Use of a Comprehensive Approach to Molecular Dynamics in Ordered Lipid Systems: Cholesterol Reorientation in Oriented Lipid Bilayers. A ^{2}H NMR Relaxation Case Study†

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Abstract: In ordered molecular systems, anisotropic deuterium (^{2}H) spin–lattice relaxation affords a method of identifying motional modes with rates on the time scale of the Larmor frequency. Such information augments significantly the more usual temperature- and frequency-dependence relaxation studies. In order to demonstrate the potential of using the specific characteristics of anisotropic ^{2}H relaxation to elucidate details of molecular dynamics in lipid bilayers, cholesterol reorientation in bilayers of dipalmitoylphosphatidylcholine (1:1 mole ratio) was investigated. The present study demonstrates that while anisotropic T_1 behavior (β-dependence) can be observed in partially relaxed line shapes of unoriented dispersions in the absence of orientational averaging, a distinct advantage of oriented systems is that they circumvent any orientational-averaging effects of rapid lateral diffusion over the curved liposomal surfaces, a physical process that would thwart any attempt to observe anisotropic relaxation in powder samples. The fused rings of cholesterol present a rigid moiety whose molecular reorientation will relax ^{2}H nuclei at different sites at rates modulated only by the respective angles (θ) between the C–^{2}H bond and the motional averaging axis. This fact is exploited to test two simple but fundamentally disparate models for cholesterol dynamics in lipid bilayers, since a correct description of this system should simultaneously predict the anisotropic (β-dependence) relaxation behavior for all labeled sites on the steroid ring system. Simulations of the angular-dependent profiles of both spin–lattice relaxation times ($T_1(\beta)$, $T_{1Q}(\beta)$), together with the observed temperature dependence, establish that cholesterol reorients by random 3-fold jumps (large-angle motion) rather than by rotational diffusion (small-angle motion). This study shows the necessity of examining the complete relaxation profile, since monitoring only the extremes ($\beta = 0°$, $\beta = 90°$) may be insufficient to differentiate between possible motional models.

Deuterium (^{2}H) solid-state NMR continues to play a leading role in studies of dynamic disorder and molecular mobility in anisotropic systems (e.g., solids, liquid crystals, and membranes). Particularly for the study of molecular dynamics, ^{2}H NMR offers two principal advantages: First, since ^{2}H NMR line shapes and relaxation are dominated by the intramolecular quadrupolar interaction, interpretation of experimental results is usually not complicated by consideration of other inter- or intramolecular spin interactions. Second, ^{2}H NMR affords a technique that permits molecular dynamics to be probed over an extremely broad dynamic range (10^0–10^{10} Hz). Thus, for example, line-shape studies of DNA,[1a] proteins,[2a] and lipid systems[3a] have elucidated motions on the 10^3–10^6-Hz time scales, while spin–lattice relaxation measurements are sensitive to motions occurring on the time scale of the Larmor frequency (10^8–10^{12} Hz) in these systems.[1a-c,2b-e,3b-e] Recently, multipulse techniques[4] and two-dimensional chemical exchange experiments[5] have extended the frequency range to motions occurring on millisecond time scales or longer.

In the case of relaxation measurements, it has been long recognized that a potentially valuable source of information on molecular dynamics in these systems could be provided by measurements of the dependence of the ^{2}H spin–lattice relaxation rates on the orientation of the motional symmetry axis with respect to the magnetic field (T_1 anisotropy). Such measurements not only would put stringent constraints on models of molecular dynamics but would also facilitate the identification of the most significant relaxation pathways for ^{2}H nuclei in such systems. Despite these obvious benefits, and despite the fact that anisotropic relaxation behavior would be anticipated even for very simple motional models, it is surprising that such measurements have only recently begun to be exploited in a variety of systems.[1b,2b-e,3d,e,6a,b,7] Of these, lipid membranes constitute an important class of anisotropic molecular assemblies that have been probed extensively and effectively by ^{2}H NMR through both line-shape and relaxation studies.[3c,d,8a,b] However, the full power of the relaxation technique has only recently been brought to bear on these systems. Measurements of relaxation time anisotropies are a potentially powerful addition to the more conventional frequency- and temperature-dependent measurements traditionally relied on in this field.

Anisotropic spin–lattice relaxation has been detected in gel-phase lipid,[3d,e] bilayers containing cholesterol,[3d] and glycolipid systems.[7,9] However, for many lipid systems in the liquid-crystalline phase, lateral diffusion over the curved surface of the liposomes is an effective mechanism for complete averaging of relaxation time anisotropies.[10] The use of lipid bilayers aligned between glass plates eliminates the latter averaging, thereby permitting the measurement of relaxation time anisotropies for

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(1) (a) Brandes, R.; Vold, R. R.; Vold, R. L.; Kearns, D. R. *Biochemistry* **1986**, *25*, 7744–7751. (b) Kintanar, A.; Huang, W.-C.; Schindele, D. C.; Wemmer, D. E.; Drobny, G. *Biochemistry* **1989**, *28*, 282–293. (c) Kintanar, A.; Alam, T. M.; Huang, W.-C.; Schindele, D. C.; Wemmer, D. E.; Drobny, G. *J. Am. Chem. Soc.* **1988**, *110*, 6367–6372.

(2) (a) Rice, D. M.; Wittebort, R. J.; Griffin, R. G.; Meirovitch, E.; Stimson, E. R.; Meinwald, Y. C.; Freed, J. H.; Scheraga, H. A. *J. Am. Chem. Soc.* **1981**, *103*, 7707–7710. (b) Batchelder, L. S.; Niu, C. H.; Torchia, D. A. *J. Am. Chem. Soc.* **1983**, *105*, 2228–2231. (c) Griffin, R. G.; Beshah, K.; Ebelhauser, R.; Huang, T. H.; Olejniczak, E. T.; Rice, D. M.; Siminovitch, D. J.; Wittebort, R. J. In *The Time Domain in Surface and Structural Dynamics*; Long, G. J., Grandjean, F., Eds.; Kluwer Academic Publishers: Dordrecht, 1988; pp 81–105. (d) Sakar, S.; Young, P. E.; Torchia, D. A. *J. Am. Chem. Soc.* **1986**, *108*, 6459–6464. (e) Beshah, K.; Olejniczak, E. T.; Griffin, R. G. *J. Chem. Phys.* **1987**, *86*, 4730–4736.

(3) (a) Huang, T. H.; Skarjune, R. P.; Wittebort, R. J.; Griffin, R. G.; Oldfield, E. *J. Am. Chem. Soc.* **1980**, *102*, 7377–7379. (b) Blume, A.; Rice, D. M.; Wittebort, R. J.; Griffin, R. G. *Biochemistry* **1982**, *21*, 6220–6230. (c) Meier, P.; Ohmes, E.; Kothe, G. *J. Chem. Phys.* **1986**, *85*, 3598–3614. (d) Siminovitch, D. J.; Ruocco, M. J.; Olejniczak, E. T.; Das Gupta, S. K.; Griffin, R. G. *Biophys. J.* **1988**, *54*, 373–381. (e) Siminovitch, D. J.; Ruocco, M. J.; Olejniczak, E. T.; Das Gupta, S. K.; Griffin, R. G. *Chem. Phys. Lett.* **1985**, *119*, 251–255.

(4) Bloom, M.; Sternin, E. *Biochemistry* **1987**, *26*, 2101–2105.

(5) Schmidt, C.; Wefing, S.; Blumich, B.; Spiess, H. W. *Chem. Phys. Lett.* **1986**, *130*, 84–90.

(6) (a) Pope, J. M.; Walker, L.; Cornell, B. A.; Separovic, F. *Mol. Cryst. Liq. Cryst.* **1982**, *89*, 137–150. (b) Jarrell, H. C.; Jovall, P. A.; Smith, I. C. P.; Mantsch, H. H.; Siminovitch, D. J. *J. Chem. Phys.* **1988**, *88*, 1260–1263.

(7) Auger, M.; Carrier, D.; Smith, I. C. P.; Jarrell, H. C. *J. Am. Chem. Soc.*, in press.

(8) (a) Kimmich, R.; Schnur, G.; Schuermann, A. *Chem. Phys. Lipids* **1983**, *32*, 271–322. (b) Williams, G. D.; Beach, J. M.; Dodd, S. W.; Brown, M. F. *J. Am. Chem. Soc.* **1985**, *107*, 6868–6873, and references cited therein.

(9) Renou, J.-P.; Giziewicz, J. B.; Smith, I. C. P.; Jarrell, H. C. *Biochemistry* **1989**, *28*, 1804–1814.

(10) Brown, M. F.; Davis, J. H. *Chem. Phys. Lett.* **1981**, *79*, 431–435.

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lipids in both the gel and liquid-crystalline states.[6a,b,7,11] Since anisotropic relaxation effects in powder line shapes are most readily detected in the region of the null in an inversion-recovery experiment, where the S/N ratio is the poorest, the quantitative evaluation of these effects in such systems is often difficult. Moreover, such behavior easily escapes detection if the set of relaxation delays, τ, does not include sufficient points in the region of the null. Even though separate experiments are required for each orientation in oriented systems, the improvement in S/N ratio, resolution, and experimental flexibility more than compensates for the additional experiments, particularly since such an approach allows an unequivocal and unambiguous detection of T_1 anisotropies. Most importantly, in systems for which overlapping powder patterns are obtained for unoriented samples, oriented samples clearly facilitate measurements of relaxation rates for each inequivalent site in the molecule.

Recently, several ^{2}H NMR studies,[6a,b] including a preliminary analysis of experimental results on deuteriated cholesterol in oriented multibilayers of dipalmitoylphosphatidylcholine (DPPC),[11] demonstrated that by facilitating the measurement of relaxation time anisotropies, oriented systems could considerably enhance the power of the ^{2}H NMR relaxation technique as a method for testing motional models. Our choice of cholesterol as a subject for testing this methodology in lipid systems was suggested by several factors: First, since cholesterol in lipid bilayers exhibits fast-limit ^{2}H NMR line shapes, relaxation studies are mandatory for the determination of motional mechanisms and rates. The fact that previous relaxation studies on this system have shown that spin–lattice relaxation is dominated by a motion with a correlation time near the Larmor frequency[12a] suggests that anisotropic relaxation behavior in this system would provide a sensitive means of discriminating between motional models. Second, the fused rings of cholesterol present a rigid moiety whose molecular motion will relax ^{2}H nuclei at different sites at rates modulated *only* by the respective angles (θ_i) between the C–^{2}H bonds and the motional-averaging axis. This modulation of the relaxation rate by the molecular geometry is an essential feature of the relaxation behavior that any successful model must predict. Third, while the motion of the steroid in a lipid bilayer is expected to be simple enough to justify the consideration of somewhat simple but nevertheless realistic models, it is apparently complex enough to remain an outstanding unsolved problem.[12a,b]

The present study extends a previous study of cholesterol dynamics in lipid bilayers.[11] The motivation behind the present work was to highlight the general utility of oriented multibilayers for the facile and routine measurement of anisotropic ^{2}H spin–lattice relaxation effects in the recovery of Zeeman order (T_1) and the loss of quadrupolar order (T_{1Q}). In particular, for the case of cholesterol, the ability to discriminate between two simple but fundamentally disparate models would be an effective demonstration that such measurements, in combination with temperature- and frequency-dependent studies, could provide a means of critically testing possible *reasonable* motional models and their rates.

Experimental Procedures

Dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylcholine (DMPC) were obtained from Sigma Chemical Co., St. Louis, MO. Specifically deuteriated cholesterols (2,2,4,4,6-$^2H_5$ and α-7-$^2H_1$) were generous gifts of Drs. E. J. Parish (Auburn University) and T. Akiyama (University of Tokyo), respectively. Aqueous multilamellar dispersions for ^{2}H NMR consisted of 30 mol % of cholesterol in DMPC (100 mg of total lipid mixture) hydrated with a 3-fold excess of deuterium-depleted water (Aldrich Chemical Co., Milwaukee, WI) in a 5-mm (o.d.) sample tube. Hydrated samples were heated cyclically to 30 °C with vortex-mixing and freeze–thawed to homogeneity (three cycles). For the preparation of oriented samples, a mixture of labeled cholesterol and DPPC (1:1 mole ratio) was dissolved in chloroform–methanol (1:1, v/v) and the mixture dropped onto 22 × 7 × 0.15 mm glass plates and allowed to dry. The plates were stacked in a 10-mm (o.d.) NMR tube and dried under vacuum for 5 h. The sample was hydrated for 6–8 h at 44 °C under a humid atmosphere of ^{2}H-depleted water. A drop of ^{2}H-depleted water was added and the tube sealed. ^{31}P and ^{2}H NMR were used to check the quality of sample alignment.

^{2}H NMR spectra were acquired at 30.7 MHz on a "home-built" solid-state spectrometer. Spin–lattice relaxation times T_1 and T_{1Q} were determined with a phase-cycled Jeener–Broekaert sequence (90_x–T–45_y–τ–45_y–AQ), from an analysis of the sum and difference magnetizations as described previously.[13] Values of T_1 were also obtained by the standard inversion–recovery–quadrupolar-echo sequence (180_x–τ–90_x–T–90_y–AQ).[12a,14] Pulse spacings in the quadrupolar echo detection sequence were typically 60 μs with $\pi/2$ pulses of either 3.5–4 μs (10-mm coil) or 2.7 μs (5-mm coil). For either sequence, the recycle time was greater than $5T_1$. Samples were enclosed in a glass jacket where the temperature was regulated to within ±0.5 °C. Typically a set of 15–20 recovery delays, τ, were used in the determination of the relaxation times. The standard error in the exponential fitting of the data was less than 5%, except for sample orientations, β, less than 20° for which the fitting errors were <15%.

Simulations of relaxation time anisotropies were performed on a VAX-11/780 or on a μVAXII computer with a slight modification of a line-shape simulation program[15] based on the general formalism of Torchia and Szabo.[16a] For each value of β the relaxation time was powder-averaged over all azimuthal angles α, where β and α are the polar and azimuthal angles, respectively, specifying the director orientation with respect to the magnetic field $\mathbf{H}_0$. Each simulated relaxation profile was defined by 100 values of β equally spaced between 0° and 90°.

Results and Discussion

The molecular dynamics of cholesterol at 30 mol % in DMPC has been investigated previously by ^{2}H NMR.[12a] While the relaxation rate was found to be dependent on the site of labeling in the rigid part of the molecule, no dependence on the orientation of the motional symmetry axis with respect to the magnetic field (angle β) was observed. However, when the inversion–recovery experiment was later repeated as part of this study for the same system containing [2,2,4,4,6-$^2H_5$] cholesterol with a set of relaxation delays, τ, judiciously chosen in the region of the null, a dependence of the relaxation rate on β is evident (Figure 1A). It is clear that, for such a multiply labeled molecule, the superposition of several powder patterns that have different relaxation rates associated with the site of labeling makes the detection of anisotropic relaxation behavior difficult. In addition, even for a singly labeled molecule, just to detect, let alone to define adequately, the nature of the anisotropy from the partially relaxed line shapes of powder spectra, requires a careful and judicious selection of τ-values near the null point. These problems, particularly that associated with the choice of τ-delays, perhaps explain why a previous study of the cholesterol–DMPC system did not detect anisotropic spin–lattice relaxation.[12a] On the other hand, a study of [3-$^2H_1$]cholesterol equimolar in *N*-palmitoylgalactosylsphingosine (NPGS) in the liquid-ordered phase reported a T_1 anisotropy qualitatively similar to that shown in Figure 1A.[3d] In the present study, we reexamine cholesterol dynamics using anisotropic relaxation in the more ordered equimolar cholesterol–DPPC system.

To circumvent the difficulties associated with overlapping powder spectra, and to extract the detailed features of the relaxation time anisotropies unobscured by the averaging effects of lateral diffusion, the cholesterol–DPPC system was aligned between glass plates. Inspection of Figure 1B reveals that the recovery rate for each molecular site is readily monitored in the partially relaxed (inversion–recovery) spectra of the multiply

(11) Bonmatin, J. M.; Smith, I. C. P.; Jarrell, H. C.; Siminovitch, D. J. *J. Am. Chem. Soc.* **1988**, *110*, 8693–8695.
(12) (a) Dufourc, E. J.; Smith, I. C. P. *Chem. Phys. Lipids* **1986**, *41*, 123–135. (b) Brainard, J. R.; Szabo, A. *Biochemistry* **1981**, *20*, 4618–4628. (c) Taylor, M. G.; Akiyama, T.; Saito, H.; Smith, I. C. P. *Chem. Phys. Lipids* **1982**, *34*, 359–379. (d) Taylor, M. G.; Akiyama, T.; Smith, I. C. P. *Chem. Phys. Lipids* **1981**, *29*, 327–339.
(13) Vold, R. L.; Dickerson, W. H.; Vold, R. R. *J. Magn. Reson.* **1981**, *43*, 213–223.
(14) Davis, J. H.; Jeffrey, K. R.; Bloom, M.; Valic, M. I.; Higgs, T. P. *Chem. Phys. Lett.* **1976**, *42*, 390–394.
(15) Wittebort, R. J.; Olejniczak, E. T.; Griffin, R. G. *J. Chem. Phys.* **1987**, *86*, 5411–5420.
(16) (a) Torchia, D. A.; Szabo, A. *J. Magn. Reson.* **1982**, *49*, 107–121. (b) Woessner, D. E. *J. Chem. Phys.* **1962**, *36*, 1–4. (c) Wallach, D. *J. Chem. Phys.* **1967**, *47*, 5258–5268.

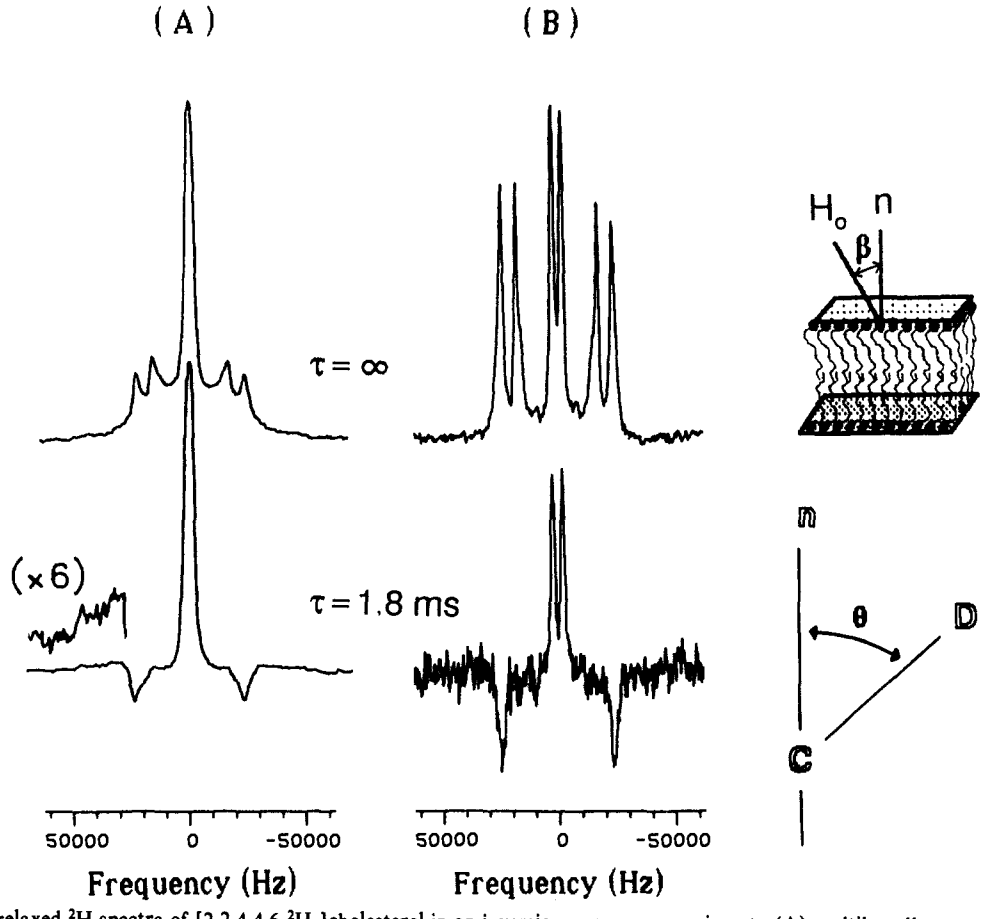

Figure 1. Partially relaxed ^2H spectra of [2,2,4,4,6-$^2H_5$]cholesterol in an inversion–recovery experiment: (A) multilamellar unoriented dispersion of cholesterol (50 mg, 30 mol %) in DMPC at 30 °C, 800K accumulations; (B) oriented multibilayers (β = 90°) of cholesterol (15 mg, 50 mol %) in DPPC at 30 °C, 30K accumulations. In both cases, spectra near the null clearly demonstrate that relaxation is not isotropic. In the powder spectrum shown in A, the anisotropy is a manifestation of two geometric effects: (i) director orientation (β) with respect to $\mathbf{H}_0$; (ii) orientation (θ_i) of each labeled site with respect to the director. For the oriented sample spectrum B, only the effects of θ_i are present (fixed β).

labeled cholesterol. Thus, for each orientation β of the sample (Figure 1) (corresponding to the director orientation), the dependence of the relaxation on molecular geometry at each site (θ_i) can be determined unambiguously; note for example when τ = 1.8 ms (Figure 1B). Then, by simply changing the sample orientation, the relaxation time dependence on the angle β (T_1 anisotropy in this case) may now be determined for each molecular position. Such measurements explicitly separate the dependences of spin–lattice relaxation on molecular geometry (θ_i) and on the orientation β of the director. In the case of an unoriented system (Figure 1A, τ = 1.8 ms), spectral overlap clearly makes such a separation difficult. Note also that the spectrum shown in Figure 1A (bottom) is the result of 800K accumulations, while the corresponding oriented sample spectrum (Figure 1B) required less than $^1/_{30}$th of this time.

For the deuterium nucleus in anisotropic systems, two spin–lattice relaxation times, T_1 and T_{1Q}, are available through the Jeener–Broekaert pulse sequence.[13] Thus, through the use of oriented samples, the anisotropies of each of these relaxation times may be readily measured. Typical results for oriented multibilayers of [α-7-$^2H_1$]cholesterol in DPPC are displayed in Figure 2. Inspection of Figure 2 reveals clearly that as β is varied from 0° to 90°, the T_1 relaxation times for the axial deuterons at the C2-, C4-, and C7-positions of cholesterol range from 2.2 to 3.8 ms, corresponding to an anisotropy factor [T_1(90°)/T_1(0°)] of ca. 1.7. Relaxation of quadrupolar order, T_{1Q}, is also anisotropic, with a corresponding anisotropy factor [T_{1Q}(90°)/T_{1Q}(0°)] of ca. 0.5. Although in principle such profiles augment previous temperature- and frequency-dependence studies of cholesterol dynamics, can such data be utilized to extract rates and types of molecular motion?

Extracting this information depends upon a judicious choice of model for the system of interest and a method of calculating

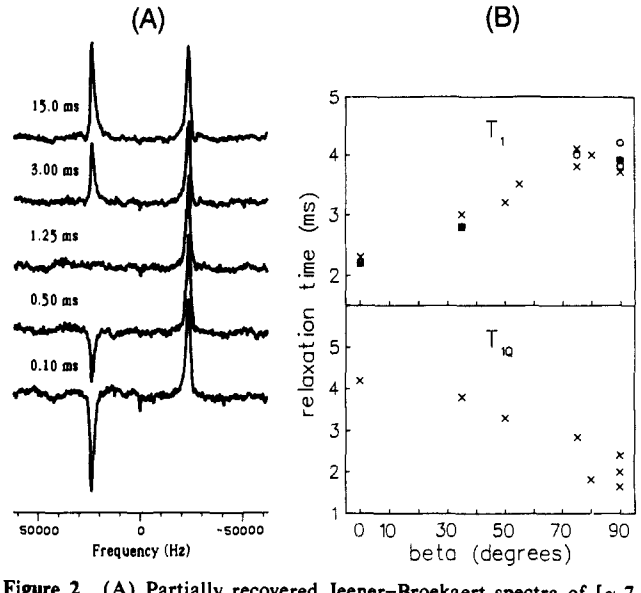

Figure 2. (A) Partially recovered Jeener–Broekaert spectra of [α-7-$^2H_1$]cholesterol (50 mol %) in DPPC bilayers oriented at β = 90°. (B) Orientation dependence of the relaxation times (T_1, T_{1Q}) for the C7 (×), C2 (axial) (○), and C4 (axial) (□) positions. The $T_1(\beta)$ data in B were obtained by both the inversion–recovery and Jeener–Broekaert sequences, demonstrating that both experiments gave similar results. The estimated error in setting the angle β is ±3° as determined from multiple measurements. Measurements on different sample preparations indicated the results are reproducible to within ±10%.

the anisotropy in the spin–lattice relaxation times for the chosen model of C–^{2}H bond reorientation, whether discrete or diffusive.

Accordingly, we begin our discussion of the cholesterol results with a brief outline of the general formalism we will use for calculating the orientation dependence of relaxation times. When the master equation for the time evolution of the density matrix is solved, the ^2H spin–lattice relaxation rates are given by[17a,b]

$$1/T_1 = \tfrac{1}{3}\omega_Q^2[J_1(\omega_0) + 4J_2(2\omega_0)] \quad (1)$$

$$1/T_{1Q} = \tfrac{1}{3}\omega_Q^2[3J_1(\omega_0)] \quad (2)$$

where $\omega_Q = {}^3/_4 e^2qQ/\hbar$ and e^2qQ/h is the quadrupolar coupling constant (170 kHz for alkane[18a] and 175 kHz for olefinic C–^{2}H[18b]). The spectral density functions $J_m(\omega)$ are related to the Fourier transform of the autocorrelation functions $G_m(t)$ by the Wiener–Khintchine theorem:[19a-c]

$$J_m(\omega) = 2\int_0^\infty G_m(t)\cos(\omega t)\,dt \quad (3)$$

The autocorrelation functions, expressed in the notation of Torchia and Szabo,[16a] are given by

$$G_m(t) = \sum_{a,a'=-2}^{2} d^{(2)}_{ma}(\beta)\, d^{(2)}_{ma'}(\beta)\exp[i(a-a')\alpha]\, G_{aa'}(t) \quad (4a)$$

where

$$G_{aa'}(t) = \langle D^{(2)}_{0a}{}^*(0,\theta(0),\phi(0))\, D^{(2)}_{0a'}(0,\theta(t),\phi(t))\rangle \quad (4b)$$

and the brackets indicate an ensemble average. In eq 4a the angles β and α are the spherical polar angles that specify the director (in this case the bilayer normal, i.e., the normal to the plane of the glass plates for the aligned sample) with respect to the external magnetic field, and $d^{(2)}_{ma}(\beta)$ are the reduced Wigner rotation matrix elements. The relaxation time anisotropies ($T(\beta,\alpha)$, $T = T_1, T_{1Q}$) are determined by those terms in eq 4a (exclusive of $G_{aa'}(t)$) that are functions of the angles β and α only, of which the angle β is under the control of the experimenter via sample rotation (Figure 2). Since there is no control over the angle α, the experimentally accessible quantities are in fact $T(\beta) = \overline{T(\beta,\alpha)}$, where the bar denotes a powder average over all azimuthal angles α. In eq 4b, the angles θ and ϕ specify the orientation of the C–^{2}H bond vector with respect to the director, and $D^{(2)}_{0a}{}^*(0,\theta,\phi)$ are the Wigner rotation matrix elements. The above general expressions simplify considerably when we consider a class of models in which C–^{2}H bond reorientation about a symmetry axis does not alter the angle θ. Such models, discussed in some detail by Torchia and Szabo,[16a] are appropriate for describing the reorientational motions of C–^{2}H bond vectors on the rigid moiety of cholesterol as it reorients about its motional-averaging axis. For these models

$$G_{aa'}(t) = d^{(2)}_{0a}(\theta)\, d^{(2)}_{0a'}(\theta)\, \Gamma_{aa'}(t) \quad (5)$$

where since θ is fixed, the ensemble-averaging problem is reduced to evaluating the *azimuthal* autocorrelation function

$$\Gamma_{aa'}(t) = \langle \exp[ia\phi(0)]\exp[-ia'\phi(t)]\rangle \quad (6)$$

Within this class of models, we shall consider two of the simplest possibilities for cholesterol reorientation about its motional-averaging axis, either random jumps with rate constant K among three equivalent sites or free diffusion with diffusion coefficient D. In either case, $\Gamma_{aa'}$ is readily evaluated, yielding the following simple expressions for $\Gamma_{aa'}(t)$:

jump[16a]

$$\Gamma^J_{aa'}(t) = e^{-\lambda_a t} \qquad a = a' \,(\mathrm{mod}3)$$

$$\Gamma^J_{aa'}(t) = 0 \qquad \text{otherwise} \quad (7)$$

where

$$\lambda_a = 4K\sin^2(\pi a/3)$$

diffusion[16b,c]

$$\Gamma^D_{aa'}(t) = \delta_{aa'}e^{-a^2Dt} \quad (8)$$

Note that

$$\lim_{N,K\to\infty}\Gamma^J_{aa'}(t) = \Gamma^D_{aa'}(t)$$

if $D = 4\pi^2K/N^2$ is kept constant.[16a] These simple forms for the azimuthal autocorrelation functions $\Gamma_{aa'}(t)$ can be used to derive closed-form expressions for the respective spin–lattice relaxation rates; these are given in ref 16a for the Zeeman relaxation rates T_1^{-1} and will not be repeated here.

The ensemble average of eq 4b, governed in general by both molecular geometry and details of the molecular motion, reduces in this class of models to a much simpler form in which the effects of molecular geometry and motion are explicitly separated (eq 5). Moreover, the effects of molecular geometry, now determined exclusively by the time-independent angle θ in the elements of the reduced Wigner matrices (eq 5), have been removed from the ensemble average, leaving only the effects of molecular motion in the time dependence of the azimuthal angle $\phi(t)$. Although the azimuthal autocorrelation function is not under direct control of the experimenter, it can nevertheless be varied through its dependence on temperature and Larmor frequency. Both of these dependences were investigated in previous studies of cholesterol.[12a,c]

Going beyond evaluation of the individual spectral densities from measurements of T_1 and T_{1Q}, further interpretation requires a description of molecular dynamics in terms of specific motional models. This is equivalent to evaluating eq 4b in the framework of a given motional description. Equations 4a and 4b can be evaluated explicitly, either by computer[15] or, for a limited number of simple cases,[2b,16a-c,20] by analytical means, to give spectral densities and their associated anisotropic (α,β) behavior. Recently, several examples relating to methyl group dynamics in solids have demonstrated that such an approach can discriminate between a 3-fold jump and diffusion about the symmetry axis.[2a-e]

The β-phase of cholesterol–DPPC mixtures, found at concentrations greater than about 22 mol % cholesterol, is characterized by highly ordered (rigid) chains undergoing rapid axially symmetric reorientation.[21] In this β-phase at 50 mol % cholesterol, where molecular ordering is nearly unity, motion of the steroid about the molecular long axis is expected to be a reasonable starting point for modeling molecular dynamics, particularly since three previous studies on this system have suggested that such a motion dominates ^2H spin–lattice relaxation in these systems.[12a-c] Henceforth, we shall assume that cholesterol reorientation about its motional averaging axis is governed by a single motion that may be either diffusive or discrete in nature. The simplest discrete motion consistent with the axial symmetry of the β-phase spectra is a stochastic jump between three equivalent sites. With use of a modified version of a line-shape relaxation program[15] based on the formalism of Torchia and Szabo[16a] (vide supra), values of T_1 and T_{1Q} for the 0° and 90° orientations were calculated for the jump and diffusion models as a function of the jump rate K and the diffusion constant D, respectively, for [α-7-$^2H_1$]cholesterol. Inspection of Figure 3 reveals that, for both models, for all correlation times, the anisotropy $T_1(90°)/T_1(0°)$ is greater than 1. However, for T_{1Q}, the corresponding anisotropy is less than unity for both models. In addition, the degree of anisotropy is sensitive to both the model and the motional rate. Although the experimental relaxation times may be reproduced by both models with motional rates on either side of the minimum, the evident sensitivity of the anisotropy to the motional rate may be used to discriminate between the two possible correlation times for each

(17) (a) Abragam, A. In *The Principles of Nuclear Magnetism*; Oxford University Press: London, 1961; Chapter 8. (b) Jeffrey, K. R. *Bull. Magn. Reson.* **1981**, *3*, 69–82.

(18) (a) Burnett, L. J.; Muller, B. B. *J. Chem. Phys.* **1971**, *55*, 5829–5831. (b) Kowalewski, J.; Lindblom, T.; Vestin, R.; Drakenberg, T. *Mol. Phys.* **1976**, *31*, 1669–1676.

(19) (a) Wiener, N. *Acta Math.* **1930**, *55*, 117–258. (b) Khintchine, A. *Math. Ann.* **1934**, *109*, 604–615. (c) McQuarrie, D. A. *Statistical Mechanics*; Harper & Row: New York, 1976.

(20) Jeffrey, K. R.; Wong, T. C.; Tulloch, A. P. *Mol. Phys.* **1984**, *52*, 307–318.

(21) Vist, M. R.; Davis, J. H. *Biochemistry*, in press.

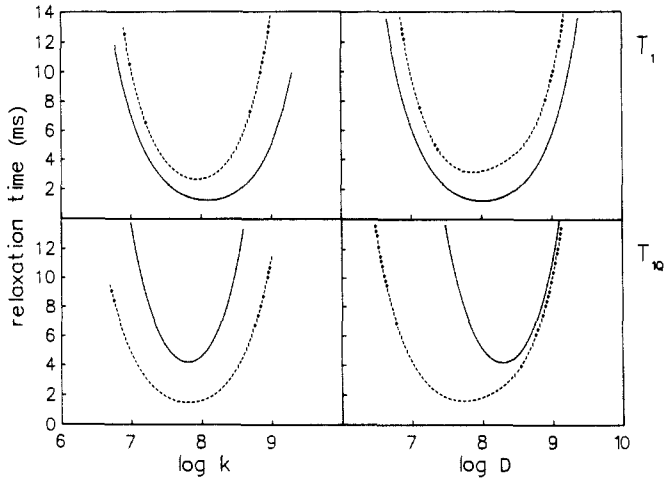

Figure 3. Simulated dependence of the spin–lattice relaxation times, T_1 and T_{1Q}, on the motional rate for the diffusion (right) and 3-fold jump (left) models. Results were simulated with e^2qQ/h = 170 kHz, a geometrical angle (θ) of 77°, and two director orientations: (···) β = 90°; (—) β = 0°. Note that the predicted anisotropy ratio T(90°)/T(0°) (T = T_1, T_{1Q}) is sensitive to motional rate for both models and may be useful in distinguishing between the two correlation times that are possible for a given relaxation time.

model. Despite the sensitivity of the magnitude of the anisotropy to correlation time, the T_1 anisotropy for both models remains greater than unity over 4 decades in motional frequencies, spanning the short correlation time regime, through the minimum, to the long correlation time regime. This behavior should be contrasted with that of the methyl group (θ = 70.3°) for which the diffusion model predicts isotropic relaxation (i.e., $T_1(90°)/T_1(0°)$ = 1) in the short correlation time limit.[2b,16a]

Anisotropy Dependence on Motional Models and Rates. A preliminary analysis of previous results from this laboratory reported that, for this equimolar cholesterol–DPPC system, the three-site jump model with a jump rate in the fast-motional regime could reproduce most of the general features of the observed anisotropic T_1 behavior (Figure 4).[11] However, for the same motional model, the slow-motional regime was not investigated. For this motional description, $T_1(\beta)$ profiles were calculated for the C7 label, varying the jump rate K, with $\omega_0/3K < 1$, to obtain the best fit to $T_1(\beta)$ (Figure 4). For this labeled position, the relaxation behavior can, in fact, be reproduced with exchange rates of either 3.2 × 10^7 or 3 × 10^8 s^{-1}. However, since the A and B rings of cholesterol represent a rigid molecular framework, deuterons on either ring must experience the same molecular motion about the motional-averaging axis and, therefore, relax at rates modulated only by the molecular geometry (angle θ) at the labeled site. Thus, the motional rate that best reproduces the $T_1(\beta)$ profile for the C7 label for the two models under consideration should also adequately reproduce the corresponding profiles for other labeled positions for which only the angle θ will differ. Accordingly, $T_1(\beta)$ profiles were calculated for the axial (C2, C4, θ = 77°), equatorial (C2, C4, θ = 68°), and olefinic (C6, θ = 56°) sites (Figure 4) with the two possible jump rates determined from fitting the C7 label profile. It is clear that whether absolute values or profiles are considered, the slower rate reproduces the experimental relaxation data with better fidelity than does the faster rate. Note in particular that, for a θ-value of 56°, the maximum in $T_1(\beta)$ at β = 55° is well reproduced. Thus, from $T_1(\beta)$ profiles for the jump model, the faster jump rate may be excluded, a conclusion supported by temperature-dependent results (vide infra). It should be noted that the results presented in Figure 4 demonstrate that measurements of spin–lattice relaxation rates at only 0° and 90° would be insufficient to critically assess anisotropic relaxation. This is particularly evident for results with the C6 label (Figure 4), where $T_1(90°)$ and $T_1(0°)$ are very similar.

Although at this point the jump model appears to adequately reproduce the relaxation data, how unique is such a model? Could

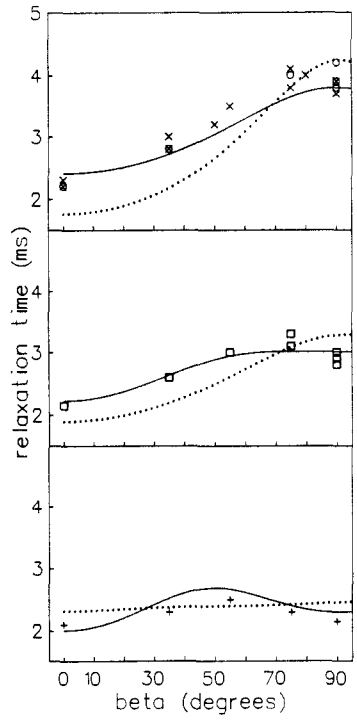

Figure 4. Experimental (symbols) and calculated (lines) ^2H spin–lattice relaxation times for oriented multibilayers of cholesterol (50 mol %) in DPPC at 30 °C as a function of the angle β. Labeled cholesterol positions are indicated as C7 (θ = 75°, ×); C2, C4 axial (θ = 75–77°, ○) (top); C2, C4 equatorial (θ = 68°, □) (middle); and C6 (θ = 56°, +) (bottom). $T_1(\beta)$ profiles were calculated for each labeled site with an axial 3-fold jump model and the following exchange rates: (—) K = 3.2 × 10^7 s^{-1}; (···) K = 3 × 10^8 s^{-1}. The angles θ_i are taken from Taylor et al.[12d]

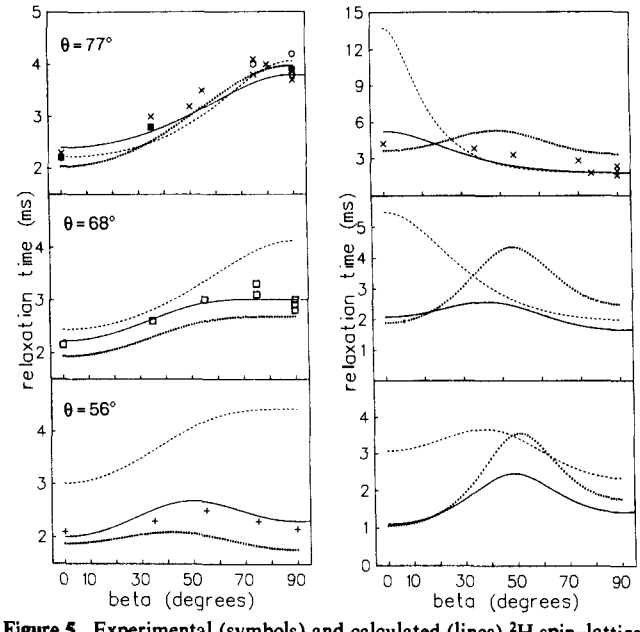

Figure 5. Experimental (symbols) and calculated (lines) ^2H spin–lattice relaxation times (T_1, left; T_{1Q}, right) for oriented multibilayers of cholesterol (50 mol %) in DPPC at 30 °C as function of the angle β. Labeled cholesterol positions are indicated as C7 (θ = 75°, ×); C2, C4 axial (θ = 75–77°, ○) (top); C2, C4 equatorial (θ = 68°, □) (middle); and C6 (θ = 56°, +) (bottom). Relaxation profiles were calculated for each labeled site with the following models: axial 3-fold jump model (—) K = 3.2 × 10^7 s^{-1}; continuous diffusion (···) D = 3.2 × 10^8 s^{-1}, (- - -) D = 3.0 × 10^7 s^{-1}.

the most reasonable alternative model, namely axial diffusion, also be an equally satisfactory motional description? To address these questions, for the two models, the complete $T_1(\beta)$ profiles were calculated for the [α-7-$^2H_1$]cholesterol (θ = 77°) system (Figure

5, top). The jump rate was set at its best fit value ($K = 3.2 \times 10^7$ s^{-1}), while for axial diffusion values of the diffusion constant D were selected on either side of the T_1 minimum such that both relaxation times and anisotropies were reproduced as well as possible. Inspection of Figure 5 (top) indicates that for both motional descriptions and, in the case of diffusion, the two possible motional rates, calculated $T_1(\beta)$ profiles reproduce experiment equally well. Thus, these data from only one labeled site cannot be used to distinguish between models or rates. While the results of Figure 3 would suggest that for either model discriminating between the two possible correlation times should be feasible from $T_1(\beta)$, in this case (near the T_1 minimum) for axial diffusion, no clear choice is possible. Alternatively, inspection of Figure 3 would suggest that, for the diffusion model, $T_{1Q}(\beta)$ profiles from different sides of the T_{1Q} minimum should be substantially different. In fact, as Figure 5 (top right) shows, the calculated $T_{1Q}(\beta)$ profiles for the diffusion model differ so substantially that the slow-motion case could be ruled out immediately. Note in particular the large discrepancy between the predicted value of $T_{1Q}(0°)$ for the slow-diffusion model and the experimental value. However, in comparison to the slow-motion case, the fast-motion counterpart for the diffusion model clearly agrees much better with experiment. Even though the profile for the slow-motion jump model represents the data somewhat better, at this point an unambiguous discrimination between the jump model (slow limit) and diffusion model (fast limit) cannot yet be made.

Since the motional rates that best reproduce the $T_1(\beta)$ profile for the C7 label for the two models under consideration should also adequately reproduce the corresponding profiles for other labeled positions on the rigid steroid rings for which only the angle θ will differ, $T_1(\beta)$ profiles of the diffusion model and the two possible diffusion rates were therefore calculated for the axial deuterons at C2 and C4 ($\theta = 77°$), equatorial positions at C2 and C4 ($\theta = 68°$), and the olefinic site at C6 ($\theta = 56°$) in Figure 5 (top, middle, and bottom, respectively). The relaxation rate is clearly sensitive to geometry and is particularly so when the director orientation β approaches 90°. Thus, for $\beta = 90°$, the observed T_1 decreases from 3.8 to 2.1 ms when θ changes from 77° to 56°, respectively. Interestingly, when $\beta = 0°$, T_1 is nearly identical for the three geometric sites. For these three sites, the anisotropy ratios are found to be 1.7, 1.4, and 1 for deuterons in the axial, equatorial, and olefinic sites, respectively. Note that, for the C6-position ($\theta = 56°$), T_1 goes through a maximum near $\beta = 55°$, a feature that distinguishes this from the other $T_1(\beta)$ profiles, which are instead characterized by monotonically increasing values of $T_1(\beta)$ over the same angular range. The calculated profiles presented in Figure 5 demonstrate that the specific nature of the profile is dependent not only on the type and rate of molecular motion but on the molecular geometry at each labeled site (θ_i). Recall that, on the basis of the predicted $T_{1Q}(\beta)$ profiles for the C7 label alone (vide supra), axial diffusion in the long correlation time regime could be rejected as a valid description of cholesterol dynamics. The $T_1(\beta)$ profiles for the other cholesterol positions (Figure 5), particularly those for the C6-position, clearly support this conclusion. Thus, while slow diffusion can reproduce T_1 anisotropies for sites having θ-values near 77°, such a model is consistent with neither the $T_{1Q}(\beta)$ profile at this position nor the $T_1(\beta)$ profiles at the other two.

It is readily apparent from a comparison of the simulated angular-dependent $T_1(\beta)$ and $T_{1Q}(\beta)$ profiles in Figure 5 that, for any labeled position, the differences between models are more pronounced for the $T_{1Q}(\beta)$ profiles than for the corresponding $T_1(\beta)$ profiles. Furthermore, for any particular model, the variations from site to site are also more pronounced for the $T_{1Q}(\beta)$ profiles. The corresponding jump and diffusion models for CH_3 group reorientation provide a similar but more striking example of differences in T_1 and T_{1Q} relaxation behavior.[16a] In the extreme narrowing limit, T_1 for a freely diffusing methyl group is isotropic,[16a] whereas T_{1Q} is anisotropic. On the other hand, for 3-fold reorientation, T_1 is predicted to be anisotropic, allowing a clear distinction between diffusive and jump models. The complete cancellation of the angular dependences of $J_1(\omega_0)$ and $J_2(2\omega_0)$ in the linear combination $\{J_1(\omega_0) + 4J_2(2\omega_0)\}$ for a freely diffusing methyl group is a somewhat extreme example of a more general behavior recognized previously by Wefing et al.,[22] who noted that because $J_1(\omega_0)$ and $J_2(2\omega_0)$ have different angular dependences that partly cancel each other, the line-shape changes of partially relaxed spectra observed in T_1 experiments may only be minor compared to those observable in T_{1Q} experiments. This difference in the angular-dependent behavior of T_1 and T_{1Q} would imply that, in powder and oriented spectra, partially relaxed Jeener–Broekaert (or spin alignment) spectra may be more useful than partially relaxed inversion–recovery spectra in discriminating between different motional models, a conclusion borne out by T_1 experiments on rotating phenyl and methyl groups.[22] The pronounced differences in the anisotropic behavior of T_1 and T_{1Q} seen here for labeled cholesterol, and previously for labeled lipid,[6b] point to a partial (if not complete) cancellation of the angular dependences of $J_1(\omega_0)$ and $J_2(2\omega_0)$, suggesting that T_{1Q} experiments may also prove to be a more useful strategy for testing models of molecular dynamics in these systems.

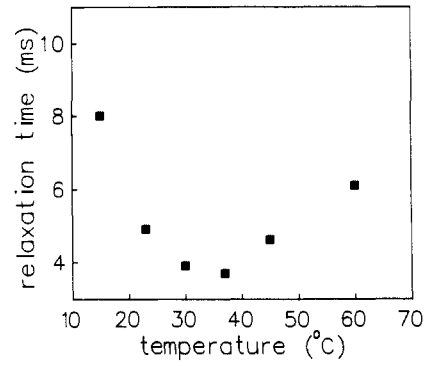

Figure 6. Temperature dependence of T_1 for oriented ($\beta = 90°$) multibilayers of [α-7-^{2}H$_1$]cholesterol (50 mol %) in DPPC.

Temperature Dependence of T_1. On the basis of β-dependences of spin–lattice relaxation times, the choice between a 3-fold jump ($K = 3.2 \times 10^7$ s^{-1}) and axial diffusion ($D = 3.2 \times 10^8$ s^{-1}) about the molecular long axis is not clear-cut, although the 3-fold jump model provides a somewhat better fit. However, the fact that their respective motional rates lie on opposite sides of the T_1 (T_{1Q}) minimum means that these models must have opposite temperature dependences, a striking and easily experimentally verifiable difference that can now be exploited to distinguish between the two models. The temperature dependence of $T_1(\beta = 90°)$ for [α-7-^{2}H]cholesterol (Figure 6) exhibits a minimum value of 3.7 ms at 37 °C. Previous ^{2}H NMR studies of cholesterol–lipid systems have reported similar temperature dependences and relaxation minima for labeled cholesterol.[12a,c] The observation of a T_1 minimum at 37 °C means that, at 30 °C, molecular motion must be in the long correlation time regime, conclusively eliminating fast diffusion as a possible model. Furthermore, Figure 6 confirms the previous discrimination by $T_1(\beta)$ (Figure 3) between the two possible exchange rates for the jump model by establishing the rate to be in the slow limit. Analysis of the T_1 temperature dependence in terms of an Arrhenius relationship leads to an activation energy of 30 ± 4 kJ·mol^{-1}, which is in close agreement with a value of 32 ± 5 kJ·mol^{-1} reported for cholesterol in DMPC at 30 mol %.[12a]

Frequency Dependence of Spin–Lattice Relaxation Times. The motional models have been subjected to several stringent criteria: First, for a single-labeled site, the model was required to reproduce the $T_1(\beta)$ profile by varying only the rate of motion. Second, the model and rate that adequately describe the T_1 relaxation rates were required to reproduce the corresponding T_{1Q} results. Third, the dynamics concluded for one site (one value of θ) had to reproduce results for other molecular sites (other θ's). Last, the model and rate of motion had to be fully consistent with the temperature dependence of the relaxation rates. Of the two

(22) Wefing, S.; Jurga, S.; Spiess, H. W. *22nd Ampere Congress Proceedings*, Zürich Ampere Committee: Zürich, 1984; pp 375–376.

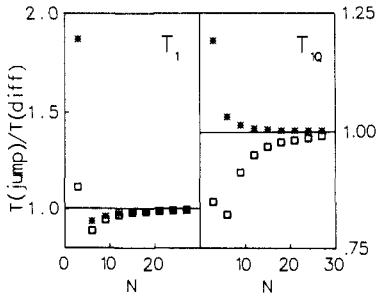

Figure 7. Relative comparison of the relaxation times predicted for the diffusion model (solid line) with those calculated as a function of the number of sites with an N-site jump model for two orientations of the motional axis: $\beta = 90°$ (*) and $\beta = 0°$ (□). Simulations were performed with $\theta = 77°$ and a rotational diffusion rate $D = 1.4 \times 10^8\ s^{-1}$. For the jump model, the exchange rates were calculated according to the relation[16a] $K_N = DN^2/4\pi^2$. Note that when $N = 3$, $K = 3.2 \times 10^7\ s^{-1}$, which is the rate found for 3-fold reorientation of cholesterol in this study.

reasonable but simple descriptions for cholesterol dynamics tested by this protocol, only a jump between three equivalent sites with a rate in the slow-motional regime survived. If the jump rate is such that $\omega_0/3K > 1$, a frequency dependence of relaxation times is expected. Frequency-dependent studies are a well-established approach to testing motional models in lipid systems.[12a,23a,b] A previous ^2H relaxation study of cholesterol in DMPC (30 mol %)[12a] reported that, for the two Larmor frequencies, 30.7 and 46.1 MHz, the ratio $T_1(46.1)/T_1(30.7)$ for sites having $\theta = 77°$ varied from 2 (15 °C) to 1.0 (50 °C). A comparison of the temperature dependence of T_1 (Figure 6) at 30.7 MHz for the cholesterol–DPPC system with that reported for the cholesterol–DMPC mixture[12a] reveals a striking similarity in relaxation behavior with the two profiles offset by approximately the difference in the phase-transition temperatures of the pure lipids. Thus, at the same relative temperatures, cholesterol in the two systems has nearly identical T_1 values (3.8 ms, DPPC; 4.1 ms, DMPC) and should exhibit the same frequency dependences. For the DMPC system the ratio $T_1(46.1)/T_1(30.7)$ was 1.9, which is in accord with a value of 2.0 calculated with the jump model and $K = 3.2 \times 10^7\ s^{-1}$. Thus, the model and its associated jump rate provide an adequate description of the frequency dependence.

Alternative Models. The preceding analysis of the anisotropic relaxation data has focused on two simple but fundamentally disparate models for cholesterol reorientation in lipid bilayers. The general problem of distinguishing between discrete and diffusive motions, reduced to its simplest but most extreme form in the consideration of 3-fold hops and free-diffusion models for cholesterol (vide supra) or methyl group reorientation,[16a] can be seen from a slightly different perspective by noting that the limiting form ($N \rightarrow \infty$) of the jump model azimuthal autocorrelation function (eq 8) is identical with that of the diffusion model. This identity raises the following questions: (a) Could jump models involving nearest-neighbor jumps among more than three sites account for all of the results? (b) At what number of sites does the jump model become indistinguishable from the diffusion model? To address these questions, (nearest-neighbor) jump model relaxation times were calculated as a function of the number of sites N (Figure 7) for two director orientations: $\beta = 0°$ and $\beta = 90°$. When the value of the axial diffusion constant D (set to $D = 4\pi^2K/9$, $K = 3.2 \times 10^7\ s^{-1}$) is kept fixed, the associated jump rates K_N were scaled according to the relation $D = 4\pi^2K_N/N^2$ as the number of sites N was increased. Figure 7 clearly shows that the significant difference between the jump and diffusion models seen for $N = 3$ is effectively eliminated for T_1 (T_{1Q}) values when $N > 6$ ($N > 9$). Since the diffusion model has been ruled out, the virtual equivalence of jump and diffusion models for higher values of N establishes an upper bound of six sites for a jump model. However, since attempts to reproduce the relaxation data with $N = 6$ gave less satisfactory fits than with $N = 3$, it would appear that cholesterol reorientation is best described by jumps of 120°.

Comparison with Previous Studies. Previous ^2H NMR relaxation studies in the β-phase[21] of cholesterol–PC mixtures reported correlation times in the range of 2–4 ns for cholesterol reorientation at 30 °C where T_1 was at its minimum value.[12a,c] In a detailed study of cholesterol–DMPC (30 mol %), relaxation times were interpreted in terms of two diffusive motions, with the slower of the two dominating.[12a,c] This slow motion was proposed to be molecular axial diffusion. In addition, an important element in the analysis of the T_1 data was the observed linear dependence of the relaxation rates on the square of the C–^2H bond order parameter at each labeled site. It is of interest to note that in the present study where molecular motion is represented by a single motion, an axial jump, this dependence of the relaxation rate on the order parameter is well reproduced. It appears that this simple description may be appropriate anywhere in the β-phase of cholesterol–PC mixtures including the steroid at 30 mol % in DMPC, where molecular order is of the order of 0.8, since for this system the T_1 anisotropy (Figure 1) is consistent with that predicted by the jump model. In this case, since molecular order is <1, the motion of the molecular long axis about the bilayer normal may lead to some averaging of the T_1 anisotropy over the angle β. Thus, in modeling this less ordered system, the effects of the latter averaging, while expected to be small, may have to be considered.

As to the question of stochastic reorientational motion via jumps or rotational diffusion, two recent magnetic resonance studies[3d,24] of cholesterol–lipid mixtures have also adopted jump-modeling approaches to the motion of cholesterol[3d] or the cholesterol spin label (CSL)[24] in these systems. A solid-state ^2H NMR study[3d] of [3-$^2H_1$]cholesterol equimolar in NPGS concluded from an analysis of anisotropic effects observed in partially relaxed powder line shapes that cholesterol executes a three-site hop about its axis of motional averaging. A line-shape analysis of slow-motion EPR spectra of CSL embedded in macroscopically oriented multibilayers of DMPC–cholesterol (2:1) has shown that the CSL molecules undergo a random rotational jump motion rather than small-step Brownian diffusion.[24] As in the present study, the analysis performed in each of these previous studies was carried out assuming that the motional-averaging axes of cholesterol or CSL are perfectly ordered along the director or bilayer normal (i.e., $S_{mol} = 1$). With such an assumption, the reorienting molecules in question have only a single rotational degree of freedom about their long axes, thereby allowing the data to be modeled with only one adjustable parameter, the motional rate. Both the present study and the EPR study[24] highlight the utility of oriented multibilayers, since anisotropic *spectral* effects (EPR) proved extremely useful in discriminating between motional mechanisms, in the same way as anisotropic *relaxation* effects (NMR) were used in this study.

Conclusions

The present study demonstrates that, for ordered systems, in addition to the well-known frequency and temperature dependences of ^2H relaxation, the specific details of anisotropic relaxation behavior may also be used to test critically various reasonable descriptions of molecular dynamics. To this end, oriented molecular systems, which enable the orientation of the motional symmetry axis with respect to the static magnetic field to be systematically varied, offer considerable potential. This is particularly true for many lipid systems whose unoriented preparations allow fast lateral diffusion to completely average anisotropic spin–lattice relaxation. This study of the dynamics of cholesterol in phospholipid bilayers illustrates that while temperature and frequency dependences of ^2H relaxation times can be utilized to extract correlation times,[12a] additional information, particularly that available from anisotropic relaxation data, is required to distinguish between proposed motional models. This information

(23) (a) Brown, M. F. *J. Chem. Phys.* **1982**, *77*, 1576–1599. (b) Brown, M. F. *J. Chem. Phys.* **1984**, *80*, 2803–2831.

(24) Dammers, A. J.; Levine, Y. K.; Balasubramanian, K.; Beth, A. H. *Chem. Phys.* **1988**, *127*, 149–160.

is readily available through the use of oriented multibilayers, which facilitate the unambiguous determination of T_1 and T_{1Q} anisotropies in detail. In particular, the relaxation times can be determined for any orientation, not just for the parallel ($\beta = 0°$) and/or the perpendicular ($\beta = 90°$) orientations, which alone are often insufficient to discriminate between models. The necessity of considering the entire relaxation profile whenever possible is evident in the $T_1(\beta)$ profiles (experiment and theory) and $T_{1Q}(\beta)$ profile (theory) for the C6-position, all of which exhibit a maximum near $\beta = 55°$. Although cholesterol motion is relatively simple on the time scale appropriate to spin-lattice relaxation, allowing a straightforward analysis of the relaxation data, even for systems whose dynamical complexity will require a more sophisticated treatment, the interpretation of anisotropic relaxation time measurements, together with their frequency and temperature dependences, will facilitate the identification of individual motional modes and their correlation times.

The principal aim of this study was to demonstrate that anisotropic spin-lattice relaxation may be used to distinguish between possible descriptions of cholesterol motion in phospholipid bilayers. For cholesterol at 50 mol % in DPPC, a large-angle jump between three equivalent sites with an exchange rate of 3.2×10^7 s^{-1} describes T_1 and T_{1Q} anisotropies for several sites on the steroid molecule, as well as temperature-dependent results. While axial diffusion or axial nearest-neighbor jumps among a larger number of sites may be equally valid descriptions a priori, they are not consistent with all of the relaxation data. Viewed in the broader context of stochastic reorientational motion of a uniaxial rotator, cholesterol reorientation is seen to be an experimental realization of the continuous random walk (CRW) model, first employed by Barnes[25] to describe hindered rotation of alkanes. In this model, the diffusive motions of the alkane methylene protons were described as a random walk on a circle containing N discrete sites. It is not surprising, therefore, that the mathematical problem of determining the rotational scattering law for quasi-elastic scattering from alkanes[25] or liquid crystals[26] performing such a random walk is tantamount to that solved by Torchia and Szabo[16a] in evaluating the NMR azimuthal autocorrelation function (eq 6) for nearest-neighbor jumps among N equivalent sites. Although large-angle hopping motions of the molecular long axis have been detected previously in a smectic liquid crystal,[27] for example, by incoherent neutron quasi-elastic scattering, to our knowledge, this is the first demonstration by NMR that such motions occur in lipid bilayers.

Acknowledgment. We gratefully acknowledge Drs. E. J. Parish and T. Akiyama for the gift of deuteriated cholesterols and Dr. R. G. Griffin for generously providing a copy of the computer program for calculating T_1 anisotropies. We thank Doug Moffatt for his skillful assistance in plotting figures and Michèle Auger for useful discussions.

Registry No. DPPC, 2644-64-6; cholesterol, 57-88-5.

(25) Barnes, J. D. *J. Chem. Phys.* **1973**, *58*, 5193-5201.
(26) Dianoux, A. J.; Nolino, F.; Hervet, H. *Mol. Phys.* **1975**, *30*, 1181-1194.
(27) Leadbetter, A. J.; Richardson, R. M.; Frost, J. C. *J. Phys., Colloq.* **1979**, *40*, C3-125.

Ab Initio Calculations on Borylphosphines: Prediction of a Synergistic Substituent Effect in Diborylphosphine

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Abstract: The results of ab initio calculations on the borylphosphines, $H_{3-n}P(BH_2)_n$, $n = 1$-3, are reported. The barrier to rotation of one BH_2 is found to increase on going from $n = 1$ to $n = 2$, so that the two BH_2 groups in diborylphosphine appear to act cooperatively, rather than competitively. The origin of the apparent synergistic substituent effect in diborylphosphine is shown to be the ability of one conjugated BH_2 group to reduce dramatically the energetic cost of planarizing phosphorus. This permits the formation of stronger P-B π-bonds in $HP(BH_2)_2$ than in H_2PBH_2.

Experimental and computational evidence indicates that π-electron-acceptor substituents compete with each other for π-electron density when attached to the same π-electron donor. Consequently, as the number of substituent groups increases, the strength of the π-bond to each substituent decreases. For example, the barrier to rotation of a formyl group has been found to decrease from 17.8 kcal/mol in H_2NCHO[1] to 12.9 kcal/mol in $HN(CHO)_2$[2] to 7.5 kcal/mol in $CH_3CON(CHO)_2$.[2] Calculations on $H_{3-n}N(BH_2)_n$ have shown the barriers to rotation about a B-N bond to decrease monotonically as n goes from 1 to 3.[3] The decrease in barrier heights was correlated with a decrease in the π-overlap populations along each B-N bond.[4]

In this paper we report the results of ab initio calculations on mono-, di-, and triborylphosphine, $H_{3-n}P(BH_2)_n$. Our calculations show that, in contrast to the case with the borylamines, in the borylphosphines the barrier to rotation about a P-B bond actually increases on going from $n = 1$ to $n = 2$. The cause of the apparent synergism between the two boron substituents in $HP(BH_2)_2$ is

Table I. RHF P-B Bond Lengths (Å) and Phosphorus Pyramidalization Angles[a] (deg) and Relative MP4SDTQ Energies (kcal/mol) of Different Geometries of $H_{3-n}P(BH_2)_n$

molecule	geometry	R(P-B)	ϕ^a	E
H_2PBH_2	equilibrium	1.905[b]	71.0	0[c]
	rotated	1.973	82.4	10.4[d]
	planar	1.808	0.0	4.5
	planar-rotated	1.961	0.0	44.6
$HP(BH_2)_2$	equilibrium	1.869[e]	45.1	0[f]
	rotated	1.900, 1.969	71.9	17.5
	planar	1.849	0.0	0.5
	planar-rotated	1.811, 1.955	0.0	21.1
$P(BH_2)_3$	equilibrium	1.873	0.0	0[g]
	rotated	1.859, 1.951	25.5	12.8
	planar-rotated	1.853, 1.950	0.0	13.0

[a] Angle between the R_2P plane and the extension of the P-R′ bond. [b] At the MP2 level $R = 1.863$ Å and $\phi = 66.4°$. [c] Relative to -367.9199 hartrees. [d] With $\phi = 71.0°$, $E = 12.8$ kcal/mol. [e] At the MP2 level, $R = 1.837$ Å and $\phi = 34.9°$. [f] Relative to -393.2757 hartrees. [g] Relative to -418.6264 hartrees.

(1) Drakenburg, T.; Forsen, S. *J. Phys. Chem.* **1970**, *74*, 1.
(2) Noe, E. A.; Raban, M. *J. Am. Chem. Soc.* **1975**, *97*, 5811.
(3) Gundersen, G. *Acta. Chem. Scand. A* **1981**, *35*, 729, and references therein.
(4) Similar results were obtained for $H_{3-n}BX_n$ for X = NH_2 and X = OH.[3]

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