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Iron-sulfur proteins: Recent developments in the field

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Summary. Iron-sulfur clusters in proteins are now recognized as among the main types of electron-transferring groups in biological systems, besides heme and flavins. Recent developments have brought forth a better understanding about the ways the protein environment modulates the potential of the cluster by placing the cluster in a more or less hydrophobic surrounding. Refinement in models, extensive studies on the kinetics of electron transfer (e.g. by measurement of the electronic spin lattice relaxation time) and the introduction of novel spectroscopic methods (EXAFS, magnetic CD and others) in the elucidation of structures in various systems are among the main developments. Other advances include EPR studies of the spatial orientation of Fe-S centers in complex membraneous systems (e.g. in mitochondria) and the recent elucidation of the nature of center X in photosystem I by Mössbauer-spectroscopy. Mössbauer studies have also been described on a number of Fe-S proteins (nitrogenase, aconitase, some ferredoxins, etc.) and revealed the existence of novel structures that enlarged the number of known basic units of Fe-S centers. These advances include: 1. the discovery of a novel non-heme Fe-protein (called desulforedoxin) of the rebredoxin type, 2. the elucidation of the nitrogenase Fe-S centers and the nitrogenase cofactor and 3. the discovery of a three-iron cluster in several enzymes and some ferredoxins. The latter 3-Fe cluster seems capable of being converted into a classical 4-Fe cluster under appropriate conditions, a phenomenon that plays a role in activation-deactivation of some enzymes (e.g. aconitase). It is now recognized that some iron-sulfur clusters may be involved in systems devoided of any oxydation-reduction reaction and may act as sensors of the surrounding redox potential, triggering the activation/deactivation of an enzyme (cf. e.g. aconitase).

Introduction

General properties of iron-sulfur proteins

During the past decade, the field of iron-sulfur proteins has witnessed an intensive development, and considerable progress has recently been achieved in this fascinating subfield of biochemistry. Thanks to a very interdisciplinary collaboration among biochemists, chemists and physicists, sophistication has rapidly brought this field to maturity. These advances have culminated in establishing the iron-sulfur clusters as the main types of electron transferring groups in proteins, together with the flavins, heme and copper. The properties of iron-sulfur proteins have been extensively reviewed in three volumes[1] and several reviews[2-6], and we shall deal here primarily with more recent developments.

The iron-sulfur proteins contain four-coordinate, non-heme iron in a tetrahedral or distorted tetrahedral geometry bound to sulfur ligands, both cysteinyl residues from the protein core and (except in the rubredoxins) inorganic 'labile' sulfide. This class of proteins is ubiquitous in living organisms and has been the object of a large number of investigations. By a combination of X-ray crystallography, resonance spectroscopy (primarily EPR ENDOR and Mössbauer spectroscopy) and synthesis of chemical analogs, it was established in the 1970's that all iron-sulfur proteins known so far contained either 1Fe, 2Fe or 4Fe clusters (fig. 1). Yet this picture looks somewhat oversimplified in view of recently discovered Fe-S centers[7-10], as will be discussed later on in this paper. The 1Fe centers are found in rubredoxins, while [2Fe-2S] and [4Fe-4S] clusters have been found in many electron transferring systems and enzymes either as single electron transferring centers (in simple Fe-S proteins) or in combination with other prosthetic groups (flavins, metals, other Fe-S centers, etc.) in cases of so-called conjugated Fe-S proteins[11]. The table summarizes some of these properties.

These electron carriers operate over a wide range of oxidation-reduction potentials, depending upon the protein core that stabilizes the cluster. In general, they operate in the low or even very low potential region (up to -700 mV in some cases), although some proteins have a high positive midpoint potential (e.g., 'High potential iron-sulfur proteins' or HiPIP). The

ways in which the protein moiety can modulate the oxidation-reduction properties of an Fe-S cluster chiefly include[12]: a) exclusion of the cluster from solvent by the polypeptide backbone (to raise the redox potential); b) hydrogen bonding from amide NH to both inorganic 'labile' sulfide and S_γ-cysteinyl groups (that stabilize negative charges and, e.g. in [4Fe-4S] clusters, favor two out of three redox states); c) NH– – –C-O bonds on basic residues (that states); negative cluster charge); and d) also aromatic amino acids located in the vicinity of the Fe-S which affects its oxidoreduction behavior by charge transfer and by their hydrophobicity[13].

The iron-sulfur proteins are usually described by their EPR-spectra, since these give a good picture of their magnetic properties. Figure 2 shows a representative selection of spectra of iron-sulfur proteins. While the oxidized 1Fe proteins have characteristically narrow EPR-signals around g=4.3, the [2Fe-2S] clusters and some of the [4Fe-4S] clusters give EPR-signals in the reduced state centered at g=1.94; other [4Fe-4S] clusters (of the 'HiPIP' type) give EPR-signals around g=2.01 in their oxidized state. Thus [4Fe-4S] clusters can either shuttle between an oxidized state that is paramagnetic and a reduced state that is diamagnetic or they can operate in nature between a reduced state that is paramagnetic and a diamagnetic oxidized state. In the first case, the midpoint potentials of the clusters are around +350 mV (e.g., in *Chromatium vinosum* HiPIP), whereas they are around −300 to −400 mV and sometimes lower in most [4Fe-4S] ferredoxins and other Fe-S proteins. The fact that the X-ray diffraction pattern of these two types of [4Fe-4S] clusters is virtually identical has been rationalized by Carter[14] who proposed that the cluster of reduced HiPIP and of the oxidized ferredoxins are in an equivalent diamagnetic state (state +2); the oxidized HiPIP type clusters represent a higher level oxydation of the cluster (state +3), while reduced ferredoxins represent a lower oxidation-reduction state of the same basic structure (state +1) (see fig. 3). This hypothesis is also supported by data from Mössbauer-spectroscopy, by study of synthetic analogs of [4Fe-4S] clusters[15] and by the observation of a ferredoxin-like EPR signal for superreduced HiPIP and a HiPIP-like EPR signal for superoxidized ferredoxins[16].

An essential feature of all Fe-S clusters in all oxidation states is spin coupling through exchange interaction[5] with a spin coupling constant $J \simeq -100\ cm^{-1}$. [2Fe-2S] clusters in the oxidized state are diamagnetic, indicative of antiferromagnetic coupling between two

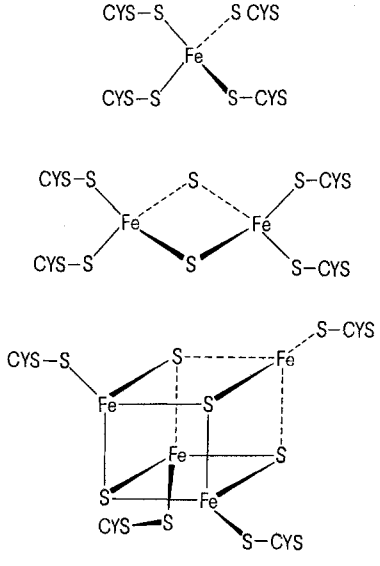

Figure 1. Structures of iron-sulfur clusters. (Top) The iron-sulfur cluster of rubredoxin, showing the iron and four cysteinyl sulfur atoms. (Middle) [2Fe-2S] clusters found in higher plant-type ferredoxin and other 2Fe proteins. (Bottom) Iron clusters found in bacterial ferredoxins and in 4Fe proteins, as derived from X-ray diffraction studies.

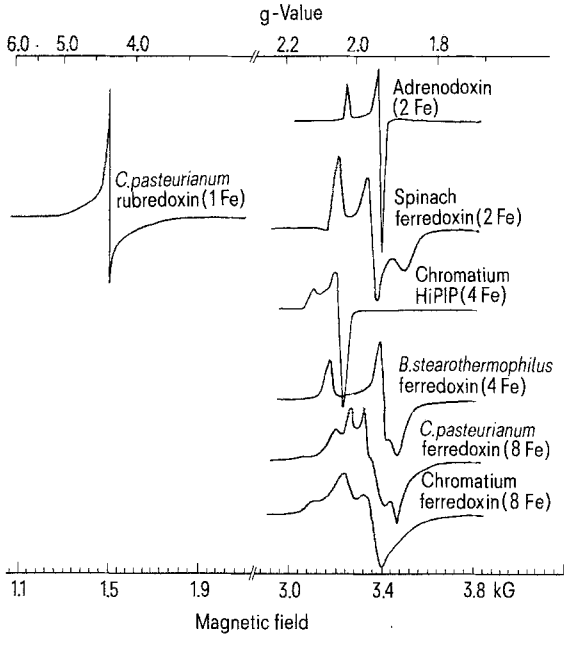

Figure 2. Electron paramagnetic resonance spectra of the different types of iron-sulfur proteins. Modified from D. O. Hall et al.[92].

	Formal oxidation states of Fe	Oxidation level
HiPIP	$4\ Fe^{3+}$	4+
	$3\ Fe^{3+} + Fe^{2+}$	3+
	$2\ Fe^{3+} + 2Fe^{2+}$	2+
Ferredoxin	$Fe^{3+} + 3Fe^{2+}$	1+
	$4Fe^{2+}$	0

Figure 3. Oxidation levels of the [4Fe-4S] cluster according to the Carter's hypothesis. Oxidized [4Fe-4S] ferredoxins or reduced HiPIP share the same oxidation level 2+. The different oxidation levels which the cluster can take in proteins partly explains the considerable difference in redox potential of 4Fe-ferredoxins (~ −300−400 mV) and HiPIP (~ +350 mV).

high-spin Fe^{3+} ions, to give an S=0 ground state. Upon reduction the antiferromagnetic coupling between high-spin Fe^{3+} ($S=\frac{5}{2}$) and high-spin Fe^{2+} (S=2) leads to a paramagnetic system with a spin ½ ground state and gives rise to slightly anisotropic EPR-spectra with $g_{average}=1.94$ (Gibson et al.[17]). The spin coupling within the [4Fe-4S] clusters in the diamagnetic state +2 results from $2Fe^{2+}$ and $2Fe^{3+}$ ions with strong electron delocalization and the resultant magnetic moment of the system is zero. In states +1 and +3 there is a net spin of ½, as evidenced from the observation of magnetic hyperfine splitting of Mössbauer-spectra in large magnetic fields (~30 kg)[18]. However, a theoretical framework such as that provided for the [2Fe-2S] clusters[17] is still lacking for [4Fe-4S] clusters due to the immensely greater complexity of the spin coupling problem between 4Fe atoms in all redox states.

In the last few years renewed interest in the field of Fe-S proteins was brought about by the discovery of novel structures, which has enlarged the number of known basic units of Fe-S clusters. Three new types of clusters have been discovered: a three-iron cluster has been found in several ferredoxins and some enzymes (aconitase and glutamate synthase); on the other hand, the elucidation of the nitrogenase Fe-S centers (the so-called P-clusters) has shown the presence of a new basic structure, probably a variant of the [4Fe-4S] cluster; and finally the nitrogenase cofactor also contains a new kind of [Fe-S] center with one molybdenum and probably six Fe atoms and labile sulfurs.

Recent developments

1Fe-centers (rubredoxin-type)

This type of protein constitutes the simplest class of iron-sulfur proteins with one iron coordinated to four cysteinyl residues and no labile sulfur. They are present in aerobic and anaerobic organisms; but despite their widespread occurrence, their general function is not yet clear. An enormous effort has been devoted to the determination of the structural features and electronic properties of the iron center [X-ray[19], Mössbauer[20], EXAFS[21], MCD[22] and studies on model compounds[23]]. A more complex rubredoxin with two domains, each containing one iron atom, has also been found in *P. olievoranus*[24] wich is involved in electron transfer to a fatty acid ω-hydroxylase system.

A new type of non-heme Fe-protein, called desulforedoxin, from sulfur reducing bacteria, has been the subject of comparative studies with rubredoxin[25]. This protein has a mol. wt of 7600 and operates in the same range of redox potentials as the rubredoxins (about −35 mV). It consists of two identical subunits and contains two iron atoms linked to the sulfur of eight cysteines with no acid-labile sulfur[26]. Yet it seems that the two irons crosslink the two monomers, each being liganded to three cysteine ligands of one monomer and to the fourth ligand in the other monomer[27]. EPR-studies of desulforedoxin[28] showed a complex pattern in the oxidized state, with g-values around 7.72, 5.73, 4.94 and 1.84 (compared to typical g-values around 9.4 and 4.27 in oxidized rubredoxin). This was interpreted in terms of high-spin ferric ions ($S=\frac{5}{2}$). The resonances at 7.7, 4.9 and 1.8 were assigned to the ground state doublet ($\pm\frac{1}{2}$) and the resonances at g=5.7 to the middle doublet[29]. Magnetic susceptibility measurements of the oxidized and reduced states in the protein suggested spin-spin interaction between the two iron centers; this is also supported by Mössbauer-data. Optical studies showed charge transfer bands at 495 and 565 nm, suggestive of transition $^6A_1 \rightarrow {}^6E$ and $^6A_1 \rightarrow {}^6A_2$[28].

Another 1Fe protein has been recently investigated and deserves to be briefly mentioned here, although it does not belong to the class of Fe-S proteins but may constitute a new class of Fe protein. This protein, purple uteroferrin (also called purple acid phosphatase), is a 1Fe protein that has an unusual EPR-spectrum with $g_{average}=1.79$. Recent magnetic susceptibility measurements between 2 and 77 °K, together with the EPR-spectrum, showed that the paramagnetic center arises from a single unpaired spin. This study is indicative for the presence of a mononuclear low spin Fe-atom with unknown ligand[30].

[2Fe-2S] clusters

A theoretical interpretation of the variation of some physical parameters within the [2Fe-2S] clusters has been proposed[31]. According to the model previously proposed by Gibson et al.[17] the 2 high-spin Fe ions are strongly coupled by an antiferromagnetic exchange interaction giving a Fe^{3+}-Fe^{3+} pair in the oxidized state and an Fe^{3+}-Fe^{2+} pair in the reduced state. However, some parameters show unexplained variations (symmetry of the g tensor, electron field gradient (EFG) tensor as measured by Mössbauer-spectroscopy, intensity of the exchange coupling and values of the electronic spin lattice relaxation time T_1). The new model proposes a variable mixing of some d-orbitals of the Fe^{2+} ion owing to rhombic distortion of the active site with the same geometrical character but different intensity for each protein. This should explain some peculiar experimental results such as the axial EPR-spectra and the EFG tensor of some ferredoxins without assuming properties drastically different from those of other ferredoxins. An accurate theoretical description of the antiferromagnetic coupling between the 2Fe atoms has also been reported (32) using Xα valence bond theory. This latter study, based on a calculation of $Fe_2S_2(SH_4)^{2-3-}$ models for the active sites of oxidized and reduced ferredoxins, shows much greater similarity between 2Fe and 1Fe clusters than was evident from previous Xα MO calculations[33].

The ferredoxins are characterized by a strong temperature-dependence of the electronic spin lattice relaxation time T_1. A recent study[34] using either a continuous saturation method or a saturating pulse method, has disclosed different relaxation processes and the corresponding vibration modes which can take place in these proteins. This may become useful in understanding the kinetics of electron transfer between oxidation-reduction centers. As already mentioned, the oxidation-reduction properties of the Fe-S cluster are greatly controlled and determined by the nature of the surrounding polypeptide chain[35]. Sequence homologies in ferredoxins have, therefore, been thoroughly investigated and discussed with a view to understanding both the electronic properties of the Fe-S clusters and the evolutionary implications of the protein backbone (see, e.g., a study on the amino acid sequence of ferredoxin from *T. aestivum*[36]). The modulation of electronic properties of the Fe-S cluster does not yet imply drastic changes in the average structural parameters of the redox center upon incorporation in the protein. EXAFS studies of protein and model compounds containing dimeric as well as tetrameric Fe-S clusters[37] clearly showed that structural changes are very small upon incorporation and that upon electron transfer these changes are small also. The redox behavior of the Fe-S cluster in proteins is partially controlled by the distribution of cysteine residues constructing the Fe-S center[38] and also by the sensitivity of aromatic residues located in the vicinity of the cluster as, e.g., a fluorescence study on a ferredoxin from *Halobacterium halobium* has clearly shown[39]. This [2Fe-2S] bacterial ferredoxin acts as electron donor to ferredoxin: 2-oxoacid oxidoreductase and has an amino acid sequence homologous to ferredoxins from cyanobacteria and plants. The fluorescence decay studies on this Fe-S protein indicate that two tryptophan residues show different fluorescence characteristics; one is probably associated with the occurrence of a higher potential in this protein when compared with chloroplast-type ferredoxins. The environment of the other tryptophan residue becomes more hydrophobic upon conformation change induced by removal of the ferric ion but is unaffected by reduction of the Fe-S cluster.

The structure of [2Fe-2S] clusters, as studied by X-ray crystallography at 2.5 Å (Fukuyama et al.[40]) in a plant-type ferredoxin from *Spirulina plantensis* also indicates the possibility of some NH − − − S hydrogen bonds. The cluster appears to be at about 8 Å from the surface of the protein. A similar result was obtained in a chloroplast-type ferredoxin. Studies on oxidized analogs of the [2Fe-2S] core have shown that the iron-thiol S-bond lengths are intermediate to those of oxidized and reduced rubredoxin analogs[21]. The analog has an exact two-fold axis but to what extent this persists in the protein is not known. The reduction of the analog proceeds with potentials much more negative than observed in proteins (e.g., − 1.5 V vs − 400 mV in the protein), so the protein has made the potential of the clusters much more positive than in an unprotected environment[41].

Conformational change, induced upon complex-formation between ferredoxin and protein has been observed by circular dichroism (e.g., on ferredoxin: NADP oxidoreductase[42] and ferredoxin: nitrite oxidoreductase) and CD-spectra of these proteins indicate both changes in the environment of the prosthetic groups of the proteins and increase in protein secondary structure. Electrochemical titrations of these complexes showed that complex formation alters the midpoint oxidation-reduction potentials of both proteins, e.g., the potential of ferredoxin in ferredoxin: NADP oxidoreductase becomes 22 mV more negative upon complex formation[43]. A change in the midpoint potential has also been generated by neutral salts[44] which modulate the interaction between the Fe-S cluster and the protein moiety. This results in thermal stabilization of the protein with increase of salt concentration as suggested from a study by differen-

Diversity of Fe-S proteins

Prosthetic Groups	Enzymes
Iron-sulfur group(s)	Hydrogenase, glutamine amidoribosyl-transferase, 4′-methoxybenzoate-0-dimethylase, ω-hydroxylase, aconitase, photosystem I
Iron-sulfur-flavin	Succinate dehydrogenase, NADH dehydrogenase ETF-ubiquinone oxidoreductase, glutamate synthase, dihydroorotate dehydrogenase, dimethylamine dehydrogenase, trimethylamine dehydrogenase, formate dehydrogenase, enoate reductase
Iron-sulfur-heme	Sulfite reductase (dissimilatory), adenylyl sulfate reductase, ubiquinone-cytochrome *c* reductase, nitrite reductase
Iron-sulfur-heme-flavin	Sulfite reductase (assimilatory), nitrite reductase
Iron-sulfur-molybdenum	Nitrogenase, formate dehydrogenase, CO_2 reductase, nitrate reductase
Iron-sulfur molybdenum-flavin	Xanthine oxidase, xanthine dehydrogenase, aldehyde oxidase
Iron-sulfur-thiamine pyrophosphate	Pyruvate-ferredoxin oxidoreductase, 2-oxoglutarate-ferredoxin oxidoreductase, 2-oxobutyrate ferredoxin oxidoreductase

tial scanning calorimetry and circular dichroism. The effect of the high negative charge of the protein on the Fe-S center has also been discussed in a study by ^{35}Cl-NMR of anion binding to halophilic ferredoxin from halobacterium[45].

A characterization of individual Fe-S centers in more complex systems has been attempted on the basis of differential electron spin relaxation. In plant mitochondria, a number of EPR-signals have been detected and assigned to Fe-S centers[46]. In complex I, signals assigned to clusters N-1b, N-2 and N-3 or N-4 behave like ferredoxins and show an EPR-signal upon reduction. In the succinate:ubiquinone reductase complex (complex II) three centers have been characterized. An attempt has been made to distinguish the overlapping signals on the basis of the electronic spin relaxation rate on the Fe-S centers deduced from microwave power saturation studies[47]. This method was also useful for detecting magnetic interactions between paramagnetic centers as between centers S-1 and S-2 of complex II and between center S-3 and ubisemiquinone molecules. In the latter case, saturation data of the complex spectrum showed that center S-3 has faster electronic spin relaxation than ubisemiquinone molecules. In contrast to plant mitochondria, a greater number of Fe-S centers have been observed in mammalian mitochondria and their physicochemical properties have recently been reviewed[48,49]. In mammalian mitochondria, centers S-1 and S-2 of complex II are [2Fe-2S] centers whose spatial relationship and structure have been characterized[50]. Membrane bound redox Fe-S centers are fixed in the mitochondrial membrane at defined angles relative to the membrane plane; the Fe-Fe axis (g_z) of binuclear Fe-S centers is in the membrane plane, whereas the tetranuclear Fe-S clusters can have their g_z axis either perpendicular or parallel to the membrane plane; intermediate orientations are not observed[51]. (A similar EPR-study has been reported with bacterial membrane particles from disrupted *E. coli*[52]; the orientation of membrane-bound Fe-S clusters is quite similar). In mitochondria, spin coupling between the two binuclear centers of complex II is manifested by broadening and splitting of spectra of reconstitutively active and inactive preparations, relief of power saturation of center S-1 spectra on reduction of center S-2 and by the observation of a half-field transition signal ('$\Delta M_s = \pm 2$') in dithionite reduced preparations. This coupling seems to induce extremely fast relaxation of center S-2 at low temperature. Aquisition of individual EPR-spectra that are characterized by nearly identical rhombic g-tensors was made possible by the fact that the two centers exhibit very large differences in their midpoint potentials (0 and -400 mV)[51].

[3Fe-3S] clusters

The possibility of the existence of a [3Fe-3S] cluster for the active center of iron-sulfur proteins came about two years ago from Mössbauer-spectroscopy of ferredoxin I from *Azotobacter vinelandii*[9]; ferredoxin II from *Desulfovibrio gigas*[8]; and simultaneously of aconitase[10], the long-known citric acid cycle enzyme. The function of the azotobacter ferredoxin has not been established; this protein also contains an additional [4Fe-4S] cluster. The *D. gigas* ferredoxin II is a tetramer and appears to mediate electron transfer between cytochrome c_3 and the sulfite reductase[53]; its amino acid sequence is identical to that of *D. gigas* ferredoxin I which is isolated as a trimer and contains only [4Fe-4S] clusters[54]. [3Fe-3S] clusters have also been found in a ferredoxin from *Thermus thermophilus*[55] (which also contains an additional [4Fe-4S] cluster in a way similar to the azotobacter ferredoxin I) and in glutamate synthase from *E. coli* or *A. vinelandii*[56]; the latter contains additional prosthetic groups (FAD, FMN and 2[4Fe-4S] clusters)[57]. In the oxidized state, these proteins exhibit a nearly isotropic EPR-signal around $g = 2.01$, which results from an $S = 1/2$ ground state. Because of the similarity of their EPR-spectra with those of oxidized HiPIP, they have sometimes been misinterpreted in the past as being tetranuclear Fe-S centers.[58,60,61] Also, cluster extrusion experiments on proteins bearing [3Fe-3S] clusters have been ambiguous[59] since it seems that these [3Fe-3S] clusters are unstable and decompose under these conditions. Recently the Mössbauer technique has contributed substantially to the discovery and characterization of the [3Fe-3S] centers and the data have been reviewed by Münck[62,63] and by Huynh and Kent[64]. X-ray diffraction studies[65,66] on the oxidized [3Fe-3S] cluster of *A. vinelandii* ferredoxin I have shown that the three iron and the three sulfur atoms are arranged in a ring in a flattened twist-boat configuration with two iron atoms residing in a tetrahedral sulfur environment made up of two sulfides and two cysteinyl sulfur residues. The third iron site has three sulfur ligands; the nature of the fourth one is not known with certainty (see fig. 4) at present. The Mössbauer-spectra of the oxidized center at 4 °K show three components of high spin ferric ions having

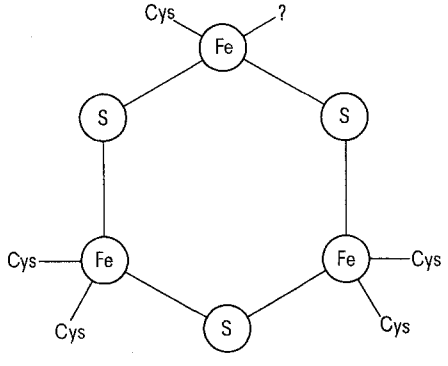

Figure 4. Structure of the [3Fe-3S] cluster in the ferredoxin from *A. vinelandii*. Adapted from data published by Stout and coworkers[12].

intensity ratios 1:1:1 and characterized by significantly different magnetic hyperfine constants. The magnetic properties of the cluster (with an observed isomer shift $\delta \simeq \pm 0.28$mm/sec relative to iron metal and a quadrupole splitting $\Delta E_Q \simeq 0.55$–0.63 mm/sec) suggests the presence of high spin ferric ions ($S = 5/2$) in a distorted tetrahedral sulfur environment[62] exchange coupled to a system spin $S = 1/2$. Since two high spin ferric ions ($S = 5/2$) cannot be coupled to yield the half integral spin observed for the oxidized center, a third half integral spin is required. The three sites have intrinsic hyperfine interactions similar to those of ferric rubredoxins; the difference in the observed interactions probably reflects the geometrical features of spin coupling[63].

Upon reduction with dithionite the [3Fe-3S] clusters usually exhibit no EPR-signal; however, under certain circumstances a ferredoxin-type signal can be observed in the [3Fe-3S] center of aconitase that accounts for up to 30% of the intrinsic cluster. The nature of this signal is not yet clear (J. L. Dreyer et al., unpublished results). Two well resolved quadrupole doublets (fig. 5b) (doublets I and II) are observed by Mössbauer-spectroscopy in dithionite-reduced [3Fe-3S] clusters which are present in the ration of 2:1. Their Mössbauer parameters suggest that the iron associated with doublet II is high-spin ferric in character, whereas the parameters of doublet I suggest a formal iron oxidation state of about $Fe^{2,5+}$, i.e., upon reduction the additional electron is shared by two of the three iron atoms. Studies in magnetic fields revealed that the reduced [3Fe-3S] cluster has non-zero integer electronic spin and that doublet II has a positive internal magnetic field, whereas the two iron sites in doublet I remain indistinguishable and experience a negative internal magnetic field. This reveals a structure with antiferromagnetic coupled spins[64]. The magnetic hyperfine coupling constants A differ drastically in sign and magnitude and this has been explained with a simple spin-coupling model[62]. The model assumes isotropic exchange and different couplings between the iron sites. The three exchange coupling constants are comparable to within a factor of two, which implies that the three-iron cluster is a single covalently linked structure and cannot be merely considered as a [2Fe-2S] cluster weakly coupled to a third iron atom. This current picture of [3Fe-3S] centers might, nevertheless, be subject to modification as more information is gathered on this type of cluster. It already appears in the [3Fe-3S] center of aconitase that several states of reduction can be achieved under different conditions, either paramagnetic or diamagnetic; in some cases EPR-signals typical of reduced ferredoxins have been observed. In aconitase the Fe-S cluster seems to undergo significant rearrangements, triggering an active conformation of the protein[67] although the Fe-S center does not seem to participate in catalysis. CD- and MCD-spectra of the cluster of aconitase are significantly different from those of either [2Fe-2S] or [4Fe-4S] cluster[68].

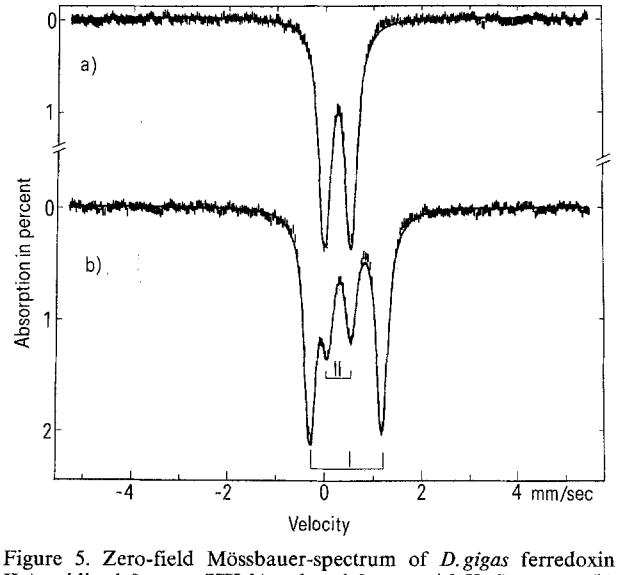

Figure 5. Zero-field Mössbauer-spectrum of *D. gigas* ferredoxin IIa) oxidized form at 77K b) reduced form at 4.2 K. Spectrum (b) is referred to as the 'fingerprint' spectrum of the new [3Fe-3S] center. Reproduced from A. V. Xavier et al.[29] by permission.

The case of aconitase is particularly interesting: this enzyme is not active when isolated aerobically and contains a [3Fe-3S] cluster[10,69]. Activity can be regained upon reduction with ferrous iron (and other reductants). Activation studies with iron enriched in ^{57}Fe or ^{56}Fe suggest strongly that the activation transforms the 3-Fe cluster into a center with a [4Fe-4S] core. This conclusion is supported by the observation that substantial EPR signals characteristic of reduced [4Fe-4S] clusters can be elicited under appropriate conditions and that the Mössbauer spectra exhibit all the unique features which have been reported for proteins with 4-Fe clusters. Thus the ferrous ion needed for activation is used to rebuild a [4Fe-4S] cluster[70]. A similar study on ferredoxin II from *Desulfovibrio gigas* showed similar results. This ferredoxin which contains one [3Fe-3S] cluster per monomer can develop EPR signals typical of reduced [4Fe-4S] clusters[71,72] by incubation of the protein with Fe^{2+} in the presence of dithiothreitol. By using iron enriched in ^{57}Fe for the incubation procedure, it was found that the iron is incorporated into 1 or possibly 2 subsites of the newly formed [4Fe-4S] core. Mössbauer studies of the oxidized[73] or reduced[72] forms of the cluster, as well as cluster reconstitutions and studies of the time course of the cluster interconversions have been recently reported[73].

[4Fe-4S] clusters

The list of Fe-S proteins bearing [4Fe-4S] centers is steadily growing. Mössbauer-spectroscopy has been used in recent studies[74] of the iron-sulfur centers of

photosystem 1 from blue green algae. This study may have confirmed the assignment of the EPR-signals of centers A and B to paramagnetic [4Fe-4S] centers similar to those found in reduced ferredoxin; an additional feature in the Mössbauer-spectra also indicated that a substantial amount of the Fe in the photosystem 1 preparations is not in the form of reducible A and B centers. Mössbauer-spectoscopy has also been used in the study of the Fe-S center of glutamine-phosphoribosyl pyrophosphate amidotransferase from *Bacillus subtilis*[75]. The presence and the function of an Fe-S center in a system like this that has no obvious electron-transfer reaction is still very puzzling; the Fe-S cluster in amidotransferase is essential for enzyme activity, since reaction with oxygen or o-phenantroline causes complete inactivation. Yet it seems unlikely that the Fe-S center is oxidized or reduced during catalysis[75] (a situation similar to that found in aconitase[67] as already mentioned). The Mössbauer-data indicated the presence of a [4Fe-4S] cluster in the +2 state which was corroborated by cluster extrusion experiments. In this latter technique[76] the protein is unfolded and the iron-sulfur cluster, stabilized by benzenethiol, is transferred to other apoproteins (from either [4Fe-4S] or [2Fe-2S] ferredoxin), which will then exhibit a characteristic absorption spectrum upon reconstitution with the extruded cluster. This technique has also been used successfully in the characterization of a ferredoxin from *Rhibozoidum japanicum* bacteroids[77], which mediates electron transfer between illuminated chloroplasts and bacteroid nitrogenase; spectroscopic examination of the extruded iron-sulfur clusters showed the presence of 2[4Fe-4S] clusters. The iron-sulfur center of spinach ferredoxin-nitrite reductase (a siroheme containing enzyme) has been reinvestigated[78]; the presence of a [4Fe-4S] center has been established, using a method for the identification of iron-sulfur clusters based on the specificity and temperature sensitivity of the EPR-spectra of dithionite-reduced clusters in dimethylsulfoxide[16]. Recent systematic studies[79,80] of chiroptical electronic spectroscopy (CD and MCD) of simple Fe-S proteins have shown that this technique can be useful in characterizing iron-sulfur cluster types, oxidation level and protein environment; chiroptical spectra at room temperature are markedly more structured and more informative than the corresponding absorption spectra. This method applied to the identification of iron-sulfur clusters of nitrogenase proteins clearly demonstrated the presence of a reduced bacterial-ferredoxin-like [4Fe-4S] center[81].

The electron exchange kinetics of tetranuclear iron-sulfur clusters has been studied on model compounds to give a picture of the intrinsic operation of 4Fe clusters in electron transfer in the absence of modulating influences of protein structures[82]. In going from $(Fe_4S_4(S(_6H_S)_4)^{2-}$ to its trianion, the [4Fe-4S] core (of idealized D_{2d} symmetry in both cases) changes from a slightly compressed cubane-like structure with eight long and four short Fe-S bonds to a somewhat elongated configuration with four long and eight short Fe-S bonds. This can be described in terms of an axial expansion along the four axes[37] as EXAFS studies seem to indicate. The structural reorganization energy of the [4Fe-4S] core upon passing from a compressed tetragonal to an elongated tetragonal geometry via a T_d transition state in an outer sphere process has been estimated. The generality of this structural change in electron transfer reactions of 4Fe cores has been demonstrated[83] by Mössbauer studies, magnetic susceptibility measurements and EPR-spectroscopy of an extensive series of compounds; the elongated, idealized D_{2d} core structure is the intrinsically stable configuration of $(Fe_4S_4(SR)_4)^{3-}$. The relatively slow electron self-exchange rates between oxidized and reduced forms of proteins containing a 4Fe site can be primarily attributed to kinetically retarding steric influences of protein structures rather than intrinsically slow reactions of the sites themselves[84]. Thus, e.g., deprotonation of vicinal histidine can result in destabilization of the reduced cluster and thus in a faster rate of oxidation[85]. Amino acid side chains play a significant but limited role in the electrostatic interactions of the cluster in electron tranfer[86]; the cluster charge is apparently distributed on the surface of the protein molecule through a network of hydrogen bonds. It, nevertheless, seems that in high-potential iron-sulfur proteins the kinetics of oxidation and reduction do not correlate with the oxidation-potential of the protein[86]. The electron transfer mechanism of these proteins has been explored via stopped-flow spectrophotometric kinetics studies[87]; previous studies already showed that the net redox charge does change with pH, assuming proper corrections are made to partition purely electrostatic effects from mechanistically significant ionization of amino acid residues.

EPR-spectroscopy has been applied to determine the energy of the low lying excited state of paramagnetic tetranuclear clusters, either oxidized $[4Fe\text{-}4S]^{(+2,+3)}$ ferredoxins (HiPIP) or reduced $[4Fe\text{-}4S]^{(+1,+2)}$ ferredoxins[88]. Relating the spectral parameters to the electronic structure of the ferredoxins, one finds increasing axial distortion in the EPR-spectra of the $[4Fe\text{-}4S]^{(+2,+3)}$ associated with higher energy differences.

EPR-spectroscopy has also proven useful in determining magnetic interactions between enzyme components and estimation of their vectorial relationships; the interactions reveal themselves as line broadening, splitting and relief from saturation of EPR-signals. Alterations in such EPR-parameters can be measured when the paramagnetic protein is exposed to exogenous paramagnetic ions such as dys-

prosium. A study of the relaxation behavior of tetranuclear HiPIP by this method[89] showed that the spin-lattice interaction is described at low temperature by a predominant second order Raman relaxation mechanism, whereas at higher temperature an Orbach process becomes dominant in either *Rhodopseudomonas gelatinosa* [4Fe-4S] HiPIP or a 2[4Fe-4S] ferredoxin from *Clostridium pasteurianum*. Cooperative interaction between clusters in the latter protein has been studied[90]; reducing one [4Fe-4S] cluster does not affect the redox potential of the other unreduced cluster, so that apparently cluster-cluster interaction is not strong enough to be functional. This has been confirmed independently by pulse-radiolysis[91] of the same 2[4Fe-4S] protein. The absence of any significant effect is surprising in view of the apparent advantage of this cooperativity for $2e^-$ transfer proteins.

Conclusion

It is now recognized that the iron-sulfur clusters are among the main types of electron-transferring groups in proteins. The protein environment modulates the energy levels by placing the cluster in a more or less hydrophobic environment. The study of the structure and function of these proteins has been one of the most challenging research fields, involving an ensemble of multidisciplinary sciences and the use of numerous techniques and sophisticated instrumentation. The recent discovery of novel structures has further stimulated researchers from all fields, as Nature gradually unveils deeper levels of its complexity.

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SPECIALIA

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Antimicrobial activity of carbazole derivatives[1]

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Summary. Glycozoline and girinimbine isomers (**IV** and **VII**) were synthesized and their activity against 2 bacterial strains, viz., *E. coli* and *S. aureus,* and 2 fungal strains, viz. *C. albicans* and *A. niger* were studied. The hydroxy synthons (**III**) were also tested.

Carbazole derivatives exhibit diverse biological activities; for example they may be antidepressant[4], antiinflammatory[5], anticonvulsant[6], antiserotonin[7], cardiotonic[8,9], analgesic[9] etc. Some of their derivatives also display bactericidal[10], antiviral[11] and antifungal[12] properties. Chowdhury et al.[13], studied the insecticidal and antimicrobial properties of some carbazole, tetrahydrocarbazole and 1-oxo-tetrahydrocarbazole derivatives. The antibiotic activities of some carbazole alkaloids, e.g. glycozoline (**I**), and related compounds have been reported by Chakraborty et al.[14]. They found that demethylated glycozoline (6-hydroxy-3-methylcarbazole) was the most active antifungal agent among the