Interference between *J*-Couplings and Cross-Relaxation in Solution NMR Spectroscopy: Consequences for Macromolecular Structure Determination

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Other than as a mechanism of line broadening, the interaction between the coherent *J*-interaction and incoherent spin relaxation is not usually explicitly considered in NMR studies of macromolecular structure, despite the fact that as early as 1967[1] it was noted that dipolar relaxation in a simple two-spin system can alter the apparent *J*-splitting whenever the rates of certain relaxation processes become comparable to the coupling constant. In essence, this effect reduces to the problem of diagonalizing a 2 × 2 matrix with both real and imaginary elements. Consider two spins, *I* and *S*, interacting via a *J*-coupling, small compared with the frequency difference between them, and simultaneously relaxing. Under these circumstances, the Liouville space can be decomposed into small subgroups. The subgroup which concerns us here contains two elements, I_1 and $2I_1S_0$; in terms of the more familiar Cartesian spin operators these are

$$I_1 = -(I_x + iI_y)/(2)^{1/2} \qquad S_0 = S_z \tag{1}$$

The equation of motion of the matrix is governed by a superoperator **U** = **L** + **R**, where **L** and **R** are the Liouville and Redfield superoperators which describe coherent evolution and relaxation, respectively.

$$\mathrm{d}/\mathrm{d}t\begin{pmatrix} I_1 \\ 2I_1S_0 \end{pmatrix} = (\mathbf{L} + \mathbf{R})\begin{pmatrix} I_1 \\ 2I_1S_0 \end{pmatrix};\quad \mathbf{R} = \begin{pmatrix} R_{11} & 0 \\ 0 & R_{22} \end{pmatrix};\quad \mathbf{L} = i\begin{pmatrix} \omega_I & -\pi J \\ -\pi J & \omega_I \end{pmatrix} \tag{2}$$

The observable NMR frequencies are obtained from the imaginary part of this matrix after diagonalization. Evolution depends critically on the diagonal matrix elements U_{11} and U_{22}. If the two operators I_1 and $2I_1S_0$ undergo relaxation at identical rates, then $U_{11} = U_{22}$, and the diagonal matrix elements transform as the identity matrix, which is transparent to diagonalization. This leaves the off-diagonal part, which depends only on the *J*-coupling. The eigenvalues are $U_{11} \pm i\pi J$—two resonances of equal width split by the normal *J*-coupling.

If, however, $U_{11} \neq U_{22}$, then **U** has a diagonal σ_z as well as an off-diagonal σ_x component. Although the σ_z part has a purely real coefficient and σ_x has a purely imaginary one, diagonalization mixes the two, leading to the eigenvalues:

$$\lambda_{1,2} = \{(U_{11} + U_{22}) \pm i[(2\pi J)^2 - (R_{11} - R_{22})^2]\}/2^{1/2},\qquad (R_{11} - R_{22})^2 \leq (2\pi J)^2 \tag{3}$$

Thus the apparent *J*-splitting explicitly depends on the differential rate of relaxation.

This effect will occur whenever I_1 and $2I_1S_0$ relax at different rates. This can occur if $2I_1S_0$ can relax significantly via decay of the S_0 component. In the two-spin case considered by Lynden-Bell,[1] such differential relaxation can occur only via T_1 relaxation of the *S* spin, which becomes comparable in magnitude to the *I* spin T_2 near or at the limit of extreme motional narrowing. This is probably of limited significance at high field, since even for the most strongly dipolar coupled systems, at its maximum $1/T_1$ is usually small compared with typical 3-bond *J*-couplings, and this maximum in turn occurs at tumbling rates much faster than typically observed for macromolecules.

In systems of more than two spins, however, an additional mechanism comes into play; $2I_1S_0$ can relax by a mutual spin-flip of the *S* spin with a third proton (*T*). This process is actually cross-relaxation; however, if for the purposes of understanding the phenomenon we regard the $2I_1T_0$ magnetization produced by such cross-relaxation as a sink (for example, if *T* in turn cross-relaxes rapidly with other spins), then we can approximate the evolution of the system as a two-spin problem.[2] We here consider explicitly the evolution of *I* and *S* spin magnetization for the 4-element subgroup (I_1, S_1, $2I_1S_0$, $2I_0S_1$); this larger subgroup was used because the mixing of the eigenbasis caused by cross-relaxation makes the weak coupling approximation invalid. The major assumptions of this model are that τ_c is in the slow-correlation limit and that direct dipolar relaxation between *I* and *T* and dipolar cross-correlation are negligible. We also ignore *J*-couplings except those between *I* and *S*. The relaxation matrix elements for the Redfield superoperator of this subgroup are[3]

$$R_{11} = -(\omega_{dd,IS})^2\tau_c/4;\quad R_{22} = R_{11} - (\omega_{dd,ST})^2\tau_c/4;\quad R_{33} = R_{11} - (\omega_{dd,ST})^2\tau_c/10;\quad R_{44} = R_{22} \tag{4a}$$

$$R_{MN} = 0, M \neq N;\quad \omega_{dd,IS} = (\mu_0/4\pi)r_{IS}^{-3}\gamma^2\hbar \tag{4b}$$

Calculated spectra of the *I* and *S* spin doublets are shown in Figure 1. These were done on a Macintosh Quadra 900 using the program *Mathematica* (Wolfram Research, Urbana, IL); the initial transverse magnetization $I_1 + S_1$ was transformed into the eigenbasis of **U** and allowed to evolve. Subsequent back-transformation gave the observable time-domain magnetization, which was Fourier transformed to give the calculated line shapes. In the calculations, we varied the ratio $f = r_{ST}/r_{IS}$, where r_{ST},r_{IS} are the relevant internuclear distances. The true *J*-coupling and chemical shifts are identical in all cases; the correlation times τ_c have been adjusted so that the line width of the *I* spin multiplet is the same in every spectrum. This is done to offset the usual illusory reduction in the multiplet splitting caused by a simple increase in Lorentzian line width. It can be seen that as the *S*–*T* dipolar coupling increases relative to the *I*–*S* coupling, the difference between the imaginary part of the eigenvalues, which we call J^*, decreases and the decrease is asymmetric; the apparent splittings of the two *J*-coupled spins become different, with the larger decrease experienced by the *I* spin doublet. In addition, there is a second, more subtle effect: because the eigenbasis coefficients as well as the eigenvalues are now complex, individual resonances acquire significant dispersive character. The sign of the dispersive part is reversed in the two halves of each doublet, and so the effect is not obvious; however, neither multiplet is now a combination of two Lorentzians. The negative dispersive contribution falls on outer side of the *I* spin doublet. This partially cancels the Lorentzian tails and in addition causes a further apparent reduction in the already attenuated splitting. In contrast, within the *S* spin multiplet the tails are enhanced but the reduced J^* is less obvious. Anet and O'Leary[4] have recently reported line shape distortions with a similar origin in a system of two nearly degenerate but uncoupled cross-relaxing spins.

The interproton distances, coupling constant, and correlation times used in these calculations are all within the range encountered for biological macromolecules. While the model is

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(1) Lynden-Bell, R. M. *Prog. Nucl. Magn. Reson.* **1967**, *2*, 163–204.

(2) A similar model, which, however, does not consider the effects of cross-relaxation, has recently been published: London, R. E. *J. Magn. Reson.* **1990**, *86*, 410–415.

(3) Derived by the methods of, and in agreement with, Spiess, H. W. *NMR: Basic Princ. Prog. Dynamic NMR Spectrosc.* **1978**, *15*, 59–214.

(4) Anet, F. A. L.; O'Leary, D. J. *J. Magn. Reson.* **1990**, *86*, 358–370.

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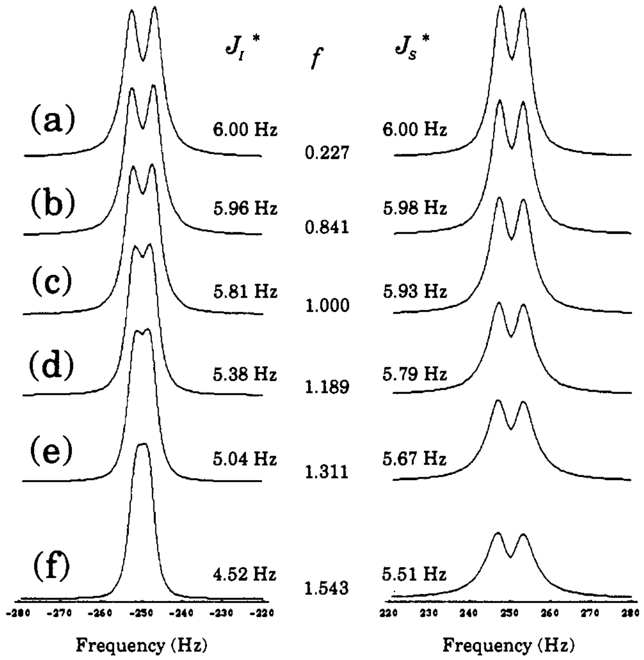

Figure 1. Calculated I (left) and S (right) spin NMR line shapes for a coupled pair of protons ($J_{IS} = 6$ Hz, $\omega_I = -\omega_S = 2\pi \times 250$ Hz), undergoing transverse dipolar relaxation in the slow correlation limit. In addition, the S spin is relaxed by a nearby third proton T. The eigenfrequency difference J^* (defined in the text) and the ratios of internuclear bond lengths $f = (r_{IS}/r_{ST})$ are also given. Specific input parameters used in the calculations: (a) $\tau_c = 1.37 \times 10^{-8}$ s, $r_{IS} = 0.227$ nm, $r_{ST} = 1.00$ nm; (b) $\tau_c = 1.16 \times 10^{-8}$ s, $r_{IS} = 0.227$ nm, $r_{ST} = 0.270$ nm; (c) $\tau_c = 0.91 \times 10^{-8}$ s, $r_{IS} = 0.227$ nm, $r_{ST} = 0.227$ nm; (d) $\tau_c = 1.60 \times 10^{-8}$ s, $r_{IS} = 0.270$ nm, $r_{ST} = 0.227$ nm; (e) $\tau_c = 1.10 \times 10^{-8}$ s, $r_{IS} = 0.270$ nm, $r_{ST} = 0.206$ nm; (f) $\tau_c = 0.50 \times 10^{-8}$ s, $r_{IS} = 0.270$ nm, $r_{ST} = 0.175$ nm.

highly simplified for heuristic reasons, it is not too far distant from spin systems of biological significance. For example, a methine proton vicinal to a methylene group about a gauche single bond, as is encountered in a deoxyribose ring, or an isolated amide proton coupled to a more rapidly relaxed α proton in a peptide backbone are cases for which this model is a first-order approximation. However, to obtain correct J-couplings for spin subsystems within macromolecules, the full **U** matrix should be explicitly diagonalized. Since the necessary rates and apparent splittings are easily available from NOESY and COSY spectra, respectively, this is not a difficult undertaking; diagonalization of the relaxation matrix alone is employed in the more careful contemporary structural determinations.[5]

It is important to emphasize that the input parameters used in Figure 1 were chosen conservatively; situations undoubtedly exist where the scaling of the J-splitting is more severe than the cases considered in Figure 1. One extreme example is the well-known situation of a proton bound to a quadrupolar nucleus,[6] where rapid T_1 relaxation of the latter scales the J-splitting to 0 (so called "self-decoupling"). Distorted multiplet patterns reported previously by Anet[7] probably also have a similar origin.

Some final points: firstly, it appears that the primary effect of this phenomenon is a reduction in apparent 3-bond J-splittings, particularly smaller ones. A cursory examination of previously published data[8] suggests that in the interpretation of NMR spectra of DNA oligomers, failure to account for such a reduction will overall tend to artifactually increase the calculated population of 3′-endo conformers. If this is so, correcting for it will improve the agreement of solution NMR results with solid-state NMR studies of highly hydrated B-DNA fibers[9] and crystalline oligomers,[10] which suggest 2′-endo populations of near 100% and little dynamic interconvertion between ring pucker conformers. Secondly, the mixing of coherent and incoherent evolution terms also changes the cross-relaxation rates. Finally, we should point out that while the eigenfrequency changes reported here will be pulse-sequence-independent, the dispersive phase contribution will depend on the detailed spin dynamics of the NMR experiment.

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