than were formed during association, result in exchange of all of these sites. This process is consistent with the observation[8] of tin-119/117 satellites for the averaged OCH_2 signal at 55 °C because here the individual monomer units stay together during the exchange process.

A different process can be observed in the NMR spectra of 1,3,2-dioxastannolane derivatives that lack a plane or axis of symmetry relating the two substituents on tin in the monomeric structure. Luchinat and Roelens[12] examined the dynamic ^{13}C NMR spectra of the chiral and racemic 4-methyl and 4-phenyl derivatives of **1**. At room temperature, the spectra of these compounds exhibit two equally intense peaks for the α-, β-, and γ-carbons of the butyl groups. The two peaks for each carbon coalesce as the temperature is raised with $\Delta G^{\ddagger}$ of about 17 kcal mol^{-1}, considerably larger than other barriers measured for these compounds. The two butyl groups on each tin atom are diastereotopic in the monomer and thus should give anisochronous signals as long as processes that result in inverting the relationship of the butyl groups on tin to the chiral center in the same 1,3,2-dioxastannolane ring are slow. No associative process could account for the observed coalescence because, during any such process, the butyl groups maintain their relationship to the chiral center. Luchinat and Roelens have proposed a mechanism for this process that involves breaking two internal Sn-O bonds in a dimer to give a ten-membered ring intermediate that reassociates to a different dimer in which the $Sn(Bu)_2$ groups have exchanged the chiral center with which they are associated inside the two 1,3,2-dioxastannolane rings of the dimer.[12]

Conclusions

2,2-Di-*n*-butyl-1,3,2-dioxastannolanes exist in solution in nonpolar solvents as mixtures of oligomers, including dimers, trimers, tetramers, and pentamers. The amount of monomer present is below the level of detection by NMR at room temperature. The constitution of the mixture present is influenced by temperature, concentration, and the nature of the substituents on the ring. The temperature dependences of the oligomerization equilibria are particularly steep. For the parent compound (**1**), the trimer and tetramer constitute the majority of the species present below -20 °C, but the dimer dominates increasingly as the temperature is raised. Two small, 4,5-trans substituents do not influence the equilibria much, but the 4,4,5,5-tetramethyl derivative exists solely as the dimer. 1,3,2-Dioxastannolanes undergo particulary complex exchange processes through a series of related association-dissociation equilibria involving dimers, trimers, tetramers, and pentamers and possibly monomers and hexamers. The lowest barriers for compounds **1-4** were observed for association of two dimer molecules to a tetramer and of a dimer and a trimer to a pentamer.

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Elucidation of Motional Modes in Glycoglycerolipid Bilayers. A ^2H NMR Relaxation and Line-Shape Study†

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Abstract: In the present study, a combination of ^2H spin-lattice relaxation and line-shape analysis demonstrates that two motions are sufficient to describe the spectral and relaxation behavior of the glycolipid 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (β-DTGL). In the gel phase, at 25 °C, the powder and oriented-sample spectral line shapes for the glycolipid, specifically labeled at the C3-position of the glycerol backbone, are characteristic of fast-limit axially asymmetric motions. In particular, the oriented-sample spectra have powder line shapes characteristic of a system with a motionally averaged asymmetry parameter η_{eff} close or equal to unity. Moreover, the Zeeman spin-lattice relaxation times were dependent on both the polar and azimuthal angles, θ and ϕ, describing the orientation of the motional axis relative to the magnetic field direction. Inspection of the partially relaxed line shapes of powder spectra of gel-phase lipid clearly revealed the θ-dependence of the spin-lattice relaxation times. Furthermore, for oriented samples with a given θ, a ϕ-dependence of the relaxation times was also observed. This effect was most evident at the magic-angle ($\theta = 54.7°$) orientation. The nature of this ϕ-dependence puts severe constraints on the motional model and the motional rates used to simulate the gel-phase line shape and T_1 anisotropy ($T_{1Z}(\theta,\phi)$). The line-shape and relaxation features were best simulated with a 3-site jump model with relative site populations of 0.46, 0.34, and 0.20 and a correlation time of 6.7×10^{-10} s. These results indicate that a single internal motion is sufficient to describe the line shape and relaxation in the gel phase. However, a second motion, namely rotation about the long axis of the molecule as a whole, is needed to account for the observed variation in the quadrupolar echo amplitude and the spectral line shape over the temperature range of 25-60 °C. This motion does not significantly influence the line shape in the gel phase at 25 °C or the spin-lattice relaxation behavior in the gel and liquid-crystalline phases. From the line-shape study, an activation energy of about 150 kJ mol^{-1} was determined for this motion.

Glycosphingolipids constitute a class of biomolecules that can assume many biological roles.[1] Of particular importance is their capacity to function as recognition sites (cell-cell recognition,[2] immune recognition,[3] and toxin receptor[4]) and as modulators of membrane structure.[5] In addition to the structure and conformation of the oligosaccharide moiety, molecular recognition at the cell membrane surface will depend on a number of factors such as surface orientation and spatial constraint imposed by the bilayer surface, which will affect "accessibility" of the carbohydrate

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residues for interaction.[6a,b] In view of the important roles played by the glycolipid head group, it is clear that probing the spatial disposition and dynamics of the carbohydrate moiety can provide valuable insight into cell surface recognition at the molecular level.

Since the systems of interest are inherently spatially anisotropic, solid-state ^2H NMR spectroscopy is ideally suited to probing such systems.[7a-e] ^2H NMR studies of glycosphingolipids[8a,b] and glycoglycerolipids[8c-h] have demonstrated that the head group undergoes motion that is spatially restricted (ordered) and that the orientation of the head group relative to the membrane plane can be elucidated. In addition, the conformation of the head group under the influence of the anisotropic interactions present at the membrane surface can be probed.[8a-h]

In addition to probing structural features such as lipid bilayers, ^2H NMR provides observation windows in the range where the frequencies of most molecular motions occur ($1-10^{10}$ s^{-1}, for lipid bilayers[9]). Spin-lattice relaxation data are a source of information about molecular dynamics on time scales near the Larmor frequency. However, the quantitative interpretation of such measurements ultimately requires choosing a motional description that is consistent with experiment. Previous studies from this laboratory have focused on glycoglycerolipids as models of glycosphingolipids in order to elucidate the orientation, conformation, and dynamics of the carbohydrate head groups at a bilayer surface and to probe the factors influencing these spatial and motional parameters.[8c-h] During the course of these studies, two observations were common to all systems studied: *anisotropic ^2H spin-lattice relaxation* (dependent on the orientation of the director with respect to the magnetic field direction) in the liquid-crystalline phase and a dramatic reduction in the ^2H quadrupolar echo signal intensity at temperatures just below the gel to liquid-crystalline phase-transition temperature[8g] (Jarrell, unpublished results). The observation of anisotropic relaxation is significant in that it puts severe constraints on the motional descriptions that may be considered to explain relaxation in these systems. Anisotropic spin-lattice relaxation has been used in biomolecular systems to extract details of molecular motion in proteins,[10a,b] DNA,[11] and lipids.[12a-c] The loss of quadrupolar signal intensity reflects motion on the 10^3-10^6 time scale and is sensitive to the specific details of the motion(s) involved.[7b]

In the present study, the glycolipid 1,2-di-*O*-tetradecyl-3-*O*-(β-glucopyranosyl)-*sn*-glycerol is studied in the gel state by ^2H spin-lattice relaxation and analysis of quadrupolar echo line shapes. The principal objective of this study is to probe the lipid dynamics in the highly ordered gel state, where molecular motion is expected to be less complicated, in order to develop a basis for describing molecular motion in the more complex but biologically more relevant liquid-crystalline phase. Our results demonstrate the considerable utility of oriented samples in relaxation and line-shape measurements.

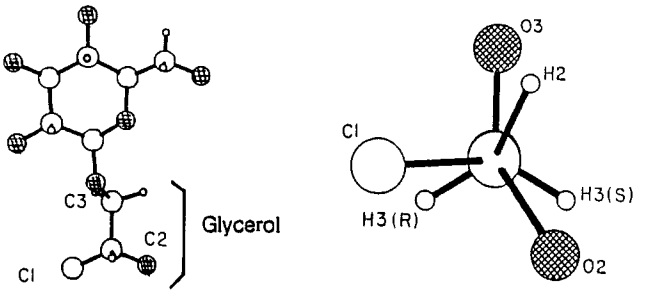

Figure 1. Left: Head-group orientation of β-DTGL relative to the bilayer normal as calculated from ^2H NMR data.[8c,e] Right: Glycerol backbone of β-DTGL. View is along the C2–C3 bond from C2 toward C3. The conformation shown is that calculated from the estimated $^1H2-^2H3(S)$ and $^1H2-^2H3(R)$ dipolar couplings.[8e]

Experimental Section

Dipalmitoylphosphatidylcholine (DPPC) was obtained from Sigma Chemical Co., St. Louis, MO, and 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-[3,3-$^2H_2$]glycerol was prepared as described previously.[8c,e] Multilamellar dispersion samples for ^2H NMR consisted of 50–100 mg of dry lipid hydrated with a 10-fold excess of deuterium-depleted water (Aldrich Chemical Co., Milwaukee, WI) in a 10-mm (o.d.) sample tube. Hydrated samples were heated cyclically to 60 °C with vortex mixing and freeze–thawed to homogeneity (four to five cycles). For the preparation of oriented samples, the required amount of each lipid (30–60 mg, total lipid) was dissolved in chloroform/methanol (2:1, v/v). The lipid mixture was dropped on 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 3 h. The sample was hydrated overnight at 55 °C under a humid atmosphere of ^2H-depleted water. A drop of ^2H-depleted water was added and the tube sealed.

^2H NMR data were acquired at 30.7 MHz on a "home-built" solid-state NMR spectrometer operated by a Nicolet 1280 computer. Spectra were recorded by means of the quadrupolar echo sequence[13] with full phase cycling[7b] and quadrature detection. Pulse spacing was typically 60 μs with $\pi/2$ pulses of 3.8–4.0 μs (10-mm coil) and a recycle time greater than $5T_{1Z}$. Longitudinal relaxation times (T_{1Z}) were obtained by the standard inversion-recovery sequence coupled with the quadrupolar echo sequence.[14] Samples were enclosed in a glass jacket where the temperature was regulated to within ±0.5 °C.

Simulations were performed on a μ-Vax II or on a Sun 4-260 computer with a line-shape simulation program[15] based on the general formalism of Torchia and Szabo.[16] Simulated line shapes were corrected for the finite width of the experimental 90° pulses in the quadrupolar echo sequence,[17a] and the partially recovered T_{1Z} spectra were further corrected for the finite width of the 180° pulse.[17b]

Results and Discussion

Deuterium nuclear magnetic resonance (^2H NMR) spectroscopy has been used extensively to examine the structural and dynamical properties of lipid bilayers in the liquid-crystalline phase (L_α) phase.[7a-e] ^2H NMR spectra of the liquid-crystalline phase are, in general, characteristic of axially symmetric motion. For a C–^2H bond executing axially symmetric motions, the observed quadrupolar splitting is given by

$$\Delta\nu_Q(\theta) = \frac{3e^2qQ}{2h} S \frac{3\cos^2\beta - 1}{2} \frac{3\cos^2\theta - 1}{2} \qquad (1)$$

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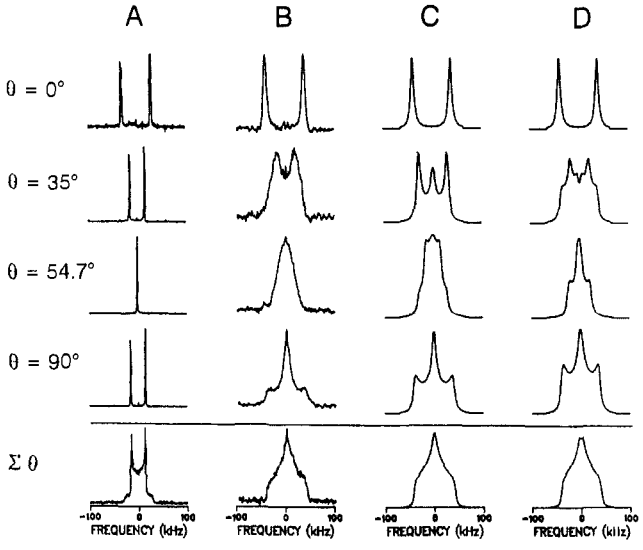

Figure 2. Bottom row: ^2H NMR spectra of multilamellar dispersions of [3,3-$^2H_2$]β-DTGL. Four top rows: Oriented-sample spectra of [3,3-$^2H_2$]β-DTGL/DPPC (4:1, molar ratio) as a function of the angle θ (eq 2) between the normal to the plane of the glass plates (taken to be coincident with the bilayer normal) and the magnetic field direction. A: Experimental spectra in the liquid-crystalline phase, 55 °C. B: Experimental spectra in the gel phase, 25 °C. C, D: Simulated spectra in the gel phase. Spectral simulations were performed with two different 3-site jump models with populations 0.50, 0.25, and 0.25 (C) and 0.46, 0.34, and 0.20 (D). The C–^2H bonds of the 3-carbon of the glycerol backbone are oriented at 69° with respect to the axis of motional averaging, and the exchange rate is 5×10^8 rad s^{-1}. A line broadening of 4 kHz was used in the simulations of gel-phase spectra in order to fit the experimental line width (8 kHz) of the 0° orientation spectrum in the gel phase.

where e^2qQ/h is the quadrupolar coupling constant (170 kHz), θ is the angle between the symmetry axis for the motion and the magnetic field direction, S is the segmental order parameter describing the anisotropic motion of the molecular unit relative to the bilayer normal (the wobbling), and β is the angle between the C–^2H bond and the molecular rotation axis.

The structural and dynamical properties of the glycolipid β-DTGL in the liquid-crystalline phase have been studied by ^2H NMR.[8c-e] Study of β-DTGL labeled in both head-group and glycerol regions has allowed the determination of the orientations of these moieties relative to the axis of motional averaging, as shown in Figure 1 (left). The ^2H NMR spectrum of a multilamellar dispersion of β-DTGL labeled at the C3-position of glycerol is shown in Figure 2 (bottom row, column A) at 55 °C, at which temperature the lipid is in the liquid-crystalline phase.[8c] This spectrum is characteristic of lipids undergoing axially symmetric motion and exhibits two quadrupolar splittings, indicating that the two C–^2H bonds at this position make slightly different angles with respect to the symmetry axis. From the spectra of oriented samples, estimates of the ^2H–^2H dipolar coupling between the deuterons at C3 as well as the ^1H–^2H dipolar couplings between the proton at C2 and the deuterons at C3 of glycerol of β-DTGL were also obtained. From these results, the dihedral angle about the C2–C3 bond, as well as the average conformation of this part of the glycerol backbone, was estimated, as shown in Figure 1 (right).[8e] Moreover, the conformation about the glycosidic bond of the glycolipid is such that the sugar ring is fully extended away from the bilayer surface.[8c-e] Segmental order parameters S of 0.45, 0.65, and 0.40 were determined for the head group, the glycerol backbone, and the hydrophobic core of β-DTGL, respectively.

In contrast to the liquid-crystalline phase, the gel phase of phospholipids and glycolipids has received much less attention. This is in part due to the technical difficulties associated with obtaining gel-state ^2H NMR spectra, which are usually a factor of 2–4 times wider, with transverse relaxation times much shorter than those of the liquid-crystalline phase. ^2H NMR studies of phospholipid specifically deuteriated in both the acyl chain and head-group regions suggested that the dominant motion present in these systems is slow-axial diffusion with a correlation time τ_c of about 10^{-5} s.[18,19a-c] However, line-shape and spin–lattice relaxation studies of acyl-chain-labeled *N*-palmitoylgalactosylsphingosine (NPGS) in the gel phase[20a,b] indicate that, unlike the phospholipid systems, axial diffusion is very slow on the ^2H NMR time scale ($\tau_c = 1/\Delta\nu_Q$) and the gel-phase spectra are the result of a simple exchange process of intermediate rate between gauche and trans conformers. To our knowledge, the gel-phase ^2H NMR spectra of the glycerol or head-group regions of glycolipids have never been investigated. If axial diffusion is effectively stopped on the ^2H NMR time scale in these systems, the gel-phase spectra of the head-group region of glycolipids should also be solely the result of internal motions. Elucidating these motional modes would provide a strong foundation upon which to examine the dynamics of these head groups in the less ordered liquid-crystalline phase. To this end, we have investigated the ^2H NMR spectral features and relaxation behavior of the glycolipid β-DTGL, labeled at the C3-position of the glycerol backbone, in both the gel and liquid-crystalline phases, in order to elucidate some of the fundamental motions of the lipid in the two phases.

The ^2H NMR spectrum (Figure 2 (bottom row, column B)) of glycerol-labeled β-DTGL in the gel phase at 25 °C, well below the gel to liquid-crystalline phase-transition temperature (52 °C),[8c] is very broad (≈90 kHz) and characteristic of fast-limit axially asymmetric motion. In such cases, the quadrupolar splitting depends on both Eulerian angles θ and ϕ defining the orientation of the *motionally averaged* electric field gradient tensor $\mathbf{V}_{ij}$ with respect to the magnetic field direction:

$$\Delta\nu_Q(\theta,\phi) = \frac{3e^2qQ}{2h}S\frac{3\cos^2\beta - 1}{2}\left(\frac{3\cos^2\theta - 1}{2} + \frac{\eta_{eff}}{2}\sin^2\theta\cos(2\phi)\right) \quad (2)$$

In this equation, η_{eff} is the motionally averaged asymmetry parameter defined as $(\bar{V}_{xx} - \bar{V}_{yy})/\bar{V}_{zz}$, where $\bar{V}_{yy} < \bar{V}_{xx} < \bar{V}_{zz}$ are the principal components of the *effective* electrostatic field gradient tensor in the molecular fixed frame. All the other parameters have the same meaning as in eq 1.

It has been shown that the observation of spectra indicative of fast-limit axially asymmetric motion requires that the motions have 2-fold or lower symmetry.[7b] This requirement is most simply satisfied with a 2-site exchange process, but a mechanism with sites of unequal populations will also satisfy that requirement. Moreover, the spectral line shapes will depend strongly on the nature of the motion, the populations of the sites, and the rates associated with the exchange process.[7b]

The line shape of the [3,3-$^2H_2$]β-DTGL powder spectrum presented in Figure 2 is invariant to the spacing, τ, between pulses in the quadrupolar echo sequence for τ-values between 40 and 120 μs. In the slow ($\omega_Q\tau_C \gg 1$, $\omega_Q = 2\pi \times \Delta\nu_Q$) or fast ($\omega_Q\tau_C \ll 1$) limit motional regimes, the transverse relaxation time T_{2e} is much greater than the delay τ in the quadrupolar echo sequence, and the line shapes are invariant to pulse spacing.[21] However, for intermediate exchange ($\omega_Q\tau_C \approx 1$), T_{2e} is anisotropic and smaller or equal to the delay τ for many orientations in the powder, resulting in distorted line shapes and in a dependence of the line shapes on the delay τ.[22a,b] Moreover, the spectral intensity is

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dramatically decreased when passing through the intermediate exchange region.[15] The absence of a pulse-spacing dependence at 25 °C for the [3,3-$^2H_2$]β-DTGL spectra therefore indicates that the axially asymmetric motion(s) giving rise to such spectra is in the fast limit relative to the time scale of ^2H NMR ($\gg 10^5$ s^{-1}).

Fast Motion. While a number of potential motional models may be envisaged to account for the β-DTGL powder spectral line shape, it is attractive to search for those that are the simplest *reasonable* descriptions that are consistent with the system under study. A previous study of a phosphatidylcholine suggested that motion about the C2–C3 bond consists of jump between three conformations.[23] With the use of a line-shape simulation program developed by Wittebort et al.[15] based on the general formalism of Torchia and Szabo,[16] the [3,3-$^2H_2$]β-DTGL powder spectral line shape in the gel phase presented in Figure 2 was simulated with several nonequally populated 3-site jump models. In these simulations, the angle β is the angle between the C–^2H bond and the axis of motional averaging. As mentioned previously, it has been shown that in the liquid-crystalline phase the two C–^2H bonds of the glycerol backbone C3 make slightly different angles relative to the axis of motional averaging. From the quadrupolar splittings obtained in the liquid-crystalline phase and the value of the segmental order parameter S of 0.65 obtained for the glycerol backbone, the two angles β were calculated to be 73.7 and 71.5°. On the other hand, in the gel phase, only one powder pattern is obtained. This may be due to a larger dipolar contribution to the intrinsic line width, which obscures the spectral differences associated with a small difference in the angle β. Alternatively, there may be a small change in the glycerol backbone orientation in the gel phase compared to that in the liquid-crystalline phase that makes the two angles β equivalent. A more exact β-value in the gel phase was calculated from the measurement of the doublet separation obtained for the 0°-oriented sample spectra (vide infra). The doublet separation in this spectrum is 80 kHz. Using eq 2 and assuming that the segmental order S is equal to unity in the gel phase (this assumption is reasonable since, as will be shown later, axial diffusion is basically stopped on the ^2H NMR time scale at that temperature; any angular fluctuations of the molecule as a whole can therefore be neglected), an angle β of 69.3° was calculated. This angle is slightly smaller than those obtained for the two C–^2H bonds in the liquid-crystalline phase. However, whether or not this is due to a change in the orientation of the glycerol backbone remains to be confirmed. An angle β of 69° was therefore used for all the gel-phase spectral simulations presented in this paper. However, the results of these simulations were not affected significantly when β was varied by ±1° about 69°.

The experimental [3,3-$^2H_2$]β-DTGL powder spectrum line shape was relatively well simulated with a variety of unequally populated 3-site jump models. Since the gel-phase spectra (Figure 2) are in the fast limit relative to the time scale of ^2H NMR line shape, simulated spectral line shapes were relatively insensitive to the jump rate k used in the simulations ($k > 5 \times 10^6$ rad s^{-1}). Two examples are shown in Figure 2 (bottom row, columns C and D) for populations of 0.50, 0.25, 0.25 and 0.46, 0.34, 0.20, respectively. It should be mentioned at this point that two main categories of 3-site jump models were investigated, the first where one of the populations was much larger than the other two and a second with two approximately equal populations and a third population much smaller than these. Interestingly, the populations of the second set are relatively close to those of the gauche-(+), gauche-(−), and trans populations about the glycerol C2–C3 bond proposed in phospholipids (0.48, 0.37, and 0.15 for dihexadecylphosphatidylcholine (DHPC) in D_2O).[23] However, from just the powder spectral line-shape simulations (Figure 2), it is not possible to elucidate unambiguously the exact motion executed about the C2–C3 bond of the glycerol backbone in the gel phase, since the experimental powder pattern can be simulated with a variety of motional models.

In order to determine accurately the asymmetry parameter η_{eff} and therefore the principal components of the motionally averaged electric field gradient tensor $\bar{V}_{xx}$, $\bar{V}_{yy}$, and $\bar{V}_{zz}$ for glycerol-labeled β-DTGL in the gel phase, measurements of oriented-sample spectra are of great use since this is analogous to a single-crystal study. This approach has previously been used in liquid crystals to determine the asymmetry parameter η of biaxial smectic phases.[24a,b] The ^2H NMR spectra of a macroscopically oriented sample of [3,3-$^2H_2$]β-DTGL (80 mol % in DPPC) are shown in Figure 2 (column B). For the preparation of oriented samples, β-DTGL was mixed 80 mol % in DPPC in order to achieve a better alignment of the sample. It is important to note that both powder and oriented-sample line-shape and relaxation features are well simulated with the same motional model (vide infra). Moreover, the motionally averaged tensor elements extracted from the powder and oriented-sample spectra are very similar, and the T_1 values determined from the oriented-sample spectra are in good agreement with the T_1 anisotropy observed in the powder spectra (vide infra). Therefore, the presence of 20 mol % DPPC in the oriented sample does not significantly affect the dynamics of β-DTGL. Spectra were measured as a function of the angle θ (eq 2) between the normal to the plane of the glass plates (taken to be coincident with the bilayer normal) and the magnetic field direction. The 0° orientation corresponds to the plane of the glass plates orthogonal to the magnetic field direction. The results shown in Figure 2 clearly demonstrate that, for all but the 0° orientation, powder spectra (over ϕ (eq 2)) are obtained since $\eta_{eff} \neq 0$. These spectra have line shapes characteristic of an asymmetry parameter close to unity (simulations not shown). Moreover, the single doublet obtained for the 0° orientation confirms that, as was previously demonstrated in the liquid-crystalline phase,[8e] the bilayer normal is the axis of motional averaging for β-DTGL in the gel phase. For the 0° orientation, the two peaks are much broader than those obtained in the liquid-crystalline phase, due mostly to increased dipolar coupling between the deuterons at C3 and the neighboring protons. It should be noted that the oriented sample used in the gel phase was the same as that from which the oriented spectra in the liquid-crystalline phase were obtained (Figure 2, column A). Therefore, the line shapes obtained for the gel-phase spectra are not due to poor orientation of the sample. Moreover, it is important to note that the gel-phase oriented-sample spectra are the result of 500K accumulations, while the corresponding liquid-crystalline phase spectra have been obtained with only 50K accumulations.

With use of a modified version of the line-shape simulation program previously used for the β-DTGL *powder* spectra (vide supra), the *oriented-sample* spectral line shapes were now simulated by several 3-site jump models distinguished only by the relative populations of the sites. The results for two sets of populations (0.50, 0.25, 0.25 and 0.46, 0.34, 0.20) are shown in Figure 2 (columns C and D, respectively) and compared to the experimental spectra for the 0, 35, 54.7, and 90° orientations. It is interesting to note that both descriptions can reproduce relatively well the experimental line shapes, with the 54.7° orientation being better simulated by the first motional model while the 35° orientation spectrum is better reproduced by the second model. It should be noted at this point that the [3,3-$^2H_2$]β-DTGL powder spectrum line shape could also be simulated by an equally populated 2-site jump model with the 2 sites separated by an angle of 120°. However, when this motional model was used, the simulated and experimental oriented-sample line shapes differed significantly. It is clear that the use of oriented samples (fixed θ, variable ϕ) can facilitate the discrimination between different motional models that could not be differentiated solely on the basis of powder (variable θ and ϕ) line shapes.

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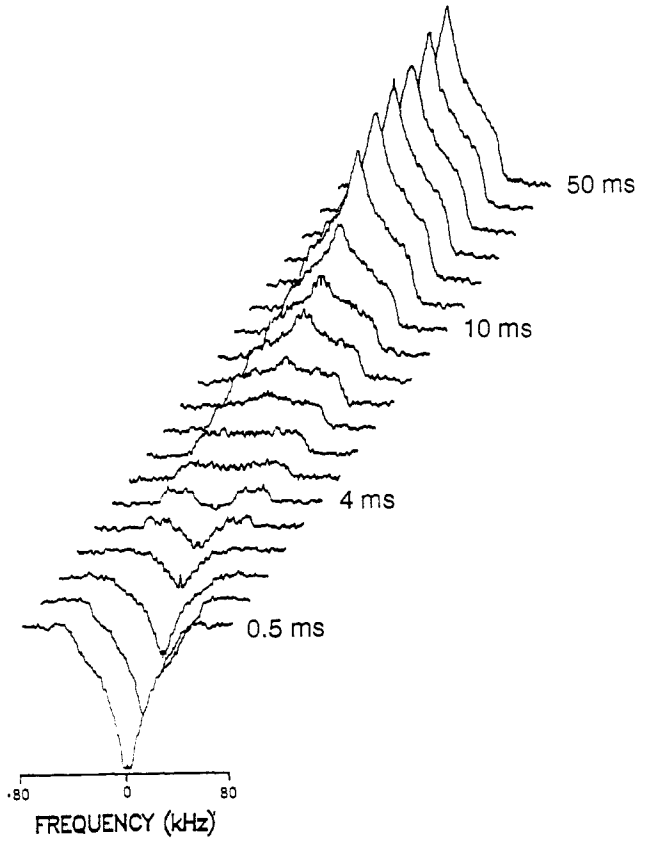

Figure 3. Experimental spectra of a multilamellar dispersion of [3,3-$^2H_2$]β-DTGL in the gel phase (25 °C) as a function of the delay T in the inversion-recovery sequence: 180_x–T–$[90_x$–τ–$90_y]$. Note that, near the null point ($T \approx 4$ ms), a θ-dependence of the relaxation times T_{1Z} is observed.

So far, we have demonstrated that a few reasonable 3-site exchange models can account for the powder and oriented-sample line shapes of [3,3-$^2H_2$]β-DTGL in the gel phase at 25 °C. However, as discussed previously, the motion giving rise to such spectra appears to be in the fast limit relative to the ^2H NMR line-shape time scale. Therefore, a unique correlation time τ_c for this motion could not be obtained solely from the spectral line-shape simulations. In the slow- or fast-limit motional regimes, other methods are necessary in order to obtain dynamic information. Ultraslow motions in solids and solid polymers, characterized by correlation times $\tau_c \gg 1$ s, have been studied by deuteron spin alignment[25a] and more recently by two-dimensional exchange NMR.[25b,c] On the other hand, for phospholipids in the liquid-crystalline phase, where correlation times for C–^2H bond motions are shorter than 10^{-7} s, molecular dynamics of the acyl chains have been widely studied by ^2H spin-lattice relaxation.[26a-d] For multilamellar dispersions of liquid-crystalline bilayers, the rapid lateral diffusion of lipid molecules over the curved two-dimensional surface of multilamellar liposomes is frequently sufficiently fast to prevent the observation of orientation-dependent relaxation.[26b] However, if the anisotropic relaxation effects are not obscured by lateral diffusion, the measurement of the orientation-dependent ^2H spin-lattice relaxation times (T_{1Z}) can be very useful in the study of molecular dynamics in lipid bilayers. In this regard, the use of macroscopically oriented samples circumvents the orientational averaging effects of rapid lateral diffusion over the curved liposomal surfaces and has proven to be of great value in the study of orientation-dependent relaxation of lipids in the liquid-crystalline phase.[12b,c27a,b] On the other hand, the study of ^2H spin-lattice relaxation in the gel phase of lipid bilayers or in the "liquid-gelatin" phase of lipid/cholesterol mixtures is possible since the lipid lateral diffusion rates are about 1 order of magnitude smaller.[12a,19b,20b,28] In particular, the study of the orientation dependence of ^2H spin-lattice relaxation for the acyl chain labeled glycolipid NPGS (vide supra) in the gel phase has allowed the determination of the correlation time for trans–gauche isomerization,[20b] information that could not be obtained from the line-shape analysis of fast-limit spectra.[19b]

Table I. Experimental and Calculated ^2H (30.7 MHz) Spin-Lattice Relaxation Times (T_{1Z}) for Oriented Samples of [3,3-$^2H_2$]β-DTGL in the Gel Phase at 25 °C[a]

	relaxation time (ms)		
		calcd for k (rad s^{-1}) =	
θ (deg)	exptl	5×10^8	1.25×10^7
0	4.04 ± 0.06	3.76	4.22
54.7	5.23 ± 0.05	5.41	5.50
90	6.19 ± 0.13	6.92	5.61

[a] The angle θ is the angle between the bilayer normal and the magnetic field direction. The 0° orientation corresponds to the plane of the glass plates orthogonal to the magnetic field direction. Simulations were performed with a 3-site jump model with populations 0.46, 0.34, and 0.20 and two different jump rates, as indicated in the table.

In order to discriminate between different 3-site exchange models that are able to reproduce the experimental powder and oriented-sample spectra of β-DTGL in the gel phase and to determine the correlation time of the motion in question, we have first performed an inversion-recovery experiment on a multilamellar dispersion of [3,3-$^2H_2$]β-DTGL at 25 °C. The results shown in Figure 3 as a function of the delay T after the inverting pulse clearly demonstrate that there is a significant orientation dependence of the ^2H spin-lattice relaxation times (T_{1Z}) in this system. From this figure, it is clear that the outer edges of the powder spectrum recover faster than does the central part. This effect is particularly visible near the null point, for T values of about 4 ms. However, since each part of the powder spectrum is not due solely to a unique angle θ describing the orientation of the bilayer normal relative to the magnetic field direction but to a combination of angles (θ, ϕ), it is not possible from the powder spectra to determine the individual spin-lattice relaxation times for each orientation and extract details about the T_1 anisotropy.

In order to determine the T_{1Z} values for a given orientation θ, as well as to examine the T_{1Z} anisotropy, if any, associated with the angle ϕ for this particular orientation, inversion-recovery experiments were performed on oriented samples of [3,3-$^2H_2$]β-DTGL at 25 °C for θ values of 0, 54.7, and 90°. The experimental T_{1Z} values for these orientations (Table I) exhibit the following angular dependence of the spin-lattice relaxation times:

$$T_{1Z}\,(0°) < T_{1Z}\,(54.7°) < T_{1Z}\,(90°)$$

It should be noted that, for each value of θ, the relaxation time presented in this table is a powder average over all azimuthal angles ϕ. The spin-lattice relaxation times obtained for [3,3-$^2H_2$]β-DTGL are very short, indicating that the correlation time(s) of the motion(s) dominating the relaxation is (are) very close to the T_1 minimum ($\omega_0\tau_C \approx 0.65$).

The experimental T_{1Z} values are compared in Table I with those calculated by the general formalism developed by Torchia and Szabo.[16] The two categories of 3-site exchange model described earlier reproduced the orientation-dependent relaxation times relatively well; calculated values with relative populations of 0.46, 0.34, and 0.20 are presented in Table I. Simulations were again performed with an angle β of 69°, and the exchange rate k was varied in order to match the experimental T_{1Z} values. The results

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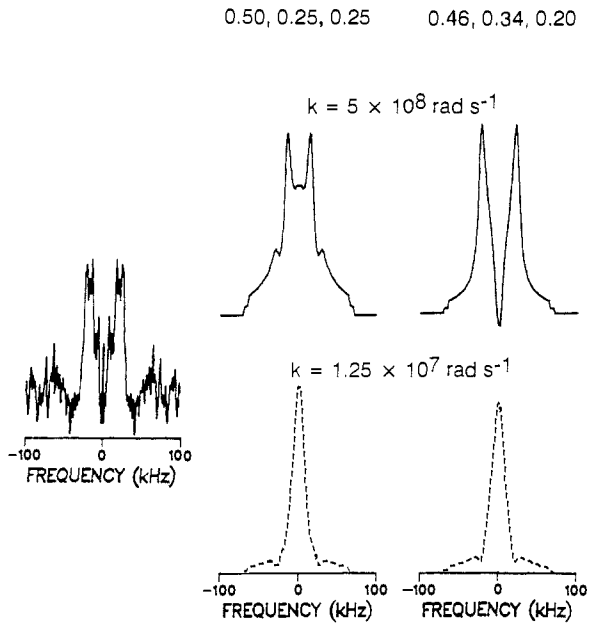

Figure 4. Left: Experimental inversion-recovery spectrum (T = 3.5 ms) of [3,3-$^2H_2$]β-DTGL/DPPC (4:1, molar ratio) oriented at θ = 54.7°. Note that, for this particular θ, a *ϕ-dependence* of the relaxation times is observed. Right: Corresponding simulated inversion-recovery spectra (T = 4 ms). Simulations were performed with the use of the two 3-site jump models described previously, with exchange rates on both sides of the T_1 minimum.

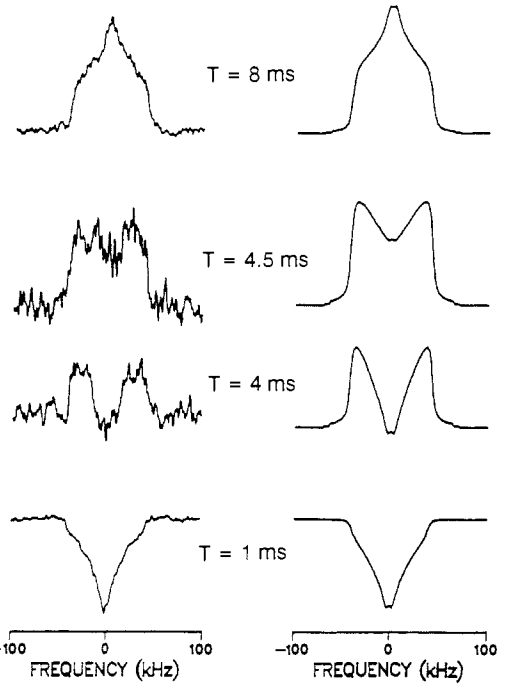

Figure 5. Experimental (left) and simulated (right) partially recovered (T_1) spectra of a multilamellar dispersion of [3,3-$^2H_2$]β-DTGL at 25 °C as a function of the delay T in the inversion-recovery sequence. Simulations were performed with a 3-site jump model with an exchange rate of 5 × 10^8 rad s^{-1} and populations 0.46, 0.34, and 0.20.

presented in Table I indicate that the orientation dependence of the spin–lattice relaxation times observed experimentally can be reproduced with exchange rates of 1.25 × 10^7 and 5 × 10^8 rad s^{-1}, corresponding to correlation times τ_c of 2.7 × 10^{-8} and 6.7 × 10^{-10} s, on both sides of the T_{1Z} minimum (where $\tau_c \approx 4 \times 10^{-9}$ s at 30.7 MHz). On the basis of these results alone, it is therefore difficult to determine unambiguously the exact correlation time of the 3-site exchange motion.

An additional striking feature of the partially relaxed spectra for the 54.7- and 90°-oriented samples is a dependence of the relaxation times for a given angle θ on the angle ϕ, a *ϕ-dependence*. Except in very restricted cases, it is generally expected that spin–lattice relaxation times will depend on both angles θ and ϕ.[16] However, in powder spectra, the ϕ-dependence is not easily distinguishable from the dependence on the angle θ. To our knowledge, this is the first time that such a ϕ-dependence of relaxation times for axially asymmetric spectra has been observed in lipid bilayers. This ϕ-dependent T_{1Z} for θ = 54.7 is particularly clear in Figure 4 (left spectrum), which shows the partially relaxed spectrum of [3,3-$^2H_2$]β-DTGL for a delay T of 3.5 ms in the inversion-recovery sequence. A ϕ-dependence was also observed for θ = 90°, in which case the central part of the spectrum relaxes faster than the outer edges.

This ϕ-dependence of the spin–lattice relaxation times observed for the 54.7° orientation serves as an additional feature of the anisotropic relaxation behavior useful in discriminating between the different motional models discussed so far. Simulated partially relaxed spectra for the 54.7° orientation near the null point, for a T value of 4 ms in the inversion-recovery sequence (Figure 4), clearly demonstrate that, for both sets of relative populations, simulations performed with the slower exchange rate (1.25 × 10^7 rad s^{-1}) give a reversed ϕ-dependence of the relaxation times, even if the average (over ϕ) T_1 value is relatively close to the experimental value. For the faster exchange rate (5 × 10^8 rad s^{-1}), a ϕ-anisotropy very similar to that observed experimentally is obtained with relative populations of 0.46, 0.34, and 0.20.

We can therefore conclude that this ϕ-dependence of the relaxation times for a given value of θ puts severe constraints on both the motional model and exchange rate used to simulate the line-shape and relaxation features observed for glycerol-labeled β-DTGL in the gel phase at 25 °C. Taking into account the powder and oriented-spectral line shapes, as well as the θ- and ϕ-dependences of the spin–lattice relaxation times T_{1Z}, the line-shape and relaxation features of [3,3-$^2H_2$]β-DTGL in the gel phase were best simulated by a 3-site jump model with relative populations of 0.46, 0.34, and 0.20 and an exchange rate k of 5 × 10^8 rad s^{-1}, which corresponds to a correlation time τ_c ($\tau_c = (3k)^{-1}$) of 6.7 × 10^{-10} s. With use of these parameters, the inversion-recovery spectra of multilamellar dispersions of [3,3-$^2H_2$]β-DTGL were simulated near the null point in the inversion-recovery sequence. As shown in Figure 5, the agreement between the experimental and simulated partially relaxed spectra is very good. It therefore appears that a combination of ^2H NMR line-shape and relaxation studies of gel-phase glycerol-labeled β-DTGL does allow a relatively unambiguous determination of the motions dominating the line shape and relaxation features in this system. Furthermore, the use of aligned samples facilitates the separation of the θ- and ϕ-contributions to $T_1(\theta,\phi)$. It is clear that the individual *subspectra or partially relaxed subspectra* are *more* sensitive to model parameters than are the corresponding powder spectra.

Thus far, we have not discussed the detailed nature of the 3-site jump motion. Are the features observed for the C3 carbon of the glycerol backbone the result of a motion of the glycolipid molecule as a whole or of an internal motion about the C2–C3 (or C1–C2) bond of the glycerol backbone? The first possibility is unlikely on the basis of previous results obtained with glycolipids in the gel phase (vide supra),[19b-20b] which have shown that the line-shape and relaxation features in these systems are dominated by internal motions. In addition, the relaxation times for the lipid in the liquid-crystalline phase (52 °C) and the gel phase (25 °C) are very similar, a surprising result given that the higher temperature phase is less ordered. Internal motion in the glycerol backbone of β-DTGL is therefore a more plausible site of the jumping motion.

The powder line-shape and relaxation features noted for glycerol-labeled β-DTGL were also obtained for the head-group-labeled (C1) glycolipid (results not shown) and for a number of other head-group-labeled mono- and disaccharide glycolipids in the gel phase (Jarrell et al., unpublished results). Whatever motion is occurring at the glycerol backbone level seems therefore to be reflected in the head-group region. Whether this motion takes

place about the C2–C3 or the C1–C2 bonds of the glycerol backbone would have to be confirmed by the analysis of β-DTGL specifically deuteriated at the C2-position of the glycerol backbone.

The possibility of an internal motion at the glycerol backbone of β-DTGL seems, however, in apparent contradiction with previous studies that indicated that the glycerol backbone in lipids is essentially rigid on the ^2H quadrupolar splitting[29] and ^{13}C chemical shift anisotropy (CSA)[30] time scales. The results of these studies suggested that the glycerol conformation is constrained to one that produces motionally inequivalent quadrupolar splittings of the deuterons at C1 and C3 of the glycerol backbone while keeping the same C1 and C3 methylene carbon orientations. Moreover, as mentioned previously, it was shown for β-DTGL that differential ^1H–^2H dipolar couplings between the two deuterons at C3 and the proton at C2 were obtained, suggesting an inequivalence of these two deuterons.[8e] However, since the correlation time of the proposed internal motion is very short (6.7×10^{-10} s) compared to the time scales of the residual ^2H quadrupolar splittings and ^{13}C CSA, the results of the present study may not be in contradiction with the earlier studies described above. If the internal motion is very fast relative to the interaction studied, only an average conformation about the C2–C3 bond will be detected and the glycerol backbone can be treated as effectively rigid relative to the time scale of interest. Additional support for the presence of a fast internal motion at the C2–C3 bond of the glycerol backbone is that the relative populations determined in the present study (0.46, 0.34, 0.20) are in good agreement with those proposed for the gauche-(+), gauche-(−), and trans conformers about the C2–C3 bond in phospholipids (0.48, 0.37, 0.15).[23] Moreover, relatively small potential energy barriers have been calculated for the rotation about the C1–C2 and C2–C3 bonds of the glycerol backbone in phospholipids.[31]

Slow Motion. The results presented so far were obtained at 25 °C, well below the gel to liquid-crystalline phase transition temperature (52 °C) for β-DTGL. As the temperature of multilamellar dispersions of [3,3-$^2H_2$]β-DTGL was varied from 25 °C (gel phase) to 60 °C (liquid-crystalline phase), a dramatic decrease in spectral intensity was observed at 50 °C, just below the gel to liquid-crystalline phase transition temperature. The results (Figure 6 (top, filled circle)) indicate that the relative intensity at 50 °C is about 5% of the intensity observed at 55 °C, in the liquid-crystalline phase. Moreover, this decrease in intensity is accompanied by a large change in the spectral line shape at 50 °C (Figure 6, bottom left). It is also interesting to note that the intensity of the gel-phase spectrum at 25 °C is about half that observed in the liquid-crystalline phase. This decrease in intensity near a phase transition has been reported for phospholipid systems.[32] In addition, a similar decrease in spectral intensity has been observed in several mono- and disaccharide glycolipid systems (Jarrell et al., unpublished results). Clearly, additional motion(s) is being activated over the temperature range between 25 and 60 °C.

The observation of axially symmetric ($\eta_{eff} = 0$) powder spectra for glycolipid in the liquid-crystalline phase implies that the motions giving rise to such spectra are axially symmetric, i.e., have a 3-fold or higher symmetry, and are in the fast limit relative to the ^2H NMR line-shape time scale. Generally, it is believed, for both phospholipid and glycolipid systems, that an axial motion of the molecule as a whole is responsible for the averaging of the electric field gradient tensor to axial symmetry in the liquid-crystalline phase. Additional averaging is also usually observed in the liquid-crystalline phase due to off-axis motion ("wobbling") of the symmetry axis.

If the axial motion of the entire β-DTGL molecule is too slow in the gel phase to influence spectral line shapes ($\omega_Q\tau_C \gg 1$) and

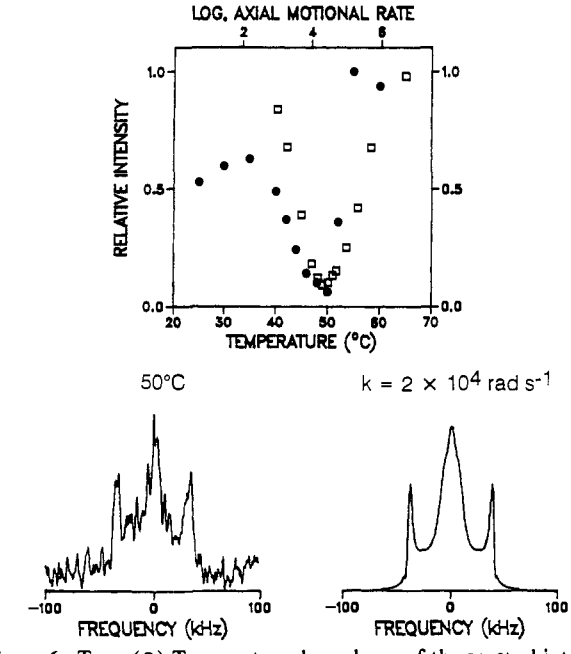

Figure 6. Top: (●) Temperature dependence of the spectral intensity for a multilamellar dispersion of [3,3-$^2H_2$]β-DTGL. Note that, just before the phase-transition temperature (52 °C), a dramatic decrease in intensity is observed. (□) Calculated spectral intensity as a function of the axial motional rate. Simulations were performed with a 9-site model, in which the axial motion was modeled by an equally populated 3-site jump. A similar decrease in spectral intensity was observed with a 36-site model, in which the axial motion was modeled by an equally populated 12-site jump. For the 9-site model, the lowest intensity occurs for an axial motional rate of 2×10^4 rad s^{-1}, which corresponds to a correlation time τ_c ($\tau_c = (3k)^{-1}$) of 1.7×10^{-5} s for the axial motion. Bottom: Comparison between the experimental line shape at 50 °C and that calculated with an axial motion rate of 2×10^4 rad s^{-1}.

fast enough in the liquid-crystalline phase to average the electric field gradient tensor to axial symmetry ($\omega_Q\tau_C \ll 1$) (for temperatures between 25 and 60 °C), the axial motion has to go through an intermediate motional regime ($\omega_Q\tau_C \approx 1$). In such cases, short anisotropic transverse relaxation times T_{2e} will result in a dramatic decrease in spectral intensity and changes in line shape (vide supra). A reasonably simple explanation for the variation in spectral intensity observed for β-DTGL at temperatures between 25 and 60 °C is that there is such a change in the rate of axial motion of the glycolipid molecule. To test this hypothesis, simulations of powder and oriented-sample line shapes were performed with a composite motional model including both the fast rotameric (unequally populated) 3-site jump model (internal mode) needed to account for the gel-phase line-shape and relaxation features, and an axial motion (whole molecule mode) with exchange rates that were varied from about 1×10^3 rad s^{-1} (slow motional limit) to 5×10^6 rad s^{-1} (fast motional limit). The axial motion was first modeled simply by an equally populated 3-site exchange model in which the 3 sites were coincident with the rotameric jump sites. The relative spectral intensities for powder spectra of glycerol-labeled β-DTGL, simulated by this 9-site model, are presented in Figure 6 (top, open squares) as a function of the log of the axial motional rate. It is clear from this figure that varying the axial motion rate in the 9-site model, while keeping the rotameric jump rate constant, can satisfactorily reproduce the experimental temperature dependence of spectral intensity. The smallest simulated spectral intensity, obtained by an axial motion rate k of 2×10^4 rad s^{-1}, is about 5% that obtained for $k = 5 \times 10^6$ rad s^{-1}. It should be noted, however, that unlike the experimental temperature dependence of spectral intensity, the simulated intensity curve is symmetric since contributions other than chemical exchange were neglected in the simulations. With use of the 9-site model, the powder spectrum line shape obtained for the axial rate giving the smallest simulated spectral intensity (2×10^4 rad s^{-1}) was simulated and compared with the experi-

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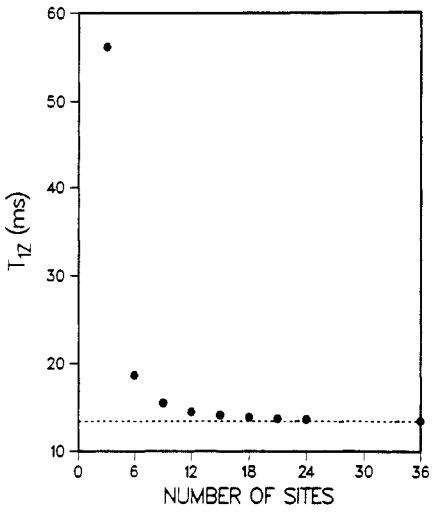

Figure 7. Calculated spin–lattice relaxation times T_{1Z} as a function of the number of sites N in a N-site exchange model. T_{1Z} were calculated from an exchange rate k determined from a constant rotational diffusion rate D of 5×10^6 rad s^{-1}, according to the expression $D = (4\pi^2 k/N^2)$.[16] (···) Free diffusion limit (T_{1Z} = 13.4 ms).

mental powder spectrum at 50 °C (Figure 6, bottom). Since the intensity of this powder spectrum is only about 5% that of the liquid-crystalline phase spectrum, the signal-to-noise ratio for the experimental spectrum is rather poor. However, the two powder spectrum line shapes compare favorably and further support the conclusions made from the temperature dependence of spectral intensities.

The quadrupolar echo amplitude decays with a time constant T_{2e}, which is sensitive to slow motions.[33a,b] For powder samples, the motion that will most likely dominate transverse relaxation is diffusion of the lipid molecules along the curved membrane surfaces.[33b-e] By using oriented samples, the orientational averaging effects of rapid lateral diffusion over the curved liposomal surfaces are circumvented. The transverse relaxation time T_{2e} for an oriented sample of [3,3-$^2H_2$]β-DTGL was measured at 52 °C for θ = 90°. The experimental value of 1.2 ms was then compared with that calculated from the 9-site model described previously, in which axial motion is modeled by an equally populated 3-site jump. A T_{2e} of 1.13 ms was calculated from an axial motional rate of 3×10^6 rad s^{-1}, which corresponds to the rate estimated for a temperature of ≈55 °C in the liquid-crystalline phase, as shown in Figure 6 (top). The good agreement between the experimental and simulated T_{2e} values confirms the presence of slow axial motion (on the ^{2}H spin–lattice relaxation time scale) in the liquid-crystalline phase of β-DTGL and indicates that contributions from any other slow motions to transverse relaxation can most likely be neglected. Note that additional contributions to the transverse decay rate from static and fluctuating dipolar interactions have been neglected in these T_{2e} calculations.

Previous line-shape simulations of phospholipid gel-phase spectra[19a] and of lipid/cholesterol "liquid-gelatin" phase spectra[12a] have shown that 3-fold (120°) rotational jumps about the director would seem to be an appropriate model for rotational diffusion. In the present system, the question remains that while modeling axial motion by large angle jumps (3 sites) is sufficient to explain the data, can a diffusive (small angle) motion be equally satisfactory? Torchia and Szabo[16] have demonstrated that the correlation function for a N-site nearest-neighbor jump model reduces to that obtained for free diffusion when $N \rightarrow \infty$ and $k \rightarrow \infty$ such that $D = 4\pi^2 k/N^2$, where D is the rotational diffusion rate. In order to investigate this point, we have determined the effect on both T_{1Z} and line-shape intensity of the number of sites used to model axial motion. These simulations were performed with a constant value of the rotational diffusion rate D ($D = 5 \times 10^6$ rad s^{-1}), and the exchange rate k for a particular value of N was calculated from the expression $k = N^2 D/4\pi^2$. The results for N varying from 3 to 36 are presented in Figure 7. From this figure, it is clear that a large difference in the T_{1Z} values is observed between a 3- and a 6-site jump model and that the T_{1Z} values approach the value calculated for free diffusion (13.4 ms) when the number of sites is equal to or greater than 12. Similar results have been obtained by Bonmatin et al.[27b] in a study of the dynamical properties of cholesterol in liquid-crystalline bilayers. In that case, it is found that large (3-fold) jumps of cholesterol can best account for the relaxation features in this system, in comparison to small-step Brownian diffusion.

The simulations presented in Figure 6 were repeated with use of a 36-site exchange model, representing a 3-site fast rotameric jump model with a jump rate of 5×10^8 rad s^{-1} and an axial motion modeled by a 12-site nearest-neighbor jump model. The results of these spectral line-shape simulations are essentially the same as those presented in Figure 6; a similar decrease in spectral intensity is observed, the lowest intensity occurring for an axial motion rate of about 5×10^4 rad s^{-1}. The temperature dependence of spectral intensity observed for β-DTGL at temperatures between 25 and 60 °C can therefore be simulated by either a 9- or a 36-site exchange model in which the axial motion of the entire molecule is modeled by either a 3- or a 12-site jump model. Thus, it is not possible from the present results to determine the exact nature of the axial motion of the β-DTGL molecule. The use of two-dimensional ^{2}H NMR exchange techniques,[25b,c] which are sensitive to the rate and mechanism of very slow motions, could provide a way of distinguishing between a large jump or a free diffusion model of axial motion for β-DTGL. It is of interest to note that a recent study suggested that, in highly ordered systems, large angle motion may be favored.[34a] Indeed, two recent studies have concluded that large-angle jumps occur rather than diffusive motion.[27b,34b]

From the comparison between the temperature dependence of spectral intensity and that obtained from the variation of the axial motional rate (Figure 6), an activation energy of about 150 kJ mol^{-1} was estimated for the axial motion of β-DTGL in the gel phase. This value is higher than the value of ≈70 kJ mol^{-1} determined by Meier et al.[32] for phospholipids in the gel phase and could explain why axial motion appears to be slower in the gel phase of glycolipid systems compared to phospholipid systems.

The presence of two motions, a fast internal motion and a slower axial motion, can therefore account quantitatively for the line-shape features of β-DTGL in both the gel and liquid-crystalline phases. As discussed previously, the rates of axial motion (≈1 $\times 10^6$ rad s^{-1}) in the liquid-crystalline phase are in the fast limit relative to the ^{2}H NMR line-shape time scale and, therefore, average the spectrum to an axially symmetric powder spectrum. However, these motional rates are relatively far from the T_1 minimum and therefore should not dominate the relaxation features in this system. To verify this point, we have calculated the spin–lattice relaxation times T_{1Z} using either a 9- or a 36-site exchange model and with variation of the axial motional rates from 1×10^2 to 5×10^6 rad s^{-1} while the rotameric jump rate was kept to a constant value of 5×10^8 rad s^{-1}. In all cases, the T_1 values were found to be identical with those presented in Table I with a 3-site rotameric jump model (with $k = 5 \times 10^8$ rad s^{-1}), indicating that the second motion needed to account for the spectral line shapes observed for β-DTGL does not contribute significantly to spin–lattice relaxation in both the gel and liquid-crystalline phases.

We have demonstrated earlier that the fast internal 3-site jump motion at the glycerol backbone provides the dominant spin–lattice relaxation mechanism for β-DTGL in the gel phase at 25 °C. Having a reasonable mechanism for spin–lattice relaxation in the

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gel phase, it is intriguing to test whether such a simple description can account for the relaxation in the less ordered liquid-crystalline phase. Preliminary investigations show that, in the liquid-crystalline phase at 52 °C, the relaxation times T_{1Z} obtained for [3,3-$^2H_2$]β-DTGL are similar to those obtained in the gel phase. Values of 4.2, 5.7, and 6.7 ms have been obtained, respectively, for the 0, 54.7, and 90° orientations at 52 °C. Comparison of these values with those presented in Table I clearly indicates similar θ-dependent relaxation times in the gel and liquid-crystalline phases. On the basis of these results, it therefore appears that a fast internal motion could also provide a dominant relaxation mechanism for glycerol-labeled β-DTGL in the liquid-crystalline phase. A more detailed analysis will, however, be necessary to take into account the effects of other motions present in the liquid-crystalline phase, such as the off-axis motion of the entire glycolipid molecule.

Comparison with Previous Studies. It is important to consider the results of the present study in the context of previous descriptions of analogous systems. It has been shown[26a-d] that the ^2H relaxation rate T_1^{-1} in the liquid-crystalline phase of saturated lipid bilayers can be written as

$$T_1^{-1} = T_{1f}^{-1} + T_{1s}^{-1}$$

where T_{1f}^{-1} represents the contribution from relatively fast reorientational motions, including isomerizations, torsional oscillations, and long-axis rotational diffusion and T_{1s}^{-1} represents the contribution from slower reorientations of the molecule with respect to the bilayer normal (director) due to collective order director fluctuations. ^2H NMR studies of perdeuteriated 1,2-diacyl-*sn*-glycero-3-phosphocholine[26d] have shown that the T_{1f}^{-1} contributions from local motions are relatively small and suggested that the dominant contribution to the ^2H spin–lattice relaxation rates arises from collective order director fluctuations (ODF). However, the T_1 anisotropy predicted by this slow collective model was not observed in the partially relaxed powder spectra of unoriented lipid/cholesterol bilayers[12a] or in the spectra of oriented bilayers of DMPC and DPPC.[12b] Therefore, it would appear that isolated rather than collective motions best describe molecular dynamics on time scales $<10^{-8}$ s in lipid bilayers. The results obtained in this study for the gel and liquid-crystalline phases of the glycolipid β-DTGL further support this hypothesis.

The present study demonstrates that the line-shape and relaxation features observed for glycerol-labeled β-DTGL can be best reproduced by a fast internal 3-site jump motion at the glycerol backbone and that a change in the axial motional rate is observed on going from the gel to the liquid-crystalline phase. It is also interesting to note that the rotameric jump constant ($k = 5 \times 10^8$ rad s^{-1}) is about 2 orders of magnitude faster than the axial motional rate estimated in the liquid-crystalline phase (5×10^6 rad s^{-1}). Similar results have been obtained by Meier et al.[32] for phospholipid bilayers where the correlation times for chain rotation and chain fluctuation were of the order of 10^{-8} s, while trans–gauche isomerization was significantly faster ($\tau_j \approx 10^{-10}$ s) in the liquid-crystalline phase. Moreover, the activation energies for local motions were determined to be about 1 order of magnitude smaller than those of the whole-molecule motions. On the other hand, for NPGS/cholesterol bilayers in the liquid-gelatin phase, it was determined that at least two modes of molecular reorientation could contribute to spin–lattice relaxation, namely trans–gauche isomerization and long-axis diffusion.[12a] The relative rates of these processes (as determined from the T_1 anisotropy of NPGS powder spectra) were calculated to be of 5×10^8 s^{-1} for rotational diffusion and 5×10^7 s^{-1} for trans–gauche isomerization. The relative rates of axial diffusion and local motion determined for NPGS/cholesterol bilayers contrast with those obtained in the present study for β-DTGL, where the axial motion was found to be about 2 orders of magnitude slower in the liquid-crystalline phase compared to the internal motion. However, there are several experimental observations to support the conclusions obtained in the present study, in particular the ϕ-dependence of the relaxation times observed in gel-phase oriented-sample spectra. Moreover, the characteristic powder line shapes observed for these oriented-sample spectra also provide a way of discriminating between different motional models. The use of oriented samples may, therefore, prove to be of great value in the study of molecular dynamics of lipid bilayers in the gel phase and may provide a better way of elucidating motional modes than an interpretation based solely on powder spectrum line shape and relaxation.

A firm basis for the analysis of motional modes in both the gel and liquid-crystalline phases of glycoglycerolipid bilayers has been established in the present study. In effect, dynamics in two well-separated motional windows were examined, one in the nanosecond range and the other in the millisecond range. In addition, the internal motion elucidated in this study suggests that similar motion should be considered in the description of the head-group motion of other lipid classes.

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Registry No. DPPC, 2644-64-6; 1,2-di-*o*-tetradecyl-3-*o*-(β-D-glucopyranoryl)-*sn*-[3,3-$^2H_2$]glycerol, 106975-34-2.