8.3. Electron Transport Proteins

Electron transport proteins are responsible for the transport of reducing equivalents (electrons) from a biological redox couple having a lower standard redox potential to one having a higher standard redox potential. Standard redox potential of an electron transport protein should be intermediate between those of the electron acceptor and the electron donor couples. Electron transporting mettallo proteins are mainly the iron-sulfur proteins, *viz.*, *ferredoxins* and the Fe (III)-heme proteins, *viz.*, *cytochromes*. Both these groups operate through their Fe (III) - Fe (II) couples. The protein parts of these substances, though essentially are organic in nature, but play crucial roles in modifying the redox potentials of these electron carriers and help passage of the electrons through the tunnels in their structures. NAD^+/ NADH, $NADP^+$ / NADPH, FAD / $FADH_2$, Q / H_2Q (Q = ubiquinone, plastoquinone) redox coenzymes also function as electron carriers in biological redox reactions (Ch-9).

(*a*) *Iron-sulfur proteins* (*ferredoxins*)**:** Iron-sulfur proteins function as electron carriers in biological redox reactions, *viz.*, photosynthesis, nitrogen fixation and mitochodrial respiration. These consist of non-heme iron, coordinated by cysteine sulfur (–SH) and acid labile inorganic sulfide sulfur (S^{2-}).

Iron-sulfur proteins are often abbreviated as $n-(Fe-S^*)$ centres, where, *n* stands for the number of iron cations per protein and S^* stands for the inorganic sulfide sulfur (S^{2-}) usually *n* in number. These labile sulfur atoms are liberated as H_2S on acidification of the protein and are readily air oxidized to elemental sulfur. Iron in these proteins are approximately tetrahedrally coordinated by four sulfur atoms, of which at least one is cysteine sulfur from the protein chain. Irrespective of their sources, Fe – S proteins having the same *n*, have same number of cysteine sulfur coordinated to iron.

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The electron transport by ferredoxins takes place *via* Fe(III) – Fe(II) couple. In contrast to the standard redox potential of 0.785 *volt* for the $Fe^{3+}(aq) / Fe^{2+}(aq)$ couple, the redox potential of Fe – S proteins are close to that of the $2H^+ / H_2$ couple and vary widely depending upon the nature of the protein environment around iron. Ferredoxin in photosynthetic bacterium, *Chromatium* has $E^{o'}$ (redox potential at 25° C, *p*H 7) = −0.49 *volt*, i.e., close to the $E^{o'}$ of the $2H^+/H_2$, couple, while Fe – S protein in beef-heart mitochondria has $E^{o'}$ = + 0.22 *volt*. Properties of some Fe – S proteins from various sources are summarised in Table 8.1.

Table 8.1. Properties of some iron sulfur proteins

Type	*M.W.* (*daltons*)	*n*(Fe – S)	$E^{o'}$ (*volts*)
Mitochondrial			
NADH Dehydrogenase	78,000		
Succinate Dehydrogenase	100,000		
Other sources			
Ferredoxin (*Chromatium*)	10,000	4	–0.49
Ferredoxin (*Pasteurianum*)	40,000	2	–0.39
Ferredoxin (*Spinach*)	12,000	2	–0.42
Adrenodoxin (*Adrenal cortex*)	16,000	2	–0.27
Putidaredoxin (*Pseudomonusputida*)	12,000	2	–0.24

(*i*) *Rubredoxin*

Rubredoxin is the simplest of the Fe – S proteins. It contains one iron but no acid labile (S^{2-}) sulfur. Iron in rubredoxin is coordinated by four cysteinyl sulfur atoms in a distorted tetrahedral geometry (Fig 8.18), in which Fe – S bonds are unusually short. It is a low molecular weight protein (*MW* ≈ 6000 *daltons*) consisting of 53-54 amino acids. Although there are variations in the amino acid sequences, the positions of the four cysteine residues remain fixed at 6, 9,39 and 42.

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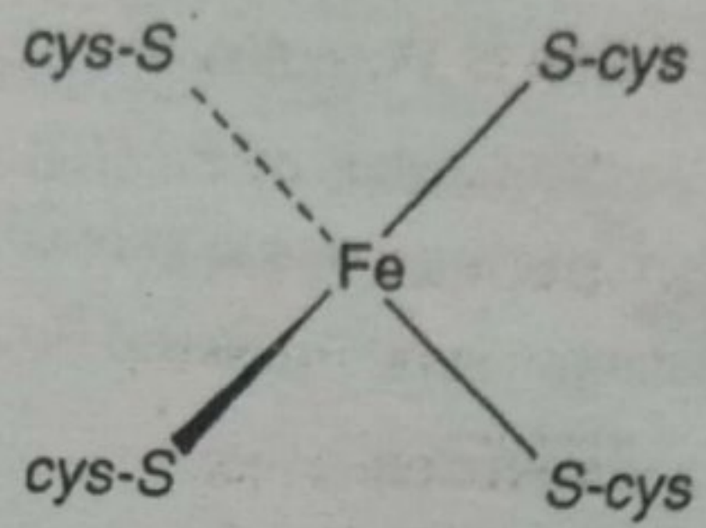

Fig. 8.18. Active site structure of rubredoxin.

The oxidized form of the protein is red coloured, contains high spin Fe (III). The reduced form is colourless and contains Fe (II) in a high spin configuration. The distorted tetrahedral structure of iron is responsible for the lower redox potential ($E^{o'} = -0.057$ *volts*) and rapid electron transfer properties of rubredoxin. It is a single electron transport protein due to the couple,

$$[Fe^{III}(S\text{-}cys)_4]^{-} + e \rightleftharpoons [Fe^{II}(S\text{-}cys)_4]^{2-}$$

Metabolic role of rubredoxin is less well known. Rubredoxin from *Pseudomonas oleovorans* is comparatively larger (*MW* ≈19,000 *daltons*). It consists of two isolated iron sites $[Fe^{III}(S-cys)_4^-\ .\ Fe^{III}(S-cys)_4^-]$. It is involved in aliphatic hydroxylation reactions.

(ii) Ferredoxins

(a) 2Fe – 2S ferredoxins : *2Fe – 2S* ferredoxins occur in the chloroplasts of many plants, in several bacteria, beef-heart mitochondria and in pig adrenal glands. These consist of single peptide chains of 98 amino acids (*MW* ≈10,500 *daltons*) . Their active sites contain two iron centres bridged by two acid labile (S^{2-}) sulfur and each iron is bound to two cysteine sulfur atoms of the protein chain in such a manner that the individual $(cys-S)_2\ Fe(S^{2-})_2$ units appear tetrahedral, providing high spin configuration to iron (Fig. 8.19)

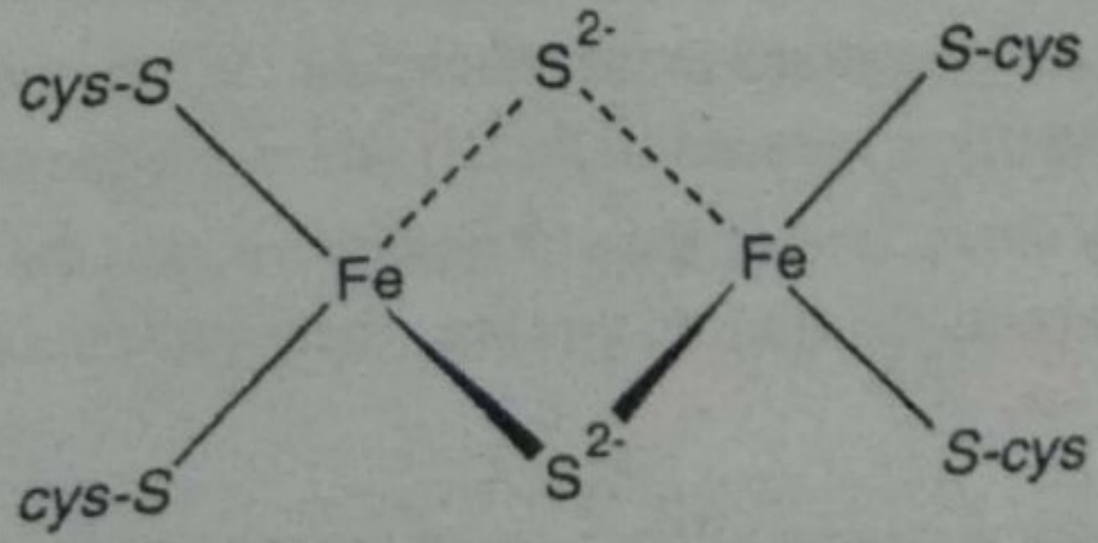

Fig. 8.19. Active site structure of 2Fe - 2S ferredoxins.

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In the oxidized form, both the iron atoms are in Fe (III) state with high spin ($S = 5/2$) configuration, yet the protein is diamagnetic and is *e.p.r.* silent due to antiferromagnetic Fe (III)...Fe (III) coupling. Iron and S^{2-} ions form a planar $[Fe(S^{2-})_2 Fe]$ cluster. The dz^2 orbital of each iron makes a σ overlap with the σ orbitals of S^{2-} ions. The d_{xz} and d_{yz} orbitals of iron make π - overlaps with the vacant $d\pi$ orbitals of the two cysteine sulfur atoms. The d_{xy} orbitals, of iron atoms remain non-binding. The bridging sulfide (S^{2-}) ligands enable the individual paramagnetic Fe (III) centres to pair up with each others spin through *super exchange interaction*. The reduced from of the protein is paramagnetic for one unpaired electron and is *e.p.r.* active, $(g_{\perp} = 1.97)$, which corresponds to reduction of one of the two Fe (III) centres to Fe (II). $2Fe-2S$ ferredoxins, therefore, function as one-electron transport proteins :

$$[(cys-S)_2 Fe^{III}(S^{2-})_2 Fe^{III}(S-cys)_2]^{2-} + e$$

$$\rightleftharpoons [(cys-S)_2 Fe^{III}(S^{2-})_2 Fe^{II}(S-cys)_2]^{3-}$$

In the reduced form, a high spin $Fe^{III}(S = 5/2)$ and a high spin $Fe^{II}(S = 4/2)$ are antiferromagnetically coupled to give a net electron spin of $S = 1/2$ in the ground state. Electron transport occurs with very small energy transfer, as the redox potential $(E^{\circ'})$ of this protein is very low, *e.g.* -0.2 to -0.4 *volt*.

Iron centres in the reduced form are non-equivalent, though they are equivalent in the oxidized form. Due to reduction, the ionic radius of four coordinated iron changes from 0.63Å in high spin Fe (III) to 0.77Å in high spin Fe (II). This distorts the planarity of $[Fe(S^{2-})_2 Fe]$ moiety and initiates non-valence (tertiary and quaternary) interactions in the protein chain. Conversely, alteration in the protein conformation may also alter the redox potential at the active site of these proteins.

(*b*) *4Fe – 4S Proteins* : *4Fe – 4S* proteins, isolated from *Chromatium* (*Strain D*) and *Rhodopseudomonas gelatinosa* have molecular weights of 9,600 and 10,000 *daltons* and redox potential of +0.35 *volt* and +0.33 *volt* respectively. These proteins can undergo one electron redox reactions, though their biochemical functions are less well known . These are not generally classed as ferredoxins, rather, these are often called '*high potential iron-proteins*', and are abbreviated as "*HIPIF*". Active sites of these proteins consist of four iron atoms, four acid labile sulfide sulfur (S^{2-}) and four cysteinyl sulfur atoms arranged in a cubic structure (Fig. 8.20).

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The mode of bonding of iron and sulfur (S^{2-}) in 4(Fe - 4S) proteins is same as that in 2Fe- 2S proteins. The cubic $[Fe_4(S^{2-})_4]$ clusters in 4Fe - 4S proteins may be visualised as a combination of two $[Fe(S^{2-})_2Fe]$ units of 2Fe- 2S ferredoxins. Each iron in these $[Fe_4(S^{2-})_4]$ clusters is tetrahedrally coordinated by three acid labile sulfide sulfur (S^{2-}) and one cysteine sulfur (*cys*- S).

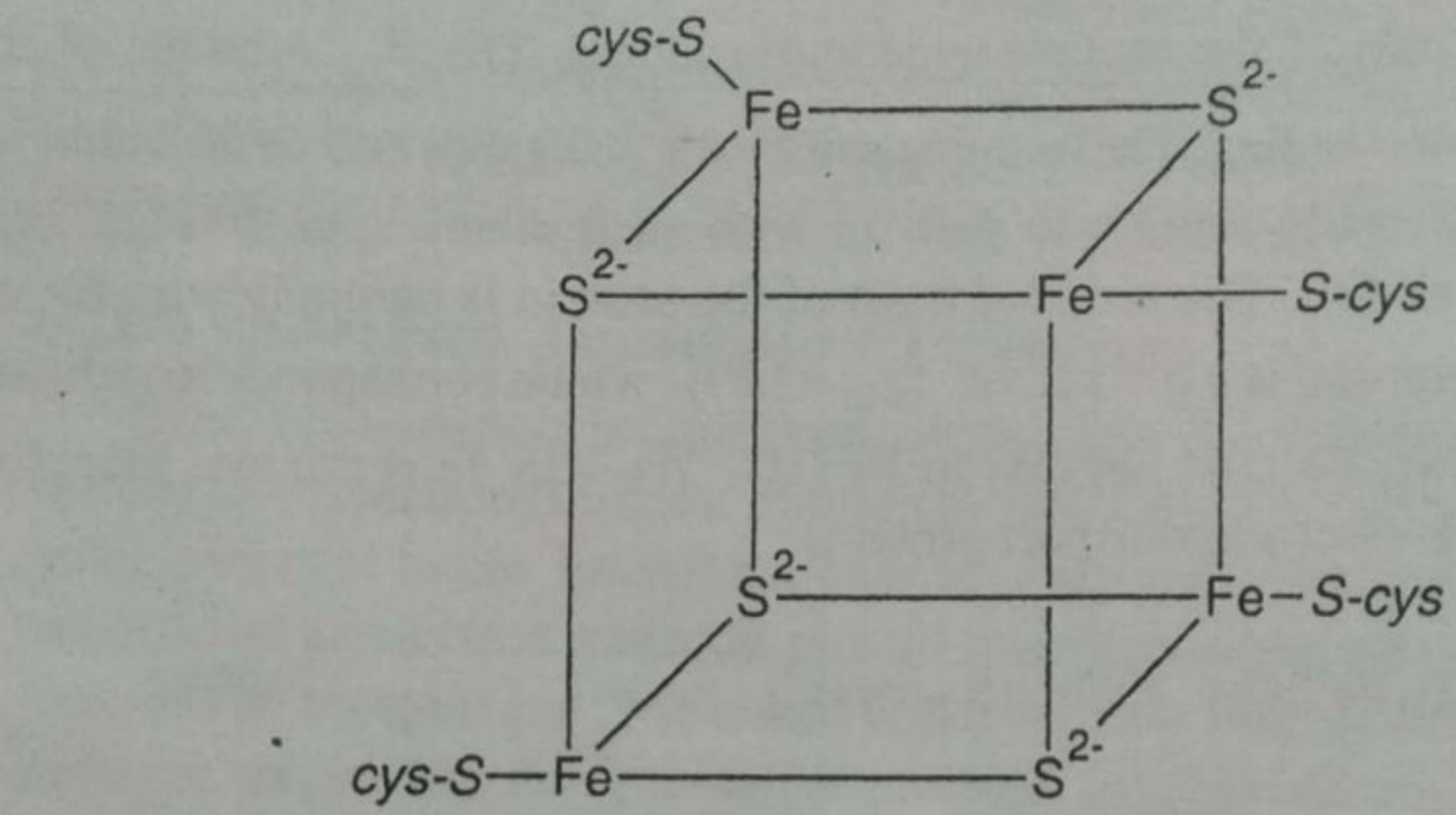

Fig. 8.20. Active site structure of 4Fe-4S proteins.

The oxidized forms of 4 Fe – 4S proteins contain three high spin Fe (III) and one high spin Fe(II) and their paramagnetism is equivalent to one unpaired electron due to antiferromagnetic interaction. The oxidized forms of *HIPIP* are *e.p.r.* active $(g_{\perp} = 2.02)$. The reduced forms contain two Fe (III) and two Fe (II) and are diamagnetic due to antiferromagnetic interaction. These proteins, therefore, may function as one-electron carriers :

$$[(Fe^{III})_3(Fe^{II})(S^{2-})_4(S-cys)_4]^- + e$$

$$\rightleftharpoons [(Fe^{III})_2(Fe^{II})_2(S^{2-})_4(S-cys)_4]^{2-}$$

Due to non-equivalence of the iron centres, the Fe_4S_4 cube distorts from its normal position to cause a variation of redox potential from about + 0.40 volt to −0.40 *volt*. Two tyrosine residues (*Tyr* -2 and *Tyr* -28), at 3.5Å from the opposite faces of the Fe_4S_4 cube, participate in electron transfer and control the redox potential.

(*c*) *8Fe – 8S Ferredoxins* : *Ferredoxins* (from *Clostridium*) function as electron carriers in the biological nitrogen fixation systems. These ferredoxins are

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small ($MW \approx 6000\,daltons$) and consist of two 4Fe – 4S clusters situated at 12Å apart (Fig. 8.21) each of which can undergo one electron change with $E^{o'} = -0.40$ *volt*. As a result, the whole protein may function as two-electron carrier. The oxidized form contains equal number of Fe (III) and Fe (II), but shows lower magnetic moment (1.2 *B.M.* per iron) due to extensive antiferromagnetic coupling and spin delocalization through $Fe(S^{2-})$ Fe and Fe ... Fe bonds. Magnetic moment of the protein increases on reduction.

$$2\{[(Fe^{III})_2(Fe^{II})_2(S^{2-})_4(S-cys)_4]^{2-} + e\} \rightleftharpoons 2\{[(Fe^{III})(Fe^{II})_3(S^{2-})_4(S-cys)_4]^{3-}\}$$

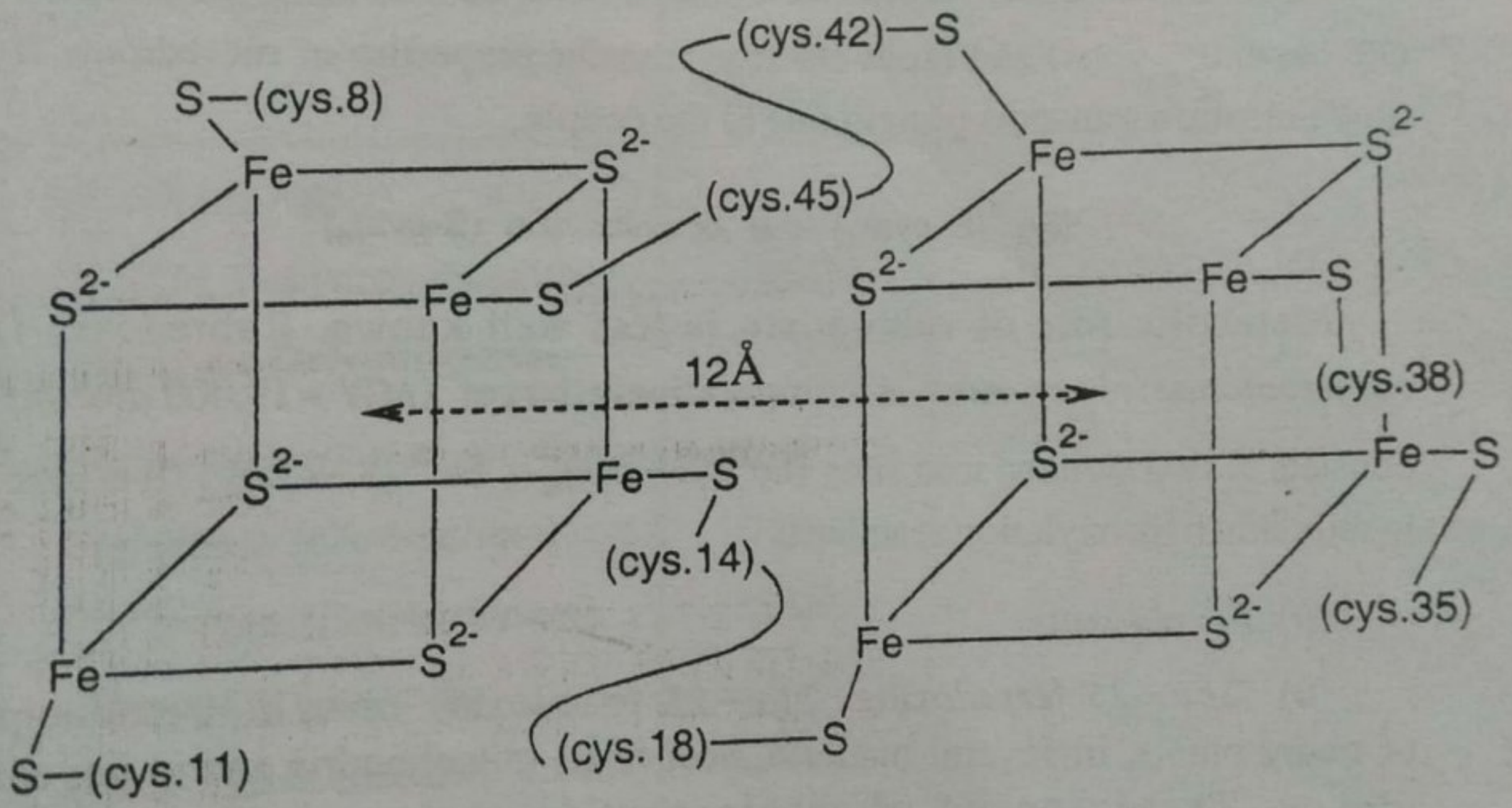

Fig. 8.21 Active site structure of 8Fe-8S ferredoxins.

The nature of bonding in the two Fe_4S_4 cubes are same as that in 4Fe-4S proteins. There is a crossover of the protein chain, as the cysteine residues at positions 8, 11, 14 and 45 coordinate iron atoms of one cube, while those at positions 18, 35, 38 and 42 coordinate iron atoms in the other cube. When one Fe_4S_4 unit accepts one electron, one Fe(III) is reduced to Fe (II) and consequent alteration in the tertiary and quarternary interactions in the protein chain makes the second Fe_4S_4 unit ready to accept the second electron. In this way these proteins function as two-electron-carriers.

(*d*) *3Fe – 4S Proteins* : Structure of this class of Fe – S proteins are similar

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to that of a Fe_4S_4 unit with one Fe removed to leave a voided cuboidal cluster (Fig. 8.22)

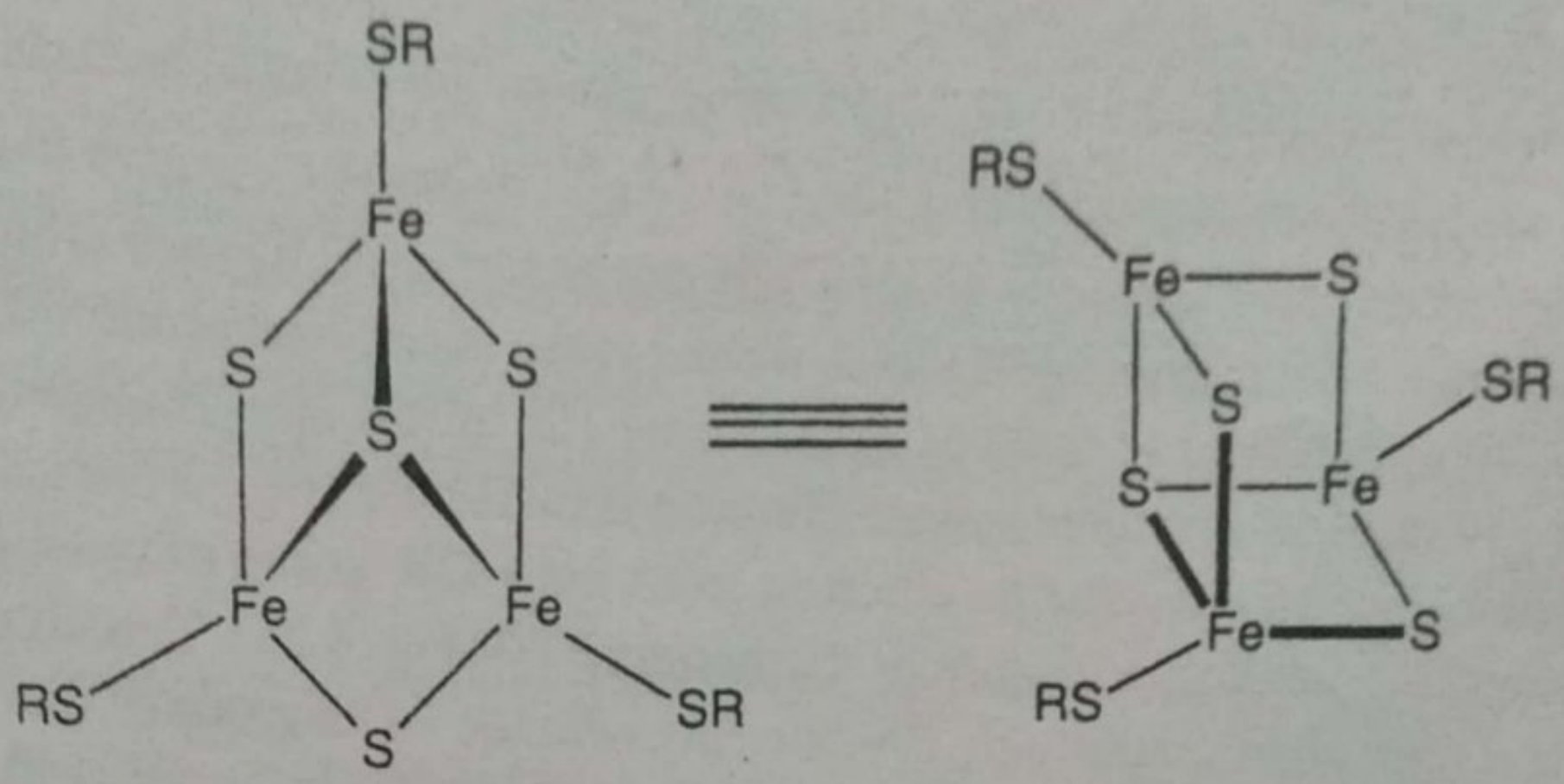

Fig. 8.22 : Active site structure of 3Fe – 4S proteins (R = cys).

The 3Fe – 4S proteins are present in the bacteria, *viz*, *Desulfovibrio gigas*, *Azotobacter vinlandii*. These are also present in the inactive form of pig heart *aconitase*, the enzyme that catalyses the conversion of citrate to isocitrate through a stereospecific dehydration reaction (p. 97). These 3Fe – 4S clusters can undergo interconversion into 4Fe – 4S clusters involving minimum structural alteration, particularly when there is a non-thiol fourth ligand available in the 4Fe – 4S form. In aconitase, a molecule of H_2O plays the role of this fourth ligand.

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