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The Effects of Dipolar Cross Correlation on ^{13}C Methyl-Carbon T_1, T_2, and NOE Measurements in Macromolecules

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The effects of dipolar cross correlation on ^{13}C T_1, T_2, and NOE values are calculated for methyl groups attached to macromolecules. Using the Woessner model to describe methyl-group internal dynamics, it is found that for macromolecules with tumbling times between 5 and 20 ns and fast internal methyl rotation characterized by a correlation time between 15 and 65 ps, the recovery of longitudinal magnetization differs by less than 10% from that of the case with no cross correlation. Therefore for a large range of values of τ_m and τ_e, cross-correlation effects on longitudinal relaxation are small, despite the fact that reorientation is highly anisotropic. In contrast, for ^{13}C spin–spin relaxation the effects of dipolar cross correlation are significant for all values of τ_m and τ_e in the range examined ($2\text{ ns} < \tau_m < 40\text{ ns}$, $0\text{ ps} < \tau_e < 100\text{ ps}$), and differences in relaxation rates by as much as a factor of 3.0 relative to predicted values based on the neglect of cross correlation are obtained for an isolated AX_3 spin system attached to a macromolecule. The effect of cross correlation is calculated to change NOE values by no more than 6–7% in the range examined. The influence of neighboring ^1H spins on ^{13}C relaxation is assessed by including random-field effects in the calculations. While the effects of cross correlation are attenuated, ^{13}C longitudinal and transverse relaxation rates can, under certain conditions, still be substantially different from rates obtained in the absence of cross correlation. Although a quantitative description of cross-correlation effects on ^{13}C relaxation depends on the details of methyl-group internal dynamics, the results derived here using the Woessner model are qualitatively the same as results obtained for different descriptions of the methyl-carbon internal motions.

NMR is a powerful technique for obtaining information on internal dynamics in proteins. The measurement of relaxation rates of heteronuclei (A = ^{13}C or ^{15}N) directly bonded to protons is particularly useful for obtaining motional information since the relaxation of these nuclei is governed predominately by dipolar interactions with directly bonded protons. Other relaxation mechanisms such as chemical-shift anisotropy (CSA) and spin–rotation often make small contributions to the heteronuclear relaxation (*1*). Moreover, the interpretation of such relaxation rates requires only that the ^1H–A bond length be known and, in general, does not require a knowledge of the overall structure of the molecule in question. The availability of nearly complete heteroatom assignments of spectra of uniformly ^{13}C- and ^{15}N-labeled proteins, due to recently developed double- and triple-resonance 3D (*2–6*) and 4D NMR (*7, 8*) techniques, has provided access to a large number of probes of internal dynamics throughout the entire molecule. This advance, coupled with 2D pulse schemes for measuring

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heteroatom T_1, T_2 (*9–11*), and NOEs (*11*) with high sensitivity and resolution, allows for the extraction of a large number of highly accurate relaxation rates.

The simplest and most common approach to the analysis of heteroatom relaxation rates is to assume that the interactions can be described in a pairwise fashion. In this way, for example, the dipolar relaxation of a carbon spin in an AX_3 spin system is the sum of each ^1H–^{13}C contribution. However, this simplification neglects the fact that the motion of the three ^1H–^{13}C interatomic vectors is correlated and studies by Werbelow and Marshall (*12*), the Volds (*13*) and Werbelow and Grant (*14*) have shown that cross-correlation effects can be significant. A recent ^{13}C NMR relaxation study of leucine residues of the protein staphylococcal nuclease (SNase, MW 17 kDa) labeled with ^{13}C in the C^δ positions indicated that in almost all of the cases examined, the decay of transverse magnetization was not monoexponential (*15*). This result has prompted us to examine the effects of dipolar cross correlation on measured T_1, T_2, and NOE values for AX_3 spin systems attached to molecules tumbling in the limit $(\omega\tau_m)^2 \gg 1$, where ω and τ_m are the spectrometer frequency and overall molecular tumbling correlation time, respectively. While the effects of cross correlation on ^{13}C T_1 and NOE measurements (*14*) and on ^{13}C T_2 measurements (*16*) have been examined in the literature for AX_3 spin systems attached to molecules tumbling in the extreme narrowing regime $[(\omega\tau_m)^2 \ll 1]$, to our knowledge studies of these effects in macromolecular systems $[(\omega\tau_m)^2 \gg 1]$ have not appeared.

The equations describing the effects of dipolar cross correlation on ^{13}C T_1 and NOE measurements in AX_3 spin systems are given in (*14*) (see Eqs. [9.30] and [9.34]). The influence of cross correlation on measured ^{13}C transverse relaxation in AX_3 spin systems attached to macromolecules has not been described, however. Such a description is most easily accomplished using Redfield theory (*17*). Using this formalism, the relaxation of elements of the density matrix corresponding to transverse A magnetization is given by

$$d\rho/dt = \mathbf{R}\rho, \qquad [1]$$

where ρ is a vector containing all density elements corresponding to transverse A magnetization and $\mathbf{R}$ is a Redfield relaxation matrix whose dipolar contribution from various pairs of interacting spins ij, kl is given by (*17*)

$$\begin{aligned} R^{ijkl}_{\alpha\alpha'\alpha''\alpha'''} = \sum_{m,n} \{ & (-1)^m \delta_{m,-n} 2 J^{00}_{ijkl}(\omega_{\alpha\alpha''}) \langle \alpha | T^m_2(ij) | \alpha'' \rangle \langle \alpha''' | T^n_2(kl) | \alpha' \rangle \\ & - \delta_{\alpha\alpha''} \sum_\beta (-1)^m \delta_{m,-n} J^{00}_{ijkl}(\omega_{\alpha'''\beta}) \langle \alpha''' | T^m_2(ij) | \beta \rangle \langle \beta | T^n_2(kl) | \alpha' \rangle \\ & - \delta_{\alpha'\alpha'''} \sum_\beta (-1)^m \delta_{m,-n} J^{00}_{ijkl}(\omega_{\alpha''\beta}) \langle \alpha | T^m_2(ij) | \beta \rangle \langle \beta | T^n_2(kl) | \alpha'' \rangle \}. \end{aligned} \qquad [2]$$

In Eq. [2], α, α', α'', α''', and β denote the various spin states for an AX_3 spin system (*18*), $T^n_2(ij)$ are the components of the second-order dipolar spin tensor with n or m running from -2 to 2, and $\delta_{\alpha\alpha'}$ is a Dirac function equal to 0 if $\alpha \neq \alpha'$ and equal to 1 otherwise. The $J^{00}_{ijkl}(\omega_{\alpha\alpha'})$ are the second-order spherical harmonic spectral densities evaluated at a frequency given by $\omega_{\alpha\alpha'} = (E_{\alpha'} - E_\alpha)/\hbar$. In the discussion that follows, $J_{ijkl}(\omega) = J^{00}_{ijkl}(\omega)$. Using the formalism of Lipari and Szabo (*19, 20*) we can express $J_{ijkl}(\omega)$ in a "model-independent" manner as

$$J_{ijkl}(\omega) = 1/(4\pi)\zeta_{ij}\zeta_{kl}\{S_f^{2(ij,kl)}\tau_m/[1 + (\omega\tau_m)^2] + [P_2(\mathbf{u}_{ij}\cdot\mathbf{u}_{kl}) - S_f^{2(ij,kl)}]\tau/[1 + (\omega\tau)^2]\}, \quad [3]$$

where we have included the effects of cross correlation. In Eq. [3], $\zeta_{ij} = (6\pi/5)^{0.5}(\gamma_i\gamma_j\hbar/r_{ij}^3)$, $S_f^{(ij,kl)}$ is an order parameter characterizing the internal motions, τ_m is the overall molecular tumbling correlation time, $\tau^{-1} = \tau_m^{-1} + \tau_e^{-1}$, where τ_e is the effective correlation time for the internal motions, $\mathbf{u}_{ij}$ is a unit vector describing the orientation of the interaction vector ij in a reference frame that is rigidly attached to the macromolecule, and P_2 is a second-order Legendre polynomial. It is straightforward to evaluate $P_2(\mathbf{u}_{ij}\cdot\mathbf{u}_{kl})$ in terms of (θ_{ij}, ϕ_{ij}) and (θ_{kl}, ϕ_{kl}), the polar angles of $\mathbf{u}_{ij}$ and $\mathbf{u}_{kl}$, respectively, by the addition theorem for spherical harmonics (*21*) to give

$$P_2(\mathbf{u}_{ij}\cdot\mathbf{u}_{kl}) = \tfrac{1}{4}(3\cos^2\theta_{ij} - 1)(3\cos^2\theta_{kl} - 1) + \tfrac{3}{4}\sin 2\theta_{ij}\sin 2\theta_{kl}\cos(\phi_{ij} - \phi_{kl}) + \tfrac{3}{4}\sin^2\theta_{ij}\sin^2\theta_{kl}\cos 2(\phi_{ij} - \phi_{kl}). \quad [4]$$

If the motions of the vectors $\mathbf{u}_{ij}$ and $\mathbf{u}_{kl}$ are axially symmetric then $S_f^{2(ij,kl)}$ can be expressed as

$$S_f^{2(ij,kl)} = P_2(\cos\beta_{ij})P_2(\cos\beta_{kl}), \quad [5]$$

where $P_2(\cos\beta_{ij}) = (3\cos^2\beta_{ij} - 1)/2$ and β_{ij} is the angle between $\mathbf{u}_{ij}$ and the axis of symmetry. Note that for the case where $ij = kl$ (autocorrelation terms) and where vector ij executes threefold jumps about some symmetry axis, Eq. [3] reduces to the Woessner model (*22*), where τ_e is the correlation time describing the three-site jump.

Equation [1] can be solved numerically using standard matrix techniques to give (*14*)

$$\rho(t) = \mathbf{Q}\exp(-\mathbf{Q}^{-1}\mathbf{R}\mathbf{Q}t)\mathbf{Q}^{-1}\rho(0), \quad [6]$$

where $\mathbf{Q}$ is a matrix of eigenvectors of $\mathbf{R}$, $\mathbf{Q}^{-1}$ is the inverse of matrix $\mathbf{Q}$, and $\rho(0)$ contains the values of the transverse A elements of the density matrix at the outset of the relaxation period. Finally, ^{13}C transverse relaxation is evaluated according to

$$A_{x(y)} \propto \mathrm{Tr}\{\rho(t)\cdot\mathbf{A}_{x(y)}\}, \quad [7]$$

where $A_{x(y)}$ is the $x(y)$ component of ^{13}C magnetization written in matrix form and Tr denotes the trace of the matrix $\rho(t)\cdot\mathbf{A}_{x(y)}$.

In the limit that $J(0)$ spectral-density terms dominate the transverse relaxation, a simple analytical solution to Eq. [1] is readily derived,

$$\frac{dA_{OUT}(t)}{dt} = \{-2J_{AHC}(0) - 4J_{CHCH}(0)\}A_{OUT}(t)$$

$$\frac{dA_{IN}(t)}{dt} = \left\{-2J_{AHC}(0) + \frac{4}{3}J_{CHCH}(0)\right\}A_{IN}(t), \quad [8]$$

where A_{OUT} is the intensity of the two outer lines of the ^{13}C quartet, A_{IN} is the intensity of the inner two lines, $J_{AHC}(0)$ is the autocorrelation spectral-density function ($\theta_{ij} = \theta_{kl} = 109.5°$, $\phi_{ij} - \phi_{kl} = 0°$ in Eq. [4]) for the internuclear ^1H–^{13}C vector evaluated

at zero frequency, and $J_{CHCH}(0)$ is the three-spin cross-correlation spectral-density function where two distinct ^1H methyl spins share the same ^{13}C spin ($\theta_{ij} = \theta_{kl} = 109.5°$, $\phi_{ij} - \phi_{kl} = 120°$ in Eq. [4]). Equation [8] describes the relaxation of the ^{13}C multiplet components of a methyl group that would be measured using a standard CPMG (*23, 24*) pulse sequence. We have assumed that the delays between successive application of 180° pulses are much shorter than $1/(2J_{XH})$, where J_{XH} is the one-bond ^1H–^{13}C coupling constant, so that the effects of scalar relaxation of the second kind (*11*) are insignificant. The solution to Eq. [8] is

$$\begin{aligned} A_{OUT}(t) &= A_{OUT}(0)\exp\{[-2J_{AHC}(0) - 4J_{CHCH}(0)]t\} \\ A_{IN}(t) &= 3A_{OUT}(0)\exp\{[-2J_{AHC}(0) + \tfrac{4}{3}J_{CHCH}(0)]t\} \\ A_x(t) &= A_{OUT}(t) + A_{IN}(t) \\ &= A_x(0)\{\tfrac{3}{4}\exp[(-2J_{AHC}(0) + \tfrac{4}{3}J_{CHCH}(0))t] \\ &\qquad + \tfrac{1}{4}\exp[(-2J_{AHC}(0) - 4J_{CHCH}(0))t]\}, \end{aligned} \quad [9]$$

where $A_x(t)$ is the x component of A (^{13}C) magnetization. Thus, in the limit that $(\omega\tau_m)^2 \gg 1$, the relaxation of $A_x(t)$ is biexponential because the outer lines relax more rapidly than the inner lines as a consequence of dipolar cross correlation. Note, however, that for short t ($t \ll \{2J_{AHC}(0) - \frac{4}{3}J_{CHCH}(0)\}$, $t \ll \{2J_{AHC}(0) + 4J_{CHCH}(0)\}$), the relaxation is independent of the effects of cross correlation.

A qualitative appreciation of the effects of cross correlation on the decay of longitudinal and transverse magnetization can be obtained by examining Eq. [3] in more detail. Substitution of Eq. [4] into Eq. [3] and using the Woessner model to describe internal dynamics give

$$\begin{aligned} J_{ijkl}(\omega) = 1/(4\pi)\zeta_{ij}\zeta_{kl}\{&[(3\cos^2\beta - 1)^2/4]\tau_m/[1 + (\omega\tau_m)^2] \\ &+ [\tfrac{3}{4}\sin^2 2\beta\cos(\phi_{ij} - \phi_{kl}) + \tfrac{3}{4}\sin^4\beta\cos 2(\phi_{ij} - \phi_{kl})]\tau/[1 + (\omega\tau)^2]\}, \end{aligned} \quad [10]$$

where β is the angle between vectors $\mathbf{u}_{ij}$ or $\mathbf{u}_{kl}$ and the symmetry axis of the methyl group and all other symbols are defined as for Eq. [3]. Note that for the case of a methyl group with $(\phi_{ij} - \phi_{kl}) = 0°$ (if $ij = kl$) and with $(\phi_{ij} - \phi_{kl}) = 120°$ (if $ij \neq kl$) it is possible to express the auto (cross) spectral-density functions as a weighted sum (difference) of slow- and fast-motion contributions via

$$\begin{aligned} J_{ijij}(\omega) &= a + 2b \\ J_{ijkl}(\omega) &= a - b \qquad (ij \neq kl), \end{aligned} \quad [11]$$

where $a = 1/(4\pi)\,\zeta_{ij}\zeta_{kl}\{[(3\cos^2\beta - 1)^2/4]\tau_m/[1 + (\omega\tau_m)^2]\}$ and $b = 1/(4\pi)\zeta_{ij}\zeta_{kl} \times \{[\frac{3}{8}\sin^2 2\beta + \frac{3}{8}\sin^4\beta]\tau/[1 + (\omega\tau)^2]\}$.

Equation [11] shows that since $a, b \geq 0$

$$J_{ijij}(\omega) \geq J_{ijkl}(\omega) \qquad (ij \neq kl), \quad [12]$$

with equality of auto- and cross-spectral-density terms occurring only in the limit that $\tau_e = 0$. In this limit and assuming further that $(\omega\tau_m)^2 \ll 1$, Werbelow and Grant (*14*) showed that the effects of cross-correlation on methyl-carbon longitudinal relaxation

are substantial. On the basis of their calculations one might suppose that cross correlation will significantly affect longitudinal methyl-carbon relaxation in macromolecules where reorientation is highly anisotropic, i.e., $\tau_m \gg \tau_e$. However, this expectation is not in general fullfilled for methyl-carbon relaxation in macromolecules for the following reason. In the macromolecular limit, $(\omega\tau_m)^2 \gg 1$, and assuming further that $(\omega\tau_e)^2 \ll 1$, it is easily shown that $J_{ijkl}(\omega)$ $(ij \neq kl)$ vanishes for certain values of the correlation times. This occurs for methyl-group cross-spectral-density terms $J_{CHCH}(\omega)$ when

$$\tau_e\tau_m \sim 1/(4\omega^2). \quad [13]$$

We note that if $\tau_e\tau_m > 1/(4\omega^2)$ cross- and auto-spectral-density terms are of opposite sign (cross-spectral-density terms are negative) while both cross- and auto-spectral-density terms are positive for $\tau_e\tau_m < 1/(4\omega^2)$. When Eq. [13] is approximately satisfied the cross-spectral densities are much smaller than the auto-spectral densities and cross-correlation effects on longitudinal relaxation are negligible, despite the fact that reorientation is highly anisotropic. In contrast, for τ_e values such that $\tau_e \ll [1/(4\omega^2\tau_m)]$, $(\omega\tau_m)^2 \gg 1$, $(\omega\tau_e)^2 \ll 1$, the decay of longitudinal magnetization is highly nonexponential since in this limit the ratio of cross- to auto-spectral densities is maximal. In the limit that $\tau_e \gg [1/(4\omega^2\tau_m)]$ and $(\omega\tau_m)^2 \gg 1$, $(\omega\tau_e)^2 < 1$, $J_{AHC}(\omega) = -2J_{CHCH}(\omega)$, and the effects of cross correlation are significant as well.

The situation is somewhat more complicated than that described above, since the effects of cross correlation depend upon the sum of cross-spectral-density terms evaluated at several different frequencies. A quantitative picture of such effects can be obtained by realizing that the limiting slope at $t = 0$ of the recovery of longitudinal or transverse magnetization is equal to the slope in the absence of cross correlation while the limiting slope at $t = \infty$ can be significantly smaller due to the influence of cross correlation. Therefore, a measure of the effect of cross correlation on methyl relaxation is the value of the ratio of the slopes of the relaxation curves at $t = 0$ and $t = \infty$. This ratio is calculated and plotted in Fig. 1A with internal methyl-group dynamics described by the Woessner model (*22*). For macromolecules with tumbling times between 5 and 20 ns and fast internal methyl rotation characterized by a correlation time between 15 and 65 ps, the slope of the recovery of longitudinal magnetization at long relaxation times ($t \rightarrow \infty$) differs by less than 10% from that in the case with no cross correlation. For shorter recovery periods the deviation is smaller. For methyl correlation times faster than 10 ps the effects of cross correlation become more severe.

Understanding the influence of cross correlation on the transverse relaxation rate for methyl carbons in macromolecules is more straightforward than that for longitudinal relaxation since in the former case the dominant contribution to relaxation originates from spectral-density terms of the form

$$J_{ijkl}(0) = 1/(4\pi)\zeta_{ij}\zeta_{kl}P_2(\cos\beta_{ij})P_2(\cos\beta_{kl})\tau_m, \quad [14]$$

where axially symmetric internal motion has been assumed. Note that both auto- and cross-spectral-density terms are equal for all values of τ_m and τ_e provided that $(\omega\tau_m)^2 \gg 1$ and $(\omega\tau_e)^2 \ll 1$. There is no sign reversal of cross-spectral-density functions and hence the ratio of cross- to auto-spectral-density terms is maximal. Not surprisingly

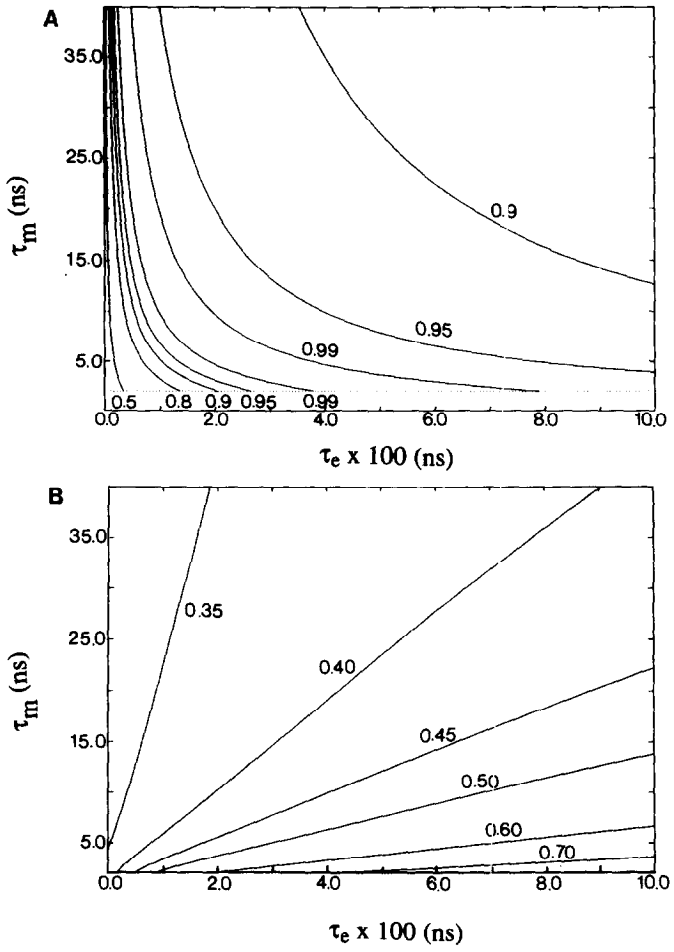

FIG. 1. Contour plots showing the effects of dipolar cross correlation on the relaxation of longitudinal (A) and transverse (B) methyl-carbon magnetization. The Woessner model (*22*) is used to describe the methyl-group dynamics. Each contour indicates the limiting ratio of the slopes (evaluated at $t = 0$ and at $t = \infty$) of the relaxation curves calculated with cross-correlation effects. The slope ratio associated with each contour line is indicated by the number adjacent to each line.

the effects of cross correlation are significant. This situation is very similar to results obtained for longitudinal relaxation in the limit that $\tau_e \rightarrow 0$. Figure 1B shows the effects of cross correlation on transverse ^{13}C methyl-group relaxation for methyl-group motion described by the Woessner model. As is illustrated, the effects on transverse relaxation are severe for the range of τ_m and τ_e values considered.

Figure 2 illustrates the influence of cross correlation on the recovery of longitudinal and transverse magnetization as well as on the NOE for particular values of τ_m and τ_e. For simplicity, the Woessner model has been used with τ_e equal to the three-site jump correlation time and $S_f^{2(ij,kl)}$ given by Eq. [5]. For $\tau_m = 10$ ns and $\tau_e = 25$ ps it is clear that the effects on longitudinal magnetization recovery are small. In this

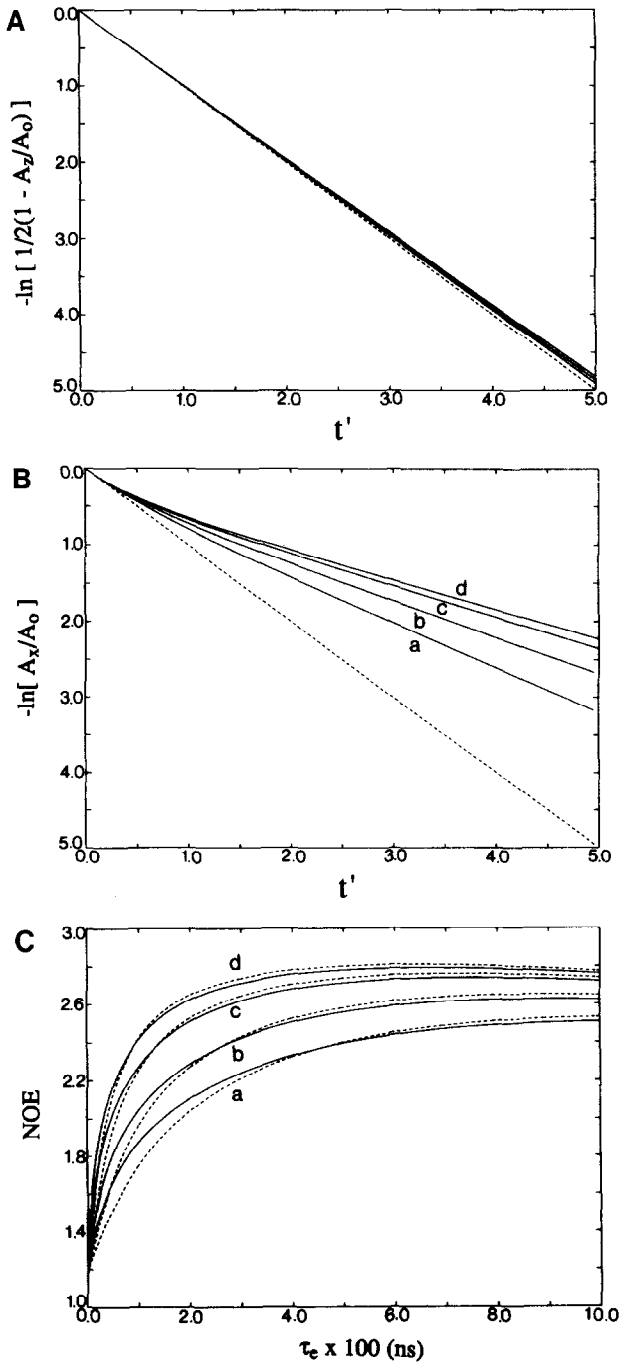

FIG. 2. The effects of dipolar cross correlation on the T_1, T_2, and NOE profiles of a ^{13}C spin in an AX_3 spin system (methyl group) attached to a macromolecule for select values of τ_m and τ_c. The Woessner model is used to describe methyl-group dynamics. The reduced variable, t', is equal to $t/T_{i=\{1,2\}}$, where T_i is the relaxation time in the absence of cross correlation or random fields. (A) T_1 recovery of ^{13}C magnetization

case, the initial and final relaxation rates differ by only 5%, while for $\tau_m = 5$ ns and $\tau_e = 25$ ps the curves derived with and without dipolar cross-correlation effects are superimposable. The effects of cross correlation on ^{13}C spin–spin relaxation rates are dramatic, as Fig. 2B illustrates. In the particular case where values of $\tau_m = 10$ ns and $\tau_e = 25$ ps are chosen, the initial and final decay rates of transverse magnetization differ by a factor of 2.4. The effect of cross correlation on the NOE is indicated in Fig. 2C. For $\tau_m = 10$ ns and for NOE values less than $\sim$2.6, neglect of cross correlation will result in an error in τ_e of at most 5 ps. However, for NOE values larger than 2.6, neglect of cross correlation can result in significant errors in the estimation of τ_e. As Fig. 2C illustrates, cross correlation does not change the absolute intensity of the NOE appreciably. For 0 ps $\leq \tau_e \leq$ 100 ps and 2 ns $\leq \tau_m \leq$ 40 ns the intensity change is never in excess of 6–7%.

The results discussed above have been derived assuming isolated AX_3 spin systems. In tightly packed macromolecules, such as proteins, this simplification is clearly not valid. For example, in the protein SNase, there are, on average, close to 100 proton spins within 4 Å of the leucine C^δ methyl protons (*25*). In order to explore the effects that dipolar interactions between the methyl protons and neighboring ^1H spins have on cross correlation we have included ^1H random-field effects in the calculation (*14*). While the use of random fields does not rigorously model the effects of dipolar interactions between spins (*13*), it nevertheless provides a framework in which a qualitative estimate of the influence of neighboring spins on ^{13}C relaxation can be obtained. Figure 3A illustrates the contribution of random-field terms of differing sizes to the transverse relaxation of ^{13}C magnetization in an AX_3 spin system for $\tau_m = 10$ ns and $\tau_e = 25$ ps using the Woessner model to describe the methyl-group internal dynamics. As can be seen, random-field contributions due to "external" ^1H spins decrease the effects of cross correlation. This is true for T_1 and NOE values as well, where for the values of τ_m and τ_e used in Fig. 3A, the curves corresponding to the situation with and without cross correlation become nearly superimposable (data not shown). We have also examined the influence of the surrounding ^1H spins by explicitly including a fourth ^1H in the calculations at a distance of 1.8 Å from the methyl protons. As expected, the effects of cross correlation decrease and for $\tau_m = 10$ ns and $\tau_e = 25$ ps the ratio of initial to final slopes of transverse magnetization decay (data not shown) decreases from 2.4 in the absence of the fourth ^1H spin to 2.1 in its presence.

The extent to which cross correlation affects ^{13}C relaxation is also a function of the motional properties of the ^{13}C spin. For example, consider a model in which, in addition

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$(-\ln[1/2(1 - A_z/A_0)])$ vs t', where A_z and A_0 are ^{13}C z magnetization and equilibrium magnetization, respectively, with full ^1H decoupling and with (solid lines) and without (dashed line) dipolar cross correlation. The solid curve closest to the curve describing relaxation in the absence of dipolar cross correlation (dashed line) was obtained with $\tau_m = 10$ ns followed by curves obtained with values of τ_m equal to 2.5 and 15 ns. The curve generated with $\tau_m = 5$ ns can be superimposed on the dashed line. The value of τ_e was set to 25 ps in all cases. All random-field terms were set to zero. (B) T_2 decay of ^{13}C magnetization ($-\ln[A_x/A_0]$ vs t') with (solid lines) and without (dashed line) cross correlation calculated in the absence of ^1H decoupling. Values of $\tau_m = 2.5$ ns (a), 5.0 ns (b), 10 ns (c), and 15 ns (d) were chosen with τ_e set to 25 ps in all cases. Random-field terms were set to zero. (C) NOE vs τ_e for the case with (solid lines) and without (dashed line) cross correlation. Value of $\tau_m = 2.5$ ns (a), 5 ns (b), 10 ns (c), and 15 ns (d) were chosen. Random-field effects were neglected.

to fast methyl rotation, an additional motion on a time scale intermediate between methyl rotation and the overall molecular tumbling is included. This is a model that might be used to describe the dynamics of a methyl group attached to a long side chain. For this case the spectral-density function of Clore *et al.* (*26*) is modified to give

$$J_{ijkl}(\omega) = 1/(4\pi)\zeta_{ij}\zeta_{kl}\{S^{2(ij,kl)}\tau_m/[1 + (\omega\tau_m)^2] + [P_2(\mathbf{u}_{ij}\cdot\mathbf{u}_{kl}) - S_f^{2(ij,kl)}]\tau_1/[1 + (\omega\tau_1)^2] + S_f^{2(ij,kl)}(1 - S_s^2)\tau_2/[1 + (\omega\tau_2)^2]\}, \quad [15]$$

where $S_f^{(ij,kl)}$ and S_s are order parameters describing the fast and slow methyl internal motions, respectively, $S^2 = S_f^{2(ij,kl)}\cdot S_s^2$, $\tau_1^{-1} = \tau_m^{-1} + \tau_f^{-1}$, $\tau_2^{-1} = \tau_m^{-1} + \tau_s^{-1}$, τ_f and τ_s are the correlation times for the rotation about the methyl symmetry axis and the slower reorientation of the symmetry axis, respectively, and all other symbols are defined as for Eq. [3]. For the case of a methyl group executing three-site jumps about a symmetry axis,

$$S_f^{2(ij,kl)} = P_2(\cos\beta_{ij})P_2(\cos\beta_{kl}), \quad [16]$$

where β_{ij} and β_{kl} are defined as before.

Figure 3B shows the effects of cross correlation and random fields on ^{13}C transverse relaxation in an AX_3 spin system using this new model to describe internal motion and for which values of $\tau_m = 10$ ns, $\tau_f = 25$ ps, $\tau_s = 0.5$ ns, and $S_s^2 = 0.5$ are chosen. [The value of S_f^2 is readily calculated from Eq. [16] assuming tetrahedral geometry so that, for example, for spectral-density terms $J_{AHC}(\omega)$ and $J_{CHCH}(\omega)$, $\beta_{ij} = \beta_{kl} = 109.5°$ and $S_f^{2(ij,kl)} = 0.111$]. Although cross correlation still has a significant effect on the rate of decay of transverse magnetization of an isolated AX_3 spin system, the ratio of the initial to the final decay rates has decreased from 2.4 for the Woessner model ($\tau_m = 10$ ns and $\tau_e = 25$ ps) to 1.9. Additionally, random fields attenuate the effects of cross correlation more significantly than in the Woessner model, as the figure illustrates.

In addition to dipolar interactions involving methyl protons and neighboring spins, competing relaxation mechanisms, such as CSA, spin–rotation, and ^1H–^{13}C dipolar interactions involving nonbonded protons can also decrease the influence of dipolar cross correlation. However, for methyl groups in proteins the effects of CSA are small; assuming an axially symmetric chemical-shift tensor with $\Delta\delta = 25$ ppm (*27*) and using the Woessner model with $\tau_m = 10$ ns and $\tau_e = 30$ ps, the contributions of CSA to T_1, T_2, and the NOE at a frequency of 500 MHz are less than 2.5% of the contribution from the ^1H–^{13}C intra-methyl-group dipolar interaction. An estimate of the influence of spin–rotation on the relaxation of ^{13}C methyl spins in macromolecules can be obtained from ^{13}C relaxation studies of the methyl group in toluene measured at temperatures between 200 and 330 K (*28*). A contribution of no more than 0.05 s^{-1} to the overall ^{13}C methyl-group longitudinal relaxation rate is obtained from spin–rotation. This value is larger than the contribution expected in proteins where the diffusion of the methyl group is considerably slower than the free diffusion experienced by the methyl group of toluene (*29*). Thus for ^{13}C relaxation in macromolecules a contribution of at most a few percent is expected from this relaxation mechanism. Finally, ^{13}C dipolar interactions with remote ^1H spins are also likely to be negligible

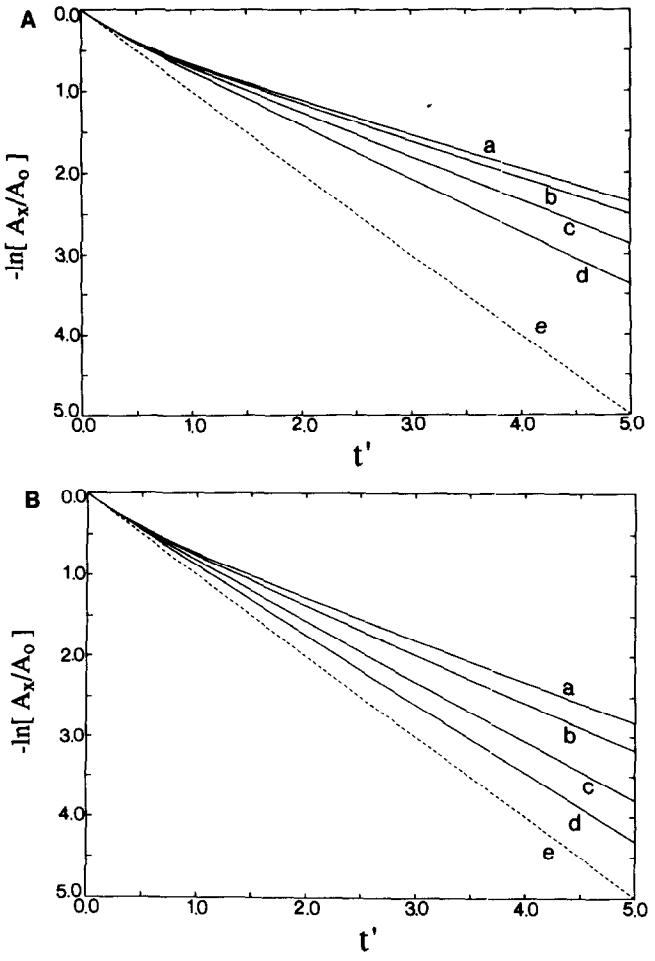

FIG. 3. The effects of ^{1}H random-field terms on ^{13}C transverse relaxation in AX_3 spin systems attached to macromolecules. Only auto-random-field terms are considered. In a–d the effects of dipolar cross correlation are included. (a) Random-field terms set to zero. (b) $j(\omega) = 5J(\omega)$, where $j(\omega)$ is the ^{1}H random-field spectral-density term and $J(\omega)$ is the autocorrelation term arising from ^{1}H–^{1}H methyl-group dipolar interactions. (c) $j(\omega) = 20J(\omega)$. (d) $j(\omega) = 50J(\omega)$. (e) Random terms set to zero and the effects of cross correlation are neglected. (A) The Woessner model (*22*) is used to describe the methyl dynamics with values for τ_m and τ_c of 10 ns and 25 ps, respectively. (B) The model described in Eq. [10] is used with $\tau_m = 10$ ns, $\tau_f = 25$ ps, $\tau_s = 0.5$ ns, and $S_s^2 = 0.5$. The reduced time variable t' is defined as in Fig. 2.

since for methyl groups the dominant ^{13}C relaxation mechanism is provided by the three ^{1}H spins located at ~1.09 Å from the ^{13}C nucleus.

Experimentally we have observed the effects of cross correlation on the decay of ^{13}C transverse magnetization of methyl groups attached to leucine residues in the protein SNase. It was found that the initial transverse relaxation rate was 10–20% faster than the relaxation rate after ^{13}C magnetization had decayed to ~20% of its original value. These experimental results indicate that, at least in this case, the effects

of cross correlation are somewhat smaller than theoretical predictions described here on the basis of simple models for the internal methyl dynamics. Nevertheless, it is clear that ^1H–^{13}C dipolar cross correlations can have important implications for the interpretation of T_1 and T_2 values within the framework of a specific motional model. It is therefore critical that such values be obtained from the initial decay of magnetization where the influence of cross correlation is smallest and that the size of such effects be estimated by comparing the initial decay rate with the rate at much longer times.

In summary, in this paper we have considered the effects of dipolar cross correlation on measured ^{13}C T_1, T_2, and NOE values in AX_3 spin systems attached to macromolecules. For 5 ns $< \tau_m <$ 20 ns, 15 ps $< \tau_e <$ 65 ps, the contributions to T_1 values are likely to be small, especially for methyl groups buried in the molecule and thus in close proximity to a large number of ^1H spins. However, for other values of τ_m and τ_e, ^1H–^{13}C dipolar cross correlation may have much more pronounced effects on measured longitudinal relaxation rates. For NOE measurements, it is calculated that, for all values of τ_m and τ_e examined in the present study (2 ns $< \tau_m <$ 40 ns, 0 ps $< \tau_e <$ 100 ps), the contribution of cross correlation is less than 6–7% of the NOE intensity predicted in the absence of such effects. Finally, the calculated effects of cross correlation on transverse relaxation rates are significant for all values of correlation times considered. Although dipolar relaxation between methyl protons and neighboring protons does attenuate the effects of cross correlation on the measured ^{13}C spin–spin relaxation rates, caution must nevertheless be exercised in the interpretation of ^{13}C T_2 values as these effects are unlikely to be attenuated completely.

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Note added in proof. Cross correlation between CSA and dipolar interactions may result in substantial errors in the measurement of heteronuclear T_1 and T_2 values. (J. Boyd, U. Hommel and I. Campbell, *Chem. Phys. Lett.* **175,** 477 (1990); L. E. Kay, L. K. Nicholson, F. Delaglio, A. Bax and D. A. Torchia, *J. Magn. Reson.* in press). We have recently developed pulse schemes to eliminate such effects (L. E. Kay, L. K. Nicholson, F. Delaglio, A. Bax and D. A. Torchia, *J. Magn. Reson.*, in press).

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