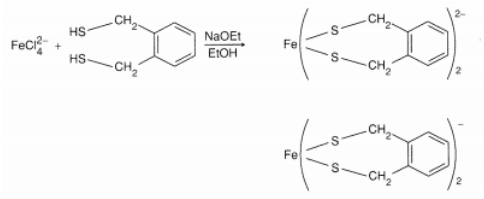
7.2: Iron-sulfur Proteins and Models (Part 2)

Rubredoxin Model Systems

The simple mononuclear tetrahedral site of Rd has been chemically modeled in both its reduced and its oxidized forms. The bidentate o-xylyl-α,α'-dithiolate ligand forms bis complexes of Fe(II) and Fe(III) that have spectroscopic features quite similar to those of the protein.[62,63] The preparative procedure is relatively straightforward (Equation 7.1).



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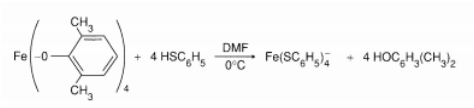
The UV-visible-NIR spectra, Mössbauer spectra, and magnetic susceptibility differ only slightly from those of oxidized and reduced rubredoxins.

The monodentate benzenethiolate (thiophenolate) ligand, $C_6H_5S^-$, similarly forms the ferrous $Fe(SC_6H_5)_4^{2-}$ complex.[64,65] Although for some time it was felt that the oxidized form, $Fe(SC_6H_5)_4^-$, was inherently unstable, the sterically hindered monothiolate ligand 2,3,5,6-tetramethylbenzenethiolate was found to form[66-68] a stable, quite symmetric Fe(III) tetrathiolate anion. Armed with this information, the preparation of the tetrakis(benzenethiolate) Fe(III) complex was reinvestigated, and the complex successfully synthesized[67] (Equation 7.2).



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The Fe(III) and Fe(II) tetrathiolate species now serve as excellent structural models for the Fe sites of both oxidized and reduced Rd.[69]

The structural parameters for the oxidized rubredoxin analogues are very similar to those of the oxidized Rd iron site. The reduced complexes reveal a lengthening of the average Fe-S bond from 2.27 to 2.36 Å, consistent with the change in oxidation state from ferric to ferrous. The addition of an electron has a more profound structural effect in this single-iron center than in some of the multiiron clusters, where electrons are more delocalized.

Clearly, for the single-Fe sites, the dominant structural feature is their near-tetrahedral tetrathiolate coordination. The dominant electronic structural feature is the presence of high-spin Fe^{3+} and Fe^{2+} sites. The important mode of chemical reactivity is a simple one-electron transfer. Each of these features carries over to the 2Fe, 4Fe, and 3Fe sites discussed below.

Fe_2S_2 Ferredoxins

The simple 2Fe-2S proteins are sometimes referred to as "plant" or "plant-type" ferredoxins. The protein from spinach, which serves as an electron acceptor in the photosynthetic apparatus,[14,15,50,70] was among the first to be wellcharacterized and widely studied, and could be considered the prototypical 2Fe-2S ferredoxin, However, 2Fe-2S proteins are also well-known in bacteria.[4] The protein from the cyanobacterium (blue-green alga) *Spirulina platensis* has been structurally elucidated by x-ray crystallography.[47] Putidaredoxin, from *Pseudomonas putida*, which serves as a donor to the P-450 camphor monooxygenase system, has been extensively studied.[28] Fe_2S_2 centers are also well-established in mammalian proteins. Adrenodoxin[29] serves as the electron donor to the P-450 monooxygenase system that carries out the 11-β-hydroxylation of steroids, The so-called "Rieske proteins" are found in the bc_1 complex of mitochondria[47] as well as in the bd complex of the photosynthetic apparatus of plants.[71] In addition, Fe_2S_2 centers are well-known constituents of such redox proteins as xanthine oxidase,[25,72] CO oxidase,[25] succinate dehydrogenase,[73-75] and putidamonooxin.[76] Table 7.1 lists some of the Fe_2S_2 proteins and their properties.

The x-ray crystal structure of only the single 2Fe-2S protein mentioned above has been determined;[70a] the 2Fe-2S ferredoxin from the blue-green alga Spirulina platensis[6,22,47,77,78] shows significant sequence identity with chloroplast ferredoxins typical of higher plants.[79,80] As Figure 7.8 shows, the Fe_2S_2 unit in this 11-kDa protein is bound by Cys-41, Cys-46, Cys-49, and Cys-79, The

binuclear iron cluster is found in a largely hydrophobic region of the protein, but is within 5 Å of the protein surface.[6] The sulfur atoms of the cluster, both inorganic and cysteinyl, are hydrogen-bonded to six peptide NH groups and one serine OH group, which presumably stabilize the cluster/protein complex, The serine involved in the H-bonding, Ser-40, is conserved in all plant and algal 2Fe-2S ferredoxins sequenced, which implies that it plays a crucial structural or functional role.

Figure 7.8 - The x-ray crystal structure of the Fe_2S_2 ferredoxin from Spirulina platensis.[70a]

The structure of the 2Fe-2S core in Figure 7.2 reveals a tetrahedron of S ligands surrounding each Fe atom. The two tetrahedra share an edge defined by the two bridging sulfide ions, and the core structure is designated $Fe_2(\mu_2\text{-}S)_2$. Fe-S distances and angles cannot be measured accurately in the structure at the present 2.5-Å resolution;[70a] so we will later discuss these details in terms of model compounds.

The Fe_2S_2 center shows nicely how spectroscopy can be used to deduce the structure of an active site. Indeed, in this case the now well-established active-site structure was deduced by a combination of chemical, spectroscopic, and magnetic methods, and the site was successfully modeled long before the first protein crystallographic study was reported. The presence of acid-labile, inorganic sulfide is a key feature of both the Fe_2S_2 and the Fe_4S_4 centers. The 1:1 stoichiometry between iron and acid-labile sulfide was eventually established analytically for Fe_2S_2 centers.[9-11] Care must be taken to ensure that both the protein and its active-site complement are homogeneous. Although protein homogeneity is usually established by electrophoretic methods, these methods may not distinguish between pure proteins and those with absent or incomplete active centers. Fortunately, absorption at 420 nm is due solely to the Fe_2S_2 cluster, whereas the 275-nm absorption is dominated by the protein. Therefore a good criterion for active-site saturation and homogeneity is the ratio of the absorbances at 420 and 275 nm, $A_{420\ nm}/A_{275\ nm}$, which is ~0.48 for pure spinach ferredoxin.[81] Once homogeneous protein is obtained, the Fe_2S_2 composition of the "plant" ferredoxins can be correctly deduced analytically.

The Fe_2S_2 center displays two redox states that differ by a single electron. The potential range for the couple is -250 to -420 mV, revealing the highly reducing nature of the ferredoxin. The correct structure of the Fe_2S_2 center was first proposed in 1966 based on EPR studies.[82] The reduced state of the cluster shows a rhombic EPR signal with g values of 1.88, 1.94, and 2.04 (Figure 7.6B) characteristic of an $S = \frac{1}{2}$ center. The oxidized state is EPR-silent. The weakness of the sulfur ligand field causes the iron atoms to be high-spin. But how can two sulfur-ligated iron atoms, each with a tendency to be high-spin, produce a state with a single unpaired electron?

The individual Fe atoms in the Fe_2S_2 cluster resemble those in rubredoxin quite closely. The two redox states of the Fe_2S_2 protein correspond to an Fe^{3+}-Fe^{3+} and an Fe^{3+}-Fe^{2+} pair, respectively, as shown in Figure 7.9. In the all-ferric oxidized state, the two Fe^{3+} sites are antiferromagnetically coupled; i.e., the spins of the five d electrons on the two iron atoms are oppositely aligned, such that their pairing produces an effective S = 0, diamagnetic ground state. In the reduced form, a single unpaired electron is present, because the $S = \frac{5}{2}$ Fe^{3+} and S = 2 Fe^{2+} sites are antiferromagnetically coupled, leaving one net unpaired spin and an $S = \frac{1}{2}$ ground state. The profound difference between the electronic properties of rubredoxin and Fe_2S_2 ferredoxin arises because the latter has two Fe atoms in close proximity, which allows for their magnetic coupling.

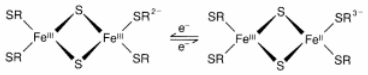


Figure 7.9 - Redox states of Fe_2S_2 proteins: (A) reduced; (B) oxidized.

Strong support for the spin-coupling model in Fe_2S_2 ferredoxins comes from a detailed analysis of their absorption and circular dichroism spectra.[83] As with rubredoxin (see Figure 7.5), we expect no low-energy spin-allowed d-d bands for the ferric site in either the oxidized or the reduced state. Indeed, the oxidized state containing all Fe^{3+} shows no low-energy bands; the reduced state

containing a single Fe^{2+} displays low-energy, low-intensity bands in the region 4,000-9,000 cm^{-1}, in close analogy to the situation in reduced rubredoxin. The combined EPR and optical spectra leave little doubt about the structural assignment: two coupled high-spin ferric ions in the oxidized state, and coupled high-spin ferric and ferrous ions in the reduced state. Moreover, the spectra are consistent only with a localized model, i.e., one in which the Fe(II) site is associated with a single iron.[83,83a] The Fe_2S_2 site is inherently asymmetric, and inequivalence of the Fe(lIl) sites is spectroscopically detectable in the all-ferric oxidized form.[84] In fact, the localized valence trapping is present in reduced model compounds that contain no ligand asymmetry.

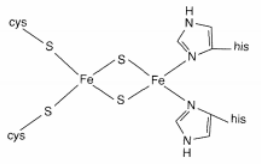
Mössbauer spectra provide additional and striking confirmation of the structural assignment. The spectrum of the oxidized ferredoxin (Figure 7.7) resembles strongly that of oxidized rubredoxin, indicating the presence of high-spin Fe^{3+}, even though the net spin is zero. In the reduced form, the Mössbauer spectrum involves the superposition of signals from a high-spin Fe^{2+} and a high-spin Fe^{3+}, i.e., a reduced and an oxidized rubredoxin, respectively. Clearly, the simplest interpretation of this result consistent with the $S = \frac{1}{2}$ spin state required by the EPR is the localized Fe^{2+}-Fe^{3+} antiferromagnetic coupling model discussed above.

NMR studies of oxidized Fe_2S_2 proteins reveal broad isotropically shifted resonances for the CH_2 protons of the cysteine ligands.[85] Despite the coupling of the irons, the net magnetism at room temperature is sufficient to lead to large contact shifts (-30 to -40 ppm downfield from TMS). The assignment of the resonance was confirmed with the synthesis and spectroscopic analysis of model compounds.[86] Extensive NMR studies of the Fe_2S_2 proteins have been reported.[87,87a]

Resonance Raman spectra of Fe_2S_2 sites[88,89,90] reveal many bands attributable to Fe-S stretching. Detailed assignments have been presented for the four bridging and four terminal Fe-S modes. A strong band at 390 cm^{-1}, which shifts on ^{34}S sulfide labeling, is assigned to the A_{1g} "breathing" mode; another band at 275 cm^{-1} is assigned to B_{3u} symmetry in point group D_{2h}.[57,88] Spectroscopic differences in the terminal, Fe-S(Cys) stretches between plant ferredoxins and adrenodoxin (which also differ somewhat in redox potential) seem to reflect different conformations of the cysteine ligands in the two classes. Evidence for asymmetry of the iron atoms is found in the intensity of the resonance enhancement of certain modes.

Rieske Centers

Within the class of Fe_2S_2 ferredoxins there is a subclass called the Rieske proteins, or the Rieske centers.[47,91,92] The Rieske iron-sulfur centers are found in proteins isolated from mitochondria and related redox chains.[47,92] In addition, the phthalate dioxygenase system from *Pseudomonas cepacia*[93,94] contains one Fe_2S_2 Rieske center as well as one additional nonheme Fe atom. Although the Rieske centers appear to contain an Fe_2S_2 core, there is extensive evidence for nonsulfur ligands coordinated to at least one of the Fe atoms. The proposed model in Scheme (7.3) has two imidazole ligands bound to one Fe atom. The nitrogen atoms are seen in ENDOR (Electron Nuclear Double Resonance) experiments,[93] and are manifest in EXAFS spectra, which are consistent with the presence of a low-Z (atomic number) ligand bound to iron.[94] The potentials for the Rieske proteins range from +350 to -150 mV,[47] in contrast to the plant-type Fe_2S_2 centers, which range from -250 to -450 mV. The strong dependence of redox potential on pH[95] suggests a possible role in coupling protonand electron-transfer processes.



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Fe_2S_2 Models

Although spectroscopic studies led to the correct deduction of the structure of the Fe_2S_2 core, the synthesis of model compounds containing this core provided unequivocal confirmation. The model compounds allowed detailed structural analysis unavailable for the proteins. Moreover, by using a uniform set of peripheral ligands, properties inherent to the Fe_2S_2 core could be discerned.

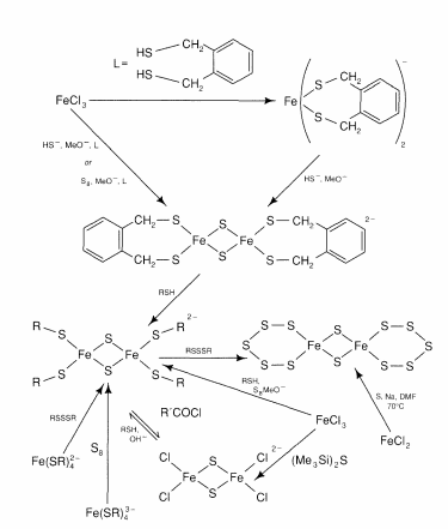


Figure 7.10: Preparative schemes leading to complexes containing the Fe_2S_2 core.[128]

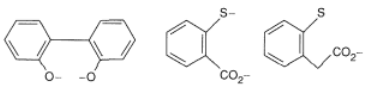
The Fe_2S_2 core has been synthesized by several routes[86,96,96a,b,c,d] (see Figure 7.10). For example, the reaction of $Fe(SR)_4^{2-}$, the ferrous rubredoxin model, with elemental sulfur produces the complex $Fe_2S_2(SR)_4^{2-}$. In this reaction the sulfur presumably oxidizes the Fe^{2+} to Fe^{3+}, being reduced to sulfide in the process. The Fe_2S_2 core has been prepared with a variety of peripheral S-donor ligands. Metrical details for $Fe_2S_2(SC_6H_4\text{-}p\text{-}CH_3)_4^{2-}$ are given in Table 7.3. Notable distances are the Fe-S (bridging) distance of 2.20 Å, the Fe-S (terminal) distance of 2.31 Å, and the Fe-Fe distance of 2.69 Å.

Table 7.3: Structural parameters for $Fe_2S_2(SC_6H_4-p-CH_3)_4^{2-}$. a) Data from Reference 211.

Atoms[a]	Distance Å	Atoms[a]	Angle[a]
Fe-Fe	2.691 (1)	Fe-S-Fe	75.3
Fe-S1 (bridge)	2.200 (1)	S-Fe-S	104.6
Fe-S2 (bridge)	2.202 (1)	S-Fe-S	115.1
Fe-S3	2.312 (1)	S-Fe-S	105.4

To date, all analogue systems structurally characterized contain the Fe^{3+}-Fe^{3+} fully oxidized form. Attempts to isolate the Fe^{3+}-Fe^{2+} form have so far failed. However, the mixed-valence Fe_2S_2 form can be generated and trapped by freezing for spectroscopic examination.[97,98] Mössbauer spectroscopy reveals the presence of distinct Fe^{2+} and Fe^{3+} ions, as found in the proteins, clearly showing that "trapped" valence states are an inherent characteristic of the $Fe_2S_2^{2+}$ core and are *not* enforced by the protein.[97,98]

The existence of noncysteine-bound Fe_2S_2 cores in Rieske-type proteins has led to attempts to synthesize complexes with oxygen and nitrogen ligands.[99-101] Characterized species include $Fe_2S_2(OC_6H_5)_4^{2-}$, $Fe_2S_2(OC_6H_4\text{-}p\text{-}CH_3)_4^{2-}$, $Fe_2S_2(C_4H_4N)_4^{2-}$, and $Fe_2S_2(L)_2^{2-}$, where L is a bidentate ligand.



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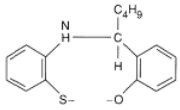
The potentially tridentate ligand



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acts in a bidentate fashion, binding through S and O but not N.

No Fe_2S_2 complexes containing mixed S,N terminal ligands, such as those suggested for the Rieske site, have been prepared. The Se^{2-} bridged analogue has been prepared for some of the complexes.[102,103]