6.1: Biological Redox Components

Three types of oxidation-reduction (redox) centers are found in biology: protein side chains, small molecules, and redox cofactors. The first class is frequently overlooked by mechanistic enzymologists. The sulfhydryl group of cysteine is easily oxidized to produce a dimer, known as cystine:

$$2R-SH \xrightarrow[-2H^+]{-2e^-} R-S-S-R \tag{6.1}$$

This type of interconversion is known to occur in several redox proteins, including xanthine oxidase, mercuric ion reductase, and thioredoxin. Other enzyme systems display spectral evidence pointing to the presence of a protein-based radical in at least one intermediate. EPR spectroscopy provides a powerful tool in studying such systems; the observation of a g = 2.0 signal that cannot be attributed to impurities or an organic redox cofactor is generally taken to be evidence for a protein-based radical. Radicals localized on tyrosine (e.g., in photosystem II and the B2 subunit of ribonucleotide reductase[1]) and tryptophan (e.g., in yeast cytochrome c peroxidase[2]) have been unambiguously identified using EPR techniques together with protein samples containing isotopically labeled amino acids (e.g., perdeuterated Tyr) or single amino-acid mutations (e.g., Trp → Phe).

A variety of small molecules, both organic and inorganic, can function as redox reagents in biological systems. Of these, only the nicotinamide and quinone coenzymes are found throughout the biosphere. Nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) participate in a wide variety of biological redox reactions. The 4-position of the pyridine ring is the reactive portion of both molecules (Figure 6.1). Both typically function as 2-electron redox reagents.

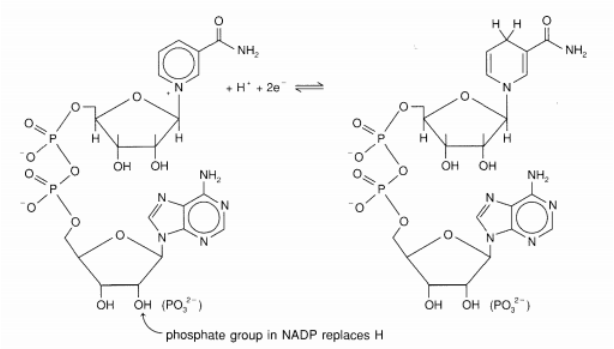

Figure 6.1 - Reduction of NAD^+ to NADH.

In contrast, quinones may function as either 1- or 2-electron carriers:

$$Q \overset{e^-,H^+}{\rightleftharpoons} QH\cdot \overset{e^-,H^+}{\rightleftharpoons} QH_2 \tag{6.2}$$

Free-radical semiquinone (QH•) intermediates have been detected by EPR spectroscopy in some electron transfers. Coenzyme Q, also called ubiquinone because it occurs in virtually all cells, contains a long isoprenoid tail that enables it to diffuse through membranes rapidly. This quinone derivative, which occurs in both free and protein-bound forms, is called ubiquinol when reduced (Figure 6.2). Other types of quinones are less frequently found in cells.

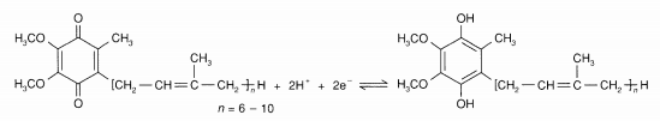

Figure 6.2 - Reduction of coenzyme Q (ubiquinone) to ubiquinol.

Metalloproteins containing a single type of redox cofactor can be divided into two general classes: electron carriers and proteins involved in the transport or activation of small molecules. Adman[3] has identified some of the factors that seem to be characteristic of electron-transfer proteins (these proteins are sometimes called "electron transferases"): (a) possession of a suitable cofactor to act as an electron sink; (b) placement of the cofactor close enough to the protein surface to allow electrons to move in and out; (c) existence of a hydrophobic shell adjacent to, but not always entirely surrounding, the cofactor; (d) small structural changes accompanying electron transfer; and (e) an architecture that permits slight expansion or contraction in preferred directions upon electron transfer.

Proteins that function as electron transferases typically place their prosthetic groups in a hydrophobic environment and may provide hydrogen bonds (in addition to ligands) to assist in stabilizing both the oxidized and the reduced forms of the cofactor. Metal-ligand bonds remain intact upon electron transfer to minimize inner-sphere reorganization[4] (discussed in Section III). Many of the complex multisite metalloenzymes (e.g., cytochrome c oxidase, xanthine oxidase, the nitrogenase FeMo protein) contain redox centers that function as intramolecular electron transferases, shuttling electrons to/from other metal centers that bind exogenous ligands during enzymatic turnover.

There are four classes[3,5] of electron transferases, each of which contains many members that exhibit important structural differences: flavodoxins, blue copper proteins, iron-sulfur proteins, and cytochromes.

The flavodoxins[6] are atypical in that they contain an organic redox cofactor, flavin mononucleotide (FMN; see Figure 6.3). These proteins have molecular weights in the 8-13 kDa range, and are found in many species of bacteria and algae. The FMN cofactor is found at one end of the protein, near the molecular surface, but only the dimethylbenzene portion of FMN is significantly exposed to the solvent (Figure 6.4). FMN can act as either a 1- or a 2-electron redox center. In solution, the semiquinone form of free FMN is unstable, and disproportionates to the quinone (oxidized) and hydroquinone (reduced) forms. Hence, free FMN functions in effect as a 2-electron reagent. FMN in flavodoxins, on the other hand, can function as a single-electron carrier. This is easily discerned by comparing reduction potentials for free and protein-bound FMN (Table 6.1). Clearly, the protein medium is responsible for this drastic alteration in oxidation-state stability. From an NMR study[7] of the *M. elsdenii* flavodoxin quinone/semiquinone and semiquinone/hydroquinone electron self-exchange rates, it was concluded that the latter is approximately 300 times faster than the former, in keeping with the view that the physiologically relevant redox couple is semiquinone/hydroquinone.

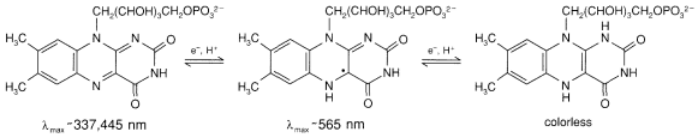

Figure 6.3 - Reduction of FMN.

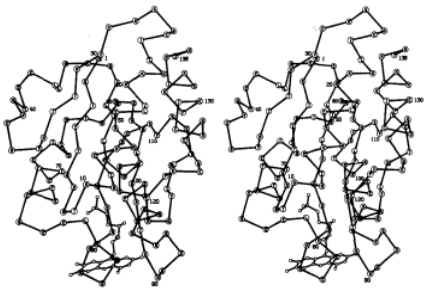

Figure kindly provided by M. L. Ludwig.

Table 6.1 - Reduction potentials of FMN couples.

Abbreviations: Q, quinone; SQ, semiquinone; HQ, hydroquinone.

	E°Q/SQ	E°SQ/HQ
Free FMN	-238 mV	-172 mV
C.M.P. flavodoxin	-92 mV	-399 mV

The blue copper proteins are characterized by intense S(Cys) → Cu chargetransfer absorption near 600 nm, an axial EPR spectrum displaying an unusually small hyperfine coupling constant, and a relatively high reduction potential.[4,8-10] With few exceptions (e.g., photosynthetic organisms), their precise roles in bacterial and plant physiology remain obscure. X-ray structures of several blue copper proteins indicate that the geometry of the copper site is approximately trigonal planar, as illustrated by the *Alcaligenes denitrificans* azurin structure (Figure 6.5).[11,12] In all these proteins, three ligands (one Cys, two His) bind tightly to the copper in a trigonal arrangement. Differences in interactions between the copper center and the axially disposed ligands may significantly contribute to variations in reduction potential that are observed[12] for the blue copper electron transferases. For example, $E^{o\prime}$ = 276 mV for *A. denitrificans* azurin, whereas that of *P. vulgaris* plastocyanin is 360 mV. In *A. denitrificans* azurin, the Cu-S(Met) bond is

0.2 Å longer than in poplar plastocyanin, and there is a carbonyl oxygen 3.1 Å from the copper center, compared with 3.8 Å in plastocyanin. These differences in bond lengths are expected to stabilize Cu^{II} in azurin to a greater extent than in plastocyanin, and result in a lower E°' value for azurin.

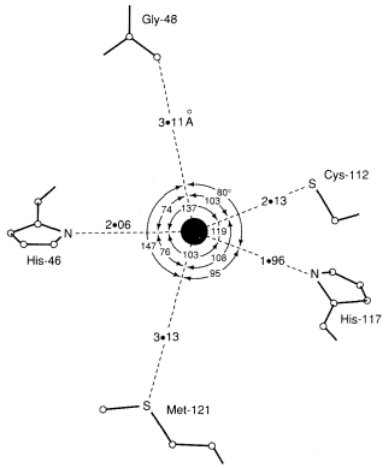

Figure 6.5 - Structure of the blue copper center in azurin.[11]

The iron-sulfur proteins play important roles[13,14] as electron carriers in virtually all living organisms, and participate in plant photosynthesis, nitrogen fixation, steroid metabolism, and oxidative phosphorylation, as well as many other processes (Chapter 7). The optical spectra of all iron-sulfur proteins are very broad and almost featureless, due to numerous overlapping charge-transfer transitions that impart red-brown-black colors to these proteins. On the other hand, the EPR spectra of iron-sulfur clusters are quite distinctive, and they are of great value in the study of the redox chemistry of these proteins.

The simplest iron-sulfur proteins, known as rubredoxins, are primarily found in anaerobic bacteria, where their function is unknown. Rubredoxins are small proteins (6 kDa) and contain iron ligated to four Cys sulfurs in a distorted tetrahedral arrangement. The E°' value for the $Fe^{III/II}$ couple in water is 770 mV; that of *C. pasteurianum* rubredoxin is -57 mV. The reduction potentials of iron-sulfur proteins are typically quite negative, indicating a stabilization of the oxidized form of the redox couple as a result of negatively charged sulfur ligands.

The [2Fe-2S] ferredoxins (10-20 kDa) are found in plant chloroplasts and mammalian tissue. The structure of *Spirulina platensis* ferredoxin[15] confirmed earlier suggestions, based on EPR and Mössbauer studies, that the iron atoms are present in a spin-coupled [2Fe-2S] cluster structure. One-electron reduction (E°' ~ -420 mV) of the protein results in a mixed-valence dimer (Equation 6.3):

$$[Fe_2S_2(SR)_4]^{2-} \underset{-e^-}{\overset{+e^-}{\rightleftharpoons}} [Fe_2S_2(SR)_4]^{3-} \tag{6.3}$$

$$Fd_{ox} \qquad Fd_{red} \tag{6.1.1}$$

$$2Fe(III) \qquad Fe(II)+Fe(III) \tag{6.1.2}$$

The additional electron in Fd_{red} is associated with only one of the iron sites, resulting in a so-called trapped-valence structure.[16] The $[Fe_2S_2(SR)_4]^{4-}$ cluster oxidation state, containing two ferrous ions, can be produced *in vitro* when strong reductants are used.

Four-iron clusters [4Fe-4S] are found in many strains of bacteria. In most of these bacterial iron-sulfur proteins, also termed ferredoxins, two such clusters are present in the protein. These proteins have reduction potentials in the -400 mV range and are rather small (6-10 kDa). Each of the clusters contains four iron centers and four sulfides at alternate comers of a distorted cube. Each iron is coordinated to three sulfides and one cysteine thiolate. The irons are strongly exchange-coupled, and the [4Fe-4S] cluster in bacterial ferredoxins is paramagnetic when reduced by one electron. The so-called "high-potential ironsulfur proteins" (HiPIPs) are found in photosynthetic bacteria, and exhibit anomalously high (~350 mV) reduction potentials. The *C. vinosum* HiPIP (10 kDa) structure demonstrates that HiPIPs are distinct from the [4Fe-4S] ferredoxins, and that the reduced HiPIP cluster structure is significantly distorted, as is also observed for the structure of the oxidized *P. aerogenes* ferredoxin. In addition, oxidized HiPIP is paramagnetic, whereas the reduced protein is EPR-silent.

This bewildering set of experimental observations can be rationalized in terms of a "three-state" hypothesis (i.e., $[4Fe\text{-}4S(SR)_4]^{n-}$ clusters exist in three physiological oxidation states).[17] This hypothesis nicely explains the differences in magnetic behavior and

redox properties observed for these iron-sulfur proteins (Equation 6.4):

$$[4Fe-4S(SR)_4]^{-} \underset{-e^-}{\overset{+e^-}{\rightleftharpoons}} [4Fe-4S(SR)_4]^{2-} \underset{-e^-}{\overset{+e^-}{\rightleftharpoons}} [4Fe-4S(SR)_4]^{3-} \quad (6.4)$$

$$HiPIP_{ox} \qquad HiPIP_{red} \qquad Ferredoxin_{red} \quad (6.1.3)$$

$$Ferredoxing_{ox} \quad (6.1.4)$$

The bacterial ferredoxins and HiPIPs all possess tetracubane clusters containing thiolate ligands, yet the former utilize the -2/-3 cluster redox couple, whereas the latter utilize the -1/-2 cluster redox couple.

The protein environment thus exerts a powerful influence over the cluster reduction potentials. This observation applies to *all* classes of electron transferases—the factors that are critical determinants of cofactor reduction potentials are poorly understood at present but are thought[18] to include the low dielectric constants of protein interiors (~4 for proteins vs. ~78 for H_2O), electrostatic effects due to nearby charged amino-acid residues, hydrogen bonding, and geometric constraints imposed by the protein.

As a class, the cytochromes[19-22] are the most thoroughly characterized of the electron transferases. By definition, a cytochrome contains one or more heme cofactors. These proteins were among the first to be identified in cellular extracts because of their distinctive optical properties, particularly an intense absorption in the 410-430 nm region (called the Soret band). Cytochromes are typically classified on the basis of heme type. Figure 6.6 displays the three most commonly encountered types of heme: heme a possesses a long phytyl "tail" and is found in cytochrome c oxidase; heme b is found in b-type cytochromes and globins; heme c is covalently bound to c-type cytochromes via two thioether linkages. Cytochrome nomenclature presents a real challenge! Some cytochromes are designated according to the historical order of discovery, e.g., cytochrome c_2 in bacterial photosynthesis. Others are designated according to the λ_{max} of the α band in the absorption spectrum of the reduced protein (e.g., cytochrome c_{551}).

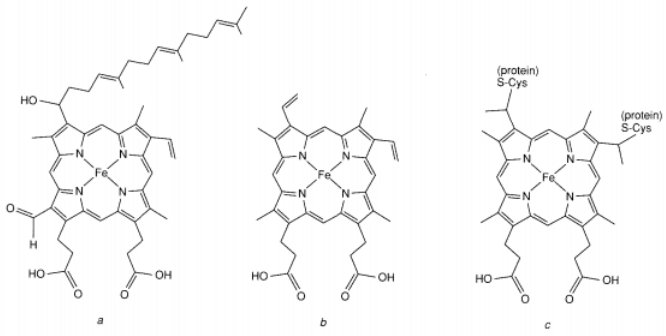

Figure 6.6 - Structures of hemes a, b, and c.

Cytochromes c are widespread in nature. Ambler[23] divided these electron carriers into three classes on structural grounds. The Class I cytochromes c contain axial His and Met ligands, with the heme located near the N-terrninus of the protein. These proteins are globular, as indicated by the ribbon drawing of tuna cytochrome c (Figure 6.7). X-ray structures of Class I cytochromes c from a variety of eukaryotes and prokaryotes clearly show an evolutionarily conserved "cytochrome fold," with the edge of the heme solvent-exposed. The reduction potentials of these cytochromes are quite positive (200 to 320 mV). Mammalian cytochrome c, because of its distinctive role in the mitochondrial electron-transfer chain, will be discussed later.

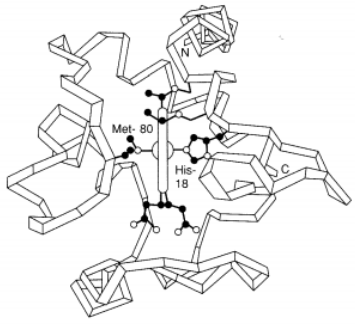

Figure 6. - Structure of tuna cytochrome c.

Class II cytochromes c ($E^{\circ\prime}$ ~ -100 mV) are found in photosynthetic bacteria, where they serve an unknown function. Unlike their Class I cousins, these c-type cytochromes are high-spin: the iron is five-coordinate, with an axial His ligand. These proteins, generally referred to as cytochromes c' , are four-α-helix bundles (Figure 6.8). The vacant axial coordination site is buried in the protein interior.

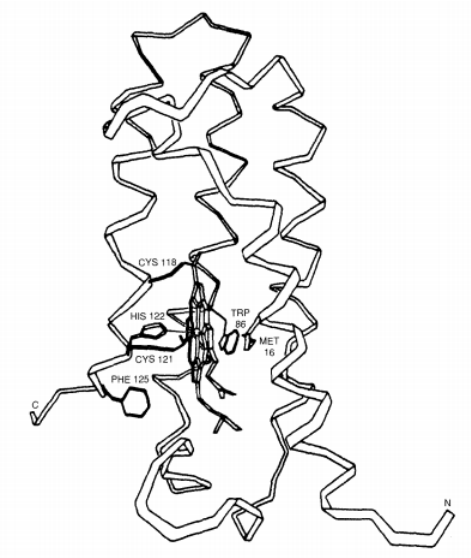

Figure 6.8 - Structure of cytochrome c'.

Finally, Class III cytochromes c, also called cytochromes c_3, contain four hemes, each ligated by two axial histidines. These proteins are found in a restricted class of sulfate-reducing bacteria and may be associated with the cytoplasmic membrane. The low molecular weights of cytochromes c_3 (~14. 7 kDa) require that the four hemes be much more exposed to the solvent than the hemes of other cytochromes (see Figure 6.9), which may be in part responsible for their unusually negative (-200 to -350 mV) reduction potentials. These proteins possess many aromatic residues and short heme-heme distances, two properties that could be responsible for their anomalously large solid-state electrical conductivity.[24]

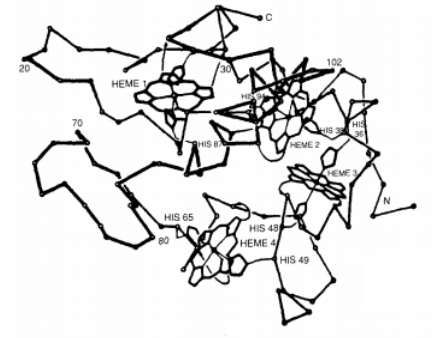

Figure 6.9 - Structure of cytochrome c_3.