c), and the activation energy for (3.5.1) depends on the activation energies for (a), (b), and (c). If there are several slow intermediate steps and several high activation energies, then (3.5.1) could depend on a combination of (a), (b), and (c). Enzymatically-mediated reactions important in living things usually reduce activation energies and greatly increase rates.

When reaction rates are determined, they can only be determined experimentally because of the inability to observe all intermediate steps. It is known that reaction rates depend on some combination of the concentrations of the reactants:

$$\text{rate} = (k)\,(\text{concentration of A})^m\,(\text{concentration of B})^n \qquad (3.5.3)$$

where k is a measured *constant specific rate* and m and n are exponents whose values must be experimentally determined. Sometimes the exponents m and n equal the coefficients in front of A and B in equation (3.5.1), sometimes they do not. For the case of a *reversible* reaction,

$$A + B \leftrightarrow C + D \qquad (3.5.4)$$

the compounds C and D are the products of the forward reaction with A and B as reactants. However, the compounds A and B are the products of the reverse reaction with C and D as reactants. Each direction of the chemical equation has its own rate. At equilibrium these two rates are equal:

$$\text{forward rate} = k_f\,(\text{concentration of A})^m\,(\text{concentration of B})^n$$

$$= \text{reverse rate} = k_r\,(\text{concentration of C})^p\,(\text{concentration of D})^q \qquad (3.5.5)$$

Thus,

$$K = \frac{k_f}{k_r} = \frac{(\text{concentration of C})^p\,(\text{concentration of D})^q}{(\text{concentration of A})^m\,(\text{concentration of B})^n} \qquad (3.5.6)$$

where K is the *equilibrium constant*, a measured quantity as given in Section 3.3. Because K is constant, changing the concentration of one or more of the constituent compounds will change the others in the system.

3.5.3 First Order Reactions

The way we name things inevitably affects how we perceive those things. *-Robert Dorit*

Reaction rates depend on concentrations of reactants that change during the reaction. Thus, the rate of a reaction will usually be greatest at the beginning of a reaction and will decrease with time until equilibrium is established. One common type of chemical reaction is the *first order reaction*, in which the rate of disappearance of a reactant is proportional to the reactant concentration. This leads to an exponentially decreasing reaction rate as time proceeds (see Section 4.3). Other types of kinetics are also possible.

3.5.4 Enzyme-Substrate Reactions

Science is by no means incompatible with finding joy, meaning, and purpose in the world. *-Robert T. Pennock*

A substrate is any substance on which an enzyme can act to form a product. Rates of simple enzyme-substrate reactions are often described by the Michaelis-Menten construction. It has been found that the rate of product formation depends directly on the substrate concentration, but that the dependence is small for low substrate concentrations and for high substrate concentrations, and the dependence is highest somewhere between. The curve

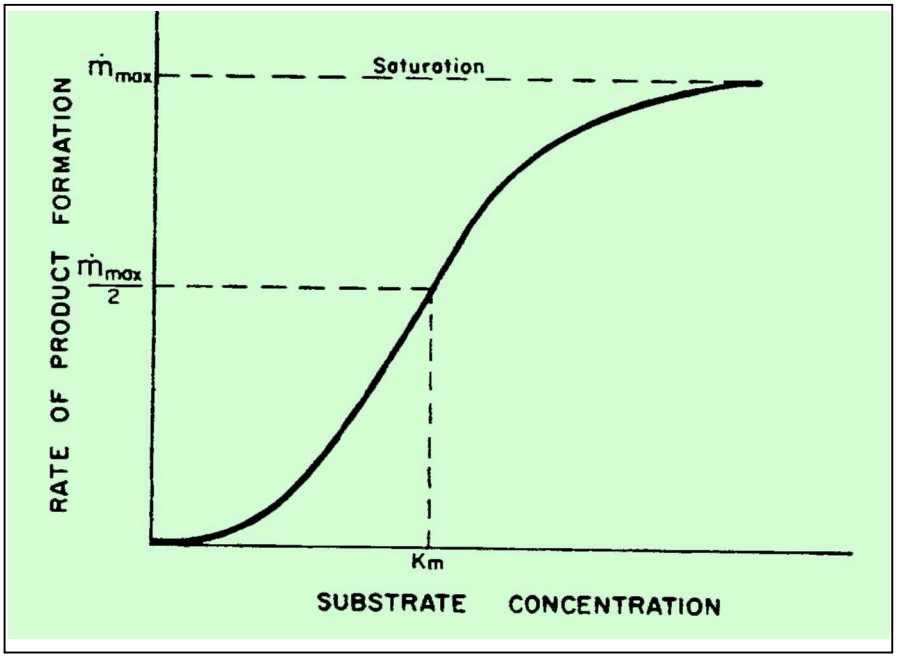


Figure 3.5.1. Michaelis-Menten reactions.

of rate of product formation plotted against substrate concentration forms an "S" shape (Figure 3.5.1). The steeper the "S", the higher is the affinity of the enzyme for the substrate. A typical enzyme-substrate system has a rate of product formation equal to half the maximum (saturation) rate at about 5 millimolar at room temperature. Changing the concentration of enzyme will affect the position of the curve.

Example 3.5.1 Faster ELISA

One quick and popular immunoassay is the enzyme-linked immunosorbent assay, or ELISA (Kemeny, 1991). In ELISA, a surface is coated with antigen or antibody and dried. An antibody is then added to the test (see Section 6.20), usually suspended in water. Chemically linked to the antibody is an enzyme, usually one of three: horseradish peroxidase, alkaline phosphatase, or β-D-galactosidase. When the sample to be tested is added, an antibody-antigen complex is formed and becomes fixed to the surface. Excess enzyme-labeled antibody is then washed from the surface. A chromogen substrate is added that combines with the enzyme linked to the antibody. When it does, it forms a colored compound that can be quantified by optical density. The optical density is thus dependent on the amount of enzyme remaining in the assay, and that amount, in turn, depends on the concentration of antibody in the sample tested.

This assay usually takes several hours to complete, but there is interest in speeding it up. The time taken by the assay is composed of diffusion time, antigen-antibody binding (very quick), and enzyme-substrate kinetics of the Michaelis-Menten type (except that the substrate concentration does not limit the rate). Thus a faster assay will come only as a result of minimizing each component of time (Johnson, 1999).

Applications and Predictions

1. To drive a reversible chemical reaction to one side, and produce the largest amount of products, greatly increase the concentration of reactants.
2. To increase the rate of a chemical reaction in aqueous solution without increasing the amounts of reactants, decrease the amount of water.
3. Enzymes will increase reaction rates by increasing local concentrations of reactants.
4. To ameliorate environmental effects of reactive chemicals, dilute them.
5. Removing the product of a chemical reaction will increase the rate of reaction.

3.6 Carbon Chemistry

Some types of molecular chains outcompeted other molecular chains for the planet's resources, and gradually they led to the kind of molecules that life depends upon – all this before the first living thing oozed forth.

-Joel Achenbach

Carbon occupies a unique place and is essential to life as we know it. More than 90% of all known compounds contain carbon, and the number of hydrocarbons and derivatives number close to one million.

Carbon has a valence of 4, and, therefore, can either gain 4 electrons or lose 4. Carbon usually forms covalent bonds where it shares electrons with another atom. What makes carbon so unique is that it readily forms covalent bonds with other carbon atoms, making long chains possible.

Diagrammatically, the sharing of a pair of electrons is usually denoted by a dash between letters standing for particular atoms. Thus, $-\overset{\text{I}}{\underset{\text{I}}{\text{C}}}-\overset{\text{I}}{\underset{\text{I}}{\text{C}}}-$ denotes a single pair of electrons shared between two carbon atoms. The lines extending radially outward from each carbon atom indicates that pairs of electrons are shared with four other atomic species. The total number of shared electrons is 8.

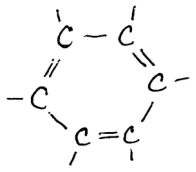
3.6.1 *Many Possible Configurations*

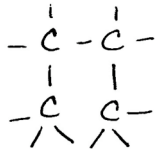
Organic chemistry is the chemistry of carbon compounds. Biochemistry is the study of carbon compounds that crawl.

-Mike Adam

Carbon can also form a double bond with another carbon, $\overset{\text{I}}{\underset{\text{I}}{\text{C}}}=\overset{\text{I}}{\underset{\text{I}}{\text{C}}}$, and a triple bond with another carbon, $-\text{C}\equiv\text{C}-$. The total number of shared electrons is still 8.

Carbon compounds can form chains, $-\overset{\text{I}}{\underset{\text{I}}{\text{C}}}-\overset{\text{I}}{\underset{\text{I}}{\text{C}}}-\overset{\text{I}}{\underset{\text{I}}{\text{C}}}-\overset{\text{I}}{\underset{\text{I}}{\text{C}}}-$; they can form branches, $-\overset{\text{I}}{\underset{\text{I}}{\text{C}}}-\overset{\text{I}}{\underset{\text{I}}{\text{C}}}-$ $\overset{\text{I}}{\underset{\text{I}}{\text{C}}}$, and they can form rings:





In all of these configurations the number of shared electrons is 8.

What's So Special About Carbon?
by Daniel Berger

Why should there be a separate field ("organic chemistry") for compounds of one element, carbon, while the other ninety-five or so chemically important elements are lumped together as "inorganic chemistry"? Worse yet, more than a thousand known compounds include carbon for each one that does not – even after more than fifty years of determined effort by synthetic inorganic chemists. And it is carbon, always and only carbon, that forms the basic molecular framework of living systems. Although there has been and continues to be speculation about inorganic biochemistries, most professional exobiologists (who search for signs of extra-terrestrial life) do not give them a moment's thought.

How can one element so dominate the provincial imaginations of chemists that exobiologists rarely bother to speculate about life based on any other element? Let's do a systematic search for *other* elements on which life might be based. They only need to fulfill a few characteristics:

1. They must be common in the universe.
2. They must be chemically reactive.
3. They must be able to form complex, branched compounds similar to those we know in organic biochemistry.
4. The two most common, chemically reactive elements in the universe are hydrogen and oxygen. Our candidates must have compounds that are reasonably stable in the presence of hydrogen and oxygen.

Let's see how many elements we can find that fill the bill.

If an element is rare, there will not be enough of it to form the chemical basis of large- or even small-scale ecosystems. As a reasonable cutoff, we will require life-elements to have at least one atom per billion hydrogen atoms. Of the eighty-five or so elements found in nature, twenty-two are common: hydrogen, helium, nitrogen, carbon, oxygen, fluorine, neon, sodium, magnesium, aluminum, silicon, phosphorus, sulfur, chlorine, argon, potassium, titanium, chromium, manganese, cobalt, nickel, and iron.

Three of our twenty-two candidates are completely inert chemically, so much so that they form no known stable compounds: Helium, neon, and argon. This pares our list to nineteen: hydrogen, nitrogen, carbon, oxygen, fluorine, sodium, magnesium, aluminum, silicon, phosphorus, sulfur, chlorine, potassium, titanium, chromium, manganese, cobalt, nickel, and iron.

To form the chemical basis of life, an element ought to be able to

What's So Special About Carbon? cont.

form large and complex molecular structures, including branched rings and chains. It would be simplest if the element were able to do this by bonding to itself.

Most elements are metals, bonding to themselves only in infinite three-dimensional arrays, and the "metallic bonds" in these arrays are not localized between pairs of atoms. This means that they cannot be selectively broken and re-formed during biochemical processes, and small molecular structures with metal-metal bonds are almost unknown. We have ten metals left in our list: sodium, magnesium, aluminum, potassium, titanium, chromium, manganese, cobalt, nickel, and iron; almost all of the sixty-three elements that we eliminated earlier as being too rare are also metals.

This leaves us nine elements: hydrogen, nitrogen, carbon, oxygen, fluorine, silicon, phosphorus, sulfur, and chlorine. All of these can bond to themselves, by localized "covalent bonds" that could be precisely manipulated by biochemical processes.

But to form branched rings and chains, an element has to be able to form more than one or two bonds. Hydrogen, fluorine, and chlorine cannot form more than one bond, and so if they bond to another atom they are done. Oxygen and sulfur can form only two bonds; they can make chains and rings such as –O-O-O-O- or –S-S-S-S-, but that is all. So, from our original list of a quarter of the elements found in nature, we are left with only four candidates for life: nitrogen, carbon, silicon, and phosphorus.

And we have to throw out nitrogen, too. When nitrogen bonds to itself, it overwhelmingly prefers to make all three bonds to one other nitrogen atom: N≡N. Nitrogen-nitrogen single and double bonds are unstable enough to make such compounds explosive.

What if nitrogen formed single bonds in which it alternated with another element? For example, single-bonded compounds with alternating boron and nitrogen atoms, or alternating nitrogen and phosphorus atoms, are stable; nitrogen forms strong bonds to carbon, silicon, and phosphorus. Unfortunately, this is essentially the same as making boron (a rare element), carbon, silicon, or phosphorus the basis of life. Besides, there is small likelihood of finding compounds lying around with the regular alternation required. So we can leave combinations of other elements with nitrogen out of consideration, at least for the moment.

We are left with only three candidates for the element of life: carbon, silicon, and phosphorus. All of our remaining candidates form bonds to themselves, and all prefer to form single bonds so that bonds are left over for rings and branched chains.

Hydrogen and oxygen are respectively the most common and third-most common elements in the universe, and are quite reactive besides. To

What's So Special About Carbon? cont.

have a fighting chance of forming any sort of life, compounds of other elements must be reasonably stable in the presence of these two elements. This stability can be approximated by bond strength: how strong are bonds to hydrogen or oxygen compared with bonds of the element to itself? If a bond is reasonably strong, there will be little if any advantage in forming a different kind of bond; and the bond will be more difficult to break in order to form a different kind of bond.

Our remaining candidates for "element of life" are carbon, silicon, and phosphorus. All of them form stronger bonds to oxygen and hydrogen than they do to themselves. But how much stronger?

Silicon's bond to oxygen is two-and-a-half times the strength of a silicon-silicon bond: so strong that in nature, silicon is *exclusively* bound to oxygen. We could conceive of a biochemistry based on silicon-oxygen polymers of the form –Si-O-Si-O-Si-O-; artificial polymers of this type (silicones) are common. Unfortunately, the silicon-oxygen bond is too strong. It is *hard* to break – as one would want to in order to work with biomolecules.

Phosphorus, like silicon, is almost always found in nature combined with oxygen. It can form stable, polymeric compounds with nitrogen (phosphazenes) that are similar to silicones. But the formation of such compounds is unlikely without strenuous help, given nitrogen's overwhelming preference for itself. (Besides, silicone and phosphazene chains and rings are oxygen-stable only if they have carbon-based side groups!)

Carbon, too, is more stable when bonded to oxygen, *but only by about 10-20 percent.* Carbon-oxygen bonds are common, but they are relatively easy to break in favor of carbon-carbon or carbon-hydrogen bonds.

Furthermore, under "reducing conditions" (an excess of hydrogen), phosphorus and especially silicon are not able to maintain bonds to themselves in favor of bonds to hydrogen: silicon-hydrogen bonds are 60 percent stronger than silicon-silicon bonds. Carbon-carbon bonds, on the other hand, are about 90 percent as strong as carbon-hydrogen bonds; hydrocarbons (compounds with both carbon-carbon and carbon-hydrogen bonds) are no more reactive than pure carbon.

To avoid infinite chains, a "chain cap" is needed, an element that forms only one bond. The capping element should form strong bonds to our life-element, but not too strong: it should be stable in the presence of oxygen, but not be too much stronger than the bonds of the element to itself. Because it is so common, hydrogen is the most likely candidate.

What's So Special About Carbon? cont.

Only carbon is able to work well with hydrogen. Carbon-carbon bonds are strong in comparison with carbon-hydrogen bonds, so that hydrogen will not break up carbon chains. And carbon-hydrogen bonds are nearly as strong as carbon-oxygen bonds, so that hydrocarbons need a considerable energy "boost" to start reacting with oxygen. Silicon and phosphorus, on the other hand, form bonds to hydrogen that are too strong – SiH_4 and PH_3 are much more stable than the same number of Si-Si or P-P bonds – *and* not strong enough- SiH_4 and PH_3 burn spontaneously in the presence of oxygen.

The other common elements suitable as chain caps are fluorine and chlorine, but they are worse than hydrogen. Carbon, silicon, and phosphorus form extremely strong bonds to fluorine – so strong that silicon-silicon and phosphorus-phosphorus bonds cannot compete. And, except for carbon, bonds to chlorine cannot compete with bonds to oxygen.

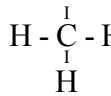
Not only does carbon fit our requirements best of all the possible elements, it often forms stronger bonds to other elements than those elements do to themselves. This allows these elements to be incorporated into carbon-based biochemical structures, and makes the organic structure of life far richer. In fact, three-quarters of the chemically active elements we began with are involved in biochemistry!

- reprinted from Phi Kappa Phi Forum (2002)

3.6.2 *Functional Groups*

The biological properties of a molecule are determined by the molecule itself - the atoms in it and the way they are bonded to one another, its flexibility and shape. *-Roald Hoffmann*

Carbon can share its electrons with any number of other elements or functional groups. Each of these must be able to share 2 electrons. For instance, carbon readily bonds with hydrogen to form hydrocarbons:



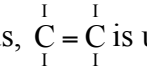
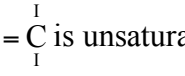
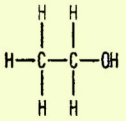
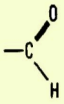
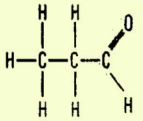
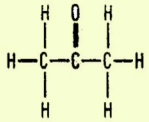
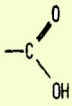
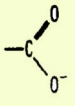
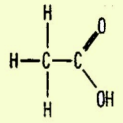
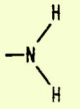
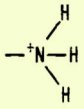
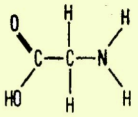
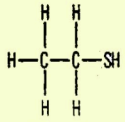
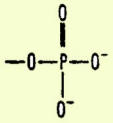
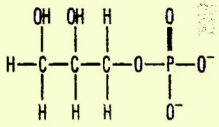
, or CH_4, is methane, the simplest hydrocarbon. Hydrocarbons may be either *saturated* or *unsaturated*, depending on whether there are additional carbon – carbon bonds that can be replaced by other elements or functional groups. Thus,  is unsaturated;  is saturated. Hydrogen is often

Table 3.6.1. Organic Functional Groups (Campbell et al, 1999).

FUNCTIONAL GROUP	FORMULA	NAME OF COMPOUNDS	EXAMPLE
Hydroxyl	—OH	Alcohols	 Ethanol (the drug of alcoholic beverages)
Carbonyl		Aldehydes	 Propanal
		Ketones	 Acetone
Carboxyl	 (non-ionized)  (ionized)	Carboxylic acids	 Acetic acid* (the acid of vinegar)
Amino	 (non-ionized)  (ionized)	Amines	 Glycine* (an amino acid)
Sulfhydryl	—SH	Thiols	 Ethanethiol
Phosphate		Organic phosphates	 Glycerol phosphate

*The ionized forms of the carboxyl and amino groups prevail in cells. However, acetic acid and glycine are represented here in their non-ionized forms.

the additional element added to the unsaturated compound to produce the saturated compound.

Instead of a simple hydrogen atom, the functional group linked to carbon may be an –OH hydroxyl group (Table 3.6.1). Addition of the –OH to carbon produces an alcohol. The alcohols act as weak acids and have polar molecules. They can dissolve both ionic and nonionic solutes.

The –CHO functional group produces an aldehyde, which is odoriferous and has various uses, including disinfection (formaldehyde). The –CO functional group produces a ketone, the most well known of which is acetone. The –COOH carboxyl functional group produces weak organic acids (CH_3COOH is acetic acid). Organic acids with few carbons are soluble in water, but the acids with more carbons are less soluble because their polarities are diluted more by the hydrocarbon remainder of their molecules. The carboxyl functional group –COO- produces esters, the product of a reaction between an organic acid and an alcohol. Esters are pleasant tasting and smelling and are the principal flavor constituents of many fruits. The –O- functional group produces an ether, which can be formed when two alcohol molecules come together so that an H from one and an OH from the other split off to form water as a byproduct. The extraction of water in this process is called a *condensation reaction,* and it does not occur spontaneously in water solution. Ethers are good organic solvents that dissolve a large variety of nonpolar substances that are only sparingly soluble in water. The amino functional group $-NH_2$ produces amines as building blocks for proteins. Additional complexity is possible considering that sulfur, phosphorus, iron, and other elements can also locate in organic compounds.

Of particular importance are the carbonyl and amino groups (Table 3.6.1). Each of these has two forms depending on pH: an ionized and nonionized form. This will be discussed further in Section 3.7.4.

3.6.3 *Amino Acids*

The best and most efficient pharmacy is within your own system.
-Robert Peale

Amino acids are extremely important to living systems because they form the bases of the many proteins necessary for life. There are 20 common amino acids that function as protein building blocks (Table 3.6.2). There are at least 31 additional amino acids not commonly part of natural proteins; among these are some hormones and neurotransmitters (Garrett and Grisham, 1999).

Amino acids polymerize to form peptides and proteins by joining amino and carboxyl groups. The structure of carbon-based organic compounds is extremely important, because functional properties depend on structure. Compounds with the same chemical composition but different structures are called *isomers*. Amino acids exist in mirror-image forms

(*stereoisomers*) that do not function identically. Only the L-isomers of amino acids commonly occur in nature (the two *chirality* designations are L, for *levorotatory*, and D, for *dextrorotatory*; levorotatory molecules rotate plane-polarized light in a counter-clockwise direction). Animals are made of proteins from L-amino acids, coded for by DNA built from D-sugars. L-amino acids and D-sugars are slightly more stable than their *enantiomers* (mirror image molecules) due to weak chemical interactions (MacDermott and Tranter, 1990).

Table 3.6.2. Twenty Common Amino Acids.

	Name	Acronym
Nonpolar		
	Leucine	Leu, L
	Proline	Pro, P
	Alanine	Ala, A
	Valine	Val, V
Polar, uncharged		
	Glycine	Gly, G
	Serine	Ser, S
	Asparagine	Asn, N
	Glutamine	Gln, Q
Acidic		
	Aspartic Acid	Asp, D
	Glutamic Acid	Glu, E
	Methionine	Met, M
	Tryptophan	Trp, W
	Phenylalanine	Phe, F
	Isoleucine	Ile, I
	Threonine	Thr, T
	Cystine	Cys, C
	Tyrosine	Tyr, Y
	Histidine	His, H
Basic		
	Lysine	Lys, K
	Arginine	Arg, R

3.6.4 *Macromolecule Types*

Assembling a review panel is analogous to finding the ingredients for a batch of minestrone soup that takes a pinch of this and a spoonful of that to taste just right. *-Thomas Cech*

The four basic types of macromolecules in living systems are:

1. carbohydrates. These serve as fuels (glucose, for example) and building materials (cellulose). Carbohydrates can exist as simple sugars, monosaccharides, and polysaccharides.
2. lipids. These are hydrophobic molecules used for dense energy storage, structural components of cell membranes, and steroidal hormones.
3. proteins. These complex molecules give structural support to cells, store energy, assist in transport of other substances, serve as signal markers, result in mechanical movement and force generation, and act as enzymes in biochemical reactions. Proteins are usually structured with a long carbon chain backbone with various organic functional groups hung like ornaments on a tree. The particular sequence of amino acids in a protein determines its shape and function (see Section 3.7).
4. nucleic acids. These molecules act as information storage units for the cell and are the basis for genetic encoding. Nucleic acids are polymers of nucleotides (complex organic compounds composed of pentose sugars, organic bases, and phosphate groups), and have the property that a single strand of nucleic acid can only form its conjugate image when duplicating (see Section 5.3).

Microbial Stoichiometry

Stoichiometry is the branch of chemistry concerned with proportions of chemical elements or compounds involved in reactions. Stoichiometry has also been associated with the use of microbes for *bioremediation* of environmental pollutants.

The composition of simple organisms such as bacteria, yeast, and algae are very similar. Microbial protoplasm has a relatively constant stoichiometric composition of (Chamberlain, 2003):

$$C_{106}\ H_{263}\ O_{110}\ N_{16}\ P_1$$

Microbial Stoichiometry (cont.)

If algal protoplasm is viewed in terms of carbon dioxide (CO_2), nitrate (NO_3^-), phosphate (HPO_4^{-2}), and water (H_2O), then the following stoichiometric balance applies (Strumm and Morgan, 1981):

$$106\ CO_2 + 16\ NO_3^- + HPO_4^{-2} + 122\ H_2O + 18\ H^+$$

$$\leftrightarrow [C_{106}\ H_{263}\ O_{110}\ N_{16}\ P_1\ ] + 138\ O_2$$

Notice that the masses of all elements and electrical charges are balanced on both sides of the equation. Energy in the form of light, and some trace elements are required on the reactant (left) side of the reaction for it to proceed. This balance simply expresses the means for algal protoplasm and oxygen to be formed from photosynthesis.

Sometimes a bioremediation design using microbes requires the addition of nutrients to promote microbial growth. The objective is to add scarce nutrients that limit microbial growth and force the microbes to use available nutrients found in pollutants. Hence, as the microbes grow and metabolize, they deplete pollutant levels. Macronutrients are often expressed in terms of carbohydrates (CH_2O, a carbon source), ammonia (NH_3, a nitrogen source), and orthophosphate (H_3PO_4, a phosphorous source). The only additional macronutrient commonly added to plant fertilizer is a potassium source, which doesn't appear in the stoichiometric formula for protoplasm. However, potassium, sodium, calcium, chloride, and other ions are necessary to maintain normal activity of living cells (see Section 4.4.3).

In terms of these macronutrients, microbial protoplasm can be expressed as:

$$[\ (CH_2O)_{106}\ (NH_3)_{16}\ (H_3PO_4)\ ]$$

and this formulation can be used to determine how much of which limiting nutrient to add to either an in situ remediation project or to a bioreactor to promote cellular growth.

Anaerobic bacteria are sometimes used in anaerobic digesters to change human, animal, or plant wastes into more useful or less toxic products. Anaerobic bacteria have a stoichiometric form of (Chamberlain, 2004):

$$C_{60}\ H_{87}\ O_{23}\ N_{12}\ P_1$$

3.6.5 Polymers

If any student comes to me and says he wants to be useful to mankind and go into research to alleviate human suffering, I advise him to go into charity instead. Research wants real egotists who seek their own pleasure and satisfaction, but find it in solving the puzzles of nature.

-Albert Szent-Györgi

Polymers are giant molecules composed of many small molecules, called *monomers*, arranged in a repetitive chain. Polymer molecular weights range from thousands to millions (Kotz and Treichel, 1999). Many polymers are formed from identical monomers; the molecules thus formed are long and thin. These compounds act as strands and can be highly elastic. Rubber, in fact, is an *elastomeric polymer*. Some polymers have *cross-linking* chemical bonds between strands or between portions of the same strand, and so have greater rigidity or dimensional stability. Hydrogen bonds or sulfur bonds are often the major stabilizing bonds present.

Polysaccharides, proteins, and nucleic acids are all polymers. Polymers such as collagen are biologically important, and the elastic properties of collagen are often used to advantage for energy storage in tendons.

3.6.6 *Melting and Boiling Points*

Glamour and rock-steady dependability do not easily walk hand-in-hand. *-Raj Tonnash*

An important physical property of organic compounds is their melting and boiling points. The smaller the organic molecule, the lower is its melting and boiling temperatures. Thus, larger molecules are less volatile and more likely to exist in solid form at room temperature. This has important implications for biological systems: those that exist at lower temperatures are more likely to contain low molecular weight fats that are more pliable (less stiff) at those temperatures than are higher molecular weight fats. Fats deposited in the appendages of the human body, with normal temperatures lower than body temperature, are usually of lower molecular weight than those deposited in the body core.

3.6.7 *Organic Reactions*

Those who have an excessive faith in their theories or in their ideas are not only poorly disposed to make discoveries, but they also make very poor observations. *-Claude Bernard*

Two important features of reactions between organic compounds are:

1) they are extremely slow, taking hours or days to complete
2) only part of the molecules take part in the reactions.

The result of the first feature is that heat and enzymes can be used to great advantage to speed the reaction so it occurs at a more useful pace. The result of the second is that organic compounds are often constructed a piece at a time in a step-by-step fashion; working on one part of the organic molecule does not usually alter the remainder. Both of these features is true whether the organic reaction takes place *in vivo* (in the living body) or *in vitro* (in glass, or outside the body).

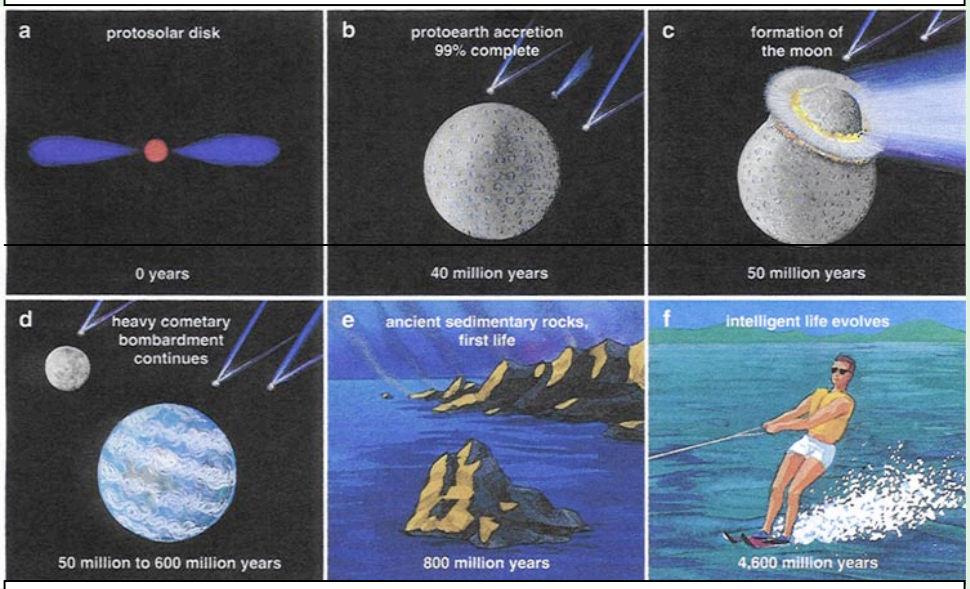
Primitive Forms of Life

There is mounting evidence that comets could have provided the young Earth with its water, atmosphere, and carbon compounds that seeded prebiotic life (Delsemme, 2001; de Duve, 1995). But prebiotic Earth would have been much different from present-day Earth. It would have been rocked by volcanic activity, and the land would have been barren. The oceans were probably acidic. The atmosphere would have been devoid of oxygen and rich in hydrogen sulfide, carbon dioxide, nitrogen, and water vapor. Atmospheric pressure would have been high enough to suppress complete loss of oceanic water through boiling, and the atmosphere would have been electrically charged. Sunlight was less strong in those days, but unfiltered ultraviolet light would have bathed the surface of the planet. It was not a place conducive to the formation of life except by the bombardment by rocks from the sky that contained primitive organic compounds.

Life, however, could have evolved under these conditions. That life is resilient has been seen in the recovery of living communities around hydrothermal vents following volcanic eruptions in the mid-Pacific. Like terrestrial biomes after a disturbance, hydrothermal vent communities develop in a succession of stages (see also Section 6.21.1). At first there was an abundant growth of chemosynthetic microbes that feed on H_2S, H_2, Fe^{++}, and other reduced species. Then followed the tubeworm *Tevria jerichonana* that lived symbiotically with the microbes (the tubeworm ingested the microbes, but did not digest them). This tubeworm was supplanted by a larger tubeworm *Riftia pachyptila*, and then the mussels *Bathymodiolus thermophilus*. It had taken eight years to reestablish a fully-functioning symbiotically-based ecological community of life (Lutz et al, 2001).

Primitive Forms of Life, cont.

Evolutionary Highlights of the Earth's Biosphere



First came dust accretion to form the protoearth, a hot, dry rock. A grazing collision between the Earth and a Mars-sized body formed the moon. All volatile substances, including water, were lost. Heavy cometary bombardment brought water, atmospheric gases, and primitive organic molecules to the planet. Primitive life began relatively recently, and now humans are the dominant feature of the Earth.

Chemical reactions involve the breaking of old bonds and the formation of new ones. When organic compounds, with their covalent bonds, are reacting, the compound A-B may break to form two ions, A^+ and B^-, or they may break to form two neutral fragments, A and B, called *radicals*, each with an odd number of electrons. A radical is a molecule with an unpaired electron in its outer shell (highest energy level). Without the requisite number of 8 electrons, each of these radicals is highly reactive. Usually one or other of these radicals is of immediate use; the other, the *free radical*, is a byproduct that must be dealt with in some way. Free radicals can be destructive to

carbohydrates and other essential biochemicals inside the cell (Capra, 1996), but do assist in defending against microbial invaders of the human body.

Example 3.6.1 Macromolecule Sieves

Some molecules important to living things are relatively small in size. H_2O, O_2, CO_2, and even insulin are among them. Other molecules, notably proteins, polycarbohydrates, and some fatty acids, are large in size. Substances that identify a particular individual, called antibodies or immunoglobulins (abbreviated as Ig, see Section 6.20.3), are among the larger macromolecules.

Molecular sizes are measured in terms of Daltons, which correspond to molecular weight. Antibodies are larger than 100 kiloDaltons, whereas proteins such as insulin are smaller.

Transplanting foreign cells into a body is a method proposed to compensate for the failure of native cells to produce essential biochemicals. Type I diabetics, for instance, lose the ability to produce sufficient amounts of insulin.

Cells taken from other people, or from other species, could be used to supply necessary insulin. These cells, however, carry markers identifying them as non-native. The body's immune system would attack them and kill them, leaving the body again without a source of insulin.

Artificial membranes can be made containing holes of a certain size. If these membranes allow water, oxygen, carbon dioxide, glucose, and insulin to pass through, but block marker proteins and antibodies from contacting each other, then non-native cells can be implanted into the body without subsequent rejection. The only concern here is to make the membrane out of a material that is itself not identified as a foreign body.

Membranes with specifically-designed pore sizes can be used to separate larger from smaller molecules for many purposes. They are used in the food industry, in wastewater treatment, in bioreactors, and in biomedicine. They act as molecular sieves, and provide relatively inexpensive solutions to many practical problems.

Applications and Predictions

1. A carbon source will be necessary for life as we know it.
2. There are many organic compounds still undiscovered.
3. Fats and oils found in trout (that thrive in cool water) will be of smaller molecular weight than those found in *tilapia* (a high temperature fish).
4. Enzymes will greatly enhance the speed of organic chemical reactions.
5. Organic compounds in living things will usually be built piece-by-piece, and the order in which they are built will be important.
6. Amino acids are necessary for life.

7. Polymers and isomers give variety and diversity to organic molecules.
8. In organic reactions, there usually will be a byproduct that affects the environment.

3.7 Physical Chemistry in Water

I believe that any bio-based engineer who doesn't appreciate how any living being (or system) interacts with, reacts to, and is affected by its total chemical, physical, and biological environment is not well prepared.
-Raj Tonnash

Water is such an active substance that it profoundly affects other chemicals it associates with. Water can tear molecules apart or greatly affect their physical properties. In this section, we will learn of some of these effects.

3.7.1 Solutions

Avogadro's Number is the fundamental physical constant that links the macroscopic world of objects we can see and feel with the submicroscopic, invisible world of atoms. *-Ronald Fox and Theodore Hill*

A great deal of the chemical activity of the cell takes place in solution. When a solid goes into solution:

1. the physical structure of the solid must be disrupted
2. the physical structure of the liquid is disrupted
3. the *solute* (or solid going into solution) must be attracted to the *solvent* (or liquid dissolving the solid).

When the process of solution is completed, solute particles are surrounded by solvent particles; the solute is hydrated (if water is the solvent) by the process called *solvation*.

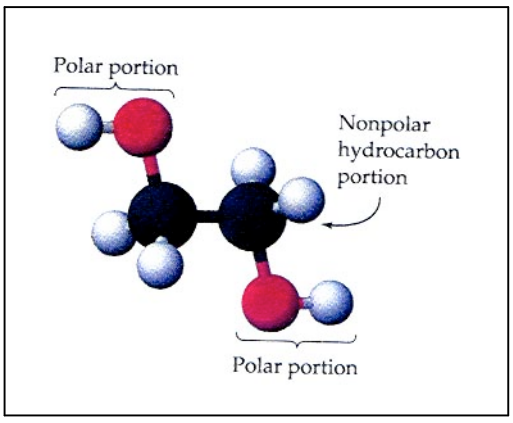


Figure 3.7.1. A molecule of ethylene glycol is a two-carbon alcohol with two hydroxyl groups that make it soluble in water.

Water as a polar molecule is attracted to other molecules with locally charged regions (Figure 3.7.1). Thus, when sugar dissolves in water, hydrogen bonds are established between water and sugar molecules. Sugar molecules thus become surrounded by water molecules bound with intermediate-strength hydrogen bonds. Substances such as lipids are not polar and do not display surface charges the way polar molecules do. Nonpolar molecules dissolve very poorly, if at all, in water or other polar solvents such as ethanol. Solutes that dissolve in polar solvents are called *hydrophilic*; solutes that do not dissolve in polar solvents are called *hydrophobic*. In general, hydrophilic solutes dissolve readily in polar solvents, whereas hydrophobic solutes dissolve readily in nonpolar solvents.

Some solutes *dissociate* when they dissolve; other don't. Salt, for example, dissociates, but sugar doesn't. Salt, NaCl, forms the ions Na^+ and Cl^-, and so forms twice as many particles in solution as does sugar (Figure 3.7.2).

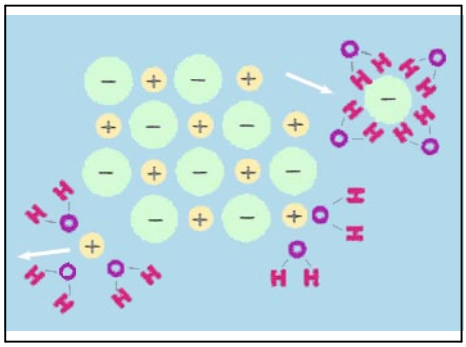


Figure 3.7.2. As NaCl is dissolved, the NaCl crystal is torn apart by water molecules.

Liquids can also dissolve in other liquids. These are termed *miscible.* When liquids do not dissolve in other liquids, they are called *immiscible*. These liquids will remain separate. Mixtures of polar and nonpolar liquids are generally immiscible.

Alcohol is a versatile solvent because part of the molecule is polar whereas the rest is not. Thus, some nonpolar solutes and some polar solutes can dissolve in alcohol. Alcohol relates both to water and oil.

Each of the three aforementioned steps to formation of a solution has associated energy requirements. Breaking the structure of the solute requires energy. Attraction of the solvent for the solute liberates energy. In addition, if the solute dissociates, energy is required for that process also. The sum of energy requirements and surpluses must be positive in order for the process of solution to succeed. Whenever more energy is liberated than is required, heat is released, and the solution heats; the solution is *exothermic*. Whenever the

required energy exceeds the amount liberated, the solution cools; the solution is *endothermic*.

The maximum amount of solute that will dissolve in a standard quantity of solvent is termed the *solubility* of the solute. Exceeding the solubility of the solute will not result in any additional amount of solute dissolved. The solubility of various solutes is affected both by temperature and pressure. Most solid solutes increase their solubilities as temperature increases (Figure 3.7.3), whereas the solubilities of gases decrease as temperature increases There is less oxygen in warm water than in cool water, so aquatic species may sometimes asphyxiate in warm water.

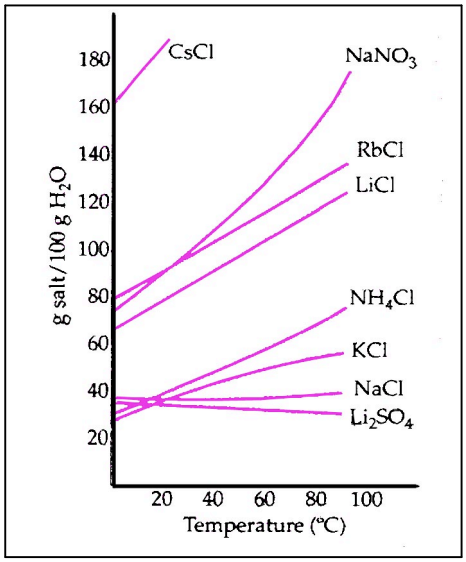


Figure 3.7.3. The temperature dependence of the solubility of some ionic compounds in water. Most compounds increase in solubility with increasing temperature.

The solubility of gases increases as pressure increases according to Henry's Law:

(gas solubility) = (Henry's Law constant) (partial pressure of the gas) (3.7.1)

Thus, large amounts of carbon dioxide can be made to dissolve in water at high pressure. If the pressure is suddenly released, the water fizzes. This is commonly utilized for carbonated beverages.

The vapor pressure of either the solvent or the solute is the pressure of that substance existing in equilibrium with the liquid phase. Thus, there is water vapor in the atmosphere above a container of pure water. The pressure exerted by the water vapor depends on the ability of the liquid water molecules on the liquid surface to evaporate, and escape into the air. At the same time as liquid is evaporating, some vapor is condensing, so an equilibrium is established where evaporation and condensation are exactly equal (Figure 3.7.4). The vapor pressure at balance is the *saturated vapor pressure* of the liquid.

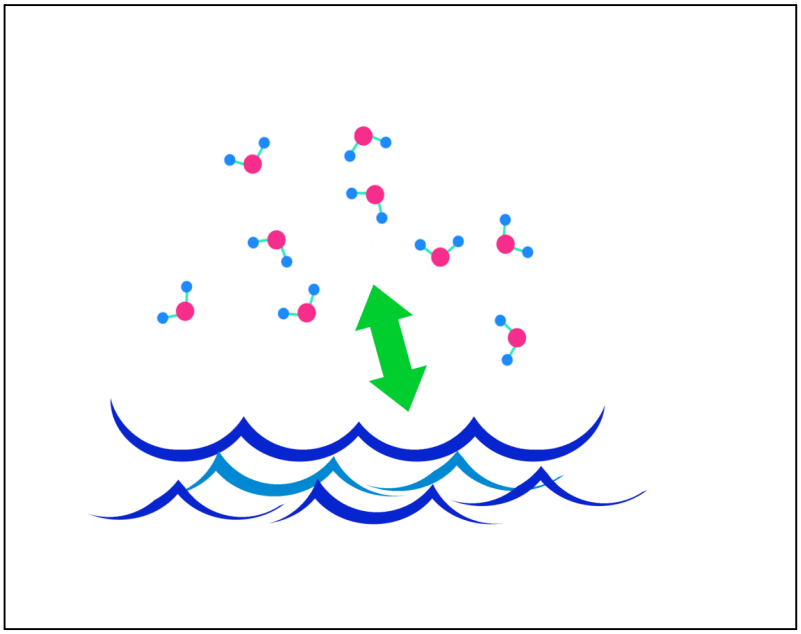


Figure 3.7.4. Net liquid evaporation or condensation depends on which physical process is greater. Both are occurring simultaneously at all times.

It takes energy to evaporate liquid. This energy comes from the kinetic energy of the liquid molecules. The higher is their temperature, the greater is the amount of kinetic energy that they possess. Hence, the saturated vapor pressure of a liquid depends on temperature (and only extremely slightly on pressure). At some temperature the saturated vapor pressure reaches atmospheric pressure, and the liquid boils.

The surface of a solution is randomly populated by solvent and solute particles. The opportunity for a solvent molecule to escape by evaporation is thus lowered by the fraction of solute molecules present in the solution. It could then be expected that the vapor pressure of the solvent above the solution is less at any given temperature than it would be for a pure solvent. This leads to Raoult's Law:

(solvent vapor pressure in solution) =
(mole fraction of solvent) (vapor pressure of pure solvent) (3.7.2)

With a lower vapor pressure for any given temperature, a higher temperature is thus required for the solution to boil than for the pure solvent. Thus, boiling food will happen at temperatures higher than 100°C, making it more likely that microbes will be killed, and also making it more likely that some of the nutritional biochemicals in the food will be altered.

At the other end of the temperature range, freezing of a solution occurs at a temperature lower than the freezing point of the pure solvent. This is because freezing requires the establishment of a solid structure for the solvent. Solute particles among the solvent particles make it more difficult to establish this structure. The more concentrated is the solute solution, the more the freezing point is depressed. Thus, most biological materials will not begin to freeze until they reach a temperature of about -3°C. Some cold water species of plants and animals contain high concentrations of glycoproteins that lower their freezing points a good deal further.

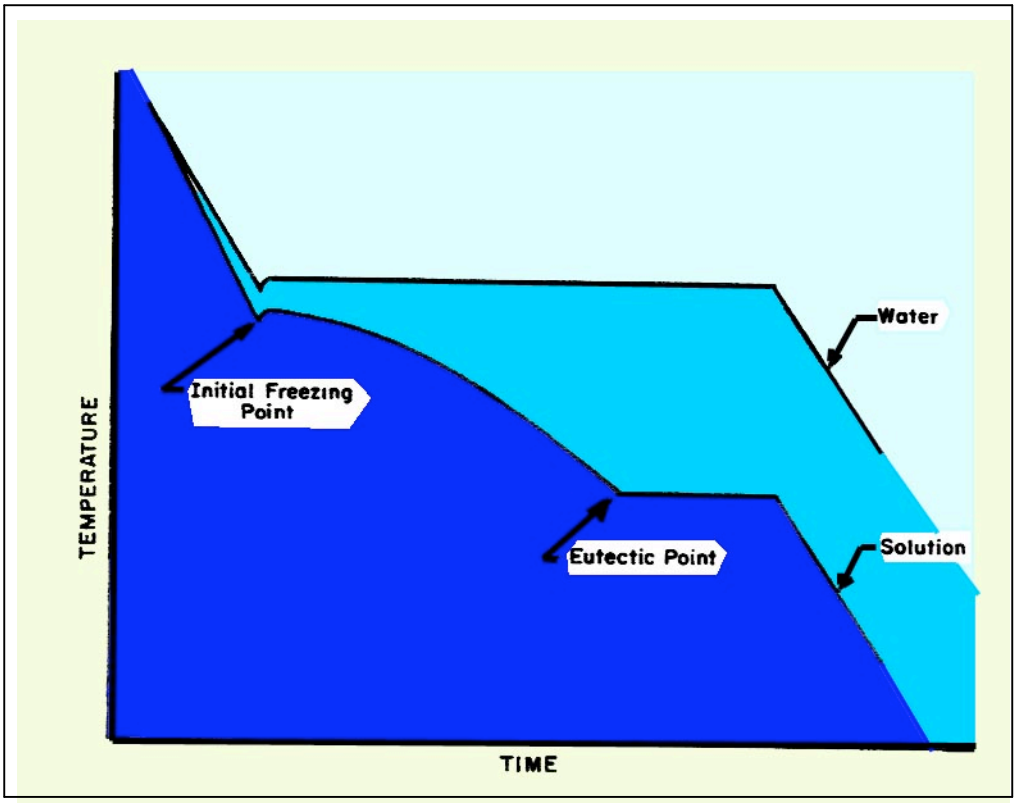


Figure 3.7.5. Schematic freezing curves for pure water and for an aqueous solution with one solute. Solutions with many solutes, found in real foods, have much more complex freezing curves.

Freezing of a mixture of substances does not occur at one temperature, but rather over a temperature range (Figure 3.7.5). In many cases, it is possible that pure water will freeze first, and then the remaining unfrozen portion would contain more concentrated solutes, which depress the freezing point even further. Thus, the temperature at which the solution begins to freeze is perhaps much higher than the temperature at which freezing is complete. The more kinds of solutes present, the more complex is the freezing process. For certain mixtures of liquids, where both liquids dissolve in each other, there may be formed a *eutectic* mixture that has a freezing temperature lower than either liquid by itself. Eutectic mixtures can be useful to protect against freezing or to promote changes of state at useful temperatures. For instance, heat can be stored in the liquid form and liberated when the substance solidifies. In order to make this heat available at the desired temperature, a eutectic mixture could be used.

3.7.2 Gels

The hypotheses we accept ought to explain phenomena which we have observed. But they ought to do more than this: our hypotheses ought to foretell phenomena which have not yet been observed.

-William Whewell

Gels are soft, solid or solid-like materials that consist of two or more components, one of which is a liquid of some abundance (Pollack, 2001). In the gels important for life, the liquid is water and the most important other component is protein.

Proteins are large complex molecules with molecular weights from 10^4 to more than 10^6. They are built of amino acids joined together by peptide bonds (Figure 3.7.6); peptide bonds are carbon-nitrogen bonds that join the carboxyl group (- C- OH) of one amino acid to the amino group (- NH_2) of another amino acid. In this way they form a long, unbranched, polypeptide chain as the primary structure. Proteins form complex folded structures that expose carbonyl groups with negative surface charges and amino groups with

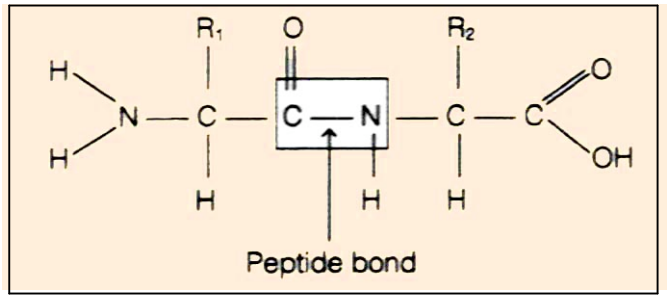


Figure 3.7.6. The peptide bond.

positive surface charges. Side chains may have either charge. As formed, the overall protein charge is neutral, but the large separation of positive and negative charges allows local regions to be anything but neutral. Protein in water dissociates somewhat, losing positive ions (see Section 3.7.4). The surrounding water spatially buffers the protein molecules, and prevents cations from reassociation. Thus, proteins in the cell carry a net negative charge.

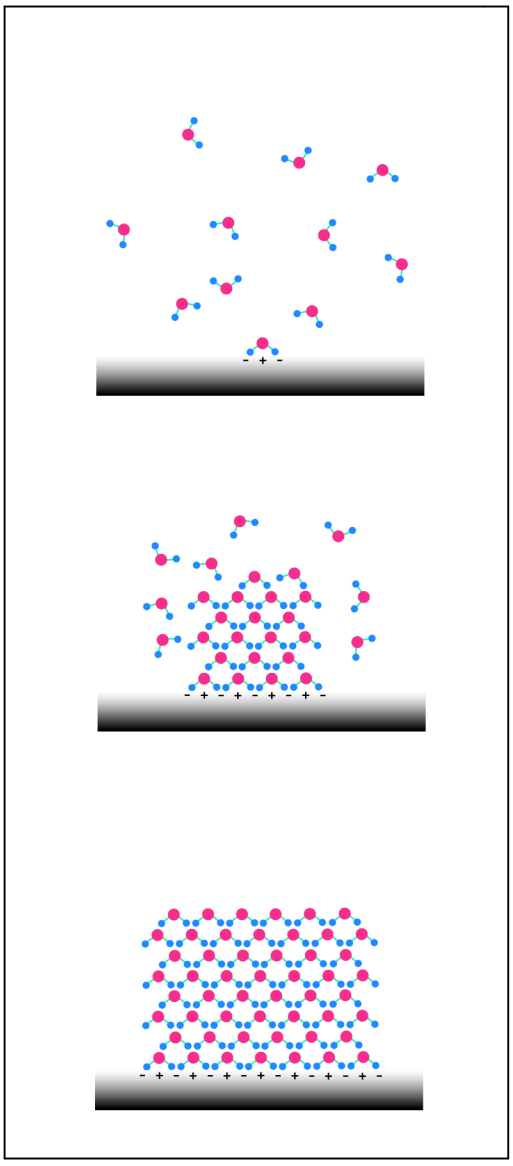


Figure 3.7.7. Surface charge on a protein strand attracts a water dipolar molecule (top). The adsorbed dipole attracts additional dipoles to form a structured organization within the liquid (middle). Additional surface charges on the protein reinforce the external dipole network (bottom) (Pollack, 2001).

Water, as we have already seen, is itself a dipolar molecule, with charge separation between its positive and negative poles. Adjacent water molecules tend to align, forming hydrogen bonds with up to four nearest neighbors. Liquid water is composed of molecules that form transient hydrogen bonds with one another on a time scale of 10^{-11} sec (Pollack, 2001). Clusters of structured water are continually forming and dissolving.

Surface charges on the protein attract water molecules, which adsorb on the protein surface. Whenever one water molecule aligns with protein surface charge, adjacent water molecules align with the first. This induces the layer of water molecules to form a loose structure with positive ends of water molecules attracting negative ends of other water molecules (Figure 3.7.7).

Because water-water hydrogen bonds are relatively weak, the influence of a single protein surface charge diminishes with distance from the charge. Proteins, however, pack many surface charges into a dense region.

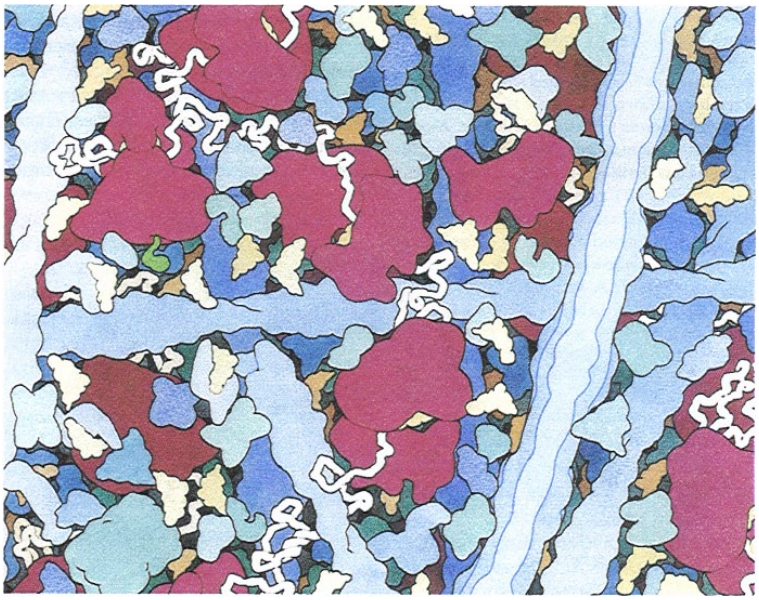


Figure 3.7.8. Cytoplasm packed with proteins and other molecules. Molecules must perform their tasks under the cell's very crowded conditions. This cross section through the cytoplasm of a typical human cell depicts macromolecules. The large purple molecules are ribosomes that are reading genetic information from the snaky white messenger RNA molecules. At the same time, orange, L-shaped transfer RNA molecules are aligned on the ribosome in order to build a new protein. Actin filaments and intermediate filaments criss-cross the space, providing support to the cell and a scaffold for hundreds of different enzymes, which are working on their metabolic tasks. the spaces between the macromolecules are also filled with small molecules and water, not depicted here for clarity, forming a very busy environment. This picture is a static snapshot of the cell, but, in reality, all of these components are in rapid motion (Goodsell, 2000).

Each charge, therefore, reinforces the influence of all other charges, and water becomes very tightly bound. The regularly repeating nature of protein surface charge tends to capture water molecules and hold them tightly to the protein surface (Pollack, 2001).

Not only does protein surface charge capture one layer of water, but, due to the dipolar nature of the water molecule, its influence extends outward through many water layers. Evidence has accumulated that indicates that water may be stratified to distances of 400-500 layers of single water molecules (Pollack, 2001). Thus, cellular water is different from bulk water; cellular water is highly stratified and immobile. The *cytoplasm* (that part of the cell not included in the nucleus) is packed with proteins (Figure 3.7.8), all of which tend to keep water structured and fixed in place.

This is likely the source of the gel-like nature of cellular contents (Pollack, 2001). Because of this gel, cellular contents do not spill out when damage to the cellular membrane occurs, nor is there loss of integrity due to the many holes that are naturally present in the membrane.

Structured water is a very poor solvent. Non-water molecules included within this structure tend to disrupt the structure. The larger the molecule or ion, the more disruption it will cause, and the less likely it will be to gain entry. The structured water state is at a minimum energy level, and any molecule forced into the structure will require a considerable amount of energy to be inserted. Thus, we would expect ions to be pretty much excluded from the cytoplasm, but, because diffusion pressure depends to a great extent on concentration, cytoplasmic ionic concentration should be related to ionic concentration outside the cell. That is, if ionic concentration of potassium (K^+) outside the cell doubles, then it is expected that the ionic concentration of K^+ inside the cell will double, although the inside concentration of K^+ will remain at a small fraction of the K^+ concentration outside the cell. Hydrated sodium ions (Na^+), because they are larger than hydrated K^+ ions, will have a smaller concentration inside the cell than will K^+.

Because protein surface charge is the basis for the gel that forms inside the cell, any change in protein structure can disrupt water structuring. Pollack (2001) has provided a hypothesis for cell activity that involves a phase transition of protein structure from long and straight to short and coiled. The long, straight protein configuration allows water to stratify regularly to maintain the gel and ion exclusion. A change of protein structure to short and coiled masks much of the protein surface charge and frees much of the surrounding water (Figure 3.7.9). The result is that the water acts much more like bulk water. It will tend to dissolve molecules and ions, and will not exclude relatively small substances from the interior of the cell.

Controlling the phase transitions allows the cell to perform its functions. Divalent ions such as Ca^{++} and the adenosine triphosphate (ATP) molecule that contains a high charge density tend to draw adjacent protein strands together and restore structure from the disordered state. Once proteins

are restored to their long configurations, water inside the cell will once again form the gel structure that apparently is required to maintain cellular integrity.

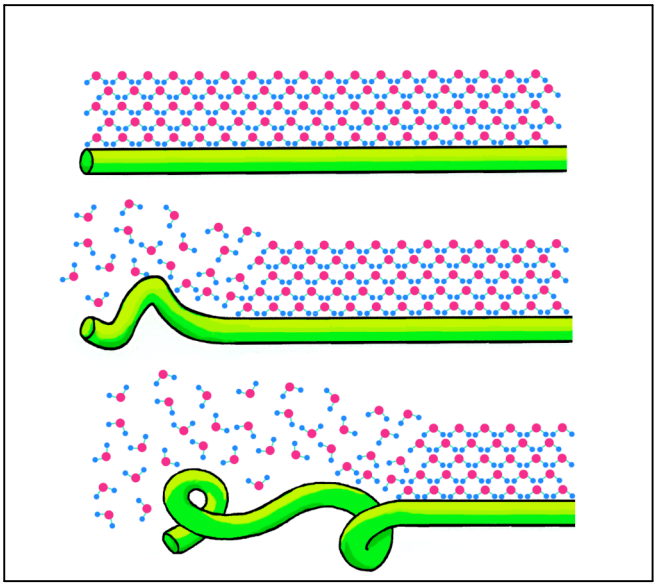
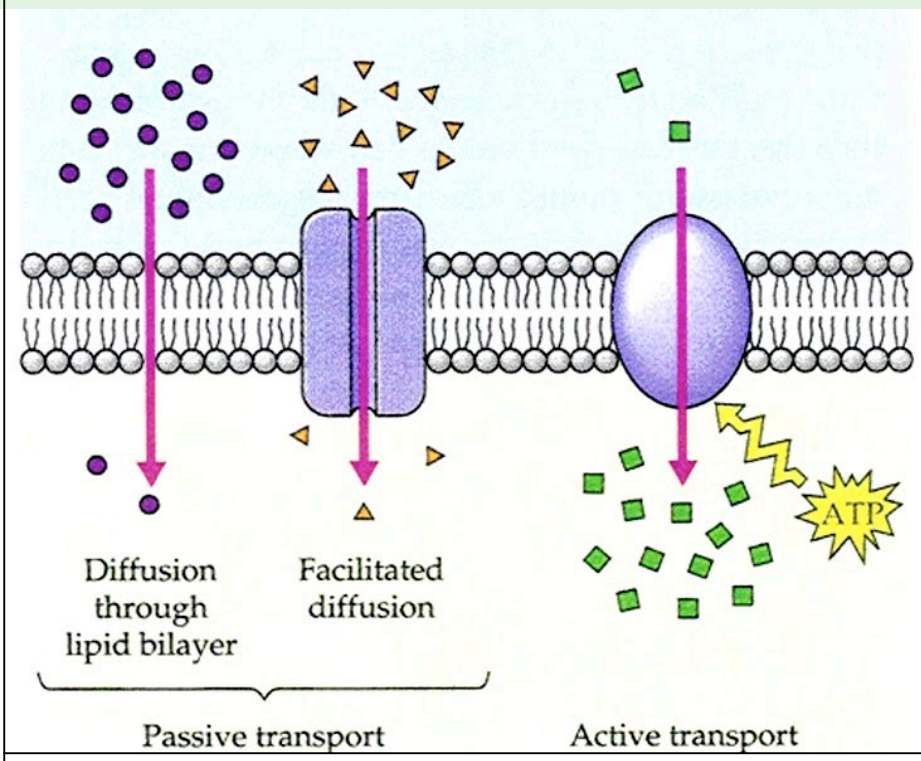


Figure 3.7.9. Protein phase transitions that cause structural changes also disrupt water layers surrounding the protein. Water structure disappears, and this allows further condensation of protein strands by coiling. The unstructured water is also free to dissolve molecules and ions (Pollack, 2001).

Cellular Resting Potential

All cells exhibit a negative internal voltage of –50 mv to –200 mv relative to the outside. This voltage, called the *resting potential*, has been explained since about 1950 by means of a cellular membrane that selectively pumps sodium ions (Na^+) out of the cell and potassium ions (K^+) into the cell (see Figure). The pump, it is thought, consists of a transport protein embedded in the cell membrane that translocates Na^+ and K^+ both against their concentration gradients. Because the concentration of Na^+ is higher outside the cell than inside the cell, Na^+ flows passively back into the cell. K^+ flows passively, too, but from inside to outside. This so-called *ion-pump* requires energy in the form of ATP to keep operating.

Cellular Resting Potential cont.



Ions move through the cell membrane by means of passive diffusion from high concentration to low. The traditional view is that hydrophobic molecules and very small uncharged polar molecules diffuse directly across the membrane. Hydrophilic substances diffuse actively, facilitated by transport proteins. Active transport occurs against a concentration gradient and requires energy (Campbell et al, 1999).

According to this explanation, the resting potential, sometimes called a membrane potential, is the net result of all the charges inside and outside the cell. Immobile proteins have a net negative charge (anions), but cannot move outside the cell, chloride ions (Cl^-) are nearly in equilibrium across the membrane, K^+ is at a higher concentration inside than out, Na^+ is at a higher concentration outside, and calcium ions (Ca^{++}) are present in concentrations that have largely been ignored.

Cellular Resting Potential cont.

The problem with this scenario is that the integrity of the membrane is not necessary to maintain the resting potential (Pollack, 2001). Large holes can and do appear in the membrane, and if the Na^+-K^+ ion pump were necessary to maintain the resting potential, it surely would be overwhelmed by passive diffusion of K^+ and Na^+. Nonetheless, the resting potential does not disappear. We can no longer call it a membrane potential if it doesn't depend on the membrane.

According to Pollack (2001), much of the negative charge inside the cell is due to the immobile proteins, with some small additional negative charge from glycoproteins, nucleic acids, and other constituents. In the gel that constitutes the cytoplasm of the cell, there is some room for relatively small hydrated K^+ ions, but hardly any room for relatively large hydrated Na^+ ions. Some Cl^- ions are present (they are relatively small), but together with the K^+ ions they do not offset the overall negative charge of the internal proteins. Additionally, the internal concentration of Na^+ is much smaller than the internal concentration of the K^+ ions, all without need to resort to an ion-pump explanation.

3.7.3 Suspensions

Jan Baptista van Helmont believed that anyone could make mice by mixing dirty rags with wheat. How could anyone doubt this? All that was needed was to put the two items together in an open container, wait an appropriate time, and sure enough, the mice would appear.

-Hal Hellman

Suspensions consist of relatively large particles in a liquid. The particles eventually settle unless disturbed by shaking or turbulent flow. Suspensions can be important in biology because the large particles sometimes scatter or reflect light necessary for aquatic species, or they may form a sediment when they settle that can either entrap smaller individuals or choke filtration mechanisms. Suspensions can sometimes be stabilized by detergents that attach the particles to water or by the formation of a gel structure.

3.7.4 Isoelectric Point

Never doubt the power of a doggedly curious mind.

-Camille Rey

Amino acids are made of an amino group (NH_2) and a carboxyl group (COOH). These are both diagrammed in Table 3.6.1. There are, in addition, other attached organic groups that give each amino acid its unique character. When there is an abundance of H^+ ions (protons) in the surrounding water (pH < 7, or acidic solution), the amino group acts as a proton acceptor and forms ionized NH_3^+. When the surrounding water contains an abundance of OH^- ions, the carboxyl group acts as a proton donor and becomes ionized COO^-. Both of these ionized forms are present in Table 3.6.1.

Amino acids can thus act as either weak acids (proton donors) or weak bases (proton acceptors), depending on pH. Such molecules are termed *amphoteric*. Weak acids or bases, as we have seen in Section 3.4.1 dissociate only partly, and can act as buffers. They can also carry a net positive or negative charge.

There is a pH value at which the positive charges on the NH_3^+ and the negative charges on the COO^- exactly balance. It is at this point where the amino acid molecule is electrically neutral. At all other pH values, the molecule carries either a net positive or net negative charge. The pH value for electrical neutrality is called the *isoelectric point*.

Proteins are composed of combinations of amino acids as well as other functional groups. Due to a preponderance of weakly acidic groups, proteins are nearly all negatively charged at neutral pH. Thus, proteins trapped inside living cells will usually carry a net negative charge.

The greater the pH deviates from the isoelectric point, the greater will be the charge on protein molecules. If proteins are suspended in water with varying pH, and an electrically negative cathode and positive anode are in place, the protein will move toward the cathode at low pH, will not move at all at the isoelectric point, and will move toward the anode at high pH values. Proteinaceous materials such as wool, silk, and gelatin will exhibit this behavior.

The solubility of the protein is minimal at the isoelectric point. Also, because protein shape is determined very much by intramolecular electrical attraction (see Section 3.8), the shapes of proteins can be dramatically altered by pH. Such abnormally-shaped proteins are called *denatured* and no longer function for their intended purposes.

Example 3.7.1 Clarifying Wine

Fermentation affects the sugars in grape juice, but the proteins remain in suspension with the result that the wine appears cloudy. To clear the wine,

a fine clay-like substance called Bentonite can be added. Bentonite attracts positively-charged particles and precipitates them. This process is called *fining*.

Wine is usually very acidic and below the isoelectric point of the suspended proteins. At a pH of 3.2, 100% of the proteins are positively charged and are capable of being removed with a Bentonite fining agent.

Isoelectric points for proteins in wine are between 3.6 and 7.1 (Zoecklin, 2000). In some years, wine pH is at or above 3.6. For those proteins above their isoelectric points, they become negatively charged and Bentonite has no effect. In that case, it becomes much more difficult to clarify the wine.

Applications and Predictions

1. Increased pressure will cause more salt to dissolve in water.
2. To dissolve a lipid, a detergent must be added to bridge between the hydrophobic lipid and polar water molecules.
3. Increased temperature will usually cause more salt or sugar to dissolve in water.
4. Suspensions will eventually settle. Using a centrifuge will speed the settling.
5. pH can have a profound effect on the net charge of a protein.
6. Constant motion of intracellular components maintains the unstructured state of water molecules, allowing water to be a better solvent.
7. The gel structure inside a cell protects cell integrity when injecting materials into a cell.
8. The resting potential of a cell will be changed based on the pH of the surrounding solution.
9. Cells with membranes partially destroyed can still function.
10. Shooting genetic material or drugs through cell membranes will not destroy the cells unless it also destroys the gel-like structure of the cytoplasm.

3.8 Protein Folding

Life, mind, and self are all constituted not by biochemistry, but by the higher-level patterns that biochemistry makes possible.

-Douglas Hofstadter

Proteins are very diverse and complex. The intestinal bacterium *Escherichia coli* contains about 2400 different proteins, with an average length of 320 amino acids (King et al, 2002). The simple nematode *Caenorhabditis elegans* has 14,261 different proteins, ranging from 40 to 2,000 amino acids long. Human beings are estimated to have 30,000 genes, each coding for a different protein. The muscle protein titin is one of the longest of these proteins, containing 10,000 amino acids.

The challenge in *proteomics* is to learn how final protein structures are formed, and the steps involved in their formations. With that knowledge, important proteins can be synthesized in the lab and in industry, improvements in protein enzyme activity can be attempted, and the proteins derived from genetic deficiencies may be able to be corrected. Protein study challenges are listed in Chen and Sivachenko (2005).

Proteins are formed by the intracellular ribosomes (see Section 5.3.2) with instructions from the DNA, where amino acids are strung together to form long chains. Depending on the organism, amino acids may be strung together at a pace of 20 per second in bacteria or 5 to 10 per second in humans (King et al, 2002). The resulting proteins begin to fold even before they are completely pieced together, but the exact nature of the processes or intermediate products are as yet unknown.

Intricate three-dimensional shapes are crucial to protein functioning. These shapes come about by cross-linking between various biochemical functional groups (mostly amino acids) on the long-chain protein molecule (King et al, 2002). It is the cross-linking among these elements that provides the motivation and the stability of protein structure.

Protein folding is the result of interactions among very weak chemical attractions. Proteins have evolved to fold in conditions present in the cells. In some cases, *chaperone proteins* are required to help other proteins to fold properly. the structural proteins actin and tubulin cannot fold without their specific chaperonins present (King et al, 2002).

This problem provides a particular challenge to biotechnologists attempting to produce functional proteins *in vitro*. Although the sequence of amino acids and side chains on the protein backbone structure may be known and duplicated, proper folding can only be achieved under conditions determined empirically. Natural conditions within the cell are extremely complex, with water, salts, other proteins, complex molecule building blocks, and other biochemicals, all at the proper temperature. Tens of thousands of interactions are probably involved in folding a single protein (King et al, 2002).

If the protein fails to fold properly, its shape is incorrect and it cannot perform its intended function. Aberrations in protein folding appear to contribute to human diseases. Among these are Alzheimer's disease, prion diseases, emphysema and cirrhosis, amyelotrophic lateral sclerosis (Lou Gehrig's disease), cystic fibrosis, some tumors, and osteogenesis imperfecta (King et al, 2002). The prion that seems to cause ovine transmissible spongiform encephalopathy, for instance, appears as a pleated sheet rather than a smooth helix.

There are four separate levels of protein structure that constitute protein shape (Figure 3.8.1). Not all proteins have all four levels.

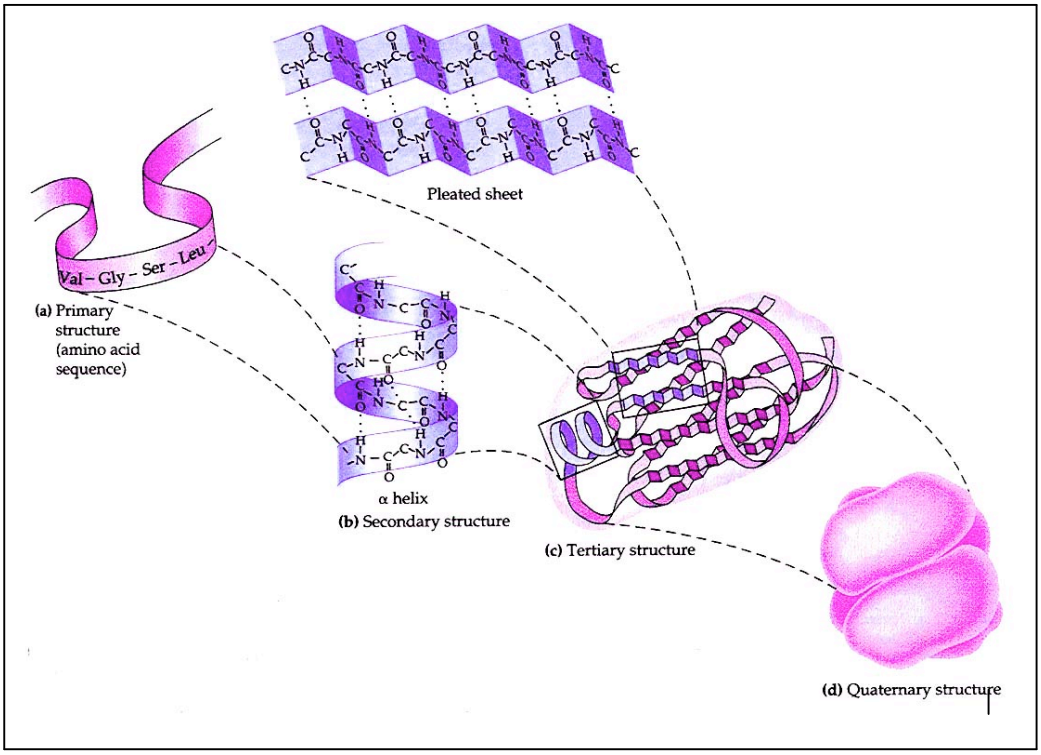


Figure 3.8.1. Diagram of four levels of protein structure (Campbell et al, 1999).

The primary protein structure is its unique sequence of a chain of amino acids (Campbell et al, 1999), called a *polypeptide backbone*. This sequence is determined by the genetic code of DNA.

Even a small change of one amino acid substituted for another can drastically alter the configuration of the protein. On the other hand, some amino acid substitutions have relatively minor effect and the protein may still be able to function nearly normally. The difference between normal red blood cells and sickle cell red blood cells is one strategically-placed amino acid in hemoglobin.

The secondary protein structure, formed once the primary structure is in place, results from hydrogen bonding between weakly positive hydrogen atoms and weakly negative oxygen and nitrogen atoms appearing along the polypeptide backbone of the protein. Although each hydrogen bond is very weak, a multitude of bonds strengthens the entire structure.

Secondary structures can take two forms: an α-helix is a spiral shape, whereas the pleated sheet looks like a piece of paper folded many times. The parallel polypeptide chains cross-linked by hydrogen bonds form an extremely tough structure. Silk is an example (Hale et al, 1995).

Tertiary protein structure is superimposed on the others, and appears as irregular contortions in protein shape. Various chemical bonds form the basis for tertiary structure. Hydrophobic reactions cause nonpolar parts of the protein to congregate in clusters inside the core of the protein away from contact with polar water molecules. Van der Waals forces between nonpolar groups strengthen the shape. Hydrophobic reactions occur almost immediately after the proteins are formed, and may be one of the primary instigators of protein folding. Hydrogen bonds between polar side chains and ionic bonds between charged elements appearing at various sites help to

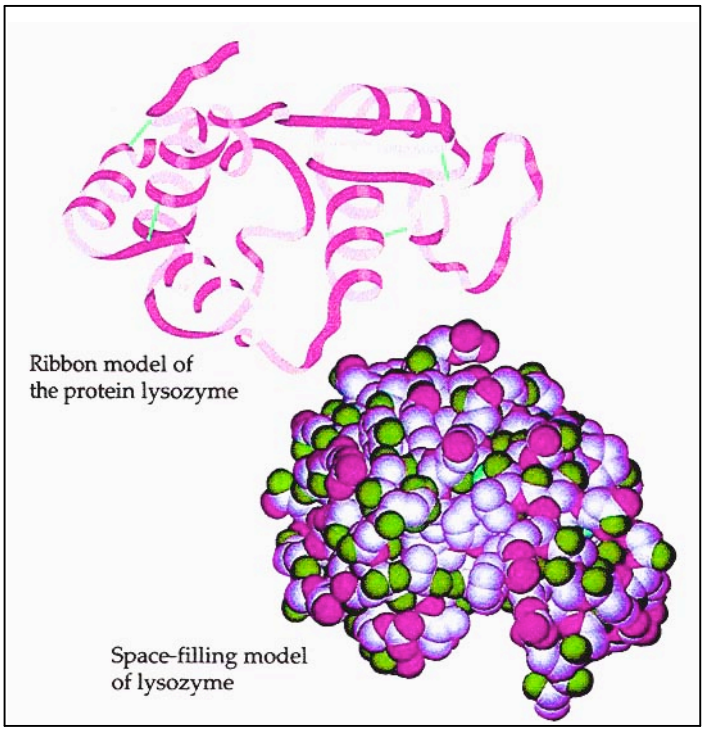


Figure 3.8.2. Two schematic models of the protein lysozyme. The ribbon model reveals the underlying protein shape (Campbell et al, 1999).

consolidate the structure. Very strong disulfide bridges form where two sulfur atoms are located adjacent to one another. The three-dimensional tertiary globular structure is represented by enzymes, antibodies, most blood proteins, and myoglobin.

Quaternary structure occurs with proteins formed from two or more polypeptide chains acting together. These chains may be coiled around one another or tangled together. An example is hemoglobin (Hale et al, 1995).

In Figure 3.8.1 are shown diagrams of these four structures. The diagram is drawn in popular ribbon form that emphasizes the polypeptide backbone of the protein. The protein itself looks more like the diagram in Figure 3.8.2, where all atoms are bunched together. It is the entire configuration of the protein that makes it functional, and defects in any part of the shape can make the entire protein unable to be useful in cellular processes.

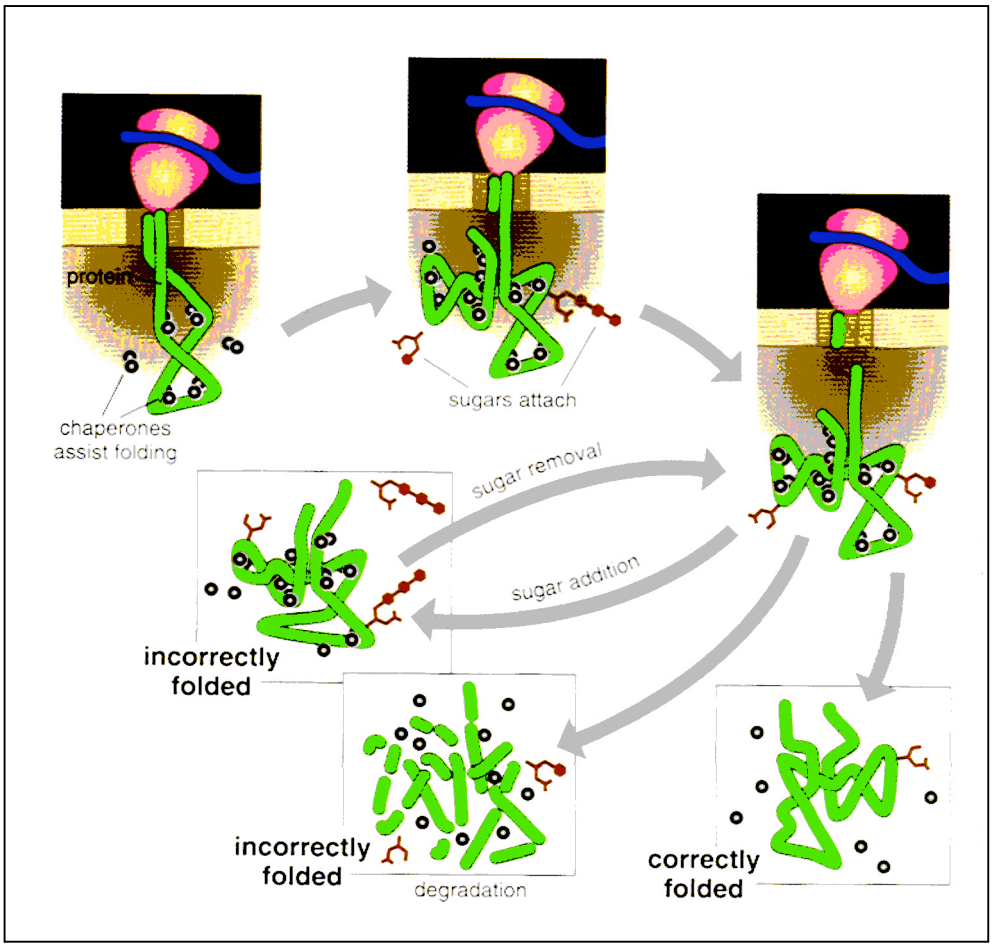


Figure 3.8.3. As proteins are formed chaperone molecules bind to them and assist them to fold in the proper way. These same chaperone molecules can recognize features of improperly-folded proteins. Addition or removal of sugar molecules aids in the diagnosis. Incorrectly-folded proteins may either be decomposed or fixed (Conn and Janovick, 2005).

Functional proteins that are folded the wrong ways are not useful. Such misfolding can result from genetic mutations or from faulty processing of the multi-amino protein backbones from which all proteins are formed. Abnormal folding can occur because of protein overabundance, temperature changes, oxidative stress, and some cellular signals (Conn and Jamovick, 2005). It is believed that proteins do not spontaneously fold into their final, active formations.

Proteins are originally synthesized inside ribosomes in the cytoplasm or ribosomes inside the *endoplasmic reticulum*. the latter synthesize proteins intended to be embedded in the cell membrane (Conn and Jamovick, 2005). When released from the ribosomes, the protein backbone first folds in groups of four or more amino acids to limit interference from bulky or charge-bearing portions of the molecule. Chaperone molecules assist the proteins to fold into their correct three-dimensional shapes. This is especially difficult among the molecules crowded inside the cell.

Mutant proteins, those incorrectly folded, are flagged by the cell's quality control mechanism and destined either for destruction or reprocessing (Figure 3.8.3). Terminally misfolded proteins are marked by the addition of many copies of a small protein called *ubiquitin*, and are then digested into fragments by *proteasomes*.

One of many characteristics of misfolded proteins is the presence of hydrophobic portions on their surfaces; normally only hydrophilic portions appear on the outside. The problem is that the cell's quality control mechanism relies on chemical, not functional, tests. Thus, a protein labeled for destruction or reprocessing may actually be functionally equivalent or superior to a normal protein.

There are many diseases caused by misfolded proteins. Among them are cystic fibrosis, retinitis pigmentosa, Alzheimer's disease, cataracts, and certain cancers. Treating these diseases at the protein level may be easier and more ethical than using gene therapy. The strategy would be to restore missing or nonfunctional proteins with pharmacological chaperone molecules inserted into the cells.

Heat Shock Proteins

Exposure to high temperatures poses a danger to living organisms because essential proteins and enzymes unfold, a process called *denaturation*. When this happens, the compounds no longer mate with complementary shape receptors or chemicals, and normal biological activities cease.

Organisms have developed a common heat stress response. There is a rapid production of *heat shock proteins* that act as molecular chaperones to ensure that newly formed proteins are folded properly, and damaged proteins

Heat Shock Proteins cont.

are either repaired or removed from the cell (Taiz and Zeiger, 1998). In addition to stabilizing unfolded protein precursors and either repairing or degrading damaged proteins, heat shock proteins translocate proteins across cellular membranes and dissolve protein aggregates (Werner it al, 2003). This is an energy-intensive process: repair of each damaged protein molecule requires as much as 100 ATP molecules.

Although the heat stress response appears to be universal across both animals and plants, it is in plants, which cannot move to escape the heat, where heat shock proteins are especially important for survival. Plants thus have more complex heat stress response components than animals. Different heat shock proteins are localized to the nucleus, mitochondria, chloroplasts, endoplasmic reticulum, and cytosol.

Heat shock proteins are not unique to high temperature stress; some are induced by other environmental stresses such as water deficit, chemical exposure, wounding, low temperature and salinity (Taiz and Zeiger, 1998). Exposure to one type of stress can cause protection against exposure to others. Therefore, production and isolation of heat shock proteins in bioreactors has commercial value when they are subsequently applied to stressed organisms.

Genes for heat shock protein production are encoded in the cell, but are not activated until other protein intermediaries sense damaging stresses and switch them on (ASAE, 2002). Thus, this is a wonderful example of the complex feedback that occurs between the environment and the basic genetic regulation of cell activity.

Example 3.8.1 Biomarkers As Indicators of Environmental Stresses

Maintaining natural populations of aquatic organisms in lakes, rivers, and streams is not simple. Human activities have so modified temperature, pH, salinity, and toxin concentrations that plants, insects, fish, and amphibians living in those bodies of water face a multitude of difficulties. The problem is especially acute for sessile organisms that cannot escape to less stressful environs.

Proper management requires that stresses be known. If a human is under stress, she or he can tell the investigator. But asking a fish is out of the question. How can environmentalists determine the level of stress in a fish?

Solution: An indirect measure of stress can be made by determining levels of all physical and chemical stressors in the environment. However, not only is this an expensive proposition, but it is not easy to translate these physical

measurements into a stress index. It would be much better to directly measure the level of stress in the living fish.

Stress proteins (also called heat shock proteins) are meant to protect organisms against damaging environmental effects. They do this by repairing damaged proteins. Stress proteins can act as biomarkers of integrated environmental stress effects.

Stress proteins are nearly the same across a wide range of species. Thus, it is not necessary to search for specific stress proteins for each type of organism. The hsp70 family of stress proteins can be monitored in the target species as an input for proper wildlife management (Werner et al, 2003).

Remark: Werner et al (2003) found that the sensitivity of fish embryos to toxic chemicals in the San Francisco Bay area is age-dependent. Very young embryos do not manufacture sufficient stress proteins to correct environmentally damaged proteins. Thus, susceptibility to environmental stress in early development is critical to survival of these species.

Example 3.8.2 Biomarkers as Indicators of Disease

A biomarker has been defined by the National Institutes of Health as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to therapeutic interventions." Biomarkers can be antecedent (indicates the proclivity to develop a disease), diagnostic (assists in the identification of a disease), or prognostic (predicts the progression or cure of the disease). Biomarkers can be as simple as the appearance of tumors in cancer or as complex as a protein profile in lupus. Advances in molecular imaging and compound detection have advanced the state of biomarker identification. When biomarker technology is sufficiently advanced, biomarkers can be used as rapid indicators of drug efficacy in the treatment of disease.

Applications and Predictions

1. Substituting a similar amino acid (polar, charged, or uncharged) in a polypeptide chain will have a much less drastic effect on the protein function than will the substitution of a much different amino acid.
2. Improperly formed proteins can cause disease.
3. Easily-measured biomarkers should be sought for any biological condition of interest.
4. Understanding of chaperone molecules can help cure disease.

3.9 Shape Effects and Enzymes

The chemical reaction should go, but refuses to do so? Bang on it – with heat, with light, with pressure. Or, so much better, find a catalyst – an ingenious partner in a reaction that cuts down an energy hill that is in the way, or gets involved with the reactant molecules , takes them in hand, so to speak, and guides them in a path around that hill.

-Roald Hoffmann

Reactions among organic compounds are extremely slow if left to themselves. They are too slow, in fact, to sustain life. Because living things exist, they have obviously found a way to circumvent the problem.

Before two compounds can react chemically, they must contact each other. Collision theory has been formulated to explain various factors on chemical reaction rates. Part of this theory is the Law of Mass Action, which states that a chemical reaction will occur at a rate proportional to the concentration of its reactants. Thus, anything that can be done to concentrate reaction precursors in the presence of each other will favor the desired chemical combination to occur.

One means to accomplish concentration is by exploiting surface effects of chemical compounds. Surface atoms do not have their electron needs totally fulfilled. They tend to be very reactive and attract other molecules with unusually strong forces. Charcoal or carbon is a substance with especially strong surface forces, although there are a large number of other substances with strong surface attractiveness as well. Many of these are used as glues or cements. What makes carbon so unique is the many channels and pores that can be formed in it to increase its surface area by orders of magnitude. Carbon tends to attract many ionic or polar molecules to its surface, and is largely nondiscriminatory. Carbon can thus be used to concentrate many different chemical compounds. At temperatures low enough to reduce the energies of gaseous particles, nonpolar atoms such as O_2 can be attracted in large numbers to carbon, probably as a result of van der Waals forces.

A more important strategy for living beings is the use of enzymes. Enzymes are proteins that are shaped by twisting and bending such that they fit extremely well with the shapes of certain chemical reactants. One part of an enzyme molecule may fit well with one reactant and another adjacent part may fit with a second reactant. With the two reactants in such close proximity, they readily combine. Once combined, the product of the reaction is removed from the enzyme and the enzyme is ready to repeat the process. The enzyme is not changed by the reaction; enzyme action is largely physical.

Enzymes are characterized by their actions. There are enzymes that link two compounds together (called *ligases*), enzymes that transfer a chemical group from one compound to another (*transferases*), enzymes that catalyze hydrolysis (*hydrolases*), enzymes that remove a chemical group from substrates (*lyases*), enzymes that oxidize some compounds while reducing

others (*oxidoreductases*), and enzymes that convert one isomer to another (*isomerases*). In general, there is an enzyme for every chemical reaction important to living systems. Some enzymes are very specific, yet others may have several functions. For instance, the enzyme maltase is specific to the sugar maltose and produces glucose as a result of its action. Chymotrypsin, on the other hand, is a general enzyme for the digestion of proteins, and can operate on many proteins.

Because enzymes are classified by their actions rather than by their actual chemical composition, many different enzymes can be identified by the same name. There are four different hexokinases that occur in tissues. Each operates on a glucose substrate, but each has a different sensitivity to glucose concentrations.

Most enzymes catalyze reactions of their substrates only in the presence of particular organic compounds called *coenzymes*. The B vitamins are important coenzymes, for instance. Coenzymes can be considered to be co-substrates, because they frequently take part in the chemical reactions, and often change form; if the substrate is oxidized, the coenzyme may be reduced, for example, or the coenzyme may act as the receptor, donor, or carrier for some functional group that is modified on the substrate.

Many enzymes also show a specific requirement for a particular metal ion. These include various ions of iron, copper, cobalt, molybdenum, zinc, manganese, magnesium, calcium, and potassium. The metal ion is thought to have a binding function for the substrate, coenzyme, and enzyme. Thus, these metals are needed in the diet in trace amounts.

Enzyme action may be decreased by various competitive and noncompetitive inhibitors. An inhibitor is a compound that combines with an enzyme, but which is not converted into a product. The result is an enzyme-inhibitor combination that changes the configuration of the enzyme enough that the enzyme is no longer effective. Competitive inhibitors are affected by the relative amounts of substrate and inhibitor; the more substrate available, the less inhibition that occurs. Noncompetitive inhibitors bear no structural resemblance to the substrates and so are not affected by substrate concentration.

Competitive inhibitors that block enzyme reactions in a parasite are potent chemotherapeutic agents. For example, sulfanilamide will block folic acid (a B vitamin) synthesis, and the resulting deficiency of this essential vitamin is fatal to the organism. Because humans cannot synthesize folic acid, but require it as a nutritional supplement, humans are not harmed by sulfanilamide ingestion. Vitamin B, however, is often in short supply in nature.

There are also enzyme inhibitors used as defenses by an organism. Pepsin and trypsin inhibitors are found in the pancreas and keep the pancreas from being digested by its own enzymes. Some enzyme inhibitors also act as antibodies against microbial infections (see Section 6.20.3).

Not all enzyme interactions are competitive. One cooperative enzyme reaction takes place when a substrate primes an enzyme with multiple reactive

sites to accept additional substrate molecules. Thus, the presence of substrate enhances the enzyme and makes it more effective.

With enzyme performance dependent on molecule configuration, anything that disrupts that configuration will interfere with enzymatic activity. The helical structure of the proteins comprising the enzyme is maintained largely through hydrogen bonding and sometimes with cross-link bonds involving elements other than hydrogen. These bonds are relatively weak compared to the covalent carbon bonds present in the carbon chain, and are therefore affected by temperature. At higher temperatures, these bonds begin to break and reduce enzyme effectiveness. The temperature at which this begins to happen depends to some extent on the normal operating temperature of the organism. An organism acclimated to a higher environmental temperature will possess enzymes that maintain their effectiveness at higher temperatures than those organisms operating normally at lower temperature environments. The actual enzyme proteins used by these two organisms would be different, although their functions (and thus their names) would be the same. At very high temperatures, these cross-link bonds cannot be reestablished and the compounds lose all enzymatic functionality. This is called *denaturation*.

One characteristic of enzyme action is that a large chemical change is usually brought about in small steps. Thus one chemical reactant is not changed all at once into a final product. Instead, a series of specific enzymes change the initial reactant into intermediate products, each closer to the final product. The *law of constant heat sums* states that the total amount of heat produced or consumed when a chemical system changes from an initial state to a final state is independent of the way in which the change is brought about. For example, glucose is oxidized to carbon dioxide and water by the following equation:

$$C_6H_{12}O_6 + 6O_2 = 6H_2O + 6CO_2 + 2.82 \times 10^6 \text{ N·m} \qquad (3.9.1)$$

which is the process by direct burning. With the enzyme zymase, glucose is oxidized in two steps:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + 0.08 \times 10^6 \text{ N·m} \qquad (3.9.2)$$

$$2C_2H_5OH + 6O_2 \rightarrow 6H_2O + 4CO_2 + 2.74 \times 10^6 \text{ N·m} \qquad (3.9.3)$$

The resulting products and energy sum is the same for both pathways.

Enzymes are the regulators of biological metabolism. Chemical reaction rates in the absence of enzymes are too slow to maintain life. Enzymes allow intracellular processes to adjust to new environmental factors and to reach the point where growth can occur. This system can be regulated in two ways: 1) the stimulation or inhibition of enzyme activity, and 2) the induction or repression of enzyme synthesis (Figure 3.9.1). The product of enzyme activity, or compounds derived from those products, can act as

inhibitors of enzyme activity. There are two possible mechanisms for this (Figure 3.9.2): 1) the presence of large amounts of product can inhibit the conversion of substrate through the law of mass action and a reversible biochemical reaction. Alternatively, 2) the product may be a potent competitive inhibitor for the substrate (called *feedback inhibition*).

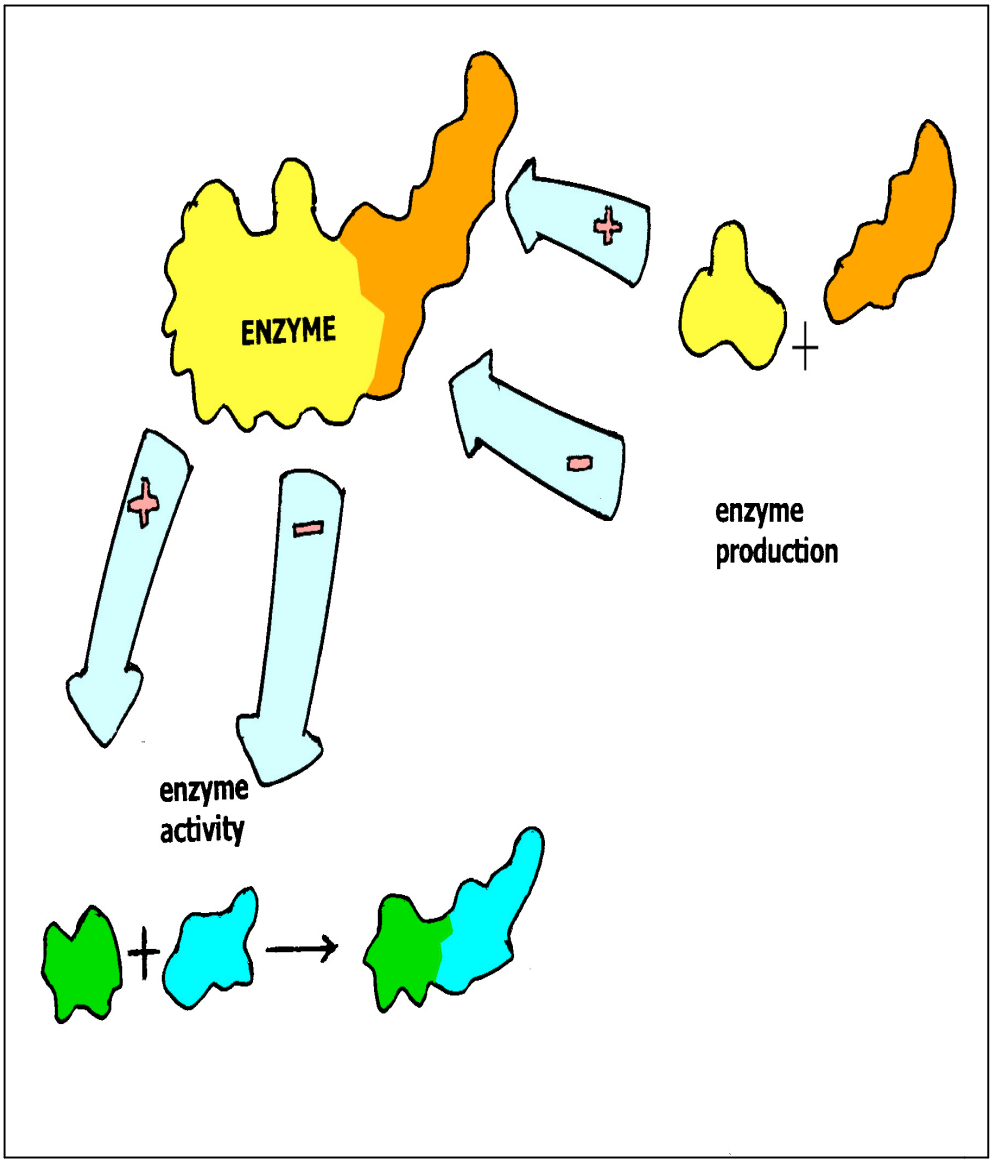


Figure 3.9.1. The two methods to regulate enzyme effectiveness are through the production or activity of enzymes.

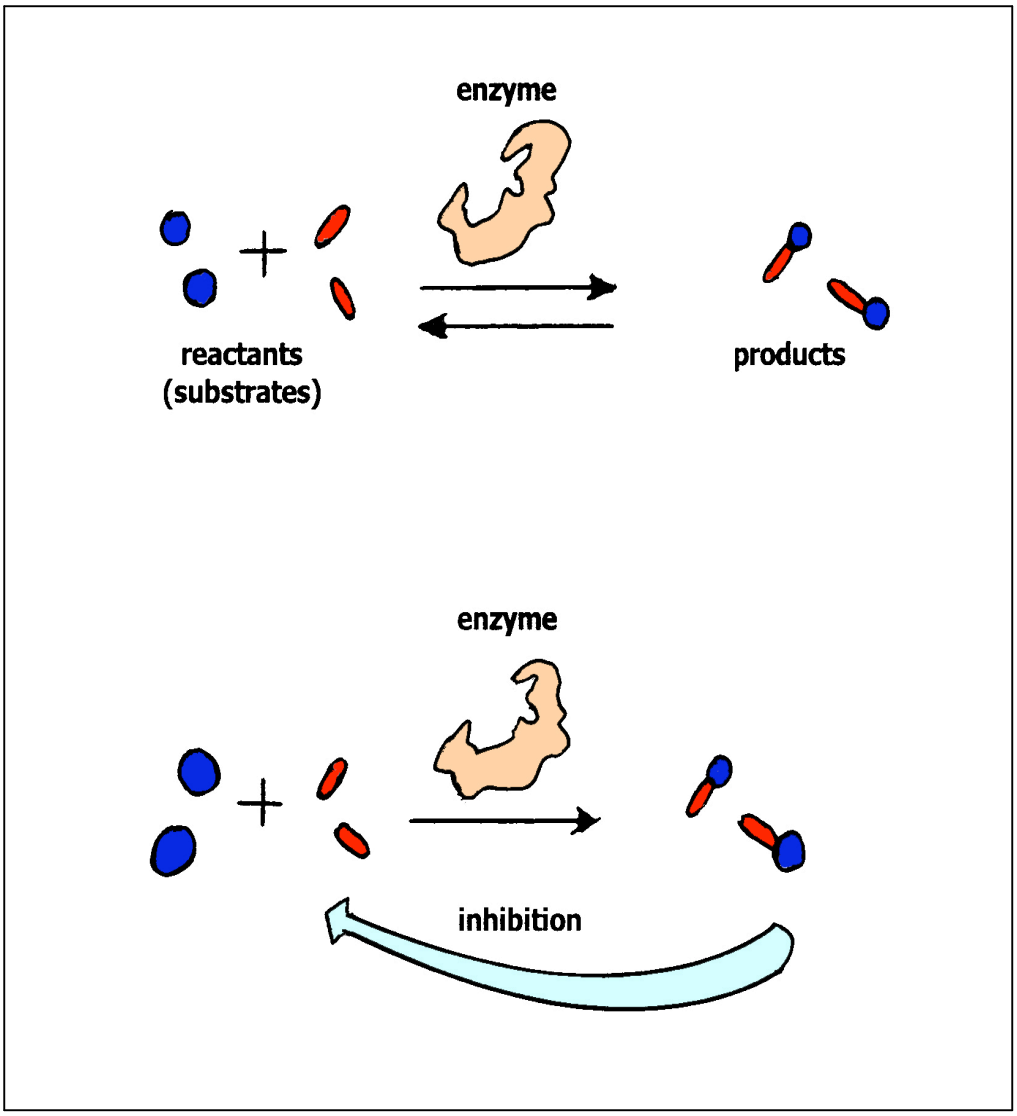


Figure 3.9.2. Inhibition of enzyme activity can be due to a high concentration of the product, or through direct inhibition of the product on the substrates, or through product repression of enzyme synthesis.

The product may also inhibit the formation of the enzyme, in a process called *repression.* In this way, a relatively small molecule (the product) has a direct effect on the production of a large molecule (the enzyme), and thereby an indirect effect on the formation of more product. Such exquisite control allows an organism to respond to a variety of substrates in a wide range of conditions to display adaptability and survival.

Applications and Predictions

1. Activated carbon can be used to enhance reaction rates.
2. The rate of a chemical reaction involving enzymes will be limited by both amounts of reactants and enzymes. Without enzymes, some reactions would practically not occur.
3. The name of an enzyme will indicate its function, but will not designate a particular compound.
4. An unwanted enzymatic reaction can be inactivated by introducing an inhibitor.
5. Bacteria will make their own folic acid. Inhibiting this synthesis can be used as an antibacterial strategy.
6. High temperatures will interfere with proper functioning of an enzyme.
7. Without inhibitors, organisms will digest themselves.
8. Enzyme regulation will be a complex process that can be disrupted for control of unwanted organisms in the environment, or production of preferred biochemicals in a reactor.
9. Enzymes will be denatured by harsh chemicals as well as by heat.
10. Vitamins will be necessary to form certain enzymes.
11. Enzymes will be able to use similar related compounds when the original compounds are not available.
12. Any substrate that bonds tightly with an enzyme will inactivate the enzyme.
13. Controlling reaction rates within limits requires the presence of inhibitors as well as enzymes.
14. Some useful drugs act as enzyme inhibitors.

3.10 Energy-Rich Compounds

If we gather huge numbers of unconscious elements together in the right way and give them the right tasks to perform, then at some point, something happens, *and consciousness emerges.*

-David Gelernter

The reason that dynamite can be used to blast through rocks and remove tree stumps is that dynamite contains an energy-rich compound. Oxidation of nitroglycerin, the active ingredient of dynamite, yields a surplus of energy that can be used to produce mechanical work. Such a chemical reaction is called *exergonic*, or *exothermic*. If the net result of a reaction is that energy is absorbed, as in photosynthesis, the reaction is said to be *endergonic,* or *endothermic*.

Nearly all Earthly life depends ultimately on energy from the sun. However, only certain photosynthetic organisms can capture the sun's energy and store it for future use. Most other life must utilize the energy that these primary energy-transforming organisms store as chemical compounds. Whereas the utilization of energy in as dramatic a fashion as with a dynamite explosion would be quite damaging to living beings, the release of energy in smaller quantities could be used to drive the many processes needed to sustain life. Thus, chemical energy of the right form and amount would be quite useful.

Long-term chemical energy storage resides mainly in carbohydrates, fats, and proteins. Liberation of energy accompanies oxidation of these compounds.

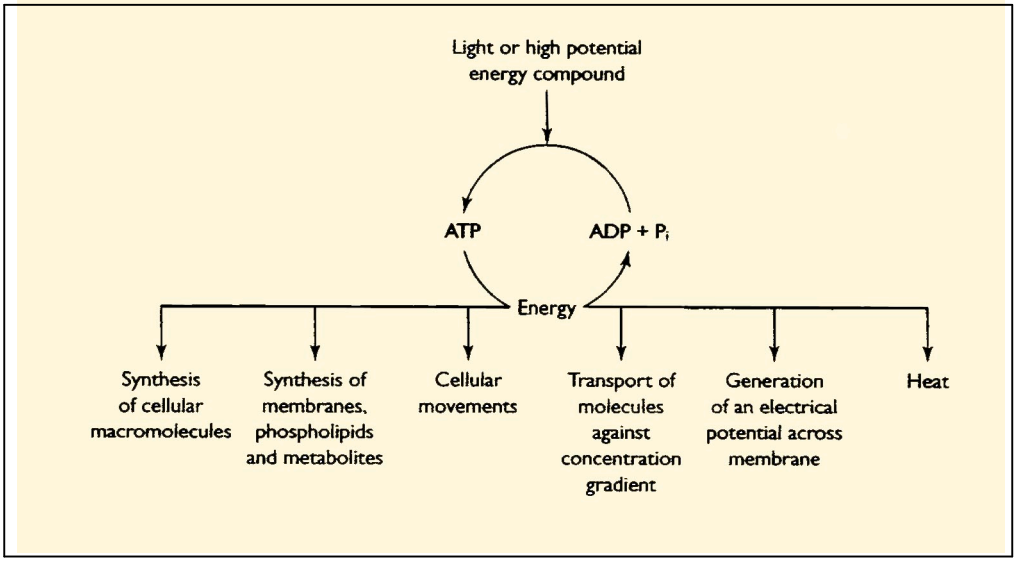


Figure 3.10.1. ATP is central to all life processes.

There is a small family of molecules that is utilized by nearly all living things to shuttle energy between the oxidative reactions of primary storage compounds and the chemical processes that maintain life (Figure 3.10.1). Most of these contain high-energy phosphate bonds. Of these, adenosine triphosphate (ATP) is the most important. ATP is formed from adenosine, which is a neucleotide formed from the double ring purine adenine with the 5-carbon sugar ribose, and three phosphate groups. The three phosphates are attached to one another (de Duve, 1995).

The two bonds linking the three phosphates are high energy bonds, called *pyrophosphate bonds*. The formation of these bonds is accompanied by the removal of a water molecule, called a *condensation reaction*. When the pyrophosphate bond is split, water is added, and this is called *hydrolysis*. Hydrolysis liberates energy (the new bond has lowered energy level) and condensation absorbs energy (the new bond has a higher amount of stored energy) in a water-filled environment. The pyrophosphate bond thus acts as a chemical storage battery, with energy available to drive other chemical reactions.

The other chemical reaction (whatever it is) will occur as long as it requires less energy than the energy stored in ATP. If, on the other hand, the other reaction takes more energy than the formation of the pyrophosphate bond, then the pyrophosphate bond will be formed. In terms of effort and flow variables, the bond energy is the effort variable, and the chemical reaction is the flow. The reaction will only occur if the effort variable goes from higher to lower.

The Physical Action of ATP

Adenosine triphosphate (ATP) is the universal energy carrier for life. It is ATP that stores energy to be used on demand, and this energy can actually take both chemical and physical forms (Pollack, 2001). The close proximity of its three phosphate complexes results in a high concentration of negative charge. Because of this, it has a high affinity for protein surfaces; ATP association for the protein myosin, for instance, is practically irreversible.

Such high charge concentration can induce structure by linking to protein strands that form the gel inside living cells. ATP negative charge can then repel negative charges on adjacent protein strands and cause them to extend to their high-energy conformation (see Section 3.8). These extended strands are the ones with structured water between them. Thus, ATP confers energy to the cell by causing order and structure.

Without ATP, muscle is in a constant state of tension. Its actin and myosin proteins are cross-linked to one another and contracted (Pollack, 2001). ATP breaks actin-myosin cross-links, and restores the proteins to their extended state. ATP does this despite the fact that its binding sites are located remotely from the sites of cross-linking and contraction. ATP drives the system to its high energy state.

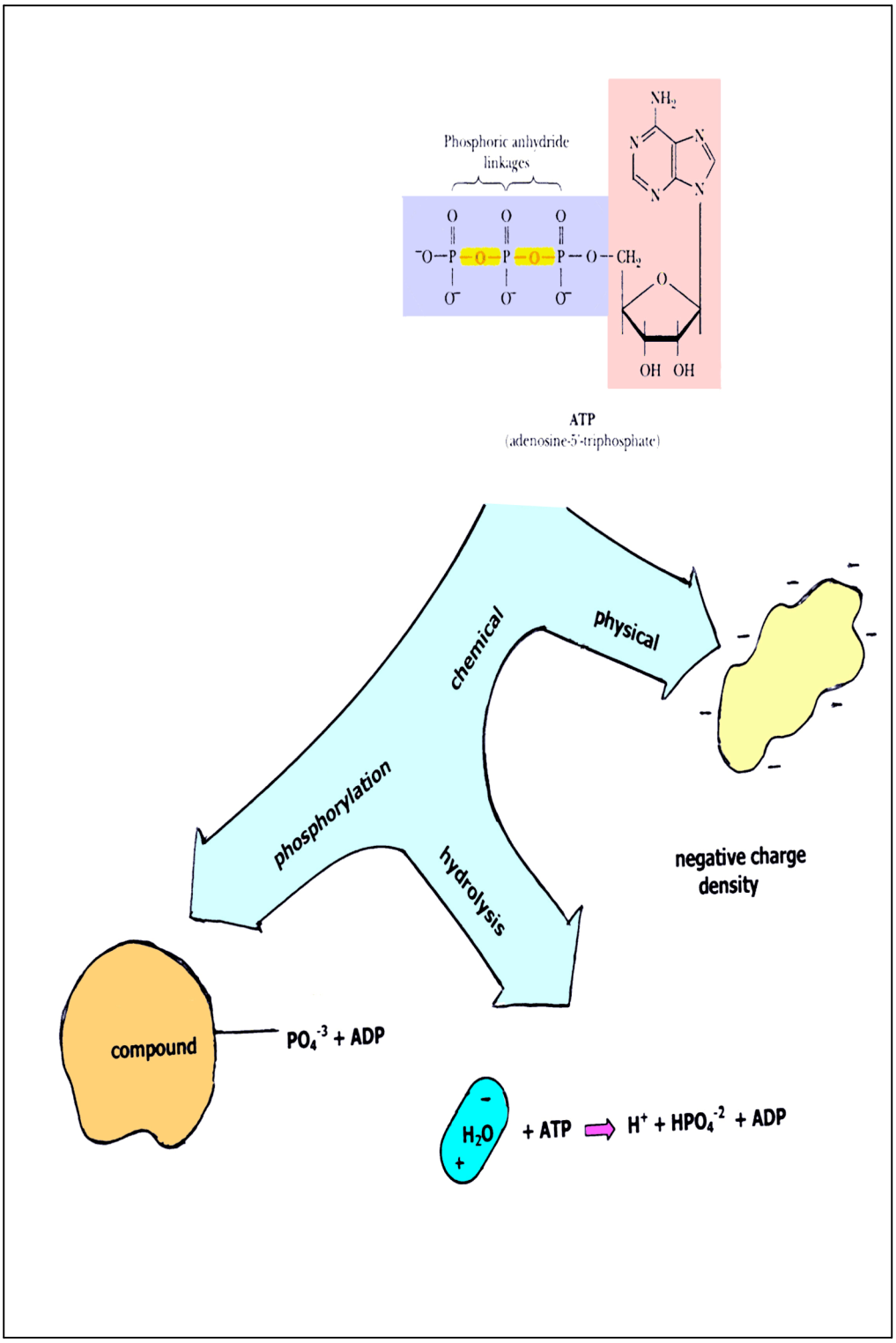


Figure 3.10.2. Energetics of ATP. There are multiple mechanisms for ATP activity, including both physical (charge density) and chemical (hydrolysis and phosphorylation).

Most of the bonds in biological substances require less energy for their formation than do the pyrophosphate bonds in ATP. The bonds of proteins and other natural substances are low energy bonds in comparison. This is why ATP is so valuable to life (de Duve, 1995).

ATP can transfer energy to another molecule in a variety of ways (Figure 3.10.2). The most common way is the transfer of a phosphate group to the molecule that requires energy (Fogiel, 1999). This reaction liberates adenosine diphosphate (ADP), and is known as a *phosphorylation reaction*. It is usually catalyzed by enzymes called *kinases*. If two phosphate groups are transferred, adenosine monophosphate (AMP) remains.

The *hydrolysis* of ATP, wherein the phosphate group is transferred to water can also be used to transfer energy to another molecule (Fogiel, 1999). In addition, the adenosine group can be transferred with the liberation of pyrophosphate and inorganic phosphate.

The source of muscular energy is in the form of ATP. Each mole of ATP represents 46,024 Joules of energy (the net energy available from each mole of ATP depends on the chemical pathway utilized), but there is not enough ATP to supply more than 0.5 seconds of muscular energy use. Creatine phosphate provides additional energy to produce more ATP as needed, but for no more than 2 seconds worth of energy. Glucose can be oxidized to produce longer- term energy, and glycogen (a storage form of glucose) provides even more energy stores. If the energy in these chemicals was not equivalent, then there could be no smooth transition from one energy source to another, but the energy is equivalent, and the muscle can produce both short-term and sustained work from these various energy sources.

Phosphate groups are not the only energy-rich chemical compounds used by living systems. Sulfur can also form high-energy bonds as part of thioesters. Sulfur was probably prevalent in the prebiotic Earth, and was probably used by the most ancient of organisms to provide energy for life (de Duve, 1995). Even today, there are primitive life forms that surround sources of sulfur-containing gases from deep within the Earth, and use these gases as their primary energy sources (see Sections 6, 6.2, and 6.5.1).

Respiration of Glucose
(Starr, 2000)

Glucose is the starting point for respiration, and energy for biochemical reactions is the end point. In between, adenosine triphosphate (ATP) is the substance that can store energy for immediate use; it is the energy currency that makes possible all the processes contributing to life.

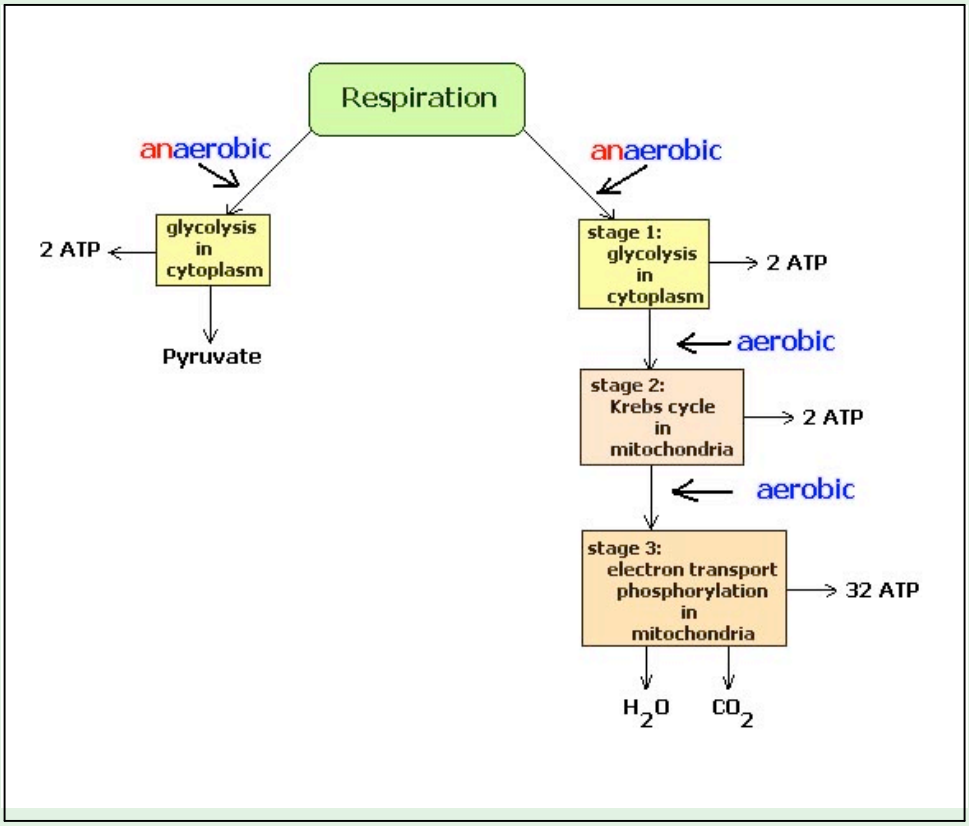
Respiration may be either aerobic (requiring oxygen) or anaerobic (proceeding in the absence of oxygen). The first stage in energy-releasing reactions is anaerobic, and takes place in the cellular cytoplasm. This is a

Respiration of Glucose cont.

very primitive respiratory stage, probably evolving in the first protists (microbes), and is still used today by organisms where oxygen is not abundant.

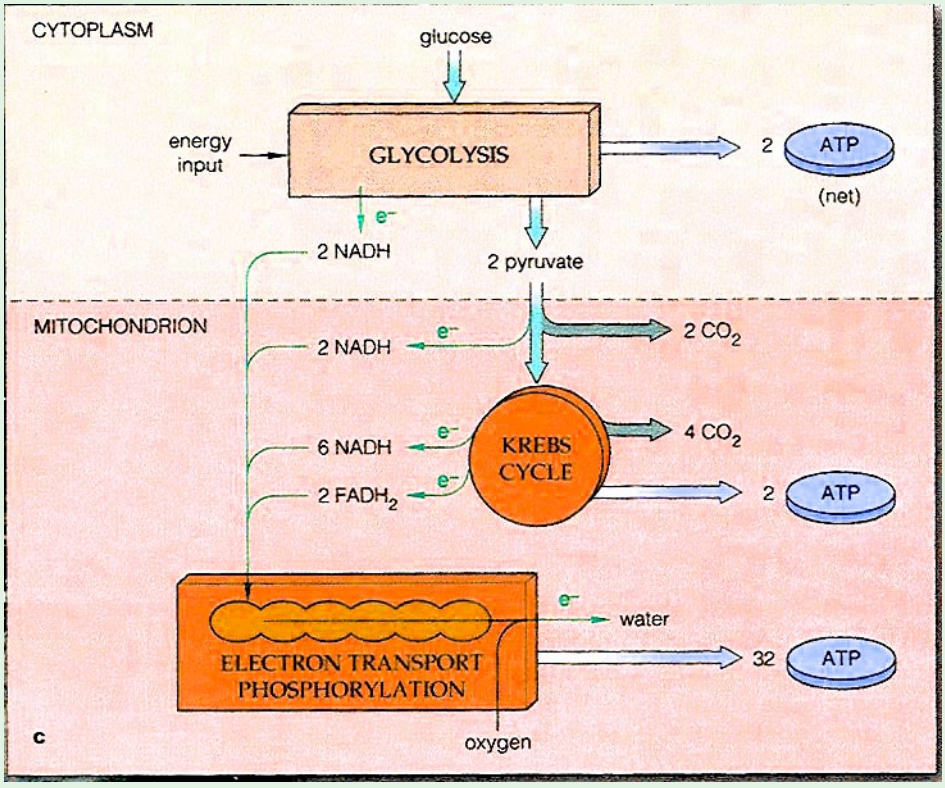
This first stage involves *glycolysis*, where enzymes split each six-carbon chain into two three-carbon molecules of pyruvate. Some energy is necessary to start this reaction, and the net result is the production of two ATP molecules.

Aerobic respiration starts with the anaerobic stage described above and adds two additional stages. The second stage in aerobic respiration proceeds according to the Krebs, or citric acid, cycle. This reaction takes place in the mitochondrion, a subcellular inclusion not present in the most primitive microbes (see Section 5.5.1). Pyruvate from glycolysis enters the mitochondrion and is shepherded through the cycle by coenzymes NADH and $FADH_2$. Carbon dioxide is formed from the two pyruvate molecules, and two more ATP molecules are formed by phosphorylation from two ADP molecules. Electrons and hydrogen are placed on various coenzyme molecules to set up the third stage.



Respiration of Glucose cont.

The third aerobic respiration stage takes place at the mitochondrial membrane, and is called electron transport phosphorylation. A hydrogen ion gradient is established and H^+ flow drives formation of ATP from ADP. Oxygen serves as the ultimate electron sink and produces water as a result. This stage yields 32 ATP molecules.



The typical yield of ATP molecules is 36, but less ATP molecules may be formed if some of the intermediate substances are in short supply for one reason or another. Each mole of glucose represents 686 kilocalories of energy. Each mole of ATP represents 7.5 kilocalories. Thus, the 36 moles of ATP coming from complete respiration of a mole of glucose contain (36) (7.5) = 270 kcal. The energy efficiency of this process is 270 / 686 = 39%.

Anaerobic organisms derive their energy only from the initial glycolysis step. Animals may also use this as a temporary energy-producing pathway when oxygen is limited. However, only two ATP molecules are produced, and this is not enough to sustain large, active, multicellular organisms.

Applications and Predictions

1. To search for evidence of biological activity, assay for ATP.
2. Microbes surrounding thermal vents will metabolize sulfur-containing compounds for their energy source.
3. Excess glucose storage capacity must be present to supply energy continuously.
4. To kill organisms, interfere with ATP production or utilization.
5. Cells deprived of oxygen will produce energy less efficiently than cells with oxygen.

3.11 Temperature and Pressure Effects

I believe that for most diseases, prevention by control of their origins is cheaper, more humane, and more effective than intervention by treatment after they occur. *-Thomas McKeown*

Temperature and pressure sometimes have profound effects on reaction rates and chemical equilibrium. One reason that biological organisms are more active and grow faster at higher temperatures is that, for every 10^oC rise in temperature, a doubling or tripling of reaction rates can generally be expected. Animals that maintain constant body temperatures (*homeotherms*) have a metabolic advantage (as long as food is not scarce) over animals whose body temperatures vary with environmental conditions (*poikilotherms*) because they can maintain higher metabolic rates. There is a limit to the body temperature that can be maintained, however, because of enzymatic inactivation or denaturation at temperatures approaching 45^oC.

Some chemical reactions are *exothermic*; that is, they produce heat, as in the oxidation of glucose to form water and carbon dioxide. Other chemical reactions require heat to proceed; these are termed *endothermic*. An increase in temperature increases the reaction rate for every reaction, whether it be endothermic or exothermic. However, the rates of endothermic reactions is always increased more than the rates of exothermic reactions for any given rise in temperature.

Chemical equilibria are also affected by temperature, and the specific changes depend upon the type of reaction. Raising the temperature of an exothermic reaction decreases the equilibrium constant, whereas the same temperature increase affects an endothermic reaction by increasing the equilibrium constant.

Increasing temperature or pressure increases the likelihood of the number and force of collision between atoms and thus favors reactions moving more rapidly to completion. The Le Chatelier principle states that if a stress is applied to a system at equilibrium, then the system readjusts, if possible, to reduce the stress. Thus, an endothermic reaction will be enhanced by an increase of temperature because the reaction absorbs heat. Increasing the concentration of one of the reactants drives a reaction toward completion because this action reduces stress on the system.

Increasing pressure on a system can have several different effects, depending on whichever action tends to reduce stress. For instance, for the chemical system that forms gaseous hydrogen iodide from hydrogen gas and gaseous iodine,

$$H_2 + I_2 \leftrightarrow 2HI \qquad (3.11.1)$$

there is no net effect of pressure on the equilibrium of this reaction because there are two molecules of gaseous product that replace two molecules of

gaseous reactants. The rate may increase, but there is no change in equilibrium.

For the reaction forming gaseous water vapor from hydrogen and oxygen,

$$2H_2 + O_2 \leftrightarrow 2H_2O \qquad (3.11.2)$$

increasing pressure tends to form more water vapor because two molecules of gaseous product are formed from three molecules of gaseous reactants. Thus, the overall pressure stress placed on the reaction is reduced by forming more water vapor. If liquid water is formed, the forward reaction is favored even more, because the volume occupied by liquid water is even less than for water vapor, thus relieving pressure stress even further.

Cricket Thermometer

Crickets make a chirping sound, called *stridulation*, when they rub their tiny wings together. The higher the temperature, the faster crickets chirp. This is an example illustrating how metabolism, and actions metabolically-based, depend on temperature. Dolbear's Law states that temperature in degrees Fahrenheit can be determined accurately from the number of chirps counted in 15 seconds:

degrees Fahrenheit = (chirps in 15 sec) + 40

Temperature in degrees Celsius can be obtained from:

degrees Celsius = (degrees Fahrenheit – 32) 5/9

Other insects also use chirps as a means for communicating. Katydids, for instance, can be used to determine temperature from:

degrees Fahrenheit = 60 + (chirps in 1 min – 19) / 3

Applications and Predictions

1. Poikilotherms should be able to survive food scarcity better than homeotherms.
2. Snakes and fish will be poikilothermic.
3. Animals with higher body temperature will produce more body heat.
4. Optimum temperatures will exist for growth, reproduction, conversion of feed to flesh, and other input-output relationships.

5. Enzymes present in poikilotherms are less sensitive to temperature changes than are those in homeotherms.

3.12 Free Energy

Facts are not science – as the dictionary is not literature.
- Howard Fabing and Ray Marr

The concept of free energy was discussed in a physical context in Section 2.5. Living systems, however, exist in an environment with contributions from both physics and chemistry (actually, there is also a biological component that has yet to be incorporated fully into the free energy concept). Chemical contributions to free energy determinations can give an overall view of the spontaneity of physicochemical processes. Living systems can exploit those processes that would occur spontaneously; they could obtain energy from them. On the other hand, there are certain physicochemical processes that are necessary, but energetically expensive. An example of these is the formation of many of the biochemical regulators of life. Living systems have developed ways to make these processes less expensive (e.g., by using specialized enzymes). Enzymes may not reduce the overall energy of formation of these compounds, but they can help to overcome energy obstacles that stand in the way of moving from one step to the next (Figure 3.12.1).

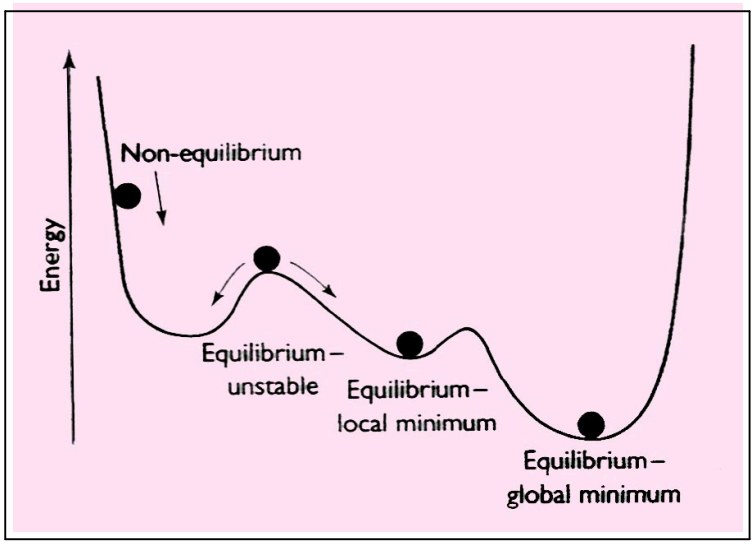


Figure 3.12.1. Before chemical reactions can take place, activation energies must usually be overcome. These appear in this diagram as energy hills between two valleys. If the reaction is exothermic, the valley on the other side of the hill is at a lower elevation, releasing energy as heat. If the reaction is endothermic, the far valley is at a higher elevation than the starting point (Haynie, 2001).

Molecules that should react with one another often do not because they lack sufficient energy. For every reaction, there is a specific activation energy, which is the minimum amount of energy that two molecules must have before a collision between them will be successful in leading to a reaction. Were it not for activation energy, all reactions would proceed quickly to equilibrium, and life as we know it would be impossible.

Enzymes enhance the rate of a reaction by lowering the energy of activation, thereby ensuring that a greater proportion of molecules will have sufficient energy to collide successfully and undergo reaction. A primary feature of an enzyme is that it is not permanently changed or consumed as the reaction proceeds.

Free energy is mainly a quantitative approach. Therefore, to calculate free energy values and determine whether a certain chemical reaction is favored, we need to have measurements of chemical reactions, and these must be made on some basis that allows comparison. Thus it is that measured *chemical activity* of compounds has been made at standard conditions of 298˚K, 1 atmosphere of pressure, 1 mole of substance, and 1 molar concentration if the reaction occurs in aqueous solution. These values can be mathematically corrected for temperature by (Haynie, 2001):

$$(\text{chemical activity at some temperature}) \propto (\text{absolute temperature})$$

Any biochemical reaction that occurs spontaneously or that emits a net amount of energy will have a negative free energy value. In this form, chemical potential and Gibbs free energy can be used interchangeably for isothermal (constant T) and isobaric (constant p) chemical processes.

It is likely that pressure and temperature can change during a chemical reaction (e.g., for products that form smaller volumes of gas than the reactants, or for exothermic reactions, respectively). These physical changes need to be taken into account, and, in some cases, can eliminate the spontaneity of the reaction.

Biocompatibility of Material Surfaces

Xenobiotic materials (materials not biological in nature) for implants must satisfy several requirements (Baier, 2002):

1. must be able to adhere
2. not diminished by biological actions
3. cannot diminish host functions
4. forms an infection-free seal
5. able to remain for a long time.

Implants must be able to function in a wet, salty, biochemically active environment.

Surfaces of these materials are critical to their successful use. For

Biocompatibility of Material Surfaces, cont.

some implants, the surface should promote healing through growth of host tissue. Artificial bones or joints are examples of these. In other cases, materials to be used in a biological environment should repel growth. Examples are surfaces of food-handling equipment, and the bottom surfaces of ships. Dental implants (artificial teeth) have both requirements: the root must promote growth to anchor it into the gum and jaw, while the top must repel plaque formation by bacteria.

The surface free energy is the energy of binding of adherent molecules to the surface of the material. Materials that promote adhesion have high surface free energies. Those that do not promote growth have low surface energies.

Materials in contact with biological environments become coated with glycoproteinaceous macromolecules in highly hydrated solution states. These bound compounds form films of considerable complexity, and can support complex bacterial colonies in the open environment (called biofilms, see Section 6.14.4). In the body, early stage attachment to the glycoproteins is by macrophages, and at later states by fibroblasts. Capsules of tissue are eventually formed around the inclusions.

An operational surface free energy of about 40 dyne-cm/cm^2 appears to divide surfaces that promote growth from those that don't. Surface energies in the neighborhood of 20-30 dyne-cm/cm^2 prevent macromolecule adhesion (Baier, 2002). These low energies are difficult to achieve. Surfaces must be cleaned either by flaming or corona discharge, held in boiled (out-gassed) triply distilled water, and coated with stearates or silicones. Coating is not necessary if the implant is to be healed into the body.

Substrate	**Typical Surface Energy** $\left(\frac{\text{dyne-cm}}{\text{cm}^2}\right)$ (Riegler and Rhodes, 2006)
Aluminum	45
Polyamide	33-46
Polycarbonate	46
Polyetherimide	40-45
Polyimide	40-50
Polymethylmethacrylate	38
Polysulfones	41
Polyurethane	43
Silicone Elastomer	24
Stainless Steel	700-1100
Titanium	>250

Applications and Predictions

1. Most biochemical reactions with a negative free energy value will not occur spontaneously because activation energies will have to be supplied before the reactions can occur.
2. Enzymes can provide surface free energy and promote binding sites for molecules.
3. Surface free energies can be selected depending whether surface growth is wanted or unwanted.
4. Enzymes lower activation energies.

QUESTIONS
Chapter 3

3.0.1 Distinguish among the different subdisciplines of chemistry.

3.0.2 In which branch of chemistry would you expect to study the following:
   a. proteins
   b. arsenic
   c. ionic bonds
   d. osmosis
   e. properties of cell membranes

3.0.3 Physical, chemical, and biological components of the environment interact with living things. Give examples of the chemical environment of organisms.

3.0.4 Why is the study of chemistry important for biological engineers?

3.0.5 Explain why the study of chemistry is necessary to understand the workings of biological systems.

3.0.6 Someone has said that the study of biology is the same as the study of chemistry. Why is this statement both true and false?

3.0.7 Describe in your own words differences among different branches of chemistry and how they relate to biology.

3.1.1 Discuss the advantages to knowledge of families of elements with similar features.

3.1.2 Estimate the number of electrons in a wart hog.

3.1.3 Will an atom of Barium (element number 56) combine most readily with Bismuth (Bi, no. 83), Polonium (Po, no. 84), Astatine (At, no. 85), or Radon (Rn, no. 86)?

3.1.4 Is carbon an alkaline or acidic element? Why?

3.1.5 What is the heaviest element with a clear function in living things? Does this mean that heavier elements are not found in living things?

3.1.6 Add to the list of Applications and Predictions.

3.2.1 Rank the following bonding types in terms of stability of bonds:
   covalent bonds

hydrogen bonds
ionic bonds
metallic bonds
Van der Waals bonds

3.2.2 Compare chemical ionic bonding to covalent bonding. Is there a clear distinction between the two?

3.2.3 The element nitrogen forms combinations with many other elements. The charges on nitrogen range from –3 to +5, inclusive. Give examples of as many compounds as you can illustrating different charges on the nitrogen atom.

3.2.4 What biological functions would you expect to be performed by compounds composed of elements with high electronegativity? Low electronegativity?

3.2.5 What are the characteristics of polar and nonpolar molecules that are exploited in living things?

3.2.6 The relatively low-density structure of ice is fortunate for the survival of aquatic species. How does the same structure harm single cells?

3.2.7 Speculate on ways in which the void spaces in hexagonal-structured ice can be exploited.

3.2.8 Where are hydrogen bonds of importance in living things?

3.2.9 If the freezing point depression of a single solute solution is known, how can the degree of dissociation of the solute in solution be determined? What other information is necessary?

3.2.10 Give an example from everyday experience that illustrates by analogy how Van der Waals forces operate.

3.2.11 For what purposes could the mechanism utilized by the Gecko be used by biological engineers?

3.2.12 Suggest other uses for Raman spectra.

3.2.13 Add to the list of Applications and Predictions.

3.3.1 Under what conditions would exothermic or endothermic reactions be advantageous?

3.3.2 If the equilibrium constant of a chemical reaction is high, what can you say about the completeness of the reaction? If the equilibrium constant is low, what can be done to drive the reaction toward higher production of products?

3.3.3 What information does the dissociation constant give about the presence of ionic forms in solution?

3.3.4 Add to the list of Applications and Predictions.

3.4.1 If the dissociation constant of an acid is small, is the acid strong or weak?

3.4.2 Why is carbonic acid such an important buffer compound in living things? What does this have to do with metabolism?

3.4.3 Why would you expect solutions in living systems to be almost devoid of strong acids and bases?

3.4.4 What information is contained in a measurement of pH? What can a measurement of pH tell about the biological state of: blood, wastewater, food, ground water, rain, bioreactor medium, air conditioner condensation, gel for growth of microbes?

3.4.5 Add to the list of Applications and Predictions.

3.5.1 Would you think fast or slow chemical reactions are important biologically? Why?

3.5.2 How is the equilibrium constant for a reversible chemical reaction related to the forward and backward rates?

3.5.3 How can the reaction rate of a first-order reaction be maintained at a high level? How can the rate be controlled at any level?

3.5.4 How do reactions described by Michaelis-Menten kinetics relate to biological processes?

3.5.5 List some specific uses of ELISA.

3.5.6 Add to the list of Applications and Predictions.

3.6.1 Suggest other elements besides carbon upon which an organic chemistry could be based.

3.6.2 For what special purposes would the substitution of another element for carbon be appropriate in certain biochemicals?

3.6.3 Make a list of biochemical functional groups and give a short description of their characteristics.

3.6.4 How does an amino acid differ from a hydrocarbon?

3.6.5 To what valuable use can a D-amino acid be put?

3.6.6 List the four basic types of macromolecules, and their purposes.

3.6.7 For what purposes can the equivalent chemical formulas for living tissues be used?

3.6.8 Why does it make sense that lower molecular weight fats are located in the peripheral areas of the body?

3.6.9 If you wanted to find a natural source of high melting point fats, would you look in a plant growing in warm or cold climates?

3.6.10 List the two important features of reactions between organic compounds. Why is each important?

3.6.11 What is a free radical? What harm can free radicals do? What good can they do?

3.6.12 Describe a process to form a membrane that can let insulin molecules through but exclude antibodies.

3.6.13 Add to the list of Applications and Predictions.

3.7.1 Why is physical chemistry in water so important for the study of biology?

3.7.2 If the solubility of a substance, say oxygen, is to limited to be useful, explore other means to carry adequate amounts of the substance to distant tissues.

3.7.3 When the vapor pressure of a liquid has reached an equilibrium, what changes of state of that liquid are still occurring?

3.7.4 How is the gel structure of water and proteins related to the integrity of a cell?

3.7.5 If a protein molecule is electroneutral, why does water adhere so firmly to it?

3.7.6 When the gel structure of water is disrupted by a protein phase change, what effect would you expect there to be on the ionic composition inside the cell?

3.7.7 How is the ordered state of water restored after a phase transition?

3.7.8 How can the resting potential of a cell be measured?

3.7.9 Suggest applications that take advantage of the isoelectric point of proteins.

3.7.10 Considering the pH effects on proteins, why would it be normally disadvantageous for the interior of a living organism to have an uncontrolled interior pH level? Would there be any unusual circumstances that would benefit from large pH changes?

3.7.11 Add to the list of Applications and Predictions.

3.8.1 Of what commercial value are heat shock proteins?

3.8.2 Describe the four types of protein folding.

3.8.3 Describe a process to deliver pharmacological chaperone proteins to treat cystic fibrosis.

3.8.4 Describe a hypothetical process that intervenes in the body's natural protein quality-control mechanism to improve health.

3.8.5 Add to the list of Applications and Predictions

3.9.1 Describe an experiment you could perform to determine the amount of energy required to liberate a chemical from the surface of carbon.

3.9.2 Hexokinase is an enzyme involved in glucose metabolism. Hexokinase in the liver has different kinetics than hexokinase in the brain. How can that be?

3.9.3 Explain how enzymes promote chemical reactions.

3.9.4 Explain how Vitamin B can be used to identify chemicals that act as competitive inhibitors.

3.9.5 What is the difference between a natural protein and a denatured protein?

3.9.6 Why does the law of constant heat sums make sense for living things?

3.9.7 The mechanisms of many modern therapeutic drugs affect enzyme actions. Some drugs work by inactivating essential enzyme action, thus depriving microbes of necessary metabolic steps. Other drugs use the enzymes to produce products toxic to unwanted cells. Discuss the possibilities to influence enzymatic actions to cure diseases.

3.9.8 Add to the list of Applications and Predictions.

3.10.1 Would you call ATP a high, low, or intermediate energy compound? Why must it be so?

3.10.2 Compare the chemical energy action of ATP to the electrical charging and discharging of a storage battery.

3.10.3 At what stage are respiration of glucose requiring oxygen and respiration not requiring oxygen the same?

3.10.4 How would you expect the activity level of anaerobic microbes to compare with that of aerobes? Why?

3.10.5 Add to the list of Applications and Predictions.

3.11.1 Use the LeChatclier Principle to predict how the following would be affected by an increase in 1) temperature, and 2) pressure (separately changed):

respiration of glucose
condensation of water
oxygen dissolved in water
photosynthesis
production of proteins

3.11.2 The other night I heard no crickets chirping. What was the temperature?

3.11.3 Add to the list of Applications and Predictions.

3.12.1 Besides using enzymes, what are other possible means to overcome activation energy requirements for a chemical reaction?

3.12.2 What is meant by surface free energy of biomaterials, and how can it be minimized?

3.12.3 Explain why a catalyst should have high surface energy.

3.12.4 Compare the surface energies of stainless steel and polyimide. Which would you use if you wished to discourage biochemical attachment to the surface? Which would you use to encourage attachment?

3.12.5 Add to the list of Applications and Predictions.