Interdoublet transitions in S = 5/2 protein systems

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Abstract

Beginning with known parameters that characterize the EMR spectra of several proteins containing high-spin ferric iron, the information content of the spectra has been examined by simulations that cover a range of magnetic fields and frequencies. Transitions between levels that are not Kramers doublet levels are particularly interesting when the applied frequency is approximately two to three times the value of the zero-field splitting parameter, D. In these cases, transitions at very low magnetic fields correspond to portions of interdoublet transitions that are well separated from all other transitions. The magnetic field is aligned at angles between the molecular principal axes for the portion of the molecules giving rise to the low-field interdoublet transitions. This provides an opportunity for unique angle-selection experiments. © 1997 Elsevier Science B.V.

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1. Introduction

Electron magnetic resonance (EMR) spectra of paramagnetic metal ions in proteins [1] potentially contain information about metal ligation that is much more detailed than can be provided by X-ray structures of these macromolecules. The appeal of the EMR applications to this type of problem can be seen by comparing EMR and X-ray structure information about the non-heme iron site in the iron transport protein, transferrin. Two of the ligands to iron in this protein are provided by oxygen atoms of an anion. High resolution X-ray structures have been completed for ferric transferrin complexed with carbonate (the natural anion) and with oxalate [2,3]. Although only slight differences in the metal-ligand bond angles and bond lengths were found from comparison of the X-

ray structures, EMR spectra of these complexes are vastly different, suggesting that there are indeed differences in geometry and/or charge distribution in the metal-ligand complex. It is interesting to examine the information content of spectra from high-spin iron samples [4–6]. Here the focus is on conditions of magnetic field and frequency in which interdoublet transitions do not overlap significantly with other transitions. At the lowest field maxima in these spectra, the magnetic field direction is not along a molecular principal axis but makes angles of 10–80° with the principal axes. The exact value of the angle corresponding to the low-field edge of the spectrum varies with the applied frequency and the values of the zero-field parameters, D and E. The relations between resonance field and axis orientation, as well as transition probability, are complex. The practical consequences of these combined effects are best illustrated by simulations of EMR spectra.

High-spin ferric iron (S = 5/2) has six magnetic

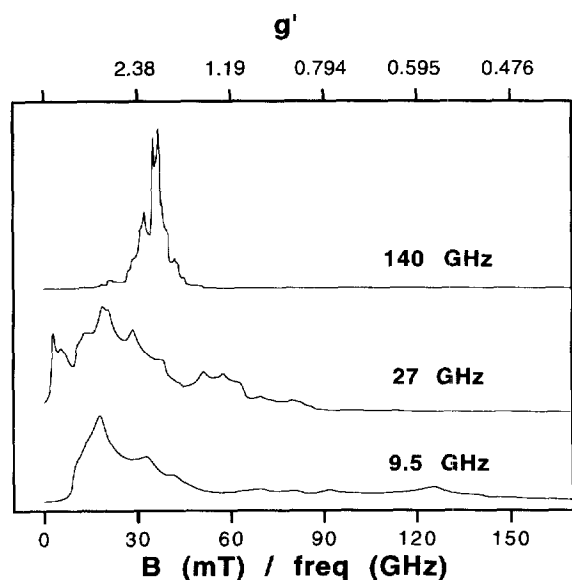* Corresponding author. Tel.: +1 904 6448739;
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Fig. 1. Simulated EMR powder pattern absorption spectra at three different frequencies with the following parameters: $D = 0.5$ cm^{-1} (equivalent to 15 GHz or 0.72 K), $E/D = 0.08$, linewidth 600 MHz, temperature 5 K. At a temperature of 5 K, the Boltzmann populations favor the lower energy levels somewhat. The calculated spectra are the sum of all 15 possible transitions, although some of these do not contribute significantly to the sum. The abscissa is given in units of mT/GHz so that spectra at different frequencies can be compared with a common scale. Effective g-values (g') are given at the top of the figure.

energy levels and 15 possible transitions between these levels. Since the lowest term of the ground configuration $(3d^5)$ for high-spin ferric iron is ^6S, with $L = 0$, the EMR observable fine structure term is quite simple. A program for simulation of EMR spectra of S = 5/2 samples has been published [1] and is based on the following spin Hamiltonian.

$$H_S = g\beta\boldsymbol{B} \cdot \boldsymbol{S} + D(S_z^2 - 1/3S^2) + E(S_x^2 - S_y^2)$$

The coordinate system we use in these calculations takes $|D_z| \geq |D_y| \geq |D_x|$. Naturally occurring samples containing this form of iron have values of the zero-field splitting parameter, D, that range from about 0.1 to 10 cm^{-1} [1,7]. Fig. 1 gives the overall effect on electron magnetic resonance (EMR) spectra of varying the ratio of the applied frequency to the value of D. Although $D = 0.5$ cm^{-1} was chosen for this illustration, the same effects would be found in cases with other values of D, but with the field and frequency scaled by D. The value of E (0.04 cm^{-1}) was chosen

so that the simulations represented spectra that might be expected of some transferrin complexes [5,6]. Because the Zeeman term is relatively small compared to the zero-field splitting term, the calculations shown in Fig. 1 employ an isotropic g-value. The g-value of Mn^{+2} ion, another S = 5/2 case, was found to be close to isotropic in recent studies at 140 GHz of some protein systems [8]. In experimental X-band studies of non-heme iron proteins, the observed lineshape is dominated by distributions in zero-field splitting terms, rather than the underlying linewidths [4,9]. In the calculations leading to the spectra in Fig. 1, distributions in zero-field splitting terms were not introduced and linewidths were similar to those used earlier in simulations of X-band spectra [5]. The overall effect illustrated in Fig. 1 is that as the applied frequency is increased relative to D, the Zeeman term plays an increasing part and the spectra shift toward $g' = 2$. The breakdown of transitions that contribute significantly to the calculated 27 GHz EMR spectrum in Fig. 1 is given in Fig. 2.

The feature at the lowest field (50–100 mT for a frequency of 27 GHz) in the spectrum shown in Fig. 2 arises from a transition between levels 2 and 3 (numbering from the lowest level of the sextet). A feature of this type well separated from all other transitions is seen in EMR spectra of ferric iron when the value of the applied frequency is near 2D (axial symmetry) to
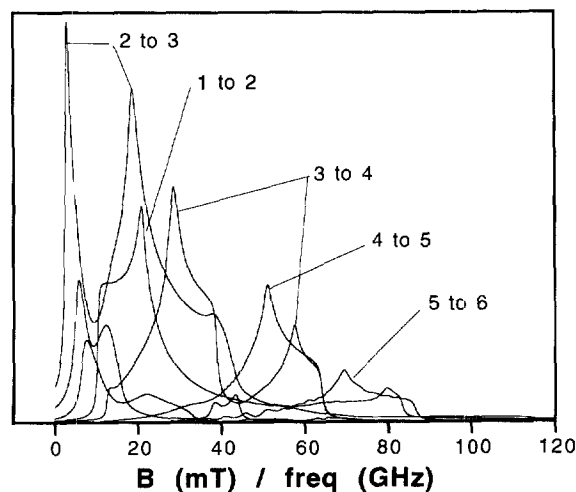

Fig. 2. Breakdown of calculated 27 GHz spectrum from Fig. 1. Transitions with significant contributions are indicated by labels. Two minor transitions (between levels 1 and 3, and 2 and 4) are shown but are not labeled.
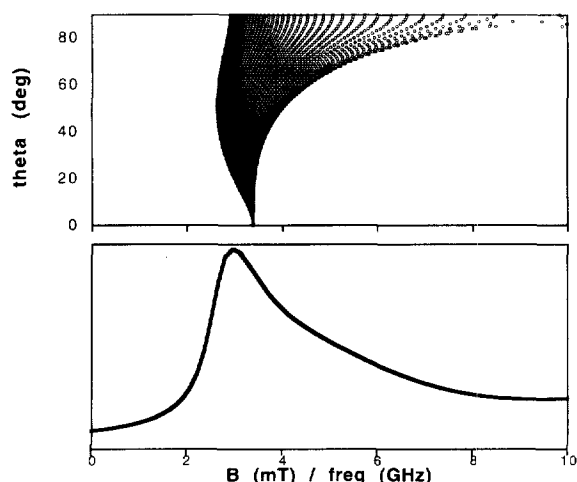
Fig. 3. The angular dependence of the low field feature of the 2–3 transition. The lower plot shows the calculated EMR absorption spectrum of the 2–3 transition and the upper plot shows the angular dependence of this absorption. The simulation parameters are the same as in Fig. 1.

3D (rhombic symmetry) and it results from a transition between levels that are not Kramers doublet levels (interdoublet transition). At higher and lower frequencies this transition is, of course, also present but it may overlap with many other transitions. In cases where a region of an interdoublet transition is separated from other transitions, as in the example of Fig. 2, this region of the spectrum might be used for angle selection in pulsed EMR experiments. Fig. 3 illustrates the angular dependence of the resonance field for this particular interdoublet transition. The lowest field edge is contributed by molecules in which the D_z axis of the zero-field tensor makes an angle, θ, of 52° with the direction of the magnetic field. Calculations of interdoublet transitions for other values of E/D and D/ν show that the low field

edges of isolated interdoublet transitions will result from angles between D_z and the magnetic field direction of 20–80°. Since the edges of the spectrum from a transition within a Kramers doublet, the 1 to 2 transition for example corresponds to $\theta = 0$ or 90°, the interdoublet transitions present unique opportunities for 'off-axis' angle selection magnetic resonance experiments [10]. For instance, calculations of W-band (95 GHz) spectra with $D = 1$ cm^{-1} and $E = 0.065$ cm^{-1} also show well separated interdoublet transitions between levels 1 and 4, 2 and 4, and 3 and 5, at low field. In that case, the 1 to 4 transition is at the lowest field and has θ approximately equal to 60° at the lowest field edge.

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