Angular anisotropy of $^2\text{H}$ NMR spectral densities in phospholipid bilayers containing cholesterol

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Spin–lattice ($R_{1z}$) and quadrupolar order ($R_{1Q}$) relaxation rates were measured for bilayers of macroscopically oriented 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC-$d_{34}$) containing cholesterol (1:1 molar ratio), using inversion recovery and broadband Jeener–Broekaert pulse sequences, respectively. Anisotropic deuterium ($^2\text{H}$) NMR spin relaxation data were obtained for the first time along the entire flexible acyl chains of the phospholipid molecules in the liquid-crystalline state. Individual spectral densities $J_1(\omega_0)$ and $J_2(2\omega_0)$ were calculated from these relaxation rates, and a strong dependence on the angle $\theta$ between the macroscopic bilayer normal and the static magnetic field was observed. The spectral densities exhibited opposite angular anisotropies, which were explained in terms of a simple rotational diffusion model for the molecular dynamics of membrane lipid constituents.

1. Introduction

Deuterium ($^2\text{H}$) NMR spectroscopy has seen extensive use recently in the study of phospholipid lamellar phases [1–3]. In addition to being of fundamental importance in the investigation of liquid-crystalline properties, lipid bilayers are also valuable as models for biological membranes. The relaxation rates measured by $^2\text{H}$ NMR experiments depend on the motional types, rates, and amplitudes of the phospholipid molecules within the bilayer, and are a unique source of experimental information about their dynamical and equilibrium properties [4]. Although models of varying complexity have been developed and used to explain NMR results, a comprehensive picture of bilayer dynamics is still not in hand. One quantity that has received attention is the dependence of nuclear spin relaxation rates on the orientation of the macroscopic bilayer normal with respect to the external static magnetic field [5–12]. Theoretical models predict specific forms of the angular dependence which can be tested by comparison with experiment. Early studies using multilamellar dispersions of phospholipids found no significant orientational anisotropy of the spin–lattice relaxation rates $R_{1z}$, due to averaging by fast lateral diffusion about the curved surface of the bilayer [5]. More recent investigations of macroscopically oriented lipid systems have yielded somewhat different results. Studies of pure phospholipids specifically labeled in the acyl chains near the glycerol backbone have reported little or no angular dependence of $R_{1z}$ [6,11,13], while the incorporation of cholesterol resulted in a strong angular anisotropy [11]. As the body of relaxation data increases, the proposed models of lipid dynamics will be critically tested in their ability to explain the experimental findings. In this Letter, we report a significant angular anisotropy of both the $R_{1z}$ and quadrupolar order relaxation rate $R_{1Q}$, and derived spectral densities $J_1(\omega_0)$ and $J_2(2\omega_0)$, for the perdeuterated acyl chains of dimyristoylphosphatidylcholine (DMPC-$d_{34}$) in bilayers containing cholesterol. Because $R_{1z}$ is a summation of two spectral densities, their individual behavior can be easily concealed; measurement of $R_{1Q}$ enables determination of $J_1(\omega_0)$ and $J_2(\omega_0)$ and thus a more revealing level of analysis. This

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is the first published evaluation of the orientational dependence of the two individual spectral densities \( J_1(\omega_0) \) and \( J_2(2\omega_0) \) for positions along the entire acyl chains in oriented bilayers.

2. Theory

The interpretation of nuclear spin relaxation data using dynamical models has been described elsewhere [1], and only a brief summary is given here. Using standard \(^2\)H NMR relaxation theory [14,15], the observable spin–lattice \( R_{1Z} \) and quadrupolar order \( R_{1Q} \) relaxation rates are given by

\[
R_{1Z} = \frac{3}{4} \pi^2 \chi^2 [J_1(\omega_0) + 4J_2(2\omega_0)],
\]

\[
R_{1Q} = \frac{3}{4} \pi^2 \chi^2 J_1(\omega_0),
\]

where the quadrupolar coupling constant \( \chi = e^2 q Q / h \), and \( \omega_0 \) is the nuclear Larmor frequency. The spectral densities \( J_m(m\omega_0) \) are Fourier-transform partners of the autocorrelation functions \( G_m(t) \) where

\[
J_m(m\omega_0) = \text{Re} \int_{-\infty}^{\infty} G_m(t) \exp(-im\omega_0 t) \, dt.
\]

The autocorrelation functions describe fluctuations of the electric field gradient (EFG) with respect to the external, static magnetic field, and are defined by

\[
G_m(t) = \langle [V_m^{(2)LAB}(0) - \langle V_m^{(2)LAB} \rangle] [V_m^{(2)LAB}(t) - \langle V_m^{(2)LAB} \rangle] \rangle / (V_m^{(2)PAS})^2.
\]

Here \( V_m^{(2)LAB} \) and \( V_m^{(2)PAS} \) are the irreducible components of the EFG tensor in the laboratory frame and principal axis system (PAS), respectively [15].

In the following paragraph a simple model for anisotropic rotations of membrane constituents will be considered [1]. For the case of rotational diffusion within a potential of mean torque (pmt), the spectral densities can be expressed by [7,9]

\[
J_m(m\omega_0) = \sum_q \sum_n \left| D_q^{(2)}(\Omega_1) - \frac{\eta}{6} \left[ D_q^{(2)}(\Omega_2) + D_q^{(2)}(\Omega_3) \right] \right|^2 \\
\times \left| \langle |D_q^{(2)}(\Omega_2)|^2 \rangle - \langle |D_q^{(2)}(\Omega_3)|^2 \rangle \delta_{q0} \delta_{n0} \rangle \right| j_q^{(2)}(m\omega_0) |D_n^{(2)}(\Omega_3)|^2,
\]

in which the asymmetry parameter, \( \eta = (V_y - V_z)/V_z \), describes the deviation of the EFG tensor from cylindrical symmetry. If the reorientational dynamics arise from segmental motions, then the fixed transfor-0mation \( \Omega_1 \) is identified with the Euler angles \( \Omega_{pl} \) describing rotation from the PAS system of the static EFG tensor to the internal motional frame of the diffusion tensor; \( \Omega_2 \) then are represented by the Euler angles \( \Omega_{ID} \) defining the time-dependent transformation from the diffusion tensor frame to the average director (i.e. macroscopic bilayer normal). The angles \( \Omega_3 \) are designated as \( \Omega_{DL} \) and describe the fixed transformation from the director to the laboratory frame (i.e. static external magnetic field), in which \( (\alpha_{DL}, \beta_{DL}, \phi, \theta) \). The general expression in eq. (5) is easily extended to other limiting types of rotational dynamics. In the case of molecular fluctuations, the internal motions produce a residual or effective EFG tensor that is modulated by the reorientations. The fixed angles \( \Omega_i \) are now identified with \( \Omega_{IM} \), representing transformation between the internal coordinate system of the diagonalized effective EFG tensor and the molecular axis system of the rotational diffusion tensor. The internal motions will yield reduced values \( \chi_{en} \) and \( \eta_{en} \) which are the effective quadrupolar coupling constant and asymmetry parameter, respectively. The angles \( \Omega_i \) now become \( \Omega_{MD} \) describing the time-dependent transformation between the molecular and director coordinate frames, and \( \Omega_1 \) are again identified with the Euler angles \( \Omega_{DL} \). The assumption of rotational symmetry about both the diffusion tensor and the director \( z \) axes removes cross-terms involving matrix elements with different indices \( q \) and \( n \); however cross-
terms involving the asymmetry parameter $\eta$ still remain [7]. The products of the rotation matrices are evaluated in terms of their Clebsch–Gordan series [16] expansions

$$D^{(2s)}_{m_1m_2}(\Omega)D^{(2s)}_{m_2m_1}(\Omega) = (-1)^{m_1-m_2}\sum_j (2j+1)\begin{pmatrix} 2 & 2 & j \nonumber \\ -m_1 & m_2 & m \nonumber \\ -m_1 & m_2 & m \nonumber \end{pmatrix} D^{(2s)}_{m'm'}(\Omega),$$

(6)

where the values of $j$ must satisfy the triangle condition $\Delta(2j)$, and in general include both even and odd $j \leq 4$ integer terms.

The spectral density functions $J_m(m\omega_0)$ for an asymmetric EFG tensor can then be written as a linear sum of individual contributions

$$J_m(m\omega_0) = J^{(4)}_m(m\omega_0) + J^{(6)}_m(m\omega_0) + J^{(8)}_m(m\omega_0) + J^{(10)}_m(m\omega_0).$$

(7)

Here

$$J^{(4)}_m(m\omega_0) = \sum_q \sum_n |D^{(2)}_{nq}(\Omega_1)|^2 F^{(2)}_{qn}(\Omega_2; m\omega_0) |D^{(2)}_{nm}(\Omega_3)|^2,$$

(8)

$$J^{(6)}_m(m\omega_0) = \sum_q \sum_n \eta^2 |D^{(2)}_{nq}(\Omega_1)|^2 F^{(2)}_{qn}(\Omega_2; m\omega_0) |D^{(2)}_{nm}(\Omega_3)|^2,$$

(9)

$$J^{(8)}_m(m\omega_0) = \sum_q \sum_n \frac{\eta^4}{36\sqrt{70}} \left[ (72 - 155q^2 + 35q^4) \cos(4\alpha) d^{(4)}_{q0}(\beta) \right] F^{(2)}_{qn}(\Omega_2; m\omega_0) |D^{(2)}_{nm}(\Omega_3)|^2,$$

(10)

$$J^{(10)}_m(m\omega_0) = -\sum_q \sum_n \frac{4\eta}{7\sqrt{6}} \cos(2\alpha) \left( (q^2 - 2) d^{(6)}_{q0}(\beta) + \frac{72 - 155q^2 + 35q^4}{8\sqrt{15}} d^{(8)}_{q0}(\beta) \right)$$

$$\times F^{(2)}_{qn}(\Omega_2; m\omega_0) |D^{(2)}_{nm}(\Omega_3)|^2,$$

(11)

and

$$F^{(2)}_{qn}(\Omega_2; m\omega_0) = \left[ \langle |D^{(2)}_{qn}(\Omega_2)|^2 \rangle - |\langle D^{(2)}_{qn}(\Omega_2) \rangle |^2 \delta_{m0}\delta_{n0} \right] F^{(2)}_{qn}(m\omega_0).$$

(12)

In eqs. (8)–(11) all terms containing $\Omega_i$ which are of odd parity cancel rigorously. The contributions of terms invariant to rotations about $\alpha_i$ (i.e. symmetric with respect to the $z$ axis of the EFG tensor PAS) are given in eqs. (8) and (9) and have been discussed previously [7]. It should be noted that when $\beta_1 = 0^\circ$, the cylindrical symmetry assumed for the diffusion tensor is now imposed on the effective EFG tensor and the asymmetric terms given by eqs. (10) and (11) vanish, with the spectral density in eq. (7) becoming independent of the angle $\alpha_i$. This is the limiting case investigated by Brown and Söderman [7]. Evaluation of the mean-squared fluctuations and the form of the reduced spectral densities $j^{(2)}_{qn}(m\omega_0)$ in eq. (12) are governed by the potential employed. A general uniaxial potential of mean torque [17] can be approximated by

$$\hat{U}(\cos \beta) = \sum_i c_i P_i(\cos \beta),$$

(13)

where $c_i$ is the coefficient of the Legendre polynomial $P_i(\cos \beta)$ in the expansion. Approximation of the potential in this manner allows the relevant order parameters to be evaluated [17] using the Boltzmann expression

$$\langle P_j \rangle = \int_0^\pi P_j(\cos \beta) \exp[-U(\cos \beta)/kT] \sin \beta d\beta \left/ \int_0^\pi \exp[-U(\cos \beta)/kT] \sin \beta d\beta \right..$$

(14)

In general the products of the rotation matrices $D^{(2)}_{nq}(\Omega_2)$ and the reduced spectral densities $j^{(2)}_{qn}(m\omega_0)$ in eq. (12) are functions of both even- and odd-rank order parameters. It can be shown that $F^{(2)}_{qn}(\Omega_2; \omega_0) = F^{(2)}_{-q-n}(\Omega_2; \omega_0)$, allowing eq. (12) to be rewritten in terms of components having even or odd parity with respect to $\beta_2 = \pi/2$, namely
Using a single Lorentzian approximation, the reduced spectral densities for rotational diffusion can be written as

\[ j^{(2)}_{q_n}(m\omega_0) = 2\tau^{(2)}_{q_n} / [1 + (m\omega_0 \tau^{(2)}_{q_n})^2] \]

The parity of eq. (12) reduces the number of correlation times \( \tau^{(2)}_{q_n} \) or reorientational modes in eq. (16) from 25 to 13 for a potential described by eq. (13). If the potential is even with respect to \( \beta_2 = \pi/2 \) only the even term in the mean-squared fluctuations are non-zero, eliminating the second term in eq. (15), and resulting in only 9 reorientational modes. It has been remarked that a phospholipid in a bilayer is polar in which the headgroup is associated with the aqueous interface. Realizing that use of an even potential to describe lipid reorientation represents an approximation, as seen from inspection of eq. (15), the evaluation of the spectral densities in eq. (7) for segmental and molecular motions is greatly simplified. For a potential of the Maier–Saupe form, \( U(\cos \beta_2) = p \cos \beta_2 \), the correlation times \( \tau^{(2)}_{q_n} \) are functions of the order parameter \( \langle P_2(\cos \beta_2) \rangle \), and for a single exponential approximation are written in terms of the correlation time \( \tau^{(1)}_{q_n} \) according to [1]

\[ \frac{1}{\tau^{(2)}_{q_n}} = \frac{1}{\tau^{(1)}_{q_n}} \frac{\alpha^{(2)}_{q_n} + (\eta_0 - 1)q^2}{\alpha^{(1)}_{q_n}} \]

In eq. (17) the values of \( \alpha^{(2)}_{q_n} \) have been previously evaluated [18,19], \( \eta_0 = D_\parallel / D_\perp \) with the principal values of the axially symmetric rotational diffusion tensor \( D \) being given by \( D_\parallel \) and \( D_\perp \), and \( \tau^{(2)}_{q_n} = 1/\alpha^{(2)}_{q_n} D \). As described elsewhere [1,7,9], use of a symmetric top approximation gives \( \alpha^{(2)}_{q_n} = 6 \), with \( j^{(2)}_{q_n}(\omega_0) \rightarrow j^{(2)}_{q}(\omega_0) \) and \( \tau^{(2)}_{q_n} \rightarrow \tau^{(2)}_{q} \), thereby removing the dependence of the correlation time on the ordering potential, and yielding only three distinct reorientational modes. Eqs. (5), (16), and (17) yield expressions in closed-form for quadrupolar relaxation due to anisotropic rotational diffusion [1], and are utilized here to interpret the anisotropic relaxation observed for oriented DMPC-d_{54}: cholesterol bilayers.

3. Results and discussion

Samples of DMPC-d_{54} were synthesized [20] and mixed with cholesterol (1:1 molar ratio) as previously described [21]. The 2H labeled lipid mixture was then dispersed in an excess of 20 mM tris buffer, pH=7.5, utilizing deuterium depleted water and was aligned on glass substrates as detailed elsewhere [22]. Relaxation experiments were carried out on macroscopically oriented bilayers of DMPC-d_{54}: cholesterol at several values of the angle \( \theta \) between the bilayer normal and the external magnetic field to determine the angular dependence of \( R_{1\perp} \) and \( R_{1Q} \). The latter relaxation rate was measured by a modified version of a broadband Jeener–Broekaert pulse sequence described by Wimperis [23]. This sequence has proved far superior to the standard Jeener–Broekaert pulse sequence [24,25] in the broadband creation of quadrupolar order [23,26]. The utility for the measurement of \( R_{1Q} \) in lipid bilayers is demonstrated here for the first time. With one experiment it was possible to determine \( R_{1Q} \) for resonances across a large spectral width (±30 kHz). The accuracy of relaxation rates obtained using this sequence was verified by performing standard Jeener–Broekaert experiments in parallel, selecting the appropriate parameters for individual quadrupolar splittings [24,25]. The values for \( R_{1Q} \) obtained by the two methods were identical within experimental error for all observable resonances. Representative partially relaxed 2H NMR spectra obtained from a standard inversion recovery experiment and the broadband Jeener–Broekaert experiment are shown in figs. 1a and 1b, respectively. Comparison of the line shapes of the fully relaxed spectra from the inversion recovery pulse sequence (upper spectra in fig. 1a) with
Fig. 1. Partially relaxed $^2$H NMR spectra of macroscopically oriented bilayers of DMPC-$d_{44}$: cholesterol (1:1 molar ratio) at 40°C in 20mM tris buffer, pH = 7.5. The spectra were obtained at a resonance frequency of $\nu_0 = 76.8$ MHz and enable determination of the (a) spin–lattice, $R_{1z}$, and (b) quadrupolar order, $R_{1Q}$, relaxation rates. The results in (a) were obtained using an inversion recovery pulse sequence, while the spectra in (b) were obtained using a broadband Jeener-Broekaert pulse sequence: $(\pi/2)_r-(3\pi/8)_{\tau_1}-(\pi/4)_{\tau_2}-(\pi/4)_{\tau_3}-(\pi/4)_{\tau_4}-(\pi/4)_{\tau_5}-(\pi/4)_{\tau_6}-(\pi/4)_{\tau_7}-(\pi/4)_{\tau_8}-(\pi/4)_{\tau_9}-(\pi/4)_{\tau_{10}}$-acquire [23]. Relaxation intervals for the representative spectra are (from bottom to top) (a): 2, 15, 24, 36, 54, 110, 440 ms; and (b): 2, 15, 54, 110, 240, 440, 800 ms. The angle between the macroscopic bilayer normal and the external magnetic field was $\theta = 90^\circ$. This figure reveals the utility of the broadband Jeener-Broekaert pulse sequence in the creation of quadrupolar order.

The least decayed spectra from the Wimperis sequence (lower spectra in fig. 1b) shows creation of quadrupolar order across the entire spectral width.

From these types of spectra, the relaxation rates $R_{1Z}$ and $R_{1Q}$ were obtained [20] and the spectral densities $J_1(\omega_0)$ and $J_2(2\omega_0)$ were determined according to eqs. (1) and (2) for different values of the angle $\theta$. The splittings of the spectra in fig. 1 can be assigned to various carbon segments in the acyl chains of the phospholipids [27,28], and profiles of $J_1(\omega_0)$ and $J_2(2\omega_0)$ versus carbon segment at four values of $\theta$ are shown in fig. 2. The data for carbons 2–6 of the acyl chains (exclusive of the C-2 segment of the sn-2 chain) correspond to the largest quadrupolar splitting, commonly referred to as the plateau region. The measured values of $R_{1Z}$ are consistent with previous studies of the angular dependence of $R_{1Z}$ for bilayers of di[6',6'-H2] DMPC containing cholesterol (40 mol%) [11]. Inspection of fig. 2 clearly reveals that the angular dependencies of the two individual spectral densities are opposite in the plateau region. The spectral density $J_1(\omega_0)$ increases from $\theta = 0^\circ$ to $90^\circ$, while $J_2(2\omega_0)$ decreases (see also fig. 3). For carbon segments of the acyl chain closer to the terminal methyl group, this dependence decreases and changes form, pointing to the additional degrees of freedom experienced by these segments. Because of the relatively high degree of ordering in the plateau region, carbon segments 2–6 provide the simplest case for analysis of the relaxation profiles, and are therefore the focus for the remainder of the discussion. The angular dependence of $J_1(\omega_0)$ and $J_2(2\omega_0)$ for carbons 2–6 of the acyl chains are shown in fig. 3 (symbols). The angular dependency of the two spectral densities exhibits a rich behavior, and exemplifies the usefulness and necessity of determining both spectral densities individually [24,25].

The data in fig. 3 can be interpreted using the rotational diffusion model by assuming as limiting cases that the relaxation arises from local segmental motions, or alternatively whole molecular motions [1,7,9]. If the
reorientation results from fast segmental dynamics, the motions will modulate a static EFG tensor with its principal symmetry axis parallel to the C–2H bond. Theoretical fits using this model and an axially symmetric ($\eta=0$) EFG tensor with $\chi=170$ kHz [29] are shown in fig. 3 by solid lines. The observed segmental order parameter $S_{CD}=\langle D_{00}^{(2)}(\Omega_{PD}) \rangle = D_{00}^{(2)}(\Omega_{pd}) < D_{00}^{(2)}(\Omega_{PD}) > =0.42$ is determined experimentally from the quadrupolar splitting, in which the closure relation of the group of rotations $R_3$ is applied. Axial symmetry of the diffusion tensor is assumed, leaving only three variable parameters, viz. $\beta_{pd}$, $\tau_{00}^{(2)}$, and $\eta_D$ (see section 2). The resulting fits for a Maier–Saupe potential and the symmetric top approximation are summarized in table 1, and both predict the shape and magnitude of the angular anisotropy for the individual spectral densities $J_1(\omega_0)$ and $J_2(2\omega_0)$ reasonably well.

Alternatively, if the relaxation arises from molecular reorientations, then the slower dynamics modulate an effective or residual EFG tensor which is pre-averaged by fast internal motions, thereby reducing its principal values and/or changing its orientation within the molecular frame. The asymmetry of the residual EFG in this case may no longer be zero [7,30]. Theoretical fits assuming a rotational diffusion model for molecular reorientation are depicted in fig. 3 by dashed lines. For an effective EFG tensor, the interaction constant $\chi$ is scaled by the estimated order parameter of the fast internal motions to obtain the effective interaction constant $\chi_{eff}=\chi < D_{00}^{(2)}(\Omega_{PD}) >$, which is obtained from the observed segmental order parameter. $S_{CD}=\langle D_{00}^{(2)}(\Omega_{PD}) \rangle = \sum_s < D_{00}^{(2)}(\Omega_{PD}) D_{00}^{(2)}(\Omega_{IM}) D_{00}^{(2)}(\Omega_{MD}) > . \text{Closure is invoked and the diffusion tensor is considered to be axially symmetric (cf. table 1). If one assumes that } < D_{00}^{(2)}(\Omega_{MD}) > \approx 0.9, \text{ which is the molecular order parameter observed for } ^2\text{H labeled cholesterol in oriented bilayers [8,9], there are six variable parameters: } \chi_{eff}, \eta_{eff}, \alpha_{IM}, \beta_{IM}, \tau_{00}^{(2)}, \text{ and } \eta_D. \text{ Theoretical fits utilizing an asymmetric } (\eta_{eff} \neq 0) \text{ effective EFG tensor}
Fig. 3. Experimentally determined and theoretically modeled spectral densities $J_1(\omega_0)$ (●) and $J_2(2\omega_0)$ (○) versus bilayer orientation ($\theta$). The experimental data are from the plateau region of DMPC-d$_{54}$:cholesterol (1:1 molar ratio) in macroscopically oriented bilayers at $\nu_0=76.8$ MHz in the L$_\alpha$ phase at 40°C. The observed segmental order parameter for this region was $S_{CD}=0.42$. Theoretical fits were performed simultaneously with the simplex method for both spectral densities using the formalism discussed in the text for: (a) Maier-Saupe potential, and (b) symmetric top approximation. For a segmental model (—), the static EFG tensor was assumed to be axially symmetric ($\eta=0; \chi=170$ kHz), with its symmetry axis along the C-2H bond. The principal symmetry axis of the diffusion tensor was set perpendicular to the C=O plane ($\beta_{eq}=90^\circ$), and the spectral densities were evaluated using eqs. (7), (8), and (16). For a molecular model (-----), the residual EFG tensor was assumed to be axially symmetric ($\eta_{res}=0$) with $\chi_{eff}=159$ kHz. Spectral densities were evaluated using eqs. (7)-(11) and (16), and fourth-rank order parameters $\langle D^{4}\rangle(D_2)$ were calculated from the second-rank order parameters $\langle D^{2}\rangle(D_2)$ using eq. (14). The remaining fitting parameters for these models are given in table 1. The theoretical fits for the segmental (—) and molecular (-----) models adequately predict both the magnitude and form of the angular anisotropy.

were not significantly different than those utilizing a symmetric effective tensor, which allows the elimination of two variable parameters ($\alpha_{IM}$ and $\eta_{eff}$). The fitting parameters are given in table 1, with the predicted angular dependence of the spectral densities shown in fig. 3. It should be noted that due to the extremely high order of segments in the plateau region, internal motions that give rise to an axially asymmetric effective EFG tensor are greatly reduced, and the parameters obtained for the segmental and molecular models are necessarily similar.

A comparison of the dynamical and equilibrium properties of the phospholipid acyl chains (this work) with the cholesterol molecule is now possible. The ordering effect of cholesterol on the configurational properties of the bilayer acyl chains has been studied in numerous previous investigations [27,31,32]. In bilayers of DMPC-d$_{54}$, the addition of a 1:1 molar ratio of cholesterol increases the observed order parameters of the acyl chain segments near the glycerol backbone by greater than twofold ($S_{CD}=0.20\rightarrow0.42$). Fourier-transform IR spectroscopy has shown that the presence of cholesterol (33 mol%) in dipalmitoylphosphatidylcholine bilayers decreases the population of existing gauche conformers from 20.7% and 32.3% to 3.7% and 3.6% for carbon segments 4 and 6, respectively [33,34]. This result suggests that the carbon segments in the plateau region move as a nearly rigid body in the presence of cholesterol. In the present work, new knowledge of the dynamics of phospholipid molecules in the presence of cholesterol has been obtained. The angular dependence of $R_{1z}$ and $R_{1Q}$ for specifically labeled cholesterol in a similar bilayer system has been reported [8,35], and discussed using the anisotropic rotational diffusion formalism [9]. The anisotropic diffusion coefficients for cholesterol in the short correlation time regime were found to be $D_1=2.4\times10^8$ s$^{-1}$ and $D_\perp=6.7\times10^6$ s$^{-1}$, while with the symmetric top approximation $D_1=4.2\times10^8$ s$^{-1}$ and $D_\perp=8.3\times10^6$ s$^{-1}$ [9]. Inspection of table 1 reveals that the apparent diffusion rates for either the segmental or molecular model are 10 to 130 times larger for the phospholipid than for cholesterol. An even larger difference exists if a comparison is made with the long correlation
Table 1
Parameters for fits of angular anisotropy of spectral densities in DMPC-d$_{44}$:cholesterol (1:1) bilayers at 40°C

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Model $^b$</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\chi$ (kHz) $^c$</td>
<td>170</td>
<td>170</td>
<td>159</td>
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<tr>
<td>$\eta$ $^c$</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>$\tau_{16}^d$ (10$^{-10}$ s)</td>
<td>1.56</td>
<td>1.29</td>
<td>3.17</td>
<td>2.69</td>
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<tr>
<td>$D_h$ (10$^9$ s$^{-1}$)</td>
<td>4.95</td>
<td>4.28</td>
<td>4.46</td>
<td>4.15</td>
<td></td>
</tr>
<tr>
<td>$D_L$ (10$^9$ s$^{-1}$)</td>
<td>0.431</td>
<td>1.29</td>
<td>0.129</td>
<td>0.620</td>
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</tr>
<tr>
<td>$D_{\parallel}$ (10$^9$ s$^{-1}$)</td>
<td>11.5</td>
<td>3.32</td>
<td>34.5</td>
<td>6.71</td>
<td></td>
</tr>
<tr>
<td>$\langle D_{16}^{01}(\Omega_{PD}) \rangle$</td>
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<td>0.42</td>
<td>0.42</td>
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</tr>
<tr>
<td>$\langle D_{16}^{01}(\Omega_{PD}) \rangle$</td>
<td>-</td>
<td>-</td>
<td>0.93</td>
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</tr>
<tr>
<td>$\beta_{\text{H}}$ (deg)</td>
<td>90 $^d$</td>
<td>90 $^d$</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td>$\beta_{\text{M}}$ (deg)</td>
<td>-</td>
<td>-</td>
<td>90</td>
<td>90</td>
<td></td>
</tr>
</tbody>
</table>

$^a$) The experimental values of $J_1(\omega_0)$ and $J_2(2\omega_0)$ were fit simultaneously using the simplex method with the constraint that the segmental C--H order parameter $S_{\text{CD}}$ was equal to the experimental value. For an axially symmetric, static EFG tensor $S_{\text{CD}} = \langle D_{16}^{01}(\Omega_{PD}) \rangle = D_{16}^{01}(\Omega_{PD}) \langle D_{16}^{01}(\Omega_{PD}) \rangle$. In the case of a non-axially symmetric, effective EFG tensor $S_{\text{CD}} = \langle D_{16}^{01}(\Omega_{PD}) \rangle = \langle D_{16}^{01}(\Omega_{PD}) \rangle [D_{16}^{01}(\Omega_{PD}) - (2/\sqrt{6})\eta_{\text{PD}}^d D_{16}^{01}(\Omega_{PD}) \cos(2\alpha_{\text{PD}})] \langle D_{16}^{01}(\Omega_{PD}) \rangle$.

$^b$) Model designations: A = segmental; B = segmental, symmetric top approximation; C = molecular; D = molecular, symmetric top approximation.

$^c$) Static or effective values for segmental and molecular models, respectively.

$^d$) Parameter not varied during fits.

The experimental values of $J_1(\omega_0)$ and $J_2(2\omega_0)$ were fit simultaneously using the simplex method with the constraint that the segmental C--H order parameter $S_{\text{CD}}$ was equal to the experimental value. For an axially symmetric, static EFG tensor $S_{\text{CD}} = \langle D_{16}^{01}(\Omega_{PD}) \rangle = D_{16}^{01}(\Omega_{PD}) \langle D_{16}^{01}(\Omega_{PD}) \rangle$. In the case of a non-axially symmetric, effective EFG tensor $S_{\text{CD}} = \langle D_{16}^{01}(\Omega_{PD}) \rangle = \langle D_{16}^{01}(\Omega_{PD}) \rangle [D_{16}^{01}(\Omega_{PD}) - (2/\sqrt{6})\eta_{\text{PD}}^d D_{16}^{01}(\Omega_{PD}) \cos(2\alpha_{\text{PD}})] \langle D_{16}^{01}(\Omega_{PD}) \rangle$.

Model designations: A = segmental; B = segmental, symmetric top approximation; C = molecular; D = molecular, symmetric top approximation.

Static or effective values for segmental and molecular models, respectively.

Parameter not varied during fits.

time regime solutions proposed for the cholesterol dynamics [8]. These differences are present although the molecular order parameters of the phospholipid chain segments are on the order of 0.9. A simple explanation for this result is that the phospholipids have internal degrees of freedom not present in the rigid frame of cholesterol. Although similar orientational order is observed, the structure of the phospholipid molecule allows for internal motions of the highly ordered acyl chains that are not present in the cholesterol ring. It should also be noted that spin–lattice relaxation rates have been reported for the glycerol backbone of phospholipids and are on the order of 75 s$^{-1}$ [36] to 200 s$^{-1}$ [37] and change very little with the incorporation of cholesterol [37]. These observations suggest that motional preaveraging and/or faster motion exists for the plateau region acyl chain segments compared to the glycerol backbone. The dynamics, however, cannot involve a substantial amount of trans-gauche isomerization [33,34], on account of the high degree of orientational order [27,31,32]. A considerable degree of internal rotation of the phospholipid acyl chains as a relatively rigid unit in the presence of cholesterol is one possibility that would fit these criteria, but further analysis is needed to evaluate this possibility.

In conclusion, a significant orientation dependence of the spectral densities $J_1(\omega_0)$ and $J_2(2\omega_0)$ has been found for the acyl chains of lipid:cholesterol bilayers with broadband excitation of Zeeman and quadrupolar order. Data for the highly ordered plateau region have been successfully explained in terms of a simple model for anisotropic rotational diffusion. The findings suggests that the acyl chains of bilayer lipids have additional degrees of freedom not present in the interacting cholesterol molecules, or within the glycerol backbone. Analysis of the spectral densities from carbon segments along the entire acyl chain will provide an additional test of dynamical models; these will be forthcoming.

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