7.3: Iron-sulfur Proteins and Models (Part 3)

Fe_4S_4 Ferredoxins (including HiPIPs)

We now turn our attention to proteins containing the Fe_4S_4 center. Historically, within this class a strong distinction was made between the "ferredoxins," which are low-potential (as low as -600 mV in chloroplasts) iron-sulfur proteins, and the "HiPIPs" = High Potential Iron Proteins, which have positive redox potentials (as high as +350 mV in photosynthetic bacteria). Although the HiPIP designation is still useful, proteins of both high and low potential are considered ferredoxins, whose key defining feature is the presence of iron and acid-labile sulfide.[13]

The Fe_4S_4 proteins participate in numerous electron-transfer functions in bacteria, and in some organisms (such as *Clostridium*) are the immediate electron donors for the nitrogenase and/or hydrogenase enzymes. The function of the HiPIPs seems obscure at present. In addition, Fe_4S_4 centers have been shown or postulated to occur in numerous microbial, plant, and mammalian redox enzymes, including nitrate reductase,[104] sulfite reductase,[24] trimethylamine dehydrogenase,[105] succinate dehydrogenase,[73,106] hydrogenase, and, possibly, in altered forms, nitrogenase. Table 7.1 lists some of the Fe_4S_4 ferredoxins and their properties.

In the Fe_2S_2 ferredoxins, combined spectroscopic, analytical, and model-system work led to an unequivocal assignment of the structural nature of the active site long before the crystallography was done. In contrast, for Fe_4S_4 systems and in particular the 8Fe-8S = $2Fe_4S_4$ systems from bacteria, the initial chemical suggestions were fallacious, and even the number and stoichiometry of the clusters were in doubt. In these cases, crystallography provided the definitive structural information.

The first indication of the presence of the "thiocubane" structure came in 1968, when a 4-Å resolution study[107] indicated a compact cluster of potentially tetrahedral Fe_4 shape in the HiPIP from *Chromatium vinosum*. This finding did not lead to much excitement, since it was not yet appreciated that HiPIPs and ferredoxins were structurally similar. In 1972, the high-resolution structure solution of both *Chromatium* HiPIP[108] and the 8Fe ferredoxin from *Peptococcus aerogenes* (formerly *Microbacter aerogenes*)[101] confirmed the presence of virtually identical thiocubane clusters in the two proteins.[108] Moreover, the structures for both oxidized and reduced HiPIP were deduced, and these revealed that the Fe_4S_4 cluster remained intact during the redox interconversion.[109] Subsequently, four-iron clusters have been crystallographically confirmed in an Fe_4S_4 ferredoxin from *Bacillus thermoproteolyticus*,[110,110a] in *Azotobacter vinelandii* ferredoxin I (also previously called Shethna Fe-S protein II), which also contains a 3Fe-4S cluster,[111,112] in the active form of aconitase,[113] and in sulfite reductase, where the cluster is probably bridged by cysteine sulfur to a siroheme.

In all the proteins characterized to date, the Fe_4S_4 clusters adopt the thiocubane structure,[108] which is discussed at greater length in the section on models. The clusters are usually bound to their proteins by four cysteine residues. As shown in Figure 7.11, in the *P. aerogenes* protein the two Fe_4S_4 clusters are bound by cysteines numbered 8, 11, 14, 18, 35, 38, 41, and 45.[101,114] The presence of the Cys-x-x-Cys unit is again apparent. However, this sequence seems prominent in all Fe-S proteins, and so is not specific for a particular Fe-S site. At first glance one might expect one cluster to be bound by cysteines 8,11,14, and 18, the other by cysteines 35, 38, 41, and 45. Actually, one cluster is bound by cysteines 8, 11, 14, and 45, the other by cysteines 35, 38, 41, and 18. The binding of a given cluster by cysteine residues from different portions of the polypeptide chain apparently helps stabilize the tertiary structure of the protein and brings the two clusters into relatively close proximity, the center-center distance being 12 Å.[114]

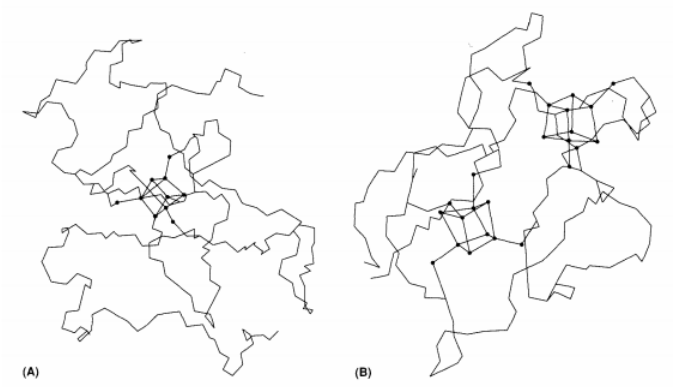

Figure 7.11 - The x-ray crystal structures of (A) *Chromatium vinosum* HiPIP[108] and (B) the 8Fe-8S ferredoxin from *Peptococcus aerogenes*.[101]

The *C. pasteurianum* protein displays weak magnetic coupling, which leads to an unusual EPR spectrum[115] consistent with the 12-Å cluster-cluster separation. However, the redox potentials for the two sites seem virtually identical at -412 mV, thus allowing the

8Fe ferredoxin to deliver two electrons at this low redox potential.[115] Significant sequence identity indicates the likelihood that other 8Fe ferredoxins, such as the well-studied one from *C. pasteurianum*,[116-118] have quite similar structures.

The thiocubane unit of Fe_4S_4 proteins can exist in proteins in at least three stable oxidation states. This so-called three-state model[74,109,119,120] contrasts dramatically with the situation for Rd(1Fe), Fe_2S_2, and Fe_3S_4 systems, in which only two oxidation states are accessible through simple electron transfer for each center. For the thiocubane structure, the three accessible states can be designated $Fe_4S_4^{3+}$, $Fe_4S_4^{2+}$, and $Fe_4S_4^{+}$, corresponding to $[Fe(III)_2Fe(II)]$, $[Fe(III)_2Fe(II)_2]$, and $[Fe(III)Fe(II)_3]$ valence-state combinations, respectively. It is crucial to note that, in sharp contrast to the Fe_2S_2 and Fe_3S_4 sites, the oxidation states are not localized in the Fe_4S_4 clusters. In most cases, each Fe atom behaves as if it had the same average oxidation level as the other Fe atoms in the cluster. The redox interconversion of the Fe4S4 sites is shown in Figure 7.12. The $Fe_4S_4^{3+} \rightleftharpoons Fe_4S_4^{2+}$ couple is the high-potential redox couple characteristic of HiPIPs; the $Fe_4S_4^{2+} \rightleftharpoons Fe_4S_4^{+}$ couple is responsible for the low-potential process characteristic of the classical ferredoxins. In any given protein under physiological conditions, only one of the two redox couples appears to be accessible and functional.

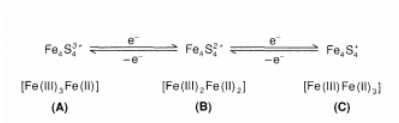

Figure 7.12 - The redox interconversions of Fe_4S_4 sites illustrating the three-state model. The states are found in (A) oxidized HiPIP; (B) reduced HiPIP and oxidized ferredoxin; (C) reduced ferredoxin.

Both the $Fe_4S_4^{+}$ and the $Fe_4S_4^{3+}$ states of the thiocubane cluster are paramagnetic and display characteristic EPR spectra (Figure 7.6C,D). The $Fe_4S_4^{3+}$ site in reduced ferredoxins[46,48,49,119] displays a rhombic EPR signal (Figure 7.6C) with g = 1.88, 1.92, and 2.06. The oxidized form of low-potential ferredoxins is EPR-silent, and attempts to "superoxidize" it to achieve the $Fe_4S_4^{3+}$ state invariably lead to irreversible cluster decomposition, probably through a 3Fe-4S structure. The $Fe_4S_4^{3+}$ signal is usually referred to as the HiPIP signal (Figure 7.6D) and shows distinct g values at 2.04($g_\perp$) and 2.10($g_\parallel$); it is present in oxidized HiPIP but absent in reduced HiPIP.[46,119] Reduction of HiPIP to a "super-reduced" state apparently occurs under partially denaturing conditions in aqueous DMSO.[108] The observed axial EPR signal with g = 1.94 and 2.05 is assigned to the $Fe_4S_4^{+}$ state characteristic of reduced ferredoxins. This result[108] is consistent with structural and spectroscopic identity of the HiPIP and Fd sites, as required by the three-state model of the Fe_4S_4 proteins (Figure 7.12).

In Fe_4S_4 centers at each level of oxidation, electronic transitions give rise to characteristic visible and UV spectra, although the delocalized nature of the electronic states makes detailed assignment difficult. MCD spectra of clusters in the three states of oxidation are clearly distinguishable from each other and from MCD of Fe_2S_2 clusters.[43,119] MCD, magnetic susceptibility, and Mössbauer spectra provide evidence that the S = $\frac{1}{2}$ state, whose EPR signal is so distinct in reduced ferredoxins, may coexist at higher T with S = $\frac{3}{2}$ and perhaps even higher spin states. Indeed, recent studies with model systems[121,122] and theoretical treatments[123,124] clearly support the ability of the Fe_4S_4 cluster to display a number of spin states that are in labile equilibria, which are influenced, perhaps quite subtly, by local structural conditions. The iron protein of nitrogenase also displays this behavior.

The Mössbauer spectra of Fe_4S_4 centers of ferredoxins reveal the equivalence of the Fe sites, and quadrupole splittings and isomer shifts at averaged values for the particular combination of oxidation states present.[3,51,52] Representative spectra are shown in Figure 7.7. Magnetic coupling is seen for the paramagnetic states.

Resonance Raman spectra (and IR spectra) have been extensively investigated in *C. pasteurianum* ferredoxin and in model compounds.[57,125] Selective labeling of either thiolate sulfur or sulfide sulfur with ^{34}S allows modes associated with the Fe_4S_4 core to be distinguished from modes associated with the FeSR ligands. The band at 351 cm^{-1} is assigned to Fe-SR stretching, and Fe_4S_4 modes occur at 248 and 334 cm^{-1} in reduced ferredoxin from *C. pasteurianum*. There is little difference between the oxidized and reduced spectra, although an extra band at 277 cm^{-1} seems present in the oxidized protein. The Fe_4Se_4 substituted protein has also been studied.[125]

As in the 1Fe and 2Fe proteins, ^1H NMR spectra reveal resonances from contact-shifted-CH_2-groups of cysteinyl residues.[125a] However, unlike the other proteins, where all states are at least weakly magnetic, only the reduced ferredoxin and the oxidized HiPIP states show contact shifts.[87a,125a,b,c]

EXAFS studies on proteins and on model compounds clearly identify the Fe-S distance of ~2.35 Å and an Fe-Fe distance of 2.7 Å. These distances, as expected, vary only slightly with state of oxidation.[125d]

Fe_4S_4 Models

Judging from the ease with which models of Fe_4S_4 are prepared under a variety of conditions and their relative stability, the $Fe_4S_4^{2+}$ core structure seems to be a relatively stable entity, a local thermodynamic minimum in the multitude of possible iron-sulfide-thiolate complexes. The initial preparation and structural characterization[126,127] of the models showed that synthetic chemistry can duplicate the biological centers in far-simpler chemical systems, which can be more easily studied in great detail.

The general synthetic scheme for Fe-S clusters is shown in Figure 7.13. Many different synthetic procedures can be used to obtain complexes with the Fe_4S_4 core.[126-138,138a,b] The multitude of preparative procedures is consistent with the notion that the $Fe_4S_4^{2+}$ core is the most stable entity present and "spontaneously self-assembles" when not limited by stoichiometric constraints.

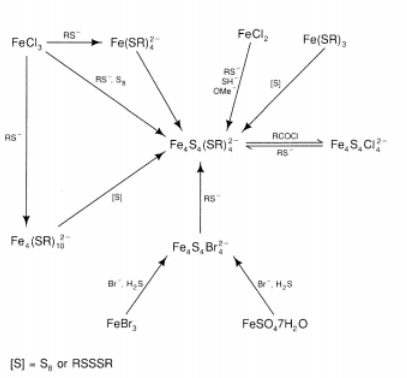

Figure 7.13 - Preparative schemes leading to complexes containing the Fe_4S_4 core.[128]

The thiocubane structure can be viewed as two interpenetrating tetrahedra of 4Fe and 4S atoms. The 4S tetrahedra are the larger, since the S-S distance is ~3.5 Å, compared with the Fe-Fe distance of ~2.7 Å. The S_4 tetrahedron encloses ~2.3 times as much volume as does the Fe_4 tetrahedron.[128] Key distances and angles for $Fe_4S_4(SCH_2C_6H_5)_4^{2-}$ given in Table 7.4 are extremely similar to those found in oxidized ferredoxin and reduced HiPIP centers in proteins.[127]

Table 7.4 - Structural parameters for $Fe_4S_4(SCH_2C_6H_5)_4^{2-}$.

a) Data from References 126 and 127.

Atoms[a]	Average Distances	Number of Bonds	Type
Fe(1)-S(3)	2.310 (3)	8	Sulfide
Fe(1)-S(2)	2.239 (4)	4	Sulfide
Fe(1)-S(5)	2.251 (3)	4	Thiolate
Fe(1)-Fe(2)	2.776 (10)	2	
Fe-Fe(other)	2.732 (5)	4	

Atoms[a]	Average Distances	Number of Bonds	Type
Fe-S-Fe	73.8 (3)	12	
S-Fe-S	104.1 (2)	12	Sulfide-Fe-Sulfide
S-Fe-S	111.7-117.3	12	Sulfide-Fe-Thiolate

The idealized symmetry of $Fe_4S_4^{2+}$ model systems is that of a regular tetrahedron, i.e., T_d. Though the distortion of the cube is quite pronounced, all known examples of the Fe_4S^{2+} core show distortion, which lowers the symmetry at least to D_{2d}. In most Fe_4S^{2+} core structures, this distortion involves a tetragonal compression, which leaves four short and eight long Fe-S bonds.

Complexes with non-S-donor peripheral ligands have been prepared and studied. The halide complexes $Fe_4S_4X_4^{2-}$ (X = Cl^-, Br^-, I^-) have been prepared, and serve as useful starting points for further syntheses.[129-133] The complex $Fe_4S_4(OC_6H_5)_4^{2-}$ can be prepared[134] from the tetrachloride (or tetrathiolate) thiocubane by reaction with $NaOC_6H_5$ (or HOC_6H_5). There are a few examples of synthetic $Fe_4S_4^{2+}$ cores in which the peripheral ligands are not identical. For example, $Fe_4S_4Cl_2(OC_6H_5)_2^{2-}$ and

$Fe_4S_4Cl_2(SC_6H_5)_2^{2-}$ have structures characterized by D_{2d} symmetry.[135] The complexes $Fe_4S_4(SC_6H_5)_2[S_2CN(C_2H_5)_2]^{2-}$ and $Fe_4S_4(SC_6H_4OH)_4^{2-}$ are similarly asymmetric, containing both four- and five-coordinate iron.[136-138] The presence of five-coordinate iron in the Fe_4S_4 cluster is notable, since it offers a possible mode of reactivity for the cluster wherever it plays a catalytic role (such as in aconitase). Complexes with Fe_4Se^{2+} and $Fe_4Te_4^{2+}$ cores have also been prepared.[138c,d]

One structural analysis of $Fe_4S_4(SC_6H_5)_4^{3-}$, which contains the reduced $Fe_4S_4^{+}$ core, revealed a tetragonal elongation[139] in the solid state. In contrast, analysis of $Fe_4S_4(SCH_2C_6H_5)_4^{3-}$ revealed a distorted structure possessing C_{2v} symmetry.[102] It would appear that the $Fe_4S_4^{+}$ clusters maintain the thiocubane structure, but are nevertheless highly deformable. Interestingly, when the solidstate C_{2v} structure, $Fe_4S_4(SCH_2C_6H_5)_4^{3-}$, is investigated in solution, its spectroscopic and magnetic behavior change to resemble closely those of the $Fe_4S_4(SC_6H_5)_4^{3-}$ cluster,[140] which does not change on dissolution. The simplest interpretation assigns the elongated tetragonal structure as the preferred form for $Fe_4S_4^{+}$ cores with deformation of sufficiently low energy that crystal packing (or, by inference, protein binding forces) could control the nature of the distortions in specific compounds.[128] The elongated tetragonal structure has four long and eight short bonds in the core structure. The terminal (thiolate) ligands are 0.03-0.05 Å longer in the reduced structure, consistent with the presence of 3Fe(ll) and 1Fe(III) in the reduced form, compared to 2Fe(II) and 2Fe(III) in the oxidized form. There is no evidence for any valence localization.[128]

The oxidized $Fe_4S_4^{3+}$ core defied isolation and crystallization in a molecular complex prior to the use of sterically hindered thiolate ligands. With 2,4,6 tris(isopropyl)phenylthiolate, the $Fe_4S_4L_4^{-}$ complex could be isolated and characterized.[141] The structure is a tetragonally compressed thiocubane with average Fe-S and Fe-SR distances 0.02 and 0.04 Å shorter than the corresponding distances in the $Fe_4S_4L_4^{2-}$ complex. Again, there is no evidence for Fe inequivalence or more profound structural distortion in this 3Fe(III)-1Fe(II) cluster. Clearly, the Fe_4S_4 clusters have highly delocalized bonding.

Evidence from model systems using sterically hindered thiolate ligands indicates the existence of an $Fe_4S_4^{4+}$, i.e., all-ferric fully oxidized cube.[142] The existence of the complete series $Fe_4S_4[(Cy)_3C_6H_2S]_4^{n}$ (Cy =cyclohexyl; n = 0, -1, -2, -3) is implied by reversible electrochemical measurements. Clearly, five different states of the Fe_4S_4 core—including the (at least) transient fully oxidized state and the all-ferrous fully reduced state—may have stable existence. Although only the central three states have been shown to exist in biological contexts, one must not rule out the possible existence of the others under certain circumstances.

Recently, specifically designed tridentate ligands have been synthesized that bind tightly to three of the four Fe atoms in the thiocubane structure.[143,143a,b] The remaining Fe atom can then be treated with a range of reagents to produce a series of subsite-differentiated derivatives and variously bridged double-cubane units. These derivatives illustrate the potential to synthesize complexes that mimic the more unusual features of Fe_4S_4 centers that are bound specifically and asymmetrically to protein sites. The recently synthesized complex ion $[(Cl_3Fe_4S_4)_2S]^{4-}$, containing two Fe_4S_4 units bridged by a single S^{2-} ligand, illustrates the potential coupling of known clusters into larger aggregates.[143c]

The model-system work has made an important contribution to our understanding of the Fe_4S_4 centers. The existence of three states, the exchange of ligands, the redox properties, the metrical details of the basic Fe_4S_4 unit, and the subtleties of structural distortion can each be addressed through the study of models in comparison with the native proteins.