13.4 Biological Oxidation-Reduction Reactions

The transfer of phosphoryl groups is a central feature of metabolism. Equally important is another kind of transfer: electron transfer in oxidation-reduction reactions, sometimes referred to as redox reactions. These reactions involve the loss of electrons by one chemical species, which is thereby oxidized, and the gain of electrons by another, which is reduced. The flow of electrons in oxidation-reduction reactions is responsible, directly or indirectly, for all work done by living organisms. In nonphotosynthetic organisms, the sources of electrons are reduced compounds (foods); in photosynthetic organisms, the initial electron donor is a chemical species excited by the absorption of light. The path of electron flow in metabolism is complex. Electrons move from various metabolic intermediates to specialized electron carriers in enzyme-catalyzed reactions. The carriers, in turn, donate electrons to acceptors with higher electron affinities, with the release of energy. Cells possess a variety of molecular energy transducers, which convert the energy of electron flow into useful work.

We begin by discussing how work can be accomplished by an electromotive force (emf), then consider the theoretical and experimental basis for measuring energy changes in oxidation reactions in terms of emf and the relationship between this force, expressed in volts, and the free-energy change, expressed in joules. We also describe the structures and oxidation-reduction chemistry of the most common of the specialized electron carriers, which you will encounter repeatedly in later chapters.

The Flow of Electrons Can Do Biological Work

Every time we use a motor, an electric light or heater, or a spark to ignite gasoline in a car engine, we use the flow of electrons to accomplish work. In the circuit that powers a motor, the source of electrons can be a battery containing two chemical species that differ in affinity for electrons. Electrical wires provide a pathway for electron flow from the chemical species at one pole of the battery, through the motor, to the chemical species at the other pole of the battery. Because the two chemical species differ in their affinity for electrons, electrons flow spontaneously through the circuit, driven by a force proportional to the difference in electron affinity, the **electromotive force**, **emf**. The emf (typically a few volts) can accomplish work if an

appropriate energy transducer—in this case a motor—is placed in the circuit. The motor can be coupled to a variety of mechanical devices to do useful work.

Living cells have an analogous biological "circuit," with a relatively reduced compound such as glucose as the source of electrons. As glucose is enzymatically oxidized, the released electrons flow spontaneously through a series of electron-carrier intermediates to another chemical species, such as O_2. This electron flow is exergonic, because O_2 has a higher affinity for electrons than do the electron-carrier intermediates. The resulting emf provides energy to a variety of molecular energy transducers (enzymes and other proteins) that do biological work. In the mitochondrion, for example, membrane-bound enzymes couple electron flow to the production of a transmembrane pH difference and a transmembrane electrical potential, accomplishing chemiosmotic and electrical work. The proton gradient thus formed has potential energy, sometimes called the proton-motive force by analogy with electromotive force. Another enzyme, ATP synthase in the inner mitochondrial membrane, uses the proton-motive force to do chemical work: synthesis of ATP from ADP and P_i as protons flow spontaneously across the membrane. Similarly, membrane-localized enzymes in *E. coli* convert emf to proton-motive force, which is then used to power flagellar motion. The principles of electrochemistry that govern energy changes in the macroscopic circuit with a motor and battery apply with equal validity to the molecular processes accompanying electron flow in living cells.

Oxidation-Reductions Can Be Described as Half-Reactions

Although oxidation and reduction must occur together, it is convenient when describing electron transfers to consider the two halves of an oxidation-reduction reaction separately. For example, the oxidation of ferrous ion by cupric ion,

$$\mathbf{Fe^{2+} + Cu^{2+} \rightleftharpoons Fe^{3+} + Cu^{+}}$$

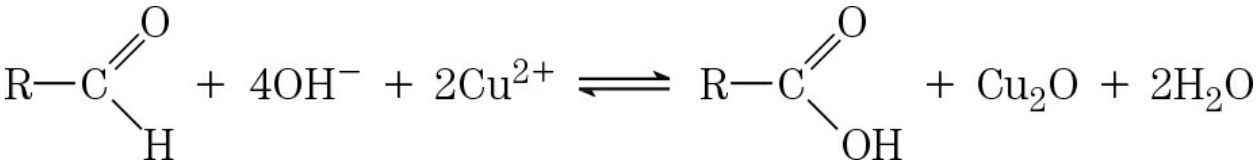
can be described in terms of two half-reactions:

$$\textbf{(1)}\quad \mathbf{Fe^{2+} \rightleftharpoons Fe^{3+} + e^-}$$

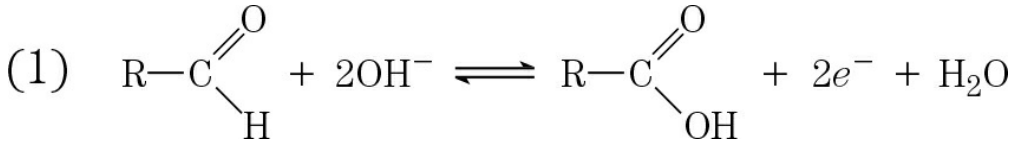
$$\textbf{(2)}\quad \mathbf{Cu^{2+} + e^- \rightleftharpoons Cu^+}$$

The electron-donating molecule in an oxidation-reduction reaction is called the reducing agent or reductant; the electron-accepting molecule is the oxidizing agent or oxidant. A given agent, such as an iron cation existing in the ferrous (Fe^{2+}) or ferric (Fe^{3+}) state, functions as a conjugate reductant-oxidant pair (redox pair), just as an acid and corresponding base function as a conjugate acid-base pair. Recall from Chapter 2 that in acid-base reactions we can write a general equation: proton donor $\rightleftharpoons H^+$ + proton acceptor. In redox reactions we can write a similar general equation: electron donor (reductant) $\rightleftharpoons e^-$ + electron acceptor (oxidant). In the reversible half-reaction (1) above, Fe^{2+} is the electron donor and Fe^{3+} is the electron acceptor; together, Fe^{2+} and Fe^{3+} constitute a **conjugate redox pair**. The mnemonic OIL RIG —*o*xidation *i*s *l*osing, *r*eduction *i*s *g*aining—may be helpful in remembering what happens to electrons in redox reactions.

The electron transfers in the oxidation-reduction reactions of organic compounds are not fundamentally different from those of inorganic species. Consider the oxidation of a reducing sugar (an aldehyde or ketone) by cupric ion:



This overall reaction can be expressed as two half-reactions:



$$\textbf{(2)}\quad \mathbf{2Cu^{2+} + 2e^- + 2OH^- \rightleftharpoons Cu_2O + H_2O}$$

Notice that because two electrons are removed from the aldehyde carbon, the second half-reaction (the one-electron reduction of cupric to cuprous ion) must be doubled to balance the overall equation.

Biological Oxidations Often Involve Dehydrogenation

The carbon in living cells exists in a range of oxidation states **(Fig. 13-22)**. When a carbon atom shares an electron pair with another atom (typically H, C, S, N, or O), the sharing is unequal, in favor of the more electronegative atom. The order of increasing electronegativity is H < C < S < N < O. In oversimplified but useful terms, the more electronegative atom "owns" the bonding electrons it shares with another atom. For example, in methane (CH_4), carbon is more electronegative than the four hydrogens bonded to it, and the C atom therefore owns all eight bonding electrons (Fig. 13-22). In ethane, the electrons in the C—C bond are shared equally, so each C atom owns only seven of its eight bonding electrons. In ethanol, C-1 is less electronegative than the oxygen to which it is bonded, and the O atom therefore owns both electrons of the C—O bond, leaving C-1 with only five bonding electrons. With each formal loss of "owned" electrons, the carbon atom has undergone oxidation—even when no oxygen is involved, as in the conversion of an alkane (—CH_2—CH_2—) to an alkene (—CH=CH—). In this case, oxidation (loss of electrons) is coincident with the loss of hydrogen. In biological systems, as we noted earlier in the chapter, oxidation is often synonymous with **dehydrogenation**, and many enzymes that catalyze oxidation reactions are **dehydrogenases**. Notice that the more reduced compounds in Figure 13-22 (top) are richer in hydrogen than in oxygen, whereas the more oxidized compounds (bottom) have more oxygen and less hydrogen.

Not all biological oxidation-reduction reactions involve carbon. For example, in the conversion of molecular nitrogen to ammonia, $6H^+ + 6e^- + N_2 \rightarrow 2NH_3$, the nitrogen atoms are reduced.

Electrons are transferred from one molecule (electron donor) to another (electron acceptor) in one of four ways:

1. Directly as *electrons*. For example, the Fe^{2+}/Fe^{3+} redox pair can transfer an electron to the Cu^+/Cu^{2+} redox pair:

$$\mathbf{Fe^{2+} + Cu^{2+} \rightleftharpoons Fe^{3+} + Cu^{+}}$$

2. As *hydrogen atoms*. Recall that a hydrogen atom consists of a proton (H^+) and a single electron (e^-). In this case we can write the general

equation

$$\mathrm{AH_2} \rightleftharpoons \mathrm{A} + 2e^- + 2\mathrm{H^+}$$

where AH_2 is the hydrogen/electron donor. (Do not mistake the abov reaction for an acid dissociation, which involves a proton and no electron.) AH_2 and A together constitute a conjugate redox pair (A/AH_2), which can reduce another compound B (or redox pair, B/BH_2) by transfer of hydrogen atoms:

$$\mathrm{AH_2} + \mathrm{B} \rightleftharpoons \mathrm{A} + \mathrm{BH_2}$$

3. As a *hydride ion* ($:H^-$), which has two electrons. This occurs in the case of NAD-linked dehydrogenases, described below.

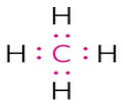
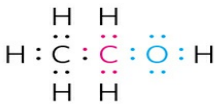
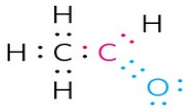
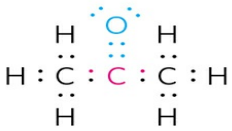
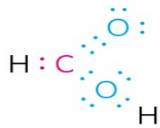
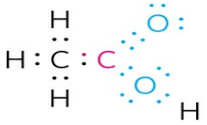
Methane		8
Ethane (alkane)		7
Ethene (alkene)		6
Ethanol (alcohol)		5
Acetylene (alkyne)		5
Formaldehyde		4
Acetaldehyde (aldehyde)		3
Acetone (ketone)		2
Formic acid (carboxylic acid)		2
Carbon monoxide		2
Acetic acid (carboxylic acid)		1
Carbon dioxide		0

FIGURE 13-22 Different levels of oxidation of carbon compounds in the biosphere. To approximate the level of oxidation of these compounds, focus on the red carbon atom and its bonding electrons. When this carbon is bonded to the less electronegative H atom, both bonding electrons (red) are assigned to the carbon. When carbon is bonded to another carbon, bonding electrons are shared equally, so one of the two electrons is assigned to the red carbon. When the red carbon is bonded to the more electronegative O atom, the bonding electrons are assigned to the oxygen. The number to the right of each compound is the number of electrons "owned" by the red carbon, a rough expression of the degree of oxidation of that compound. As the red carbon undergoes oxidation (loses electrons), the number gets smaller.

4. 4. Through direct *combination with oxygen.* In this case, oxygen combines with an organic reductant and is covalently incorporated in the product, as in the oxidation of a hydrocarbon to an alcohol:

$$R - CH_3 + \tfrac{1}{2}O_2 \rightarrow R - CH_2 - OH$$

The hydrocarbon is the electron donor and the oxygen atom is the electron acceptor.

All four types of electron transfer occur in cells. The neutral term **reducing equivalent** is commonly used to designate a single electron equivalent participating in an oxidation-reduction reaction, no matter whether this equivalent is an electron per se or is part of a hydrogen atom or a hydride ion, or whether the electron transfer takes place in a reaction with oxygen to yield an oxygenated product.

Reduction Potentials Measure Affinity for Electrons

When two conjugate redox pairs are together in solution, electron transfer from the electron donor of one pair to the electron acceptor of the other may proceed spontaneously. The tendency for such a reaction depends on the relative affinity of the electron acceptor of each redox pair for electrons. The **standard reduction potential**, $\mathbf{E^\circ}$, a measure (in volts) of this affinity, can be determined in an experiment such as that described in **Figure 13-23**. Electrochemists have chosen as a standard of reference the half-reaction

$$\mathrm{H^+} + e^- \rightarrow \tfrac{1}{2}\mathrm{H_2}$$

The electrode at which this half-reaction occurs (called a half-cell) is arbitrarily assigned an $E°$ of 0.00 V. When this hydrogen electrode is connected through an external circuit to another half-cell in which an oxidized species and its corresponding reduced species are present at standard concentrations (at 25 °C, each solute at 1 M, each gas at 101.3 kPa), electrons tend to flow through the external circuit from the half-cell of lower $E°$ to the half-cell of higher $E°$. By convention, a half-cell that takes electrons from the standard hydrogen cell is assigned a positive value of $E°$, and one that donates electrons to the hydrogen cell, a negative value. When any two half-cells are connected, that with the larger (more positive) $E°$ will be reduced; it has the greater reduction potential.

The reduction potential of a half-cell depends not only on the chemical species present but also on their activities, approximated by their concentrations. About a century ago, Walther Nernst derived an equation that relates standard reduction potential ($E°$) to the actual reduction potential (E) at any concentration of oxidized and reduced species in a living cell:

$$E = E° + \frac{RT}{nF} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]} \quad \textbf{(13-5)}$$

where R and T have their usual meanings, n is the number of electrons transferred per molecule, and F is the Faraday constant, a proportionality constant that converts volts to joules (Table 13-1). At 298 K (25 °C), this expression reduces to

$$E = E° + \frac{0.026\ \text{V}}{n} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]} \quad \textbf{(13-6)}$$

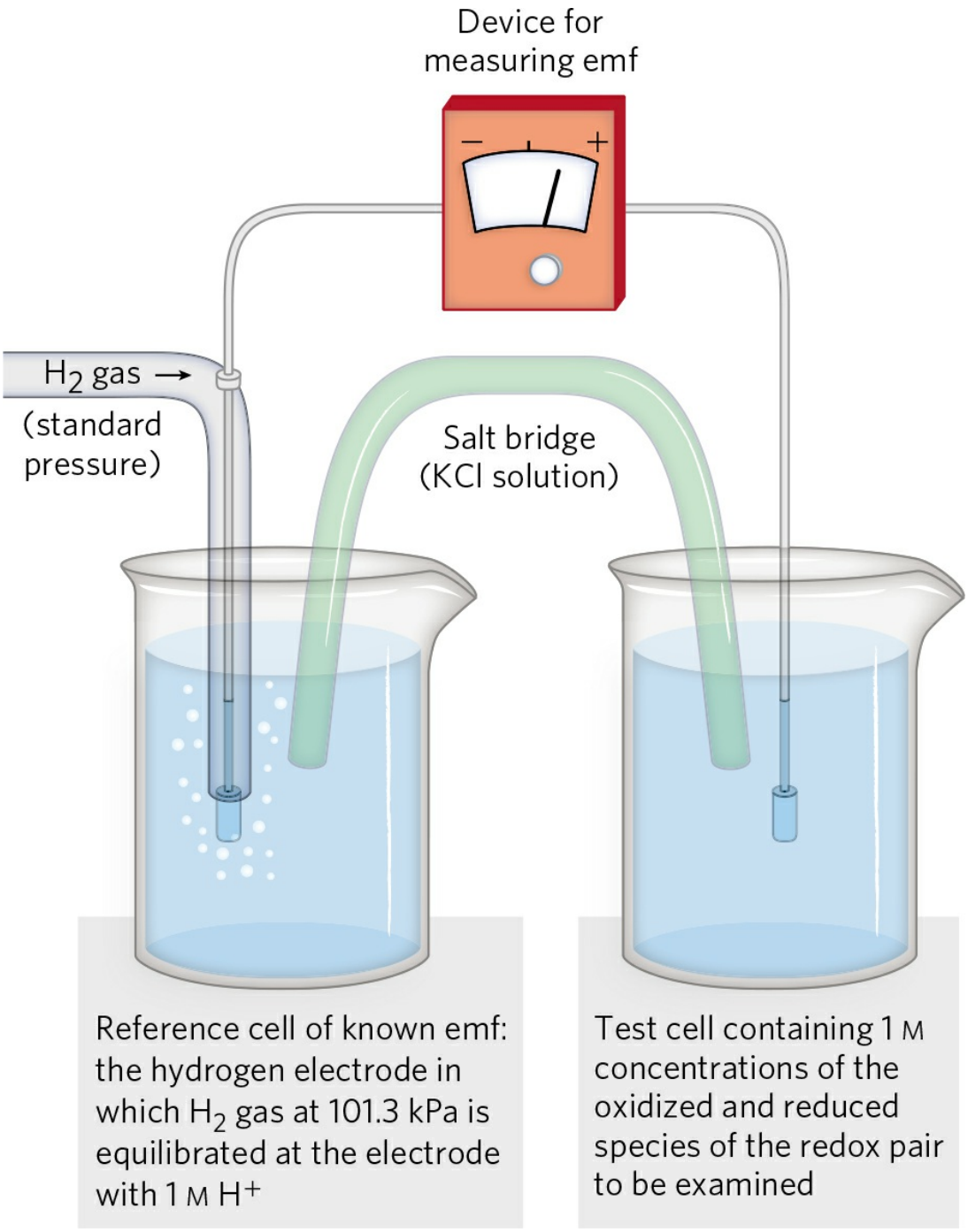


FIGURE 13-23 Measurement of the standard reduction potential (E′°) of a redox pair. Electrons flow from the test electrode to the reference electrode, or vice versa. The ultimate reference half-cell is the hydrogen electrode, as shown here, at pH 0. The electromotive force (emf) of this electrode is designated 0.00 V. At pH 7 in the test cell (at 25 °C), $E'°$ for the hydrogen electrode is −0.414 V. The direction of electron flow depends on the relative electron "pressure" or potential of the two cells. A salt bridge containing a saturated KCl solution provides a path for counter-ion movement between the test cell and the

reference cell. From the observed emf and the known emf of the reference cell, the experimenter can find the emf of the test cell containing the redox pair. The cell that gains electrons has, by convention, the more positive reduction potential.

›› **Key Convention:** Many half-reactions of interest to biochemists involve protons. As in the definition of $\Delta G'^\circ$, biochemists define the standard state for oxidation-reduction reactions as pH 7 and express a standard transformed reduction potential, E'°, the standard reduction potential at pH 7 and 25 °C. By convention, $\Delta E'^\circ$ for any redox reaction is given as E'° of the electron acceptor minus E'° of the electron donor. ‹‹

The standard reduction potentials given in Table 13-7 and used throughout this book are values for E'° and are therefore valid only for systems at neutral pH. Each value represents the potential difference when the conjugate redox pair, at 1 M concentrations, 25 °C, and pH 7, is connected with the standard (pH 0) hydrogen electrode. Notice in Table 13-7 that when the conjugate pair $2H^+/H_2$ at pH 7 is connected with the standard hydrogen electrode (pH 0), electrons tend to flow from the pH 7 cell to the standard (pH 0) cell; the measured E'° for the $2H^+/H_2$ pair is −0.414 V.

TABLE 13-7 Standard Reduction Potentials of Some Biologically Important Half-Reactions

Half-reaction	E'° (V)
$\frac{1}{2}\,O_2 + 2H^+ + 2e^- \rightarrow H_2O$	0.816
$Fe^{3+} = e \rightarrow Fe^{2+}$	0.771
$\mathbf{NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O}$	0.421
Cytochrome f $(Fe^{3+}) + e^- \rightarrow$ cytochrome f (Fe^{2+})	0.365
$\mathbf{Fe(CN)_6^{3-}}$ $\mathbf{(\textit{ferricyanide}) + e^- \rightarrow Fe(CN)_6^{4-}}$	0.36
Cytochrome a_3 $(Fe^{3+}) = e^- \rightarrow$ cytochrome a_3 (Fe^{2+})	0.35

$O_2 = 2H^+ = 2e \rightarrow H_2O_2$	0.295
Cytochrome a (Fe^{3+}) = e^- → cytochrome a (Fe^{2+})	0.29
Cytochrome c (Fe^{3+}) = e^- → cytochrome c (Fe^{2+})	0.254
Cytochrome c_1 (Fe^{3+}) = e^- → cytochrome c_1 (Fe^{2+})	0.22
Cytochrome b (Fe^{3+}) = e^- → cytochrome b (Fe^{2+})	0.077
Ubiquinone = $2H^+ = 2e$ → ubiquinol	0.045
Fumarate^{2-} → = $2H^+ = 2e^-$ → succinate^{2-}	0.031
$2H^+ = 2e^- \rightarrow H_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA = $2H^+ = 2e^-$ → butyryl-CoA	−0.015
Oxaloacetate^{2-} + $2H^+ = 2e^-$ → malate^{2-}	−0.166
Pyruvate$^-$ + $2H^+ = 2e^-$ → lactate$^-$	−0.185
Acetaldehyde + $2H^+ + 2e^-$ → ethanol	−0.197
FAD + $2H^+ + 2e^- \rightarrow FADH_2$	−0.219[a]
Glutathione = $2H^+ = 2e^-$ → 2 reduced glutathione	−0.23
$S + 2H^+ + 2e^- \rightarrow H_2S$	−0.243
Lipoic acid + $2H^+ + 2e^-$ → dihydrolipoic acid	−0.29
$NAD^+ + H^+ + 2e^- \rightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \rightarrow NADPH$	−0.324
Acetoacetate + $2H^+ + 2e^-$ → β-hydroxybutyrate	−0.346
α-Ketoglutarate + $CO_2 + 2H^+ + 2e^-$ → isocitrate	−0.38
$2H^+ + 2e^- \rightarrow H_2$ (at pH 7)	−0.414
Ferredoxin (Fe^{3+}) + e^- → ferredoxin (Fe^{2+})	−0.432

Source: Data mostly from R. A. Loach, in *Handbook of Biochemistry and Molecular Biology,* 3rd edn (G. D. Fasman, ed.), *Physical and Chemical Data*, Vol. 1, p. 122, CRC Press, 1976.

[a]This is the value for free FAD; FAD bound to a specific flavoprotein (e.g., succinate dehydrogenase) has a different E′° that depends on its protein environment.

Standard Reduction Potentials Can Be Used to Calculate Free-Energy Change

Why are reduction potentials so useful to the biochemist? When E values have been determined for any two half-cells, relative to the standard hydrogen electrode, we also know their reduction potentials relative to each other. We can then predict the direction in which electrons will tend to flow when the two half-cells are connected through an external circuit or when components of both half-cells are present in the same solution. Electrons tend to flow to the half-cell with the more positive E, and the strength of that tendency is proportional to ΔE, the difference in reduction potential. The energy made available by this spontaneous electron flow (the free-energy change, ΔG, for the oxidation-reduction reaction) is proportional to ΔE:

$$\Delta G = -nF\Delta E \text{ or } \Delta G'^{\circ} = -nF\Delta E'^{\circ} \quad (13\text{-}7)$$

where n is the number of electrons transferred in the reaction. With this equation we can calculate the actual free-energy change for any oxidation-reduction reaction from the values of $\Delta E'^{\circ}$ in a table of reduction potentials (Table 13-7) and the concentrations of reacting species.

WORKED EXAMPLE 13-3 Calculation of ΔG′° and ΔG of a Redox Reaction

Calculate the standard free-energy change, $\Delta G'^{\circ}$, for the reaction in which acetaldehyde is reduced by the biological electron carrier NADH:

$$\text{Acetaldehyde} + \text{NADH} + \text{H}^+ \rightarrow \text{ethanol} + \text{NAD}^+$$

Then calculate the *actual* free-energy change, ΔG, when [acetaldehyde] and [NADH] are 1.00 M, and [ethanol] and [NAD^+] are 0.100 M. The relevant half-reactions and their $E'°$ values are:

$$(1)\quad \text{Acetaldehyde} + 2\text{H}^+ + 2e^- \rightarrow \text{ethanol}\quad E'^\circ = -0.197\,\text{V}$$

$$(2)\quad \text{NAD}^+ + 2\text{H}^+ + 2e^- \rightarrow \text{NADH} + \text{H}^+\quad E'^\circ = -0.320\,\text{V}$$

Remember that, by convention, $\Delta E'°$ is $E'°$ of the electron acceptor minus $E'°$ of the electron donor. It represents the difference between the electron affinities of the two half-reactions in the table of reduction potentials (Table 13-7). Note that the more widely separated the two half-reactions in the table, the more energetic the electron-transfer reaction when the two half-reactions occur together. By convention, in tables of reduction potentials, all half-reactions are represented as reductions, but when two half-reactions occur together, one of them must be an oxidation. Although that half-reaction will go in the opposite direction from that shown in Table 13-7, we *do not change the sign* of that half-reaction before calculating $\Delta E'°$, because $\Delta E'°$ is *defined* as a difference of reduction potentials.

Solution: Because acetaldehyde is accepting electrons ($n = 2$) from NADH, $\Delta E'° = -0.197\ \text{V} - (-0.320\ \text{V}) = 0.123\ \text{V}$. Therefore,

$$\begin{aligned}\Delta G'^\circ &= -nF\Delta E'^\circ = -2\,(96.5\,\text{kJ/V}\cdot\text{mol})\ (0.123\,\text{V})\\ &= -23.7\,\text{kJ/mol}\end{aligned}$$

This is the free-energy change for the oxidation-reduction reaction at 25 °C and pH 7, when acetaldehyde, ethanol, NAD^+, and NADH are all present at 1.00 M concentrations.

To calculate ΔG when [acetaldehyde] and [NADH] are 1.00 M, and [ethanol] and [NAD^+] are 0.100 M, we can use Equation 13-4 and the standard free-energy change calculated above:

$$\begin{aligned}\Delta G &= \Delta G'^{\circ} + RT \ln \frac{[\text{ethanol}][\text{NAD}^+]}{[\text{acetaldehyde}][\text{NADH}]} \\ &= -23.7\,\text{kJ/mol} + (8.315\,\text{J/mol}\cdot\text{K})(298\,\text{K})\, ln \frac{(0.100\,\text{M})(0.100\,\text{M})}{(1.00\,\text{M})(1.00\,\text{M})} \\ &= -23.7\,\text{kJ/mol} + (2.48\,\text{J/mol}) \ln 0.01 \\ &= -35.1\,\text{kJ/mol}\end{aligned}$$

This is the actual free-energy change at the specified concentrations of the redox pairs.

Cellular Oxidation of Glucose to Carbon Dioxide Requires Specialized Electron Carriers

The principles of oxidation-reduction energetics described above apply to the many metabolic reactions that involve electron transfers. For example, in many organisms, the oxidation of glucose supplies energy for the production of ATP. The complete oxidation of glucose:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

has a $\Delta G'^{\circ}$ of −2,840 kJ/mol. This is a much larger release of free energy than is required for ATP synthesis in cells (50 to 60 kJ/mol; see Worked Example 13-2). Cells convert glucose to CO_2 not in a single, high-energy-releasing reaction but rather in a series of controlled reactions, some of which are oxidations. The free energy released in these oxidation steps is of the same order of magnitude as that required for ATP synthesis from ADP, with some energy to spare. Electrons removed in these oxidation steps are transferred to coenzymes specialized for carrying electrons, such as NAD^+ and FAD (described below).

A Few Types of Coenzymes and Proteins Serve as Universal Electron Carriers

The multitude of enzymes that catalyze cellular oxidations channel electrons from their hundreds of different substrates into just a few types of universal electron carriers. The reduction of these carriers in catabolic processes results

in the conservation of free energy released by substrate oxidation. NAD, NADP, FMN, and FAD are water-soluble coenzymes that undergo reversible oxidation and reduction in many of the electron-transfer reactions of metabolism. The nucleotides NAD and NADP move readily from one enzyme to another; the flavin nucleotides FMN and FAD are usually very tightly bound to the enzymes, called flavoproteins, for which they serve as prosthetic groups. Lipid-soluble quinones such as ubiquinone and plastoquinone act as electron carriers and proton donors in the nonaqueous environment of membranes. Iron-sulfur proteins and cytochromes, which have tightly bound prosthetic groups that undergo reversible oxidation and reduction, also serve as electron carriers in many oxidation-reduction reactions. Some of these proteins are water-soluble, but others are peripheral or integral membrane proteins (see Fig. 11-6).

We conclude this chapter by describing some chemical features of nucleotide coenzymes and some of the enzymes (dehydrogenases and flavoproteins) that use them.

.

NADH and NADPH Act with Dehydrogenases as Soluble Electron Carriers

Nicotinamide adenine dinucleotide (NAD; NAD^+ in its oxidized form) and its close analog nicotinamide adenine dinucleotide phosphate (NADP; $NADP^+$ when oxidized) are composed of two nucleotides joined through their phosphate groups by a phosphoanhydride bond **(Fig. 13-24a)**. Because the nicotinamide ring resembles pyridine, these compounds are sometimes called **pyridine nucleotides**. The vitamin niacin is the source of the nicotinamide moiety in nicotinamide nucleotides.

Both coenzymes undergo reversible reduction of the nicotinamide ring (Fig. 13-24). As a substrate molecule undergoes oxidation (dehydrogenation), giving up two hydrogen atoms, the oxidized form of the nucleotide (NAD^+ or $NADP^+$) accepts a hydride ion ($:H^+$, the equivalent of a proton and two electrons) and is reduced (to NADH or NADPH). The second proton removed from the substrate is released to the aqueous solvent. The half-reactions for these nucleotide cofactors are

$$NAD^+ + 2e^- + 2H^+ \rightarrow NADH + H^+$$
$$NADP^+ + 2e^- + 2H^+ \rightarrow NADPH + H^+$$

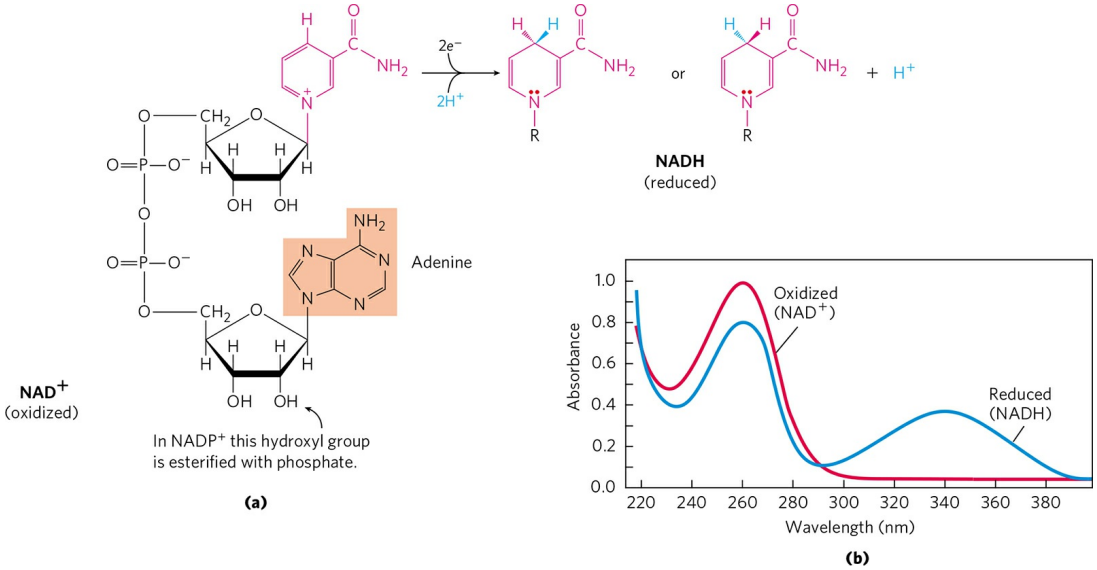


FIGURE 13-24 NAD and NADP. (a) Nicotinamide adenine dinucleotide, NAD^+, and its phosphorylated analog, $NADP^+$, undergo reduction to NADH and NADPH, accepting a hydride ion (two electrons and one proton) from an oxidizable substrate. The hydride ion is added to either the front or the back of the planar nicotinamide ring. **(b)** The UV absorption spectra of NAD^+ and NADH. Reduction of the nicotinamide ring produces a new, broad absorption band with a maximum at 340 nm. The production of NADH during an enzyme-catalyzed reaction can be conveniently followed by observing the appearance of the absorbance at 340 nm (molar extinction coefficient ε_{340} = 6,200 M^{-1} cm^{-1}).

Reduction of NAD^+ or $NADP^+$ converts the benzenoid ring of the nicotinamide moiety (with a fixed positive charge on the ring nitrogen) to the quinonoid form (with no charge on the nitrogen). The reduced nucleotides absorb light at 340 nm; the oxidized forms do not (Fig. 13-24b). Biochemists use this difference in absorption to assay reactions involving these coenzymes. Note that the plus sign in the abbreviations NAD^+ and $NADP^+$ does *not* indicate the net charge on these molecules (in fact, both are negatively charged); rather, it indicates that the nicotinamide ring is in its oxidized form, with a positive charge on the nitrogen atom. In the abbreviations NADH and NADPH, the "H" denotes the added hydride ion.

To refer to these nucleotides without specifying their oxidation state, we use NAD and NADP.

The total concentration of NAD^+ + NADH in most tissues is about 10^{-5} M; that of $NADP^+$ + NADPH is about 10^{-6} M. In many cells and tissues, the ratio of NAD^+ (oxidized) to NADH (reduced) is high, favoring hydride transfer from a substrate *to* NAD^+ to form NADH. By contrast, NADPH is generally present at a higher concentration than $NADP^+$, favoring hydride transfer *from* NADPH to a substrate. This reflects the specialized metabolic roles of the two coenzymes: NAD^+ generally functions in oxidations—usually as part of a catabolic reaction; NADPH is the usual coenzyme in reductions—nearly always as part of an anabolic reaction. A few enzymes can use either coenzyme, but most show a strong preference for one over the other. Also, the processes in which these two cofactors function are segregated in eukaryotic cells: for example, oxidations of fuels such as pyruvate, fatty acids, and α-keto acids derived from amino acids occur in the mitochondrial matrix, whereas reductive biosynthetic processes such as fatty acid synthesis take place in the cytosol. This functional and spatial specialization allows a cell to maintain two distinct pools of electron carriers, with two distinct functions.

More than 200 enzymes are known to catalyze reactions in which NAD^+ (or $NADP^+$) accepts a hydride ion from a reduced substrate, or NADPH (or NADH) donates a hydride ion to an oxidized substrate. The general reactions are

$$\mathbf{AH_2 + NAD^+ \rightarrow A + NADH + H^+}$$
$$\mathbf{A + NADPH + H^+ \rightarrow AH_2 + NADP^+}$$

where AH_2 is the reduced substrate and A is the oxidized substrate. The general name for an enzyme of this type is **oxidoreductase**; they are also commonly called dehydrogenases. For example, alcohol dehydrogenase catalyzes the first step in the catabolism of ethanol, in which ethanol is oxidized to acetaldehyde:

$$\underset{\text{Ethanol}}{\mathbf{CH_3CH_2OH}} + \mathbf{NAD^+} \rightarrow \underset{\text{Acetaldehyde}}{\mathbf{CH_3CHO}} + \mathbf{NADH + H^+}$$

Notice that one of the carbon atoms in ethanol has lost a hydrogen; the

compound has been oxidized from an alcohol to an aldehyde (refer again to Fig. 13-22 for the oxidation states of carbon).

Most dehydrogenases that use NAD or NADP bind the cofactor in a conserved protein domain called the Rossmann fold (named for Michael Rossmann, who deduced the structure of lactate dehydrogenase and first described this structural motif). The Rossmann fold typically consists of a six-stranded parallel β sheet and four associated α helices **(Fig. 13-25)**.

The association between a dehydrogenase and NAD or NADP is relatively loose; the coenzyme readily diffuses from one enzyme to another, acting as a water-soluble carrier of electrons from one metabolite to another. For example, in the production of alcohol during fermentation of glucose by yeast cells, a hydride ion is removed from glyceraldehyde 3-phosphate by one enzyme (glyceraldehyde 3-phosphate dehydrogenase) and transferred to NAD^+. The NADH produced then leaves the enzyme surface and diffuses to another enzyme (alcohol dehydrogenase), which transfers a hydride ion to acetaldehyde, producing ethanol:

$$\begin{aligned}
(1)\quad & \text{Glyceraldehyde 3-phosphate} + \text{NAD}^+ \\
& \rightarrow \text{3-phosphoglycerate} + \text{NADH} + \text{H}^+ \\
(2)\quad & \text{Acetaldehyde} + \text{NADH} + \text{H}^+ \rightarrow \text{ethanol} + \text{NAD}^+ \\
\hline
\textit{Sum}: \quad & \text{Glyceraldehyde 3-phosphate} + \text{acetaldehyde} \\
& \rightarrow \text{3-phosphoglycerate} + \text{ethanol}
\end{aligned}$$

Notice that in the overall reaction there is no net production or consumption of NAD^+ or NADH; the coenzymes function catalytically and are recycled repeatedly without a net change in the total amount of NAD^+ + NADH.

Both reduced and oxidized forms of NAD and NADP serve as allosteric effectors of proteins in catabolic pathways. As we describe in later chapters, the ratios NAD^+/NADH and $NADP^+$/NADPH serve as sensitive gauges of a cell's fuel supply, allowing rapid, appropriate changes in energy-yielding and energy-dependent metabolism.

NAD Has Important Functions in Addition to Electron Transfer

Some key cellular functions are regulated by enzymes that use NAD^+ not as a redox cofactor but as a substrate in a coupled reaction in which the availability of NAD^+ can be an indicator of the cell's energy status. In DNA replication and repair, the enzyme DNA ligase is adenylylated and then transfers the AMP to a 5′ phosphate in a nicked DNA (see Fig. 25-16); in bacteria, NAD^+ serves as the source of the activating AMP group. A family of proteins called sirtuins regulate the activity of proteins in diverse cellular pathways by deacetylating the ε-amino group of an acetylated Lys residue. The deacetylation is coupled to NAD^+ hydrolysis, yielding *O*-acetyl-ADP-ribose and nicotinamide. Among the cellular processes regulated by sirtuins are inflammation, apoptosis, aging, and DNA transcription; deacetylation by a sirtuin alters the charge on histones, influencing which genes are expressed (see p. 1149). The availability of NAD^+ for these types of reactions may indicate that the cell is undergoing stress and that pathways designed to respond to stress should be activated.

NAD^+ also plays an important role in cholera infections (see Box 12-1). Cholera toxin has an enzymatic activity that transfers ADP-ribose

from NAD^+ to a G protein involved in regulating ion fluxes in the cells lining the gut. This ADP-ribosylation blocks water retention, causing the diarrhea and dehydration characteristic of cholera. ■

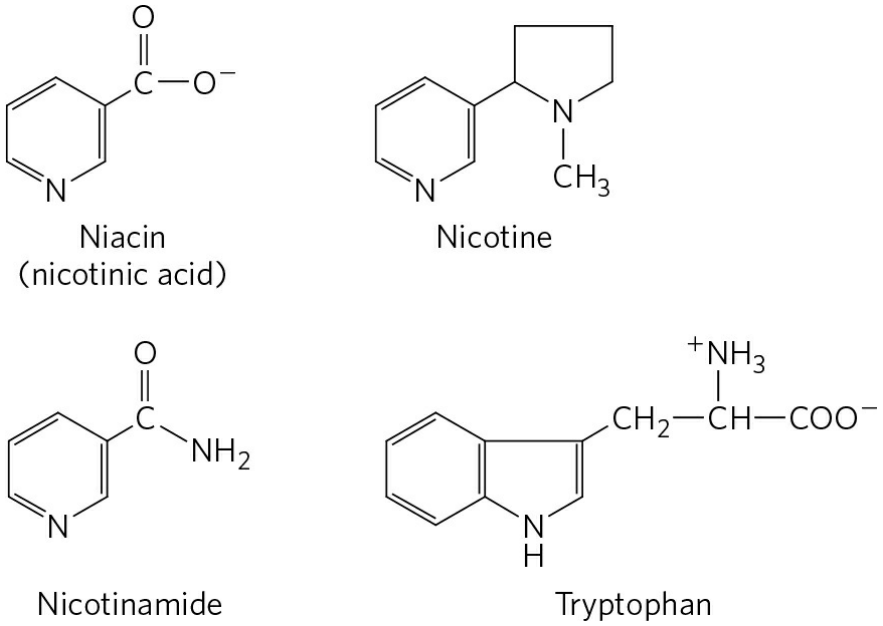


FIGURE 13-26 Niacin (nicotinic acid) and its derivative nicotinamide. The biosynthetic precursor of these compounds is tryptophan. In the laboratory, nicotinic acid was first produced by oxidation of the natural product nicotine—thus the name. Both nicotinic acid and nicotinamide cure pellagra, but nicotine (from cigarettes or elsewhere) has no curative activity.

Dietary Deficiency of Niacin, the Vitamin Form of NAD and NADP, Causes Pellagra

As we noted in Chapter 6 and will discuss further in later chapters, most coenzymes are derived from the substances we call vitamins. The pyridine-like rings of NAD and NADP are derived from the vitamin **niacin** (nicotinic acid; **Fig. 13-26**), which is synthesized from tryptophan. Humans generally cannot synthesize sufficient quantities of niacin, and this is especially so for individuals with diets low in tryptophan (maize, for example, has a low tryptophan content). Niacin deficiency, which affects all the NAD(P)-dependent dehydrogenases, causes the serious human disease pellagra (Italian for "rough skin") and a related disease in dogs, blacktongue. Pellagra is characterized by the "three Ds": dermatitis, diarrhea, and dementia, followed in many cases by death. A century ago, pellagra was a

Flavin Nucleotides Are Tightly Bound in Flavoproteins

Flavoproteins are enzymes that catalyze oxidation-reduction reactions using either flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as coenzyme **(Fig. 13-27)**. These coenzymes, the **flavin nucleotides**, are derived from the vitamin riboflavin. The fused ring structure of flavin nucleotides (the isoalloxazine ring) undergoes reversible reduction, accepting either one or two electrons in the form of one or two hydrogen atoms (each atom an electron plus a proton) from a reduced substrate. The fully reduced forms are abbreviated $FADH_2$ and $FMNH_2$. When a fully oxidized flavin nucleotide accepts only one electron (one hydrogen atom), the semiquinone form of the isoalloxazine ring is produced, abbreviated $FADH^{\bullet}$ and $FMNH^{\bullet}$. Because flavin nucleotides have a slightly different chemical specialty from that of the

nicotinamide coenzymes—the ability to participate in either one- or two-electron transfers—flavoproteins are involved in a greater diversity of reactions than the NAD(P)-linked dehydrogenases.

Like the nicotinamide coenzymes (Fig. 13-24), the flavin nucleotides undergo a shift in a major absorption band on reduction (again, useful to biochemists who want to monitor reactions involving these coenzymes). Flavoproteins that are fully reduced (two electrons accepted) generally have an absorption maximum near 360 nm. When partially reduced (one electron), they acquire another absorption maximum at about 450 nm; when fully oxidized, the flavin has maxima at 370 and 440 nm.

The flavin nucleotide in most flavoproteins is bound rather tightly to the protein, and in some enzymes, such as succinate dehydrogenase, it is bound covalently. Such tightly bound coenzymes are properly called prosthetic groups. They do not transfer electrons by diffusing from one enzyme to another; rather, they provide a means by which the flavoprotein can temporarily hold electrons while it catalyzes electron transfer from a reduced substrate to an electron acceptor. One important feature of the flavoproteins is the variability in the standard reduction potential ($E'°$) of the bound flavin nucleotide. Tight association between the enzyme and prosthetic group confers on the flavin ring a reduction potential typical of that particular flavoprotein, sometimes quite different from the reduction potential of the free flavin nucleotide. FAD bound to succinate dehydrogenase, for example, has an $E'°$ close to 0.0 V, compared with −0.219 V for free FAD; $E'°$ for other flavoproteins ranges from −0.40 V to +0.06 V. Flavoproteins are often very complex; some have, in addition to a flavin nucleotide, tightly bound inorganic ions (iron or molybdenum, for example) capable of participating in electron transfers.

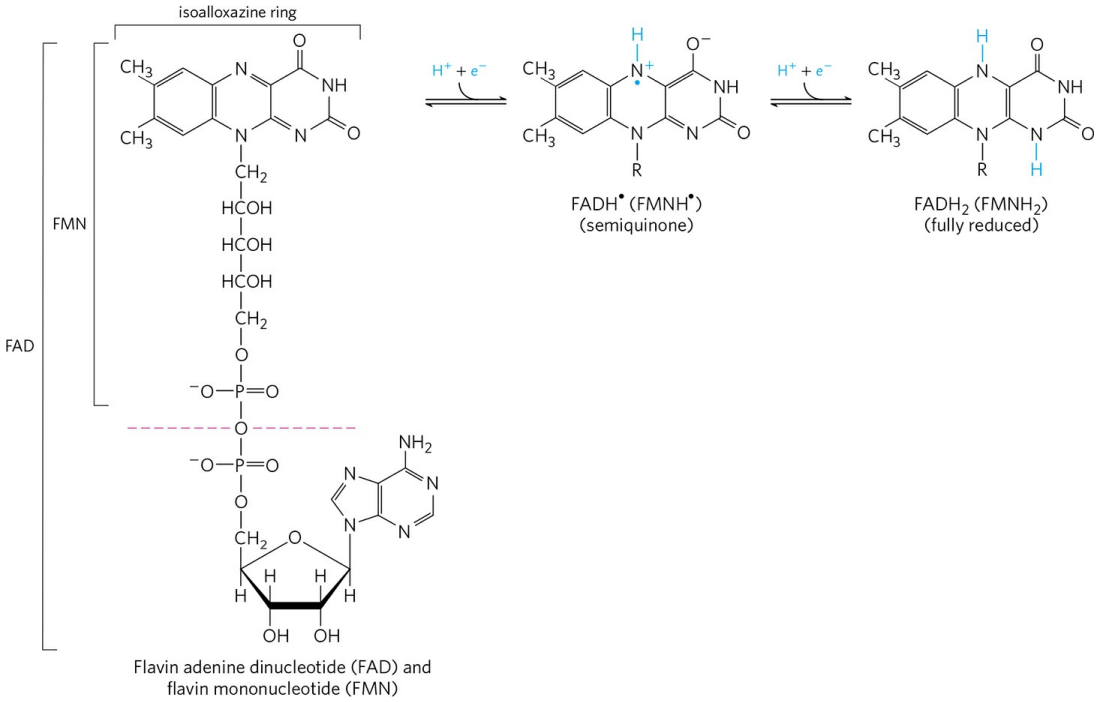


FIGURE 13-27 Oxidized and reduced FAD and FMN. FMN consists of the structure above the dashed red line across the FAD molecule (oxidized form). The flavin nucleotides accept two hydrogen atoms (two electrons and two protons), both of which appear in the flavin ring system (isoalloxazine ring). When FAD or FMN accepts only one hydrogen atom, the semiquinone, a stable free radical, forms.

Certain flavoproteins have a distinctly different role, as light receptors. **Cryptochromes** are a family of flavoproteins, widely distributed in the eukaryotic phyla, that mediate the effects of blue light on plant development and the effects of light on mammalian circadian rhythms (oscillations in physiology and biochemistry, with a 24-hour period). The cryptochromes are homologs of another family of flavoproteins, the photolyases. Found in both bacteria and eukaryotes, **photolyases** use the energy of absorbed light to repair chemical defects in DNA.

We examine the function of flavoproteins as electron carriers in Chapters 19 and 20, when we consider their roles in oxidative phosphorylation (in mitochondria) and photophosphorylation (in chloroplasts), and we describe the photolyase reactions in Chapter 25.

SUMMARY 13.4 Biological Oxidation-Reduction Reactions

■ In many organisms, a central energy-conserving process is the stepwise oxidation of glucose to CO_2, in which some of the energy of oxidation is conserved in ATP as electrons are passed to O_2.

■ Biological oxidation-reduction reactions can be described in terms of two half-reactions, each with a characteristic standard reduction potential, $E'°$.

■ When two electrochemical half-cells, each containing the components of a half-reaction, are connected, electrons tend to flow to the half-cell with the higher reduction potential. The strength of this tendency is proportional to the difference between the two reduction potentials (ΔE) and is a function of the concentrations of oxidized and reduced species.

■ The standard free-energy change for an oxidation-reduction reaction is directly proportional to the difference in standard reduction potentials of the two half-cells: $\Delta G'° = -nF\Delta E'°$.

■ Many biological oxidation reactions are dehydrogenations in which one or two hydrogen atoms ($H^+ + e^-$) are transferred from a substrate to a hydrogen acceptor. Oxidation-reduction reactions in living cells involve specialized electron carriers.

■ NAD and NADP are the freely diffusible coenzymes of many dehydrogenases. Both NAD^+ and $NADP^+$ accept two electrons and one proton. In addition to its role in oxidation-reduction reactions, NAD^+ is the source of AMP in the bacterial DNA ligase reaction and of ADP-ribose in the cholera toxin reaction, and is hydrolyzed in the deacetylation of proteins by some sirtuins.

■ FAD and FMN, the flavin nucleotides, serve as tightly bound prosthetic groups of flavoproteins. They can accept either one or two electrons and one or two protons. Flavoproteins also serve as light receptors in cryptochromes and photolyases.

pyridine nucleotide
oxidoreductase
flavoprotein
flavin nucleotides
cryptochrome
photolyase

Problems

1. Entropy Changes during Egg Development Consider a system consisting of an egg in an incubator. The white and yolk of the egg contain proteins, carbohydrates, and lipids. If fertilized, the egg is transformed from a single cell to a complex organism. Discuss this irreversible process in terms of the entropy changes in the system, surroundings, and universe. Be sure that you first clearly define the system and surroundings.

2. Calculation of $\Delta G'^{\circ}$ from an Equilibrium Constant Calculate the standard free-energy change for each of the following metabolically important enzyme-catalyzed reactions, using the equilibrium constants given for the reactions at 25 °C and pH 7.0.

(a) Glutamate + oxaloacetate $\overset{\text{aspartate aminotranferase}}{\rightleftharpoons}$ aspartate + α-ketogluta

(b) Dihydroxyacetone phosphate $\overset{\text{triose phosphate isomerase}}{\rightleftharpoons}$ glyceraldehyde 3-phosphate

(c) Fructose 6-phosphate + ATP $\overset{\text{phosphofructokinase}}{\rightleftharpoons}$ fructose 1,6-bisphosphate + ADP

3. Calculation of the Equilibrium Constant from $\Delta G'^{\circ}$ Calculate the equilibrium constant K'_{eq} for each of the following reactions at pH 7.0 and 25 °C, using the $\Delta G'^{\circ}$ values in Table 13-4.

$$\text{(a)}\quad \text{Glucose 6-phosphate} + H_2O \xrightleftharpoons{\text{glucose 6-phosphatase}} \text{glucose} + P_i$$

$$\text{(b)}\quad \text{Lactose} + H_2O \xrightleftharpoons{\beta\text{-galactosidase}} \text{glucose} + \text{galactose}$$

$$\text{(c)}\quad \text{Malate} \xrightleftharpoons{\text{fumarase}} \text{fumarate} + H_2O$$

4. Experimental Determination of K'_{eq} and $\Delta G'^{\circ}$ If a 0.1 M solution of glucose 1-phosphate at 25 °C is incubated with a catalytic amount of phosphoglucomutase, the glucose 1-phosphate is transformed to glucose 6-phosphate. At equilibrium, the concentrations of the reaction components are

$$\underset{4.5 \times 10^{-3}\ \text{M}}{\text{Glucose 1-phosphate}} \rightleftharpoons \underset{9.6 \times 10^{-2}\ \text{M}}{\text{glucose 6-phosphate}}$$

Calculate K'_{eq} and $\Delta G'^{\circ}$ for this reaction.

5. Experimental Determination of $\Delta G'^{\circ}$ for ATP Hydrolysis A direct measurement of the standard free-energy change associated with the hydrolysis of ATP is technically demanding because the minute amount of ATP remaining at equilibrium is difficult to measure accurately. The value of $\Delta G'^{\circ}$ can be calculated indirectly, however, from the equilibrium constants of two other enzymatic reactions having less favorable equilibrium constants:

$$\text{Glucose 6-phosphate} + H_2O \rightarrow \text{glucose} + P_i \qquad K'_{eq} = 270$$

$$\text{ATP} + \text{glucose} \rightarrow \text{ADP} + \text{glucose 6-phosphate} \qquad K'_{eq} = 890$$

Using this information for equilibrium constants determined at 25 °C, calculate the standard free energy of hydrolysis of ATP.

6. Difference between $\Delta G'^{\circ}$ and ΔG Consider the following interconversion, which occurs in glycolysis (Chapter 14):

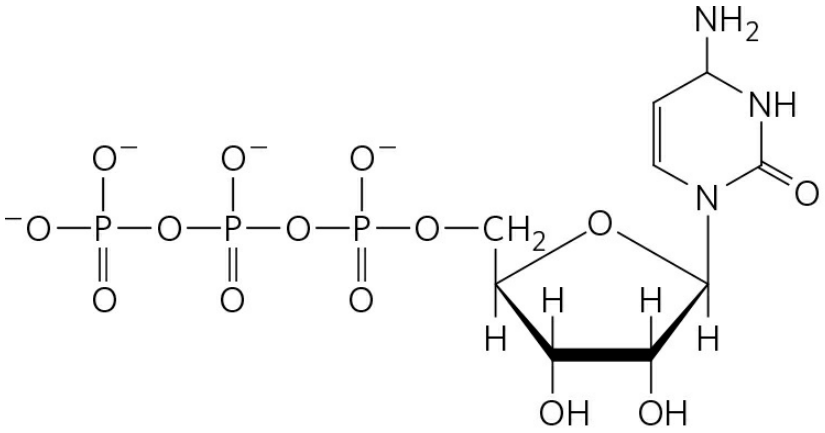
$$\text{Fructose 6-phosphate} \rightleftharpoons \text{glucose 6-phosphate} \qquad K'_{eq} = 1.97$$

(a) What is $\Delta G'^{\circ}$ for the reaction (K'_{eq} measured at 25 °C)?

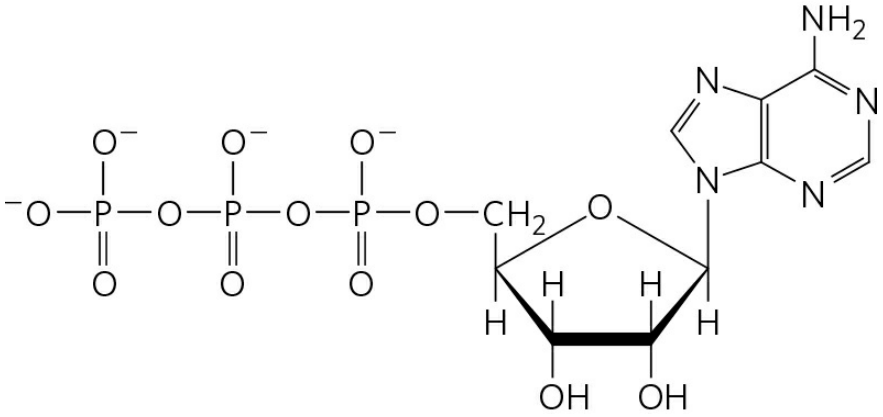
(b) If the concentration of fructose 6-phosphate is adjusted to 1.5 M and that of glucose 6-phosphate is adjusted to 0.50 M, what is ΔG?

(c) Why are $\Delta G'^{\circ}$ and ΔG different?

7. Free Energy of Hydrolysis of CTP Compare the structure of the nucleoside triphosphate CTP with the structure of ATP.



Cytidine triphosphate (CTP)



Adenosine triphosphate (ATP)

Now predict the K'_{eq} and $\Delta G'^{\circ}$ for the following reaction:

$$\text{ATP} + \text{CDP} \rightarrow \text{ADP} + \text{CTP}$$

8. Dependence of ΔG on pH The free energy released by the hydrolysis of ATP under standard conditions is −30.5 kJ/mol. If ATP is hydrolyzed under standard conditions except at pH 5.0, is more or less free energy released? Explain.

9. The $\Delta G'^{\circ}$ for Coupled Reactions Glucose 1-phosphate is converted into fructose 6-phosphate in two successive reactions:

$$\text{Glucose 1-phosphate} \rightarrow \text{glucose 6-phosphate}$$
$$\text{Glucose 6-phosphate} \rightarrow \text{fructose 6-phosphate}$$

Using the $\Delta G'^{\circ}$ values in Table 13-4, calculate the equilibrium constant, K'_{eq}, for the sum of the two reactions:

$$\text{Glucose 1-phosphate} \rightarrow \text{fructose 6-phosphate}$$

10. Effect of [ATP]/[ADP] Ratio on Free Energy of Hydrolysis of ATP Using Equation 13-4, plot ΔG against ln Q (mass-action ratio) at 25 °C for the concentrations of ATP, ADP, and P_i in the table below. $\Delta G'^{\circ}$ for the reaction is −30.5 kJ/mol. Use the resulting plot to explain why metabolism is regulated to keep the ratio [ATP]/[ADP] high.

	Concentration (mM)				
ATP	5	3	1	0.2	5
ADP	0.2	2.2	4.2	5.0	25
P_i	10	12.1	14.1	14.9	10

11. Strategy for Overcoming an Unfavorable Reaction: ATP-Dependent Chemical Coupling The phosphorylation of glucose to glucose 6-phosphate is the initial step in the catabolism of glucose. The direct phosphorylation of glucose by P_i is described by the equation

$$\text{Glucose} + P_i \rightarrow \text{glucose 6-phosphate} + H_2O \quad \Delta G'^{\circ} = 13.8\ \text{kJ/mol}$$

(a) Calculate the equilibrium constant for the above reaction at 37 °C. In the rat hepatocyte, the physiological concentrations of glucose and P_i are maintained at approximately 4.8 mM. What is the equilibrium concentration of glucose 6-phosphate obtained by the direct phosphorylation of glucose by P_i? Does this reaction represent a reasonable metabolic step for the catabolism of glucose? Explain.

(b) In principle, at least, one way to increase the concentration of glucose 6-phosphate is to drive the equilibrium reaction to the right by increasing the intracellular concentrations of glucose and P_i. Assuming a fixed concentration of P_i at 4.8 mM, how high would the intracellular concentration of glucose have to be to give an equilibrium concentration of glucose 6-phosphate of 250 μM (the normal physiological concentration)? Would this route be physiologically reasonable, given that the maximum solubility of glucose is less than 1 M?

(c) The phosphorylation of glucose in the cell is coupled to the hydrolysis of ATP; that is, part of the free energy of ATP hydrolysis is used to phosphorylate glucose:

$$\begin{array}{lll} (1)\ \text{Glucose} + \text{P}_\text{i} \rightarrow \text{glucose 6-phosphate} + \text{H}_2\text{O} & \Delta G'^\circ = 13.8\ \text{kJ/} \\ (2)\ \text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{P}_\text{i} & \Delta G'^\circ = -30.5 \\ \hline \textit{Sum}: \ \text{Glucose} + \text{ATP} \rightarrow \text{glucose 6-phosphate} + \text{ADP} \end{array}$$

Calculate K'_{eq} at 37 °C for the overall reaction. For the ATP-dependent phosphorylation of glucose, what concentration of glucose is needed to achieve a 250 μM intracellular concentration of glucose 6-phosphate when the concentrations of ATP and ADP are 3.38 mM and 1.32 mM, respectively? Does this coupling process provide a feasible route, at least in principle, for the phosphorylation of glucose in the cell? Explain.

(d) Although coupling ATP hydrolysis to glucose phosphorylation makes thermodynamic sense, we have not yet specified how this coupling is to take place. Given that coupling requires a common intermediate, one conceivable route is to use ATP hydrolysis to raise the intracellular concentration of P_i and thus drive the unfavorable phosphorylation of glucose by P_i. Is this a reasonable route? (Think about the solubility product, K_{sp}, of metabolic intermediates.)

(e) The ATP-coupled phosphorylation of glucose is catalyzed in hepatocytes by the enzyme glucokinase. This enzyme binds ATP and glucose to form a glucose-ATP-enzyme complex, and the phosphoryl group is transferred directly from ATP to glucose. Explain the advantages of this route.

12. Calculations of $\Delta G'^\circ$ for ATP-Coupled Reactions From data in Table 13-6, calculate the $\Delta G'^\circ$ value for the following reactions:

(a) Phosphocreatine + ADP → creatine + ATP

(b) ATP + fructose → ADP + fructose 6-phosphate

13. Coupling ATP Cleavage to an Unfavorable Reaction To explore the consequences of coupling ATP hydrolysis under physiological conditions to a thermodynamically unfavorable biochemical reaction, consider the hypothetical transformation X → Y, for which $\Delta G'^\circ$ = 20.0 kJ/mol.

(a) What is the ratio [Y]/[X] at equilibrium?

(b) Suppose X and Y participate in a sequence of reactions during which ATP is hydrolyzed to ADP and P_i. The overall reaction is

$$\text{X} + \text{ATP} + \text{H}_2\text{O} \rightarrow \text{Y} + \text{ADP} + \text{P}_\text{i}$$

Calculate [Y]/[X] for this reaction at equilibrium. Assume that the temperature is 25 °C and the equilibrium concentrations of ATP, ADP, and P_i are 1 M.

(c) We know that [ATP], [ADP], and [P_i] are *not* 1 M under physiological conditions. Calculate [Y]/[X] for the ATP-coupled reaction when the values of [ATP], [ADP], and [P_i] are those found in rat myocytes (Table 13-5).

14. Calculations of ΔG at Physiological Concentrations Calculate the actual, physiological ΔG for the reaction

$$\text{Phosphocreatine} + \text{ADP} \rightarrow \text{creatine} + \text{ATP}$$

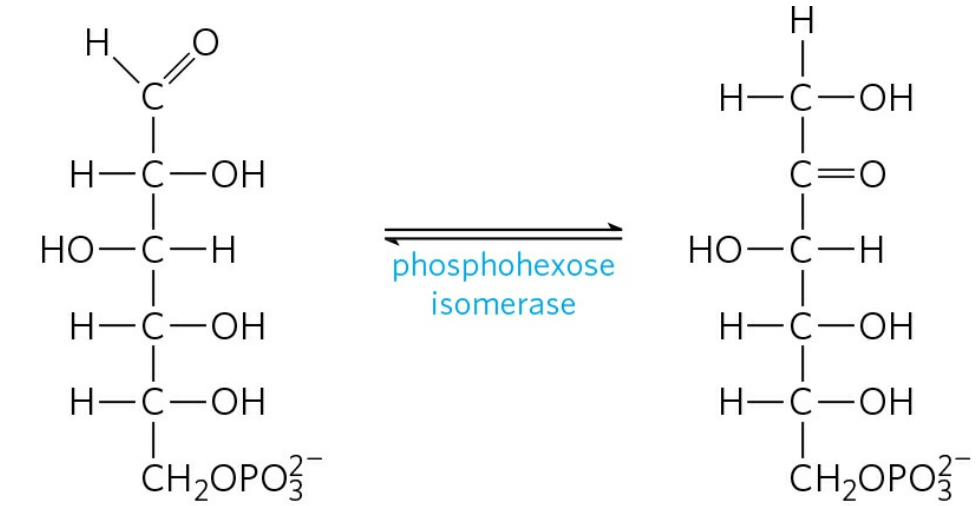
at 37 °C, as it occurs in the cytosol of neurons, with phosphocreatine at 4.7 mM, creatine at 1.0 mM, ADP at 0.73 mM, and ATP at 2.6 mM.

15. Free Energy Required for ATP Synthesis under Physiological Conditions In the cytosol of rat hepatocytes, the temperature is 37 °C and the mass-action ratio, Q, is

$$\frac{[\text{ATP}]}{[\text{ADP}][\text{P}_\text{i}]} = 5.33 \times 10^2\ \text{M}^{-1}$$

Calculate the free energy required to synthesize ATP in a rat hepatocyte.

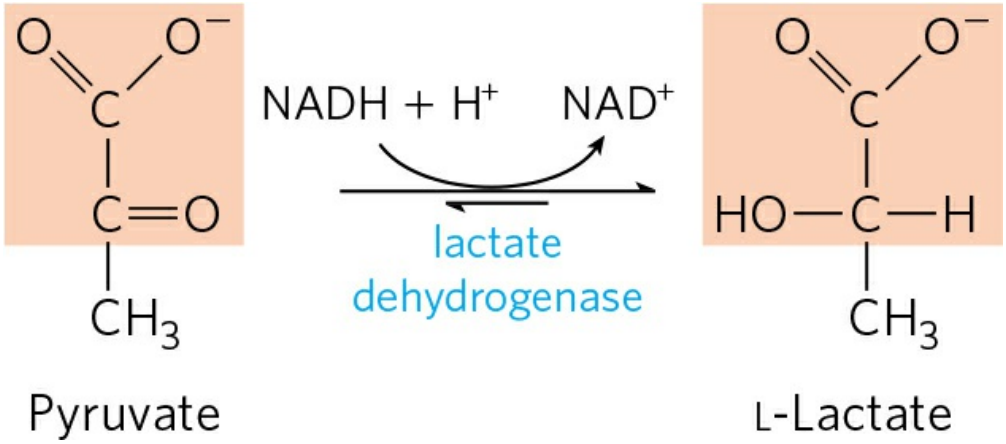
16. Chemical Logic In the glycolytic pathway, a six-carbon sugar (fructose 1,6-bisphosphate) is cleaved to form two three-carbon sugars, which undergo further metabolism (see Fig. 14-6). In this pathway, an isomerization of glucose 6-phosphate to fructose 6-phosphate (shown below) occurs two steps before the cleavage reaction (the intervening step is phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate (p. 539)).



Glucose 6-phosphate Fructose 6-phosphate

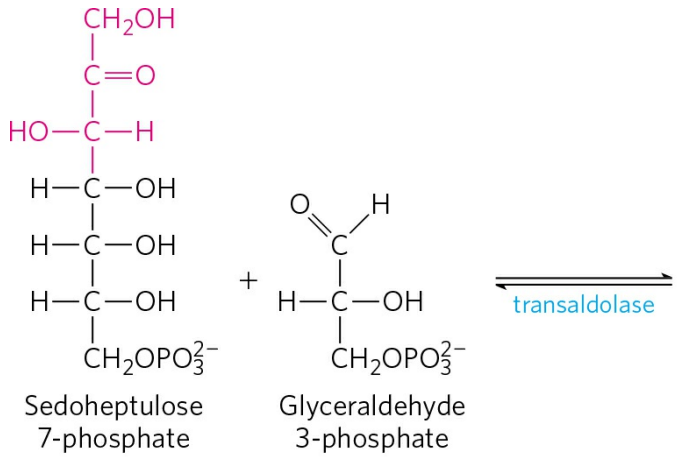
What does the isomerization step accomplish from a chemical perspective? (Hint: Consider what might happen if the C—C bond cleavage were to proceed without the preceding isomerization.)

17. Enzymatic Reaction Mechanisms I Lactate dehydrogenase is one of the many enzymes that require NADH as coenzyme. It catalyzes the conversion of pyruvate to lactate:



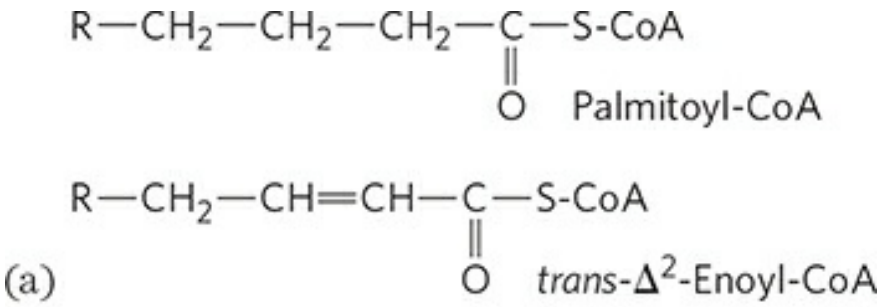
Draw the mechanism of this reaction (show electron-pushing arrows). (Hint: This is a common reaction throughout metabolism; the mechanism is similar to that catalyzed by other dehydrogenases that use NADH, such as alcohol dehydrogenase.)

18. Enzymatic Reaction Mechanisms II Biochemical reactions often look more complex than they really are. In the pentose phosphate pathway (Chapter 14), sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate react to form erythrose 4-phosphate and fructose 6-phosphate in a reaction catalyzed by transaldolase.

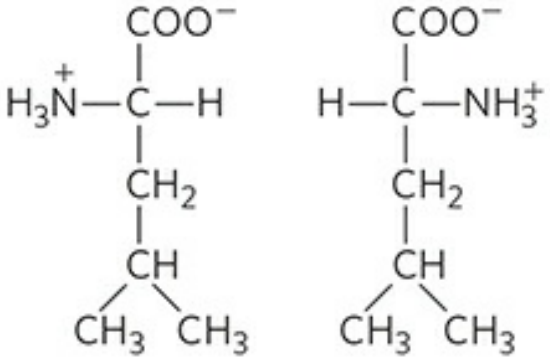


Draw a mechanism for this reaction (show electron-pushing arrows). (Hint: Take another look at aldol condensations, then consider the name of this enzyme.)

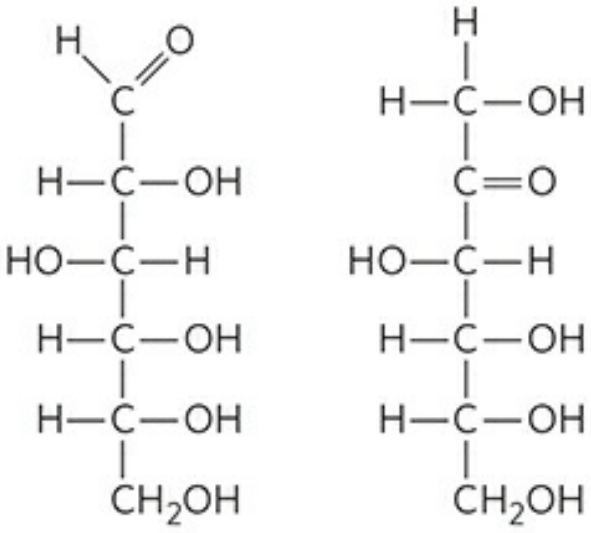
19. Recognizing Reaction Types For the following pairs of biomolecules, identify the type of reaction (oxidation-reduction, hydrolysis, isomerization, group transfer, or internal rearrangement) required to convert the first molecule to the second. In each case, indicate the general type of enzyme and cofactor(s) or reactants that would be required, and any other products that would result.



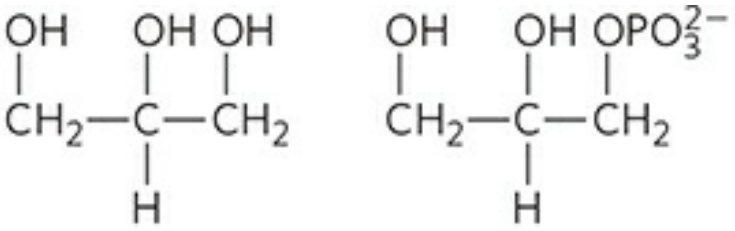
(a)



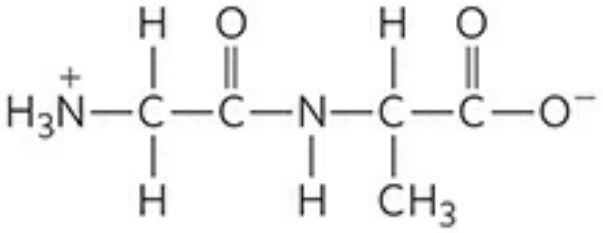
(b) L-Leucine D-Leucine



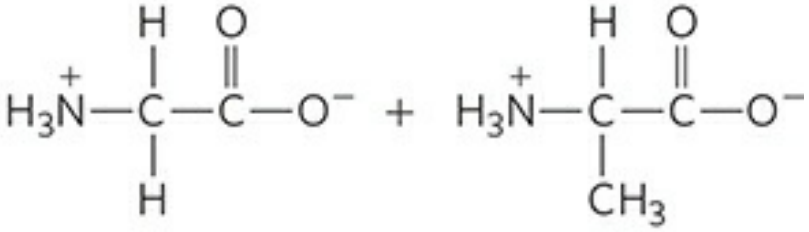
(c) Glucose Fructose



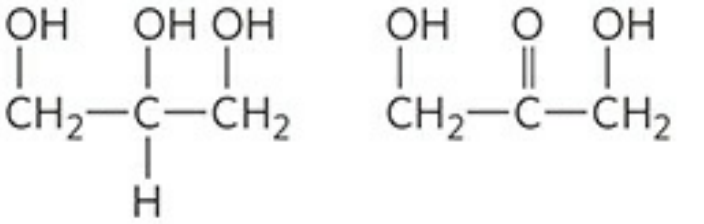
(d) Glycerol Glycerol 3-phosphate



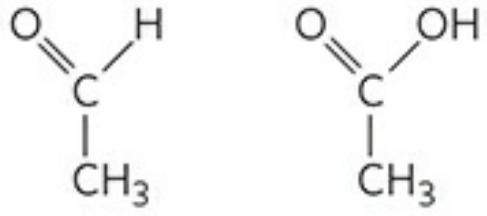
Glycylalanine



(e) Glycine Alanine

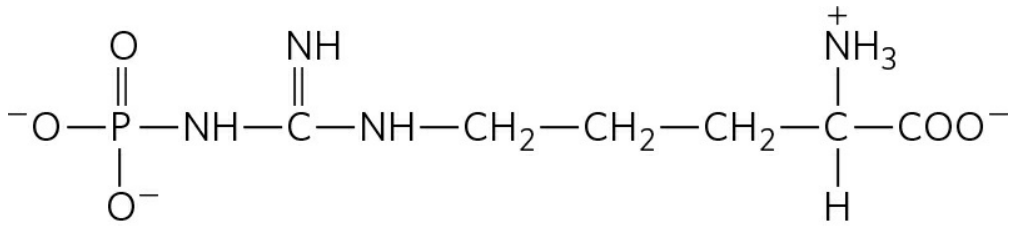


(f) Glycerol Dihydroxyacetone



(g) Acetaldehyde Acetic acid

20. Effect of Structure on Group Transfer Potential Some invertebrates contain phosphoarginine. Is the standard free energy of hydrolysis of this molecule more similar to that of glucose 6-phosphate or of ATP? Explain your answer.



Phosphoarginine

21. Polyphosphate as a Possible Energy Source The standard free energy of hydrolysis of inorganic polyphosphate (polyP) is about −20 kJ/mol for each P_i released. We calculated in Worked Example 13–2 that, in a cell, it takes about 50 kJ/mol of energy to synthesize ATP from ADP and P_i. Is it feasible for a cell to use polyphosphate to synthesize ATP from ADP? Explain your answer.

22. Daily ATP Utilization by Human Adults

(a) A total of 30.5 kJ/mol of free energy is needed to synthesize ATP from ADP and P_i when the reactants and products are at 1 M concentrations and the temperature is 25 °C (standard state). Because the actual physiological concentrations of ATP, ADP, and P_i are not 1 M, and the temperature is 37 °C, the free energy required to synthesize ATP under physiological conditions is different from $\Delta G'°$. Calculate the free energy required to synthesize ATP in the human hepatocyte when the physiological concentrations of ATP, ADP, and P_i are 3.5, 1.50, and 5.0 mM, respectively.

(b) A 68 kg (150 lb) adult requires a caloric intake of 2,000 kcal (8,360 kJ) of food per day (24 hours). The food is metabolized and the free energy is used to synthesize ATP, which then provides energy for the body's daily chemical and mechanical work. Assuming that the efficiency of converting food energy into ATP is 50%, calculate the weight of ATP used by a human adult in 24 hours. What percentage of the body weight does this represent?

(c) Although adults synthesize large amounts of ATP daily, their body weight, structure, and composition do not change significantly during this period. Explain this apparent contradiction.

23. Rates of Turnover of γ and β Phosphates of ATP If a small amount of ATP labeled with radioactive phosphorus in the terminal position, [γ-^{32}P]ATP, is added to a yeast extract, about half of the ^{32}P activity is found in P_i within a few minutes, but the concentration of ATP remains unchanged. Explain. If the same experiment is carried out using ATP labeled with ^{32}P in the central position, [β-^{32}P]ATP, the ^{32}P does not appear in P_i within such a short time. Why?

24. Cleavage of ATP to AMP and PP_i during Metabolism Synthesis of the activated form of acetate (acetyl-CoA) is carried out in an ATP-dependent process:

$$\text{Acetate} + \text{CoA} + \text{ATP} \rightarrow \text{acetyl-CoA} + \text{AMP} + PP_i$$

(a) The $\Delta G'^\circ$ for hydrolysis of acetyl-CoA to acetate and CoA is −32.2 kJ/mol and that for hydrolysis of ATP to AMP and PP_i is −30.5 kJ/mol. Calculate $\Delta G'^\circ$ for the ATP-dependent synthesis of acetyl-CoA.

(b) Almost all cells contain the enzyme inorganic pyrophosphatase, which catalyzes the hydrolysis of PP_i to P_i. What effect does the presence of this enzyme have on the synthesis of acetyl-CoA? Explain.

25. Energy for H^+ Pumping The parietal cells of the stomach lining contain membrane "pumps" that transport hydrogen ions from the cytosol (pH 7.0) into the stomach, contributing to the acidity of gastric juice (pH 1.0). Calculate the free energy required to transport 1 mol of hydrogen ions through these pumps. (Hint: See Chapter 11.) Assume a temperature of 37 °C.

26. Standard Reduction Potentials The standard reduction potential, E'°, of any redox pair is defined for the half-cell reaction:

$$\text{Oxidizing agent} + n\,\text{electrons} \rightarrow \text{reducing agent}$$

The E'° values for the NAD^+/NADH and pyruvate/lactate conjugate redox pairs are −0.32 V and −0.19 V, respectively.

(a) Which redox pair has the greater tendency to lose electrons? Explain.

(b) Which pair is the stronger oxidizing agent? Explain.

(c) Beginning with 1 M concentrations of each reactant and product at pH 7 and 25 °C, in which direction will the following reaction proceed?

$$\text{Pyruvate} + \text{NADH} + H^+ \rightleftharpoons \text{lactate} + NAD^+$$

(d) What is the standard free-energy change ($\Delta G'^\circ$) for the conversion of pyruvate to lactate?

(e) What is the equilibrium constant (K'_{eq}) for this reaction?

27. Energy Span of the Respiratory Chain Electron transfer in the mitochondrial respiratory chain may be represented by the net reaction equation

$$\text{NADH} + H^+ + \tfrac{1}{2}O_2 \rightleftharpoons H_2O + NAD^+$$

(a) Calculate $\Delta E'^\circ$ for the net reaction of mitochondrial electron transfer. Use E'° values in Table 13-7.

(b) Calculate $\Delta G'^\circ$ for this reaction.

(c) How many ATP molecules can *theoretically* be generated by this reaction if the free energy of ATP synthesis under cellular conditions is 52 kJ/mol?

28. Dependence of Electromotive Force on Concentrations Calculate the electromotive force (in volts) registered by an electrode immersed in a solution containing the following mixtures of NAD^+ and NADH at pH 7.0 and 25 °C, with reference to a half-cell of E'° 0.00 V.

(a) 1.0 mM NAD^+ and 10 mM NADH

(b) 1.0 mM NAD^+ and 1.0 mM NADH

(c) 10 mM NAD^+ and 1.0 mM NADH

29. Electron Affinity of Compounds List the following in order of increasing tendency to accept electrons: (a) α-ketoglutarate + CO_2 (yielding isocitrate); (b) oxaloacetate; (c) O_2; (d) $NADP^+$.

30. Direction of Oxidation-Reduction Reactions Which of the following reactions would you expect to proceed in the direction shown, under standard conditions, in the presence of the appropriate enzymes?

(a) Malate + NAD^+ → oxaloacetate + NADH + H^+

(b) Acetoacetate + NADH + H^+ → β-hydroxybutyrate + NAD^+

(c) Pyruvate + NADH + H^+ → lactate + NAD^+

(d) Pyruvate + β-hydroxybutyrate → lactate + acetoacetate

(e) Malate + pyruvate → oxaloacetate + lactate

(f) Acetaldehyde + succinate → ethanol + fumarate