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EPR DETERMINATION OF THE Co(II)-FREE RADICAL MAGNETIC GEOMETRY OF THE “DOUBLET” SPECIES ARISING IN A COENZYME B-12-ENZYME REACTION

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Summary

The physical significance of the observed structure of the EPR signal, commonly known as the “doublet” spectrum, is that it contains information not only about the exchange coupling but also about the geometry of the magnetic dipole-dipole spin-spin coupling. We can show this because we have developed a general method of analysis applicable to this type of system and because we demand a quantitative fit of theory to experiment at two microwave frequencies. We have chosen the “doublet” free radical signal, which arises in the ribonucleotide reductase-5'-deoxyadenosylcobalamin system (from *Lactobacillus leichmannii*, see Hamilton et al., *Biochemistry* 11, 4696–4705 (1972)), for study, for the particular reason that the 35 GHz “doublet” spectrum has three components (in this case) rather than two, which provides an important test of the recently proposed model of isotropic exchange coupling by Schepler et al. ((1975) *Biochim. Biophys. Acta* 397, 510–518). We find that a quantitative fit to the EPR “doublet” lineshape can be obtained with a model of isotropic exchange, and a “point” magnetic dipole-dipole interaction acting over a distance of 9.9 Å with the radical located approx. 34° off the principal g_{zz} axis and <1° off the principal g_{xx} axis of the Co(II) in the corrin ring. Quantitative fits of the doublet portion of the observed lineshape at both 9 and 35 GHz were achieved with this model, assuming an axially symmetric free radical signal and a Gaussian spin-packet lineshape with isotropic linewidth.

Introduction

The “doublet” EPR spectrum of an intermediate in several enzyme systems requiring B-12 coenzyme is believed to arise from an interacting substrate radical and a low-spin Co(II). The evidence supporting this model has recently been summarized by Schepler et al. [1]. The splitting is believed to be the

result of a spin-spin interaction. The magnitude of the splitting, its independence of microwave frequency, and isotopic substitution studies show that the interaction is a result of an electron spin-electron spin coupling as opposed to an electron spin-nuclear spin coupling.

Schepler et al. [1] have proposed that the coupling between the unpaired spins of the Co(II) and the radical is the result of a weak isotropic exchange interaction, and they have demonstrated that this model can produce a qualitative fit of the experimentally observed EPR spectra using perturbation theory-dependent lineshape simulations. The magnetic dipole-dipole coupling is proposed to be absent due to the "magic angle" effect.

Now, the relative amount of exchange coupling (either direct or superexchange), compared to the magnetic dipole-dipole coupling, is of importance with respect to the structure of the molecular complex. This importance is not restricted to this system alone. The relative amount of mixing of each spin-spin coupling mechanism involves such fundamental quantities as the distance between the unpaired spins, their relative angular orientation, and the amount of overlap of occupied molecular orbitals. Thus, an accurate assessment of the amount of each mechanism will better characterize the nature of the two interacting electron spin distributions, as well as the range of the spin-spin interaction.

By analysis of the observed EPR lineshape at two frequencies we are able to extract the magnetic dipole-dipole information present in the spectra and thus determine the geometrical arrangement of the two coupled spin systems.

Theory

The spin-Hamiltonian for two interacting $S = 1/2$ spin systems may be written as

$$\mathcal{H} = \mathcal{H}_1 + \mathcal{H}_2 + \mathcal{H}_{12} \quad (1)$$

where \mathcal{H}_1 and \mathcal{H}_2 contain the Zeeman and hyperfine terms for components 1 and 2, respectively, and \mathcal{H}_{12} contains the exchange (EX) and magnetic dipole-dipole (MDD) interactions. We have investigated a special case of Eqn. 1:

$$\mathcal{H} = \beta \vec{B}^t \cdot g_1 \cdot \vec{S}_1 + \beta \vec{B}^t \cdot g_2 \cdot \vec{S}_2 + \mathcal{H}_{\text{EX}} + \mathcal{H}_{\text{MDD}} \quad (2)$$

where the first two terms are the interactions of the electronic magnetic moments with the external magnetic field, the third term is the general exchange interaction, and \mathcal{H}_{MDD} is the through-space magnetic dipole-dipole interaction. (We use conventional matrix notation: \vec{S} is a column vector with components S_x, S_y, S_z ; g is a 3×3 matrix, assumed symmetric; \vec{B}^t is a row vector with magnetic field elements B_x, B_y, B_z ; the superscript t represents the transpose operation which converts a column vector into a row vector or the matrix $\{T_{ij}\}$ into $\{T_{ji}\}$); β is the Bohr magneton. The coordinate system for spin 1 is chosen such that g_1 is diagonal. The matrix of the g tensor for spin 2, g_2 , is then generally non-diagonal, but can be made diagonal by a rotation through three successive Eulerian angle rotations (α', β', γ') to a coordinate system in which g'_2 is diagonal. Then $g_2 = T \cdot g'_2 \cdot T^t$, where T is the total rotation matrix for all three successive rotations. As this set of rotations is an orthogonal

transformation the transpose of T , T^t , is equal to T^{-1} . The magnetic dipole-dipole interaction,

$$\mathcal{H}_{\text{MDD}} = \frac{1}{r^3} [\vec{\mu}_1^t \cdot \vec{\mu}_2 - 3(\vec{\mu}_1^t \cdot \hat{e}_r)(\vec{\mu}_2^t \cdot \hat{e}_r)], \quad (3)$$

in addition to the six components of the diagonal g tensors for spins 1 and 2, then depends on these six quantities: r , the radial distance between the spins; θ, ϕ the spherical angles giving the direction of the unit vector \hat{e}_r from spin 1 to spin 2; and α', β', γ' describing the orientation of the diagonal g'_2 tensor with respect to the reference frame of spin 1. The explicit dependence on the Eulerian angles (usually ignored) is evident on substitution for $\vec{\mu}_1$ and $\vec{\mu}_2$ in terms of g_1 and g'_2 : $\vec{\mu}_1 = -\beta g_1 \cdot \vec{S}_1$ and $\vec{\mu}_2 = -\beta T \cdot g'_2 \cdot T^{-1} \cdot \vec{S}_2$.

The most general form for the exchange interaction between two spins, S_1 and S_2 , which is bilinear in the spin operators is

$$\mathcal{H}_{\text{EX}} = \sum_{i,j} S_{1i} J_{ij} S_{2j} \quad (4)$$

This is often written in the form [2,3]

$$\mathcal{H}_{\text{EX}} = J \vec{S}_1^t \cdot \vec{S}_2 + \vec{D}^t \cdot (\vec{S}_1 \times \vec{S}_2) + \vec{S}_1^t \cdot \Gamma \cdot \vec{S}_2, \quad (5)$$

where J is the isotropic exchange, D is the Dzyaloshinski (antisymmetric) exchange, and Γ the symmetric anisotropic exchange. If spin-orbit effects are small when compared to the crystal field splitting, as is typically the case with the first row transition elements, then the following approximations (due to Moriya [4]) hold:

$$D_i \approx \left(\frac{\Delta g}{g}\right) J, \quad \Gamma_{ij} \approx \left(\frac{\Delta g}{g}\right)^2 J \quad (6)$$

where $\Delta g = |g - 2|$. Operators arising from biquadratic spin-exchange effects, such as exchange striction, have been assumed to be negligible.

The total interaction may now be written, with all spin operators expressed in the same reference frame, as:

$$\begin{aligned} \mathcal{H} = & \beta \vec{B}^t \cdot g_1 \cdot \vec{S}_1 + \beta \vec{B}^t \cdot (T \cdot g'_2 \cdot T^t) \cdot \vec{S}_2 + \vec{S}_1^t \cdot J \cdot \vec{S}_2 \\ & + \frac{\beta^2}{r^3} (g_1 \cdot \vec{S}_1)^t \cdot (T \cdot g'_2 \cdot T^t) \cdot \vec{S}_2 - 3[(g_1 \cdot \vec{S}_1)^t \cdot \hat{e}_r] \\ & \cdot [(T \cdot g'_2 \cdot T^t \cdot \vec{S}_2) \cdot \hat{e}_r] \end{aligned} \quad (7)$$

A general accounting of the total number of degrees of freedom for Eqn. 7 is in order. There are six g tensor components, three Eulerian angles, three spherical coordinates, r, θ, ϕ and nine components of the general exchange tensor. Thus there may be as many as 21 independent parameters required to specify completely the sum of the two operators $\mathcal{H}_{\text{EX}} + \mathcal{H}_{\text{MDD}}$.

The observed EPR lineshape for a molecular system consisting of randomly oriented molecules arises as a result of the summation over all orientations, Ω , of the contribution to the absorption function due to each transition, and may be written [5] as:

$$S(\omega, B) = \int d\Omega \sum_{i < j} P_{ij}(B, \Omega) G[\omega - \omega_{ij}(B, \Omega)]. \quad (8)$$

Here, $P_{ij}(B, \Omega)$ is the probability of the $|i\rangle$ to $|j\rangle$ transition occurring, and $G[\omega - \omega_{ij}(B, \Omega)]$ is the frequency-dependent shape function for this same transition where $\omega_{ij}(B, \Omega)$ is the field and angular-dependent frequency of the transition. In this calculation, the transition frequencies and relative intensities are found by diagonalization of the complex Hamiltonian matrix. The spin-packet lineshape is taken to be Gaussian and the effects of inhomogeneous broadening are ignored. While population effects may easily be included, this is nevertheless a rigid lattice approximation.

The particular advantage of this method is that we can accurately investigate the magnetic dipole-dipole exchange nature of the "doublet" portion of the spectrum (which exhibits no hyperfine structure) without being limited by the non-convergence properties of a perturbation theory approach. Thus, we may vary the magnitudes of both types of bilinear spin operators in Eqn. 2 from less than, to equal to, or greater than the Zeeman field-dependent operators, and obtain converged lineshapes at any field value. We have also shown convergence with respect to the angular integrations for 1, 2, or 4 octants of the unit sphere.

It is important to establish just what we may learn (or not learn) from a lineshape simulation using the operator of Eqn. 2. Now both the magnetic dipole-dipole and exchange interactions of Eqn. 2 may be expanded in the same set of nine bilinear spin operators: $S_{1x}S_{2x}$, $S_{1x}S_{2y}$, . . . $S_{1z}S_{2z}$. Thus the general operator when both dipole-dipole and exchange terms are present and significant is:

$$\mathcal{H}_{\text{MDD}} + \mathcal{H}_{\text{EX}} = C(1)S_{1x}S_{2x} + C(2)S_{1x}S_{2y} + \dots + C(5)S_{1y}S_{2y} + \dots + C(9)S_{1z}S_{2z} \quad (9)$$

where the coefficients $C(i)$ must, in general, be decomposed into a magnetic dipole-dipole and an exchange part: $C(i) = C_{\text{MDD}}(i) + C_{\text{EX}}(i)$, for example. Experiment clearly determines only the $C(i)$ coefficients, so the decomposition into dipole-dipole and exchange parts depends on other information (other than resonance lineshape), or is arbitrary. An experimental approach to this decomposition could be based on the basically different radial and/or temperature dependence of the dipole-dipole and exchange effects, while a theoretical approach must depend on the differences of the dipole-dipole and exchange tensor coefficients, predicted by a known structure and an approximate ground state wave-function. A special case in which one or the other interaction or some parts thereof may be assumed to be of negligible magnitude may be solved by lineshape analysis techniques.

Results

We chose to examine the 35 GHz EPR spectrum of the doublet signal obtained from the vitamin B-12 coenzyme-enzyme system of *Lactobacillus leichmanii* studied previously [6] for the reason that this spectrum exhibits not two components of the "doublet", but rather three. This additional feature may be useful in establishing the validity of any particular magnetic dipole-dipole exchange model. It is not a property that results directly from an isotropic exchange interaction.

We have investigated the behavior of both the absorption and absorption derivative lineshapes calculated according to Eqns. 7 and 8 as the parameter values were changed. We find that the splittings and ratios of the heights of the derivative components of the doublet at 35 GHz depend in an interesting way on the anisotropies of the spin-Hamiltonian parameters. The ratio of the heights of the two components of the doublet at 35 GHz is controlled principally by the anisotropies in the spin-spin coupling parameters. Both reasonable and unreasonable changes were made in the values of the cobalt g tensor, but only minor changes were produced in the radical doublet intensity ratios at 35 GHz. With an isotropic exchange model, the height of the high field component doublet was always too large when g_{xx} and g_{yy} of the cobalt were in the range of 2.30–2.20. But, as expected, these same changes produced large variations in the calculated intensity ratios of the doublet at 9 GHz. However, large changes in both the 9 and 35 GHz doublet intensity ratios could be produced by allowing the spin-spin interaction to be anisotropic. In fact, the intensity ratio of the radical doublet can even be inverted by an anisotropy in the interaction such that $|C(9)| < |C(5)| < |C(1)|$. The splitting of the smaller component of the doublet to give the additional shoulder observed at high field in the 35 GHz signal is controlled by the anisotropy of the free radical g tensor. An axial free radical also accounts for the difference observed in the linewidth within the "doublet" in the 9 GHz experiment.

The most interesting discovery was that the spin-spin interaction must be anisotropic. Now, the four possibilities which may introduce anisotropy into the spin-spin coupling are: (i) The spin-spin coupling consists of a magnetic dipole-dipole interaction only, and no exchange is present. (ii) The spin-spin coupling is principally the result of an anisotropic exchange with essentially no magnetic dipole-dipole interaction present. (iii) The anisotropy arises from both an anisotropic exchange and a magnetic dipole-dipole interaction, both being equally important. (iv) The observed anisotropy arises principally from a magnetic dipole-dipole coupling with an isotropic exchange interaction also present.

The first possibility was ruled out because the shape of the doublet (as also pointed out by Schepler et al. [1]), especially at 9 GHz, was not typical of the asymmetric lineshapes produced by a magnetic dipole-dipole coupling of two spin systems. The nearly symmetric shapes observed suggest the presence of an isotropic exchange coupling as proposed by Schepler et al. [1]. To test the other models, the 35 GHz spectrum was subjected to a least squares procedure [7]. To verify the parameter values determined by the least squares procedure, a comparison of the 9 GHz calculated and experimental spectra was also made. In the final stages of the fitting process, the standard "eyeball" test was no longer able to detect significant improvements in the fit. So, as a measure of quality of fit, a convenient form of the R factor (see ref. 7) was used. (The smaller the R factor, the better the fit.) No significant improvements for the various parameter values were found when the R factor was reduced to approx. 6–7%. Although there is not enough information in the 35 GHz spectrum to uniquely determine all parameter values, we were able to gain information that helped to distinguish which is the correct model by requiring a simultaneous fit at 9 GHz. A unique solution for a system consisting of overlapping spectra, due

to two interacting spin systems, can only be achieved when a simultaneous fit of two spectra at distinctly different frequencies is obtained [7].

To test the anisotropic exchange model, we assumed a diagonal interaction which is a physically reasonable assumption, especially in light of Eqn. 6. We were able to obtain a good fit (R factor $\approx 8\%$, see Fig. 1) at 35 GHz with $g_{xx} \approx g_{yy} \approx 2.30$ for the cobalt, and the calculated 9 GHz spectrum resulted in an intensity ratio of approx. 3 : 1, a little larger than the 2.5 : 1 ratio observed. However, the depth of the high field component at 35 GHz was not as experiment showed. This feature was improved (R factor $\approx 6\%$) by allowing the g_{xx} and g_{yy} of the cobalt to vary in the least squares procedure. The improved 35 GHz fit resulted in $g_{xx} = 2.28$, $g_{yy} = 2.16$. But with these g values, the intensity ratio of the doublet at 9 GHz went to approx. 4.3 : 1, as opposed to the approx. 2.5 : 1 ratio observed experimentally (see Fig. 2). Using Eqn. 6 to make an estimate, one would at most predict an anisotropy of only 5% for this system. However, the anisotropy required by a pure exchange model is of the order of 30–35%. Thus a pure exchange model fails on two counts: (1) line-shape simulation, and (2) theoretical estimate of the exchange anisotropy.

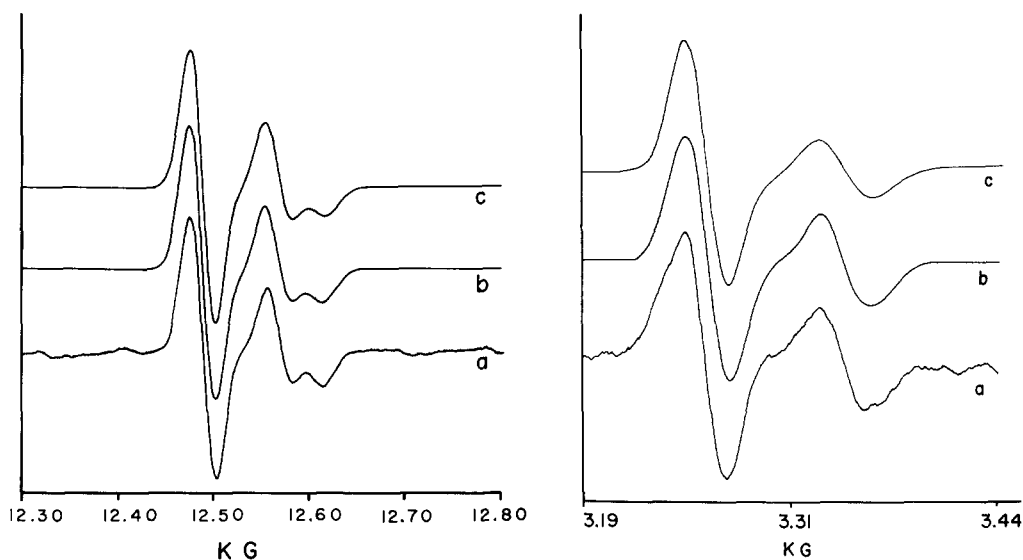


Fig. 1. (a) Experimental 35 GHz EPR spectrum of the ribonucleotide reductase system. The reaction mixture was as given in ref. 6. Microwave frequency, 35.166 GHz; microwave power, 0.5 mW; modulation amplitude, 16 G; time constant, 0.3 s; temperature, approx. 145°K. (b) Computed best fit with parameter values as given in Table I. This shape is essentially identical to that calculated using the parameters that produced shape C of Fig. 2. (c) A computed fit using a diagonal exchange model. The cobalt g values are, $g_{xx} = g_{yy} = 2.30$, $g_{zz} = 1.98$; the radical g values are as in Table I; the exchange parameters are $J_{xx} = -0.00565$, $J_{yy} = -0.00904$, $J_{zz} = -0.01243$ (cm^{-1}). The dipolar interaction has been turned off by using $r = 1000$ Å.

Fig. 2. (a) Experimental 9 GHz EPR spectrum of the ribonucleotide reductase system. Microwave frequency, 9.258 GHz; microwave power, 10 mW; modulation amplitude, 2 G; time constant, 3 s; scan rate, 25 G/min; temperature, 150°K. (b) Computed best fit with parameters values as given in Table I. (c) Computed fit using a diagonal exchange model. The cobalt g values are $g_{xx} = 2.28$, $g_{yy} = 2.16$, $g_{zz} = 1.98$; the radical g values are as in Table I; the exchange parameters are $J_{xx} = -0.00562$, $J_{yy} = -0.00917$, $J_{zz} = -0.01162$ (cm^{-1}). The dipolar interaction has been turned off by using $r = 1000$ Å.

even model (iii), in which both sources of the anisotropy are equally important, fails on these same grounds.

The isotropic exchange and magnetic dipole-dipole model (iv; above) is able to fit the 35 GHz doublet spectrum exactly, including the depth of the high field component (see Fig. 1), and simultaneously reproduce the approx. 2.5 : 1 intensity ratio observed at 9 GHz (see Fig. 2). The parameter values which produced the match are summarized in Table I. Although there is a large uncertainty in the g values for the cobalt, we were able to determine $g_{xx} \approx 2.30$ and $g_{yy} \approx 2.23$ by requiring a simultaneous fit at two frequencies. These values are in agreement with those determined by Pilbrow and Winfield [8] for the cobalt in vitamin B-12 (Cob(II)alamin) in the enzyme ribonucleotide reductase.

The final match was achieved by employing the following special case of the spin-Hamiltonian of Eqn. 2:

$$\mathcal{H} = \beta \vec{B}^t \cdot g_1 \cdot \vec{S}_1 + \beta \vec{B}^t \cdot g_2 \cdot \vec{S}_2 + J \vec{S}_1^t \cdot \vec{S}_2 + \frac{\beta^2}{r^3} [\vec{\mu}_1^t \cdot \vec{\mu}_2 - 3(\vec{\mu}_1^t \cdot \hat{e}_r)(\vec{\mu}_2^t \cdot \hat{e}_r)]. \quad (10)$$

Even though the uncertainties in the g values of the Co(II) are large, (within the limits of these uncertainties), no chemically significant change in the position of the radical with respect to the cobalt g tensor is introduced. After the best dipolar parameters were determined, a least squares run was made to determine how large an anisotropy could arise from the exchange interaction. A 0.75% improvement in the R factor was realized, but the anisotropies introduced in the exchange interaction were only between 1 and 3%, well within the estimates of Eqn. 6.

Of the 21 parameters which are present in the general form of Eqn. 2, only nine were required for this particular problem. The three Eulerian rotations were not needed, as rotations of the nearly isotropic radical produced changes that occurred under the linewidth, and therefore were not observable. Of the nine possible exchange parameters only one was necessary for this problem. One g value was eliminated because of the axial nature of the radical. The shape calculation required a linewidth parameter and the least squares procedure used

TABLE I

The errors estimates are based upon the range of values of the parameters for which the fit is acceptable.

Parameter	
Co(II) g values	$g_{xx} = 2.30 \pm 0.04$ $g_{yy} = 2.23 \pm 0.04$ $g_{zz} = 1.998 \pm 0.01$
Radical g values	$g_{xx} = g_{yy} = 2.00613 \pm 0.0003$ $g_{zz} = 2.00108 \pm 0.0003$
Exchange *	$J = -75.6 \pm 0.5 \cdot 10^{-4} \text{ cm}^{-1}$
Dipolar parameters	$r = 9.92 \pm 0.4 \text{ \AA}$ $\theta = 33.7^\circ \pm 2^\circ$ $\phi = 0.3^\circ \pm 2^\circ$
Linewidth (derivative peak to peak)	$\Delta H_{pp} = 25.5 \text{ G}$

* “—” sign used in the calculations, but otherwise (experimentally) unknown.

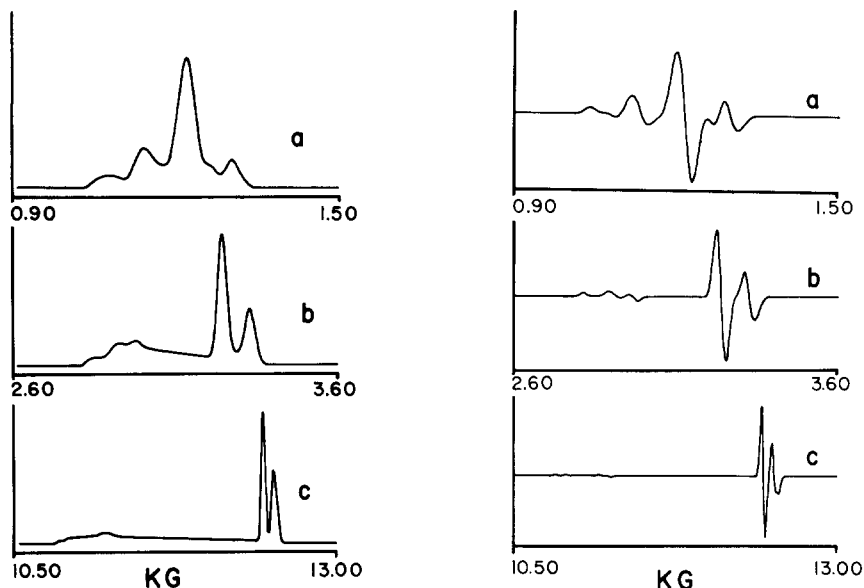


Fig. 3. Calculated absorption lineshapes using the parameters of Table I for (a) 3.50 GHz; (b) 9.20 GHz; (c) 35.00 GHz.

Fig. 4. Calculated absorption derivative lineshapes using the parameters of Table I for (a) 3.50 GHz; (b) 9.20 GHz; (c) 35.00 GHz.

an adjustable factor to scale the calculated shape to the observed shape. Thus, only 11 parameters needed to be varied in the least squares procedure. 47 data points were used, so the problem was overdetermined by a factor of 4^{\dagger} .

We have used the parameters derived here to investigate the behavior of the spectrum versus microwave frequency. Calculated absorption and absorption derivative lineshapes at 3.50, 9.20, and 35.00 GHz are presented in Figs. 3 and 4. When it is realized that the actual relative height in the derivative spectrum of the cobalt, as compared to the free radical, will be about 10% of what is shown because of hyperfine splitting, it is clear that the lower frequency would be best for studying the composite spectrum of Co(II) plus radical. Although at higher frequencies the anisotropy of the radical is enhanced, information regarding the nature of the Co(II) is lost.

Conclusions

The exact calculation, omitting hyperfine splitting, of the doublet free radical component, reveals interesting differences from the perturbation theory calculation, especially in regard to the calculated absorption intensities. The source of this difference is that we compute the spectrum directly from the spin-Hamiltonian. In this way, the typical errors of the usual perturbation method, i.e. convolution of a stick-diagram and the failure of the perturbation theory expansion to be convergent after a finite number of terms of the infinite series, are avoided. As both the position and the intensity of the doublet must be accurate, these errors are compounded in a perturbation theory calculation.

Thus, the simple model developed by Schepler et al. [1] is relatively easy to solve but is difficult to generalize, since the perturbation theory equations then become algebraically complicated, particularly when hyperfine splitting is included [9]. By contrast, the numerically exact model developed here is comparatively simple to program, has high flexibility, and uses only moderate amounts of computer time. The principal limits to accuracy appear to be the limitations of the model itself, such as its neglect of inhomogeneous broadening and unresolved hyperfine splitting. Schepler's calculation included the cobalt hyperfine splitting which we have here neglected. This approximation is justified by the observation that the unpaired spin of the cobalt(II) makes a negligible contribution to the doublet part of the spectrum (especially at the higher frequencies; see Fig. 3), and we could demonstrate that the doublet line-shape dependence on the cobalt g_{\parallel} was indeed very weak.

Our investigations on this system reveal that the dipolar information needed to locate the radical is present in the observed EPR spectra. The information lies in the observed intensity ratios of the doublet. But in order to extract that information, it is necessary to simultaneously fit the experimental spectral at two frequencies, such as 9 and 35 GHz. Using an isotropic exchange and a point magnetic dipole-dipole model, this has been done for the doublet free radical signal, which arises in the ribonucleotide reductase-5'-deoxyadenosyl-cobalamin system of *L. leichmanii*. As shown in Fig. 5, the radical has been located at 9.9 Å from the Co(II), at a position approx. 34° off the principal g_{zz} axis, and $<1^{\circ}$ off the principal g_{xx} axis of the Co(II). Although we have no information as to how the principal axis system of the Co(II) g tensor is related to a molecular axis system, it seems quite reasonable to assume that the g_{xx} and g_{yy} axes of the Co(II) lie somewhere in or near the plane of the corrin ring, with the principal g_{zz} axis of the Co(II) then being perpendicular or nearly so to the ring. This is in agreement with the findings of Pilbrow and Winfield [8] for the vitamin B-12r system. Thus, the model, as shown in Fig. 5, appears to be quite reasonable for this system.

Our dipolar parameters have assumed point dipoles for the two spin systems. But in the real system there will be a spatial distribution of spin density as well

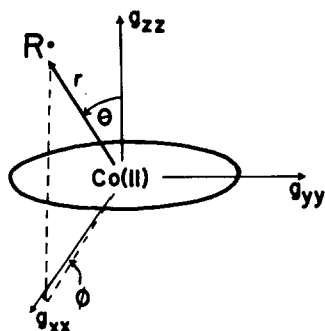


Fig. 5. Schematic diagram of the Co(II) ion and the corrin ring showing the location of the radical with respect to the principal axis system of the cobalt g tensor. The positioning of this principal axis system was chosen to be consistent with the findings of Pilbrow and Winfield [8].

as some covalent transfer of the spin-magnetic moments to neighboring groups. Although we cannot yet speculate as to the nature of the radical, such covalent effects will most certainly occur in the cobalt-corrin spin system. Smith and Owen [10] and Harris [11] have investigated the changes in the dipolar anisotropy energy that results from these effects in solids. They have observed both increases and decreases in this energy ranging from a 10% change for Mn^{2+} in MgO , to a 34% change for Mn^{2+} in NiO . More drastic effects of covalency than are likely to be applicable to this system have been reported by Shields [12] for tetracyanoquinodimethane (TCNQ) sandwich complexes. It is not possible to determine whether an increase or decrease should occur in the observed dipolar anisotropy energy in the doublet system, but a change by as much as 30% in the dipolar anisotropy energy would result in only an approximate 1 Å change in the effective r . Thus, even though the magnetic dipole-dipole model is not exact, it still yields considerable chemical information. Our investigations of the "magic-angle effect" nature of the magnetic dipole-dipole interaction for the case of general g tensors of both spins shows clearly that the "magic-angle" result of $54^\circ 53'$ is peculiar to the case of isotropic g values for both g_1 and g_2 . We find that the angles for which there is a minimum in the splitting observed for the radical need not be the same set of angles for which the splitting in the cobalt region of the spectrum is minimal. Even in the cases where the dipolar splittings are minimal, asymmetries are introduced into the shapes from the dipolar anisotropies present in the energy matrix; thus the "magic-angle effect" may not even be present in a general case.

A study of the properties of systems with long-range exchange coupling shows that when $r \geq 5$ Å, the mechanism of exchange coupling can only be superexchange (which may be either positive or negative), or some higher order long-range coupling. It can not be direct exchange, which is always ferromagnetic [13] as it arises from the $1/r_{ij}$ electron repulsion matrix elements. In known cases where long-range coupling such as superexchange has been identified and the structures have been determined, the pathway for the exchange is through a π -electron network of delocalized electrons.

We have seen that similar shapes can be obtained for various combinations of dipole-dipole and exchange interactions but the best fit was obtained with a model consisting of isotropic exchange and a magnetic dipole-dipole interaction. Another approach to unraveling the exchange and dipole-dipole interactions in this system may be to use the difference in dependence of the two mechanisms on distance. An exchange interaction has an exponential dependence on distance, whereas the radial dependence of the dipolar coupling varies as $1/r^3$. Thus, some other experiment must be done to take advantage of these different dependencies. Another approach may lie in the unusual temperature dependence of the coupling as observed by Hamilton et al. [6]. This behavior is much like that observed by Kurzynski [14] for exchange-coupled Jahn-Teller systems. The results of a detailed study of the effects of temperature on the spin-spin coupling of this system will be forthcoming.

In summary, we have confirmed the presence of a low spin Co(II) and an organic free radical in this system. Our analysis shows that there is not only an exchange interaction but also a magnetic dipole-dipole interaction present in the "doublet" EPR lineshape. As our method of analysis is general, it may be

applied to other systems in which both a magnetic dipole-dipole and exchange interaction may be present, such as the 8Fe-8S* ferredoxins [15]. There is, however, a definite limitation to this type of analysis which must be observed in all treatments of systems exhibiting spin-spin coupling effects: if the exchange tensor is not isotropic (as assumed here), then the anisotropic exchange operator is pseudo-dipolar in nature, and cannot be uniquely separated from the magnetic dipolar operator unless independent knowledge on the nature of one of the coupling mechanisms is available.

The biochemical significance of our result, in terms of this system, is that the Co(II) in the "doublet" spectrum appears to be quite similar to that of enzyme-bound Cob(II)alamin. As seen in Figs. 3 and 4, an experiment at S-band may provide the data necessary for a more meaningful comparison of the environments of the Co(II) in each of these systems. Since our analysis provides geometric information on the relative location of the free radical, it may be informative to repeat the analysis (including temperature dependence) on other systems exhibiting "doublet" spectra. The relationship of the organic radical present in this system to the mechanism of enzymatic catalysis is yet another question which is beyond the scope of this work.

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