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Antisymmetric Exchange in [2Fe−2S]$^{1+}$ Clusters: EPR of the Rieske Protein from *Thermus thermophilus* at pH 14

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Proteins containing reduced [2Fe−2S]$^{1+}$ clusters exhibit a characteristic EPR signature, the so-called $g = 1.94$ signal. This type of signal has one g-value just above $g = 2$ (range 2.01−2.05) and two g-values below $g = 2$, typically between 1.85 and 1.95, to yield a $g_{av} = (g_x + g_y + g_z)/3 \approx 1.96$.[1] First recognized by Gibson et al.,[2] these signals are the result of antiferromagnetic exchange interactions, $H = J\mathbf{S_1}\cdot\mathbf{S_2}$, between a high-spin ($S_1 = {}^5/_2$) Fe^{3+} and a high-spin ($S_2 = 2$) Fe^{2+} site;[3,4] the $S = {}^1/_2$ ground state of the coupled pair yields the $g = 1.94$ signal. Bertrand and co-workers[1] have classified the signals of many ferredoxins with a crystal field model, among them the Rieske proteins, which have $g_{av} \approx 1.91$. For the Rieske protein from *Thermus thermophilus (Tt)* a non-cysteine coordination was demonstrated nearly 20 years ago;[5,6] this ferredoxin exhibits g-values at 1.80, 1.90, and 2.02, $g_{av} = 1.91$ (Figure 1A) and the ferrous site of its [2Fe−2S]$^{1+}$ cluster is coordinated by two histidines.[7] It has been proposed that protonation of one of the Fe-bound imidazole rings is essential for the biological function of this protein,[8] and protein film voltammetric studies have shown that both imidazole rings are deprotonated in the reduced protein above pH ≈ 13.5.[9]

Here we report on the novel EPR spectrum of the reduced *Tt* Rieske protein at pH 14. The signal is characterized by unusual g-values and line-widths that are highly similar to those reported for the signal II associated with some [2Fe−2S]$^{1+}$ clusters in proteins of the xanthine oxidase family (Table 1 of ref 10). These clusters exhibit $S = {}^1/_2$ EPR spectra for which one g-value is substantially above $g = 2.00$, up to 2.16 for CO dehydrogenase from *Hydrogenophaga pseudoflava*.[10] The nature of signal II has remained unexplained for more than 20 years; however, we now realize that antisymmetric[11] (or Dzyaloshinskii−Moriya, D−M) exchange may be involved. We have shown previously that D−M exchange affects the Mössbauer spectra of oxidized methane monooxygenase[12] and the Mössbauer and EPR spectra of [Fe_3S_4]$^{1+}$ clusters.[13] In this communication we demonstrate that antisymmetric exchange is the cause of the unusual upward shift in signals of this type.

Figure 1 shows EPR spectra of the *Tt* Rieske protein prepared at pH 7 and 14 (samples R7 and R14, respectively).[14] The spectrum of R14, shown in (B), yields g-values at ∼1.81, 1.94, and 2.14, with $g_{av} \approx 1.97$. To explain the unusually large g-value at $g_z = 2.14$ we consider the Hamiltonian

$$H = J\mathbf{S}_1\cdot\mathbf{S}_2 + \mathbf{d}\cdot\mathbf{S}_1\times\mathbf{S}_2 + \sum_{i=1,2}\{\beta\mathbf{S}_i\cdot\mathbf{g}_i\cdot\mathbf{B} + \mathbf{S}_i\cdot\mathbf{D}_i\cdot\mathbf{S}_i\} \quad (1)$$

where $i = 1$ and 2 designate the ferric and ferrous sites, respectively. The terms in the sum describe the Zeeman interactions and zero-field splittings (ZFS) of the two sites, respectively. For [2Fe−2S]$^{1+}$ clusters J is larger than 150 cm^{-1} ,[1,15] and for the ZFS parameters we may consider those of Fe^{3+} and Fe^{2+} rubredoxin,[16,17] $D_1 = 1.9$ cm^{-1} and $D_2 = 5.7$ cm^{-1}. The effects of the ZFS terms on the

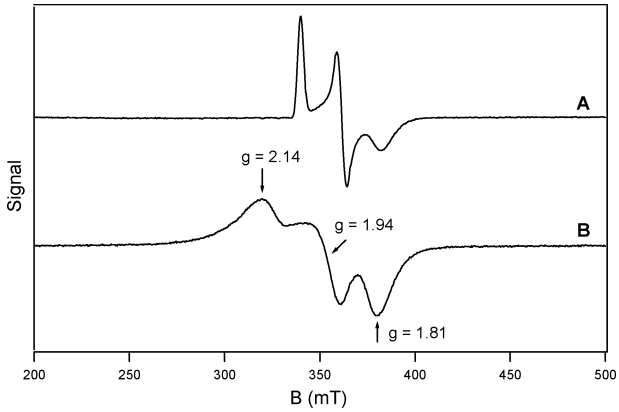

Figure 1. EPR spectra of *Tt* Rieske protein at pH 7 (A) and pH 14 (B). Conditions: $T = 18$ K; 9.62 GHz; 0.2 mW (A) and 2 mW (B) microwave power, 1 mT modulation.

g-values have been estimated to be $|\Delta g| < 0.01$,[18] and for $D_1 = D_2 = 0$ the g-tensor of the $S = {}^1/_2$ ground state is given by $\mathbf{g} = ({}^7/_3)\mathbf{g_1} - ({}^4/_3)\mathbf{g_2}$.[2] The g-values of the ferric site can be taken between 2.01 and 2.04,[19] while two components of $\mathbf{g_2}$ may be as large as 2.10−2.15, yielding two principal values of $\mathbf{g}$ below 2.0. If the ZFS and J terms are of comparable magnitude, the g-values of the $S = {}^1/_2$ ground doublet are significantly modified by admixture of $S = {}^3/_2$ and ${}^5/_2$ states of the spin ladder. This situation occurs for some diiron proteins, for which correction formulas have been reported.[18,20,21] Examination of the published expressions for $\mathbf{g}$, as well as exact solutions of eq 1 for $\mathbf{d} = 0$, show that mixing by ZFS terms shifts the g-values *downward* and thus cannot explain $g_z = 2.14$. (For some combination of parameters, g_z is shifted upward, but these shifts are <0.01 for reasonable choices of ZFS parameters.) However, inclusion of D−M exchange,[11] $\mathbf{d}\cdot\mathbf{S_1}\times\mathbf{S_2}$, combined with a small value of J provides an explanation for the large g_z of R14; $\mathbf{d}$ is a vector representing the three antisymmetric components of the J tensor in $\mathbf{S_1}\cdot\mathbf{J}\cdot\mathbf{S_2}$.[22] The D−M term of eq 1 mixes the $S = {}^1/_2$ doublet with the $S = {}^3/_2$ manifold. The desired shifts occur for $|d|/J \approx 0.18$. For J values ∼40 cm^{-1} (see below) mixing by the ZFS terms can be substantial. In this case, the number of unknowns is unmanageably large: there are six local g-values, four ZFS parameters, the three components of $\mathbf{d}$, and since low symmetries favor D−M exchange,[11] the various tensors may not be collinear. However, the essential argument can be made with some simplifying assumptions, namely that the g-values of the ferric site are all 2.02, that $D_1 = D_2 = 0$, and that one of the ferrous g-values, g_{2z}, is equal to 2.0. Further, the effect of the D−M term can be illustrated by directing the $\mathbf{d}$ along the x-direction. This leaves g_{2x}, g_{2y}, and $d = d_x$ as the critical parameters.

We have diagonalized eq 1 for $g_{2x} = 2.18$, $g_{2y} = 2.15$, and variable d/J, and the results are plotted in Figure 2. It can be seen that the d/J term shifts g_y and g_z while preserving g_x. For $d/J \approx$

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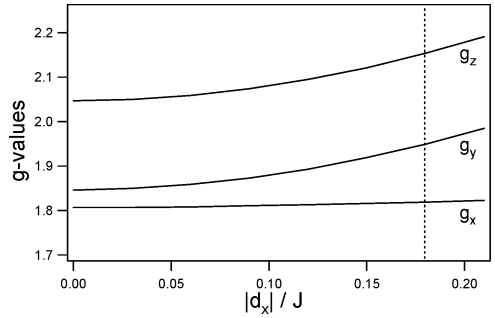

Figure 2. Plot of g-values of the $S = {}^1/_2$ ground state versus $|d_x|/J$ based on eq 1. Fixed parameters were $g_{1x} = g_{1y} = g_{1z} = 2.02$; $g_{2x} = 2.18$, $g_{2y} = 2.15$, $g_{2z} = 2.00$; $D_1 = D_2 = 0$. The dashed line is drawn at $|d_x|/J = 0.18$. When the **d** vector is directed along x, g_y and g_z will shift upward. The value of $|d_x|/J$ is a rough estimate; thus, while ZFS terms alone cause little upshifts of g_z, $\Delta g_z < 0.01$, ZFS terms in conjunction with D−M exchange cause increased upshifts, and thus somewhat smaller d values may be indicated.

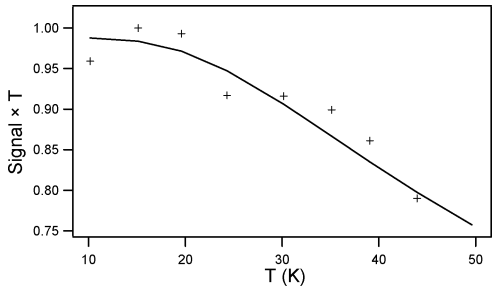

Figure 3. Plot of (Signal × T) of the EPR signal of the Rieske protein at pH 14. Solid line gives the population of the $S = {}^1/_2$ ground state for $J = 43$ cm^{-1}. The data points were obtained by double integration of the spectra. Spectra at each data point were checked for saturation effects. Relaxation broadening commences at about 40 K, most conspicuously for the $g = 1.81$ feature. Above 50 K the spectra have broadened to an extent that integration is not practical.

0.18 the resonance at $g_z = 2.046$ for $d = 0$, moves to $g = 2.14$.[23] The upshifts occur because the D−M term mixes the $M_S = \pm{}^3/_2$ states of the $S = {}^3/_2$ multiplet into the $S = {}^1/_2$ ground state, thereby increasing the magnetic moment. The upward shift is also evident in the value of g_{av} which shifts from 1.91 at pH 7 to 1.97 at pH 14. Because R14 has deprotonated imidazole rings which exert a ligand field different from that of the protonated ligands, the g-values of R14, for $d = 0$, do not have to match those of R7. We illustrate in Figure S1 that the use of realistic ZFS parameters does not affect the major conclusion.

Between 20 and 50 K the intensity of the $S = {}^1/_2$ signal of R14 deviates substantially from Curie behavior, indicating that the $S = {}^3/_2$ multiplet, at energy $\Delta = ({}^3/_2)J$, is populated. A fit to the temperature dependence of the signal (Figure 3) yields $J = 43 \pm 10$ cm^{-1}, suggesting that $|d| \approx 8$ cm^{-1}. A similar J value has been reported for signal II of xanthine oxidase by Caldeira et al.[10] who obtained $J = 40$ cm^{-1} from the temperature dependence of signal II and $J = 30$ cm^{-1} from relaxation studies. The EPR signal of R14 shares with signal II the following properties: (1) one g-value is substantially above $g = 2.0$; (2) the $S = {}^1/_2$ signal deviates from the Curie law above ∼20 K; (3) above ∼40 K the spectra broaden by spin lattice relaxation (shown for signal II to proceed by an Orbach process[10]); (4) the EPR spectra are unusually broad, attributable perhaps to distributions[24,25] of the parameters of eq 1. Properties (2−4) are a consequence of the small J values, while (1) results from a sizable $|d|/J$. The value quoted for $|d|$ should be considered as an upper limit as 0.02−0.08 upshifts of g_z could result from the $({}^7/_3)\mathbf{g_1}$ term. Interestingly, the [2Fe−2S] clusters of the xanthine oxidase family of proteins yielding signal II have Fe−S_{cys}−C_β dihedral angles (Protein Data Bank) that differ substantially from those of the ferredoxins of the $g = 1.94$ family (Figure S2), and it will thus be interesting to see whether the unusual geometries are at the root of the small J-values. It is possible that many $[2Fe-2S]^{1+}$ clusters have d-values as large as in R14 and signal II clusters; however, unless $|d|/J > 0.1$ the effects of D−M exchange would be difficult to distinguish from upshifts caused by the $({}^7/_3)g_{1z}$ contribution to g_z.

Finally, density functional theory calculations[26] suggest that at pH 14 the energy difference between adding an electron to the histine- or cysteine-coordinated site is quite small, raising the possibility of thermally assisted electron hopping between the sites. We are presently studying this question using Mössbauer spectroscopy.

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Supporting Information Available: Effect of ZFS parameters on the EPR signal of R14 and comparison of torsion angles of clusters I and II of xanthine oxidase (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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