7.1: Iron-sulfur Proteins and Models

Iron sulfide proteins involved in electron transfer are called *ferredoxins* and *rubredoxins*.* The ferredoxins were discovered first, and were originally classified as bacterial (containing Fe_4S_4 clusters) and plant (containing Fe_2S_2 clusters) ferredoxins. This classification is now recognized as being not generally useful, since both Fe_2S_2 and Fe_4S_4 ferredoxins are found in plants,[14,15] animals,[2,6,16] and bacteria.[4] Ferredoxins are distinguished from rubredoxins by their possession of acid-labile sulfide; i.e., an inorganic S^{2-} ion that forms H_2S gas upon denaturation at low pH. Rubredoxins have no acid-labile sulfide, and generally have a single iron in a more or less isolated site. Despite their lack of acid-labile sulfide, rubredoxins are included in this chapter because they have sequences much like those of the ferredoxins, and because their simple mononuclear Fe^{2+} and Fe^{3+} sites provide convenient illustrations of key structural and spectroscopic features.

In most ferredoxins, and in all rubredoxins, the protein ligands are cysteines, which provide four thiolate donors to the 1Fe, 2Fe, or 4Fe center. Additionally, the existence of 3Fe centers and of Fe-S sites that contain a second metal (i.e., heteronuclear clusters) make the Fe-S class a broad and multifunctional one.

Simple cytochromes and simple iron-sulfide proteins are similar, in that both can undergo one-electron transfer processes that are generally uncoupled from proton-, atom-, or group-transfer processes. Some of these proteins, such as cytochrome c_3 from *Desulfovibrio* with four hemes[17] or ferredoxin from *Clostridium pasteurianum* with two Fe_4S_4 centers,[6] can transfer more than one electron, because they have multiple copies of a one-electron transfer group. The cytochromes were discovered in 1886 by McMunn,[18] and their role in metabolism was discovered in the 1920s by Keilin (Chapter 6). The intense optical absorbance of these heme-containing proteins contributed singularly to their discovery and biochemical characterization. In contrast, the iron-sulfur proteins, although red to red-brown, absorb far more weakly in the visible region than do the cytochromes. Their presence is sometimes obscured by the cytochromes, and their frequent air instability made their initial recognition and isolation more difficult. It was not until the early 1960s that discoveries by several research groups[19] led to the isolation, recognition, and characterization of the ferredoxins. The use of EPR spectroscopy and its application to biological systems had a profoundly stimulating effect on the field (see below).

Although cytochromes were discovered first, the ferredoxins are likely to be the older proteins from an evolutionary perspective.[20] Ferredoxins have relatively low-molecular-weight polypeptide chains, require no organic prosthetic group, and often lack the more complex amino acids. In fact, the amino-acid composition in clostridial ferredoxin is close to that found in certain meteorites.[21]

The various Fe-S sites found in electron-transfer proteins (ferredoxins) are also found in many enzymes,[6,11,22.23] where these centers are involved in intraor interprotein electron transfer. For example, sulfite reductase contains a siroheme and an Fe_4S_4 center,[24] which are strongly coupled and involved in the sixelectron reduction of SO_3^{2-} to H_2S. Xanthine oxidase (see Figure 7.1) has two identical subunits, each containing two different Fe_2S_2 sites plus molybdenum and FAD sites. In xanthine oxidase, the Mo(VI) site carries out the two-electron oxidation of xanthine to uric acid, being reduced to Mo(IV) in the process.[25] The Mo(VI) site is regenerated by transferring electrons, one at a time, to the Fe_2S_2 and flavin sites, thereby readying the Mo site for the next equivalent of xanthine. Although the Fe_2S_2 sites do not directly participate in substrate reactions, they are essential to the overall functioning of the enzyme system. The Fe_2S_2 centers in xanthine oxidase play the same simple electron-transfer role as the Fe_2S_2 ferredoxins play in photosynthesis.

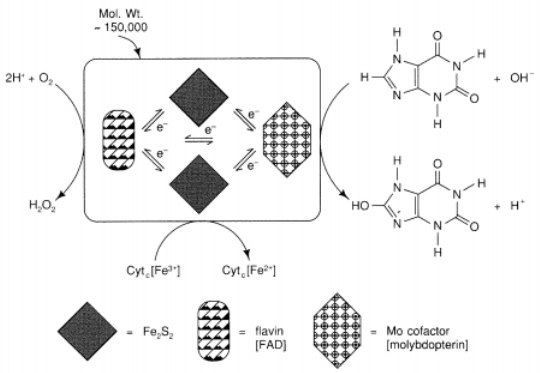

Figure 7.1 - A schematic drawing of xanthine oxidase illustrating the Mo, flavin, and Fe_2S_2 sites and interaction of the enzyme with substrate and oxidant(s).

Structurally, all the iron-sulfur sites characterized to date are built up of (approximately) tetrahedral iron units (see Figure 7.2). In rubredoxins the single iron atom is bound in tetrahedral coordination by four thiolate ligands provided by cysteine side chains. In two-iron ferredoxins the Fe_2S_2 site consists of two tetrahedra doubly bridged through a pair of sulfide ions, i.e., $Fe_2(\mu_2\text{-}S)_2$, with the tetrahedral coordination of each Fe completed by two cysteine thiolates. In four-iron or eight-iron ferredoxins, the 'thiocubane' Fe_4S_4 cluster consists of four tetrahedra sharing edges with triply bridging S^{2-} ions, i.e., $Fe_4(\mu_3\text{-}S)_4$, with each Fe completing its tetrahedron by binding to a single cysteine thiolate. Finally, for Fe_3S_4 clusters, which are now being found in more and more proteins, the well-established structure has one triply bridging and three doubly bridging sulfide ions, $Fe_3(\mu_3\text{-}S)(\mu_2 S)_3$. The Fe_3S_4 unit can be thought of as derived from the 'thiocubane' Fe_4S_4 unit by the removal of a single iron atom.

In what follows we will introduce these structures in the order 1Fe, 2Fe, 4Fe, and 3Fe. For each, we will first discuss the physiological role(s) of the particular proteins, then the structural features, followed by the spectroscopic properties and model systems.

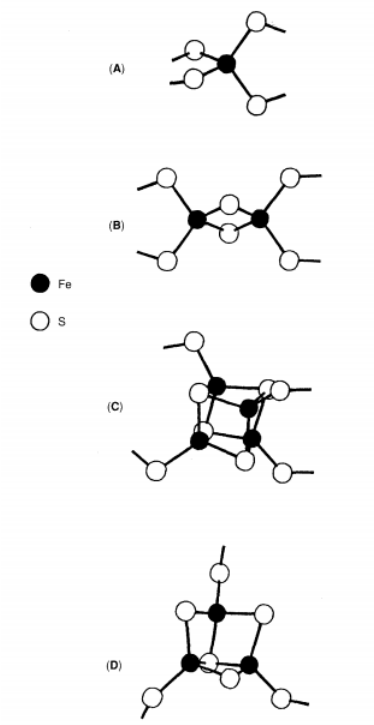

Figure 7.2 - Structural systematics of Fe-S units found in proteins: (A) rubredoxin single Fe center; (B) Fe_2S_2 unit; (C) Fe_4S_4 unit; (d) Fe_3S_4 unit.

* For review articles, see References 1-11. For a discussion of nomenclature, see References 12 and 13.