

## THE IRON COMPLEX IN SPINACH FERREDOXIN

By J. F. GIBSON,\* D. O. HALL,† J. H. M. THORNLEY,‡ AND F. R. WHATLEY†

BOTANY DEPARTMENT, KING'S COLLEGE, LONDON, CHEMISTRY DEPARTMENT,  
IMPERIAL COLLEGE, LONDON, AND CLARENDON LABORATORY, OXFORD

*Communicated by Sir Hans H. Krebs, June 20, 1966*

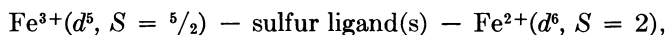
In an earlier communication<sup>1</sup> we reported the observation of electron spin resonance (ESR) in spinach ferredoxin, a nonheme iron protein isolated from spinach. An ESR signal centered on  $g = 1.94$  was observed after reducing the protein with sodium dithionite; this signal was just visible as a broad line at 150°K, while on cooling to 90°K the line intensified and structure appeared on the wings. We now wish to report the behavior of this spectrum at temperatures down to 2°K, to discuss the model recently put forward by Brintzinger, Palmer, and Sands,<sup>2</sup> and to suggest an alternative explanation of the spectrum.

The experimental procedures used were essentially those described by Hall, Gibson, and Whatley,<sup>1</sup> who followed the method of Hill and Bendall to prepare the spinach (*Spinacea oleracea*) ferredoxin. For ESR experiments at liquid hydrogen and liquid helium temperatures, the samples were placed in silica tubes 2 cm long with narrow neck and containing a little sodium dithionite to reduce the ferredoxin. The tubes were quickly plugged with silicone grease, shaken, and then frozen. The very low temperature experiments were performed on a transmission X-band spectrometer of conventional design.

Our low temperature data show that the spectra previously observed at 90°K<sup>1</sup> and 40°K<sup>2</sup> remain essentially the same down to 2°K, and can be attributed to a ground state doublet with  $g_x = 1.88$ ,  $g_y = 1.94$ , and  $g_z = 2.04$ . We have observed that at the lowest temperatures the spectrum is easily saturated, implying that some strongly temperature-dependent relaxation mechanism operates. In this connection, it would be most interesting to measure the temperature dependence of the relaxation process, for, if it were due predominantly to the so-called two-phonon resonant mechanism,<sup>3</sup> valuable information about the energy levels of the complex might be obtained. We shall come back to this point later, as the relaxation behavior supports our model.

Brintzinger *et al.*<sup>2</sup> have recently postulated that the iron in reduced spinach ferredoxin is low-spin ferric in a tetrahedral environment. However, their model has some features that are not too satisfactory. First, a very large tetrahedral ligand field splitting (of order at least 20,000 cm<sup>-1</sup>) is required to cause spin-pairing, and also to give the positive  $g_z$ -shift observed; this is much larger than is observed in a wide variety<sup>4</sup> of complexes (2000–5000 cm<sup>-1</sup>). Further, such a strong tetrahedral field with spin-pairing might be expected to cause a much more intense optical spectrum than is in fact observed, as both spin-allowed and Laporte-allowed transitions are likely to occur.

We wish to suggest that the two iron atoms in the spinach ferredoxin molecule strongly interact with one another through one or more ligands, which in this case are likely to be sulfur. If the reduced complex is of the schematic type



then an antiferromagnetic exchange interaction between the two spins will couple

them<sup>5</sup> to give a total spin of  $1/2$  in the ground state. If spin  $S_1$  with  $g$ -value  $g_1$  is coupled to spin  $S_2$  with  $g$ -value  $g_2$ , it can be shown that the effective  $g$ -value  $g$  of the coupled state with spin  $S$  is given by

$$g = \frac{g_1 + g_2}{2} + \frac{(g_1 - g_2)}{2S(S+1)} [S_1(S_1 + 1) - S_2(S_2 + 1)]. \quad (1)$$

For anisotropic  $g$ -values, this expression will hold for the separate components of  $g$ , provided that the principal axes of  $g_1$  and  $g_2$  are the same. In our case we take  $S_1 = 5/2$ ,  $S_2 = 2$ , and  $S = 1/2$ , obtaining

$$g = (7g_1 - 4g_2)/3. \quad (2)$$

To fit the  $g$ -values in detail, an orbital energy-level scheme must be chosen. It is to be emphasized that there are several ways of obtaining the experimental  $g$ -values with various energy-level schemes and ligand fields (both octahedral and tetrahedral). We shall content ourselves with giving the scheme which at this time seems most acceptable and most in accord with other properties of spinach ferredoxin. In order to simplify the analysis, it will be assumed that any crystal field splittings of the spin degeneracies (6 and 5) of the ground states ( $S_1 = 5/2$  and  $S_2 = 2$ ) are small compared with the exchange interaction, although this approximation is by no means essential.

High-spin  $\text{Fe}^{3+}$  is expected to have an isotropic  $g$ -value, and we take  $g_1 = 2.019$ , as Title<sup>6</sup> observed this value for  $\text{Fe}^{3+}$  ions in  $\text{ZnS}$  where  $\text{Fe}^{3+}$  is tetrahedrally coordinated to sulfurs. For the  $\text{Fe}^{2+}$  ion, we shall assume that the ligand field has some tetrahedral character and gives rise to the energy-level scheme below:

$$\begin{array}{l} d_{xy} \quad \text{-----} \quad \uparrow \\ d_{zz} \quad \text{-----} \quad \uparrow \quad 6000 \text{ cm}^{-1} \\ d_{yz} \quad \text{-----} \quad \uparrow \quad 3750 \text{ cm}^{-1} \\ \\ d_{x^2-y^2} \quad \text{-----} \quad \uparrow \\ d_{3z^2-r^2} \quad \text{-----} \quad \uparrow \downarrow \quad 0 \text{ cm}^{-1} \end{array} \quad (3)$$

The orbitals  $d_{xy}$  and  $d_{x^2-y^2}$  do not enter the present analysis, and it is not intended that this diagram represent the real positions of these orbitals. The  $g$ -values in this scheme are easily calculated and are found to be (with  $S_2 = 2$ )

$$\begin{aligned} g_x &= g_e + 3\zeta/2(E_{yz} - E_{3z^2-r^2}) \\ g_y &= g_e + 3\zeta/2(E_{zx} - E_{3z^2-r^2}) \\ g_z &= g_e \end{aligned} \quad (4)$$

where  $g_e$  is the free electron  $g$ -value,  $\zeta$  is the spin-orbit coupling constant for  $\text{Fe}^{2+}$ , and  $E_i$  is energy of the  $i$ th state. If we write  $g_1 = g_e + \Delta g_1$ , then application of equations (2) and (4) gives

$$\begin{aligned} g_x &= g_e + 7/3 \Delta g_1 - 2\zeta/(E_{yz} - E_{3z^2-r^2}) \\ g_y &= g_e + 7/3 \Delta g_1 - 2\zeta/(E_{zx} - E_{3z^2-r^2}) \\ g_z &= g_e + 7/3 \Delta g_1. \end{aligned} \quad (5)$$

For the free ferrous ion  $\text{Fe}^{2+}$ , the spin-orbit coupling constant  $\zeta$  is  $400 \text{ cm}^{-1}$  (ref. 7), and reducing this to allow for the covalency of the complex,<sup>8</sup> we choose  $\zeta = 300 \text{ cm}^{-1}$ . Applying equations (5) to the experimental  $g$ -values, we find the energy levels indicated in the diagram (3). In conclusion, let us list the many advantages of the present model.

(a) The  $g$ -values are explained without having to assume that the iron complex in spinach ferredoxin has any unusual properties which are not found in other systems.

(b) Integration of the ESR spectrum indicated a smaller number of unpaired spins than there are iron atoms.<sup>9</sup>

(c) ESR is not observed in the oxidized state because of the strong interaction of the two magnetic ions. (We assume here that the ESR signal in spinach ferredoxin at  $g = 4.27^1$  may be due to an impurity.)

(d) The proximity of the two iron atoms suggests a reason why, on reduction, the molecule only accepts a single electron.<sup>10</sup> The extra electron of the ferrous complex will be in a molecular orbital which will take it onto the ferric complex, reducing the electron affinity of the ferric complex,<sup>11</sup> and thus preventing it from also accepting an electron.

(e) The anomalous temperature dependence of the spectrum may be explained in terms of a two-phonon relaxation process,<sup>3</sup> in this case occurring via the low-lying states of higher total spin, e.g.,  $S = 3/2, 5/2$ , etc.

This model has the virtue of explaining much experimental data in a reasonable way, but then there are other possible models which will also do this. It is known, for example, that  $[\text{Ru}(\text{NH}_3)_6]^{3+} 4d^5$  (low spin) has  $g$ -values of 1.80, 1.90, and 2.06.<sup>12</sup> If a low-spin ferric ion placed next to a diamagnetic ferrous ion showed similar behavior, a model of this type would have all the advantages (a)–(e) listed above. Another plausible model can be constructed by assuming a very strong interaction between two iron atoms (our model might in these terms be described as a weak interaction case). The final choice between the various possible models must be made by further experimentation.

Of course, it is possible that similar models in which an electron is “shared” between two or more iron atoms are applicable to other ferredoxins. Indeed, it is entertaining to speculate about possible evolutionary relationships between such ferredoxins. Clostridial ferredoxin has 5–6 irons and may possibly have two groupings of iron, each of which is similar to the spinach ferredoxin. This type of ferredoxin is thus able to accept two electrons;<sup>13</sup> this is important since it is primarily concerned with pyruvate metabolism which involves 2-electron transport. However, in higher plants it is possible for only one electron at a time to be accepted in the photoact (system 1), and thus only one grouping of iron atoms is necessary.

It would be interesting to measure the susceptibility of spinach ferredoxin at different temperatures, since this information could support one or other of the models mentioned. Our model suggests that at  $300^\circ\text{K}$  the susceptibility would be about  $1250 \times 10^{-6} \text{ emu/mole}$  if the ground state ( $S = 1/2$ ) only were populated. A susceptibility in excess of this would be expected if the higher excited states ( $S = 3/2, 5/2$ , etc.) were partially populated at  $300^\circ\text{K}$ .

The authors are indebted to Mr. J. J. Davies for help with experiments and the loan of his ESR spectrometer, to Dr. R. J. P. Williams for helpful comments, and to Mrs. S. Druery for technical assistance.

\* Chemistry Department, Imperial College, London.

† Botany Department, King's College, London.

‡ Clarendon Laboratory, Oxford.

<sup>1</sup> Hall, D. O., J. F. Gibson, and F. R. Whatley, *Biochem. Biophys. Res. Commun.*, **23**, 81 (1966).

<sup>2</sup> Brintzinger, H., G. Palmer, and R. H. Sands, these PROCEEDINGS, **55**, 397 (1966).

<sup>3</sup> Orbach, R., *Proc. Roy. Soc. (London)*, **A264**, 458 (1961).

<sup>4</sup> Jørgensen, C. K., *Absorption Spectra and Chemical Bonding in Complexes* (Pergamon Press, 1962), p. 110.

<sup>5</sup> Bleaney, B., and K. D. Bowers, *Proc. Roy. Soc. (London)*, **A214**, 451 (1952); Owen, J., *J. Appl. Phys.*, **32**, 213S (1961).

<sup>6</sup> Title, R. S., *Phys. Rev.*, **131**, 623 (1963).

<sup>7</sup> Dunn, T. M., *Trans. Faraday Soc.*, **57**, 1441 (1961).

<sup>8</sup> Owen, J., *Proc. Roy. Soc. (London)*, **A227**, 183 (1955), Table 3.

<sup>9</sup> Beinert, H., in *I.U.B. Symposium on Flavins and Flavoproteins*, ed. E. C. Slater (New York: Elsevier, 1965).

<sup>10</sup> Whatley, F. R., K. Tagawa, and D. I. Arnon, these PROCEEDINGS, **49**, 266 (1963).

<sup>11</sup> For example, R. G. Shulman and S. Sugano [*J. Chem. Phys.*, **42**, 39 (1965)] conclude that the effective charges on ferrous and ferric in octahedral cyanide complexes are much the same.

<sup>12</sup> Griffiths, J. H. E., J. Owen, and I. M. Ward, *Proc. Roy. Soc. (London)*, **A219**, 526 (1953).

<sup>13</sup> Sobel, B. E., and W. Lovenberg, *Biochemistry*, **5**, 6 (1966).