CHAPTER 1

The Chemical Foundations of Biochemistry

Chemistry in Context

An archeologist who has discovered a lost civilization would be confronted with numerous questions. How did that civilization function? How did it store food and confront challenges to survive? How did it archive and pass information from one generation to the next? If there were some way to translate the writings found into something understandable, we would stand a better chance of discovering what went on in that civilization.

This story is an analogy for where we begin with biochemistry, the study of life at the molecular level. In this analogy, the civilization is any system we choose to study, from the bacterium *Escherichia coli* to humans. From the perspective of the biochemist, the language that unites these systems and explains how and why things occur is chemistry.

This chapter focuses on several topics that will be helpful in defining and explaining biochemical reactions throughout the rest of the text. These topics include thermodynamics, equilibrium, organic reaction mechanisms, and some of the basics of structure and function. The chapter then goes on to discuss water, the properties of acids and bases, and buffers (systems resistant to changes in pH). It is intended to be a review of specific topics pertinent to biochemistry.

The dialect of chemistry that we speak in biochemistry is specialized. Biochemistry deals in aqueous systems; in small, multifunctional organics; and in large polymers. The systems have been optimized by eons of natural selection and will continue to be altered throughout time as conditions and selective pressures change. However, the foundational laws that all systems must abide by are chemical in nature.

Chapter Outline

Common Themes

Evolution's outcomes are conserved.	• Evolution takes advantage of the basic laws of chemistry, and it must abide by those laws as systems change.
Structure determines function.	• The rules that dictate the behavior of small chemical structures also apply to macromolecules. • The properties and reactivity of organic functional groups also apply to biochemical systems.
Biochemical information is transferred, exchanged, and stored.	• Information is stored in polymers of organic compounds. • Flow, exchange, and storage of information all follow the basic laws of chemistry.
Biomolecules are altered through pathways involving transformations of energy and matter.	• The basic laws of chemistry and thermodynamics apply to biochemical systems as they do to classical chemical systems.

1.1 General Chemical Principles

Chemistry is the study of matter. We observe molecules and how they behave, we create new ways to synthesize molecules, we characterize molecules using light or other wavelengths of the electromagnetic spectrum, and we determine how molecules form and break down. General chemistry, the introductory subject taught in high school and the first year of college, is a combination of several different subdisciplines of chemistry, including:

- *organic* chemistry—the study of carbon-containing molecules
- *inorganic* chemistry—the study of compounds comprised of elements other than carbon
- *analytical* chemistry—the identification, quantitation, and precise analysis of compounds
- *physical* chemistry—the study of the interactions of matter with energy

General chemistry covers several topics that are highly pertinent to biochemistry and is a good place to begin a review of chemical principles. Most of the topics covered in general chemistry are important to biochemistry and can help to explain many important biological principles, but they are not often discussed in a biochemical context in a general chemistry course. Few, if any, biochemical examples are used, and students are sometimes left with the impression that the material lacks relevance. Nothing could be further from the truth. Biochemical examples can be used to illustrate any chemical principle. Freed from this bias, you can begin to think of biological questions as having answers written in the language of chemistry.

1.1.1 Principles of thermodynamics describe all systems

Thermodynamics is the study of energy, and it is an important concept in biochemistry. Studying the thermodynamics of a reaction or system gives us valuable information about how and why a reaction can occur. In some cases, it can also provide information about the conditions required for a reaction to occur: for example, whether energy is required, if the reaction sits on the knife edge of an equilibrium constant, or if the loss of a group such as carbon dioxide (CO_2) helps

to drive the reaction forward. Most important, it can tell us whether a reaction will proceed in the forward direction. Thus, understanding the basics of thermodynamics helps to paint a rich chemical picture of what is happening in a biological process.

Fundamental laws describe energy in thermodynamics

This section briefly reviews the three fundamental laws of thermodynamics in the context of biochemical systems.

The first law of thermodynamics states that all energy is conserved. That is, energy cannot be created or destroyed but only converted from one form to another. This law of thermodynamics is one of the easiest for people to understand, yet it can be one of the most complex when dissected. When we discuss the conservation of energy in a strictly physical sense, we often think of potential and kinetic energy; this can be illustrated by the example of a roller coaster. At the top of a hill, the roller coaster has potential energy, but as it moves down the hill, some of that potential energy is converted to kinetic energy (the speed of the roller coaster). For biological systems, we often think of potential energy in terms of the energy trapped in the chemical bonds of food or other energy-storage molecules such as adenosine triphosphate (ATP) (**Figure 1.1**). The kinetic energy of a biological system could be the locomotion of that organism or the movement of fluids or gases in chambers of the organism, but there are many other ways in which energy can be used. For example, energy in biological systems can be used to generate electrochemical potentials by pumping ions across an otherwise impermeable barrier, heat energy can be released in biological systems to increase the temperature of the organism or the immediate surroundings, and chemical energy can be converted into a photon of light by bioluminescent organisms. There is no shortage of ways that energy can be used, but all of the ways obey the first law of thermodynamics.

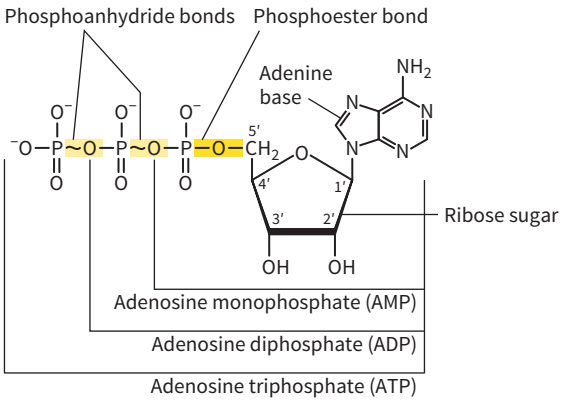

FIGURE 1.1 Structure of ATP. Adenosine triphosphate contains three phosphate groups linked via a phosphoanhydride or phosphoester bond to adenosine.

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When we discuss the energetics of any reaction, we can describe the energy as being exchanged in terms of heat or in terms of work. Heat is the amount of thermal energy that is dissipated or absorbed by a system, whereas work is generally defined as a force applied over a distance or as a change in pressure or volume. Because most biochemical systems operate under constant pressure, the heat of a reaction is a close approximation of the energy of that system. We define this energy as the **enthalpy**, ***H***.

The second law of thermodynamics states that the ***entropy, S****, of the universe is always increasing and that the entropy of any system tends toward increasing randomness and disorder.* This definition should be familiar to us on a macroscopic scale. If we consider gas molecules distributed throughout a flask, these molecules are randomly distributed. Similarly, the air we breathe has a statistically random sample of gas molecules. It would be unthinkable to walk down the street and encounter a pocket of carbon dioxide molecules that had randomly and spontaneously assembled out of the mixture. A better definition is the number of potential ways we could arrange those molecules or, to put it more scientifically, "the number of microstates a system can achieve." This definition is somewhat more detailed than disorder, and it can be used to explain a greater variety of phenomena. Imagine a system containing two objects, one of which is cool, the other warm. The atoms that make up the warmer object have more kinetic energy, more molecular motion, and more microstates, while the cooler atoms have less motion and therefore fewer microstates. According to the second law, it is impossible to trap and segregate the thermal energy from the cool object and use it to further heat the warm object. Therefore, it is not possible to use an ice cream cone to cook a hot dog, and a metal bar will not spontaneously segregate energy from one end of the bar to the other, cooling one end while warming the other.

The third law of thermodynamics states that the entropy of a perfect crystalline system at 0 Kelvin is 0. Consider an absolutely pure and perfect crystal of a monomolecular solid. As the crystal cooled to lower and lower temperatures, it would have less and less vibration. As the crystal approached 0 K, the molecular motion would decrease and the number of possible microstates would also approach zero. Of the three laws of thermodynamics, this one seemingly has the least impact on biochemistry because living systems never operate at such low temperatures. However, this

definition of the third law allows us to define entropy (0 K = no entropy) and therefore merits mention here.

Free energy relates both enthalpy and entropy in a single useful term Building from the three laws of thermodynamics, there are two key parameters to discuss: enthalpy and entropy. Although enthalpy is useful in predicting the direction of a reaction, not all of the energy released can be used as enthalpy because some of it is consumed in the generation of entropy. Likewise, while entropy is important, the amounts of entropy are typically much smaller than enthalpy for a given reaction. While neither term alone can accurately describe the energetics of a reaction, the **Gibbs free energy** or simply **free energy** relates these two parameters in a simple expression:

$$G = H - TS$$

Often, free energies are compared to one another. Due to experimental constraints, these are expressed not as ultimate values (G) but rather as the change in the value (ΔG). The Gibbs free energy expression thus becomes:

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

where the change in the free energy is related to the change in the enthalpy of the reaction, minus the change in the entropy multiplied by the temperature, measured in Kelvin (Table 1.1).

The equilibrium constant of a reaction can also be expressed in terms of ΔG. Although a negative value of ΔG is often thought of as meaning that a reaction is favorable, it really means that it is favorable in the forward direction. Hence, a ΔG value of zero indicates that a system is at equilibrium, a negative value indicates that the reaction proceeds in the forward direction, and a positive value indicates that the reaction is favorable in the reverse direction.

Although ΔG can predict the net direction of a reaction, it does not affect $\Delta G^{\ddagger}$**, the free energy of the transition state** or the free energy of activation. In other words, ΔG gives the energy difference between the reactants and products, but not the energetic "hill" that has to be climbed to go from one to the other. Thus, ΔG does not predict reaction rate. Reactions with ΔG values of −30, −0.3, +0.3, or +30 kJ/mol may all proceed at the same rate.

The Gibbs free energy describes the amount of energy available to do work at constant temperature and pressure, or the amount of energy that is unavailable because it is lost to increasing

TABLE 1.1 Common Reactions with a Strongly Negative $\Delta G^{\circ\prime}$ Found in Biochemistry

Compound and Hydrolysis Reaction	$\Delta G^{\circ\prime}$ (kJ/mol)
Phosphoenolpyruvate → Pyruvate + P_i	−62.2
1,3-bisphosphoglycerate → 3-phosphoglycerate + P_i	−49.6
Creatine phosphate → Creatine + P_i	−43.3
Acetyl phosphate → Acetate + P_i	−43.3
Adenosine-5′-triphosphate → ADP + P_i	−35.7
Adenosine-5′-triphosphate → ADP + P_i (with excess Mg^{2+})	−30.5
Adenosine-5′-diphosphate → AMP + P_i	−35.7
Pyrophosphate → P_i + P_i (in 5 mM Mg^{2+})	−33.6
Adenosine-5′-triphosphate → AMP + PP_i (excess Mg^{2+})	−32.3
Uridine diphosphoglucose → UDP + glucose	−31.9
Acetyl-coenzyme A → Acetate + CoA	−31.5
S-adenosylmethionine → Methionine + adenosine	−25.6
Glucose-1-phosphate → Glucose + P_i	−21.0
Glycerol-3-phosphate → Glycerol + P_i	−9.2
Adenosine-5′-monophosphate → Adenosine + P_i	−9.2

entropy. Because biochemical systems are defined as operating under constant pressure rather than constant volume, the Gibbs free energy is used in most biochemical applications.

The standard state in biochemical systems

In all chemical systems, including biochemistry, scientists define reference states or standard states. The **standard state** is defined by a temperature at 25°C (298 K), a pressure of 1 atmosphere, and solutes with an activity of 1 (roughly corresponding to their molar concentration). These criteria are used to define the **free energy of the standard state, $\Delta G°$**. The biochemical standard state is $\Delta G°'$. This includes the activity of water (the value used in free energy calculations) as 1 and the pH as 7.0.

The free energy of the standard state is important to know because it can provide important information regarding the favorability of a reaction, that is, whether or not it will proceed in the forward direction under standard conditions. However, by itself the free energy is not enough to predict the favorability of a reaction. To do so requires that the standard free energy change be related to the conditions in the reaction. Specifically, for any reaction where:

$$a\,A + b\,B \rightarrow c\,C + d\,D$$

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

For any reaction, the direction of the reaction (the free energy) is a function of the free energy of the standard state, the temperature, T, the ideal gas constant, R, and the natural log of the ratio of the products to reactants in the reaction. While this final term may look similar to an equilibrium constant, recall that these concentrations are the ones found in the cell and are not at equilibrium. The final term is the reaction quotient, sometimes denoted as Q.

Some important biological molecules have a large negative free energy of hydrolysis

Some molecules have groups that will hydrolyze, cleaving the bond through the addition of water, with large, negative free energy values. This happens because these molecules have certain properties such as charge–charge repulsion, bond enthalpy, and differences in hydration. Adenosine triphosphate (ATP) is the best known of these molecules (**Figure 1.2**), but there are others. Any of the nucleoside triphosphates (the other building blocks of ribonucleic acid, RNA) can and do serve as molecules that donate energy to reactions. Cleavage of the phosphoanhydride bond also increases the number of available resonance states and results in an overall increase in entropy. When these molecules are hydrolyzed, typically one or two phosphate groups are cleaved away from the nucleoside phosphate. The energy of this reaction is used to drive reactions that would otherwise be energetically unfavorable in the forward direction.

Many other phosphorylated groups, for example, phosphoenolates, also serve as a temporary repository of energy (**Figure 1.3**). The energy liberated in these reactions is also used to drive reactions. Recall that enols are tautomeric forms of a ketone. Phosphoenolates can be generated by

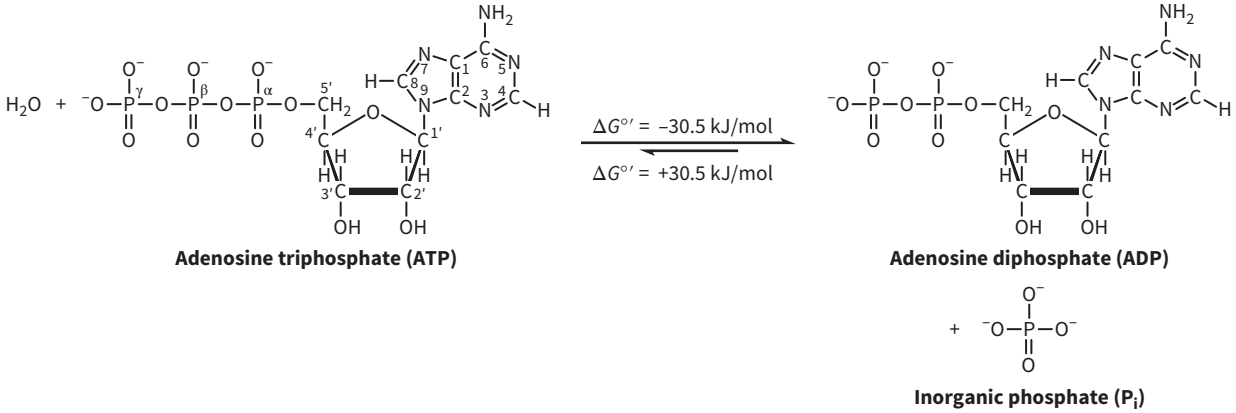

FIGURE 1.2 Hydrolysis of ATP. ATP can either lose one phosphate (P_i) or two phosphates together (termed pyrophosphate, PP_i, not shown). The hydrolysis of these phosphates is highly exergonic with a large negative ΔG value (a thermodynamically favorable reaction).

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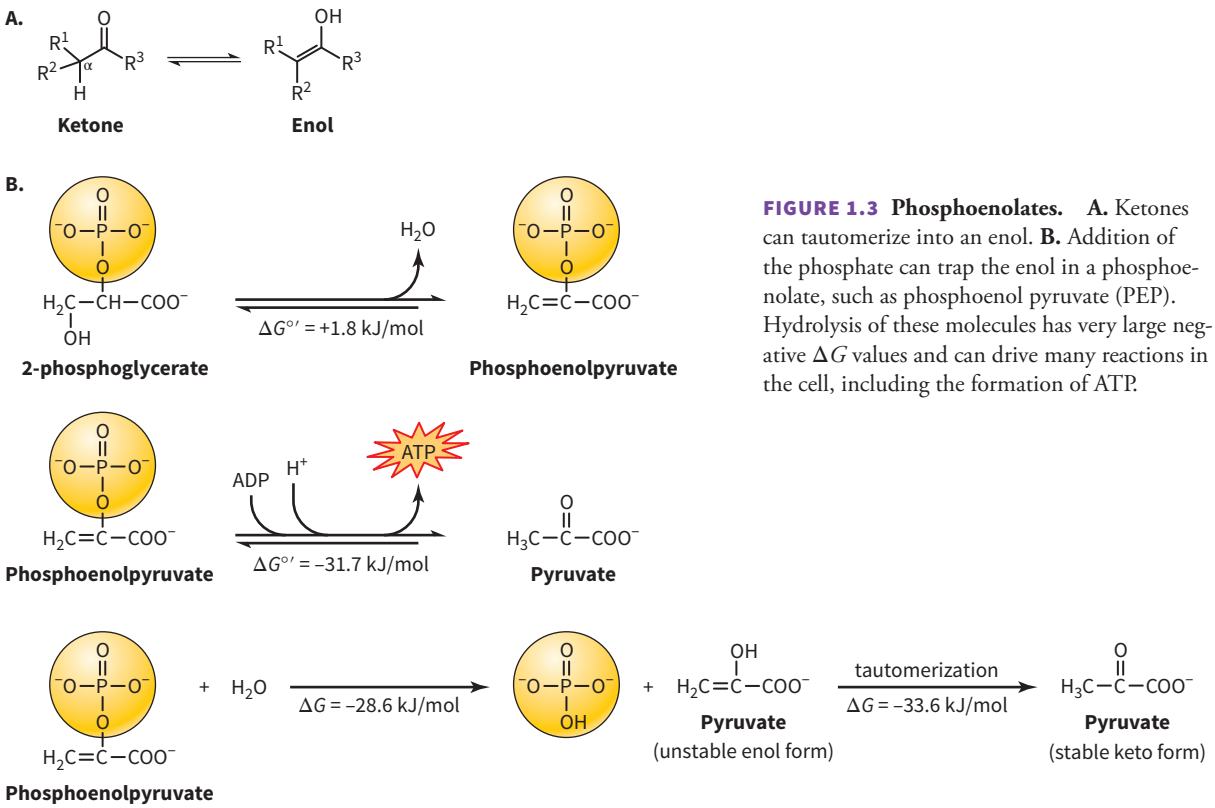


FIGURE 1.3 Phosphoenolates. **A.** Ketones can tautomerize into an enol. **B.** Addition of the phosphate can trap the enol in a phosphoenolate, such as phosphoenol pyruvate (PEP). Hydrolysis of these molecules has very large negative ΔG values and can drive many reactions in the cell, including the formation of ATP.

phosphorylating a hydroxyl group, followed by generation of a double bond via dehydration of a neighboring hydroxyl group. This phosphate has a much more negative free energy of hydrolysis and therefore a much higher transfer potential. These groups are used by the cell as one means of synthesis of ATP. The free energy of transfer from the enol to ADP is negative. Once the phosphate is lost, the enol tautomerizes into its keto form.

Knowing that these molecules hydrolyze spontaneously and have large negative ΔG values provides no information about rate. In the absence of a **catalyst** to accelerate the rate of breakdown, ATP can be isolated and stored indefinitely. In the instance of ATP, there is a relatively large activation energy barrier ($\Delta G^{\ddagger}$) that must be overcome for these molecules to react.

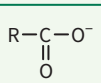
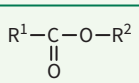
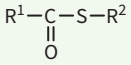
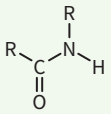
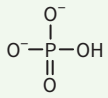
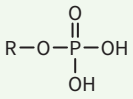
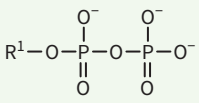
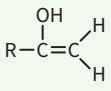
Unfavorable reactions can be made energetically possible by coupling them to favorable ones

On their own, many biochemical reactions are thermodynamically unfavorable in the forward direction ($\Delta G > 0$). For example, forming an ester between a phosphate group and a hydroxyl group generally has a positive free energy of reaction. In biochemistry, these reactions can still proceed in the forward direction by being coupled to thermodynamically favorable reactions (**Figure 1.4**). A common thermodynamically favorable reaction is the hydrolysis of ATP to ADP and Pi (inorganic phosphate), which has a $\Delta G^{\circ\prime}$ of −30.5 kJ/mol (**Table 1.2**). Instead of being hydrolyzed, the phosphate of ATP could be transferred to the hydroxyl group of another molecule. From a thermodynamic perspective, we could separate this

Reaction 1	Glucose + P_i ⟶	Glucose-6-phosphate	+18.0 kJ/mol	(unfavorable)
Reaction 2	ATP + H_2O ⟶	ADP + P_i	−30.5 kJ/mol	(favorable)
Overall reaction:	**Glucose + ATP ⟶**	**Glucose-6-phosphate + ADP**	**−12.5 kJ/mol**	**(overall favorable)**

FIGURE 1.4 Coupling favorable and unfavorable reactions. The formation of glucose-6-phosphate in the cell is energetically unfavorable ($\Delta G^{\circ\prime} = 18$ kJ/mol). The hydrolysis of ATP, reaction 2, is large and negative ($\Delta G^{\circ\prime} = -30.5$ kJ/mol). When these two reactions are coupled together and the phosphate from ATP is used to form glucose-6-phosphate, the energies of the individual reactions are additive, resulting in an overall favorable reaction ($\Delta G^{\circ\prime} = -12.5$ kJ/mol).

TABLE 1.2 Common Organic Functional Groups Found in Biochemistry

Functional Group	Structure	Notes
Oxygen-containing groups		
Hydroxyl		Hydroxyl groups are common to all classes of biological molecules. They participate in numerous reactions including esterifications, dehydrations, and oxidations.
Carbonyl (aldehydes and ketones)		The polar carbonyl group participates in numerous biochemical reactions. Frequently carbonyl carbons are subjected to nucleophilic attack by hydroxy or amino groups.
Carboxyl		Carboxyl groups are weak acids. They are common functional groups and participate in the formation of several other groups, including esters and amides.
Ester		Esters are the result of a hydroxyl and carboxylic acid reacting with one another. Esters are commonly found in many lipids.
Sulfur analogs		
Thiol		Thiol groups can be thought of as hydroxyl analogs. They are found in the amino acid cysteine.
Disulfide		Disulfides result from the reaction of two thiol groups together. Disulfide reducing agents cleave the disulfide and restore the two thiols.
Thioester		Thioesters have a large negative free energy of hydroylsis.
Nitrogen analogs		
Amino		Amino groups are common to all classes of biological molecules. The lone pair of electrons on the amino group can acquire a proton resulting in a positively charged amino group.
Amide		Amides are formed through the reaction of an amine and a carboxylic acid. Peptides are the amide linkages found in proteins, but other amide bonds are found throughout biochemistry.
Phosphorous-containing groups		
Phosphates		Phosphate, also known as inorganic phosphate, Pi, is commonly found in all biological systems.
Phosphoesters		Phosphoesters are the result of the addition of a phosphate group to a hydroxyl group.
Phosphanhydrides		Phosphanhydrides are commonly found in nucleoside triphosphates like ATP and have a large negative free energy of hydrolysis.
Other groups		
Enol		Enols are tautomers of carbonyl groups. In biochemistry they are often trapped as phosphoenolates.

reaction into two separate reactions: the hydrolysis of ATP to ADP and inorganic phosphate (P_i), and the esterification of phosphate to the hydroxyl of the new molecule. The energies of the two reactions are added together to find the net ΔG for the entire reaction.

ATP is used to drive many reactions, but in theory, any molecule with a favorable free energy of hydrolysis ($\Delta G < 0$) can be used.

1.1.2 Equilibrium concepts of specific thermodynamic state

Equilibrium refers to the chemical state when the rates of the forward and reverse state of a reaction are equal. The rates can be fast or slow, provided the net rate of product formed or reactant used is zero. Likewise, the concentrations of reactant and product do not need to be the same (and often are not). While it may seem confusing at first that a system can still be at equilibrium when the ratio of products to reactants is 1×10^{9} or 1×10^{-9}, values commonly span this range.

We define the **equilibrium constant, K_{eq}**, as the ratio of products to reactants at equilibrium. For the given reaction at equilibrium:

$$a\,A + b\,B \rightleftarrows c\,C + d\,D$$

$$K_{eq} = [C]^{c}\,[D]^{d}/[A]^{a}\,[B]^{b}$$

Combining this equation with our previous definition of the free energy of the reaction,

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

we obtain an expression that defines the free energy of any reaction as the sum of the standard free energy and the temperature and gas law constant, multiplied by the natural log of the ratio of concentrations in the reaction.

Because equilibrium is defined here as the point at which there is no net reaction, ΔG at equilibrium is zero. As a result, ΔG° becomes:

$$\Delta G^{\circ} = -RT \ln K_{eq}$$

Rearranging this equation describes the equilibrium constant in terms of the other parameters:

$$K_{eq} = e^{-\Delta G^{\circ}/RT}$$

Effectively, this means that the free energy of a reaction can be used in the standard state to describe the equilibrium constant, or vice versa. The free energy is therefore simply another way to describe the equilibrium constant.

All systems tend toward equilibrium, despite the ratio of reactants to products. If reactants are mixed in a beaker in the absence of product, they will begin to react to form product. Likewise, if the products of this same reaction are mixed together in a beaker and allowed to react, reactants will eventually appear. If a system at equilibrium is in some way perturbed, it will react to return to equilibrium. This observation is known as **Le Châtelier's principle** and is a feature of all systems, including biochemical systems. If the concentrations of reactants are increased above the equilibrium concentration, the reaction will proceed in the forward direction to compensate by making more products. Likewise, if the concentration of product is increased, the reaction will proceed in the reverse direction to generate more reactant. A biochemical example of this occurs in erythrocytes (red blood cells). In tissues, erythrocytes acquire carbon dioxide from muscle and quickly convert it into carbonic acid (H_2CO_3) via the enzyme carbonic anhydrase. In the lung, carbon dioxide concentrations are low and the process moves in the reverse direction, generating carbon dioxide from carbonic acid.

Enzymes, or any catalyst for that matter, will not perturb or in any way alter equilibrium concentrations; they will only help a reaction to reach equilibrium more quickly.

Although often used interchangeably in the popular literature, equilibrium should not be confused with **homeostasis**, the ability of an organism to live within its energetic and chemical means. An organism living in homeostasis is not consuming more energy than it is using or using its energy stores; however, it is not at chemical equilibrium with its environment. An organism that has reached equilibrium with its environment is, in all biochemical ways of thinking, dead.

1.1.3 Kinetics approaches of the rate of chemical reactions

Kinetics is the field of study that analyzes the rates of chemical reactions. Some reactions involve a single reactant being transformed to a single product, while others involve multiple reactants leading to multiple products. The rate of a reaction depends on general factors including the concentration of products and reactants, temperature, and factors specific to that individual reaction. The rate of any chemical reaction can be described using a mathematical expression termed a rate law. These laws link the rate, the change in concentration over time, to the concentration of the starting materials multiplied by a constant, k, the rate constant. The rate constant takes into account the temperature and factors such as how often a collision between reactants leads to products.

$$A + B \rightarrow C$$

$$\text{Rate} = d[C]/dt = k[A][B]$$

In the simple example shown above, two molecules, A and B, react to form the molecule C. Based on the rate law given above, the rate of this reaction is linearly dependent on the concentration of both A and B; thus, doubling the concentration of A doubles the rate, and the same is true for B. The rate needs to be experimentally determined: the rate law for that reaction is then derived from experimental data; however, once a rate law has been determined, it is possible to calculate reaction rates from given concentrations, or concentrations from observed rates.

1.1.4 Thermodynamics, equilibrium, and kinetics are used together to describe biochemical reactions and systems

The direction of any reaction depends on the initial and final energies of the starting materials and products (ΔG), and the concentrations of the reactants. The rate of a reaction is related to the activation energy ($\Delta G^{\ddagger}$), not to the initial or final states. When a catalyst acts on a reaction, it lowers the activation energy, affecting the rate constant k. The catalyst does not affect ΔG, nor does it affect the equilibrium concentrations of reactants and products, which are also ΔG dependent. The lowering of the energetic barrier between products and reactants is the same if the reaction is going in the forward or reverse direction; therefore, the rates of *both* reactions are increased. Hence, a catalyst can help a system to reach equilibrium more quickly, but it cannot change equilibrium concentrations.

When considering biochemical systems, the same rules apply, but nearly all biochemical reactions are catalyzed by specific protein catalysts termed **enzymes**. Enzymes lower activation energy by forming more stable states and facilitating collisions between reactants (**Figure 1.5**).

Worked Problem 1.1 Using thermodynamics to examine reactions

Examine the following two reactions.

$$ATP + H_2O \rightarrow ADP + P_i \qquad \Delta G^{\circ\prime} = -30.5\ \text{kJ/mol}$$

$$\text{Glucose} + P_i \rightarrow \text{Glucose-6-phosphate} + H_2O \qquad \Delta G^{\circ\prime} = +18.0\ \text{kJ/mol}$$

What is the standard free energy change for the formation of glucose-6-phosphate from glucose and ATP? That is, what is the standard free energy change for the reaction:

$$ATP + \text{glucose} \rightarrow \text{Glucose-6-phosphate} + ADP$$

Strategy The energies of thermodynamic properties, such as free energy, are additive. Combine the energies to find the overall energy of the reaction.

Solution Adding the free energies of the first two reactions, we see that the overall free energy for the two reactions combined (−30.5 kJ/mol + 18.0 kJ/mol) is negative and exergonic (−12.5 kJ/mol). Therefore, the reaction will proceed in the forward direction.

The formation of glucose-6-phosphate from glucose and phosphate is energetically unfavorable in the forward direction. Rather, it is endergonic with a positive $\Delta G^{\circ\prime}$ value. Nevertheless, glucose-6-phosphate is found in the cell because of the presence of ATP. The large negative $\Delta G^{\circ\prime}$ of hydrolysis of ATP allows it to act as a phosphate donor. More important, it provides the energy needed for this reaction to happen.

Follow-up question The ΔG values used in the previous problem were in the standard state ($\Delta G^{\circ\prime}$). What factors will modify ΔG in the cell? How could ΔG be made more negative, and would this affect how quickly the reaction proceeds?

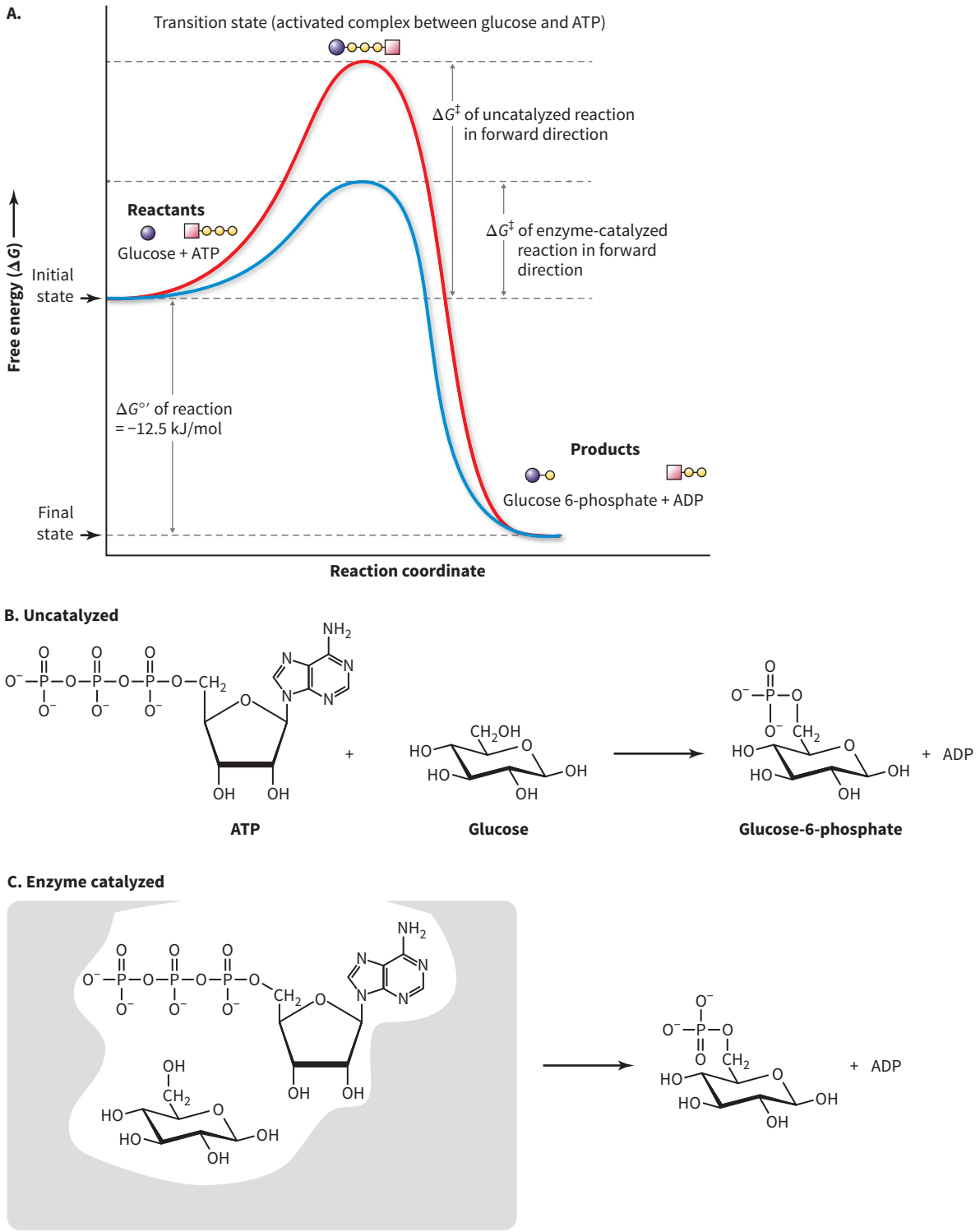


FIGURE 1.5 Reaction coordinates and catalysis. **A.** Enzymes do not alter the initial or final states but rather lower the activation energy by stabilizing the transition state ($\Delta G^{\ddagger}$), to increase rate. **B.** and **C.** This is done in part by constraining the reactants in the active site of the enzyme.

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It may be hard to conceptualize how "lower activation energy" translates to actual chemistry. Imagine two molecules such as ATP and glucose that may react together to form a glucose phosphate and ADP. Although these molecules could react with each other in the absence of a catalyst, simple random collisions between the molecules will rarely have the proper direction of collision and enough energy for the reaction to occur. Now imagine these two molecules held

in an enzyme in the correct orientation to react with one another, or brought together so that a reaction is more favorable; this gives a picture of what the lowering of the transition-state energy looks like on a physical level.

1.1.5 ATP-the energy carrier

Biological systems use many forms of organic molecules as a source of metabolic energy. Among them, ATP is the most used molecule as the "energy carrier". Although other ribonucleotides like GTP contain the same amount of energy, ATP has been preferably used as the "energy currency" by living systems to power metabolism. Living cells synthesize ATP in three ways: substrate-level phosphorylation, oxidative phosphorylation, and photophosphorylation. The synthesis of ATP by transferring a phosphate from a substrate to ADP during a metabolic reaction is known as substrate-level phosphorylation, e.g., ATP production in the reaction catalyzed by pyruvate kinase (Section 6.3). In oxidative phosphorylation, ATP synthesis is coupled to the flow of electrons from reduced coenzymes produced during the oxidation of metabolic fuels via a chain of electron transport complexes (Figure 7.12). Photophosphorylation is the synthesis of ATP using solar energy by photosynthetic cells only. This mode of ATP production is very much like that of oxidative phosphorylation. The significant difference is that photophosphorylation uses sunlight as the ultimate energy source to eject electrons from water.

Summary

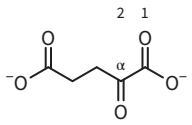
- There are three fundamental laws of thermodynamics:
  - Energy is conserved. In general, the energy of a biochemical system can be defined as its enthalpy.
  - The entropy of a spontaneous reaction is always increasing.
  - $S = 0$ at 0 Kelvin.
- Both enthalpy and entropy contribute to biochemical processes. We can aggregate these terms into one expression: the Gibbs free energy, ΔG.
- The free energy of a reaction describes the equilibrium state ($\Delta G = 0$) and whether a reaction will move in the forward ($\Delta G < 0$) or reverse direction ($\Delta G > 0$).
- The equilibrium state is one in which the rates of the forward and reverse reactions are equal; hence, there is no net production or loss of product or starting material. It can be expressed in terms of the equilibrium constant, K_{eq} (a ratio of the equilibrium concentrations) or in terms of free energy.
- Le Châtelier's principle states that all reactions seek to move toward equilibrium from other states.
- Kinetics is the study of chemical rates. Most biochemical reactions employ protein catalysts called enzymes to lower activation energies and increase reaction rates.
- ATP is the common energy molecule for driving metabolic reactions. Cells synthesize ATP through substrate-level phosphorylation, oxidative phosphorylation, or photophosphorylation.

Concept Check

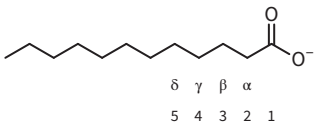
1. Describe the basic concepts of thermodynamics, equilibrium, and kinetics in your own words.
2. Use these concepts to describe a basic biochemical problem.
3. Why is ATP called the energy currency of cells?

1.2 Fundamental Concepts of Organic Chemistry

Biochemistry draws from all fields of chemistry to help answer questions, but organic chemistry has been the classical gateway into this field. This happens largely because most biological molecules contain carbon. This section focuses on four aspects of organic chemistry to prepare for the study of biochemistry: functional groups, solubility and polarity, mechanism, and polymer chemistry.



α-ketoglutarate



Fatty acid

FIGURE 1.6 Organic nomenclature using the Greek lettering system. In α-ketoglutarate the carbonyl (keto) group is α to the higher priority carboxyl group. Fatty acids are broken down through a process termed β-oxidation. The oxidation occurs on the β-carbon.

1.2.1 Chemical properties and reactions can be sorted by functional groups

Functional groups are fragments of a molecule, such as a hydroxyl or carbonyl group. Understanding how each group reacts, as well as its properties, makes it possible to apply that information to other molecules. Likewise, if a functional group does not behave as expected, the hypothesis needs to be re-examined, and theories or understanding of the system may need to change.

Many of the common functional groups found in biochemistry will be familiar (Table 1.2). They include hydroxyl groups, carbonyl groups, amines, and carboxylic acids. Others, such as thiols and phosphates may be less familiar. These groups combine to generate more complex molecules such as amides and disulfides.

Many of the molecules studied in biochemistry were identified before systematic methods of nomenclature were adopted, and therefore they have common names. Other molecules use the older Greek lettering system instead of numbering to identify the location of functional groups in a molecule (**Figure 1.6**). Although this is not the International Union of Pure and Applied Chemistry (IUPAC) nomenclature system that is used in organic chemistry, dissecting the name of a molecule can still help provide clues to its structure and function. For example, as the name implies, α-ketoglutarate contains a carbonyl group (keto) α to a carboxylate (ending in the suffix "-ate"). Without knowing other information, such as the structure of glutarate, it is not possible to draw out the full structure, but the name still provides a guide. Likewise, fatty acids are broken down through a process termed β-oxidation. In this example, the oxidation occurs at the β-carbon.

1.2.2 The solubility and polarity of a molecule can be determined from its structure

All biochemistry occurs in aqueous systems. Water is the common denominator, and it is required in some way and at some level for all life to occur. When the National Aeronautics and Space Administration (NASA) searches for life on other planets, the first molecule they often look for is water. Water is the most common solvent used in biochemistry; hence, most biological molecules need to be soluble in water. Molecules can be made more water soluble by incorporating more polar groups, like hydroxyl groups and thiols, or charged groups, such as amine and carboxylate salts; phosphates; and, in some instances, sulfates.

Although all living systems require water, not all biochemical reactions occur in water. There are microenvironments in the cell or within individual macromolecules that exclude water. These systems are hydrophobic and nonpolar. Reactions that might not occur in an aqueous system can occur within a hydrophobic microenvironment in an enzyme. Likewise, although a cell is largely a watery place, hydrophobic groups on an otherwise water-soluble molecule can tether or anchor that molecule in a hydrophobic location; for example, a water soluble protein can be anchored within a hydrophobic layer of a cell membrane.

In addition to the structure and polarity of a molecule, stereochemistry also plays a key role in biochemical systems. This is discussed in **Societal and Ethical Biochemistry: The relevance of stereochemistry**.

Societal and Ethical Biochemistry

The relevance of stereochemistry

Stereochemistry plays a critical but subtle role in biochemistry. Due to the nature of biological molecules—mostly organic, often with multiple functional groups—many of them are chiral. As we build larger molecules from smaller chiral building blocks, these macromolecules are also chiral. Being chiral themselves, enzymes often catalyze chiral or stereospecific reactions. Overall, most of biochemistry is chiral or contains molecules that are chiral in at least one carbon center. We may refer to these pairs of isomers using the *R* and *S* system, in which stereoisomers are assigned by priority of functional groups, or the D and L system, in which chiral molecules rotate plane polarized light.

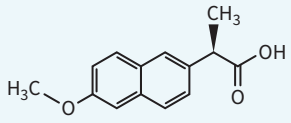
Fortunately, through evolution, nature uses a conserved set of molecules; with few exceptions, these have the same stereochemistry. Therefore, humans can obtain the amino acids they need to build new proteins from their diet (from animal, plant, or even microbial sources) and not worry about the chirality of those amino acids. The same is not true, however, for molecules made in the laboratory.

It can be difficult and expensive to produce stereochemically pure substances. Instead, when creating molecules where chirality may be important, the effort is spent on removing chiral centers or preventing their formation in the first place. A good illustration of this is in pharmaceutical development. Many pharmaceuticals bind to, and thus block or activate, either enzymes or receptors, leading to downstream biological effects. Although many pharmaceuticals have been designed to avoid chiral centers as far as possible, a lot of drugs still have them.

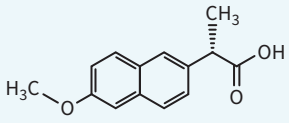
Having different stereoisomers of a drug can have a variety of effects. For example, the over-the-counter pain reliever ibuprofen has a single chiral center; this drug is supplied as a racemic mixture or mixture of the two stereoisomers, but only the *S* isomer is biologically active. The *R* isomer has no known effect. Conversely, for the drug naproxyn, the *S* enantiomer is the over-the-counter pain reliever, whereas the *R* enantiomer causes liver toxicity or hepatotoxicity. Therefore, naproxyn is synthesized and sold as the stereochemically pure S enantiomer. Similarly, dextromethorphan is a common over-the-counter cough suppressant, or antitussive, whereas levomethorphan, its enantiomer, is a powerful narcotic opioid analgesic.

Pharmaceutical companies are now aware that racemic drugs are likely to have an active and an inactive component, or one isomer that is causing the desired effect and one that is causing other effects or no effect. Advances in synthetic chemistry have made it possible to produce and test enantiomerically pure compounds, that is, only the *R* or *S* isomers. Often, when the pure drug is tested, it is found that a lower dosage of the drug can be used, and adverse effects, due to the other isomer or to metabolites of the drug, can be avoided. For example, citalopram is a selective serotonin reuptake inhibitor (SSRI), an antidepressant supplied as a racemic mixture. Although the adverse effects of this drug are relatively mild, there are common effects of SSRIs, such as weight gain and decreased libido. The stereochemically pure L isomer (escitalopram) minimizes these adverse effects. This raises some interesting scientific questions, such as whether the D isomer is responsible for the adverse effects, and if so, why and how? A smaller amount of the pure drug (the L isomer) is needed, which provides more options for delivery systems, and this puts less of a metabolic burden on the liver to detoxify other stereoisomers.

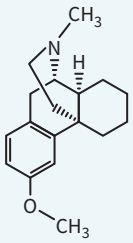
There are other advantages for pharmaceutical companies in pursuing stereochemically pure compounds. Because the effectiveness and safety of the parent compound has already been demonstrated, it is often easier and quicker to bring these drugs to market. Also, drugs that are stereochemically pure are considered new in terms of patent protection. Thus a drug company can theoretically extend patent protection on a drug, up to another 17 years (the length of a patent on a pharmaceutical compound).



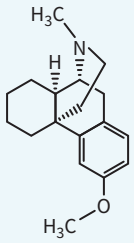
***R*-Naproxen**



***S*-Naproxen**



Dextromethorphan



Levomethorphan

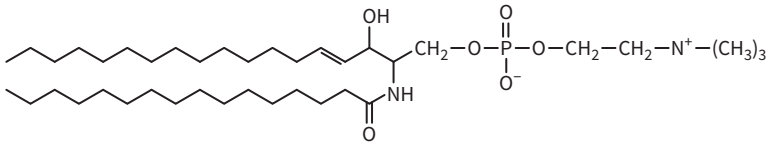
1.2.3 Reaction mechanisms attempt to explain how a reaction occurs

An organic reaction mechanism is an evidence-based explanation of how a reaction occurs. In a mechanism, we follow the flow of electrons from electron-rich nucleophiles to electron-poor nuclei. In reaction mechanisms, the direction of the arrow indicates the direction of electron flow. The headedness of the arrow indicates the number of electrons. A double-headed arrow (⟶) means two electrons are flowing, whereas a single-headed arrow (⇀) indicates one electron, or radical transfer.

The reaction mechanisms covered in organic chemistry are generally relatively simple in comparison to the enzymatically catalyzed reaction mechanisms in biochemistry. Biochemical reaction mechanisms can be quite complex, employing multiple steps, such as the mechanism of triose

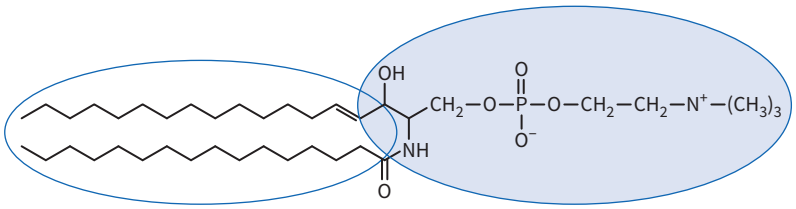
Worked Problem 1.2 Properties of a biological molecule

Shown below is the structure of sphingomyelin, a molecule that is involved in cell-to-cell communication and has a structural role in the cell membrane. Which functional groups can you identify in sphingomyelin? Would you anticipate that this molecule would be soluble in water or in a nonpolar solvent? Which functional groups in this molecule would interact with water molecules and how?



Strategy Compare the figure of sphingomyelin to Table 1.2. Which functional groups can you identify? Are those groups polar or nonpolar?

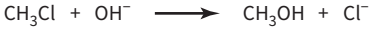
Solution There are multiple functional groups in sphingomyelin: a quaternary amine, a phosphate in a phosphodiester, an amide, a hydroxyl, and an alkene. The charged moieties (the phosphate and amine) and the hydroxyl will help make that end of the molecule water soluble. The amide has lone pairs of electrons on both the oxygen and nitrogen; these can participate in hydrogen bonding as hydrogen-bond acceptors but not as hydrogen-bond donors. The hydrocarbon tails, on the other hand, are not going to be water soluble, making the entire molecule **amphipathic**—literally, a molecule with affinities for both water and oil.



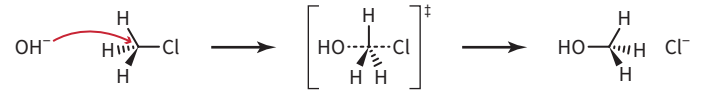
Follow-up question What would be the products if the amide bond of the molecule were hydrolyzed?

phosphate isomerase (**Figure 1.7**). In either instance, the same basic rules apply. A reaction mechanism explains the making and breaking of bonds at the molecular level. Arrows indicate the number of electrons and direction of electron flow, and electrons flow from regions of high electron density to regions of low electron density. Frequently in biochemistry these mechanisms employ nucleophiles and electrophiles similar or identical to ones that you have seen in organic chemistry.

A. Reaction

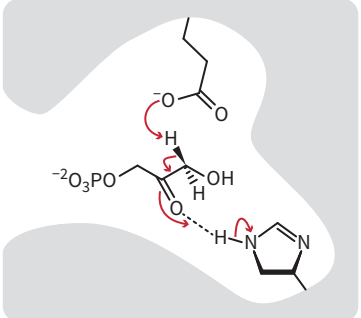


B. Organic reaction mechanism



Nucleophile attacks alkyl halide | **Transition state** | **Substitution product**

C. Enzyme reaction mechanism

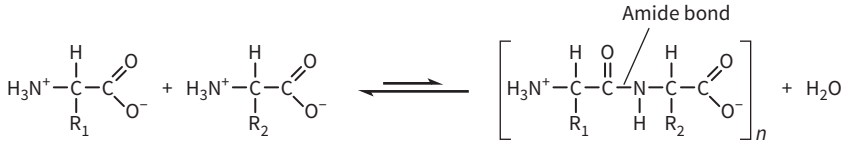


1.2.4 Major constituent biomolecules in living organisms

Many of the molecules discussed so far are small, with molecular weights below 500 g/mol. However, many if not all of these small molecules have functional groups such as alcohols, amines, and carboxylic acids that facilitate the formation of **polymers**. Polymers are macromolecular assemblies of smaller building blocks termed **monomers**,

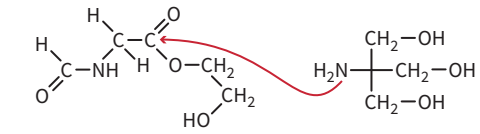
FIGURE 1.7 Reaction mechanisms. **A**. A typical substitution reaction studied in organic chemistry. **B.** The mechanism of the example shown in A (an S_N2 substitution reaction). **C.** A biochemical reaction mechanism, while more complicated, illustrates the underlying principles, such as electron flow, intermediates, and nucleophilic attack, are the same.

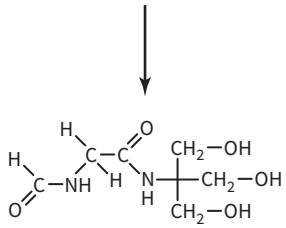
and they may be connected in a linear fashion, like the cars of a train, or in a branched fashion, like the branches of a tree.



Worked Problem 1.3 Creating a polymer

The ethylene glycol ester of *N*-formylglycine reacts with tris(hydroxymethyl)aminomethane (referred to as TRIS) to form an amide bond. The reaction is shown below:

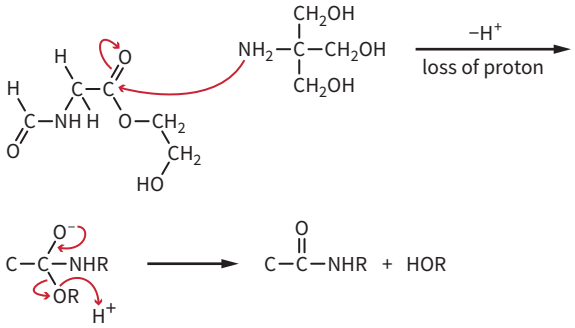




Propose a possible mechanism for this reaction. Can these compounds polymerize?

Strategy Examine the given reaction. What are the reactants and products? How might these starting materials react together to yield the final product?

Solution The mechanism of amide bond formation in the ribosome, the protein assembly complex in the cell, involves an amino acyl ester as the donor group for adding amino acids to the growing protein. Ester aminolysis is also a common organic synthetic technique.



These compounds cannot polymerize. To simplify and facilitate study of this reaction, a formyl protecting group (CHO) is coupled to the amino group of the glycine glycol ester. The formyl group ensures that only one reaction can occur—the reaction between the formylglycine glycol ester and the amino group of TRIS. If that formyl group was missing, the glycine groups could probably polymerize.

Follow-up question Would you anticipate that this reaction would proceed more favorably at an acidic pH or a basic pH?

While biochemistry is often thought of as the study of small organic molecules, it is equally a study in polymer chemistry and macromolecules. DNA, RNA, proteins, and enzymes are polymers, as are molecules such as glycogen, starch, and cellulose. Other molecules, for instance, some lipids, are not polymers, but they are assembled in a modular fashion, similar to polymers. Common themes are found throughout the biosynthesis and degradation of these molecules.

Polymers in biochemistry can be broadly categorized as those that have been coded for by some sort of template, for example DNA, RNA, and proteins, and those that have their structures determined by other means, such as carbohydrates. In both instances, the bonds that hold the polymer together generating amides, phosphodiesters, or acetals have a higher energy than the starting materials. That is, formation of a polymer requires an input of chemical energy. Polymers are formed using activated intermediates, that is, compounds that can "donate" a monomer to the growing polypeptide chain. Whereas the synthesis of a polymer requires the input of energy, the net reactions of the breakdown of a polymer releases energy. This release of energy comes not from breaking chemical bonds but in the formation of new, more stable products. Therefore, polymers can be used to store energy and drive otherwise unfavorable reactions.

Summary

- In organic chemistry molecules are categorized by functional groups. Many of the same functional groups are used in biochemistry.
- All biological systems are aqueous, and water is the solvent most commonly associated with biochemistry; however, certain microenvironments in a cell or macromolecule may exclude water. Polar or charged functional groups make organic molecules more soluble in water and less soluble in a hydrophobic environment.
- An organic chemical reaction can be partially explained using an organic reaction mechanism. The reaction mechanism shows how electrons flow, to explain the chemistry observed. Such diagrams are useful in dissecting how complex molecules such as enzymes function.
- Many biological molecules are polymers of simpler building blocks. Proteins are made from amino acids, nucleic acids from nucleotides, and complex carbohydrates from simple sugars. Other molecules including some lipids are not polymers but are made in a similar modular fashion.

Concept Check

1. Identify polar and nonpolar functional groups.
2. Identify which functional groups make a molecule more soluble in water, and which make it more soluble in a nonpolar environment, such as hexane or olive oil.
3. Describe the basic guidelines for any organic reaction mechanism.
4. What does a reaction mechanism illustrate? How do the reaction mechanisms covered in organic chemistry compare with those in biochemical reactions?

1.3 The Chemistry of Water

Life is amazingly diverse and robust. It can exist without oxygen, at extreme temperatures, and in environments ranging from dilute to nearly saturated with salts and dissolved inorganic molecules. However, one common denominator to all of life is the need for water.

1.3.1 The structure of water provides clues about its properties

Water is so common on Earth that it is easy to forget that it is unique among solvents (**Figure 1.8**). Water has two lone pairs of electrons on the oxygen atom and two polar H–O bonds. The polarity in the oxygen–hydrogen bond comes from the unequal sharing of electrons between the weakly electronegative hydrogen and the highly electronegative oxygen atom. The lone

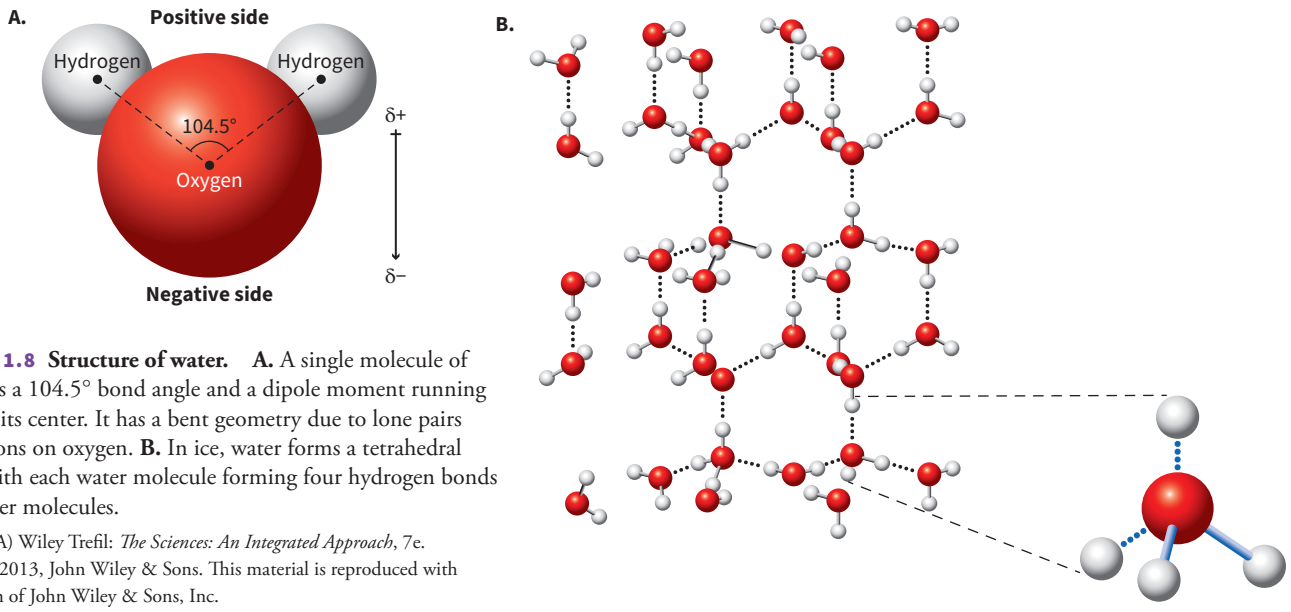


FIGURE 1.8 Structure of water. **A.** A single molecule of water has a 104.5° bond angle and a dipole moment running through its center. It has a bent geometry due to lone pairs of electrons on oxygen. **B.** In ice, water forms a tetrahedral lattice with each water molecule forming four hydrogen bonds with other molecules.

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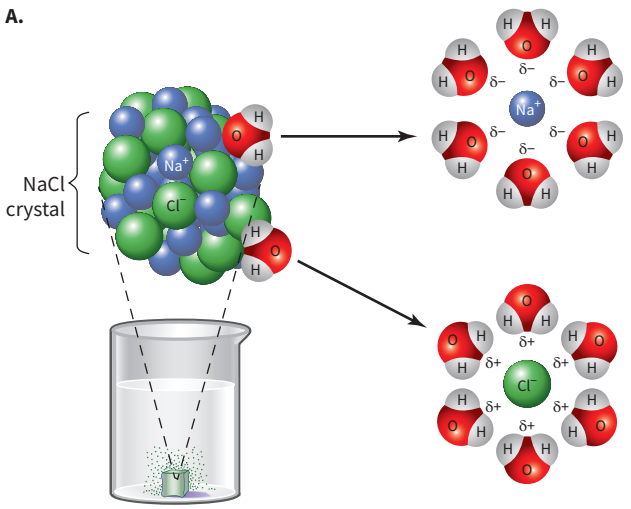
A.



B. Dielectric Constants of Common Laboratory Liquids

Solvent	Dielectric Constant (D)
Water	78.5
Ethyl alcohol	24.3
Acetone	20.7
Acetic acid	6.2
Benzene	2.3
Hexane	1.9

FIGURE 1.9 Coulombic interactions. Coulombic interactions describe the forces that hold ions together. **A.** Sodium and chloride ions in a crystal are disrupted and dissolved by water molecules. The water molecules form hydration shells around each of the ions. **B.** The dielectric constant of some common solvents. Water has one of the highest dielectric constants known.

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pairs of electrons give water a bent shape, with a nearly tetrahedral bond angle (104.5° in water compared to 109.5° in a truly tetrahedral center). Water's bent shape allows its polar H–O bonds to contribute to an overall dipole moment which runs through the entirety of the water molecule.

The polarity of water influences the strength of ionic interactions

Chemists categorize the polarity of a solvent using a constant known as the **dielectric constant**, the ability of an insulating liquid to carry current, measured in Debye units, D. Vacuum and air both have dielectric constants close to 1 D. Typical nonpolar solvents used in organic chemistry (hexane, benzene, or oils) have dielectric constants of 2 to 4 D (**Figure 1.9**). Ethanol and acetone have dielectric constants in the low 20s. Water, on the other hand, has a dielectric constant of 80 D, one of the highest values known.

The dielectric constant comes into play in coulombic interactions, defined by an equation that describes the interactions between charged species. Coulomb's law is:

$$F = kQ_1Q_2/r^2\mathrm{D}$$

where F is the force between the two charges, k is the Coulomb's law constant, Q_1 and Q_2 are the charges, r^2 is the square of the distance between the forces, and D is the dielectric constant of the medium containing the charges.

Coulomb's law states that the strength of an ionic interaction is the product of the charge of the two charges and a constant, divided by the square of the distance between those ions and the dielectric constant. This means that the force holding the two ions together decreases as the distance between the ions increases, or as the strength of the dielectric constant increases.

The high dielectric constant of water means that it can readily solubilize many ionic solids and significantly decrease ionic interactions. As a simple example, table salt can dissolve in water but not in vegetable oil. The interactions of water molecules with the sodium and chloride ions contribute more than the interactions of those ions with one another. Vegetable oil, with a far lower dielectric constant, is unable to overcome the interactions of the ions in the crystal and, as a result, the salt will not dissolve.

Although water can decrease the strength of ionic interactions, this observation should be used cautiously. Biochemistry is rich with microenvironments that may exclude water and strengthen ionic interactions.

1.3.2 Hydrogen bonding among water molecules is one of the most important weak forces in biochemistry

Hydrogen bonds are a weak force resulting from a strongly electronegative atom (typically oxygen, nitrogen, or fluorine) bound to a hydrogen atom (**Figure 1.10**). The polarity of the bond between

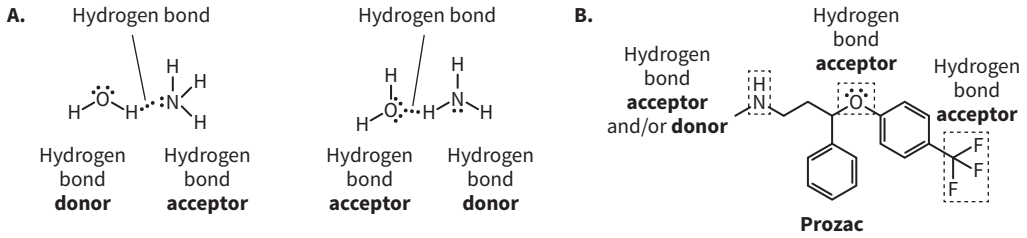


FIGURE 1.10 Hydrogen bonding. **A.** Hydrogen bonding is common between water molecules or between water and other molecules found in biological systems. **B.** While fluorine is not commonly found in biochemistry, it is an important element in numerous drugs, including the antidepressant Prozac.

these atoms results in a partial positive charge on the hydrogen. The hydrogen bond is the weak interaction between one of these hydrogen atoms and the lone pair of electrons on another electronegative atom. The presence of a hydrogen atom in a molecule is not sufficient for formation of a hydrogen bond; for example, methane (CH_4) does not exhibit hydrogen bonding.

Water has two hydrogen atoms bound to oxygen and two lone pairs of electrons; thus, it can form up to four hydrogen bonds at any one time. Water is unique in this regard, having equal numbers of hydrogen bond donors (hydrogen atoms bound to oxygen) and hydrogen bond acceptors (lone pairs). In liquid water, it has been calculated that any individual water molecule is involved in 3.7 hydrogen bonds at any one time.

The properties of hydrogen bonds

Hydrogen bonds are considered a weak force (10 to 40 kJ/mol in biological systems) when compared to a covalent bond (200 to 600 kJ/mol). The strength of a hydrogen bond is based partially on the angle between the electronegative hydrogen and the lone pair. A linear (180°) alignment produces the strongest bond. As with other bonds, hydrogen bonds have an optimal bond distance. For a hydrogen bond between a hydroxyl proton and the lone pair of electrons on another oxygen atom, the optimal length is 2.0 Å, but this can vary from 1.5 to 2.2 Å. The distance between the two non-hydrogen atoms is 2.5 to 3.2 Å.

Hydrogen bonds are not as strong as covalent bonds, but there are many hydrogen bonds within biological macromolecules. Water molecules and many biological molecules contain functional groups such as hydroxyl, amine, carbonyl, and carboxyl groups that can participate in hydrogen bonding, with tens to billions of hydrogen bonds occurring at all times in biological molecules. For example, hydrogen bonds lend strength to the cellulose in wood and plant fibers and proteins in wool and silk. Hydrogen bonds are also necessary to form the double helix of DNA.

Hydrogen bonds influence the properties of bulk water

Hydrogen bonds influence the properties of water in other ways. Water exhibits a property known as surface tension. The surface tension of water, which is illustrated by its ability to bead on a hydrophobic surface and resist an opposing force, is in part a product of the high degree of hydrogen bonding at the air–water interface. Biological systems take advantage of surface tension, but they also produce molecules to eliminate the tension. For example, water striders use surface tension to skim the surface of water, whereas in the lung, detergent-like molecules disrupt the surface tension making it easier for our lungs to expand, allowing us to breathe.

Water also has a high heat capacity, which is defined as the amount of energy needed to raise 1 gram of material by 1°C. At 4.18 J/g · K, water has a much higher heat capacity than most compounds. This is in part due to the energy needed to disrupt the hydrogen-bonding network of water to generate molecular motion—in other words, to raise the water's temperature. The heat capacity of water means that, in nature, water is relatively slow to heat up or cool down, and water is therefore liquid over a relatively broad temperature range. In effect, the heat capacity of water serves as a natural thermal buffer, helping to regulate the temperature of biochemical systems.

1.3.3 Water can ionize to acids and bases in biochemical systems

Acids, bases, and buffer systems are also important in biochemistry. There are several ways to describe acids and bases. Using the Arrhenius definition, acids are H^+ donors and bases generate

an increased concentration of OH^-. Although limited, this definition works well in many cases, especially when examining strong acids or bases. For compounds containing ammonia or an amine, the Arrhenius definition fails to explain the molecule's behavior. The **Brønsted-Lowry** definition of acids and bases categorizes acids as proton donors and bases as proton acceptors. Using this latter definition, we see that ammonia and most other amines accept a proton from water, making the solution more basic.

When examining biochemical systems, it is important to consider the protonation state of an acid or base in biochemical systems. A protonated amine is not a base; rather, it is an acid because it can act as a proton donor but not an acceptor.

The acid ionization constant and pK_a describe the strength of an acid

The acidity of a proton can be measured using its acid ionization constant, K_a:

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

where H^+ is the proton concentration, A^- is the conjugate base of the acid, and [HA] is the concentration of the acid at equilibrium, expressed in units of molarity.

Strong acids are almost 100% ionized in solution and hence have high K_a values. Conversely, weak acids are poorly ionized, often 1 molecule in 100 to 1 in 10 million, leading to small K_a values. These values are difficult to conceptualize, but taking the negative logarithm gives a whole number, the **pK_a** value, which is about 2.0 to 7.0 for weak acids (**Table 1.3**). The higher the pK_a is, the less acidic is the proton. Although in general chemistry we often think of the acidic proton in a weak acid as coming from a carboxylic acid, it is possible to determine a pK_a value for any proton. Hydroxyl protons have pK_a values of 14.0 to 16.0, whereas alkane protons may have values in the mid- to upper 30s, which is far beyond the values found in biochemistry. The pK_a value is a logarithmic rather than a linear value, so a change of three pK_a units is actually a 1,000-fold change in value. In addition, pK_a values can be modified significantly by neighboring atoms within a molecule. Introduction of electron withdrawing groups, or resonance states within the same molecule, or alterations to the local environment around a molecule of interest can change

TABLE 1.3 K_a and pK_a Values for Weak Acids Used in Biochemistry

Acid	K_a	pK_a
H_3PO_4	7.08×10^{-3}	2.15 (pK_1)
Succinic acid	6.17×10^{-5}	4.21 (pK_1)
Oxalate$^-$	5.37×10^{-5}	4.27 (pK_2)
Acetic acid	1.74×10^{-5}	4.76
Succinate$^-$	2.29×10^{-6}	5.64 (pK_2)
H_2CO_3	4.47×10^{-7}	6.35 (pK_1)
$H_2PO_4^-$	1.51×10^{-7}	6.82 (pK_2)
3-(*N*-Morpholino)propanesulfonic acid (MOPS)	7.08×10^{-8}	7.15
N-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)	3.39×10^{-8}	7.47
Tris(hydroxymethyl)aminomethane (TRIS)	8.32×10^{-9}	8.08
Boric acid	5.75×10^{-10}	9.24
NH_4^+	5.62×10^{-10}	9.25
Glycine (amino group)	1.66×10^{-10}	9.78
HCO_3^-	4.68×10^{-11}	10.33 (pK_2)
HPO_4^{2-}	4.17×10^{-13}	12.38 (pK_3)

Source: Dawson, R.M.C., Elliott, D.C., Elliott, W.H., and Jones, K.M., *Data for Biochemical Research* (3rd ed.), pp. 424–425, Oxford Science Publications (1986); *and* Good, N.E., Winget, G.D., Winter, W., Connolly, T.N., Izawa, S., and Singh, R.M.M., *Biochemistry* **5**, 467 (1966).

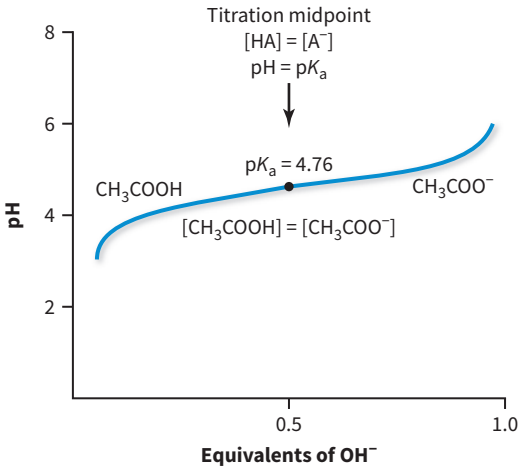


FIGURE 1.11 Buffers. Weak acids and bases will either ionize to release a proton or absorb one. Solutions buffer best over a range of ±1 pH unit around the pK_a value (the pH at which there are equal concentrations of conjugate acid and conjugate base).

a pK_a value radically. For example, molecules that would normally not deprotonate in aqueous solution often do so readily when bound to an enzyme.

The pH scale is used to measure hydrogen ion concentration

The hydrogen ion concentration of a system can range from 1 to 1×10^{-14} M. The pH scale transforms these concentrations into values that are easier to comprehend. Again, because these values are small, taking the logarithm of the value helps to generate numbers that are easier to work with. The **pH** is the negative logarithm of the hydrogen ion concentration, providing a scale that runs from 0 to 14.

In biochemistry, the pH of a reaction is critical; many important macromolecular interactions in biochemistry are dictated by weak forces that may be sensitive to changes in pH. Likewise, many catalytic mechanisms employ simple acid–base chemistry that fail to react outside a narrow pH range.

Biochemical systems are buffered from changes in pH

Buffers are chemical systems that are resistant to changes in pH. They are mixtures of weak acids, and their conjugate base (**Figure 1.11**). The mixture buffers the pH by acting like a proton sponge, releasing or absorbing protons to resist changes in pH. Buffers made with weak acids work best at pH values nearest the pK_a value of the weak acid in the system. The expression that is used to calculate the pH of a buffer system is the **Henderson–Hasselbalch** equation:

$$\text{pH} = \text{p}K_a + \log[\text{A}^-]/[\text{HA}]$$

where pH is the pH in question, the pK_a is the pK_a of the weak acid, $[A^-]$ is the molar concentration of the conjugate base of the weak acid, and [HA] is the concentration of the un-ionized weak acid, also expressed in units of molarity, M. The Henderson–Hasselbalch equation illustrates why buffers are only effective at pH values near the pK_a of the acid or base. If the pH is more than 1 pH unit from these values, the logarithmic term begins to dominate the equation, and the ratio of acid to conjugate base in the buffer fails to regulate pH. However, at pH values near the pK_a value, up to a 10-fold change in acid or base concentration will be blunted by the effect of the buffer.

It is often useful to see how an equation is derived. The derivation of the Henderson–Hasselbalch equation is shown in **Biochemistry: The derivation of the Henderson–Hasselbalch equation**.

Biochemistry

The derivation of the Henderson–Hasselbalch equation

The Henderson–Hasselbalch equation is a valuable expression in biochemistry. It relates the pH of a solution to the pK_a value of the weak acid in question, and the ratio of the concentration of weak acid to that of the conjugate base of the weak acid. The equation is useful for performing calculations with buffer solutions and pH; it is also relatively easy to derive. Although it is probably easier to simply know or look up the Henderson–Hasselbalch equation, deriving it illustrates several other important pieces of acid–base chemistry and how the equations are put together, a useful tool when examining or deriving other equations.

A weak acid (HA) will partially ionize in aqueous solution to give a proton (H^+) and the conjugate base of the weak acid (A^-). Expressing these as molar quantities at equilibrium allows us to write an acid ionization constant, K_a:

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$$

Taking the base-10 logarithm of both sides gives:

$$\log_{10} K_a = \log_{10}\left(\frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}\right)$$

The right-hand side of the equation can be rearranged to isolate the log of the hydrogen ion concentration. Adding logarithms is the equivalent of multiplying integer values:

$$\log_{10} K_a = \log_{10}[\text{H}^+] + \log_{10}\left(\frac{[\text{A}^-]}{[\text{HA}]}\right)$$

Next, we can simplify the equation. The abbreviation used when taking the negative log of a term, is p (as in pH and pK_a). The above cases use the log (not the negative log), so the equation can be simplified to:

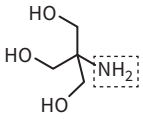
$$-\mathrm{p}K_a = -\mathrm{pH} + \log_{10}\left(\frac{[\mathrm{A}^-]}{[\mathrm{HA}]}\right)$$

Rearranging the pK_a and pH terms gives the familiar form of the Henderson–Hasselbalch equation:

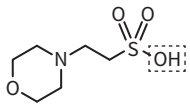
$$\mathrm{pH} = \mathrm{p}K_a + \log_{10}\left(\frac{[\mathrm{A}^-]}{[\mathrm{HA}]}\right)$$

The pH at which a weak acid or conjugate base system will buffer is a function of its pK_a, but the total amount of acid or base that can be consumed is a function of the concentration. Thus, if two potassium phosphate buffers have been prepared in the laboratory, both at pH 7.3, and one buffer is 10 mM and the other 100 mM, the latter will have the ability to consume more acid or base. This is termed the **buffer capacity** of a system.

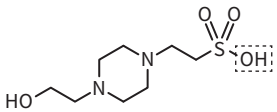
Biochemical systems are buffered by several different combinations of weak acids and their conjugate bases or weak bases and their conjugate acids. The most common buffering system in the blood is the bicarbonate/carbonate buffer. Cells use a combination of bicarbonate and phosphate to buffer against pH changes. Common biological buffers used in the laboratory include bicarbonate, phosphate, and acetate. Both carbonate and phosphate are polyprotic acids, acids that can donate more than one proton. Generally, only one of these ionizations plays a role in buffering of biochemical systems. In the laboratory, small organic molecules are often chosen to buffer near a specific pH; these include TRIS, MOPS, and HEPES.



TRIS
tris(hydroxymethyl)aminomethane



MOPS
3-(*N*-Morpholino)propanesulfonic acid



HEPES
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

In an experiment, if there is no means of buffering, it is easy for the pH to change rapidly. In the early days of experimental biochemistry, the lack of control of factors such as buffering and pH made it difficult to obtain consistent results from repeated experiments, and this generated spurious and false results (referred to as artifacts). Buffers help regulate and control biochemical experiments, and standardize results. Biochemical systems are buffered for similar reasons; the inability to regulate pH can result in wide fluctuations, many of which could be harmful to the organism. Altering the pH of a system can have implications on the molecular level such as the ability of a protein to bind to a small molecule. This can have significant effects on the organism, for example, on the ability to carry oxygen to tissues that need it.

The buffering capacity of the blood is discussed in **Medical Biochemistry: Buffers in blood**.

Medical Biochemistry

Buffers in blood

Buffers play a central role in biochemistry. In the laboratory, students are advised to always use a buffered solution for a biochemical experiment. Naturally occurring bodies of water, including streams, lakes, and even oceans, have low millimolar levels of dissolved compounds such as carbon dioxide and phosphate, which provide minor amounts of buffering. The dissolved compounds are also partially responsible for the observed pH. However, the necessity and effect of buffering are most evident inside an organism. In the blood, the bicarbonate ion (HCO_3^-, pK_a 6.3) is the primary molecule responsible for buffering and maintaining a tight pH range around 7.35 (±0.1 pH units). Other ions, such as phosphate, ammonia, or organic ions, play minor roles in blood buffering but are found at a much lower concentration than bicarbonate (17 to 24 mM in healthy humans), or have pK_a values too far from 7.35 to be effective. At physiological pH we are outside the pH range at which bicarbonate would buffer a solution: ±1 pH unit around the pK_a value or, for bicarbonate,

5.3 to 7.3. At pH 7.35 the ratio of bicarbonate to carbonic acid is over 10:1.

Several factors account for the observed pH. First, the organism can regulate pH through the release or reabsorption of CO_2 either in the lung or kidney. Second, while less important than bicarbonate, other molecules, such as phosphate and proteins, can also participate in buffering. It may seem paradoxical that the organism operates outside the optimal buffering range; however, the bicarbonate buffering system has several advantages. Tissues produce acidic waste products, and this system provides a means by which the organism can rapidly respond to increased acid levels. Carbon dioxide is generated when carbon-containing molecules are oxidized for energy, and this CO_2 must be eliminated from the body. Carbon dioxide typically diffuses out of cells and into the bloodstream. There, dissolved carbon dioxide enters red blood cells or erythrocytes, where it is acted on by the enzyme carbonic anhydrase, catalyzing the formation of carbonic acid (H_2CO_3) from carbon dioxide and water. The carbonic acid generated is mildly acidic ($pK_{a1} = 6.35$), and it rapidly ionizes to form a bicarbonate anion and a hydronium ion. The second pK_a (pK_{a2}) is 10.33 and is not typically encountered in biological systems. The bicarbonate that is generated in this reaction accumulates in the erythrocyte. These reactions would rapidly reach equilibrium were it not for the chloride–bicarbonate exchange protein, which is found in the membrane of the erythrocyte and exchanges a bicarbonate ion for a chloride ion. This process ensures that there is no significant accumulation of bicarbonate in the erythrocyte, and keeps the reaction moving in the forward direction in carbon dioxide generating tissues.

In the lung, there is a low concentration or partial pressure of carbon dioxide. Thus, as blood passes through the lung, the described reactions occur in reverse: chloride rushes out of the erythrocyte and bicarbonate rushes in. The latter protonates and is rapidly degraded to carbon dioxide, which is free to diffuse out of the erythrocyte and blood, and is exhaled and eliminated by the lung. The erythrocyte demonstrates several chemical principles including equilibrium, Le Châtelier's principle, solubility, permeability, and catalysis. In addition, the bicarbonate generated serves as a buffer for the blood, bathing tissues in a constant pH close to 7.35.

In times of excessive acid production, such as during anaerobic muscle exertion or in several disease states, more acid is produced than the buffer system can compensate for, and blood pH levels drop below pH 7.2. This condition, known as acidosis, can have serious consequences. At pH 6.8, the level of acidity is such that it becomes difficult for the blood to deliver oxygen to tissues that need it. Damage can quickly result to tissues, cells, and the molecules that make up those cells. In the clinic, sodium bicarbonate is used to raise the pH of the blood until the underlying cause of the acidosis can be determined.

In other conditions, the blood may become dangerously alkaline (above pH 7.45). This may happen, for example, when a person hyperventilates, in which case too much carbon dioxide is exhaled, depleting the body of its buffering capacity. In this instance, carbon dioxide can be recovered by having a patient breathe an environment enriched in carbon dioxide, such as a paper bag, to increase the amount of carbon dioxide and bicarbonate dissolved in blood.

Worked Problem 1.4 Working with buffers

You have been asked to prepare 1 liter of a 50 mM buffer (pH 5.2) for an experiment. In the laboratory are the following buffers: TRIS, MOPS, phosphate, and acetate.

Which buffer will you choose to make, and how will you make it?

Strategy Examine the pK_a values for each buffer in Table 1.3. How does that information help in choosing which buffer to use?

Solution Buffers only work over a range of ±1 pH unit around the pK_a of the acid or base in the buffer. Of the four buffers available, only one, acetate, has a pK_a near 5.2 (4.7). To make the buffer, you would measure out enough acetate to make 1 liter of final solution but dilute it into less than the final volume. You would then adjust the pH of this solution with a solution of base like NaOH to the desired pH (5.2). Finally, you would then dilute the buffer to the final volume (1 liter).

Follow-up question If you have a solution of 50 mM acetic acid and 50 mM sodium acetate, how much of each would you need to make 1 liter of pH 5.5 buffer?

Summary

- Water is:
  - necessary for life
  - the central solvent in biochemistry
  - highly polar
  - particularly effective at making hydrogen bonds—bonds between a hydrogen attached to an electronegative atom and another electronegative atom.
- All biological systems require water, and it is the solvent most commonly associated with biochemistry; however, microenvironments in a cell or macromolecule may exclude water. Polar or charged functional groups make organic molecules more soluble in water and less soluble in a hydrophobic environment.

- Water has the ability to ionize, forming H^+ and OH^-, affecting pH.
- Buffers are pairs of weak acids and their conjugate base (or weak bases and their conjugate acid).
- Buffers act to resist changes in pH. The compositions of a buffer and the pH can be calculated using the Henderson–Hasselbalch equation.

Concept Check

1. Discuss how biological processes can be understood in the context of water and its interaction at molecular level.
2. Describe hydrogen bonding and how it imparts to water many of the properties it exhibits.
3. Perform calculations with both strong and weak acids and bases, including pH calculations. What is the approximate pK_a of a weak acid HA if a solution of 0.1 M HA and 0.3 M A^- has a pH of 6.5?
4. Perform calculations using buffers and the Henderson–Hasselbalch equation. What would be the resulting pH if one drop (0.05 mL) of 1.0 M HCl was added to one liter of pure water?

Bioinformatics Exercises

Exercise 1 The Periodic Table of the Elements and Domains of Life

Exercise 2 Organic Functional Groups and the Three-Dimensional Structure of Vitamin C

Exercise 3 Structure and Solubility

Exercise 4 Amino acids, Ionization, and pK values

Problems

1.1 General Chemical Principles

1. State the three laws of thermodynamics and explain their significance in context of biochemical systems.

2. What is the difference between ΔG, $\Delta G°$, $\Delta G°'$ and $\Delta G^‡$? Can any of these be used interchangeably, and if so, when?

3. All biochemical macromolecules are folded into complex shapes held together in part by weak forces. Discuss the thermodynamics of these processes. Are they favorable or unfavorable? Which thermodynamic terms (ΔG, ΔH, ΔS, and others) are important in folding into a stable conformation? What other phenomena (bond breaking or forming, changes in entropy, and so on) contribute to the larger thermodynamic parameters?

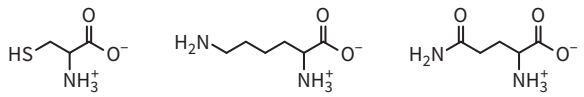
4. Phosphoglucomutase catalyzes the reaction in which a phosphate group is transferred from the C-1 of glucose to the C-6 of glucose (G1P → G6P). A student incubates a 0.2 M solution of glucose-1-phosphate overnight with a small amount of the enzyme. At equilibrium the concentration of glucose-1-phosphate is 9.0×10^{-3} M and the concentration of glucose-6-phosphate is 19.1×10^{-2} M. Calculate the equilibrium constant (K_{eq}) and the standard state free energy ($\Delta G°'$) for this reaction at 25°C.

5. If we consider the polymerization of a water soluble monomer into a polymer, is this process entropically positive or negative? What types of interactions need to be considered?

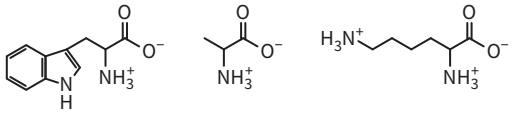
6. In organisms, β-hydroxybutyrate, found in some metabolic states, will spontaneously decarboxylate into acetone and carbon dioxide. Write a rate law for this reaction. What factors will contribute to this rate?

1.2 Fundamental Concepts of Organic Chemistry

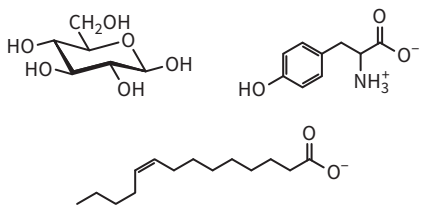
7. Examine these amino acids. Which functional groups can you identify? What types of bonds can these groups form?



8. Amino acids are the building blocks of proteins. Can you put these amino acids into order of increasing solubility in water?



9. Which of these molecules would you anticipate has the highest solubility in water? Which would be most soluble in hexane?

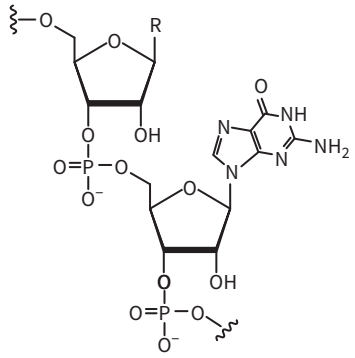


10. Examine the structures given in problem 9. If each were dissolved in water, how would the water molecules surround these solutes? What sort of interactions would occur and where?

11. Propose a mechanism for the base catalyzed cleavage of an ester.

12. If a polymer is 1,000 monomers long and includes four different types of monomers, how many different combinations of the monomers are possible?

13. Examine the structure of the polymer shown. What are the monomeric units of that polymer?



1.3 The Chemistry of Water

14. Examine the functional groups in Table 1.2. Which of these can hydrogen bond with water? How and where will these bonds form?

15. Which of these bonds would be the shortest H bond, and why? OH–O, OH–N, OH–F

16. A molecule of water is said to participate in 3.7 hydrogen bonds at any one time. How could you calculate this number? How could you measure it?

17. What is the pH value of each of these solutions?

a. 0.1 M HCl
b. 3 M H_2SO_4
c. 12 mM HCl
d. 2 M KOH
e. 100 mM $Ca(OH)_2$
f. 0.3 M acetic acid
g. 43 mM H_2CO_3
h. 25 mM ammonia
i. 80 mM TRIS base
j. 0.5 M KH_2PO_4

18. What percentage of a solution of acetic acid is ionized at pH 5.6? What percentage of a solution of HCl is ionized at the same pH?

19. What is the pH of a solution that contains three parts acetic acid and one part sodium acetate? The pK_a for acetic acid is 4.76.

20. What is the final pH if you have 500 mL of a sodium acetate buffer (50 mM, pH 5.0) and you add 5 mL of 1 M NaOH?

21. How much 6 M HCl do you need to add if you have 800 mL of 100 mM TRIS, pH 10.2 and you wish to lower the pH to 7.0?

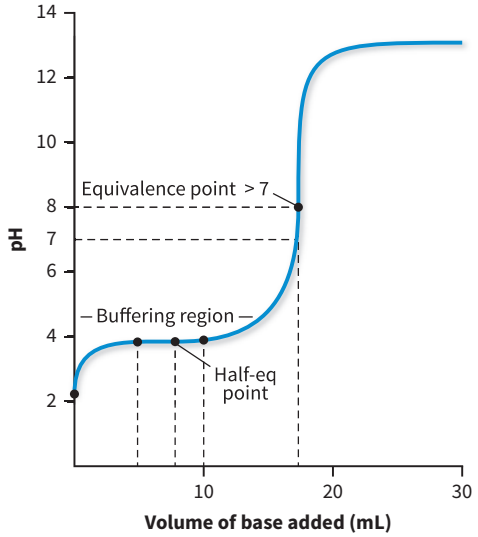
22. The pK_a of acetic acid is 4.76. If 20% of the acetic acid in a 0.1 M solution is in the protonated state, what is the pH of the solution?

23. Buffering capacity is the property of a buffer to resist changes in pH. Which has more buffering capacity: a liter of 50 mM MOPS, pH 6.9 or 100 mL of 1 M MOPS at the same pH?

24. Normal blood pH is approximately 7.35 and the concentration of carbonate and bicarbonate ions (total) is approximately 20 mM. What is the ratio of carbonate to bicarbonate ions in the blood under these conditions?

Data Interpretation

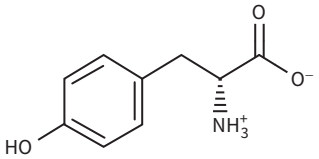
25. In the titration curve of a weak acid with a strong base, where is the equivalence point? What is the pK_a of this acid? Over what pH range would this solution act as a buffer?



Experimental Design

26. Based on your knowledge of organic chemistry, how could you increase the solubility of an amine or a carboxylic acid in an aqueous solution?

27. Tyrosine is an amino acid, one of the building blocks of proteins. It has a hydrophobic side chain. Design an experiment to test the solubility of tyrosine in can be changed to 250 mM NaCl.



28. Based on your experiences in organic chemistry, how would you identify an unknown molecule? What organic techniques would you use? What are the limitations of those techniques? Based on your current knowledge of biochemistry, what are the strengths and drawbacks of employing organic techniques to identify biological molecules?

29. If you were asked to make 1 liter of 250 mM KCl, how would you do it? Give a detailed description, including the steps in the process and the pieces of glassware you would use.

30. How would you go about making 500 milliliters of 100 mM TRIS buffer, pH 7.2? Describe the steps involved, including which apparatus and glassware you would use and when.

Ethics and Social Responsibility

31. As you begin your studies in biochemistry, what do you think are the important ethical and social questions in this field?

32. All professional organizations have codes of conduct. These include the codes of conduct for the American Society for Biochemistry and Molecular Biology, the International Union for Biochemistry and Molecular Biology, the American Chemical Society, the American

Medical Association, and others. Using the Internet, examine one of these organization's codes of conduct. Is there anything that you find surprising?

33. Various governmental agencies regulate the sale of certain biomolecules, ranging from drugs to foods to fuels. Is this regulation justified, and if so, why?

34. If you were to examine the effect that table sugar has on the body, you could find many ways in which it elicits physiological and metabolic changes. Why is sugar not considered a drug? Find a reliable resource that defines drugs and pharmaceuticals. What differentiates drugs from other molecules?

35. Drug companies have patent protection for drugs that they have developed and are on the market. If an enantiomerically pure drug can be developed from a racemate, the company is given a new patent on the stereochemically pure compound. Do you feel this situation is justified?

Suggested Readings

1.1 General Chemical Principles

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TIMELINE CHAPTER 1