THE INTERPRETATION OF THE EPR AND MÖSSBAUER SPECTRA OF TWO-IRON, ONE-ELECTRON, IRON-SULPHUR PROTEINS

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Received March 17, 1971

Summary. Previous experiments by other workers have shown that the iron-sulphur proteins adrenodoxin and putidaredoxin, on enrichment with ^{57}Fe isotope, show a hyperfine splitting of the EPR* signal of the reduced form into three lines with intensities in the ratio 1:2:1. This was taken as evidence that on reduction an electron becomes associated with two iron atoms. In this paper it is demonstrated that the EPR splitting is also consistent with a model previously proposed for plant ferredoxins in which only one iron atom is reduced, but is antiferromagnetically coupled to the other iron atom. This model is consistent with results from Mössbauer spectroscopy, and suggests that the mechanism of oxidation and reduction is the same for all two-iron, one-electron, iron-sulphur proteins.

The simplest iron-sulphur proteins are those that contain two atoms of iron and two of labile sulphur per molecule, and on reduction accept one electron. Two types of these proteins are known (see review of Hall and Evans, 1969): the plant ferredoxins, and the iron-sulphur proteins associated with hydroxylase systems. Examples of the former type that have been studied are the ferredoxins from spinach, Scenedesmus and Euglena; of the latter type, adrenodoxin from the adrenal steroid hydroxylases, and putidaredoxin from the camphor hydroxylase of Pseudomonas putida. Although these two types of Fe-S proteins have dissimilar amino acid sequences (Tanaka et al, 1970) they are similar in their optical absorption, optical rotatory dispersion, circular dichroism and Mössbauer spectra. Both types are virtually non-magnetic in their oxidized forms, and in the reduced forms have EPR* signals centred around g = 1.94.

On the basis of the EPR data a model was proposed by Gibson, Hall, Thornley and Whatley (1966) to describe the state of the iron atoms in plant

* Abbreviation : EPR, electron paramagnetic resonance.

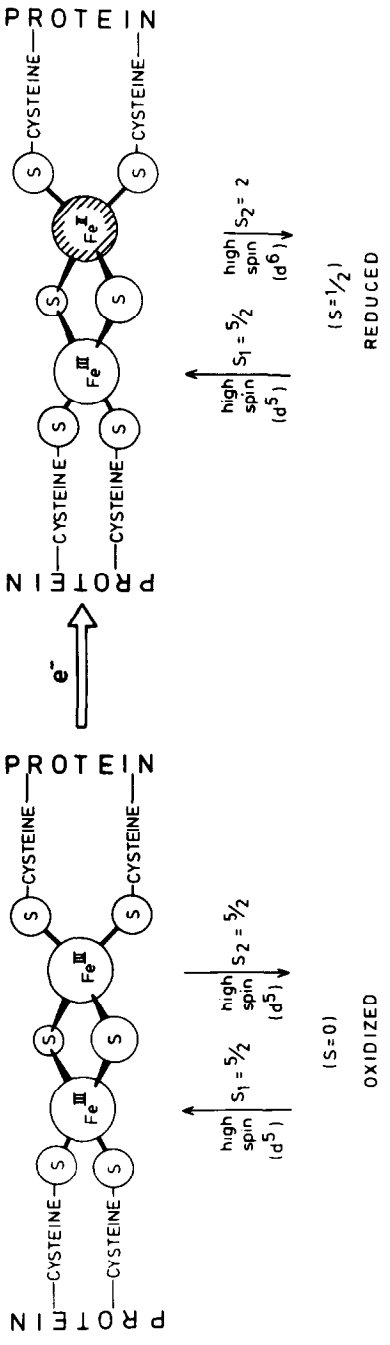

Fig. 1. Proposed model for the active centre of a two-iron, one-electron iron-sulphur protein.

ferredoxins. This model was later confirmed by Mössbauer spectroscopy (Rao, Cammack, Hall and Johnson, 1971) and is illustrated in Fig. 1. In the oxidized state, two high-spin ferric atoms ($S = \frac{5}{2}$) are coupled antiferromagnetically to give no net spin. In the reduced state, one particular iron atom is reduced to a high-spin ferrous state ($S = 2$) with the result that the molecule now has a net spin $S = \frac{1}{2}$.

On replacement of the native ^{56}Fe (nuclear spin = 0) in the proteins by ^{57}Fe (nuclear spin = $\frac{1}{2}$) a hyperfine splitting was observed in the EPR signal of the reduced form. Thus Palmer (1967) observed a broadening of the EPR

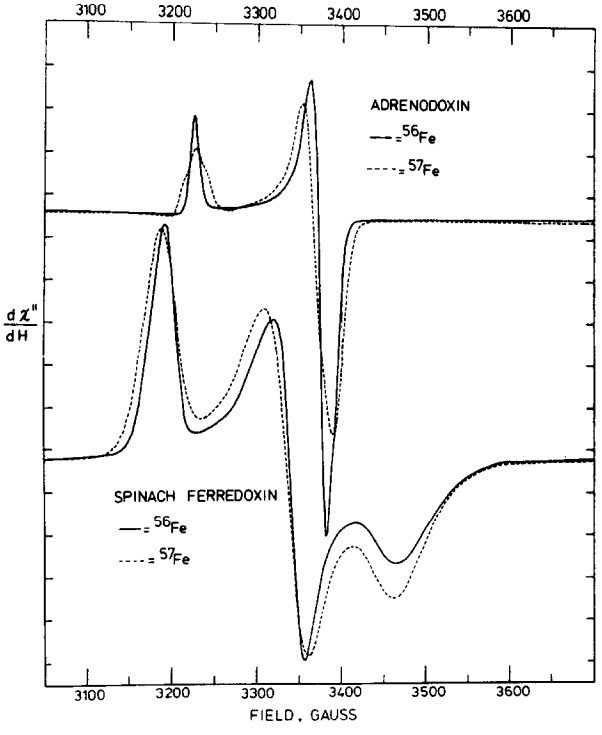

Fig. 2. EPR spectra of spinach ferredoxins and adrenodoxins, native (^{56}Fe) and reconstituted with 88% ^{57}Fe, showing hyperfine splitting. The apparent shifts in g_y and g_z on enrichment with ^{57}Fe were consistently observed with a number of different preparations of plant ferredoxins and of adrenodoxin. Preparation and reconstitution of the proteins were as described elsewhere. (Rao et al., 1971; Cammack et al., 1971). The spectra were recorded on a Varian E4 spectrometer, under the following conditions: spinach ferredoxin, temperature 25°K, microwave power 4 mW, frequency 9.170 GHz, modulation amplitude 4 gauss; adrenodoxin, temperature 77°K, microwave power 20 mW, frequency 9.170 GHz, modulation amplitude 4 gauss.

signal of spinach ferredoxin; Tsibris et al. (1968) and Orme-Johnson and Beinert (1969) observed hyperfine splitting in putidaredoxin and adrenodoxin, respectively. As can be seen from Fig. 2, which illustrates our experiments, the broadening was similar in magnitude for both spinach ferredoxin and adrenodoxin. In the case of spinach ferredoxin, the linewidth was too great to determine the number of hyperfine lines, but for putidaredoxin and adrenodoxin the hyperfine structure was clearly resolved into three lines of relative intensities 1:2:1, indicating that the two iron atoms in the molecule were somehow coupled magnetically together. One possible interpretation of this suggested by Orme-Johnson and Beinert (1969) is that in the reduced protein the iron atoms are identical, and therefore the electron is shared between them. However our Mössbauer spectra of spinach and Scenedesmus ferredoxins (Fig. 3) (Johnson, 1971) and of adrenodoxin (Cammack, Rao, Hall and Johnson, 1971) clearly show that the two iron atoms are different.

It is the purpose of this note to point out that the observed hyperfine splitting in the EPR spectrum of reduced adrenodoxin and putidaredoxin into

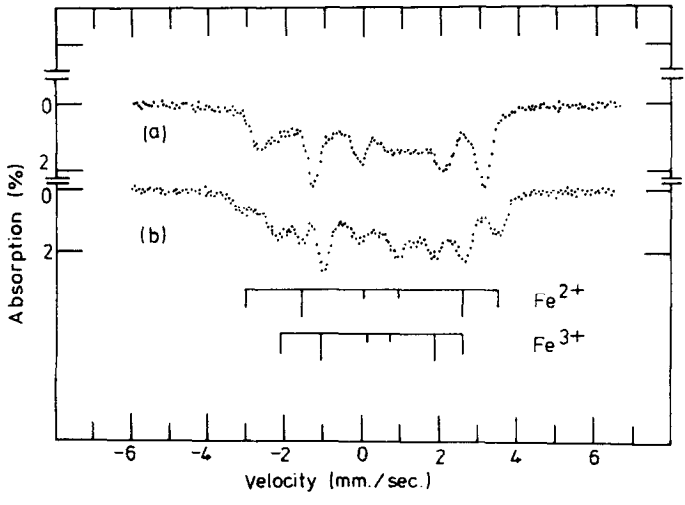

Fig. 3. Mossbauer spectrum of reduced spinach ferredoxin at 4.2°K, (a) in a field of 0.3 kG applied perpendicular to the gamma rays, (b) in a field of 30 kG applied perpendicular to the gamma rays. The ferredoxin was reconstituted with 88% enriched ^{57}Fe, and the concentration was 1.0 mM. Details of the experiment were as described by Rao et al. (1971).

3 lines is consistent with the model proposed for plant ferredoxins (Fig. 1). In this model only one iron atom becomes reduced, but the antiferromagnetic coupling between the iron atoms results in a hyperfine interaction of the electron with both iron nuclei.

Using the model of Gibson, Hall, Thornley and Whatley (1966) the spin-hamiltonian for the two-iron unit of the reduced protein in a magnetic field $\underline{H}$ may be written:

$$\mathcal{H} = \beta\, \underline{H}\cdot(g_1\underline{S}_1 + g_2\underline{S}_2) + J\, \underline{S}_1\cdot\underline{S}_2 + \underline{S}_1\cdot\underline{A}_1\cdot\underline{I}_1 + \underline{S}_2\cdot\underline{A}_2\cdot\underline{I}_2 + g_n\beta_n\, \underline{H}\cdot(\underline{I}_1 + \underline{I}_2) \qquad (1)$$

Where J is the exchange coupling, S_1 ($= \frac{5}{2}$) is the spin of the Fe^{3+} atom, S_2 ($= 2$) is the spin of the Fe^{2+} atom, A_1 and A_2 are the magnetic hyperfine couplings and I_1 and I_2 are the nuclear spins of the ^{57}Fe nuclei of the Fe^{3+} and Fe^{2+} ions, respectively; g_1 and g_2 are the g-values of the ions and g_n is the nuclear g-value for the ^{57}Fe state involved; β and β_n are the Bohr and nuclear magneton respectively. The nuclear quadrupole interaction must also be added, but its effect is relatively small and only affects the Mössbauer spectrum. The exchange term J is by far the largest term in the hamiltonian and it couples the electron spins to give a resultant spin state $\underline{S} = \underline{S}_1 + \underline{S}_2$ of which effectively only the lowest in energy is populated at ordinary temperatures and has $S = 1/2$. The spin-hamiltonian for this state may then be written

$$\mathcal{H} = \beta\, \underline{H}\cdot g\, \underline{S} + \underline{S}\cdot\underline{A}_1'\cdot\underline{I}_1 + \underline{S}\cdot\underline{A}_2'\cdot\underline{I}_2 + g_n\beta_n\, \underline{H}\cdot(\underline{I}_1 + \underline{I}_2) \qquad (2)$$

with $S = 1/2$. Gibson et al. (1966) showed that the resulting g-value is

$$g = \frac{\underline{S}_1\cdot\underline{S}}{S^2}\, g_1 + \frac{\underline{S}_2\cdot\underline{S}}{S^2}\, g_2 \qquad (3)$$

Physically the factors $\frac{\underline{S}_1\cdot\underline{S}}{S^2}$ and $\frac{\underline{S}_2\cdot\underline{S}}{S^2}$ project the g-tensors of the individual ions on the resultant spin direction to give the resultant g-tensor. For $S = 1/2$, $\frac{\underline{S}_1\cdot\underline{S}}{S^2} = \frac{7}{3}$ and $\frac{\underline{S}_2\cdot\underline{S}}{S^2} = -\frac{4}{3}$ so $g = \frac{7}{3}\, g_1 - \frac{4}{3}\, g_2$

and if $g_1 \simeq g_2 \simeq 2$ a net g of 2 results. While g_1 would be expected to be isotropic and equal to 2, since the Fe^{3+} ion is in a spherically symmetrical S-state, the g-value of the Fe^{2+} ion (g_2) would in general be anisotropic. For a d orbital it is usual to find that $g_z \simeq 2$ and $g_{x,y} > 2$. Hence in the exchange coupled state, where the spin S_2 of the Fe^{2+} ion is antiparallel to the total spin S, the effective g-values are $g_z \simeq 2$, and $g_{x,y} < 2$.

Similarly the effective hyperfine interaction tensors of the coupled state are the projections A_1' and A_2' of the single ion tensors A_1 and A_2 on to $\underline{S}$. In terms of the original single ion tensors A_1 and A_2 this is written

$$A_1' = \frac{\underline{S}_1 \cdot \underline{S}}{S^2} A_1$$

and $$A_2' = \frac{\underline{S}_2 \cdot \underline{S}}{S^2} A_2$$

and the effective hamiltonian becomes

$$\mathcal{H} = \beta \underline{H} \cdot g \underline{S} + \frac{7}{3} \underline{S} \cdot \underline{A}_1 \cdot \underline{I}_1 - \frac{4}{3} \underline{S} \cdot \underline{A}_2 \cdot \underline{I}_2 + g_n \beta_n \underline{H} \cdot (\underline{I}_1 + \underline{I}_2)$$

The EPR spectra and the Mössbauer spectra in which two separate hyperfine contributions from the two iron atoms are observed are measured in magnetic fields which are large compared with the nuclear interactions. This means that only the components of the g and A tensors parallel to the field are important and the effective spin hamiltonian is

$$\mathcal{H}_{eff} = g_z \beta H S_z + \frac{7}{3} A_{1z} S_z I_{1z} - \frac{4}{3} A_{2z} S_z I_{2z} + g_n \beta_n H (I_{1z} + I_{2z}) \quad (4)$$

where z is the direction of the applied field.

This hamiltonian is sufficient to explain both the observed EPR and Mössbauer spectra of reduced spinach ferredoxin. Consider the Mössbauer spectra first. Here we are observing nuclear transitions and the effect on the nuclear energies of the electronic magnetic moments. The effective hamiltonian for the nuclei is

$$\mathcal{H}\ \text{nuclear} = \tfrac{7}{3}A_{1z}S_zI_z - \tfrac{4}{3}A_{2z}S_zI_{2z} + g_n\beta_n H\,(I_{1z} + I_{2z})$$

$$= (\tfrac{7}{3}A_{1z}S_z + g_n\beta_n H)\,I_{1z} + (-\tfrac{4}{3}A_{2z}S_z + g_n\beta_n H)\,I_{2z}$$

$$= g_n\beta_n\,(H_{1nz} + H)\,I_z - g_n\beta_n\,(H_{2nz} - H)\,I_{2z} \qquad (5)$$

where the hyperfine fields are

$$H_{1z} = \frac{7}{3}\frac{A_1 S}{g_n\beta_n}$$

$$H_{2z} = -\frac{4}{3}\frac{A_2 S}{g_n\beta_n} \qquad (6)$$

Since the hyperfine fields H_{1z} and H_{2z} are negative for iron atoms, the external field H subtracts from the internal field (H_{1z}) of the Fe^{3+} ions and adds to that (H_{2z}) of the Fe^{2+} ions. This is just what has been observed in the Mössbauer spectra of spinach ferredoxin and Scenedesmus ferredoxin at 4.2°K in fields of up to 30 kG. (Fig. 3). In small fields just sufficient to overcome the hyperfine coupling so that equation (4) is valid, it is seen that the spectra from the two ions overlap, i.e. the components of the hyperfine fields along the direction of the applied field happen to be equal in magnitude, though opposite in sign, i.e. $\frac{7}{3}A_1 = -\frac{4}{3}A_2 = A$ (say). In a field then the splitting of the Mössbauer spectra corresponding to effective magnetic fields of $H_n \pm H$ at the nuclei, is observed.

In the EPR spectra electronic transitions are observed and we measure the way the nuclear magnetic momemt perturbs them. The direct interaction between the field and the nuclei is negligible and the effective electronic hamiltonian is

$$\mathcal{H}\ \text{electron} = g\beta HS_z + (\tfrac{7}{3}A_1I_{1z} - \tfrac{4}{3}A_2I_{2z})\,S_z \qquad (7)$$

$$= g\beta HS_z + A\,(I_{1z} - I_{2z})\,S_z \qquad (8)$$

The energy levels of the two-iron unit are then

$$W_{\text{electron}}(M,m) = [g\beta H + A(m_1 - m_2)]\,M \qquad (9)$$

where M is the projection of S on the z-axis, and m_1 and m_2 are the projections of I_1 and I_2. Transitions are observed when $\Delta M = \pm 1$, $\Delta m_1 = \Delta m_2 = 0$, and the resulting spectrum shows a splitting into three lines with relative intensities 1:2:1.

Note that this splitting occurs because of the antiferromagnetic coupling between the electronic spins and the fact that the resulting effective hyperfine interaction has a component along the field direction which is equal in magnitude but opposite in sign for the two iron ions. A similar EPR spectrum could arise in principle from hyperfine coupling of equal magnitude and sign between the two nuclei and a single electron spin, e.g. as a result of a spin hamiltonian $\mathcal{H} = g\beta HS_z + A\,(I_{1z} + I_{2z})\,S_z$ (note the change in sign of I_{2z} compared with (8)). However, this would produce a Mössbauer spectrum for which the shift produced by a large applied magnetic field would be the same (i.e. a reduction in total field) for both iron atoms, in disagreement with experiment. Hence (8) is applicable to describe the EPR spectrum; this equation is a special case of eq. (1), which describes our model (Fig. 1) of the state of the iron in reduced two-iron, one-electron, iron-sulphur proteins.

Acknowledgements

We are grateful to Mr. L.W. Becker, Mrs. M.M. Frick and Mrs. L. Turner for expert technical assistance. We thank Dr. M. Blume, Brookhaven National Laboratory, for a valuable discussion. This work was supported by grants from the Science Research Council.

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