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Iron–sulfur proteins: Spin-coupling model for three-iron clusters

(ferredoxins/exchange interactions/Mössbauer spectroscopy)

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ABSTRACT **Recent Mössbauer and EPR studies of two ferredoxins and of aconitase have given evidence for a three-iron cluster, probably of a [3Fe–3S] type. The studies of the oxidized EPR-active centers have shown that the three iron sites are characterized by significantly different magnetic hyperfine coupling constants. For the ferredoxin from *Azotobacter vinelandii*, for instance, we have observed $A_1 = -41$ MHz, $A_2 = +18$ MHz, and $|A_3| = 5$ MHz. We demonstrate here that the magnetic properties of the clusters can be explained with a simple model of three high-spin ferric ions ($S = 5/2$) exchange-coupled to a system spin $S = 1/2$. The model assumes isotropic exchange and different couplings between the iron sites. The results show that the three sites have intrinsic hyperfine interactions similar to those of ferric rubredoxin; the differences in the observed interactions reflect the geometrical features of spin coupling. Furthermore, the three exchange coupling constants are equal within a factor of 2. This implies that the three-iron cluster is a single covalently linked structure and should not be considered as a [2Fe–2S] cluster weakly coupled to a third iron atom.**

Mössbauer and electron paramagnetic resonance (EPR) data have recently been presented as evidence for a different type of metal cluster in a ferredoxin from *Azotobacter vinelandii* (1). The same structure has also been reported for a ferredoxin (Fd II) from *Desulfovibrio gigas* (2) and for aconitase from beef heart mitochondria (3). The structure contains three spin-coupled iron atoms (1, 2). X-ray crystallographic evidence (4) and chemical data (3) suggest that the center contains iron and sulfide in equimolar amounts; thus, a [3Fe–3S] cluster is anticipated.

The function of the *Azotobacter* ferredoxin has not yet been established. The *D. gigas* ferredoxin appears to mediate electron transfer between cytochrome c_3 and the sulfite reductase system (5), whereas a regulatory function has been proposed for the aconitase cluster (6). In the three proteins the new cluster can be prepared in two stable oxidation states. In the oxidized form the centers yield a EPR signal around $g = 2.01$ suggesting a system (cluster) spin $S = 1/2$. Upon a one-electron reduction the cluster becomes EPR-silent. According to the Mössbauer data (1, 2), the reduced cluster has electronic spin $S \geq 1$. High-field Mössbauer studies have shown conclusively the presence of spin-exchange interactions among the iron atoms; spin coupling is evident in both oxidation states. Mössbauer studies on the oxidized centers gave the following intriguing results: In the limit of fast electronic spin relaxation (at temperatures above 40 K) one sharp quadrupole doublet is observed, suggesting three indistinguishable iron sites. The observed isomer shift ($\delta = +0.27$ mm/s relative to iron metal) and quadrupole splitting ($\Delta E_Q = 0.63$ mm/s for the *Azotobacter* ferredoxin and $\Delta E_Q = 0.54$ mm/s for Fd II) are typical of a high-spin ferric ion ($S = 5/2$) in a distorted tetrahedral environment of sulfur atoms. The prototype for such a coordination is rubredoxin (7). In the limit of slow electronic relaxation ($T \leq 4.2$ K) the three iron sites exhibit spectra characterized by magnetic hyperfine coupling constants, A, that differ drastically in sign and magnitude. For the ferredoxin from *A. vinelandii* we have observed $A_1 = -41$ MHz, $A_2 = +18$ MHz, and $|A_3| = 5$ MHz. Do the observed A values reflect sites with different covalencies or do they express the effects of spin coupling? In this communication we will argue that the sites are intrinsically rubredoxin-like and we will show that the signs and magnitudes of the observed A values can be explained with a simple spin-coupling model.

SPIN-COUPLING MODEL

Our model for the oxidized state of the three-iron centers is based on the observation that the iron atoms are high-spin ferric in character, as indicated by the quadrupole splittings and the isomeric shifts. This suggests that we couple

$$\mathbf{S}_1 + \mathbf{S}_2 + \mathbf{S}_3 = \mathbf{S},$$

in which $S_1 = S_2 = S_3 = 5/2$, and S is the system spin. From the observation of an (almost) isotropic EPR signal at $g = 2.01$, we infer that the system ground state has $S = 1/2$. (The symmetry of iron sites in proteins is usually less than octahedral or tetrahedral. Thus, the effects of zero-field splittings would yield very anisotropic g values if $S \geq 3/2$.) We will describe the magnetic properties of the system by the Hamiltonian

$$\hat{H} = \sum_{i=1}^{3} (g_i \beta \mathbf{H}\cdot\mathbf{S}_i + a_i \mathbf{S}_i\cdot\mathbf{I}_i) + \hat{H}_c, \qquad [1]$$

in which $S_i = 5/2$ and i designates the iron sites. To a very good approximation the electron Zeeman interaction ($g_i = 2.0$) and the magnetic hyperfine interaction are isotropic for high-spin ferric ions. We can ignore zero-field splitting terms in Eq. 1 because we will consider only ground multiplets with $S = 1/2$. In order to describe the exchange coupling among the three iron atoms we assume an interaction

$$\hat{H}_c = J_{12}\mathbf{S}_1\cdot\mathbf{S}_2 + J_{23}\mathbf{S}_2\cdot\mathbf{S}_3 + J_{13}\mathbf{S}_1\cdot\mathbf{S}_3. \qquad [2]$$

The exchange interactions are assumed to be isotropic. Furthermore, terms quadratic in the S_i are not considered. In order to account for the large differences of the observed magnetic hyperfine interactions (without invoking unreasonable covalencies) three distinct coupling constants are required.

The data obtained for the two ferredoxins (1, 2) show that $\hat{H}_c$ is the dominant term in Eq. 1 and, therefore, we will consider Eq. 2 first. Because only isotropic exchange is considered, $\hat{H}_c$ can mix only states with the same total spin—i.e., S is a good quantum number. For $S_1 = S_2 = S_3 = 5/2$ only two configurations with $S = 1/2$ occur in the coupled system. These may be obtained by coupling S_2 and S_3 to an intermediate spin $S' = 2$ or 3, and then coupling S' with S_1 to obtain the system spin $S = 1/2$. Using the kets $|S_2S_3\,(S')\,S_1;\,SM\rangle$ as basis states, we write the most general state with $S = 1/2$, $M = +1/2$ as

$$|+\rangle = \sqrt{1-\alpha^2}\left|S_2S_3(2)S_1;\frac{1}{2}\frac{1}{2}\right\rangle + \alpha\left|S_2S_3(3)S_1;\frac{1}{2}\frac{1}{2}\right\rangle, \qquad [3]$$

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Abbreviation: Fd II, ferredoxin II.

in which α is a mixing parameter, $-1 \leq \alpha \leq 1$. The choice of which two spins couple to form S' is arbitrary. Our present labeling scheme was chosen so that the spins labeled 1, 2, and 3 in the results given below correspond, respectively, to the iron sites labeled 1, 2, and 3 in our previous experimental papers (1, 2).

The EPR data of the three-iron centers (1, 2) have been evaluated with the spin Hamiltonian $\hat{H} = g\beta\mathbf{H}\cdot\mathbf{S}$, in which $S = \frac{1}{2}$. In order to express the observed g factors in terms of the g values of the uncoupled ions we evaluate the Zeeman term with $\mathbf{H}$ along z.

$$\langle +|g\beta HS_z|+\rangle = \left\langle +\left|\sum_{i=1}^{3} g_i\beta HS_{iz}\right|+\right\rangle, \qquad [4]$$

which yields $g = 2.0$ because $S_z = S_{1z} + S_{2z} + S_{3z}$. Thus, the observation of an isotropic EPR signal at $g = 2.01$ reflects the fact that the constituent iron atoms have isotropic $g_i = 2$. The magnetic hyperfine coupling constants in the coupled system (denoted by A_i) are related to the a_i of the uncoupled representation by

$$\langle +|A_iS_z|+\rangle = \langle +|a_iS_{iz}|+\rangle,$$

which yields

$$A_i = 2a_i\langle +|S_{iz}|+\rangle = 2a_i\langle S_{iz}\rangle. \qquad [5]$$

Using standard techniques (8) to evaluate the matrix elements of S_{iz}, we obtain

$$\langle S_{1z}\rangle = \frac{7}{6} - 2\alpha^2,$$

$$\langle S_{2z}\rangle = \alpha^2 - \frac{1}{3} - \sqrt{3}\alpha\sqrt{1-\alpha^2}, \qquad [6]$$

and

$$\langle S_{3z}\rangle = \alpha^2 - \frac{1}{3} + \sqrt{3}\alpha\sqrt{1-\alpha^2}.$$

In Fig. 1 we have plotted the $\langle S_{iz}\rangle$ versus the square of the mixing parameters ($\alpha = 0$ results when $J_{12} = J_{13}$). Inspection of the figure reveals that the theory provides an understanding for the observation of A values of opposite sign. Moreover, it contains solutions for which $\langle S_{3z}\rangle$ and, thus, A_3 are small. In Fig. 1 the range $0 \leq \alpha^2 \leq 0.25$ contains all solutions; values of α^2 outside this range correspond to a relabeling of the sites.

Magnetic hyperfine coupling constants have been determined for a variety of ferric compounds with tetrahedral sulfur

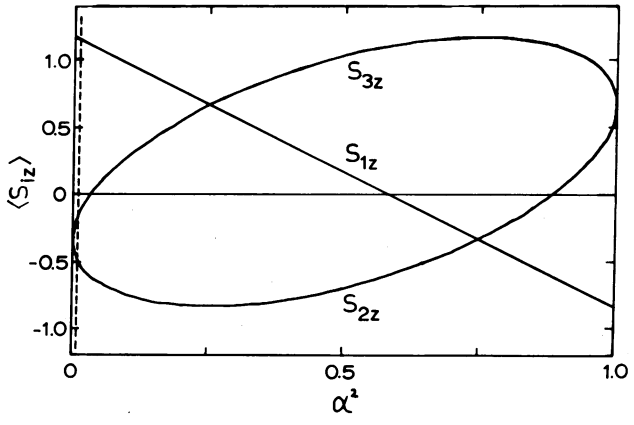

FIG. 1. Expectation values $\langle S_{iz}\rangle$ for the three iron sites as the function of the square of the mixing parameter α, according to Eqs. 6. The broken line at $\alpha^2 = 0.01$ marks the values that fit the data obtained for the ferredoxin from *A. vinelandii*. The graph corresponds to the state $S = \frac{1}{2}, M = +\frac{1}{2}$; i.e., $\langle S_{1z}\rangle + \langle S_{2z}\rangle + \langle S_{3z}\rangle = \frac{1}{2}$.

coordinations. For rubredoxin, desulforedoxin, and [2Fe–2S] clusters the a values were found to range in magnitude from 20 to 22 MHz (we discuss this in the next section). If we assume that each site of the oxidized three-iron cluster has an (uncoupled) a_i value of -20 MHz, then the value $\alpha^2 = 0.01$ yields $A_1 = -45$ MHz, $A_2 = +20$ MHz, and $A_3 = +6$ MHz. Thus, by assuming that all three sites are intrinsically rubredoxin-like—i.e., have a tetrahedral sulfur coordination—the simple one-parameter model reproduces the observed A values of the *Azotobacter* ferredoxin, $A_1 = -(41 \pm 3)$ MHz, $A_2 = +(18 \pm 3)$ MHz, and $|A_3| = (5 \pm 3)$ MHz.*

The value found for α^2 places restrictions on the coupling constants J_{ij}. Because $\alpha \approx 0$, it is instructive to explore the case in which site 1 is equally exchange-coupled to sites 2 and 3. For $J_{12} = J_{13}$ the intermediate spin S' commutes with the Hamiltonian of Eq. 2 and the energies of the spin multiplets are given by

$$E(S,S') = \frac{1}{2}J_{12}[S(S+1) - S'(S'+1) - S_1(S_1+1)] + \frac{1}{2}J_{23}[S'(S'+1) - S_2(S_2+1) - S_3(S_3+1)]. \qquad [7]$$

From this expression we learn that the state $|S_2S_3\,(2)\,S_1;\frac{1}{2}M\rangle$ is the ground state if $J_{12} > 0$ and $\frac{4}{7} < J_{12}/J_{23} < 1$; i.e., when the coupling constants are roughly equal and positive.

For the more general case in which all the coupling constants are different there is no analytical solution. In order to compute the energies of the multiplets and the wave function of the ground state we used the matrix elements listed in Griffith's article (10) on the spin coupling of polynuclear transition metal compounds. Because there are only two $S = \frac{1}{2}$ configurations, we may obtain by diagonalization of a 2×2 matrix a simple expression relating the mixing coefficient α to the exchange integrals J_{ij},

$$\alpha^2 = \frac{1}{2}\left(1 - \frac{1}{\sqrt{1+x^2}}\right),$$

$$\text{in which} \quad x = \sqrt{3}\,\frac{J_{12} - J_{13}}{2J_{23} - J_{12} - J_{13}}. \qquad [8]$$

With α already determined, it is straightforward to investigate with a computer program the ranges of the J_{ij} that yield the desired ground state. We have found that the coupling constants are restricted as follows: $J_{23} > J_{12} > J_{13} > 0$; $0.5 < J_{13}/J_{23} < 1$; $0.6 < J_{12}/J_{23} < 1$. Furthermore, J_{12} and J_{13} have to be the same to within 20%. The important result is that the coupling constants have to be about equal in magnitude.

DISCUSSION

The magnetic properties of the oxidized three-iron centers can be explained with a simple model that involves isotropic exchange coupling among three high-spin ferric ions. With only one adjustable parameter, α, the model accounts for the observed magnetic hyperfine coupling constants remarkably well. The positive sign of A_2 is seen to reflect the antiparallel orientation of S_2 relative to the system spin S, as suggested previously (1, 2). The vanishingly small hyperfine field observed for site 3 does not reflect a site with a small intrinsic coupling constant a_3. Rather, A_3 is small because S_3 is oriented almost perpendicular to the net magnetic moment, the axis of reference. [Sands and Dunham (11) have discussed a vector model for [2Fe–2S] centers in some detail.]

* The quoted experimental values are our best current estimates of the isotropic parts of the magnetic hyperfine interactions. We have attempted the difficult task of determining the sign of A_3 for Fd II from *D. gigas*; it appears that $A_3 > 0$ (see ref. 9).

In the previous section we have assumed for each site an (uncoupled) a_i value of −20 MHz. For rubredoxin, the prototype for Fe^{3+} with a distorted tetrahedral sulfur coordination, Schulz and Debrunner (12, 13) have reported $a = -22.2$ MHz (ferric proteins with oxygenic or nitrogeneous ligands typically have a values around 27–30 MHz). For desulforedoxin from *D. gigas*, which is thought to have Fe^{3+} coordinated to four cysteine residues, an a value of −21.1 MHz was determined (14). Sands and coworkers [11] have used electron–nuclear double resonance to study a variety of ferredoxins containing a [2Fe–2S] cluster. The ferric sites of adrenodoxin and putidaredoxin were found to have $a = -20.8$ MHz. The proteins from parsley and spinach have a slightly reduced a value, −19.9 MHz. These studies have also revealed sizable (10–15%) anisotropies of the magnetic hyperfine interactions; the values quoted here are averages, $a = (a_x + a_y + a_z)/3$. By assuming an intrinsic a value of −20 MHz for each site of the oxidized three-iron centers we have adopted a value at the lower end of the spectrum of observed a values. In principle, we could use $a_i = -(18–19)$ MHz, arguing that the ferric sites of the three-iron centers are slightly more covalent than the corresponding sites of [2Fe–2S] clusters. The experimental data, however, have large uncertainties due to the presence of anisotropies and microheterogeneities. Thus, no refinements to the theory are warranted at present. One major conclusion drawn from the present work is that the three-iron sites are intrinsically rubredoxin-like; i.e., a roughly tetrahedral environment of sulfur atoms is suspected for each iron site. Some distortion, however, towards square-planar (4) or a site with one nonsulfur ligand cannot be excluded.

A second conclusion of this work relates to the arrangement of the iron atoms. The finding of three comparable exchange-coupling constants argues for a fairly symmetric structure. We consider it unlikely that the three-iron centers consist essentially of a [2Fe–2S] center to which a third iron site is weakly attached. This comment is in order in light of core extrusion experiments (1, 15), which have elicited [2Fe–2S] cores in high, almost stoichiometric, yields. It appears that the three-iron centers are unstable (1) under the conditions used to extract standard [2Fe–2S] or [4Fe–4S] cores.

The considerations presented in the preceding section have yielded information concerning the relative magnitude of the exchange coupling constants. The determination of their absolute values requires that the energies of excited multiplets be measured. For [2Fe–2S] centers such information has been obtained from the temperature dependence of the magnetic susceptibility [see, for instance, Palmer (16)]. Eq. **7** shows that for $0.8 < J_{12}/J_{13} < 1$ the first excited state also has $S = \frac{1}{2}$. Because both $S = \frac{1}{2}$ states have the same magnetic moment, caution is advised for the interpretations of (future) susceptibility studies of oxidized three-iron centers.

Throughout this communication we have assumed that the g_i and the a_i in Eq. **1** are scalars. Previously (1, 2) we pointed out that the electronic Zeeman interactions of the two ferredoxins are slightly anisotropic (about 1%). Also, for the spectral simulations of the *Azotobacter* ferredoxin Mössbauer spectra (1) we used $A_x = -37$ MHz, $A_y = -42$ MHz, and $A_z = -45$ MHz for site 1. (A_1 quoted above is the average.) These anisotropies can easily be accommodated by assuming that the g_i and a_i are tensors. Anisotropies of the required magnitude have been reported for the g values of Fe^{3+} in FeS_2Se_2 (17). Rubredoxin has been shown to have an anisotropic (about 5%) a tensor (12), and electron–nuclear double resonance studies of the ferric site of [2Fe–2S] centers have revealed even larger anisotropies (11).

The model described here fits the data obtained for *D. gigas* Fd II quite well also. We have pointed out (2) that the quoted Fd II hyperfine parameters are tentative. The reported anisotropies in A_1 and A_2 were introduced to account for the broad absorption features of the low-temperature Fd II spectra. Because heterogeneities and magnetic anisotropies can broaden the spectra in similar ways, further studies are required to differentiate between the two cases. (The high-field tail observed for the Fd II EPR spectra suggests the presence of heterogeneities; see figure 2 of ref. 9.)

Finally, a spin-coupling model for the reduced three-iron cluster is not yet available. The Mössbauer data (1, 2) have shown clearly that the electronic ground state of the reduced centers has $S \geq 1$. Some important experimental information is available, but the most crucial quantity, the value for S, is as yet undetermined. Furthermore, the effects of zero-field splitting and the presence of two (identical) iron sites with formal oxidation level $Fe^{2.5+}$ need to be considered. A model involving resonance of two configurations, each with two formal high-spin ferric sites and one high-spin ferrous site, is being contemplated.

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