Journal of Magnetic Resonance **152,** 103–108 (2001)
doi:10.1006/jmre.2001.2378, available online at http://www.idealibrary.com on IDEAL®

Cross Correlation between the Dipole–Dipole Interaction and the Curie Spin Relaxation: The Effect of Anisotropic Magnetic Susceptibility

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Received January 16, 2001; revised May 11, 2001; published online August 6, 2001

Cross-correlated relaxation caused by the interference of nuclear dipole–dipole interaction and the Curie spin relaxation (DD–CSR cross relaxation) is generalized to treat the case of anisotropic magnetic susceptibility, including the important case where the latter originates from zero-field splitting. It is shown that the phenomenon of DD–CSR cross relaxation is absolutely general and to be expected under any electronic configuration. The results of the generalization are presented for a model system, and the consequences for paramagnetic metalloproteins are illustrated with an example of cerium(III)-substituted calbindin. The effects of the magnetic anisotropy are found to be substantial. © 2001 Academic Press

Key Words: **cross correlation; magnetic anisotropy; zero-field splitting; Curie relaxation; dipolar interaction.**

1. INTRODUCTION

The cross-correlation or interference effects between various interactions involving nuclear spins contribute to nuclear spin relaxation phenomena in an intriguing way, as was recognized many years ago (*1–3*). From being a spectroscopic curiosity, these phenomena have during the past decade evolved into important tools for structural studies of biological macromolecules (*4–13*). One category of interference effects is that between the dipole–dipole, DD, interaction and the chemical shielding anisotropy, CSA, (*2*) which can lead to transfer between the Zeeman order and the two-spin order (*14, 15*), to differential line broadening of spin–spin-coupled doublet components (*16, 17*) and to relaxation-allowed coherence transfer (*18*). A very clever application of this DD–CSA interference phenomenon is realized in transverse relaxation-optimized spectroscopy (TROSY) (*19*). Another interesting cross-correlation effect is that involving a pair of dipolar interactions in a three-spin *AKS* system (*1*). A special case arises if the *S* spin is that of an unpaired electron (or electrons), with an efficient relaxation mechanism and a large Zeeman splitting. The combined effect of these two properties is the occurrence of a net, thermal equilibrium magnetic moment oriented along the magnetic field. This net magnetic moment (the Curie spin) can interact with nuclear spins and, as a result of the reorientation of the spin-carrying molecule with respect to the magnetic field, can provide an extra relaxation mechanism for each of the spins *A* and *K*. This relaxation mechanism has been proposed by Gueron (*20*), who called it Curie spin relaxation, and by Vega and Fiat (*21*), who denoted it as a susceptibility mechanism. More recently, it has been shown that the Curie spin relaxation (CSR) can cross-correlate with the dipolar *AK* interaction and that this DD–CSR interference can lead to exactly the same experimental signatures as the DD–CSA interference (*22–27*).

During the past few years, the DD–CSR interference effects have begun finding applications as possible structural constraints in studies of paramagnetic proteins, along with the usual NOE constraints (*28, 29*), paramagnetic pseudocontact shifts (*30*), and residual dipolar couplings induced by the anisotropy of magnetic susceptibility (*31–34*). In most of the earlier work on the DD–CSR cross correlation, it has been assumed that the system is isotropic in two ways: the overall reorientation of the (macro)molecule is isotropic (dynamic isotropy) and the magnetic susceptibility is isotropic (magnetic isotropy). The case of magnetic anisotropy has been treated briefly (and not fully correctly) by Desvaux and Gochin (*35*). In this article, we treat the case of magnetic anisotropy in a different way. We describe the theory in Section 2 of this paper. Illustrative simulations and an example of the consequences of the use of the present theory for a lanthanide-substituted calcium-binding protein are presented in Section 3.

2. THEORETICAL

We build this discussion on two important results of earlier work. First, we use the theory of paramagnetic shifts in systems characterized by magnetic susceptibility of a general form by

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1090-7807/01 $35.00

Bleaney (*36*) and Kurland and McGarvey (*37*). Second, we apply Goldman's formulation (*2*) of the DD–CSA cross correlation for the case of a rhombic shielding tensor.

Following Bleaney (*36*) and Kurland and McGarvey (*37*), we note that the paramagnetic shift of the resonance frequency of a nuclear spin dipole-coupled to an electron spin can be expressed in terms of the rank 2 susceptibility tensor, in general of rhombic symmetry, and the position of the nucleus in the coordinate frame of the susceptibility tensor with the origin at the paramagnetic metal center. This shift, which we shall in the following call the dipolar shift, can be expressed as a second-rank Cartesian tensor, with in principle nine independent elements. Following Vega and Fiat (*21*), the dipolar shift Hamiltonian is written in the form

$$H = \gamma_A \boldsymbol{I}_A \cdot \sigma \cdot \boldsymbol{B}_0, \qquad [1]$$

where γ_A is the magnetogyric ratio for spin A with the spin operator $\boldsymbol{I}_A$, $\boldsymbol{B}_0$ is the magnetic field, and the shift tensor can be expressed as

$$\sigma = \frac{1}{4\pi} \times \begin{pmatrix} (3x^2 - r^2)\chi_x r^{-5} & 3xy\chi_y r^{-5} & 3xz\chi_z r^{-5} \\ 3xy\chi_x r^{-5} & (3y^2 - r^2)\chi_y r^{-5} & 3yz\chi_z r^{-5} \\ 3xz\chi_x r^{-5} & 3yz\chi_y r^{-5} & (3z^2 - r^2)\chi_z r^{-5} \end{pmatrix}. \qquad [2]$$

Here, χ_x, χ_y, and χ_z are the principal components of the susceptibility tensor, x, y, and z are the coordinates of the nucleus with respect to that principal frame, and r is the distance between the nucleus A and the metal S. The shift tensor in Eq. [2] can be treated as any other shift or shielding tensor. It can be decomposed into rank 0, rank 1, and rank 2 irreducible tensors (*38, 39*). The rank zero component corresponds to the spatially averaged pseudocontact shift (*39*):

$$\sigma_{pc} = \frac{1}{4\pi} \frac{(3x^2 - r^2)\chi_x + (3y^2 - r^2)\chi_y + (3z^2 - r^2)\chi_z}{3r^5}. \qquad [3]$$

The rank 1 irreducible tensor (corresponding to the antisymmeric part of the shielding) is not interesting in the present context. The rank 2 irreducible tensor is given by

$$\sigma = \frac{1}{4\pi} \begin{pmatrix} (3x^2 - r^2)\chi_x r^{-5} - \sigma_{pc} & 3xyr^{-5}(\chi_x + \chi_y)/2 & 3xzr^{-5}(\chi_x + \chi_z)/2 \\ 3xyr^{-5}(\chi_x + \chi_y)/2 & (3y^2 - r^2)\chi_y r^{-5} - \sigma_{pc} & 3yzr^{-5}(\chi_y + \chi_z)/2 \\ 3xzr^{-5}(\chi_x + \chi_z)/2 & 3yzr^{-5}(\chi_y + \chi_z)/2 & (3z^2 - r^2)\chi_z r^{-5} - \sigma_{pc} \end{pmatrix}. \qquad [4]$$

The shift tensor components given in this form can be directly substituted into the CSA Hamiltonian given by Goldman (*2*).

The formulation so far is very general and independent of the origin and symmetry of the susceptibility tensor. We can discuss two different cases. First, we can assume that the susceptibility tensor is isotropic and given by (*40*)

$$\chi_x = \chi_y = \chi_z = \chi_{ave} = \mu_0 \mu_B^2 g_e^2 \frac{S(S+1)}{3kT}. \qquad [5]$$

The spatial average of the dipolar shift, the pseudocontact shift, vanishes in this case as discussed, for example, by Bleaney (*36*). This does not imply, however, that the Curie spin relaxation or the DD–CSR cross correlation does not occur. In fact, the work on the Curie spin relaxation by Gueron (*20*) assumes isotropic susceptibility. Also, most of the work on the DD–CSR cross correlation reported so far makes use of this isotropic assumption. In addition, we assume that the reorientation of the paramagnetic complex can be described as isotropic rotational diffusion, with the rank 2 rotational correlation time equal to τ_c. Setting $\chi_x = \chi_y = \chi_z = \chi_{ave}$ in Eq. [4] and using the equations from the paper by Goldman (*2*), we obtain an axially symmetric shift tensor with the $\Delta\sigma$ given by

$$\Delta\sigma = \sigma_\parallel - \sigma_\perp = \frac{\mu_0}{4\pi} \mu_B^2 g_e^2 \frac{S(S+1)}{r^3 kT}. \qquad [6]$$

Following Goldman (*2*) we then obtain the following results for the difference in the linewidth (in Hz) of the two components for the A nucleus of the AK doublet:

$$\Delta\nu = \frac{2}{15\pi} \left(\frac{\mu_0}{4\pi}\right)^2 \frac{B_0 \gamma_A^2 \gamma_K \mu_B^2 g_e^2 \hbar S(S+1)}{r^3 r_{AK}^3 kT} \times \left(4\tau_c + \frac{3\tau_c}{1 + \omega_0^2 \tau_c^2}\right) \frac{3\cos^2\theta_{SAK} - 1}{2}. \qquad [7]$$

In the terminology of Goldman (*2*), this corresponds to $2\eta/\pi$. This equation agrees with the result of Ghose and Prestegard (*27*) (with $g_e \mu_B = \hbar\gamma_S$).[2] The angle θ_{SAK} is that between the AK axis and the AS axis. Assuming the electron spin to be localized on the metal ion (the point-dipole approximation (*41, 42*)), this latter axis is identical to the nucleus A–metal axis. Another assumption inherent in Eq. [7] is that of isotropic, rigid-body reorientational motion. More realistic models are certainly available (*43*) but we judge that this approximation is adequate for the purpose of present work.

The main topic of this paper is the case of anisotropic magnetic susceptibility. Using Eq. [4] in general form, we obtain the

[2] Note that Eq. [7] gives the differential linewidth in hertz. In the literature, equations for the additional contribution to the transverse relaxation rate of one resonance line are commonly reported (*27*).

following counterpart of Eq. [7]:

$$\Delta\nu = \frac{1}{15\pi}\left(\frac{\mu_0}{4\pi}\right)\frac{B_0\gamma_A^2\gamma_K\hbar}{r_{AK}^3}\left(4\tau_c + \frac{3\tau_c}{1+\omega_0^2\tau_c^2}\right) \cdot (\sigma_{X'}(3\cos^2\theta_{X'AK} - 1)\sigma_{Y'}(3\cos^2\theta_{Y'AK} - 1) + \sigma_{Z'}(3\cos^2\theta_{Z'AK} - 1)). \quad [8]$$

The angles $\theta_{X'AK}$, $\theta_{Y'AK}$, and $\theta_{Z'AK}$ specify the directions of the principal axes X', Y', and Z' of the shift tensor of nucleus A with respect to the AK axis. The principal axes for the shift tensor are obtained by first calculating the tensor in the principal frame of the susceptibility tensor and then diagonalizing the symmetric part which corresponds to the rank 2 irreducible tensor in Eq. [4].

One case that has been discussed by Desvaux and Gochin (*34*), and which can be now fully addressed, is that in which the magnetic susceptibility anisotropy originates from zero-field splitting. Indeed, as discussed by Bleaney (*36*) and Kurland and McGarvey (*37*), the magnetic susceptibility can, in the terminology of a general spin Hamiltonian, have its origin either in the anisotropy of the electronic g tensor or in the zero-field splitting. In terms of more fundamental interactions, both these phenomena are related to the effects of spin–orbit coupling. The case of anisotropic g tensor is not really controversial, and the relation between the g and χ anisotropy is well understood (*40*).

The case of the ZFS interaction is more complicated. Desvaux and Gochin state in their work (*35*) that if the ZFS splitting is so strong that it defines the principal quantization axis of the electron spin in the molecular frame, then the DD–CSR cross correlation vanishes. However, their argument is valid in the limit of zero magnetic field, a limit which is not relevant in the case of NMR experiments carried out at finite field, independently of the ratio of the strength of the Zeeman and the ZFS interactions. Another proof of this statement using the spin Hamiltonian formalism is presented in the Appendix. In summary, it is theoretically demonstrated that the DD–CSR cross correlation in a given magnetically anisotropic system is equally strong, independent of the origin of the magnetic anisotropy.

The discussion so far is valid for the case of a single paramagnetic center in a molecule. In the case when a protein contains several paramagnetic metals, each metal center contributes to the total magnetic susceptibility tensor. A reasonable generalization of our approach to that case is to consider individual susceptibility tensors associated with each site and to express the dipolar shift tensor associated with each site by its own term such as given by Eq. [2]. There will thus still be one total dipolar shift tensor, but it will now be given as a sum of contributions from each metal. This total shift tensor will contain a rank 2 component, consisting of a sum of contributions such as given by Eq. [4]. Also the contributions from each metal to the cross-correlation effect (the differential line broadening) will be additive, with each metal contributing a term such as given by Eq. [7] or [8].

3. RESULTS AND DISCUSSION

In Fig. 1, we use Eq. [8] to simulate the dependence of the differential linewidth for the A components of the AK doublet on the angle θ_{zAS} between the principal axis of the susceptibility tensor and the AS axis. The susceptibility components used in the simulation are highly anisotropic, $\chi_z = 3\chi_{ave}/2$ and $\chi_x = \chi_{ave}/2$. The angle θ_{SAK} between the AK and the AS axes is set to three values: zero, the magic angle, and 90°. $\theta_{SAK} = 0$ corresponds to a collinear arrangement and the maximum cross-correlation effect, while the magic angle corresponds to $3\cos^2\theta_{SAK} - 1 = 0$ and no differential linewidth for the isotropic case. $\theta_{SAK} = 90°$ changes the sign of the cross-correlation spectral density and of the differential linewidth. The isotropic model results do not depend on θ_{zAS}; the corresponding straight lines are displayed in the figure for reference. We can see that the effects of the large magnetic anisotropy are clearly nonnegligible. An interesting exception occurs at $\theta_{SAK} = 90°$ when the AK vector is within the xz plane: the "anisotropic" calculation coincides with the "isotropic."

We find it interesting to investigate the consequences of the present extension of the theory for a realistic protein example, where the differential linewidths could in principle be used as structural constraints. The protein is the cerium(III)-substituted calcium-binding protein, calbindin D_{9k}. Parameters characterizing the magnetic properties of the substituted protein are given in Table 1. The magnetic anisotropy parameters have been obtained from measurements of a large number of pseudocontact shifts for protein protons (*44*) and from the χ_{ave} value obtained from Eq. [5]. The values of J and g_J must replace the values of S and g_e, respectively, in Eqs. [5] and [6]. We combine the data in Table 1 with structural information from NMR data

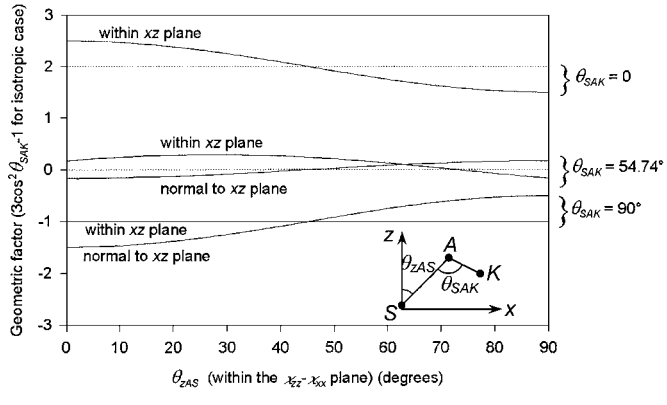

FIG. 1. Effect of magnetic susceptibility anisotropy on the angular part of the differential line-broadening effect originated by the DD–CSR cross correlation (Eq. [8]). The effect is shown as a function of the angle θ_{zAS} between the z axis of the χ tensor and the nucleus–metal AS vector when the A nucleus moves from along the z axis to along the x axis of the χ tensor, i.e., from the largest to the smallest principal value of χ. The three situations corresponding to $\theta_{SAK} = 0°$, 54.74°, and 90° are shown. For the latter two, the two extreme situations of AK being within or perpendicular to the xz plane are also shown.

TABLE 1
Magnetic Parameters Characterizing the Cerium(III) Ion Bound to the Calcium-Binding Protein Calbindin D_{9k} (*44*)

J_{Ce}	$g_{J(Ce)}$	χ_x	χ_y	χ_z
5/2	6/7	0.46×10^{-31} m^3	0.53×10^{-31} m^3	0.70×10^{-31} m^3

(including NOEs and pseudocontact shifts). For every amide ^{1}H–^{15}N spin pair ($^1\mathrm{H} = A$, $^{15}\mathrm{N} = K$), we calculate the proton coordinates in the metal-fixed susceptibility frame and then the dipolar shift tensor, using Eq. [2]. We symmetrize and diagonalize the tensor and calculate the differential linewidth for every proton resonance. We then repeat the calculation assuming the susceptibility to be isotropic and plot the "anisotropic" versus "isotropic" shifts in Fig. 2A. The figure shows only small deviations from a linear relation, and almost no anisotropy effect. The reason for that is the fact that different protons are at different distances from the metal, and the r^{-3} dependence, common in both models, is by far the most important factor in determining the cross-correlation effect.

In Fig. 2B, we show the calculated differential linewidths multiplied by r^3, in order to suppress the radial dependence and to enhance the role of the angular effects. The effect of the anisotropy of the magnetic susceptibility becomes now rather clear, even if it is still modest. In order to stress further the shortcomings of the isotropic model, we show in Fig. 2C the relative percentage deviations of the anisotropic model with respect to the isotropic model as a function of the residue number in the protein. One should be aware of the fact that, when we get close to the magic angle ($3\cos^2\theta_{SAK} - 1 = 0$), the differential linewidths become small and even small absolute effects can become large on the relative scale. For this reason, the residues with θ_{SAK} within $\pm 5^\circ$ from the magic angle are not included in Fig. 2C. The deviations are still substantial, at least for some residues. As a consequence, the use of the isotropic susceptibility model in the context of differential linewidths applied as a structural constraint may lead to some systematic errors.

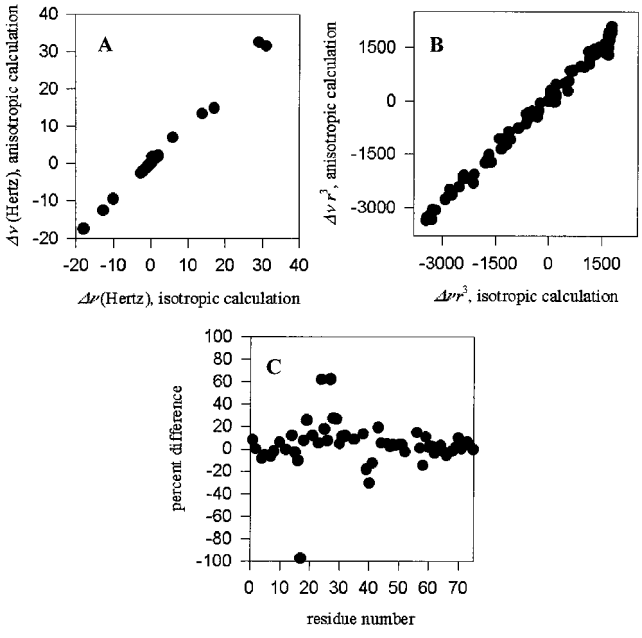

FIG. 2. DD–CSR cross-correlation effects on ^{1}H differential line broadening predicted for the peptide NH groups of the cerium(III)-substituted calcium-binding protein calbindin D_{9k}. A direct comparison of the differential line broadening predicted using the known anisotropy (*44*) versus that predicted in the isotropic approximation is shown in (A), while the corresponding comparison for the angular part of the effect is shown in (B). Panel (C) illustrates the percentage deviation of the isotropic model with respect to the anisotropic model as a function of the residue number (excluding residues with $|\Delta\nu r^3| < 500$ Å^3/s, which would give even larger deviations).

4. CONCLUSIONS

A general theoretical treatment has been developed here to account for cross-correlated relaxation caused by interference between nuclear dipole–dipole interaction and the Curie spin relaxation (DD–CSR cross correlation) in the presence of magnetic anisotropy of any origin. The main finding is that the DD–CSR cross correlation in a given magnetically anisotropic system is equally strong independently of the origin of magnetic anisotropy. A consequence of this finding is that lanthanide ions, whose anisotropy arises mostly from zero-field splitting effects, are expected to yield substantial DD–CSR cross correlation, at variance with previous predictions. This conclusion is particularly relevant in view of the growing interest in the use of lanthanide ions as paramagnetic substitutes for calcium ions in calcium-binding proteins.

Sample calculations based on the known magnetic properties of a cerium(III)-substituted calcium-binding protein show that (i) the DD–CSR cross-correlation effect is predicted to be relevant and (ii) the additional perturbation due to the magnetic anisotropy is not negligible when DD–CSR cross-correlation data are to be used for quantitative purposes.

APPENDIX

Consider the case when there is only one metal center and the electron spin Hamiltonian contains both the Zeeman and the ZFS interaction:

$$H = \mu_B \boldsymbol{S} \cdot \boldsymbol{g} \cdot \boldsymbol{B_0} + \boldsymbol{S} \cdot \boldsymbol{D} \cdot \boldsymbol{S}. \qquad [A1]$$

If the ZFS vanishes, then the eigenstates become the usual Zeeman eigenstates, $|S, m_S\rangle$, and the quantization axis of the electron spin is the laboratory z axis, defined by the direction of the magnetic field. If the Zeeman interaction vanishes (at the limit of zero magnetic field), the eigenstates of the ZFS Hamiltonian can still be labeled with the m_S quantum number, but this refers now to the projection of the spin on a molecule-fixed axis, which acts as a quantization axis. We denote the eigenstates

of the ZFS Hamiltonian $|\hat{S}, \hat{m}_S\rangle$. An important feature of the ZFS Hamiltonian is that the $\pm\hat{m}_S$ states are degenerate. Thus, the expectation value of $\hat{S}_z$ for a $|\hat{S}, \hat{m}_S\rangle$ state is $\hat{m}_S$, but when summing over equilibrium populations of the ZFS-split levels, the contributions of pairs of degenerate levels cancel exactly. In other words, the ZFS does not lead to any permanent magnetic moment.

The Hamiltonian in Eq. [A1] mixes the Zeeman and ZFS eigenstates. The expectation value of S_z (in the laboratory frame), averaged over thermally populated levels, is given by

$$\langle S_z\rangle = \frac{\sum_j \langle S_{z,j}\rangle e^{-E_j/kT}}{\sum_j e^{-E_j/kT}}, \qquad [A2]$$

where j labels the $2S+1$ eigenstates of the Hamiltonian. This expectation value is not identical to the Zeeman-only case, $\langle S_z\rangle = -g_e\mu_B S(S+1)B_0/3kT$, because the ZFS may displace the relative energies of different $\pm m_S$ doublets. $\langle S_z\rangle$ is related to the magnetic susceptibility by (*39*)

$$\chi = -\frac{g_e\mu_0\mu_B}{B_0}\langle S_z\rangle. \qquad [A3]$$

Desvaux and Gochin state in their paper (*35*) that if the ZFS dominates over the Zeeman interaction, then the cross-time correlation function between the AK dipolar interaction and the Curie spin vanishes because of the fact that the S spin is locked in the molecular frame, which leads to a product of the Wigner rotation matrices of different ranks. While this is true at the limit of zero magnetic field, it is not true if the magnetic field is present. If the field is present, then there will be a nonzero $\langle S_z\rangle$ along the field direction, given by Eq. [A2], whose interaction with the nuclear spin is modulated by rotation of the molecules in a similar way as in the Zeeman-only case and, assuming isotropic reorientation, in the same way as the AK dipolar interaction. This argument leads, after some straightforward algebra, to Eq. [8] for the differential linewidth due to DD–CSR cross correlation.

ACKNOWLEDGMENTS

This work has been supported by Murst ex 40%, Italy, the European Union, Contract HPRI-CT-1999-00009 and Contract HPRN-CT-2000-00092, CNR, Progetto Finalizzato Biotecnologie Contract 990039349, Italy, and the Swedish Natural Science Research Council.

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