J. Am. Chem. Soc. 1994, 116, 8400–8401

Unusual NMR Multiplet Structures of Spin-$^1/_2$ Nuclei Coupled to Spin-1 Nuclei

R. E. London,† D. M. LeMaster,‡ and L. G. Werbelow*,§

Laboratory of Molecular Biophysics, NIEHS, Box 12233
Research Triangle Park, North Carolina 27709
Department of Biochemistry, Molecular Biology, and
Cell Biology, Northwestern University
2153 Sheridan Road, Illinois 60208-3500
Laboratoire des Méthodes Spectroscopiques
Centre St. Jerome, Case 541
13397 Marseille, Cedex 13, France

Received June 1, 1994

NMR analysis of biological macromolecules has been dramatically stimulated by the development of multidimensional heteronuclear correlation experiments combined with the facile introduction of extensive isotopic substitution with ^{13}C, ^{15}N, and ^{2}H.[1] The practical utility of a combined ^{2}H and ^{13}C enrichment pattern has awaited the development of ^{2}H lock gating circuitry to allow for high-power ^{2}H decoupling to collapse the ^{2}H scalar broadening of the ^{13}C resonances.[2] We[3] earlier observed that, in the absence of ^{2}H decoupling, a correlation time-dependent asymmetry of the $^{13}C_\alpha$ triplets occurs in the ^{1}H–^{13}C correlation spectra of monodeuterated glycine residues in a small protein. An f$_1$ slice of a representative glycine residue in *Escherichia coli* thioredoxin is shown in Figure 1.

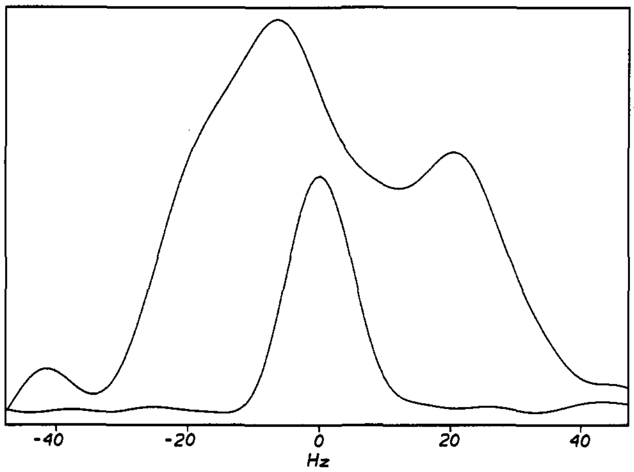

Figure 1. Upper trace: f$_1$ slice of a ^{2}H-coupled ^{1}H–^{13}C HSQC spectrum of [2-^{2}H$_R$, 2-^{13}C]glycine enriched *E. coli* thioredoxin[3] drawn through the Gly^{74} resonance. Lower trace: The corresponding f$_1$ slice obtained using broad-band ^{2}H decoupling (attenuated 6-fold relative to the ^{2}H-coupled spectrum). These spectra were obtained at 14.1 T under conditions corresponding to an 8.1 ns correlation time.[4] Contour plots of these well-resolved resonances have been published previously.[3]

Obviously, the most remarkable feature of this spectrum is the asymmetric positioning of the central component relative to the outermost components. In this communication, this unusual feature is explained.

To appreciate the essential feature involved, consider the scalar coupled two-spin system where spin $I = {}^1/_2$ and spin $S = 1$ (e.g., ^{13}C–^{2}H). For simplicity, assume that the relaxation of spin S is governed by the quadrupolar interaction, whereas spin I is relaxed directly by an I–S dipolar interaction.

The spin-$^1/_2$ line shape function,

$$\mathbf{F}(\omega) = Re\int_0^\infty \langle \mathrm{I}_+(t)\mathrm{I}_-(0)\rangle \exp(-\mathrm{i}\omega t)\,\mathrm{d}t$$

is calculated from the expression[5]

$$\mathbf{F}(\omega) = Re\sum_{m,m'}(\mathbf{A}^{-1})_{m,m'}$$

where

$$\mathbf{A} = \begin{pmatrix} i(\omega_I - \omega + J + \delta\omega_1) - (1/T_2)_1 & 4J^{\mathrm{Q}}(\omega_S) - J^{\mathrm{D}}(\omega_S) & 8J^{\mathrm{Q}}(2\omega_S) \\ 4J^{\mathrm{Q}}(\omega_S) - J^{\mathrm{D}}(\omega_S) & i(\omega_I - \omega + \delta\omega_0) - (1/T_2)_0 & 4J^{\mathrm{Q}}(\omega_S) - J^{\mathrm{D}}(\omega_S) \\ 8J^{\mathrm{Q}}(2\omega_S) & 4J^{\mathrm{Q}}(\omega_S) - J^{\mathrm{D}}(\omega_S) & i(\omega_I - \omega - J + \delta\omega_{-1}) - (1/T_2)_{-1} \end{pmatrix} \tag{1}$$

J is the I–S scalar coupling, and

$$(1/T_2)_m = 4(2 - m^2)J^{\mathrm{Q}}(\omega_S) + 8m^2J^{\mathrm{Q}}(2\omega_S) + (2 - m^2)[(1/3)J^{\mathrm{D}}(\omega_I - \omega_S) + J^{\mathrm{D}}(\omega_S) + 2J^{\mathrm{D}}(\omega_I + \omega_S)] + 2m^2[(4/3)J^{\mathrm{D}}(0) + J^{\mathrm{D}}(\omega_I)] \tag{2}$$

The dynamic frequency shift,[6,7] $\delta\omega_m$, is defined as

$$\delta\omega_m = 4(2 - 3m^2)L^{\mathrm{D\cdot Q}}(\omega_S) \tag{3}$$

For isotropic motions characterized by the correlation time, τ_2, the various spectral densities appearing in eqs 1–3 are defined as

$$J^{\mathrm{Q}}(\omega) = (3/160)(e^2qQ_S/\hbar)^2\tau_2/(1 + \omega^2\tau_2^{\,2})$$
$$J^{\mathrm{D}}(\omega) = (3/10)(\gamma_I\gamma_S\hbar/r_{IS}^{\,3})^2\tau_2/(1 + \omega^2\tau_2^{\,2}) \tag{4}$$
$$L^{\mathrm{D\cdot Q}}(\omega) = (3/40)(e^2qQ_S/\hbar)(\gamma_I\gamma_S\hbar h/r_{IS}^{\,3})\omega\tau_2^{\,2}/(1 + \omega^2\tau_2^{\,2})$$

These spectral densities are defined in terms of standard interaction constants such as internuclear distance (r_{IS}) and electric field gradient (q). It is assumed that the quadrupolar interaction is axially symmetric. Collinearity between the I–S internuclear vector and the principal axis of the electric field gradient is assumed. A dipolar dynamic frequency shift term, $L^{\mathrm{D}}(\omega)$, also enters the calculation but is quantitatively negligible compared with $\mathrm{L}^{\mathrm{D\cdot Q}}(\omega)$. In Figure 2, the behavior of eq 1 is explored using a set of parameters appropriate for the ^{13}C–^{2}H system. Plots 2a–d are generated for τ_2 = 0.1, 1.0, 8.1, and 30 ns, respectively.

In extreme narrowing (Figure 1a), the anticipated symmetric triplet is observed.[5,8] Because line widths across the spin-$^1/_2$ triplet are determined primarily by T_1 processes corresponding to the coupled, quadrupolar nucleus, the multiplet structure collapses in the neighborhood of the T_1 minimum (Figure 2b). However,

---

† NIEHS.

‡ Northwestern University.

§ Centre St. Jerome.

(1) Clore, G. M.; Gronenborn, A. M. *Prog. Nucl. Magn. Reson. Spectrosc.* **1990**, *23*, 43. Bax, A.; Grzesiek, S. *Acc. Chem. Res.* **1993**, *26*, 131. LeMaster, D. M. *Prog. Nucl. Magn. Reson. Spectrosc.* **1994**, *26*, 371.

(2) Grzesiek, S.; Anglister, J.; Ren, H.; Bax, A. *J. Am. Chem. Soc.* **1993**, *115*, 4369.

(3) Kushlan, D. M.; LeMaster, D. M. *J. Biomol. NMR* **1993**, *3*, 701.

(4) Kushlan, D. M.; LeMaster, D. M. *J. Am. Chem. Soc.* **1993**, *115*, 11026.

(5) Abragam, A. *Principles of Nuclear Magnetism*; Oxford University Press: Oxford, U.K., 1961; Chapter XI. Martin, J. F.; Vold, R. L.; Vold, R. R. *J. Magn. Reson.* **1983**, *51*, 164.

(6) Werbelow, L. G. *J. Chem. Phys.* **1979**, *70*, 5381. Fouques, C. E. M.; Werbelow, L. G. *Can. J. Chem.* **1979**, *57*, 2329. Bjorkstam, J. L.; Villa, M. *Phys. Rev. B.* **1980**, *22*, 5025. Werbelow, L. G.; Pouzard, G. *J. Phys. Chem.* **1981**, *85*, 3887. Bjorkstam, J. L. *J. Mol. Struct.* **1983**, *111*, 135.

(7) Werbelow, L. G. In *Nuclear Magnetic Probes of Molecular Dynamics*; Tycko, R., Ed.; Plenum: New York, 1994. Recent experimental observations of dynamic frequency shifts include the following: Butler, A.; Eckert, H. *J. Am. Chem. Soc.* **1989**, *111*, 2802. Aramini, J. M.; Germann, M. W.; Vogel, H. J. *J. Am. Chem. Soc.* **1993**, *115*, 9750.

(8) Pople, J. A. *Mol. Phys.* **1957**, *1*, 198.

0002-7863/94/1516-8400$04.50/0

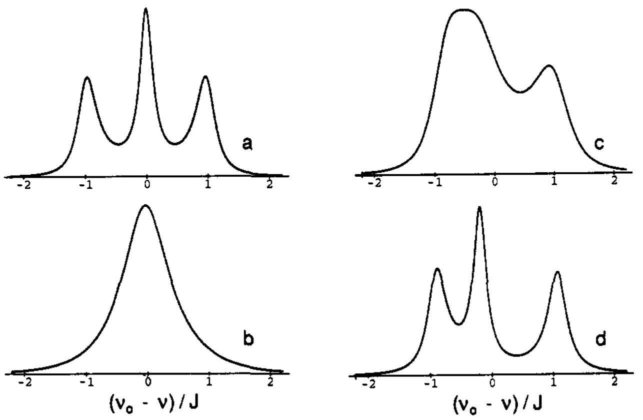

Figure 2. Plot of F(ν') (arbitrary units) versus reduced frequency ($\nu' = (\nu_0 - \nu)/J$), assuming $J = 136$ s^{-1} (21.6 Hz); ($\gamma_I\gamma_S\hbar/r_{IS}^3) \approx 2.2 \times 10^4$ s^{-1}; $(e^2qQ_S/\hbar) \approx 1.1 \times 10^6$ s^{-1}; $\omega_S = 5.8 \times 10^8$ s^{-1}. The simulations a, b, c and d, correspond to τ_2 = 0.1, 1.0, 8.1, and 30 ns, respectively. Relative vertical scaling factors for a, b, c, and d are 1.0, 1.2, 1.8, and 1.0, respectively.

upon passing into a slower correlation time domain, multiplet structure reappears bearing a markedly noncentrosymmetric line shape.

The observation of these dynamic frequency shifts in the present case reflects the absence of a contribution to the ^{13}C spectrum corresponding to the dominant adiabatic term, $J^Q(0)$. Further, the weaker adiabatic dipolar term $J^D(0)$, which becomes increasingly significant for the outer resonances of the multiplet as the motion slows, does not contribute to the central component. Further broadening of the central component will in general arise due to other interactions such as the shielding anistropy of spin I. These arguments nicely illustrate the care which must be exercised whenever adibatic contributions from dominant interactions are suppressed.[9]

The dynamic frequency shift being discussed arises only through interference (cross-correlation) between relaxation pathways operative at spin S. However, dipolar–quadrupolar interferences do not contribute to the dissipation of single quantum coherence. Conversely, shielding anisotropy–quadrupolar interferences provide important modifications to dissipative terms,[10] $(1/T_2)_m$, yet these latter interferences do not contribute to the dynamic frequency shift.

Whenever multiplet structure emerges on the slow motion side of the T_1 minimum, a sizable differential shift of the central and outermost components is expected. These shifts are 3 orders of magnitude larger than any dynamic shift normally associated with a spin system containing only spin-$^1/_2$ nuclei. Since the relative shift differences between the components of a multiplet are subject to detailed quantitative analysis, the present observations introduce the possibility of deriving dynamic information from this type of multiplet spectral data. Additionally, $L^{\text{D-Q}}(\omega_S)$, which represents the sine transform of the dipolar–quadrupolar cross-correlation function, introduces a very different functional dependence on τ compared with the more usual $J(\omega)$ (autocorrelation) and $K(\omega)$ (cross-correlation) spectral density terms (eqs 4), so that different motional regimes can be explored. Of course, the deuteron/proton substitution also enhances the sensitivity of the measurements to motions with lower frequency components near ω_D. The introduction of the additional information available from these dynamic frequency shift data should be of particular value in dealing with the generally underdetermined problem of complex macromolecular motions.

Although Figure 2c mimics well the primary features of the experimental spectra, quantitative agreement must include other interactions such as effects attributed to anisotropic chemical shieldings or additional dipolar couplings. A more detailed discussion of these features will be published elsewhere.

Acknowledgment. Stimulating conversations with Professor J. T. Gerig are gratefully acknowledged.

---

(9) Werbelow, L. G.; Thevand, A. *J. Magn. Reson. Ser. A* **1993**, *105*, 88.

(10) Werbelow, L. *J. Magn. Reson.* **1986**, *67*, 66. Werbelow, L. G.; Allouche, A.; Pouzard, G. *J. Chem. Soc., Faraday Trans. 2* **1987**, *83*, 891.