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Iron-sulfur clusters—new features in enzymes and synthetic models

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Abstract Mössbauer spectroscopy is very important for the characterization of iron sulfur clusters in biological and synthetic molecules. The electric and magnetic hyperfine parameters obtained for ^{57}Fe provide valuable information about the electronic structure of the different iron sites occurring in Fe:S clusters. Although known since more than four decades, research in this field is very active, revealing unexpected functions, structures and redox states. In this overview, new aspects of double exchange and vibronic coupling in a structurally well-characterized two-iron model compound are discussed, the electronic structure of extremely reduced clusters with all iron in ferrous or even in iron(I) state is elucidated, and an exciting new type of cubane cluster occurring in oxygen-insensitive hydrogenases is presented. The latter cluster involves structural changes during function and it supports more than one redox transition, which may be essential for oxygen protection of the enzymes.

Keywords Iron-sulfur cluster · FeS · Double exchange · Super-reduced · Low valent iron · Low coordination · Hydrogenases · Magnetic measurements · Applied field Mössbauer · Paramagnetic properties

1 Introduction

Iron sulfur proteins have been discovered in the 1960s during studies on photosynthetic and nitrogen-fixing bacteria and submitochondrial parts of mammalian cells [1]. They are distinguished by the presence of inorganic clusters of iron and sulfide (Fe:S clusters) with one to eight ferric or ferrous ions and about the same number of 'acid-labile' sulfides (S^{2-}). The clusters are covalently bound to the protein, mostly by coordination to cysteinate sulfur (S^{-}). They represent one of the most ubiquitous

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and versatile prosthetic groups found in biological molecules, abundant in all present and ancient forms of life [1–4]. Even an inorganic origin or prelude of life with an iron- and sulfide-based primordial metabolism was proposed [5]. The appearance of Fe:S clusters, however, is not protein dependent, since vigorous research in bio-inspired inorganic chemistry has provided a large variety of synthetic monomeric to tetrameric, or oligomeric iron-sulfur clusters [6], many of which are accurate analogs of the natural systems.

Iron-sulfur clusters play various physiological roles, with more than 120 distinct types of iron-sulfur enzymes and proteins known [1, 4, 7]. This is possible partly because sulfur can occur in different oxidation states, and forms highly covalent bonds with little energy necessary to make and break bonds. Iron, of course is a 'fitting partner' with a large variety of electronic configurations and close-lying redox states [2]. The chemical versatility allows Fe:S clusters easily to accept, store and donate electrons, in some cases employing long transport chains like in the linear assembly of 8–9 clusters in compound 1 of the respiratory chain for aerobic cell metabolism. Beyond such simple electron transfer, Fe:S cluster can couple electron and proton transfer. Others have structural roles, or more remarkable, serve as sensors for H_2, O_2, NO, or the iron level in the cell. Some clusters are directly involved in catalytic steps, in which they bind and activate substrates at a unique iron site. Recently, the role of cluster formation and enzyme maturation for gene expression and genetic disorder has captured much attention [8], not least due to the medical implications [9, 10].

2 Common types of clusters and electronic structures

Iron in the most common Fe:S clusters has the formal oxidation state iron(II) or iron(III) with quasi-tetrahedral coordination environment; therefore it is high-spin throughout. Mononuclear, dinuclear and trinuclear clusters occur in two oxidations states, supporting the red/ox couples $[1Fe]^{3+}$/ $[1Fe]^{2+}$, $[2Fe–2S]^{2+}$/ $[2Fe–2S]^{1+}$, and $[3Fe–4S]^{1+}$/ $[3Fe–4S]^{0+}$, respectively (see Table 1). In the oxidized states, these clusters are all-ferric, whereas the reduced $[1Fe]^{2+}$ cluster is ferrous and the reduced $[2Fe–2S]^{1+}$ and $[3Fe–4S]^{0+}$ cluster have one ferrous ion each (mixed valences) [11]. Four-iron, [4Fe–4S], clusters occur in three oxidation state (3+, 2+, 1+), although in a particular protein never both redox pairs are supported (they function either as a so-called low-potential or a high-potential Fe:S protein). The clusters of higher nuclearity (four and more) are mixed-valent in all oxidation states and show valence delocalization, predominantly occurring in pairs of formal Fe(II)–Fe(III) ions. The underlying double-exchange mechanism [12–14] accomplishes a substantial contribution to the redox potential of the systems [15].

The Mössbauer parameters of Fe:S clusters are clearly distinct from those of other biological iron centers, like hemes and non-heme centers with 'hard' oxygen and nitrogen ligands. Particularly the isomer shifts are characteristically low, due to the short and covalent Fe-S bonds caused by four-coordination and high covalency of sulfur [21]. Table 1 summarized the basic Mössbauer parameters of Fe:S clusters with one to four iron sites, whereas comprehensive and detailed descriptions also of the highly interesting magnetic properties are found in recent reviews [11, 22–24], including those of the more specific eight-iron and composite clusters.

Table 1 Typical properties of most common Fe:S clusters

Type	Formal valences[a]	Spin	δ/mms^{-1}(4.2K)	$\lvert\Delta E_Q\rvert$/mms^{-1}	Ref.
$[1Fe]^{3+}$	III	5/2	0.32	0.5	[16]
$[1Fe]^{2+}$	II	2	0.70	3.25	[16]
$[2Fe–2S]^{2+}$	2xIII	0	0.27	0.6	[17]
$[2Fe–2S]^{1+}$	III,II[b]	$\frac{1}{2}$	0.35, 0.65	0.6, 2.7	[17]
$[3Fe–4S]^{1+}$	3xIII	$\frac{1}{2}$	0.27	0.63	[18]
$[3Fe–4S]^{0}$	{II/III}, III[c]	2	0.46, 0.32	1.47, 0.52	[14]
$[4Fe–4S]^{1+}$	{II/III}, 2xII[d]	$\frac{1}{2}$	0.5, 0.58	1.32, 1.89	[19]
$[4Fe–4S]^{2+}$	2x{II/III}	0	0.42	1.12[e]	[19]
$[4Fe–4S]^{3+}$	{II/III}, 2xIII	$\frac{1}{2}$	0.4, 0.29	1.03, 0.88[e]	[20]

[a]Mixed-valence pairs are indicated by brackets {–}

[b]Two subspectra for localized Fe(III) and Fe(II) sites

[c]Two distinct subspectra with intensity ratio 2:1

[d]Two subspectra with intensity ratio 1:1

[e]Average value from non-resolved subspectra

3 Exchange and double-exchange in a synthetic [2Fe–2S] compound

Synthetic analogues for biological [2Fe–2S] cores are well established in bioinorganic chemistry [6], although terminal ligands other than thiolates, like the histidine-nitrogens in so-called Rieske clusters, are still rare (see ref. in [25]). Mössbauer spectra of a Rieske-type model complex with an asymmetric set of ligands have been published only recently [26]. Most reported analogues of [2Fe–2S] sites have been synthesized exclusively in the all-ferric state, while the mixed valent $[2Fe–2S]^{1+}$ state could, if at all, only be accessed by electrochemical methods in solution [27]; Gibson and Beardwood generated the first such reduced [2Fe–2S] cluster [28]. Although neither the molecular structures nor the magnetic susceptibilities could be determined, the electronic structure could be studied in solution by EPR and Mössbauer spectroscopy. That compound was the first Fe:S dimer with partial valence delocalization and competing exchange and double exchange interaction [29]. Now we could investigate a similar complex with mixed valent $[2Fe–2S]^{1+}$ core, which was crystallized, so that metric details could be related with the electronic structure; and also magnetic susceptibilities could be measured with a powder sample [25].

Two distinct iron sites are found, but the isomer shifts and quadrupole splitting ($\delta = 0.47$ mm/s, $\Delta E_Q = 1.41$ mm/s, and $\delta = 0.69$ mm/s, $\Delta E_Q = 2.90$ mm/s) deviated from typical values known for ferric and ferrous ions (see also Table 1). Comparisons with suitable reference systems, as well as the empirical correlation [30] $\delta(x) = [1.43–0.40{\cdot}x]$ mm/s, found for δ and the oxidation number (x) of FeS_4 units, revealed a mixing coefficient of 20% for the electronic configurations $[Fe^{2+}–Fe^{3+}]$ ("A") and $[Fe^{3+}–Fe^{2+}]$ ("B") (i.e. $a^2 = 0.8$, $b^2 = 0.2$ for $\psi = a{\cdot}\psi\text{“}_A\text{”} + b{\cdot}\psi\text{“}_B\text{”}$). Magnetic susceptibility measurements reveal antiferromagnetic coupling of the iron sites. The effective coupling constant ($J_{eff} = -134$ cm^{-1}; for $H = -2JS_1S_2$), however, comprises the combined and competing effects of exchange interaction, intrinsic electron transfer (double exchange) [12–14] and charge localization [15] due to static and vibronic coupling to the environment [31–36].

The values of J_{eff} and a^2 can be rationalized by using a phenomenological model that describes the energies of the spin states of a mixed-valent iron dimer in terms of the exchange coupling constant J, a double exchange parameter B to account for delocalization, and an effective energy difference Δ_{AB} of the configurations "A" and "B" that summarizes the charge-localizing contributions from static site differences as well as vibronic coupling. The eigenvalues of the double-exchange Hamiltonian are given as [14, 15, 29, 37]:

$$E_{\pm}(S) = -JS(S+1) \pm 1/2\sqrt{\Delta_{AB}^2 + B^2(2S+1)^2} \quad (1)$$

where the subscripts (±) denote the 'gerade' and 'ungerade' solutions, the splitting of which corresponds to the energy of the intervalence band, $\Delta_{iv} = |E_+(S) - E_-(S)|$ for spin $S = 1/2$ here, whereas the effective coupling constant is given by the energy difference of the ground state doublet and the excited quartet, $J_{eff} = 1/3|E_-(1/2) - E_-(3/2)|$. Unfortunately, the intervalence band cannot be directly detected neither here nor for other $[2Fe–2S]^{1+}$ clusters, presumably because of unfavorably low energy.

The mixing coefficient a^2 can be given in closed form as a function of Δ_{AB}/J and B/J [29]. However, with the input of only two experimental variables, J_{eff} and a^2, the equations cannot be readily solved. Hence we adopted $B = 700\ cm^{-1}$ for the double exchange parameter, as determined by DFT calculations [38], which enabled us to obtain from equation (1) the true exchange interaction $J = -341\ cm^{-1}$, and the double-exchange splitting $\Delta_{AB} = 1{,}050\ cm^{-1}$. The J value is remarkably large, but it is consistent with the result of ligand-K-edge XAS [39, 40] and DFT [38] investigations. Moreover, the set of parameters predicts for the intervalence band an energy of $\Delta_{iv} = 1{,}750\ cm^{-1}$, or $\lambda_{iv} = 5{,}714$ nm. Apparently such transitions are difficult to detect, but in particular the result rules out previous tentative assignments of bands around 540 nm. The corresponding large splitting of 'gerade' und 'ungerade' total spin states, S_+ and S_-, would not be consistent with the observed $S = 1/2$ ground state of $[2Fe–2S]^{1+}$ clusters. In summary, the data resolve a long-lasting debate in Fe:S cluster chemistry.

4 Super-reduced Fe:S clusters

In the known Fe:S clusters, the iron ions are in the +2 and +3 oxidation states, even in synthetic systems, which provide a much broader range of supporting ligands than amino-acid residues in biology. Clusters in which all the iron ions are in the Fe^{2+} state are rare, with the only known biological examples being the Fe protein [41–44] and the [8Fe–7S] P-cluster [3, 45, 46] of nitrogenase, and the super-reduced activator component of the enzyme system 2-hydroxyglutaryl-CoA dehydratase [47]. The all-ferrous state, $[4Fe–4S]^0$, of the four-iron cluster in the activator protein was established by the Mössbauer parameters, $\delta = 0.65$ mm/s and $\Delta E_Q = 1.51$–2.19 mm/s, which are typical of $Fe(II)S_4$ sites. Parallel-mode electron paramagnetic resonance (EPR) spectra showed sharp signals at $g = 16$ and 12, indicating an integer-spin system. EPR spectra and magnetic Mössbauer spectra could be consistently simulated with total spin $S_t = 4$ with weak zero-field splitting parameters $D = -0.66\ cm^{-1}$ and $E/D = 0.17$. The putative consequences of the all-ferrous $[4Fe–4S]^0$ state for the physiological role of the clusters are still under discussion [3, 47].

The so-called Rieske proteins from the bacterial and mitochondrial phosphorylation systems (for chemical energy storage) have a unique [2Fe–2S] cluster in which one of the two Fe atoms is coordinated by two histidine rather than two cysteine residues. The all-ferrous state $[2Fe–2S]^0$ was produced in solution by chemical reduction with a europium salt (Eu^{II}DTPA), and has been characterized by protein-film voltammetry and UV– spectroscopy. We have measured EPR and Mössbauer spectra to explore the electronic structure of iron [48]. The two ferrous ions are both high spin ($S_{Fe} = 2$, $\delta = 0.70$ mm/s, $\Delta E_Q = 2.76$ mm/s for 2-Cys-coordination, $\delta = 0.81$ mm/s, $\Delta E_Q = 2.32$ mm/s for 2-His-coordination). They are antiferromagnetically coupled ($-J > 30$ cm^{-1}) to give a diamagnetic ($S = 0$) ground state, as could be inferred from applied-field Mössbauer spectra. The ability of the Rieske cluster to exist in three oxidation states (2+, 1+, and 0) without an accompanying coupled reaction, such as a conformational change or protonation, is highly unusual. A combination of experimental data and calculations based on density functional theory suggested strongly that a proton binds to one of the cluster μ_2-sulfides, which demonstrates the coupling of electron and proton transfer in these systems [48].

There are no reports of iron-sulfide systems in which the iron ions could be reduced to the Fe^{1+} level. However, that oxidation state has been suggested for some sites of the FeMoco cluster of nitrogenase to explain certain intermediates. Recently the first example of an iron(I)-sulfide compound has been isolated [49], which suggests that iron(I) is in fact feasible in biological iron sulfide chemistry. The compound is a di-iron complex with two terminal β-diketiminate ligands (N-coordinating) and an S^{2-} bridge, i.e. the iron site are three-coordinated. The zero-field Mössbauer spectrum is a symmetric doublet with parameters $\delta = 0.67$ mm/s, $\Delta E_Q = 2.17$ mm/s [49]. The values are distinctly different from those of the starting compound with three-coordinate Fe(II) site ($\delta = 0.86$ mm/s, $\Delta E_Q = 0.58$ mm/s), and they resemble those of other three coordinate (non-sulfido) Fe(I) complexes like LFe(I)(HCCPh) ($\delta = 0.50$ mm/s, $\Delta E_Q = 2.05$ mm/s) [50]. The iron ions are antiferromagnetically coupled ($J = -122$ cm^{-1}, $S_{Fe} = 3/2$), showing the usual temperature dependence of the effective moments as expected from the Heisenberg Hamiltonian. Apparently the exchange interaction supersedes the effects of spin-orbit coupling observed for the only known other mononuclear three-coordinate Fe(I) system [50].

5 Unusual four-iron cluster in an O_2-tolerant [NiFe]-hydrogenase

The microorganism *Aquifex aeolicus* (lat. water maker) is a hyperthermophilic Knallgas bacterium with optimum growth at 85°C [51]. It has three distinct [NiFe] hydrogenases, among which Hydrogenase I (Hase I) is an integral part of a respiration pathway for the reduction of O_2 to water by using hydrogen [51, 52]. According to its function, the enzyme exhibits enhanced tolerance for dioxygen, as compared to 'normal' [NiFe] hydrogenases [53, 54]. This is a highly interesting feature with respect to research projects aiming at the connection of the energy-harvesting photosystem of green plant and the energy conversion catalyzed by hydrogenases (hydrogenases can operate in both directions, i.e. use or produce hydrogen).

Hase I consists of two subunits; the large subunit contains the catalytic [NiFe] site and the small subunit has three iron-sulfur clusters as electron relais system [51].

According to the amino-acid residues available for cluster binding, spectroscopic data, redox titration experiments and upcoming structural data obtained from single-crystal X-ray diffraction data, there are a [3Fe–4S], a 'normal' [4Fe–4S] and an unusual "[4Fe–4S]" cluster. The latter is located proximal to the [NiFe] center and appears to have a very unusual coordination with six cysteine residues [52].

Surprisingly, the three Fe:S clusters mediate four redox transitions. Analyses of the electrochemical results, in combination with EPR spectroscopy, show that the proximal "[4Fe–4S]" cluster is associated with two single-electron steps in a very small potential range, which has never been observed so far for any biological system. A proof that this fourth oxidation is in fact localized on the cluster and not related to a ligand oxidation could be obtained from Mössbauer spectroscopy. The system is at the resolution limit of Mössbauer spectroscopy as it contains twelve individual iron sites, the contributions of which are partially overlapping. However, not all of them must be disentangled because Fe:S cluster exhibit valence and charge delocalization so that many iron sites are similar even when they have different formal valences. The key feature for a successful interpretation of such data is the fact that specific coordination at some individual sites, particularly of the so-called proximal cluster, results in distorted charge distribution, as can be seen by unusual Mössbauer parameters.

The Mössbauer analysis was focused on the extreme redox states, which are the hydrogen-reduced and a chemically super-oxidized one; only under these conditions the samples are homogenous. In other preparations, superpositions of different redox states are expected according to the individual redox potentials of the clusters, as found by EPR titration experiments [52, 55]. The zero-field Mössbauer spectra of *H_2-reduced* samples are asymmetric quadrupole spectra, which can be fitted as expected for full reduction of the three clusters, except that one quadrupole doublet (accounting for 1/12 intensity) sticks out because of an extraordinarily large quadrupole splitting. Based on global simulations of applied-field spectra that were done to assign particularly the magnetic hyperfine tensors, this iron site can be best described as a $Fe^{2.5+}$ site of the proximal "[4Fe–4S]" cluster, which has an unusually distorted coordination. Another iron of that cluster appears to show predominant localized Fe(II) character ($\delta = 0.72$ mm/s). Particularly the signs of the A-tensors were helpful in this program, since they reveal the local spin orientation. We treated the proximal cluster in analogy with the coupling known for ferrous and ferric site in cubane Fe:S clusters [54].

The Mössbauer spectra of *super-oxidized* Hase I also show a unique subspectrum with large quadrupole splitting (2.42 mm/s). The data set could be consistently fitted by adopting one oxidized $[3Fe–4S]^{1+}$ cluster ($S = 1/2$, medial), one oxidized classical $[4Fe–4S]^{2+}$ cluster ($S = 0$, distal), the unusual (proximal) four-iron cluster ($S = 1/2$) and the low-spin Fe ($S = 0$, [NiFe] site). Applied-field analyses, however, indicate that the unusual coordination may have changed the face of the cuboid proximal cluster. Details of the interpretation shall be worked out [56] in conjunction with the upcoming crystal structure of Hase I.

In summary, the unprecedented plasticity of the proximal cluster of Hase I appears to be related to the two extra cysteins in its near environment. Structural data and Mössbauer spectra show that both these cysteins are involved in cluster coordination, forming a very unusual coordination sphere. This new type of cluster appears to occur in several oxygen tolerant [NiFe] hydrogenases, as is reported in two recent

molecular structure studies [57, 58]. Its extraordinary properties suggest a role for sequestering reactive oxygen species in order to endow oxygen tolerance to the system.

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