**Biological Redox Reactions**

Redox reactions involve the transfer of \( n \) reducing equivalents (either e- or H) from a donor to an acceptor:

\[
\text{Donor}_{\text{red}} + \text{Acceptor}_{\text{ox}} \rightarrow \text{Donor}_{\text{ox}} + \text{Acceptor}_{\text{red}}
\]

The free energy change for this reaction is

\[
\Delta G_{\text{redox}} = nF E_{\text{donor}} - nF E_{\text{acceptor}}
\]

\[
= nF (E^{\circ}_{\text{donor}} - E^{\circ}_{\text{acceptor}}) + RT \ln \left( \frac{[\text{Donor}]_{\text{ox}}[\text{Acceptor}]_{\text{red}}}{[\text{Donor}]_{\text{red}}[\text{Acceptor}]_{\text{ox}}} \right)
\]

The free energy change will be less than zero when \( E_{\text{donor}} < E_{\text{acceptor}} \). Therefore, reducing equivalents will be transferred from carriers with lower reduction potentials to carriers with higher reduction potentials and this will generally be in the direction of increasing \( E^{\circ} \).

At equilibrium, \( \Delta G_{\text{redox}} = 0 \) and the ratio of products to reactants is equal to the equilibrium constant \( K_{12} \). Therefore,

\[
K_{12} = \exp\{(E^{\circ}_{\text{acceptor}} - E^{\circ}_{\text{donor}})(nF/RT)\}
\]

The mechanisms of biological redox reactions have significant implications, because biology uses both hydrogen atom carriers and electron carriers and exploits the difference between them. Although it is common to speak of electron transfer chains and electron transfer reactions in biology, typical “outer-sphere” electron transfer reactions are rare. Outer-sphere electron transfer reactions occur spontaneously at rates determined only by the relative reduction potentials of the donor and acceptor and by the intrinsic reactivity of each reactant. The Marcus theory for electron transfer reactions in solution defines the rate constant for electron transfer from compound 1 to compound 2 (\( k_{12} \)) in terms of self-exchange rate constants for each compound (\( k_{11} \) and \( k_{22} \)) and the equilibrium constant for the reaction:

\[
k_{12} = \{k_{11}k_{22}K_{12}f_{12}\}^{1/2}
\]

\( f_{12} \) is a collision factor which, in practice, is usually about equal to 1. A significant implication of the Marcus theory is that electron transfer is an intrinsically non-specific process and will occur most rapidly between the lowest-potential electron donor and the highest-potential electron acceptor. Electron transfer, therefore, is not compatible with the sequential redox reactions of a redox chain. In fact, electron carriers tend to be avoided in biological systems because their reactions are uncontrolled.

Instead, biological redox reactions employ organic compounds that donate or accept hydrogen atoms. These compounds do not react spontaneously so their redox
reactions can be controlled by enzymes. The enzymes that react with these organic hydrogen-atom carriers, however, are generally metalloproteins. The metal ligand will accept and donate only electrons, not hydrogen atoms. The active site of the metalloenzyme, therefore, must provide its substrate (the hydrogen-atom carrier) with a way to exchange a proton as well as an electron. We will call this phenomenon concerted proton/electron transfer.

Concerted proton/electron transfer implies that biological redox reactions will naturally release or consume $\text{H}^+$. The generation of proton gradients, therefore, is a natural consequence of the vectorial organization of these reactions. Because electron transfer reactions are avoided in biology, terminology such as “electron transfer chain” is misleading. Instead, we will refer to the respiratory chain in mitochondria or to redox chains.

**The Secretory-Vesicle Ascorbic Acid-Regenerating Chain**

A good, simple example of a biological redox chain is the ascorbic acid-regenerating system in secretory vesicles. The function of this system is to provide reducing equivalents for redox reactions occurring inside the secretory vesicles. Two reactions, those catalyzed by dopamine $\beta$-monooxygenase (D$\beta$M) and peptidylglycine $\alpha$-amidating monooxygenase (PAM), are especially significant. D$\beta$M converts dopamine to norepinephrine and mediates catecholamine biosynthesis in adrenal chromaffin cells, in peripheral sympathetic nerve endings and in noradrenergic neurons in the central nervous system. PAM and an associated lyase lead to amidation of the carboxyl termini of many peptide hormones including vasopressin, oxytocin, VIP, neuropeptide Y, $\alpha$-MSH, substance P, calcitonin and gastrin.

![Figure 1. Mechanism of ascorbic acid regeneration in secretory vesicles. 1) semidehydroascorbate reductase; 2) cytochrome b$_{561}$; 3) dopamine $\beta$-monooxygenase, 4) $\text{H}^+$-translocating ATPase.](image)
The monooxygenases both incorporate one atom from O\textsubscript{2} into the substrate and reduce the second oxygen atom to H\textsubscript{2}O. The reducing equivalents are provided by intravesicular ascorbic acid (vitamin C) which in turn is oxidized to the radical anion, semidehydroascorbate. The intravesicular ascorbate is recycled by importing reducing equivalents across the vesicle membrane through cytochrome b\textsubscript{561} (Figure 1). Cytochrome b\textsubscript{561}, which spans the secretory-vesicle membrane, is reduced in turn by cytosolic ascorbic acid. This transport of electrons into the vesicles is driven by both the membrane potential (interior positive) and the pH gradient (interior acidic) generated by a H\textsuperscript{+}-translocating ATPase in the vesicle membrane. The pH-gradient favors inward electron flow because the midpoint potential of ascorbate is pH-dependent being higher at the lower internal pH. Thus, contrasting with the mitochondrial respiratory chain in which H\textsuperscript{+} is transported using energy from redox reactions, the secretory-vesicle system drives the redox reaction using energy from the proton gradient.

To understand how this system works, it is important to understand that ascorbic acid functions as a donor of single hydrogen atoms. At physiological pH, ascorbic acid exists predominantly as a monoanion (AH\textsuperscript{-}). Similarly, the oxidized form occurs as a radical anion (A\textsuperscript{-}) because this form is stabilized by its capacity to distribute the unpaired electron over a number of atoms. The fully oxidized form, dehydroascorbate, is not normally formed because that requires formation of a very unfavorable reaction intermediate. Consequently, cytochrome b\textsubscript{561} must react with ascorbic acid and its radical anion by exchanging the equivalent of a single hydrogen atom. Because the metal ligand (Fe) will only accept an electron, there must be a mechanism to facilitate ascorbate deprotonation. This implies that cytochrome b\textsubscript{561} reacts with ascorbate/semidehydroascorbate by concerted proton/electron transfer. Because the reaction involves only one reducing equivalent and causes no other chemical changes, it is an especially simple example of this kind of redox reaction.

The reaction of cytochrome b\textsubscript{561} with external ascorbate requires a histidine residue. (Histidine modification inhibits reduction of cytochrome b\textsubscript{561} by external ascorbate.) It is likely that this histidine residue hydrogen bonds to ascorbate and functions as the proton acceptor in the concerted proton/electron transfer reaction.

A probable but unproven implication of concerted H\textsuperscript{+}/e\textsuperscript{-} transfer is that the substrate binding site is sufficient to make the substrate reactive. By creating a mechanism for proton transfer, the binding site:substrate complex becomes capable of engaging in electron transfer reactions. This means that metalloproteins can be thought of as two (or more) separate electron transfer centers: the metal and the bound substrate(s). The bound substrate will normally exchange electrons with the metal because that pathway is available, but the bound substrate may also react with other redox centers that may happen to be in the vicinity. Consequently electrons may occasionally go astray, for example, reducing O\textsubscript{2} to superoxide (O\textsubscript{2}\textsuperscript{-}).

References
