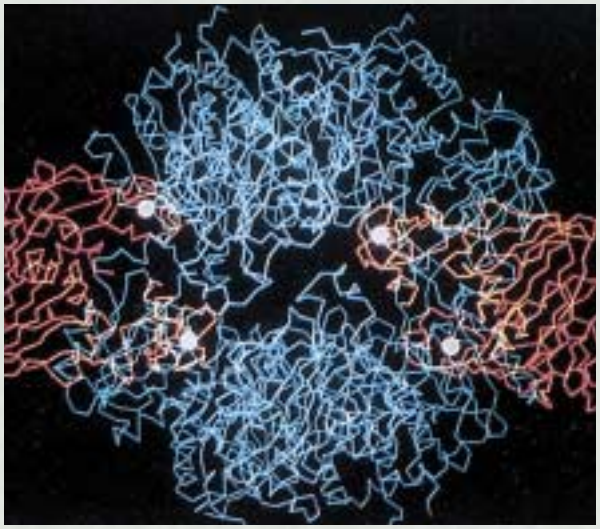
PART III

Microbial Metabolism

CHAPTER 8

Metabolism: Energy, Enzymes, and Regulation



This diagram shows *E. coli* aspartate carbamoyltransferase in the less active T state. The catalytic polypeptide chains are in blue and the regulatory chains are colored red.

Outline

Concepts

1. Energy is the capacity to do work. Living organisms can perform three major types of work: chemical work, transport work, and mechanical work.
2. Most energy used by living organisms originally comes from sunlight trapped during photosynthesis by photoautotrophs. Chemoheterotrophs then consume autotrophic organic materials and use them as sources of energy and as building blocks.
3. An energy currency is needed to connect energy-yielding exergonic reactions with energy-requiring endergonic reactions. The most commonly used currency is ATP.
4. All living systems obey the laws of thermodynamics.
5. When electrons are transferred from a reductant with a more negative reduction potential to an oxidant with a more positive potential, energy is made available. A reversal of the direction of electron transfer—for example, during photosynthesis—requires energy input.
6. Enzymes are protein catalysts that make life possible by increasing the rate of reactions at ambient temperatures. Enzymes do not change chemical equilibria or violate the laws of thermodynamics but accelerate reactions by lowering their activation energy.

7. Metabolism is regulated in such a way that (a) cell components are maintained at the proper concentrations, even in the face of a changing environment, and (b) energy and material are conserved.
8. The localization of enzymes and metabolites in separate compartments of a cell regulates and coordinates metabolic activity.
9. The activity of regulatory enzymes may be changed through reversible binding of effectors to a regulatory site separate from the catalytic site or through covalent modification of the enzyme. Regulation of enzyme activity operates rapidly and serves as a fine-tuning mechanism to adjust metabolism from moment to moment.
10. A pathway's activity is often controlled by its end products through feedback inhibition of regulatory enzymes located at the start of the sequence and at branch points.

Living cells are self-regulating chemical engines, tuned to operate on the principle of maximum economy.

—*A. L. Lehninger*

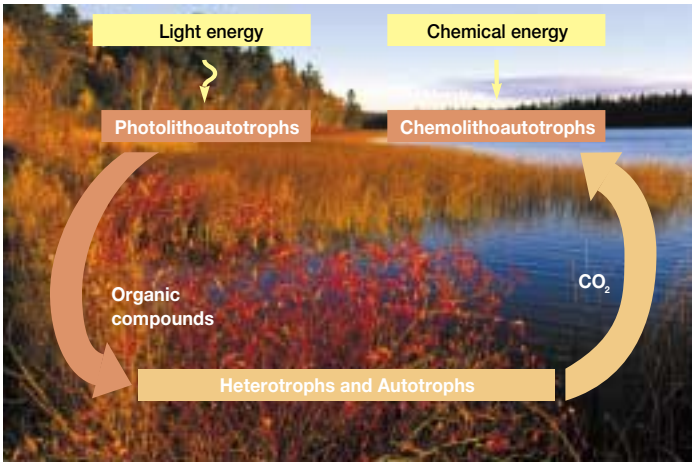


Figure 8.1 The Flow of Carbon and Energy in an Ecosystem. This diagram depicts the flow of energy and carbon in general terms. See text for discussion.

Chapters 3 and 4 contain many examples of an important principle: that a cell's structure is intimately related to its function. In each instance one can readily relate an organelle's construction to its function (and vice versa). A second unifying principle in biology is that life is sustained by the trapping and use of energy, a process made possible by the action of enzymes. Because this is so crucial to our understanding of microbial function, considerable attention is given to energy and enzymes in this chapter.

The organization of microbial metabolism will be briefly described in chapters 8 to 10. Metabolic pathways are treated as a sequence of enzymes functioning as a unit, with each enzyme using as its substrate a product of the preceding enzyme-catalyzed reaction. This picture of metabolic pathways is incomplete because we will usually ignore the regulation of pathway operation for the sake of space and simplicity. However, one should keep in mind that both regulation of the activity of individual pathways and coordination of the action of separate sequences are essential to the existence of life. Cells become disorganized and die without adequate control of metabolism, and regulation is just as important to life as is the efficient use of energy. Thus the last part of this chapter will be devoted to the regulation of metabolism as a foundation for the subsequent discussion of pathways.

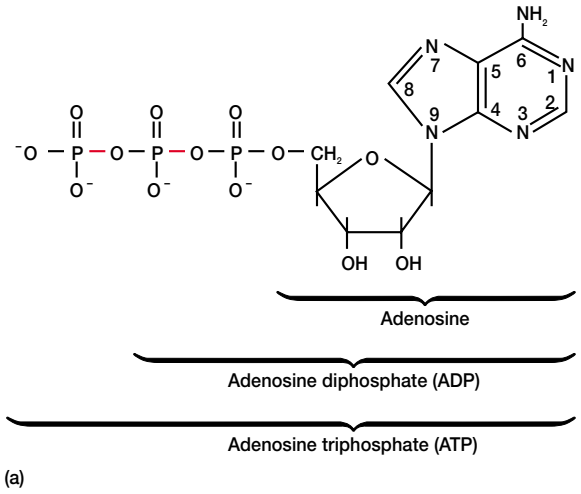
This chapter begins with a brief survey of the nature of energy and the laws of thermodynamics. The participation of energy in metabolism and the role of ATP as an energy currency is considered next. An introduction to the nature and function of enzymes follows. The chapter ends with an overview of metabolic regulation, including an introduction to metabolic channeling and the regulation of the activity of critical enzymes.

8.1 Energy and Work

Energy may be most simply defined as the capacity to do work or to cause particular changes. Thus all physical and chemical processes are the result of the application or movement of energy. Living cells carry out three major types of work, and all are essential to life processes. **Chemical work** involves the synthesis of complex biological molecules required by cells from much simpler precursors; energy is needed to increase the molecular complexity of a cell. Molecules and ions often must be transported across cell membranes against an electro-chemical gradient. For example, a molecule sometimes moves into a cell even though its concentration is higher internally. Similarly a solute may be expelled from the cell against a concentration gradient. This process is **transport work** and requires energy input in order to take up nutrients, eliminate wastes, and maintain ion balances. The third type of work is **mechanical work,** perhaps the most familiar of the three. Energy is required to change the physical location of organisms, cells, and structures within cells.

The ultimate source of most biological energy is the visible sunlight impinging on the earth's surface. Light energy is trapped by phototrophs during **photosynthesis,** in which it is absorbed by chlorophyll and other pigments and converted to chemical energy. As noted in chapter 5, chemolithoautotrophs derive energy by oxidizing inorganic compounds rather than obtaining it from light absorption. Chemical energy from photosynthesis and chemolithotrophy can then be used by photolithoautotrophs and chemolithoautotrophs to transform CO_2 into biological molecules such as glucose (**figure 8.1**). Nutritional types (pp. 97–98)

The complex molecules manufactured by autotrophic organisms (both plant and microbial producers) serve as a carbon source for chemoheterotrophs and other consumers that use complex organic molecules as a source of material and energy for building their own cellular structures (it should be remembered that autotrophs also use complex organic molecules). Chemoheterotrophs often employ O_2 as an electron acceptor when oxidizing glucose and other organic molecules to CO_2. This process, in which O_2 acts as the final electron acceptor and is reduced to water, is called **aerobic respiration.** Much energy is released



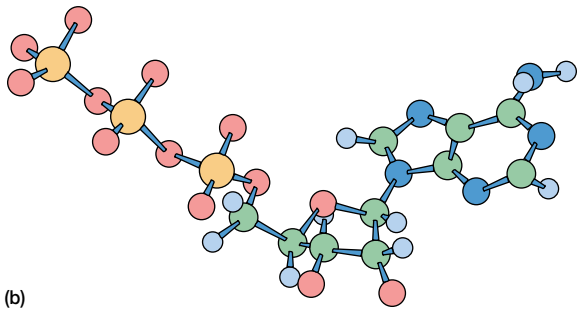


Figure 8.2 Adenosine Triphosphate and Adenosine Diphosphate. (**a**) Structure of ATP and ADP. The two red bonds are more easily broken or have a high phosphate group transfer potential (see text). The pyrimidine ring atoms have been numbered. (**b**) A model of ATP. Carbon is in green; hydrogen in light blue; nitrogen in dark blue; oxygen in red; and phosphorus in orange.

during this process. Thus, in the ecosystem, energy is trapped by photoautotrophs and chemolithoautotrophs; some of this energy subsequently flows to chemoheterotrophs when they use nutrients derived from autotrophs (figure 8.1; *see also figure 28.32*). The CO_2 produced during aerobic respiration can be incorporated again into complex organic molecules during photosynthesis and chemolithoautotrophy. Clearly the flow of carbon and energy in the ecosystem is intimately related.

Cells must efficiently transfer energy from their energy-generating or trapping apparatus to the systems actually carrying out work. That is, cells must have a practical form of energy currency. In living organisms the major currency is **adenosine 5′-triphosphate (ATP; figure 8.2)**. When ATP breaks down to **adenosine diphosphate (ADP)** and orthophosphate (P_i), energy is made available for useful work. Later, energy from photosynthesis, aerobic respiration, anaerobic respiration, and fermentation is used to resynthesize ATP from ADP and P_i. An energy cycle is created in the cell (**figure 8.3**). Fermentation (pp. 179–81); Anaerobic respiration (pp. 190–91)

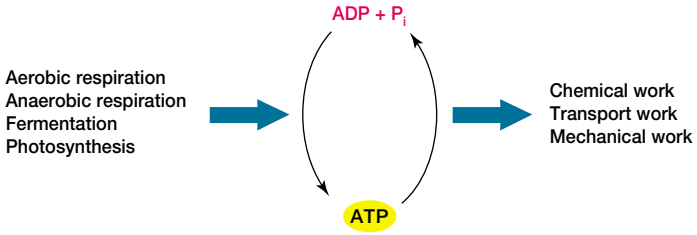


Figure 8.3 The Cell's Energy Cycle. ATP is formed from energy made available during aerobic respiration, anaerobic respiration, fermentation, and photosynthesis. Its breakdown to ADP and phosphate (P_i) makes chemical, transport, and mechanical work possible.

8.2 The Laws of Thermodynamics

To understand how energy is trapped or generated and how ATP functions as an energy currency, some knowledge of the basic principles of thermodynamics is required. The science of **thermodynamics** analyzes energy changes in a collection of matter (e.g., a cell or a plant) called a system. All other matter in the universe is called the surroundings. Thermodynamics focuses on the energy differences between the initial state and the final state of a system. It is not concerned with the rate of the process. For instance, if a pan of water is heated to boiling, only the condition of the water at the start and at boiling is important in thermodynamics, not how fast it is heated or on what kind of stove. Two important laws of thermodynamics must be understood. The **first law of thermodynamics** says that energy can be neither created nor destroyed. The total energy in the universe remains constant although it can be redistributed. For example, many energy exchanges do occur during chemical reactions (e.g., heat is given off by exothermic reactions and absorbed during endothermic reactions), but these heat exchanges do not violate the first law.

It is necessary to specify quantitatively the amount of energy used in or evolving from a particular process, and two types of energy units are employed. A **calorie** (cal) is the amount of heat energy needed to raise one gram of water from 14.5 to 15.5°C. The amount of energy also may be expressed in terms of **joules** (J), the units of work capable of being done. One cal of heat is equivalent to 4.1840 J of work. One thousand calories or a kilocalorie (kcal) is enough energy to boil 1.9 ml of water. A kilojoule is enough energy to boil about 0.44 ml of water, or enable a person weighing 70 kg to climb 35 steps. The joule is normally used by chemists and physicists. Because biologists most often speak of energy in terms of calories, this text will employ calories when discussing energy changes.

Although it is true that energy is conserved in the universe, the first law of thermodynamics does not account for many physical and chemical processes. A simple example may help make this clear. Suppose a full gas cylinder is connected to an empty one by a tube with a valve (**figure 8.4**). If the valve is opened, gas flows from the full to the empty cylinder until the gas pressure is equal on

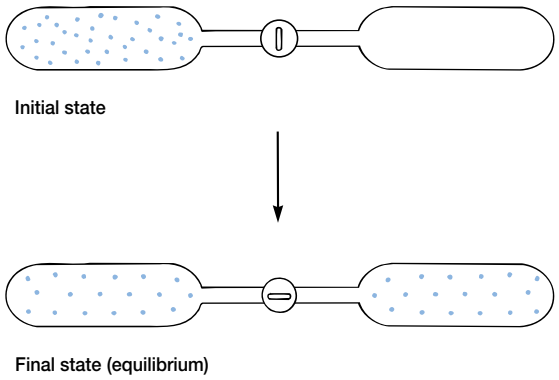


Figure 8.4 A Second Law Process. The expansion of gas into an empty cylinder simply redistributes the gas molecules until equilibrium is reached. The total number of molecules remains unchanged.

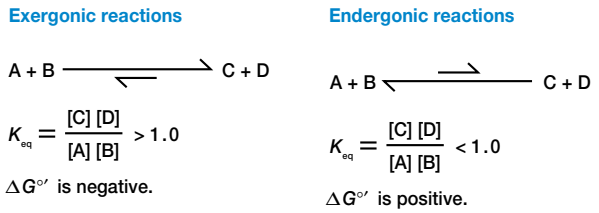


Figure 8.5 $\Delta G^{o\prime}$ and Equilibrium. The relationship of $\Delta G^{o\prime}$ to the equilibrium of reactions. Note the differences between exergonic and endergonic reactions.

both sides. Energy has not only been redistributed but also conserved. The expansion of gas is explained by the **second law of thermodynamics** and a condition of matter called entropy. **Entropy** may be considered a measure of the randomness or disorder of a system. The greater the disorder of a system, the greater is its entropy. The second law states that physical and chemical processes proceed in such a way that the randomness or disorder of the universe (the system and its surroundings) increases to the maximum possible. Gas will always expand into an empty cylinder.

8.3 Free Energy and Reactions

The first and second laws can be combined in a useful equation, relating the changes in energy that can occur in chemical reactions and other processes.

$$\Delta G = \Delta H - T{\cdot}\Delta S$$

ΔG is the change in free energy, ΔH is the change in enthalpy, T is the temperature in Kelvin (°C + 273), and ΔS is the change in entropy occurring during the reaction. The change in **enthalpy** is the change in heat content. Cellular reactions occur under conditions of constant pressure and volume. Thus the change in enthalpy is about the same as the change in total energy during the reaction. The **free energy change** is the amount of energy in a system available to do useful work at constant temperature and pressure. Therefore the change in entropy is a measure of the proportion of the total energy change that the system cannot use in performing work. Free energy and entropy changes do not depend on how the system gets from start to finish. A reaction will occur spontaneously at constant temperature and pressure if the free energy of the system decreases during the reaction or, in other words, if ΔG is negative. It follows from the equation that a reaction with a large positive change in entropy will normally tend to have a negative ΔG value and therefore occur spontaneously. A decrease in entropy will tend to make ΔG more positive and the reaction less favorable.

The change in free energy has a definite, concrete relationship to the direction of chemical reactions. Consider the following simple reaction:

$$A + B \rightleftharpoons C + D$$

If the molecules A and B are mixed, they will combine to form the products C and D. Eventually C and D will become concentrated enough to combine and produce A and B at the same rate as they are formed from A and B. The reaction is now at **equilibrium:** the rates in both directions are equal and no further net change occurs in the concentrations of reactants and products. This situation is described by the **equilibrium constant** (K_{eq}), relating the equilibrium concentrations of products and substrates to one another.

$$K_{eq} = \frac{[C][D]}{[A][B]}$$

If the equilibrium constant is greater than one, the products are in greater concentration than the reactants at equilibrium—that is, the reaction tends to go to completion as written.

The equilibrium constant of a reaction is directly related to its change in free energy. When the free energy change for a process is determined at carefully defined standard conditions of concentration, pressure, pH, and temperature, it is called the **standard free energy change** (ΔG^{o}). If the pH is set at 7.0 (which is close to the pH of living cells), the standard free energy change is indicated by the symbol $\Delta G^{o\prime}$. The change in standard free energy may be thought of as the maximum amount of energy available from the system for useful work under standard conditions. Using $\Delta G^{o\prime}$ values allows one to compare reactions without worrying about variations in the ΔG due to differences in environmental conditions. The relationship between $\Delta G^{o\prime}$ and K_{eq} is given by the following equation:

$$\Delta G^{o\prime} = -2.303RT{\cdot}\log K_{eq}$$

R is the gas constant (1.9872 cal/mole-degree or 8.3145 J/mole-degree), and T is the absolute temperature. Inspection of this equation shows that when $\Delta G^{o\prime}$ is negative, the equilibrium constant is greater than one and the reaction goes to completion as written. It is said to be an **exergonic reaction** (**figure 8.5**). In an **endergonic reaction** $\Delta G^{o\prime}$ is positive and the equilibrium constant is less than one. That is, the reaction is not favorable, and little product will be formed at equilibrium under standard conditions. Keep in mind

that the $\Delta G^{o\prime}$ value shows only where the reaction lies at equilibrium, not how fast the reaction reaches equilibrium.

1. What is energy and what kinds of work are carried out in a cell? Describe the energy cycle and ATP's role in it.
2. What is thermodynamics? Summarize the first and second laws. Define free energy, entropy, and enthalpy.
3. How is the change in standard free energy related to the equilibrium constant for a reaction? What are exergonic and endergonic reactions?

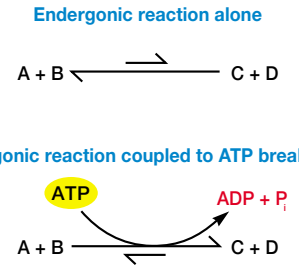


Figure 8.6 ATP as a Coupling Agent. The use of ATP to make endergonic reactions more favorable. It is formed by exergonic reactions and then used to drive endergonic reactions.

8.4 The Role of ATP in Metabolism

Many reactions in the cell are endergonic and will not proceed far toward completion without outside assistance. One of ATP's major roles is to drive such endergonic reactions more to completion. ATP is a **high-energy molecule.** That is, it breaks down or hydrolyzes almost completely to the products ADP and P_i with a $\Delta G^{o\prime}$ of -7.3 kcal/mole.

$$ATP + H_2O \longrightarrow ADP + P_i$$

In reference to ATP the term high-energy molecule does not mean that there is a great deal of energy stored in a particular bond of ATP. It simply indicates that the removal of the terminal phosphate goes to completion with a large negative standard free energy change, or the reaction is strongly exergonic. In other words, ATP has a high **phosphate group transfer potential;** it readily transfers its phosphate to water. The phosphate group transfer potential is defined as the negative of $\Delta G^{o\prime}$ for the hydrolytic removal of phosphate. A molecule with a higher group transfer potential will donate phosphate to one with a lower potential.

Thus ATP is ideally suited for its role as an energy currency. It is formed in energy-trapping and -generating processes such as photosynthesis, fermentation, and aerobic respiration. In the cell's economy, exergonic ATP breakdown is coupled with various endergonic reactions to promote their completion (**figure 8.6**). In other words ATP links energy-generating reactions, which liberate free energy, with energy-using reactions, which require free energy input to proceed toward completion. Facilitation of chemical work is the focus of the preceding example, but the same principles apply when ATP is coupled with endergonic processes involving transport work and mechanical work (figure 8.3).

8.5 Oxidation-Reduction Reactions and Electron Carriers

Free energy changes are not only related to the equilibria of "regular" chemical reactions but also to the equilibria of oxidation-reduction reactions. The release of energy normally involves oxidation-reduction reactions. **Oxidation-reduction (redox) reactions** are those in which electrons move from a donor, the **reducing agent** or **reductant,** to an electron acceptor, the **oxidizing agent** or **oxidant.** By convention such a reaction is written with the reductant to the right of the oxidant and the number (n) of electrons (e^-) transferred.

$$\text{Oxidant} + ne^- \rightleftharpoons \text{reductant}$$

The oxidant and reductant pair is referred to as a redox couple (table 8.1). When an oxidant accepts electrons, it becomes the reductant of the couple. The equilibrium constant for the reaction is called the **standard reduction potential** (E_0) and is a measure of the tendency of the reducing agent to lose electrons. The reference standard for reduction potentials is the hydrogen system with an E'_0 (the reduction potential at pH 7.0) of -0.42 volts or -420 millivolts.

$$2H^+ + 2e^- \rightleftharpoons H_2$$

In this reaction each hydrogen atom provides one proton (H^+) and one electron (e^-).

The reduction potential has a concrete meaning. Redox couples with more negative reduction potentials will donate electrons to couples with more positive potentials and greater affinity for electrons. Thus electrons will tend to move from reductants at the top of the list in **table 8.1** to oxidants at the bottom because they have more positive potentials. This may be expressed visually in the form of an electron tower in which the most negative reduction potentials are at the top (**figure 8.7**). Electrons move from donors to acceptors down the potential gradient or fall down the tower to more positive potentials. Consider the case of the electron carrier **nicotinamide adenine dinucleotide (NAD^+).** The NAD^+/NADH couple has a very negative E'_0 and can therefore give electrons to many acceptors, including O_2.

$$NAD^+ + 2H^+ + 2e^- \rightleftharpoons NADH + H^+ \qquad E'_0 = -0.32 \text{ volts}$$

$$1/2O_2 + 2H^+ + 2e^- \rightleftharpoons H_2O \qquad E'_0 = +0.82 \text{ volts}$$

Because NAD^+/NADH is more negative than 1/2 O_2/H_2O, electrons will flow from NADH (the reductant) to O_2 (the oxidant) as shown in figure 8.7.

$$NADH + H^+ + 1/2O_2 \longrightarrow H_2O + NAD^+$$

When electrons move from a reductant to an acceptor with a more positive redox potential, free energy is released. The $\Delta G^{o\prime}$ of the reaction is directly related to the magnitude of the difference

Table 8.1 Selected Biologically Important Redox Couples

Redox Couple	E'_0 (Volts) [a]
$2H^+ + 2e^- \longrightarrow H_2$	–0.42
Ferredoxin(Fe^{3+}) + $e^- \longrightarrow$ ferredoxin (Fe^{2+})	–0.42
$NAD(P)^+ + H^+ + 2e^- \longrightarrow NAD(P)H$	–0.32
$S + 2H^+ + 2e^- \longrightarrow H_2S$	–0.274
Acetaldehyde + $2H^+ + 2e^- \longrightarrow$ ethanol	–0.197
Pyruvate$^-$ + $2H^+ + 2e^- \longrightarrow$ lactate^{2-}	–0.185
$FAD + 2H^+ + 2e^- \longrightarrow FADH_2$	–0.18[b]
Oxaloacetate^{2-} + $2H^+ + 2e^- \longrightarrow$ malate^{2-}	–0.166
Fumarate^{2-} + $2H^+ + 2e^- \longrightarrow$ succinate^{2-}	0.031
Cytochrome *b* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *b* (Fe^{2+})	0.075
Ubiquinone + $2H^+ + 2e^- \longrightarrow$ ubiquinone H_2	0.10
Cytochrome *c* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *c* (Fe^{2+})	0.254
$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$	0.421
$NO_2^- + 8H^+ + 6e^- \longrightarrow NH_4^+ + 2H_2O$	0.44
$Fe^{3+} + e^- \longrightarrow Fe^{2+}$	0.771
$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$	0.815

[a] E'_0 is the standard reduction potential at pH 7.0.

[b] The value for $FAD/FADH_2$ applies to the free cofactor because it can vary considerably when bound to an apoenzyme.

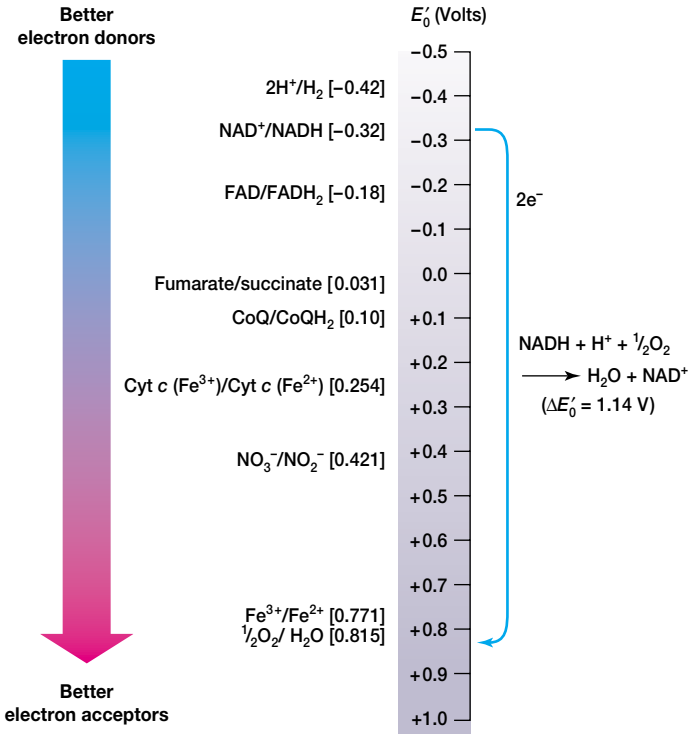


Figure 8.7 **Electron Movement and Reduction Potentials.** The vertical electron tower in this illustration has the most negative reduction potentials at the top. Electrons will spontaneously move from donors higher on the tower (more negative potentials) to acceptors lower on the tower (more positive potentials). That is, the reductant is always higher on the tower than the oxidant. For example, NADH will donate electrons to oxygen and form water in the process. Some typical donors and acceptors are shown on the left, and their redox potentials are given in brackets.

between the reduction potentials of the two couples ($\Delta E'_0$). The larger the $\Delta E'_0$, the greater the amount of free energy made available, as is evident from the equation

$$\Delta G^{o'} = -nF \cdot \Delta E'_0$$

in which n is the number of electrons transferred and F is the Faraday constant (23,062 cal/mole-volt or 96,494 J/mole-volt). For every 0.1 volt change in $\Delta E'_0$, there is a corresponding 4.6 kcal change in $\Delta G^{o'}$ when a two-electron transfer takes place. This is similar to the relationship of $\Delta G^{o'}$ and K_{eq} in other chemical reactions—the larger the equilibrium constant, the greater the $\Delta G^{o'}$. The difference in reduction potentials between NAD^+/NADH and $1/2O_2/H_2O$ is 1.14 volts, a large $\Delta E'_0$ value. When electrons move from NADH to O_2 during aerobic respiration, a large amount of free energy is made available to synthesize ATP (**figure 8.8**). If energy is released when electrons flow from negative to positive reduction potentials, then an input of energy is required to move electrons in the opposite direction, from more positive to more negative potentials. This is precisely what occurs during photosynthesis (figure 8.8). Light energy is trapped and used to move electrons from water to the electron carrier **nicotinamide adenine dinucleotide phosphate ($NADP^+$).**

The cycle of energy flow discussed earlier and illustrated in figure 8.1 can be understood from a different perspective, if the preceding concept is kept in mind. Photosynthetic organisms capture light energy and use it to move electrons from water (and other electron donors such as H_2S) to electron acceptors, such as

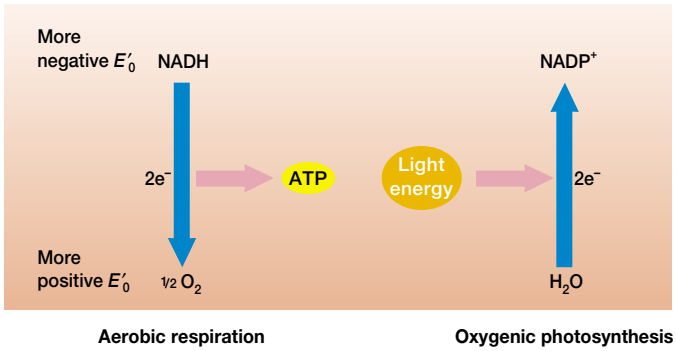
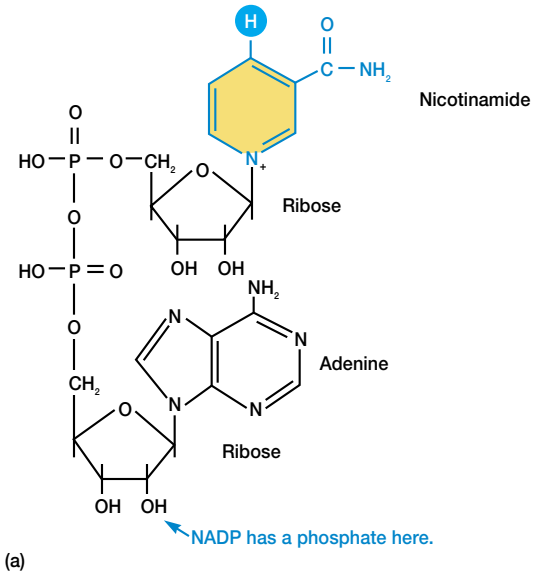


Figure 8.8 **Energy Flow in Metabolism.** Examples of the relationship between electron flow and energy in metabolism. Oxygen and $NADP^+$ serve as electron acceptors for NADH and water, respectively.



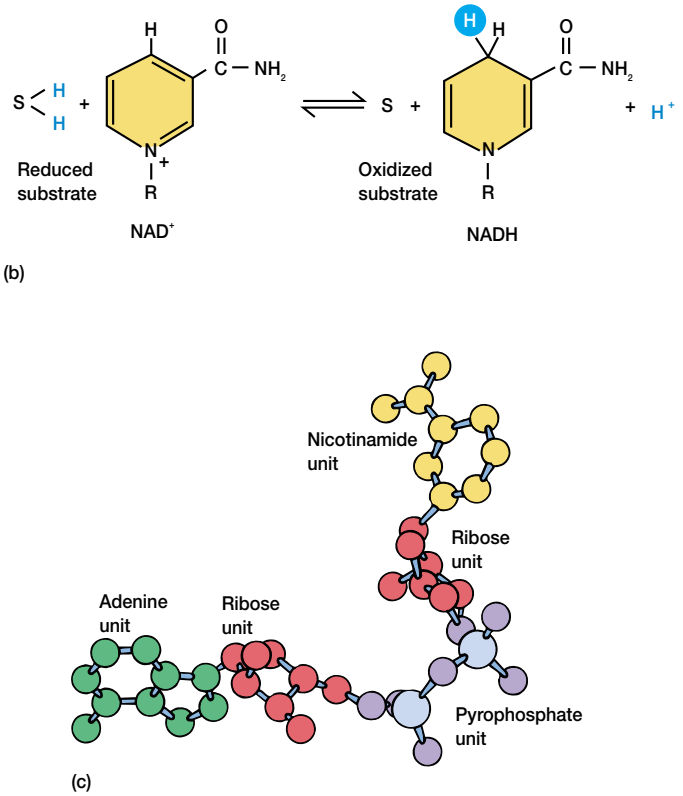


Figure 8.9 The Structure and Function of NAD. (**a**) The structure of NAD and NADP. NADP differs from NAD in having an extra phosphate on one of its ribose sugar units. (**b**) NAD can accept electrons and a hydrogen from a reduced substrate (SH_2). These are carried on the nicotinamide ring. (**c**) Model of NAD^+ when bound to the enzyme lactate dehydrogenase.

$NADP^+$, that have more negative reduction potentials. These electrons can then flow back to more positive acceptors and provide energy for ATP production during photosynthesis. Photoautotrophs use ATP and NADPH to synthesize complex molecules from CO_2 (*see section 10.2*). Chemoheterotrophs also make use of energy released during the movement of electrons by oxidizing complex nutrients during respiration to produce NADH. NADH subsequently donates its electrons to O_2, and the energy released during electron transfer is trapped in the form of ATP. The energy from sunlight is made available to all living organisms because of this relationship between electron flow and energy. Photosynthesis (pp. 195–201); Respiration and electron transport (pp. 184–89)

Electron transport is important in aerobic respiration, anaerobic respiration, chemolithotrophy, and photosynthesis. Electron movement in cells requires the participation of carriers such as NAD^+ and $NADP^+$, both of which can transport electrons between different locations. The nicotinamide ring of NAD^+ and $NADP^+$ (**figure 8.9**) accepts two electrons and one proton from a donor, while a second proton is released. There are several other electron carriers of importance in microbial metabolism (table 8.1), and they carry electrons in a variety of ways. **Flavin adenine dinucleotide (FAD)** and **flavin mononucleotide (FMN)** bear two electrons and two protons on the complex ring system shown in **figure 8.10.** Proteins bearing FAD and FMN are often called flavoproteins. **Coenzyme Q (CoQ)** or **ubiquinone** is a quinone that transports electrons and protons in many respiratory electron transport chains (**figure 8.11**). **Cytochromes** and several other carriers use iron atoms to transport electrons by reversible oxidation and reduction reactions.

$$Fe^{3+}\ (\text{ferric iron}) + e^- \rightleftharpoons Fe^{2+}\ (\text{ferrous iron})$$

In the cytochromes these iron atoms are part of a heme group (**figure 8.12**) or other similar iron-porphyrin rings. Several different cytochromes, each of which consists of a protein and an iron-porphyrin ring, are a prominent part of respiratory electron transport chains. Some iron containing electron-carrying proteins lack a heme group and are called **nonheme iron proteins. Ferredoxin** is a nonheme iron protein active in photosynthetic electron transport and several other electron transport processes. Even though its iron atoms are not bound to a heme group, they still undergo reversible oxidation and reduction reactions. Although all the previously discussed molecules function in electron transport chains, some bear two electrons (NAD, FAD, and CoQ) while others carry only one electron at a time (cytochromes and nonheme iron proteins). This difference in the number of electrons carried is of great importance in the operation of electron transport chains (*see pp. 184–87*).

1. Why is ATP called a high-energy molecule? What is its role in the cell and how does it fulfill this role?
2. Write a generalized equation for a redox reaction. Define reductant, oxidant, and standard reduction potential.
3. How is the direction of electron flow between redox couples related to the standard reduction potential and the release of free energy? Name and briefly describe the major electron carriers found in cells.

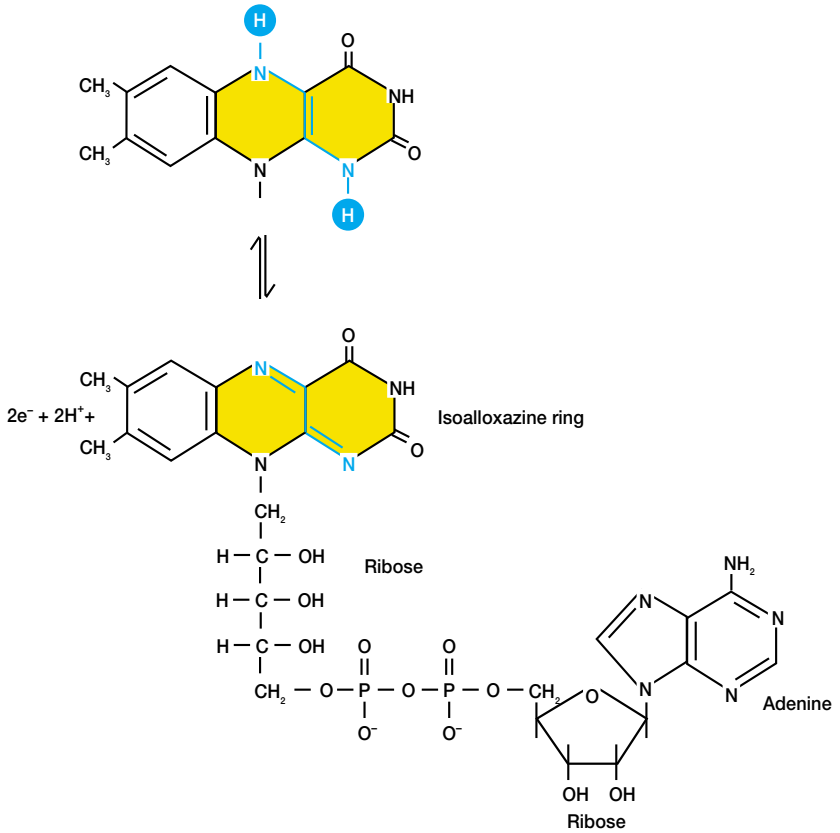


Figure 8.10 The Structure and Function of FAD. The vitamin riboflavin is composed of the isoalloxazine ring and its attached ribose sugar. FMN is riboflavin phosphate. The portion of the ring directly involved in oxidation-reduction reactions is in color.

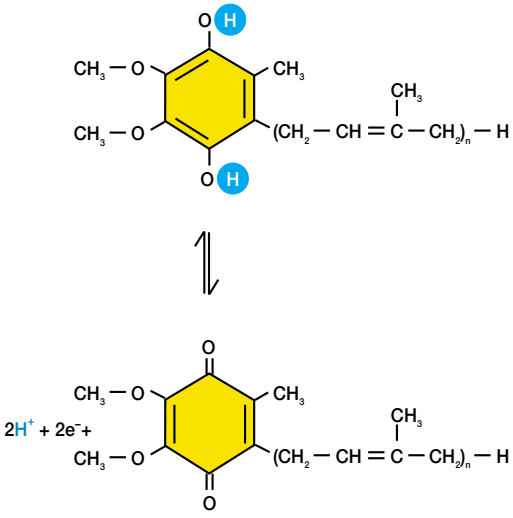


Figure 8.11 The Structure and Function of Coenzyme Q or Ubiquinone. The length of the side chain varies among organisms from n = 6 to n = 10.

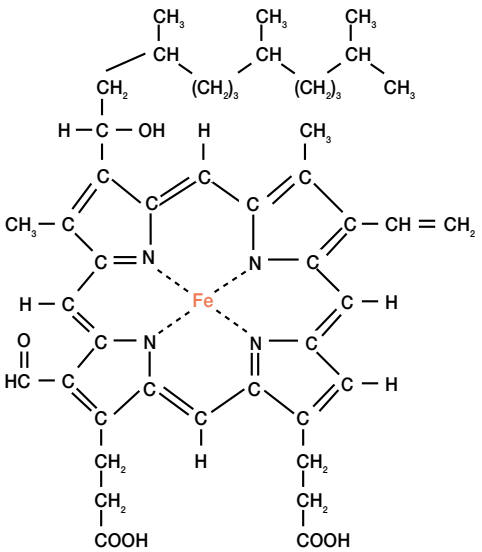
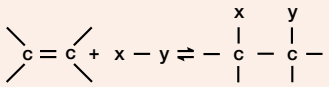


Figure 8.12 The Structure of Heme. Heme is composed of a porphyrin ring and an attached iron atom. It is the nonprotein component of many cytochromes. The iron atom alternatively accepts and releases an electron.

Table 8.2 Enzyme Classification

Type of Enzyme	Reaction Catalyzed by Enzyme	Example of Reaction
Oxidoreductase	Oxidation-reduction reactions	Lactate dehydrogenase: Pyruvate + NADH + H^+ $\rightleftharpoons$ lactate + NAD^+
Transferase	Reactions involving the transfer of groups between molecules	Aspartate carbamoyltransferase: Aspartate + carbamoylphosphate $\rightleftharpoons$ carbamoylaspartate + phosphate
Hydrolase	Hydrolysis of molecules	Glucose-6-phosphatase: Glucose 6-phosphate + H_2O $\rightarrow$ glucose + P_i
Lyase	Removal of groups to form double bonds or addition of groups to double bonds 	Fumarate hydratase: L-malate $\rightleftharpoons$ fumarate + H_2O
Isomerase	Reactions involving isomerizations	Alanine racemase: L-alanine $\rightleftharpoons$ D-alanine
Ligase	Joining of two molecules using ATP energy (or that of other nucleoside triphosphates)	Glutamine synthetase: Glutamate + NH_3 + ATP $\rightarrow$ glutamine + ADP + P_i

8.6 Enzymes

Recall that an exergonic reaction is one with a negative $\Delta G^{o\prime}$ and an equilibrium constant greater than one. An exergonic reaction will proceed to completion in the direction written (that is, toward the right of the equation). Nevertheless, one often can combine the reactants for an exergonic reaction with no obvious result, even though products should be formed. It is precisely in these reactions that enzymes play their part.

Structure and Classification of Enzymes

Enzymes may be defined as protein catalysts that have great specificity for the reaction catalyzed and the molecules acted on. A **catalyst** is a substance that increases the rate of a chemical reaction without being permanently altered itself. Thus enzymes speed up cellular reactions. The reacting molecules are called **substrates,** and the substances formed are the **products.** Protein structure and properties (appendix I)

Many enzymes are indeed pure proteins. However, many enzymes consist of a protein, the **apoenzyme,** and also a nonprotein component, a **cofactor,** required for catalytic activity. The complete enzyme consisting of the apoenzyme and its cofactor is called the **holoenzyme.** If the cofactor is firmly attached to the apoenzyme it is a **prosthetic group.** Often the cofactor is loosely attached to the apoenzyme. It can even dissociate from the enzyme protein after products have been formed and carry one of these products to another enzyme (**figure 8.13**). Such a loosely bound cofactor is called a **coenzyme.** For example, NAD^+ is a coenzyme that carries electrons within the cell. Many vitamins that humans require serve as coenzymes or as their precursors. Niacin is incorporated into NAD^+ and riboflavin into FAD. Metal ions may also be bound to apoenzymes and act as cofactors.

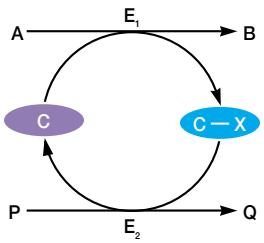


Figure 8.13 **Coenzymes as Carriers.** The function of a coenzyme in carrying substances around the cell. Coenzyme C participates with enzyme E_1 in the conversion of A to product B. During the reaction, it acquires X from the substrate A. The coenzyme can donate X to substrate P in a second reaction. This will convert it back to its original form, ready to accept another X. The coenzyme is not only participating in both reactions, but is also transporting X to various points in the cell.

Despite the large number and bewildering diversity of enzymes present in cells, they may be placed in one of six general classes (**table 8.2**). Enzymes usually are named in terms of the substrates they act on and the type of reaction catalyzed. For example, lactate dehydrogenase (LDH) removes hydrogens from lactate.

$$\text{Lactate} + NAD^+ \overset{\text{LDH}}{\rightleftharpoons} \text{pyruvate} + NADH + H^+$$

Lactate dehydrogenase can also be given a more complete and detailed name, L-lactate: NAD oxidoreductase. This name describes the substrates and reaction type with even more precision.

The Mechanism of Enzyme Reactions

It is important to keep in mind that enzymes increase the rates of reactions but do not alter their equilibrium constants. If a reaction is endergonic, the presence of an enzyme will not shift its equilibrium

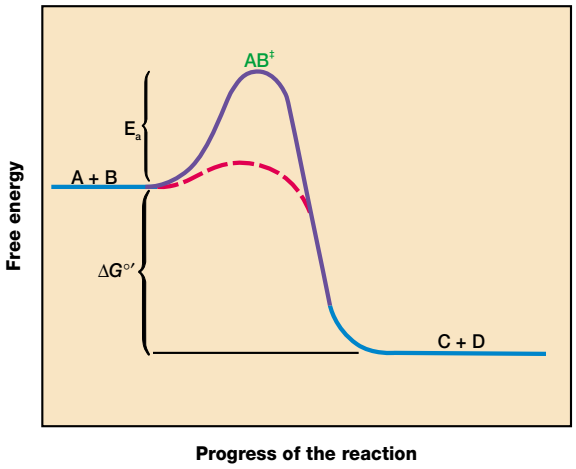


Figure 8.14 Enzymes Lower the Energy of Activation. This figure traces the course of a chemical reaction in which A and B are converted to C and D. The transition-state complex is represented by $AB^{\ddagger}$, and the activation energy required to reach it, by E_a. The colored line represents the course of the reaction in the presence of an enzyme. Note that the activation energy is much lower in the enzyme-catalyzed reaction.

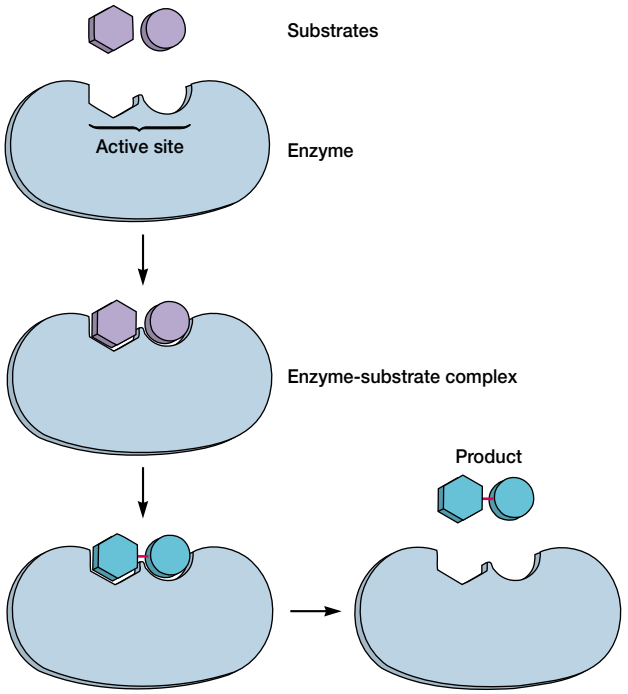


Figure 8.15 Enzyme Function. The formation of the enzyme-substrate complex and its conversion to products is shown.

so that more products can be formed. Enzymes simply speed up the rate at which a reaction proceeds toward its final equilibrium.

How do enzymes catalyze reactions? Although a complete answer would be long and complex, some understanding of the mechanism can be gained by considering the course of a normal exergonic chemical reaction.

$$A + B \rightleftharpoons C + D$$

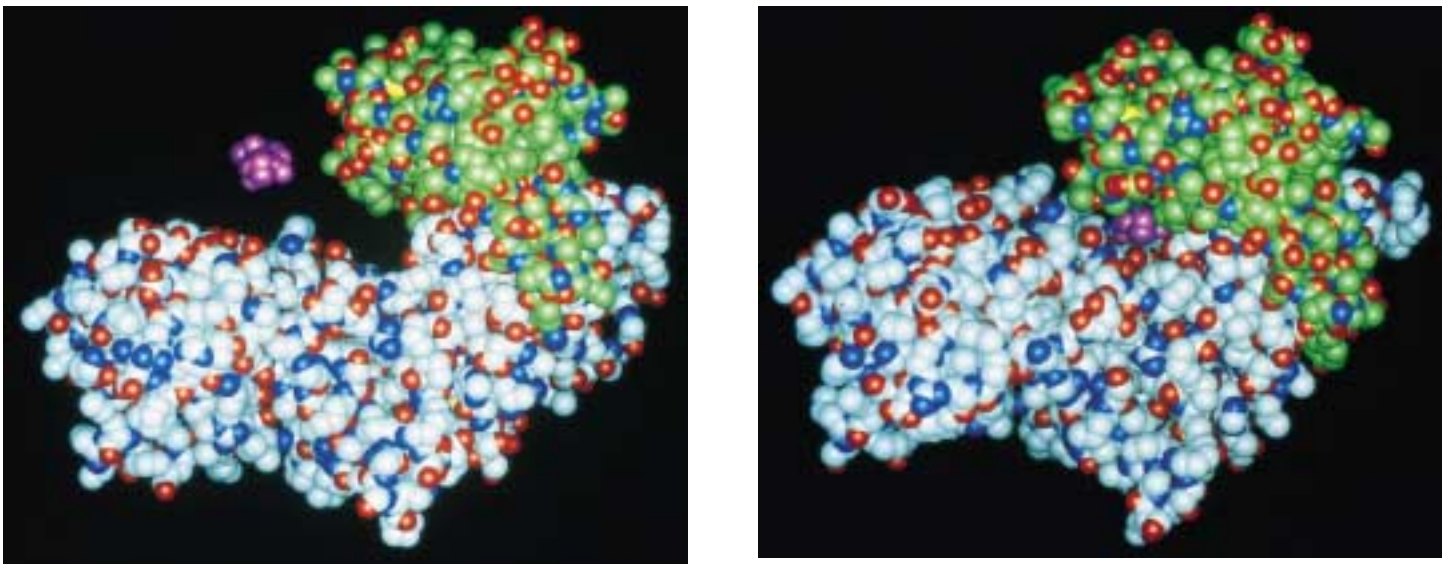
When molecules A and B approach each other to react, they form a **transition-state complex,** which resembles both the substrates and the products (**figure 8.14**). The **activation energy** is required to bring the reacting molecules together in the correct way to reach the transition state. The transition-state complex can then decompose to yield the products C and D. The difference in free energy level between reactants and products is $\Delta G^{o\prime}$. Thus the equilibrium in our example will lie toward the products because $\Delta G^{o\prime}$ is negative (i.e., the products are at a lower energy level than the substrates).

Clearly A and B will not be converted to C and D in figure 8.14 if they are not supplied with an amount of energy equivalent to the activation energy. Enzymes accelerate reactions by lowering the activation energy; therefore more substrate molecules will have sufficient energy to come together and form products. Even though the equilibrium constant (or $\Delta G^{o\prime}$) is unchanged, equilibrium will be reached more rapidly in the presence of an enzyme because of this decrease in the activation energy.

Researchers have expended much effort in discovering how enzymes lower the activation energy of reactions, and the process is becoming clearer. Enzymes bring substrates together at a special place on their surface called the **active site** or **catalytic site** to form an **enzyme-substrate complex** (**figures 8.15, 8.16;** *see also AI.19*). The enzyme can interact with a substrate in two general ways. It may be rigid and shaped to precisely fit the substrate so that the correct substrate binds specifically and is positioned properly for reaction. This mechanism is referred to as the lock-and-key model. An enzyme also may change shape when it binds the substrate so that the active site surrounds and precisely fits the substrate. This has been called the induced fit model and is used by hexokinase and many other enzymes (figure 8.16). The formation of an enzyme-substrate complex can lower the activation energy in many ways. For example, by bringing the substrates together at the active site, the enzyme is, in effect, concentrating them and speeding up the reaction. An enzyme does not simply concentrate its substrates, however. It also binds them so that they are correctly oriented with respect to each other in order to form a transition-state complex. Such an orientation lowers the amount of energy that the substrates require to reach the transition state. These and other catalytic site activities speed up a reaction hundreds of thousands of times, even though microorganisms grow between −20°C and approximately 113°C. These temperatures are not high enough to favor most organic reactions in the absence of enzyme catalysis, yet cells cannot survive at the high temperatures used by an organic chemist in routine organic syntheses. Enzymes make life possible by accelerating specific reactions at low temperatures.

The Effect of Environment on Enzyme Activity

Enzyme activity varies greatly with changes in environmental factors, one of the most important being the substrate concentration. As will be emphasized later, substrate concentrations are

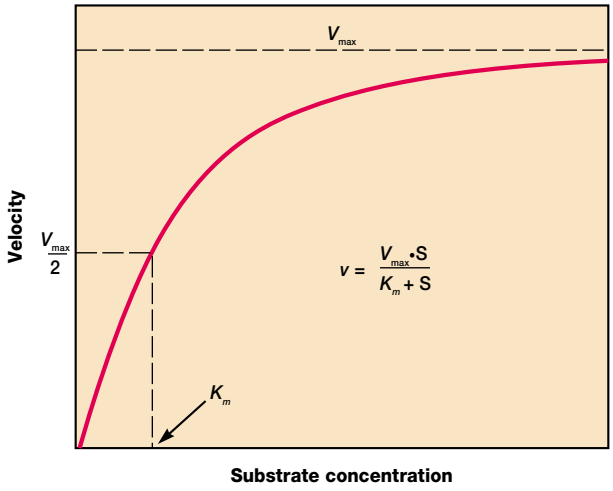


(a) (b)

Figure 8.16 An Example of Enzyme-Substrate Complex Formation. (**a**) A space-filling model of yeast hexokinase and its substrate glucose (purple). The active site is in the cleft formed by the enzyme's small lobe (green) and large lobe (gray). (**b**) When glucose binds to form the enzyme-substrate complex, hexokinase changes shape and surrounds the substrate.

usually low within cells. At very low substrate concentrations, an enzyme makes product slowly because it seldom contacts a substrate molecule. If more substrate molecules are present, an enzyme binds substrate more often, and the reaction velocity (usually expressed in terms of the rate of product formation) is greater than at a lower substrate concentration. Thus the rate of an enzyme-catalyzed reaction increases with substrate concentration (**figure 8.17**). Eventually further increases in substrate concentration do not result in a greater reaction velocity because the available enzyme molecules are binding substrate and converting it to product as rapidly as possible. That is, the enzyme is saturated with substrate and operating at maximal velocity (V_{max}). The resulting substrate concentration curve is a hyperbola (figure 8.17). It is useful to know the substrate concentration an enzyme needs to function adequately. Usually the **Michaelis constant (K_m),** the substrate concentration required for the enzyme to achieve half maximal velocity, is used as a measure of the apparent affinity of an enzyme for its substrate. The lower the K_m value, the lower the substrate concentration at which an enzyme catalyzes its reaction.

Enzymes also change activity with alterations in pH and temperature (**figure 8.18**). Each enzyme functions most rapidly at a specific pH optimum. When the pH deviates too greatly from an enzyme's optimum, activity slows and the enzyme may be damaged. Enzymes likewise have temperature optima for maximum activity. If the temperature rises too much above the optimum, an enzyme's structure will be disrupted and its activity lost. This phenomenon, known as **denaturation,** may be caused by



K_m = the substrate concentration required by the enzyme to operate at half its maximum velocity

V_{max} = the rate of product formation when the enzyme is saturated with substrate and operating as fast as possible

Figure 8.17 Michaelis-Menten Kinetics. The dependence of enzyme activity upon substrate concentration. This substrate curve fits the Michaelis-Menten equation given in the figure, which relates reaction velocity (v) to the substrate concentration (S) using the maximum velocity and the Michaelis constant (K_m).

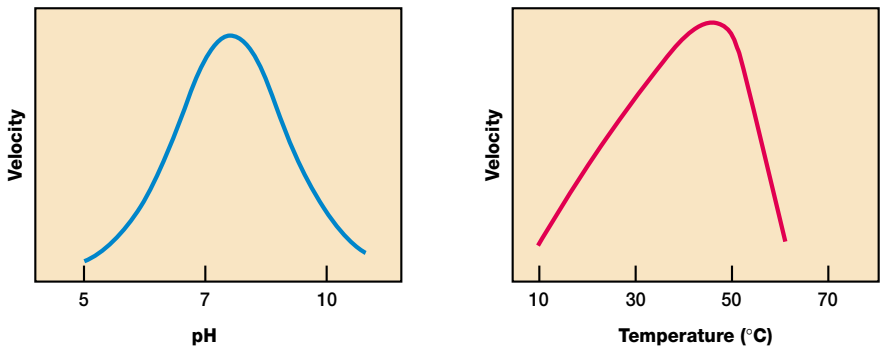


Figure 8.18 pH, Temperature, and Enzyme Activity. The variation of enzyme activity with changes in pH and temperature. The ranges in pH and temperature are only representative. Enzymes differ from one another with respect to the location of their optima and the shape of their pH and temperature curves.

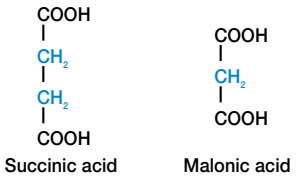


Figure 8.19 Competitive Inhibition of Succinate Dehydrogenase. A comparison of succinic acid and the competitive inhibitor, malonic acid. The colored atoms indicate the parts of the two molecules that differ.

extremes of pH and temperature or by other factors. The pH and temperature optima of a microorganism's enzymes often reflect the pH and temperature of its habitat. Not surprisingly bacteria growing best at high temperatures often have enzymes with high temperature optima and great heat stability. Temperature and growth (pp. 125–27); Heat stable enzymes in biotechnology (p. 626)

Enzyme Inhibition

Microorganisms can be poisoned by a variety of chemicals, and many of the most potent poisons are enzyme inhibitors. A **competitive inhibitor** directly competes with the substrate at an enzyme's catalytic site and prevents the enzyme from forming product. A classic example of this behavior is seen with the enzyme succinate dehydrogenase, which catalyzes the oxidation of succinate to fumarate in the tricarboxylic acid cycle (*see section 9.4*). Malonic acid is an effective competitive inhibitor of succinate dehydrogenase because it so closely resembles succinate, the normal substrate (**figure 8.19**). After malonate binds to the enzyme, it cannot be oxidized and the formation of fumarate is blocked. Competitive inhibitors usually resemble normal substrates, but they cannot be converted to products.

Competitive inhibitors are important in the treatment of many microbial diseases. Sulfa drugs like sulfanilamide resemble *p*-aminobenzoate, a molecule used in the formation of the coenzyme folic acid. The drugs compete with *p*-aminobenzoate for the catalytic site of an enzyme involved in folic acid synthesis. This blocks the production of folic acid and inhibits bacterial growth (*see section 35.6*). Humans are not harmed because they cannot synthesize folic acid and must obtain it in their diet. Destruction of microorganisms by physical and chemical agents (chapter 7)

Inhibitors also can affect enzyme activity by binding to the enzyme at some location other than at the active site. This alters the enzyme's shape, rendering it inactive or less active. These inhibitors are often called **noncompetitive** because they do not directly compete with the substrate. Heavy metal poisons like mercury frequently are noncompetitive inhibitors of enzymes.

1. What is an enzyme and how does it speed up reactions? How are enzymes named? Define apoenzyme, holoenzyme, cofactor, coenzyme, prosthetic group, active or catalytic site, and activation energy.
2. How does enzyme activity change with substrate concentration, pH, and temperature? Define the terms Michaelis constant, maximum velocity, and denaturation.
3. What are competitive and noncompetitive inhibitors and how do they inhibit enzymes?

8.7 The Nature and Significance of Metabolic Regulation

The task of the regulatory machinery is exceptionally complex and difficult. Pathways must be regulated and coordinated so effectively that all cell components are present in precisely the correct amounts. Furthermore, a microbial cell must be able to respond effectively to environmental changes by using those nutrients present at the moment and by switching on new catabolic pathways when different nutrients become available. Because all chemical components of a cell usually are not present in the surroundings, microorganisms also must synthesize unavailable components and alter biosynthetic activity in response to changes in nutrient availability. The chemical composition of a cell's surroundings is constantly changing, and these regulatory processes are dynamic and continuously responding to altered conditions.

Regulation is essential for the cell to conserve microbial energy and material and to maintain metabolic balance. If a particular energy source is unavailable, the enzymes required for its use are not needed and their further synthesis is a waste of carbon, nitrogen, and energy. Similarly it would be extremely wasteful for a microorganism to synthesize the enzymes required to manufacture a certain end product if that end product were already pres-

ent in adequate amounts. Thus both catabolism and anabolism are regulated in such a way as to maximize efficiency of operation. Catabolism and anabolism (p. 173)

The drive to maintain balance and conserve energy and material is evident in the regulatory responses of a bacterium like *E. coli.* If the bacterium is grown in a very simple medium containing only glucose as a carbon and energy source, it will synthesize the required cell components in balanced amounts. Addition of a biosynthetic end product (the amino acid tryptophan, for example) to the medium will result in the immediate inhibition of the pathway synthesizing that end product; synthesis of the pathway's enzymes also will slow or cease. If *E. coli* is transferred to medium containing only the sugar lactose, it will synthesize the enzymes required for catabolism of this nutrient. In contrast, when *E. coli* grows in a medium possessing both glucose and lactose, glucose (the sugar supporting most rapid growth) is catabolized first. The culture will use lactose only after the glucose supply has been exhausted.

The flow of carbon through a pathway may be regulated in three major ways.

1. The localization of metabolites and enzymes in different parts of a cell, a phenomenon called **metabolic channeling,** influences pathway activity.
2. Critical enzymes often are directly stimulated or inhibited to alter pathway activity rapidly.
3. The number of enzyme molecules also may be controlled. The more catalyst molecules present, the greater the pathway's activity. In bacteria regulation is usually exerted at the level of transcription. Control of mRNA synthesis is slower than direct regulation of enzyme activity but does result in the saving of much energy and raw material because enzymes are not synthesized when not required.

Each of these mechanisms is described in detail. This chapter introduces the first two: metabolic channeling and direct control of enzyme activity. The discussion of gene expression regulation follows a description of DNA, RNA, and protein synthesis in chapters 11 and 12. Regulation of gene expression (pp. 275–83)

8.8 Metabolic Channeling

One of the most common channeling mechanisms is that of **compartmentation,** the differential distribution of enzymes and metabolites among separate cell structures or organelles. Compartmentation is particularly important in eucaryotic microorganisms with their many membrane-bound organelles. For example, fatty acid oxidation is located within the mitochondrion, whereas fatty acid synthesis occurs in the cytoplasmic matrix. The periplasm in procaryotes can also be considered an example of compartmentation (*see p. 55*). Compartmentation makes possible the simultaneous, but separate, operation and regulation of similar pathways. Furthermore, pathway activities can be coordinated through regulation of the transport of metabolities and coenzymes between cell compartments. Suppose two pathways in different cell compartments require NAD for activity. The distribution of NAD between the two compartments will then determine the relative activity of these competing pathways, and the pathway with access to the most NAD will be favored.

Channeling also occurs within compartments such as the cytoplasmic matrix. The matrix is a structured dense material with many subcompartments. In eucaryotes it also is subdivided by the endoplasmic reticulum and cytoskeleton (*see chapter 4*). Metabolites and coenzymes do not diffuse rapidly in such an environment, and metabolite gradients will build up near localized enzymes or enzyme systems. This occurs because enzymes at a specific site convert their substrates to products, resulting in decreases in the concentration of one or more metabolites and increases in others. For example, product concentrations will be high near an enzyme and decrease with increasing distance from it.

Channeling can generate marked variations in metabolite concentrations and therefore directly affect enzyme activity. Substrate levels are generally around 10^{-3} moles/liter (M) to 10^{-6} M or even lower. Thus they may be in the same range as enzyme concentrations and equal to or less than the Michaelis constants (K_m) of many enzymes. Under these conditions the concentration of an enzyme's substrate may control its activity because the substrate concentration is in the rising portion of the hyperbolic substrate saturation curve (**figure 8.20**). As the substrate level increases, it is converted to product more rapidly; a decline in substrate concentration automatically leads to lower enzyme activity. If two enzymes in different pathways use the same metabolite, they may directly compete for it. The pathway winning this competition—the one with the enzyme having the lowest K_m value for the metabolite—will operate closer to full capacity. Thus channeling within a cell compartment can regulate and coordinate metabolism through variations in metabolite and coenzyme levels. Enzyme kinetics and the substrate saturation curve (pp. 162–63)

1. Give three ways in which the flow of carbon through a pathway may be regulated.
2. Define the terms metabolic channeling and compartmentation. How are they involved in the regulation of metabolism?

8.9 Control of Enzyme Activity

Adjustment of the activity of regulatory enzymes controls the functioning of many metabolic pathways. This section describes these enzymes and discusses their role in regulating pathway activity.

Allosteric Regulation

Usually regulatory enzymes are **allosteric enzymes.** The activity of an allosteric enzyme is altered by a small molecule known as an **effector** or **modulator.** The effector binds reversibly by noncovalent forces to a **regulatory site** separate from the catalytic site and causes a change in the shape or conformation of the enzyme (**figure 8.21**). The activity of the catalytic site is altered as

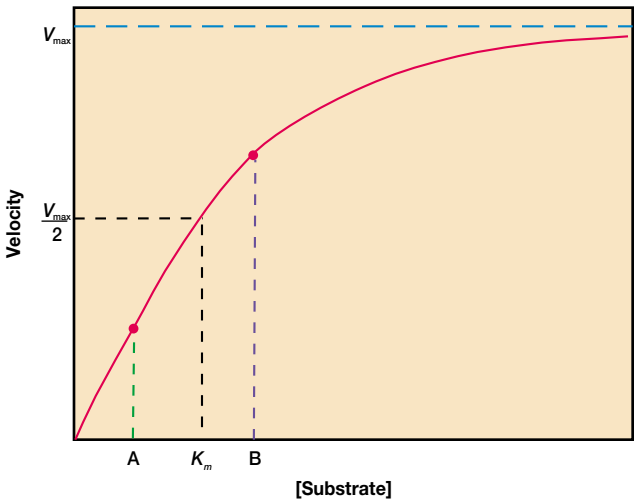


Figure 8.20 Control of Enzyme Activity by Substrate Concentration. An enzyme-substrate saturation curve with the Michaelis constant (K_m) and the velocity equivalent to half the maximum velocity (V_{max}) indicated. The initial velocity of the reaction (v) is plotted against the substrate concentration [Substrate]. The maximum velocity is the greatest velocity attainable with a fixed amount of enzyme under defined conditions. When the substrate concentration is equal to or less than the K_m, the enzyme's activity will vary almost linearly with the substrate concentration. Suppose the substrate increases in concentration from level A to B. Because these concentrations are in the range of the K_m, a significant increase in enzyme activity results. A drop in concentration from B to A will lower the rate of product formation.

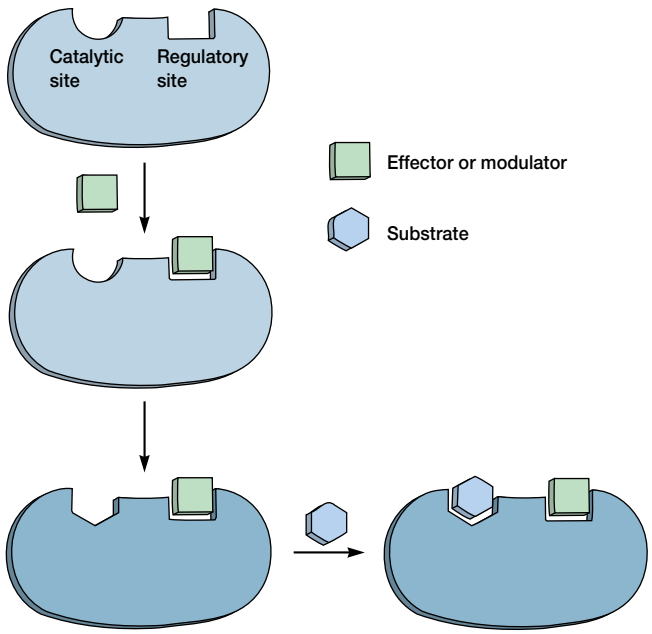


Figure 8.21 Allosteric Regulation. The structure and function of an allosteric enzyme. In this example the effector or modulator first binds to a separate regulatory site and causes a change in enzyme conformation that results in an alteration in the shape of the active site. The active site can now more effectively bind the substrate. This effector is a positive effector because it stimulates substrate binding and catalytic activity.

a result. A positive effector increases enzyme activity, whereas a negative effector decreases activity or inhibits the enzyme. These changes in activity often result from alterations in the apparent affinity of the enzyme for its substrate, but changes in maximum velocity also can occur.

The kinetic characteristics of nonregulatory enzymes show that the Michaelis constant (K_m) is the substrate concentration required for an enzyme to operate at half its maximal velocity. This constant applies only to hyperbolic substrate saturation curves, not to the sigmoidal curves often seen with allosteric enzymes (figure 8.23). The substrate concentration required for half maximal velocity with allosteric enzymes having sigmoidal substrate curves is called the $[S]_{0.5}$ or $K_{0.5}$ value.

One of the best-studied allosteric regulatory enzymes is the aspartate carbamoyltransferase (ACTase) from *E. coli*. The enzyme catalyzes the condensation of carbamoyl phosphate with aspartate to form carbamoylaspartate (**figure 8.22**). ACTase catalyzes the rate-determining reaction of the pyrimidine biosynthetic pathway in *E. coli*. The substrate saturation curve is sigmoidal when the concentration of either substrate is varied (**figure 8.23**). The enzyme has more than one active site, and the binding of a substrate molecule to an active site increases the binding of substrate at the other sites. In addition, cytidine triphosphate (CTP), an end product of pyrimidine biosynthesis, inhibits the enzyme and the purine ATP activates it. Both effectors alter the $K_{0.5}$ value of the enzyme but not its maximum velocity. CTP inhibits by increasing $K_{0.5}$ or by shifting the substrate saturation curve to higher values. This allows the enzyme to operate more slowly at a particular substrate concentration when CTP is present. ATP activates by moving the curve to lower substrate concentration values so that the enzyme is maximally active over a wider substrate concentration range. Thus when the pathway is so active that the CTP concentration rises too high, ACTase activity decreases and the rate of end product formation slows. In contrast, when the purine end product ATP increases in concentration relative to CTP, it stimulates CTP synthesis through its effects on ACTase. Pyrimidine and purine biosynthesis (pp. 216–18)

E. coli aspartate carbamoyltransferase provides a clear example of separate regulatory and catalytic sites in allosteric en-

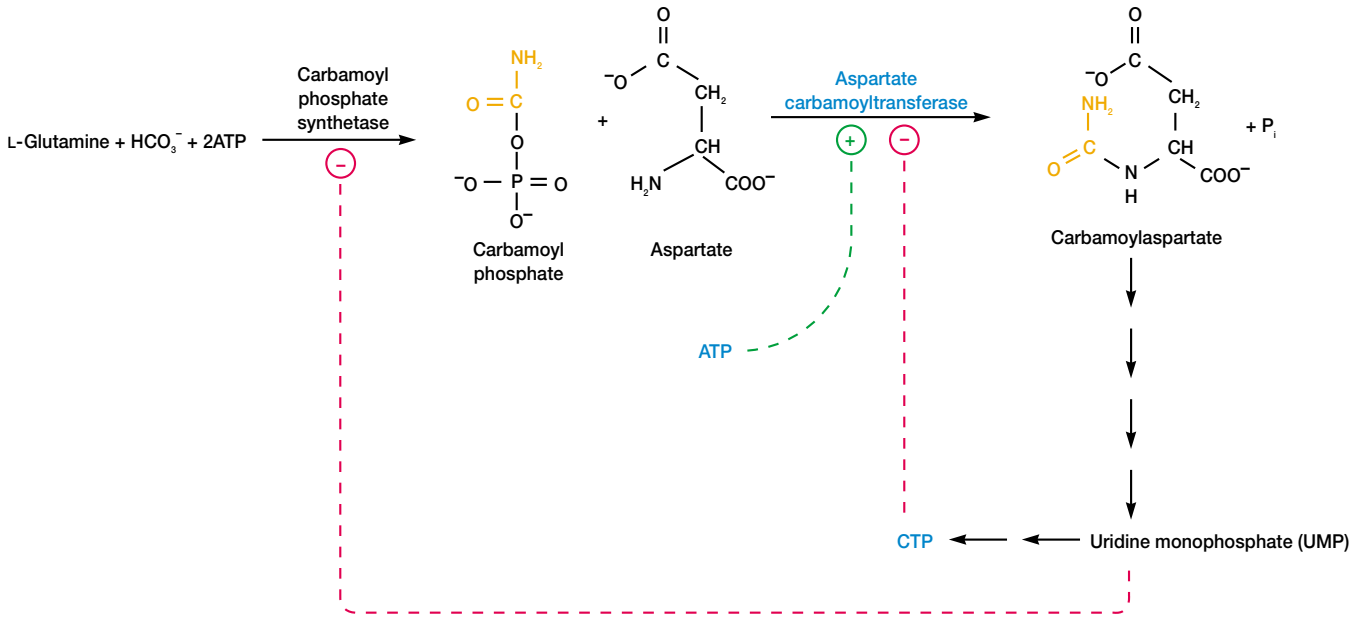


Figure 8.22 ACTase Regulation. The aspartate carbamoyltransferase reaction and its role in the regulation of pyrimidine biosynthesis. The end product CTP inhibits its activity (−) while ATP activates the enzyme (+). Carbamoyl phosphate synthetase is also inhibited by pathway end products such as UMP.

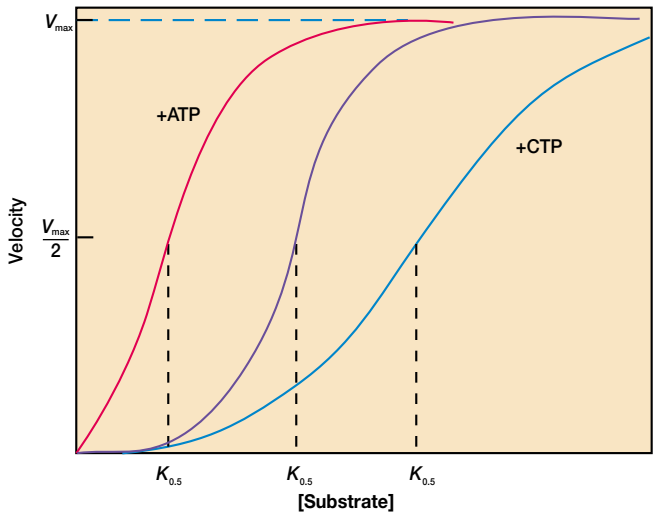


Figure 8.23 The Kinetics of *E. coli* Aspartate Carbamoyltransferase. CTP, a negative effector, increases the $K_{0.5}$ value while ATP, a positive effector, lowers the $K_{0.5}$. The V_{max} remains constant.

zymes. The enzyme is a large protein composed of two catalytic subunits and three regulatory subunits (**figure 8.24*a***). The catalytic subunits contain only catalytic sites and are unaffected by CTP and ATP. Regulatory subunits do not catalyze the reaction but do possess regulatory sites to which CTP and ATP bind. When these effectors bind to the complete enzyme, they cause conformational changes in the regulatory subunits and subsequently in the catalytic subunits and their catalytic sites. The enzyme can change reversibly between a less active T form and a more active R form (figure 8.24*b,c*). Thus the regulatory site influences a catalytic site about 6.0 nm distant.

Covalent Modification of Enzymes

Regulatory enzymes also can be switched on and off by **reversible covalent modification.** Usually this occurs through the addition and removal of a particular group, one form of the enzyme being more active than the other. For example, glycogen phosphorylase of the bread mold *Neurospora crassa* exists in phosphorylated and dephosphorylated forms called phosphorylase *a* and phosphorylase *b,* respectively (**figure 8.25**). Phosphorylase *b* is inactive

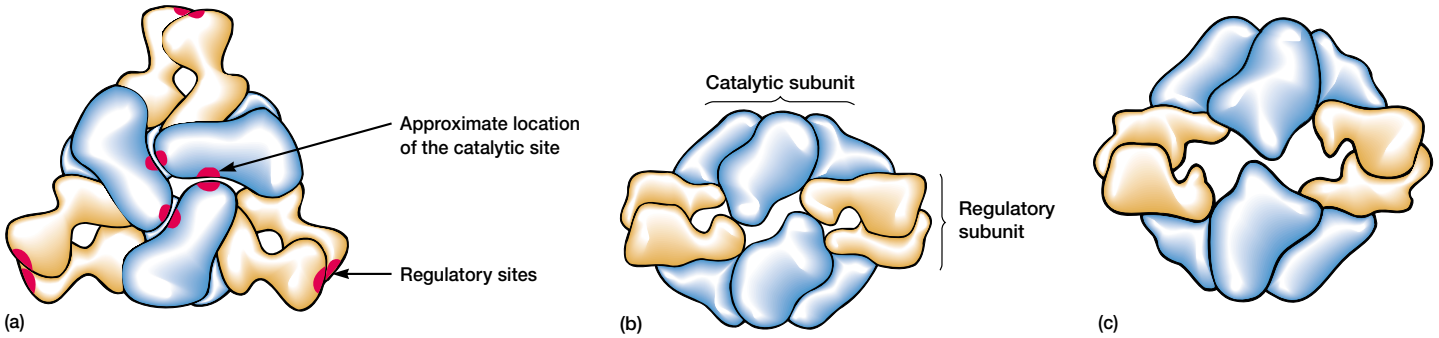


Figure 8.24 The Structure and Regulation of *E. coli* Aspartate Carbamoyltransferase. (**a**) A schematic diagram of the enzyme showing the six catalytic polypeptide chains (blue), the six regulatory chains (orange), and the catalytic and regulatory sites. The enzyme is viewed from the top. Each catalytic subunit contains three catalytic chains, and each regulatory subunit has two chains. (**b**) The less active T state of ACTase viewed from the side. (**c**) The more active R state of ACTase. The regulatory subunits have rotated and pushed the catalytic subunits apart.

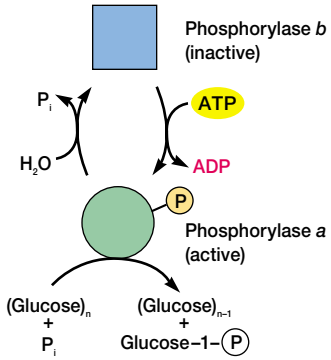
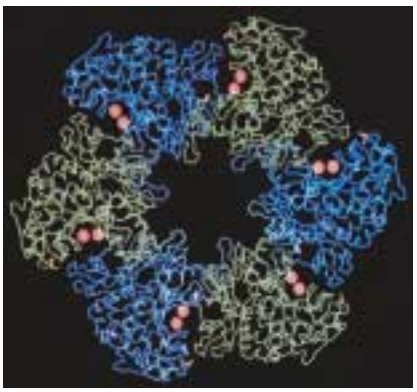


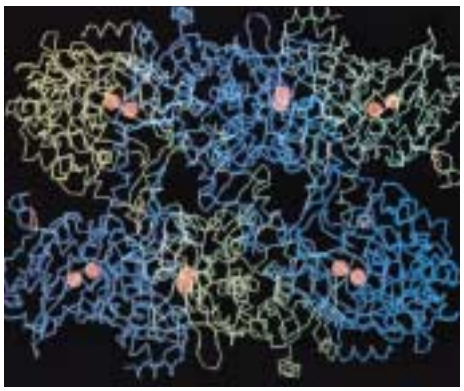
Figure 8.25 Reversible Covalent Modification of Glycogen Phosphorylase. The active form, phosphorylase *a,* is produced by phosphorylation and is inactivated when the phosphate is removed hydrolytically to produce inactive phosphorylase *b.*

because its required activator AMP is usually not present at sufficiently high levels. Phosphorylase *a,* the phosphorylated form, is active even without AMP. Glycogen phosphorylase is stimulated by phosphorylation of phosphorylase *b* to produce phosphorylase *a.* The attachment of phosphate changes the enzyme's conformation to an active form. Phosphorylation and dephosphorylation are catalyzed by separate enzymes, which also are regulated. Phosphorylase and glycogen degradation (p. 192)

Enzymes can be regulated through the attachment of groups other than phosphate. One of the most intensively studied regulatory enzymes is *E. coli* glutamine synthetase, a large, complex enzyme existing in two forms (**figure 8.26**). When an adenylic acid residue is attached to each of its 12 subunits forming an adenyly-



(a)



(b)

Figure 8.26 The Structure of *E. coli* Glutamine Synthetase. The enzyme contains 12 subunits in the shape of a hexagonal prism. For clarity the subunits are colored alternating green and blue. Each of the six catalytic sites has a pair of Mn^{2+} ions (red). The tyrosine residues to which adenyl groups can be attached are colored red. (**a**) Top view of molecule. (**b**) Side view showing the six nearest subunits.

lated enzyme, glutamine synthetase is not very active. Removal of AMP groups produces more active deadenylylated glutamine synthetase, and glutamine is formed. The glutamine synthetase system differs from the phosphorylase system in two ways: (1) AMP is used as the modifying agent, and (2) the modified form of glutamine synthetase is less active. Glutamine synthetase also is allosterically regulated. Glutamine synthetase and its role in nitrogen metabolism (pp. 211–12)

There are some advantages to using covalent modification for the regulation of enzyme activity. These interconvertible enzymes often are also allosteric. Because each form can respond differently to allosteric effectors, systems of covalently modified enzymes are able to respond to more stimuli in varied and sophisticated ways. Regulation can also be exerted on the enzymes that catalyze the covalent modifications, which adds a second level of regulation to the system.

Feedback Inhibition

The rate of many metabolic pathways is adjusted through control of the activity of the regulatory enzymes described in the preceding section. Every pathway has at least one **pacemaker enzyme** that catalyzes the slowest or rate-limiting reaction in the pathway. Because other reactions proceed more rapidly than the pacemaker reaction, changes in the activity of this enzyme directly alter the speed with which a pathway operates. Usually the first step in a pathway is a pacemaker reaction catalyzed by a regulatory enzyme. The end product of the pathway often inhibits this regulatory enzyme, a process known as **feedback inhibition** or **end product inhibition.** Feedback inhibition ensures balanced production of a pathway end product. If the end product becomes too concentrated, it inhibits the regulatory enzyme and slows its own synthesis. As the end product concentration decreases, pathway activity again increases and more product is formed. In this way feedback inhibition automatically matches end product supply with the demand. The previously discussed *E. coli* aspartate carbamoyltransferase is an excellent example of end product or feedback inhibition.

Frequently a biosynthetic pathway branches to form more than one end product. In such a situation the synthesis of pathway end products must be coordinated precisely. It would not do to have one end product present in excess while another is lacking. Branching biosynthetic pathways usually achieve a balance between end products through the use of regulatory enzymes at branch points (**figure 8.27**). If an end product is present in excess, it often inhibits the branch-point enzyme on the sequence leading to its formation, in this way regulating its own formation without affecting the synthesis of other products. In figure 8.27 notice that both products also inhibit the initial enzyme in the pathway. An excess of one product slows the flow of carbon into the whole pathway while inhibiting the appropriate branch-point enzyme. Because less carbon is required when a branch is not functioning, feedback inhibition of the initial pacemaker enzyme helps match the supply with the demand in branching pathways. The regulation of multiple branched pathways is often made even more sophisticated by the presence of **isoenzymes,** different enzymes that catalyze the same reaction. The initial pacemaker step may be catalyzed by several isoenzymes, each under separate and independent control. In such a situation an excess of a single end product reduces pathway activity but does not completely block pathway function because some isoenzymes are still active.

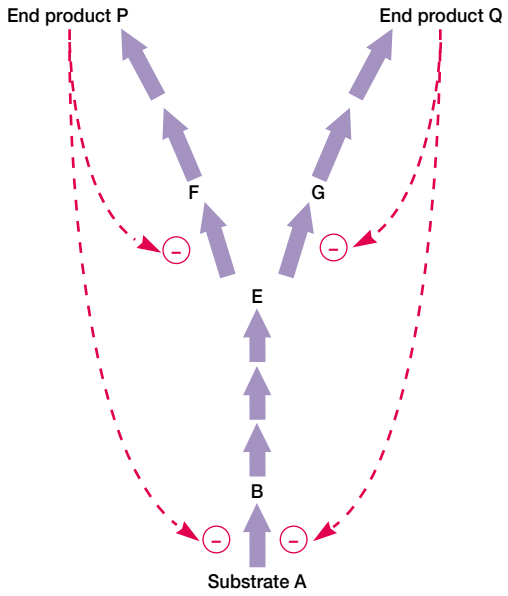


Figure 8.27 Feedback Inhibition. Feedback inhibition in a branching pathway with two end products. The branch-point enzymes, those catalyzing the conversion of intermediate E to F and G, are regulated by feedback inhibition. Products P and Q also inhibit the initial reaction in the pathway. A colored line with a minus sign at one end indicates that an end product, P or Q, is inhibiting the enzyme catalyzing the step next to the minus. See text for further explanation.

1. Define the following: allosteric enzyme, effector or modulator, and $[S]_{0.5}$ or $K_{0.5}$.
2. How can regulatory enzymes be influenced by reversible covalent modification? What groups are used for this purpose with glycogen phosphorylase and glutamine synthetase, and which forms of these enzymes are active?
3. What is a pacemaker enzyme? Feedback inhibition? How does feedback inhibition automatically adjust the concentration of a pathway end product? What are isoenzymes and why are they important in pathway regulation?

Summary

1. Energy is the capacity to do work. Living cells carry out three major kinds of work: chemical work of biosynthesis, transport work, and mechanical work.
2. The ultimate source of energy for most microbes is sunlight trapped by autotrophs and used to form organic material from CO_2. Photoautotrophs are then consumed by chemoheterotrophs.
3. ATP is the major energy currency and connects energy-generating processes with energy-using processes (**figure 8.3**).
4. The first law of thermodynamics states that energy is neither created nor destroyed.
5. The second law of thermodynamics states that changes occur in such a way that the randomness or disorder of the universe increases to the maximum possible. That is, entropy always increases during spontaneous processes.
6. The first and second laws can be combined to determine the amount of energy made available for useful work.

$$\Delta G = \Delta H - T{\cdot}\Delta S$$

In this equation the change in free energy (ΔG) is the energy made available for useful work, the change in enthalpy (ΔH) is the change in heat content, and the change in entropy is ΔS.

7. The standard free energy change ($\Delta G^{o\prime}$) for a chemical reaction is directly related to the equilibrium constant.
8. In exergonic reactions $\Delta G^{o\prime}$ is negative and the equilibrium constant is greater than one; the reaction goes to completion as written. Endergonic reactions have a positive $\Delta G^{o\prime}$ and an equilibrium constant less than one (**figure 8.5**).
9. In oxidation-reduction (redox) reactions, electrons move from a donor, the reducing agent or reductant, to an acceptor, the oxidizing agent or oxidant. The standard reduction potential measures the tendency of the reducing agent to give up electrons.
10. Redox couples with more negative reduction potentials donate electrons to those with more positive potentials, and energy is made available during the transfer (**figure 8.7**).
11. Some most important electron carriers in cells are NAD^+, $NADP^+$, FAD, FMN, coenzyme Q, cytochromes, and the nonheme iron proteins.
12. Enzymes are protein catalysts that catalyze specific reactions.
13. Enzymes consist of a protein component, the apoenzyme, and often a nonprotein cofactor that may be a prosthetic group, a coenzyme, or a metal activator.
14. Enzymes speed reactions by binding substrates at their active sites and lowering the activation energy (**figure 8.14**).
15. The rate of an enzyme-catalyzed reaction increases with substrate concentration at low substrate levels and reaches a plateau (the maximum velocity) at saturating substrate concentrations. The Michaelis constant is the substrate concentration that the enzyme requires to achieve half maximal velocity (**figure 8.17**).
16. Enzymes have pH and temperature optima for activity.
17. Enzyme activity can be slowed by competitive and noncompetitive inhibitors.
18. The regulation of metabolism keeps cell components in proper balance and conserves metabolic energy and material.
19. The localization of metabolites and enzymes in different parts of the cell, called metabolic channeling, influences pathway activity. A common channeling mechanism is compartmentation.
20. Regulatory enzymes are usually allosteric enzymes, enzymes in which an effector or modulator binds reversibly to a regulatory site separate from the catalytic site and causes a conformational change in the enzyme to alter its activity (**figure 8.21**).
21. Aspartate carbamoyltransferase is an allosteric enzyme that is inhibited by CTP and activated by ATP.
22. Enzyme activity also can be regulated by reversible covalent modification. Two examples of such regulation are glycogen phosphorylase (phosphate addition) and glutamine synthetase (AMP addition).
23. The first enzyme in a pathway and enzymes at branch points often are subject to feedback inhibition by one or more end products. Excess end product slows its own synthesis (**figure 8.27**).

Key Terms

activation energy *162*
active site *162*
adenosine diphosphate (ADP) *155*
adenosine 5′-triphosphate (ATP) *155*
aerobic respiration *154*
allosteric enzymes *165*
apoenzyme *161*
calorie *155*
catalyst *161*
catalytic site *162*
chemical work *154*
coenzyme *161*
coenzyme Q or CoQ (ubiquinone) *159*
cofactor *161*
compartmentation *165*
competitive inhibitor *164*
cytochrome *159*
denaturation *163*
effector or modulator *165*
end product inhibition *169*
endergonic reaction *156*
energy *154*
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enzyme-substrate complex *162*
equilibrium *156*
equilibrium constant (K_{eq}) *156*
exergonic reaction *156*
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ferredoxin *159*
first law of thermodynamics *155*
flavin adenine dinucleotide (FAD) *159*
flavin mononucleotide (FMN) *159*
free energy change *156*
high-energy molecule *157*
holoenzyme *161*
isoenzymes *169*
joule *155*
mechanical work *154*
metabolic channeling *165*
Michaelis constant (K_m) *163*
nicotinamide adenine dinucleotide (NAD^+) *157*
nicotinamide adenine dinucleotide phosphate ($NADP^+$) *158*
noncompetitive inhibitor *164*
nonheme iron protein *159*
oxidation-reduction (redox) reaction *157*
oxidizing agent (oxidant) *157*
pacemaker enzyme *169*
phosphate group transfer potential *157*
photosynthesis *154*
product *161*
prosthetic group *161*
reducing agent (reductant) *157*
regulatory site *165*
reversible covalent modification *167*
second law of thermodynamics *156*
standard free energy change *156*
standard reduction potential *157*
substrate *161*
thermodynamics *155*
transition-state complex *162*
transport work *154*

Questions for Thought and Review

1. Describe in general terms how energy from sunlight is spread throughout the biosphere.
2. What sources of energy, other than sunlight, are used by microorganisms?
3. Under what conditions would it be possible to create more order in a system without violating the second law of thermodynamics?
4. Do living cells increase randomness or entropy within themselves? In the environment?
5. Suppose that a chemical reaction had a large negative $\Delta G^{\circ\prime}$ value. What would this indicate about its equilibrium constant? If displaced from equilibrium, would it proceed rapidly to completion? Would much or little free energy be made available?
6. Will electrons ordinarily move in an electron transport chain from cytochrome *c* ($E'_0 = +210$ mV) to O_2 ($E'_0 = +820$ mV) or in the opposite direction?
7. If a person had a niacin deficiency, what metabolic process might well be adversely affected? Why?
8. Draw a diagram showing how enzymes catalyze reactions by altering the activation energy. What is a transition-state complex? Use the diagram to discuss why enzymes do not change the equilibria of the reactions they catalyze.
9. What special properties might an enzyme isolated from a psychrophilic bacterium have? Will enzymes need to lower the activation energy more or less in thermophiles than in psychrophiles?
10. How might a substrate be able to regulate the activity of the enzyme using it?
11. Describe how *E. coli* aspartate carbamoyltransferase is regulated, both in terms of the effects of modulators and the mechanism by which they exert their influence.
12. What is the significance of the fact that regulatory enzymes often are located at pathway branch points?

Critical Thinking Questions

1. How could electron transport be driven in the opposite direction? Why would it be desirable to do this?
2. Take a look at the structures of macromolecules (appendix I). Which type has the most electrons to donate? Why are carbohydrates usually the primary source of electrons for nonautotrophic bacteria?
3. Most enzymes do not operate at their biochemical optima inside cells. Why not?

Additional Reading

General

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