Iron Sulfur Proteins and their Synthetic Analogues: Structure, Reactivity and Redox Properties

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A number of non-heme proteins contain iron-sulfur clusters as their core. The various structural types that have been characterised so far are discussed. Attempts to understand their properties and functions at a molecular level through model systems are described.

Introduction

The understanding of structures and functions of iron sulfur proteins is an area of bio-inorganic chemistry which has developed into a subject of great significance over the last two decades. This group of non-heme iron-sulfur (Fe-S) compounds are involved in electron transfer reactions in biological systems and are thus ubiquitous in nature. They are thought to be fundamental to such processes as photosynthesis, nitrogen fixation and various metabolic reactions. Yet the precise nature of their composition and stereochemistry was not established until very recently. In fact, it was only as the result (unintentionally, as all significant discoveries tend to be) of advances made in the field of anaerobic fermentative metabolism and photosynthesis during the period 1952–63 that these Fe-S compounds were first recognised.

The main reason for such a relatively common and important class of compounds remaining undiscovered for so long is that the Fe-S redox centres, which form the active sites of these proteins, could not be extracted from the proteins until recently, and examined chemically. All the previous attempts to do so had caused the disintegration of the active site of the protein and this resulted in the complete loss of biological activity.

Optical absorption spectroscopy produces weak, broad spectra

Box 1. Heme and Non-heme Proteins

'Heme' is an iron chelate of a substituted porphyrin and is a non-protein part of a haloenzyme. Iron could be present as Fe(II) or Fe(III). For example 'heme' is the prosthetic group in haemoglobin, myoglobin, and cytochrome. 'Non-heme' proteins are thus the ones which do not contain Fe^{II}/Fe^{III} chelate of porphyrin as a non-protein in a haloenzyme.

from Fe-S proteins. So the method which finally became appropriate to their detection was low temperature electron paramagnetic resonance spectroscopy. It was only after X-ray crystallographic results became available during 1968–71 that the exact composition and stereochemistry of Fe-S protein active sites were established.

In this article, we begin with a brief survey of the types of Fe-S centres which have been characterised in proteins. Next, the synthetic analogues which have been prepared to understand the reactions and properties of the various structural types are discussed. Since Fe-S proteins are involved in electron transfer processes, numerous studies have been carried out on the redox properties of the models. To give a flavour of current research involving these systems, typical electrochemical examinations of iron-sulfur clusters are described.

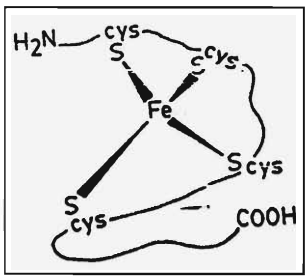
Types of Fe-S Protein Centres

Iron sulfur proteins are found to be of low molecular weight (6000–20,000) and contain iron, cysteinyl sulfurs (coming from the amino acid cysteine in the protein chain) and 'inorganic sulfur', the so called non-cysteinyl sulfur atoms. The sulfur ligands are arranged tetrahedrally about the iron atoms. The presence of inorganic sulfur is indicated through the release of H_2S gas when these proteins are treated with a mineral acid. The cluster of iron and inorganic sulfur atoms in the form $Fe_nS_{n'}$ or (nFe-n'S) where n and n' are 1, 0; 2, 2; 3, 4; 4, 4 respectively constitutes the core group in these proteins and are their redox centres. These redox centres are wrapped in the protein chain with the cysteinyl sulfurs of the protein coordinating to the

irons of the core group. These proteins may be categorised into five accepted classes of Fe-S centres, according to the numbers of iron and inorganic sulfur atoms in the cluster.

Classification of Iron-Sulfur Proteins

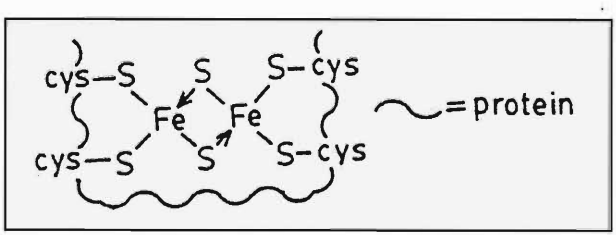
1. Rubredoxins (Fe-oS)

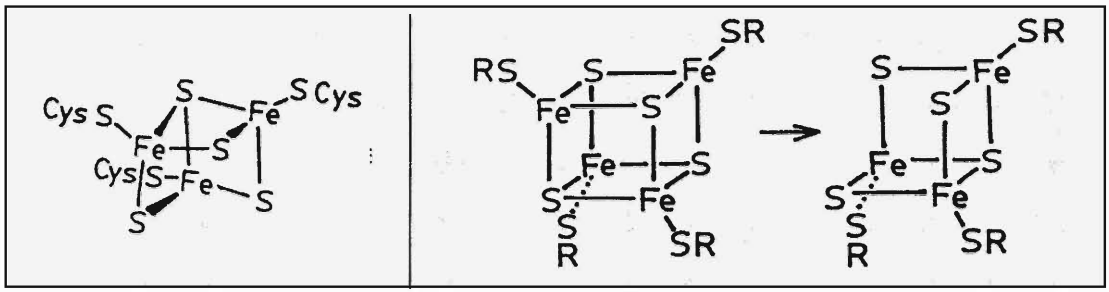


This protein is of bacterial origin and was originally isolated from *Clostridium pasteurianum* but has since been found in a number of anaerobic micro-organisms. All have one iron atom which is tetrahedrally coordinated to four sulfur atoms from the four cysteine residues present in the protein chain of 55 amino acids. The redox centre is made up of only high spin Fe(III) and there are no inorganic sulfur atoms in the core group.

2. Two-Iron Ferredoxins [2Fe-2S]

A number of these proteins (Fd) have been isolated from plant and non-plant sources. Unlike in rubredoxin, the redox centre is a cluster of equal numbers of iron and inorganic sulfur atoms represented as (2Fe-2S). This redox centre is wrapped around the protein chain, through the coordination of iron atom with the cysteinyl sulfurs. The structure of the (2Fe-2S) proteins can best be regarded as a bitetrahedral one, with the iron atoms bridged by two sulfide groups and bound additionally by two sulfurs of the cysteine groups per iron atom as represented schematically alongside. Since the iron centres are four coordinate, the crystal-field splitting is weak and thus the metals are in high spin state in both oxidised and reduced forms. One major class of (2Fe-2S) proteins is the chloroplast ferredoxin, which as the name suggests is fundamental in the photosynthetic process in plants.



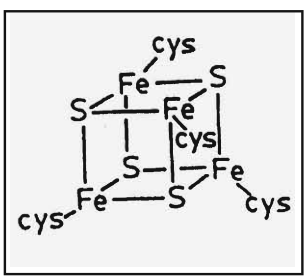


3. Three-Iron Ferredoxin [3Fe-4S]

Originally the X-ray crystal structure of the 7-iron ferredoxin from *Azotobacter vinelandii* was interpreted as having a (4Fe-4S) and (3Fe-3S) cluster. However, an error was detected in the analysis of this structure and the tri-iron cluster was corrected as having a non planar Fe_3S_4 geometry. This structure can be derived from (4Fe-4S) core (see next) by removing an Fe-S (cysteine) unit from one of the eight corners of the cube; a formal transformation is illustrated above. This cluster composition has been supported from EXAFS data on the *A. vinelandii* Fd, 3Fe-4S Fd from *Desulfovibro gigas*. Their behaviour is at present poorly understood and the biological significance is still being debated.

4. Four-Iron Ferredoxins (4Fe-4S)

This is the most ubiquitous iron-sulfur cluster in biology and has both plant and bacterial origins. The cluster contains (4Fe-4S) as the redox centre. The iron and sulfur atoms are arranged in a cubane structure, with each iron linked to the protein via a cysteinyl sulfur. The result is two interpenetrating concentric tetrahedra of four iron and four sulfide atoms with mean Fe—Fe and S—S distances being 2.75Å and 3.55Å respectively. It may be seen that the structure can be considered as a pair of interacting Fe_2S_2 groups.



5. Eight-Iron Ferredoxin [8Fe-8S]

It is of bacterial origin *(Peptococcus aerogenes)* and contains two Fe_4S_4 cubes separated from each other by 12Å.

Synthetic Models of Iron-Sulfur Protein Centres

A major part of the work leading to the current understanding of Fe-S centres in natural proteins has been the synthesis and subsequent study of synthetic analogues. Synthesis of a 4Fe-Ferredoxin centre was first achieved by Holm and his group. Treatment of a methanolic solution of iron(III) chloride and sodium hydrogen sulfide with three equivalents of $Na(SCH_2Ph)$ results in an intensely coloured solution of $[Fe_4S_4(SCH_2Ph)_4]^{-2}$ cluster. In this reaction some of the thiolate ligand is consumed to reduce half of the iron formally to the +2 oxidation level required for the $[4Fe\text{-}4S]^{2+}$ unit.

$$4FeCl_3 + 12RS^- \longrightarrow 4Fe(SR)_3 + 12Cl^-$$

$$4Fe(SR)_3 \xrightarrow[4HS^-]{4OMe^-} [Fe_4S_4(SR)_4]^{2-} + RSSR + 6RS^- + 4MeOH$$

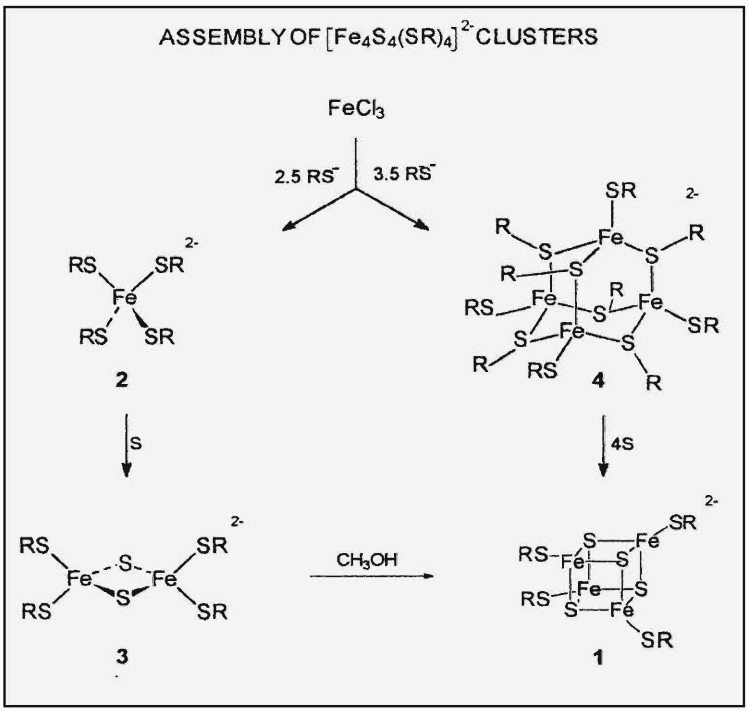
Alternatively and in slight modification to this synthetic route, Garner and his group used $FeCl_3/FeCl_2$ together with elemental sulfur instead of NaHS in the presence of thiolate. The thiolate serves as a reducing agent to yield the tetranuclear species in a 'self-assembly' reaction:

$$4FeCl_3/FeCl_2 + 4S + 14[SR]^- \longrightarrow [Fe_4S_4(SR)_4]^{2-} + 12Cl^- + 5RSSR$$

Synthetic routes to the analogous [2Fe-2S] family were discovered shortly after the preparation of tetranuclear cluster, and the first characterisation of mononuclear rubredoxin models subsequently followed. Typically in these model systems ligation of the iron centre(s) by simple alkyl- or aryl-thio groups replaces that of the cysteine residues of the protein polypeptide.

Behaviour of Synthetic Fe-S Centres and Core Extrusion from Natural Proteins

The study of properties of core clusters in proteins was greatly helped by the successful synthesis of their isostructural analogues. The investigations of the core cluster in natural proteins were

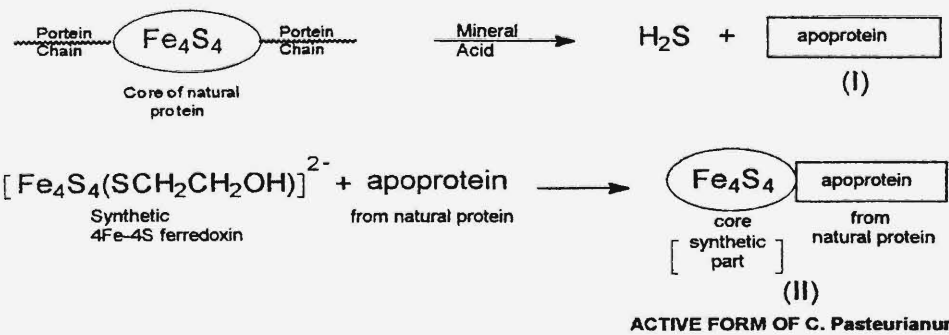


Depiction of the course of reactions resulting in assembly of $Fe_4S_4(SR)_4^{2-}$ clusters (1) via the intermediates $Fe(SR)_4^{2-}$ (2) $Fe_2S_2(SR)_4^{2-}$ (3) and $Fe_4(SR)_{10}^{2-}$ (4).

Interchange between Natural and Synthetic Clusters

Orme-Johnson has evolved parallel reactions with proteins in which the iron-sulfur core of one haloprotein was extracted and bound to another apoprotein, the iron-sulfur core of the latter having been removed by acidification (I). The reconstitution of active *C. pasteurianum* from the apoprotein and the synthetic $[Fe_4S_4(SCH_2CH_2OH_4]^{2-}$ demonstrates the complete interchange between natural and synthetic clusters (II).

Reconstitution of active *C. pasteurianum*



ACTIVE FORM OF C. Pasteurianum

These experiments show that the redox core in both the synthetic and natural clusters are isoelectronic and isodimensional.

greatly hampered because of long polypeptide chains into which these were embedded.

One reaction that proved to be of practical significance is ligand substitution in synthetic core clusters in which the terminal thiolates are replaced in a stepwise fashion.

$$[Fe_4S_4(SR)_4]^{2-} + nR'SH <-----> [Fe_4S_4(SR)_{4-n}(SR')_n]^{2-} + nRSH$$

with the equiiibrium favoured to the right if R'SH is more acidic than RSH. This facile thiol exchange together with the observation that the low molecular weight iron - sulfur proteins can be reversibly unfolded by solvents such as dimethyl sulfoxide, led to the idea that it should be possible to extrude iron-sulfur cores from natural proteins using an appropriate thiol. This approach has been applied successfully to distinguish 2Fe- and 4Fe-ferrodoxin centres in natural systems.

Redox Properties of Natural and Synthetic Centres

One area of Fe-S proteins which is closely linked with their function is their redox behaviour. It might be useful to consider this aspect and to compare with the synthetic centres. With the exception of high potential iron protein (HiPIP), all Fe-S proteins show fairly low redox potentials and generally function as electron carriers in conjugation with some other high molecular weight protein that has enzymatic activity. With all except the 8Fe-8S proteins, it appears that just one electron is involved and the synthetic Fe-S centres show analogous behaviour.

High Potential Iron Proteins (HiPIP)

A confusing part of the early studies of iron-sulfur proteins was the fact that the proteins with apparently the same core (4Fe-4S) and having isostructural cubane geometry exhibited different potentials. Such proteins called HiPIP are of bacterial origin (*Chromatium vinosum*) and have redox potentials about 0.75 V more positive than those of the normal 4-Fe ferrodoxin (Fd). It has also been established that $HiPIP_{reduced}$ is isoelectronic with $Fd_{oxidised}$ and that both are diamagnetic in these states. Though $HiPIP_{red.}$ and $Fd_{ox.}$ are both isostructural and isoelectronic, the reason for the difference in their redox behaviour has not been as yet clearly understood.

Fe-oS rubredoxin centres are not at all well investigated in terms of functions but are found in anaerobes and are definitely known to be involved in fatty acid ω-hydroxylation.

2Fe-ferredoxins transfer one electron at potentials from +250 mV to –400 mV. In the reduced state, Mössbauer spectra show that one iron atom is in the high spin ferrous state and one is in the high spin ferric state. When oxidised, both iron atoms of the 2Fe-2S centre are in the ferric state. It is difficult to assign formal oxidation states to the iron atoms in the reduced state. Ferredoxins may thus be regarded as a delocalised complex of iron with sulfur ligands. The unpaired electron in reduced ferredoxin can be regarded as being localised largely on the ligands utilising the π-acceptor properties of sulfur donor atoms. Further investigations show that the oxidised/reduced-ferredoxin couple has potentials of –300 mV difference from the corresponding 2-/3-potentials of synthetic peptide complexes. This has been attributed to the well known effect of protein structure, environment and charge distribution on the redox potentials. Nevertheless care must be taken not to take the findings of the synthetic analogue Fe-S centres too far without direct supporting evidence from natural centres.

4Fe-4S proteins do not appear to be as widespread as 2Fe-ferredoxins, but their redox chemistry seems to be a little more complex. The Fe_4S_4 cluster in the protein has a D_{2d} symmetry and the synthetic model undergoes one electron redox reaction characteristic of the cluster in natural ferredoxin. No evidence has yet been produced for localised Fe(II) and Fe(III) in synthetic 4Fe-4S clusters and it seems probable that the electrons involved in redox processes are delocalised over the whole system.

4Fe-ferredoxin can attain three oxidation states (1-, 2-, 3-) as opposed to the two of Fe-oS ad 2Fe-2S centres (2-, 3-). The existence of four oxidation states (1-, 2-, 3-, 4-) by the corresponding synthetic centres shows the complexity of the redox chemistry of 4Fe-ferredoxins. Considerable discrepancy is in fact found between the potentials of the analogue reactions

EPR (Electron Paramagnetic Resonance, also referred to as Electron Spin Resonance): A spectroscopic technique by which unpaired electrons can be detected in ions and molecules.

EXAFS (Extended X-ray Absorption Fine Structure): This is based on the back scattering of photoelectrons off ligands, and provides information on radial distances, number and types of bonded atoms.

Mössbauer Spectroscopy (named after its discoverer): This spectroscopic technique, in which fluorescence resulting from the absorption and emission of a γ-ray is observed, provides information about the electronic environment at the nucleus of an atom.

Redox Potentials: The redox potential of a metal atom/ion/molecule essentially tells us about the ease with which the species can gain or lose electrons. The redox potentials of metal ions can be significanlty attenuated by ligands. Redox potential can be measured by a variety of techniques, the most common one being Cyclic Voltammetry.

	Potential/mV (vs SHE)	Oxidised	Reduced
Rubredoxin	–40	Fe^{III}	Fe^{II}
2Fe-2S	–250 to –420	$2Fe^{III}$	Fe^{II} Fe^{III}
3Fe-4S	–130 to –420	$3Fe^{III}$	$2Fe^{2.25}$ Fe^{III}
4Fe-4S	–280 to –460	$4Fe^{2.5}$	$4Fe^{2.25}$
4Fe-4S HiPIP	+280 to +360	$4Fe^{2.75}$	$4Fe^{2.5}$

Table 1. Redox Behaviour of Fe - S Clusters

and those of the proteins but this is probably attributable to a combination of factors already mentioned. Important 4Fe-4S centre proteins (in so far as most information is available about them) are the 8Fe-8S and HiPIP proteins. The HiPIP is exceptional in more ways than its high redox potential – the Fe-S cube in HiPIP shrinks a little upon oxidation. The shape of the polyhedron in the reduced state is very close to that of the oxidised state of bacterial ferredoxin. The electronic properties of the synthetic dianion indicate that the oxidation level is equivalent to that of $HiPIP_{red}$ and Fd_{ox}, while the electronic properties of the reduced species $[Fe_4S_4\ (SR)_4]^{3-}$ are closely similar to those of Fd_{red} and include a $g=1.94$ EPR signal. Clearly the redox properties of the synthetic model are similar to those of ferredoxin rather than HiPIP.

Finally, the two identical 4Fe-centres found in 8Fe-8S proteins can be a highly efficient arrangement in terms of electron transport since there is a high 'cluster to protein weight ratio'. As yet such centres have only been found in anaerobic bacteria and the understanding of the way in which the two discrete clusters interact in Fe-S proteins is limited. This also reflects the inconsequential progress made in the synthesis of their analogues in the laboratory. However their function in these

bacteria is known to be extensive and they are thought to be involved in key metabolic processes such as hydrogen uptake, NAD reduction, ATP formation, pyruvate metabolism and nitrogen fixation.

It thus seems clear that in each type of ferredoxins, there exists at least two accessible redox states per active site. They function primarily in electron transport sequences, rather than acting as a site for substrate binding and conversion.

Conclusions

The fact that iron-sulfur clusters occur as an integral component of electron transfer proteins is an exciting area of bioinorganic chemistry.

Now, more than about thirty years after their initial discovery, Fe-S proteins are being understood to a certain extent and with more refined techniques and many more years of work, the exact nature of the ways in which they operate may be understood. The work which has so far been done on Fe-S proteins is really only a beginning in terms of thorough understanding. This beginning has been achieved largely with the help of synthetic analogues which have been used to provide supporting evidence in some areas of biochemical studies. This will result in many openings in biochemistry, not least of which are the exact nature of nitrogen fixation and chloroplast function and the implications which will follow if these are initiated in the laboratory.

Suggested Reading

- **R H Holm, S Ciurli and J A Weigel. *Progress in Inorganic Chemistry*. 38.1, 1990.**
- **P J Stephens, D R Jollie and A Warshel. *Chemical Reviews*. 96.2491, 1996.**

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After you have learned discipline and learned it well, you are free.

Thomas Aquinas Daly