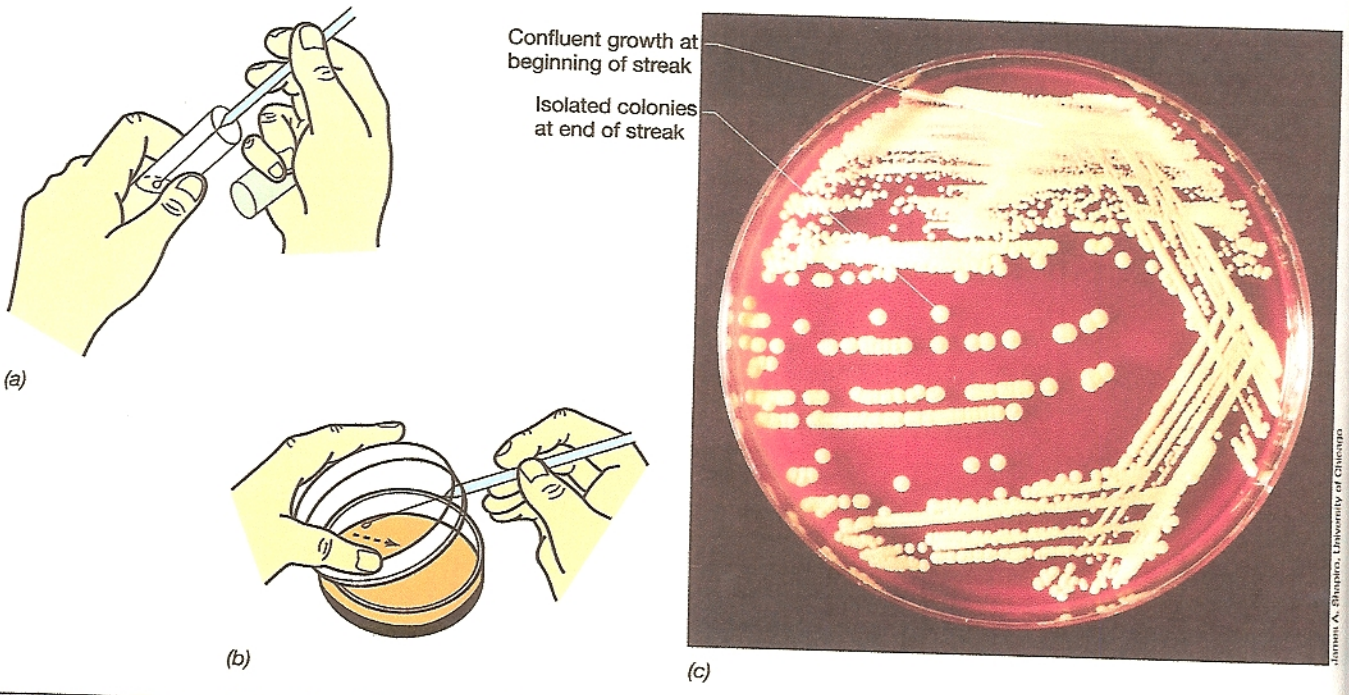


Figure 5.4 Method of making a streak plate to obtain pure cultures. (a) Loop is sterilized, and then a loopful of inoculum is removed from tube. (b) Streak is made over a sterile agar plate, spreading out the organisms. Following the initial streak, subsequent streaks are made at angles to it, the loop being resterilized between streaks. (c) Appearance of the streaked plate after incubation. Colonies of the bacterium *Micrococcus luteus* grown on blood agar plates. It is from such well-isolated colonies that pure cultures can usually be obtained.

develop from the growth and division of single cells. Picking and restreaking from an isolated colony is a major method of obtaining pure cultures from microbial communities containing many different organisms.

✓ 5.3 Concept Check

Microorganisms can be grown in the laboratory in culture media containing the nutrients they require. Successful cultivation of pure cultures of microorganisms can be done only if aseptic technique is practiced.

- ✓ What is meant by the word *sterile?* What would happen if freshly prepared culture media were not sterilized?
- ✓ Why is aseptic technique necessary for successful cultivation of pure cultures in the laboratory?

II ENERGETICS AND ENZYMES

We learned in Chapter 2 of the different energy classes of microorganisms, *chemoorganotrophs, chemolithotrophs,* and *phototrophs* (Section 2.4). However, regardless of how an organism makes a living, it must be able to obtain energy from either chemical compounds or from light, and then conserve the energy as ATP. Here we discuss the principles of energy conservation, using some simple laws of chemistry and physics to guide our understanding, and then consider the action of enzymes, the cell's biocatalysts.

5.4 Bioenergetics

Energy is defined as the ability to do work. In microbiology, energy is measured in units of *kilojoules* (kJ), a measure of heat energy. Chemical reactions are accompanied by changes in energy. Although in any chemical reaction some energy is lost as heat, in microbiology we are interested in **free energy** (abbreviated *G*), which is defined as the energy released *that is available to do useful work.* The change in free energy during a reaction is expressed as $\Delta G^{0'}$, where the symbol Δ should be read "change in." The "0" and "prime" mean that the free-energy value was obtained under "standard" conditions: pH 7, 25°C, all reactants and products initially at 1 *M* concentration.*

*Free energy calculations using standard conditions are estimations of the free energy changes that actually occur when a reaction takes place in nature, despite the fact that nutrient concentrations of 1 *M* rarely occur in nature. Although for now, calculations of $\Delta G^{0'}$ are reasonable estimates, we will see later that the actual concentration of products and reactants can occasionally alter the bioenergetics of reactions in microbiologically important ways (Sections 17.21 and 19.10). These issues are also discussed in more detail in Appendix 1.

If in the reaction:

$$A + B \rightarrow C + D$$

the $\Delta G^{0'}$ is *negative*, the reaction will proceed with the *release* of free energy, energy that the cell may be able to conserve in the form of ATP. Such energy-yielding reactions are called **exergonic**. However, if $\Delta G^{0'}$ is *positive*, the reaction *requires* energy in order to proceed; such reactions are called **endergonic**. Thus, from the standpoint of the microbial cell, exergonic reactions *yield* energy while endergonic reactions *require* energy.

Free Energy of Formation and Calculating $\Delta G^{0'}$

In order to calculate the free energy yield of a reaction, one needs to know the free energy of its reactants and products. This is the *free energy of formation*, the energy yielded or energy required for the *formation* of a given molecule from its constituent elements. By convention, the free energy of formation (G^0_f) of the elements (for instance, C, H_2, N_2) is zero. If the formation of a *compound* from elements proceeds exergonically, then the free energy of formation of the compound is negative (energy is released), whereas if the reaction is endergonic (energy is required), then the free energy of formation of the compound is positive.

A few examples of free energies of formation are given in Table 5.5. For most compounds G^0_f is *negative*, reflecting the fact that compounds tend to form spontaneously from elements. However, the positive G^0_f for nitrous oxide (+104.2 kJ/mol) tells us that this molecule does not form spontaneously, but rather decomposes to nitrogen and oxygen. The free energies of formation of a variety of compounds of microbiological interest are given in Appendix 1.

Using free energies of formation, it is possible to calculate the *change* in free energy occurring in a given reaction. For a simple reaction such as $A + B \rightarrow C + D$, $\Delta G^{0'}$ is calculated by subtracting the *sum* of the free energies of formation of the reactants (in this case A and B) from that of the products (C and D). Thus,

$$\Delta G^{0'} \text{ of } A + B \rightarrow C + D$$
$$= G^0_f[C + D] - G^0_f[A + B]$$

The phrase "products minus reactants" encompasses the necessary steps for calculating changes in free energy during chemical reactions. However, it is first necessary to balance the reaction chemically before free-energy calculations can be made. Appendix 1 details the steps involved in calculating free energies for any hypothetical reaction.

TABLE 5.5 Free energy of formation for a few compounds of biological interest

Compound	Free energy of formation[a]
Water (H_2O)	−237.2
Carbon dioxide (CO_2)	−394.4
Hydrogen gas (H_2)	0
Oxygen gas (O_2)	0
Ammonium (NH_4^+)	−79.4
Nitrous oxide (N_2O)	+104.2
Acetate ($C_2H_3O_2^-$)	−369.4
Glucose ($C_6H_{12}O_6$)	−917.3
Methane (CH_4)	−50.8
Methanol (CH_3OH)	−175.4

[a]The free energy of formation values (G_f^0) are in *kJ/mol*.

✓ 5.4 Concept Check

The chemical reactions of the cell are accompanied by changes in energy, expressed in kJ. A chemical reaction can occur with the release of free energy (exergonic) or with the consumption of free energy (endergonic).

- ✓ What is free energy?
- ✓ In general, are *catabolic* reactions exergonic or endergonic?
- ✓ Using the data in Table 5.5, calculate $\Delta G^{0'}$ for the reaction $CH_4 + \frac{1}{2}O_2 \rightarrow CH_3OH$.

5.5 Catalysis and Enzymes

A free-energy calculation tells us only whether energy is released or required in a given reaction; it tells us nothing about the *rate* of the reaction. Consider the formation of water from gaseous oxygen and hydrogen. The energetics of this reaction is quite favorable: $H_2 + \frac{1}{2}O_2 \rightarrow H_2O$, $\Delta G^{0'} = -237$ kJ. However, if we were to simply mix O_2 and H_2 together, no measurable formation of water would occur for many years. This is because the rearrangement of oxygen and hydrogen atoms to form water requires that the chemical bonds of the reactants be broken first. The breaking of bonds requires energy, and this energy is referred to as **activation energy**. Activation energy is the amount of energy required to bring all molecules in a chemical reaction to the reactive state. For a reaction that proceeds with a net release of free energy (that is, an exergonic reaction), the situation is as diagrammed in Figure 5.5.

Enzymes

The idea of activation energy leads us to the concept of catalysis. A **catalyst** is a substance that *lowers* the activation energy of a reaction, thereby *increasing* the rate of reaction. Catalysts facilitate reactions but are themselves not consumed or transformed by these reactions. It is important to note that catalysts do not affect the energetics or the equilibrium of a reaction; catalysts affect only the *speed* at which reactions proceed.

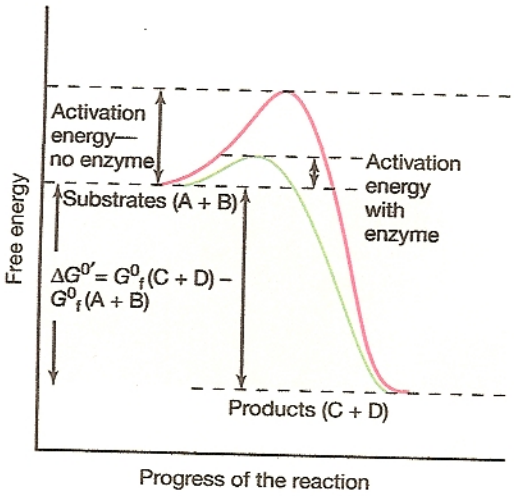


Figure 5.5 Progress of a hypothetical exergonic reaction: A + B → C + D and the concept of activation energy. Chemical reactions may not proceed spontaneously even though energy would be released, because the reactants must first be activated. Once activation has occurred, the reaction then proceeds spontaneously. Catalysts such as enzymes lower the required activation energy.

Most reactions in living organisms would not occur at appreciable rates without catalysis. The catalysts of biological reactions are proteins called **enzymes**. Enzymes are highly specific in the reactions that they catalyze. That is, each enzyme catalyzes only a *single type* of chemical reaction, or in the case of certain enzymes, a *class* of closely related reactions. This specificity is related to the precise three-dimensional structure of the enzyme molecule. In an enzyme-catalyzed reaction, the enzyme temporarily combines with the reactant, which is termed a **substrate** (S) of the enzyme, forming an **enzyme-substrate complex**. Then, as the reaction proceeds, the **product** (P) is released and the enzyme (E) is returned to its original state:

$$E + P \rightleftharpoons E{-}S \rightleftharpoons E + P$$

The enzyme is generally much larger than the substrate(s), and the combination of enzyme and substrate(s) usually depends on weak bonds, such as hydrogen bonds, van der Waals forces, and hydrophobic interactions (Section 3.1) to join the enzyme to the substrate. The small portion of the enzyme to which substrates bind is referred to as the **active site** of the enzyme.

Enzyme Catalysis

The catalytic power of enzymes is impressive. Enzymes typically increase the rate of chemical reactions from 10^8 to 10^{20} times the rate that would occur spontaneously. To catalyze a specific reaction, an enzyme must do two things: (1) bind the correct substrate, and (2) position the substrate relative to the catalytically active groups at the enzyme's active site. Binding of substrate to enzyme produces the enzyme-substrate complex (Figure 5.6). This serves to align reactive groups and places strain on specific bonds in the substrate(s). The result of en-

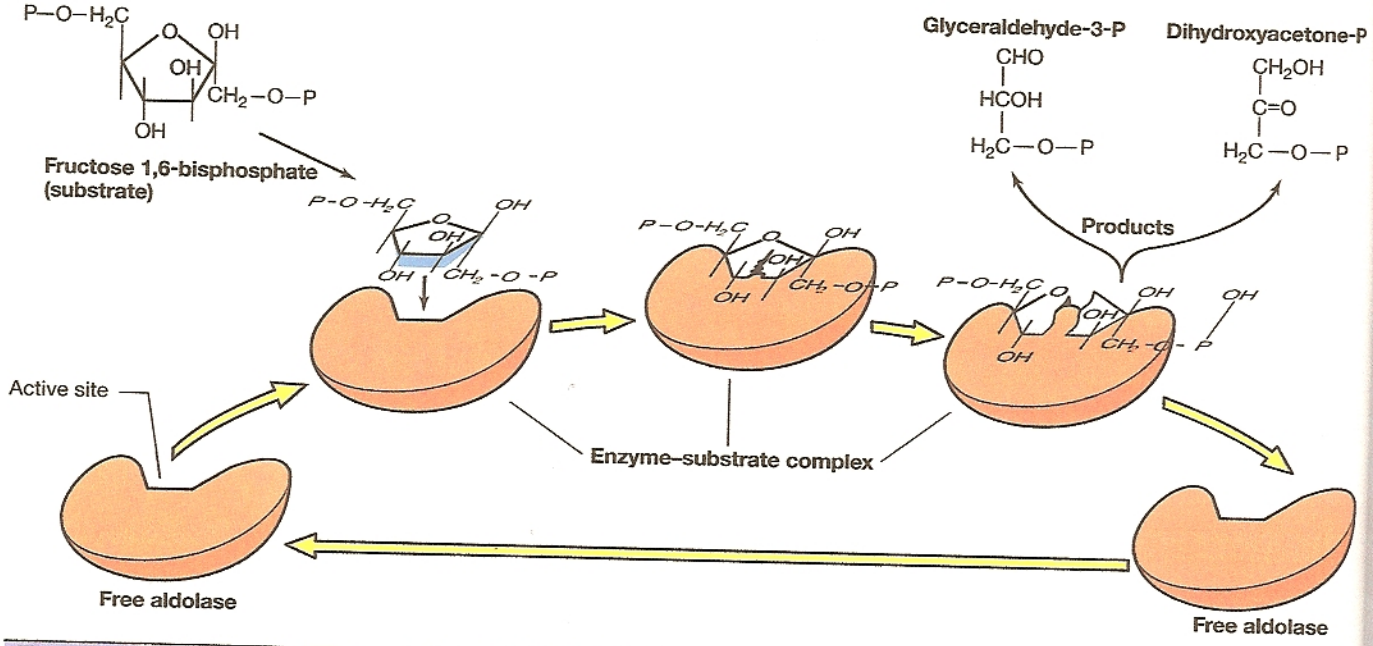


Figure 5.6 The catalytic cycle of an enzyme as depicted for the enzyme fructose bisphosphate aldolase. This enzyme catalyzes the reaction: fructose 1,6-bisphosphate → glyceraldehyde 3-phosphate + dihydroxyacetone phosphate in glycolysis (see Figure 5.14). Following binding of fructose 1,6-bisphosphate in the formation of the enzyme–substrate complex, the conformation of the enzyme is altered, placing strain on certain bonds of the substrate, which break and yield the two products.

zyme-substrate complex formation is a reduction in the activation energy required to make the reaction proceed (Figure 5.5) with the conversion of substrate(s) to product(s). These steps are summarized diagrammatically in Figure 5.6 for the glycolytic enzyme *fructose bisphosphate aldolase* (see Section 5.10).

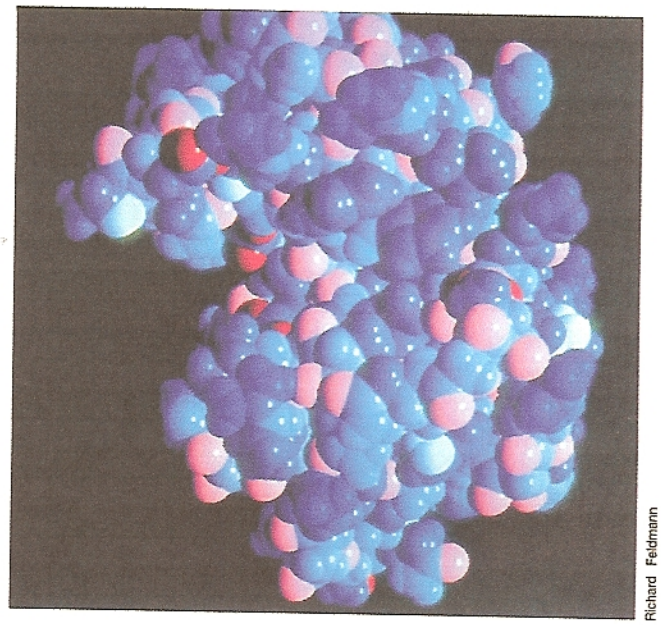
Note that the reaction depicted in Figure 5.5 is exergonic because the free energy of formation of the substrate is *greater* than that of the product; that is, product formation proceeds with the release of energy. Enzymes can also catalyze energy-requiring reactions, converting energy-poor substrates to energy-rich products. In this case, not only must an activation energy barrier be overcome, but sufficient free energy must also be put *into* the system to raise the energy level of the substrates to that of the products. Although theoretically all enzymes are reversible in their action, in practice, enzymes catalyzing highly exergonic or highly endergonic reactions are essentially unidirectional. If a particularly exergonic reaction needs to be reversed during cellular metabolism, a distinctly different enzyme is frequently involved in the reaction.

Structure of Enzymes

As we have discussed, enzymes are proteins, polymers of amino acids (Sections 3.7–3.8). Each enzyme has a specific three-dimensional shape. The linear array of amino acids (primary structure) folds and twists into a specific configuration to achieve secondary and tertiary structure. A specifically folded protein thus assumes specific binding and physical properties. The precise conformation of an enzyme may be seen more easily in a computer-generated space-filling model (Figure 5.7). In this example of the peptidoglycan-cleaving enzyme *lysozyme* (Section 4.8), the large cleft is the site where the substrate binds (the active site).

Many enzymes contain small nonprotein molecules that participate in the catalytic function but are not considered substrates in the usual sense. These small enzyme-associated molecules are divided into two categories on the basis of the nature of their association with the enzyme: *prosthetic groups* and *coenzymes*. **Prosthetic groups** are bound very tightly to their enzymes, usually permanently. The heme group present in cytochromes is an example of a prosthetic group, and cytochromes will be described in detail later in this chapter. **Coenzymes** are bound rather loosely to enzymes, and a single coenzyme molecule may associate with a number of different enzymes at different times during growth. Coenzymes serve as intermediate carriers of small molecules from one enzyme to another (see Figure 5.11). Most coenzymes are derivatives of vitamins (see Table 5.3).

Enzymes are named either for the substrate they bind or for the chemical reaction they catalyze, by addition of the combining form *-ase*. Thus, cellul*ase* is an enzyme that attacks cellulose, glucose oxid*ase* is an enzyme that catalyzes the oxidation of glucose, and ribonucle*ase* is an enzyme that decomposes ribonucleic acid. A more formal nomenclature system employing a specific numbering system is used to classify enzymes more precisely.



Richard Feldmann

Figure 5.7 Computer-generated space-filling model of the enzyme lysozyme. The substrate (peptidoglycan) binding site (active site) is in the large cleft on the left side of the model (Section 4.8).

✓ 5.5 Concept Check

The reactants in a chemical reaction must first be activated before the reaction can take place, and this often requires a catalyst. Enzymes are catalytic proteins that speed up the rate of biochemical reactions. Enzymes are highly specific in the reactions they catalyze, and this specificity resides in the folding pattern of the polypeptide(s) in the protein.

- ✓ What is the function of a *catalyst*?
- ✓ What *class* of macromolecules are enzymes?
- ✓ Where on an enzyme does its substrate bind?
- ✓ What is *activation energy*?

III OXIDATION–REDUCTION AND HIGH-ENERGY COMPOUNDS

In biological systems, energy conservation involves oxidation–reduction reactions. The result of energy released in these reactions is the production of *high-energy* compounds such as ATP. We now consider oxidation–reduction reactions and the major electron carriers

present in both the cytoplasm and the cytoplasmic membrane. Finally, we examine the nature of the high-energy compounds that actually conserve the energy released in oxidation–reduction reactions.

MEDIA TUTORIAL Oxidation–Reduction

5.6 Oxidation–Reduction

The conservation of energy from chemical reactions in living organisms involves **oxidation–reduction (redox)** reactions. Chemically, an oxidation is defined as the *removal* of an electron or electrons from a substance. A reduction is defined as the *addition* of an electron (or electrons) to a substance. In biochemistry—the chemistry of cells—oxidations and reductions frequently involve the transfer of not just electrons, but whole hydrogen atoms. A hydrogen atom (H) consists of an electron plus a proton. When its electron is removed, the hydrogen atom becomes a *proton* (or hydrogen ion, H^+). We will, on occasion, need to distinguish between oxidation–reduction reactions involving electrons only or hydrogen atoms only, but reserve this distinction for the appropriate time (see Sections 5.11 and 5.12).

Electron Donors and Acceptors

Oxidation–reduction reactions involve electrons being donated by an electron donor and being accepted by an electron acceptor. For example, hydrogen gas, H_2, can release electrons and hydrogen ions (protons) and become oxidized:

$$H_2 \rightarrow 2\,e^- + 2\,H^+$$

However, electrons cannot exist alone in solution; they must be part of atoms or molecules. The equation as drawn provides chemical information but does not itself represent a real reaction. The above reaction is only a *half reaction*, a term that implies the need for a second half reaction. This is because for any *oxidation* to occur, a subsequent *reduction* must also occur. For example, the oxidation of H_2 could be coupled to the reduction of many different substances including O_2 in a second reaction:

$$\tfrac{1}{2}O_2 + 2\,e^- + 2\,H^+ \rightarrow H_2O$$

This half reaction, which is a reduction, when coupled to the oxidation of H_2 above, yields the following overall balanced reaction:

$$H_2 + \tfrac{1}{2}O_2 \rightarrow H_2O$$

In reactions of this type, we will refer to the substance *oxidized*, in this case H_2, as the **electron donor**, and the substance *reduced*, in this case O_2, as the **electron acceptor** (Figure 5.8). The key to understanding biological oxidations and reductions is to keep straight the proper

$H_2 \rightarrow 2\,e^- + 2\,H^+$

Electron-donating half reaction

$\tfrac{1}{2}O_2 + 2\,e^- \rightarrow O^{2-}$

Electron-accepting half reaction

$2\,H^+ + O^{2-} \rightarrow H_2O$

Formation of water

Electron donor — $H_2 + \tfrac{1}{2}O_2 \rightarrow H_2O$ — Electron acceptor

Net reaction

Figure 5.8 Example of an oxidation–reduction reaction: The formation of H_2O from the electron donor H_2 and the electron acceptor O_2.

half reactions: There must always be one reaction involving an electron *donor* and another reaction involving an electron *acceptor*.

Reduction Potentials

Substances vary in their tendency to become oxidized or to become reduced. This tendency is expressed as the **reduction potential** (E_0', standard conditions) of the substance. This potential is measured electrically in units of volts in reference to a standard substance, H_2. By convention, reduction potentials are expressed for half reactions written as *reductions*. Thus, oxidized form + $e^- \rightarrow$ reduced form. If protons are involved in the reaction, as is often the case, then the reduction potential is to some extent influenced by the hydrogen ion concentration (pH). By convention in biology, reduction potentials are given for neutrality (pH 7) because the cytoplasm of most cells is neutral or nearly so. Using these conventions, at pH 7 the reduction potential (E_0') of

$$\tfrac{1}{2}O_2 + 2\,H^+ + 2\,e^- \rightarrow H_2O$$

is +0.816 volts (V), and that of

$$2\,H^+ + 2\,e^- \rightarrow H_2$$

is –0.421 V.

Oxidation–Reduction Couples and Complete Redox Reactions

Most molecules can be either electron donors or electron acceptors under different circumstances, depending on the substances with which they react. The same atom on each side of the arrow in the half reactions can be thought of as representing a *redox couple*, such as $2\,H^+/H_2$ or $\tfrac{1}{2}O_2/H_2O$. When writing a redox couple, the *oxidized* form is always placed on the left.

In constructing complete oxidation–reduction reactions from their constituent half reactions, it is simplest to remember that the reduced substance of a redox couple whose reduction potential is more negative *donates*

electrons to the oxidized substance of a redox couple whose potential is more positive. Thus, in the couple $2\,H^+/H_2$, which has a potential of –0.42 V, H_2 has a great tendency to *donate* electrons. On the other hand, in the couple $\frac{1}{2}\,O_2/H_2O$, which has a potential of +0.82 V, H_2O has a very slight tendency to donate electrons, but O_2 has a great tendency to *accept* electrons. It follows then that in a reaction of H_2 and O_2, H_2 will be the electron *donor* and become oxidized, and O_2 will be the electron *acceptor* and become reduced (Figure 5.8). Even though by chemical convention both half reactions are written as reductions, in an actual redox reaction one of the two half reactions must be written as an oxidation and therefore proceeds in the reverse direction. Thus, note that in the reaction shown in Figure 5.8, the oxidation of H_2 to $2H^+ + 2e^-$ is reversed from the formal half reaction, written as a reduction.

The Electron Tower

A convenient way of viewing electron transfer in biological systems is to imagine a vertical tower (Figure 5.9). The tower represents the range of reduction potentials for redox couples from the most negative at the top to the most positive at the bottom. The reduced substance in the redox pair at the top of the tower has the greatest tendency to donate electrons, whereas the oxidized substance in the couple at the bottom of the tower has the greatest tendency to accept electrons.

As electrons from the electron donor at the top of the tower fall, they can be "caught" by acceptors at various levels. The difference in potential between two substances is expressed as $\Delta E_0'$. The farther the electrons drop from a donor before they are caught by an acceptor, the greater the amount of energy released; that is, *$\Delta E_0'$ is proportional to $\Delta G^{0\prime}$* (Figure 5.9). O_2, at the bottom

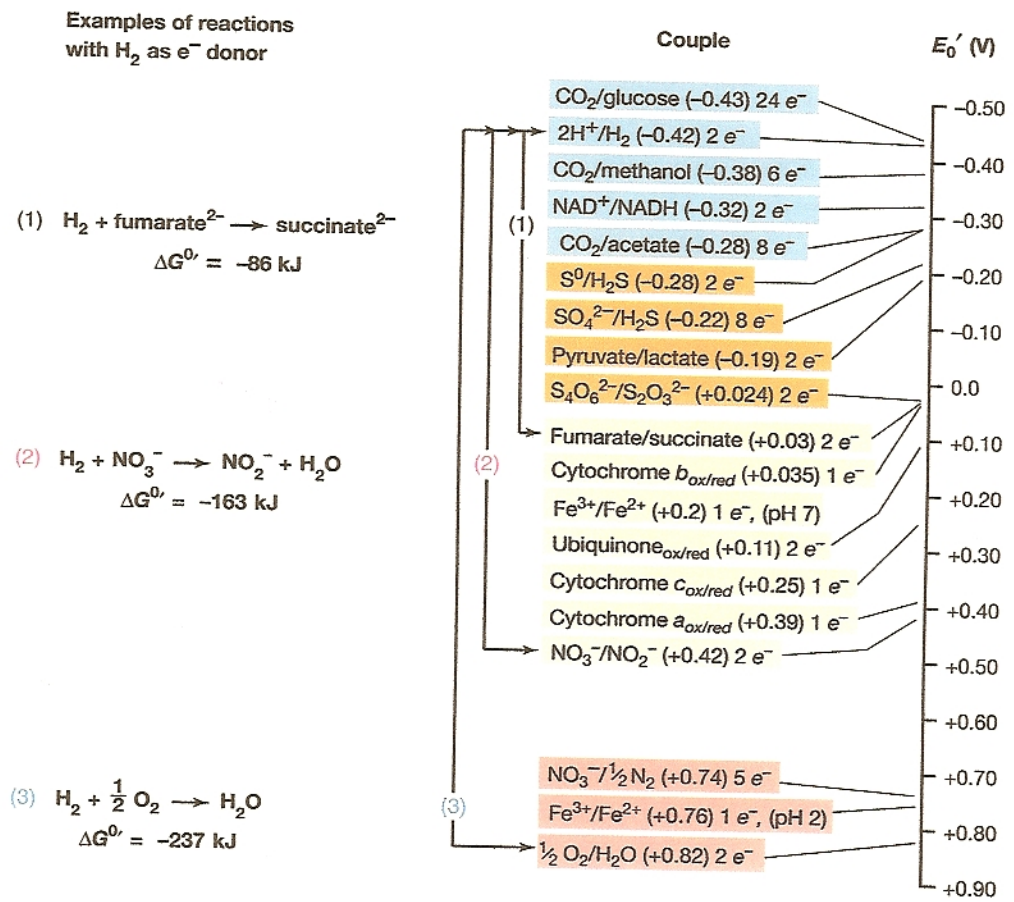


Figure 5.9 The electron tower. Redox couples are arranged from the strongest reductants (negative reduction potential) at the top to the strongest oxidants (positive reduction potentials) at the bottom. As electrons are donated from the top of the tower, they can be "caught" by acceptors at various levels. The farther the electrons fall before they are caught, the greater the difference in reduction potential between electron donor and electron acceptor and the more energy that is released. As an example of this, on the left is shown the differences in energy released when a single electron donor, H_2, reacts with any of three different electron acceptors, fumarate, nitrate, and oxygen.

of the tower, is the most favorable electron acceptor used by organisms. In the middle of the tower, redox couples can act as either electron donors or acceptors. For instance, the 2 H^+/H_2 couple has a reduction potential of –0.42 V. The fumarate–succinate couple has a potential of +0.02 V. Hence, the oxidation of hydrogen (the electron donor) can be coupled to the reduction of fumarate (the electron acceptor):

$$H_2 + \text{fumarate}^{2-} \rightarrow \text{succinate}^{2-}$$

On the other hand, the oxidation of succinate to fumarate can be coupled to the reduction of NO_3^- or $\frac{1}{2}O_2$

$$\text{Succinate}^{2-} + NO_3^- \rightarrow \text{fumarate}^{2-} + NO_2^- + H_2O$$
$$\text{Succinate}^{2-} + \tfrac{1}{2}O_2 \rightarrow \text{fumarate}^{2-} + H_2O$$

Hence, under conditions where oxygen is absent (called *anoxic*) in the presence of H_2, fumarate can be an electron acceptor (producing succinate), and under other conditions (for example, anoxic in the presence of NO_3^-, or aerobic) succinate can be an electron donor (producing fumarate). Indeed, all the transformations involving fumarate and succinate described here are actually carried out by various microorganisms under certain nutritional and environmental conditions.

Electron Donor ⟺ Energy Source

In catabolism the electron donor is often referred to as an **energy source**. Many potential electron donors exist in nature (Chapters 17 and 19), but for now it is essential to understand that it is not the electron donor *per se*, that contains energy, but it is the *chemical reaction* in which the electron donor gets oxidized, that actually releases energy. As discussed in the context of the electron tower, the amount of energy released in a redox reaction depends on the nature of *both* the electron donor and the electron acceptor: The greater the difference between reduction potentials of the two half reactions, the more energy there will be released when they react (Figure 5.9) (see also Appendix 1).

✓ 5.6 Concept Check

Oxidation–reduction reactions, which are involved in the energy-yielding reactions of cells, involve the transfer of electrons from one substance to another. The tendency of a compound to accept or release electrons is expressed quantitatively by its reduction potential.

- ✓ In the reaction $H_2 + \frac{1}{2}O_2 \rightarrow H_2O$, what is the electron *donor* and what is the electron *acceptor*?
- ✓ What is the E_0' of the 2 H^+/H_2 couple?
- ✓ Why is NO_3^- a better electron acceptor than fumarate?

5.7 NAD as a Redox Electron Carrier

In the cell, the transfer of electrons in an oxidation-reduction reaction from donor to acceptor usually involves one or more intermediates referred to as **carriers**. When such carriers are used, we refer to the initial donor as the **primary electron donor** and to the final acceptor as the **terminal electron acceptor**. The net energy change of the complete reaction sequence is determined by the *difference* in reduction potentials between the primary donor and the terminal acceptor.

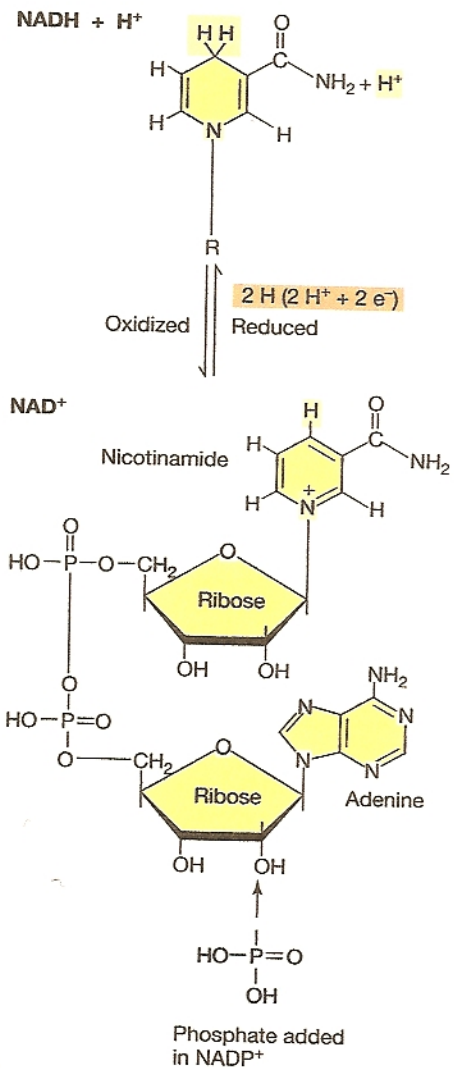


Figure 5.10 Structure of the oxidation–reduction coenzyme nicotinamide adenine dinucleotide (NAD⁺). In NADP⁺, a phosphate group is present, as indicated. Both NAD⁺ and NADP⁺ undergo oxidation–reduction as shown, are freely diffusable, and are hydrogen atom carriers.