

SUMMARY 13.2 Phosphoryl Group Transfers and ATP

- ATP is the chemical link between catabolism and anabolism. It is the energy currency of the living cell. The exergonic conversion of ATP to ADP and P_i , or to AMP and PP_i , is coupled to many endergonic reactions and processes.
- Direct hydrolysis of ATP is the source of energy in the conformational changes that produce muscle contraction but, in general, it is not ATP hydrolysis but the transfer of a phosphoryl, pyrophosphoryl, or adenylyl group from ATP to a substrate or enzyme molecule that couples the energy of ATP breakdown to endergonic transformations of substrates.
- Through these group transfer reactions, ATP provides the energy for anabolic reactions, including the synthesis of informational molecules, and for the transport of molecules and ions across membranes against concentration gradients and electrical potential gradients.
- Cells contain other metabolites with large, negative, free energies of hydrolysis, including phosphoenolpyruvate, 1,3-bisphosphoglycerate, and phosphocreatine. These high-energy compounds, like ATP, have a high phosphoryl group transfer potential; they are good donors of the phosphoryl group. Thioesters also have high free energies of hydrolysis.
- Inorganic polyphosphate, present in all cells, may serve as a reservoir of phosphoryl groups with high group transfer potential.

13.3 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. 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 contain a variety of molecular energy transducers, which convert the energy of electron flow into useful work.

We begin our discussion with a description of the general types of metabolic reactions in which electrons are transferred. After considering the theoretical and experimental basis for measuring the energy changes in oxidation reactions in terms of electromotive force, we discuss the relationship between this force, expressed in volts, and the free-energy change, expressed in joules. We conclude by describing 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 electromotive force (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 accomplish 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 electromotive force 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, accomplishing osmotic 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 electromotive force to proton-motive force, which is then used to power flagellar motion.

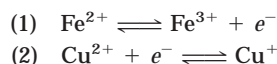
The principles of electrochemistry that govern energy changes in the macroscopic circuit with a motor and battery apply with equal validity to the molecular processes accompanying electron flow in living cells. We turn now to a discussion of those principles.

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,

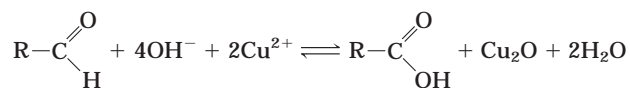


can be described in terms of two half-reactions:

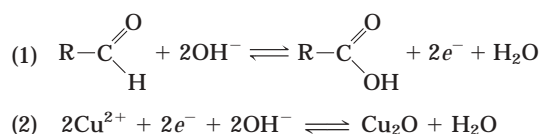


The electron-donating molecule in an oxidation-reduction reaction is called the reducing agent or reductant; the electron-accepting molecule is the oxidizing agent or oxidant. A given agent, such as an iron cation existing in the ferrous (Fe^{2+}) or ferric (Fe^{3+}) state, functions as a conjugate reductant-oxidant 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 $\rightleftharpoons \text{H}^+$ + proton acceptor. In redox reactions we can write a similar general equation: electron donor $\rightleftharpoons e^-$ + electron acceptor. 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 electron transfers in the oxidation-reduction reactions of organic compounds are not fundamentally different from those of inorganic species. In Chapter 7 we considered the oxidation of a reducing sugar (an aldehyde or ketone) by cupric ion (see Fig. 7-10a):



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



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. 13-13). 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 $\text{H} < \text{C} < \text{S} < \text{N} < \text{O}$. 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. 13-13). 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 electrons, the carbon atom has undergone oxidation—even when no oxygen is involved, as in the conversion of an alkane ($-\text{CH}_2-\text{CH}_2-$) to an alkene ($-\text{CH}=\text{CH}-$). In this case, oxidation (loss of electrons) is coincident with the loss of hydrogen. In biological systems, oxidation is often synonymous with **dehydrogenation**, and many enzymes that catalyze oxidation reactions are **dehydrogenases**. Notice that the more reduced compounds in Figure 13-13 (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, $6\text{H}^+ + 6e^- + \text{N}_2 \rightarrow 2\text{NH}_3$, the nitrogen atoms are reduced.

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

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



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



where AH_2 is the hydrogen/electron donor.

(Do not mistake the above reaction for an acid dissociation; the H^+ arises from the removal of a hydrogen atom, $\text{H}^+ + e^-$.) 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:



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

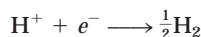


The hydrocarbon is the electron donor and the oxygen atom is the electron acceptor.

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, a hydrogen atom, or a hydride ion, or whether the electron transfer takes place in a reaction with oxygen to yield an oxygenated product. Because biological fuel molecules are usually enzymatically dehydrogenated to lose *two* reducing equivalents at a time, and because each oxygen atom can accept two reducing equivalents, biochemists by convention regard the unit of biological oxidations as two reducing equivalents passing from substrate to oxygen.

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, E°** , a measure (in volts) of this affinity, can be determined in an experiment such as that described in Figure 13–14. Electrochemists have chosen as a standard of reference the half-reaction



The electrode at which this half-reaction occurs (called a half-cell) is arbitrarily assigned a standard reduction

potential 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 (each solute at 1 M, each gas at 101.3 kPa), electrons tend to flow through the external circuit from the half-cell of

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 13–13 Oxidation states of carbon in the biosphere. The oxidation states are illustrated with some representative 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 oxidation state of that carbon. When the red carbon undergoes oxidation (loses electrons), the number gets smaller. Thus the oxidation state increases from top to bottom of the list.

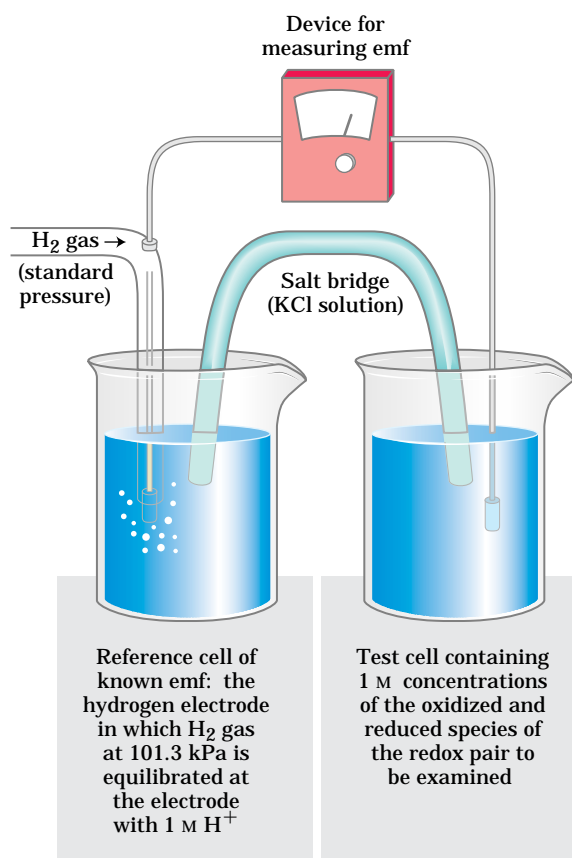


FIGURE 13-14 Measurement of the standard reduction potential (E°) of a redox pair. Electrons flow from the test electrode to the reference electrode, or vice versa. The ultimate reference half-cell is the hydrogen electrode, as shown here, at pH 0. The electromotive force (emf) of this electrode is designated 0.00 V. At pH 7 in the test cell, 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.

lower standard reduction potential to the half-cell of higher standard reduction potential. By convention, the half-cell with the stronger tendency to acquire electrons is assigned a positive value of E° .

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 reduction potential (E) at any concentration of oxidized and reduced species in the cell:

$$E = E^\circ + \frac{RT}{n\mathcal{F}} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]} \quad (13-4)$$

where R and T have their usual meanings, n is the number of electrons transferred per molecule, and \mathcal{F} is the Faraday constant (Table 13-1). At 298 K (25 °C), this expression reduces to

$$E = E^\circ + \frac{0.026 \text{ V}}{n} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]} \quad (13-5)$$

Many half-reactions of interest to biochemists involve protons. As in the definition of ΔG° , biochemists define the standard state for oxidation-reduction reactions as pH 7 and express reduction potential as E'° , the standard reduction potential at pH 7. 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 and pH 7, is connected with the standard (pH 0) hydrogen electrode. Notice in Table 13-7 that when the conjugate pair $2\text{H}^+/\text{H}_2$ 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 $2\text{H}^+/\text{H}_2$ pair is -0.414 V.

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

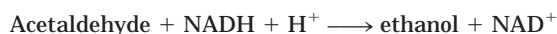
The usefulness of reduction potentials stems from the fact that when E values have been determined for any two half-cells, relative to the standard hydrogen electrode, their reduction potentials relative to each other are also known. We can then predict the direction in which electrons will tend to flow 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 the difference in reduction potentials, ΔE .

The energy made available by this spontaneous electron flow (the free-energy change for the oxidation-reduction reaction) is proportional to ΔE :

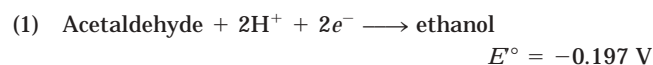
$$\Delta G = -n\mathcal{F} \Delta E \quad \text{or} \quad \Delta G^\circ = -n\mathcal{F} \Delta E^\circ \quad (13-6)$$

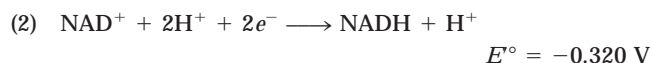
Here n represents the number of electrons transferred in the reaction. With this equation we can calculate the free-energy change for any oxidation-reduction reaction from the values of E° in a table of reduction potentials (Table 13-7) and the concentrations of the species participating in the reaction.

Consider the reaction in which acetaldehyde is reduced by the biological electron carrier NADH:



The relevant half-reactions and their E° values are:





By convention, ΔE° is expressed as E° of the electron acceptor minus E° of the electron donor. Because acetaldehyde is accepting electrons from NADH in our example, $\Delta E^\circ = -0.197 \text{ V} - (-0.320 \text{ V}) = 0.123 \text{ V}$, and n is 2. Therefore,

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

This is the free-energy change for the oxidation-reduction reaction at pH 7, when acetaldehyde, ethanol, NAD^+ , and NADH are all present at 1.00 M concentrations. If, instead, acetaldehyde and NADH were present at 1.00 M but ethanol and NAD^+ were present at 0.100 M, the value for ΔG would be calculated as follows. First,

the values of E for both reductants are determined (Eqn 13-4):

$$E_{\text{acetaldehyde}} = E^\circ + \frac{RT}{nF} \ln \frac{[\text{acetaldehyde}]}{[\text{ethanol}]} \\ = -0.197 \text{ V} + \frac{0.026 \text{ V}}{2} \ln \frac{1.00}{0.100} = -0.167 \text{ V} \\ E_{\text{NADH}} = E^\circ + \frac{RT}{nF} \ln \frac{[\text{NAD}^+]}{[\text{NADH}]} \\ = -0.320 \text{ V} + \frac{0.026 \text{ V}}{2} \ln \frac{1.00}{0.100} = -0.350 \text{ V}$$

Then ΔE is used to calculate ΔG (Eqn 13-5):

$$\Delta E = -0.167 \text{ V} - (-0.350 \text{ V}) = 0.183 \text{ V} \\ \Delta G = -nF \Delta E \\ = -2(96.5 \text{ kJ/V} \cdot \text{mol})(0.183 \text{ V}) \\ = -35.3 \text{ kJ/mol}$$

TABLE 13-7 Standard Reduction Potentials of Some Biologically Important Half-Reactions, at pH 7.0 and 25°C (298 K)

Half-reaction	E° (V)
$\frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2e^- \longrightarrow \text{H}_2\text{O}$	0.816
$\text{Fe}^{3+} + e^- \longrightarrow \text{Fe}^{2+}$	0.771
$\text{NO}_3^- + 2\text{H}^+ + 2e^- \longrightarrow \text{NO}_2^- + \text{H}_2\text{O}$	0.421
Cytochrome <i>f</i> (Fe^{3+}) + $e^- \longrightarrow$ cytochrome <i>f</i> (Fe^{2+})	0.365
$\text{Fe}(\text{CN})_6^{3-}$ (ferricyanide) + $e^- \longrightarrow \text{Fe}(\text{CN})_6^{4-}$	0.36
Cytochrome <i>a</i> ₃ (Fe^{3+}) + $e^- \longrightarrow$ cytochrome <i>a</i> ₃ (Fe^{2+})	0.35
$\text{O}_2 + 2\text{H}^+ + 2e^- \longrightarrow \text{H}_2\text{O}_2$	0.295
Cytochrome <i>a</i> (Fe^{3+}) + $e^- \longrightarrow$ cytochrome <i>a</i> (Fe^{2+})	0.29
Cytochrome <i>c</i> (Fe^{3+}) + $e^- \longrightarrow$ cytochrome <i>c</i> (Fe^{2+})	0.254
Cytochrome <i>c</i> ₁ (Fe^{3+}) + $e^- \longrightarrow$ cytochrome <i>c</i> ₁ (Fe^{2+})	0.22
Cytochrome <i>b</i> (Fe^{3+}) + $e^- \longrightarrow$ cytochrome <i>b</i> (Fe^{2+})	0.077
Ubiquinone + $2\text{H}^+ + 2e^- \longrightarrow$ ubiquinol + H_2	0.045
$\text{Fumarate}^{2-} + 2\text{H}^+ + 2e^- \longrightarrow \text{succinate}^{2-}$	0.031
$2\text{H}^+ + 2e^- \longrightarrow \text{H}_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA + $2\text{H}^+ + 2e^- \longrightarrow$ butyryl-CoA	-0.015
$\text{Oxaloacetate}^{2-} + 2\text{H}^+ + 2e^- \longrightarrow \text{malate}^{2-}$	-0.166
$\text{Pyruvate}^- + 2\text{H}^+ + 2e^- \longrightarrow \text{lactate}^-$	-0.185
Acetaldehyde + $2\text{H}^+ + 2e^- \longrightarrow$ ethanol	-0.197
$\text{FAD} + 2\text{H}^+ + 2e^- \longrightarrow \text{FADH}_2$	-0.219*
Glutathione + $2\text{H}^+ + 2e^- \longrightarrow$ 2 reduced glutathione	-0.23
$\text{S} + 2\text{H}^+ + 2e^- \longrightarrow \text{H}_2\text{S}$	-0.243
Lipoic acid + $2\text{H}^+ + 2e^- \longrightarrow$ dihydrolipoic acid	-0.29
$\text{NAD}^+ + \text{H}^+ + 2e^- \longrightarrow \text{NADH}$	-0.320
$\text{NADP}^+ + \text{H}^+ + 2e^- \longrightarrow \text{NADPH}$	-0.324
Acetoacetate + $2\text{H}^+ + 2e^- \longrightarrow \beta$ -hydroxybutyrate	-0.346
α -Ketoglutarate + $\text{CO}_2 + 2\text{H}^+ + 2e^- \longrightarrow$ isocitrate	-0.38
$2\text{H}^+ + 2e^- \longrightarrow \text{H}_2$ (at pH 7)	-0.414
Ferredoxin (Fe^{3+}) + $e^- \longrightarrow$ ferredoxin (Fe^{2+})	-0.432

Source: Data mostly from Loach, P.A. (1976) In *Handbook of Biochemistry and Molecular Biology*, 3rd edn (Fasman, G.D., ed.), Physical and Chemical Data, Vol. I, pp. 122-130, CRC Press, Boca Raton, FL.

* This is the value for free FAD; FAD bound to a specific flavoprotein (for example succinate dehydrogenase) has a different E° that depends on its protein environments.