6.2: Coupling Electron Transfers and Substrate Activation

Electron transfers are key steps in many enzymatic reactions involving the oxidation or reduction of a bound substrate. Relevant examples include cytochrome c oxidase ($O_2 \rightarrow 2H_2O$) and nitrogenase ($N_2 \rightarrow 2NH_3$). To reinforce the claim that electron-transfer steps are of widespread importance, several other redox systems, representative of diverse metabolic processes, will be mentioned here.

Xanthine oxidase (275 kDa; α_2 dimer) catalyzes the two-electron oxidation[37-39] of xanthine to uric acid (Equation 6.7).

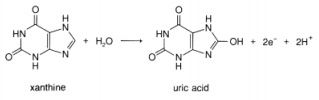

Figure 6.17 displays the cofactors in a subunit: a Mo-pterin, termed MoCo; two [2Fe-2S] centers; and one FAD. The binuclear iron-sulfur sites serve to shuttle electrons between the reduced substrate (XH) and O_2.

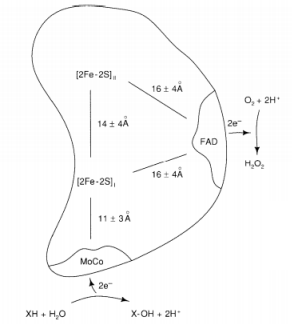

Figure 6.17 - Representation of the cofactors in one subunit of xanthine oxidase.

The first step in the biosynthesis of DNA involves the reduction of ribonucleotides (Equation 6.8) catalyzed by ribonucleotide reductase.[40] The *E. coli* enzyme is an $\alpha_2\beta_2$ tetramer composed of a B1 protein (160 kDa) and a B2 protein (78 kDa). The B1 protein (a dimer) contains redox-active dithiol groups, binding sites for ribonucleotide substrates, and regulatory binding sites for nucleotide diphosphates. Protein B2, also a dimer, possesses a phenolate radical (Tyr-122) that is stabilized by an antiferromagnetically coupled binuclear iron center (Figure 6.18). This radical is essential for enzyme activity, and is ~10 Å from the protein-B1/protein-B2 interface. Hence it cannot directly participate in an H-atom abstraction from the substrate (bound to protein B1). Instead, the x-ray structure of the B2 protein[41] suggests that a long-range electron transfer from the Tyr radical to a residue (perhaps Trp-48) on the B1 protein is operative during enzyme turnover.

(6.8)

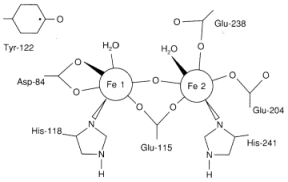

Figure 6.18 - Schematic of the binuclear iron center and Tyr-122 radical in the B2 protein of E. coli ribonucleotide reductase.[41]

Most of the presently known metal-containing mono- and dioxygenases are multicomponent, requiring the involvement of additional proteins (electron transferases) to shuttle electrons from a common biological reductant (usually NADH or NADPH) to the metallooxygenase. Cytochrome P-450, whose substrate oxidation chemistry was discussed in detail in Chapter 5, serves as an excellent example. Figure 5.10 presented a catalytic cycle for cytochrome P-450-dependent hydroxylations[42] that begins with substrate (RH) binding to the ferric enzyme (RH is camphor for *Pseudomonas putida* cytochrome P-450). To hydroxylate the camphor substrate, the monooxygenase must be reduced via the electron-transport chain in Equation (6.9).

(6.9)

The ferredoxin reductase receives two electrons from NADH and passes them on, one at at time, to putidaredoxin, a [2Fe-2S] iron-sulfur protein. Thus, two single-electron-transfer steps from reduced putidaredoxin to cytochrome P-450 are required to complete one enzyme turnover.

The activity of the enzyme appears to be regulated at the first reduction step.[43] In a 1:1 putidaredoxin-cytochrome P-450 complex, the reduction potential of putidaredoxin is -196 mV, but that of cytochrome P-450 is -340 mV in the absence of camphor; reduction of the cytochrome P-450 is thus thermodynamically unfavorable ($k \sim 0.22\ s^{-1}$). Upon binding camphor, the reduction potential of cytochrome P-450 shifts to -173 mV, and the electron-transfer rate in the protein complex accordingly increases to $41\ s^{-1}$. "Costly" reducing equivalents are not wasted, and there are no appreciable amounts of noxious oxygen-reduction products when substrate is not present.

In the third step, molecular oxygen binds to the camphor adduct of ferrous cytochrome P-450. This species, in the presence of reduced putidaredoxin, accepts a second electron, and catalyzes the hydroxylation of the bound camphor substrate. The turnover rate for the entire catalytic cycle is 10-20 s^{-1}, and the second electron-transfer step appears to be rate-determining.[44]

The bulk of the interest in electron-transfer reactions of redox proteins has been directed toward questions dealing with long-range electron transfer and the nature of protein-protein complexes whose structures are optimized for rapid intramolecular electron transfer. Before we undertake a discussion of these issues, it is worth noting that studies of the reactions of redox proteins at electrodes are attracting increasing attention.[45-47] Direct electron transfer between a variety of redox proteins and electrode surfaces has been achieved. Potential applications include the design of substrate-specific biosensors, the development of biofuel cells, and electrochemical syntheses. An interesting application of bioelectrochemical technology is the oxidation of p-cresol to p-hydroxybenzaldehyde (Figure 6.19).[48]

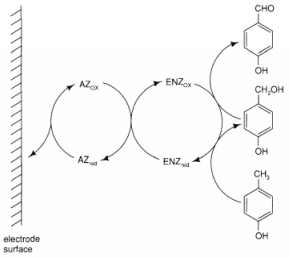

Figure 6.19 - Enzyme-catalyzed electrochemical oxidation of p-cresol to p-hydroxybenzaldehyde. AZ is azurin, and ENZ is p-cresol methylhydroxylase.[48]