Glycerolipids: Common Features of Molecular Motion in Bilayers[†,‡]

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ABSTRACT: In the present study, analysis of ^{2}H NMR line-shape and spin–lattice relaxation behavior has been used to investigate the dynamics of several glycolipid and phospholipid bilayers. The gel-phase spectra of these lipids labeled at the C3 position of the glycerol backbone are broad (≈90 kHz) and characteristic of fast-limit axially asymmetric motion. Moreover, anisotropic spin–lattice relaxation is observed in all of these systems. The line-shape and relaxation features of the lipids in the gel phase were best simulated by using a fast-limit three-site jump model, with relative site populations of 0.46, 0.34, and 0.20. This motion is associated with an internal jump about the C2–C3 bond of the glycerol backbone. A second motion, rotation about the long axis of the molecule, is needed to account for the observed temperature dependence of the quadrupolar echo amplitude and the spectral line shape above and below the gel to liquid-crystalline phase transition temperature. On the other hand, the gel-phase spectra of phospholipids labeled at the C2 position of the glycerol backbone are also characterized by a fast internal motion, which is simulated by a two-site librational jump. The results indicate that the glycerol backbone dynamics of the glycolipid and phospholipid systems investigated in this study can be described in terms of common fast internal motions and a slower whole molecule axial motion. These results are compared with previous dynamic studies of similar systems.

Considerable effort has been expended over the last 20 years on probing physicochemical properties of lipid bilayers. The principal motive behind most of these studies has been to achieve a better understanding of the physical properties of biological membranes. While the structural characteristics of lipid membranes have been the subject of much study, the dynamical aspects of these systems are increasingly recognized as an important element in the description of these assemblies. Although there have been numerous studies of molecular motion in lipid membranes (Seelig, 1977; Griffin, 1981; Brown, 1982; Marsh, 1981), frequently experimental results cannot be unambiguously interpreted in terms of rates and mode of molecular motion. Recent studies have begun to unravel the complex dynamics of these systems (Auger et al., 1990; Bonmatin et al., 1988, 1990; Griffin et al., 1988; Meier et al., 1986; Siminovitch et al., 1985, 1988; Speyer et al., 1989). Interest in characterizing the types and rates of motions of the constituent molecules in bilayers has focused not so much on their absolute nature and values, respectively, but more so on how they change as a function of membrane composition, temperature, and other factors. With such information, a more complete understanding of the nature of membrane systems should be forthcoming.

Since membrane systems often exhibit complex motions, it is important to characterize molecular motion over a broad frequency range in order to achieve as complete a description of bilayer dynamics as possible. Establishing the time scales and amplitudes of molecular motions in the anisotropic environment of lipid bilayers is a problem well suited to solid-state ^{2}H NMR spectroscopy (Seelig, 1977; Griffin, 1981; Davis, 1983, 1986; Bloom & Smith, 1985; Smith, 1984). A recent ^{2}H NMR spin–lattice relaxation and line-shape study from our laboratory (Auger et al., 1990) has demonstrated that a simple motional model involving two motions, a fast internal jump about the C2–C3 bond of the glycerol backbone and a slow rotation about the long axis of the molecule as a whole, is 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)[1] in the gel phase. It seemed natural to ask if such a simple dynamical description was unique to the β-DTGL system or if it is applicable to other lipid systems.

A comparison of ^{2}H NMR results from previous studies from this laboratory (Jarrell et al., 1986, 1987a,b; Carrier et al., 1989; Renou et al., 1989) and others (Huang et al., 1980; Siminovitch et al., 1985; Blume et al., 1982a,b; Meier et al., 1986) suggested some common features. Similar axially asymmetric line shapes have been observed for the gel-phase spectra of the glycerol backbone region of a number of glycerolipids. In addition, the echo intensity of the ^{2}H NMR spectra exhibited a minimum as a function of temperature, with the minimum occurring near the gel to liquid-crystalline phase transition. Since the glycerol backbone is a structural feature common to all the lipids involved in these studies, are there also common features in their dynamical description? More specifically, since an adequate motional description for the glycolipid β-DTGL has been elaborated (Auger et al., 1990), one might speculate that the same simple motional model elucidated for this glycerol-labeled glycolipid could then be generalized to other glycolipids as well as to phospholipid bilayers. Therefore, we have investigated the ^{2}H NMR line-shape and relaxation features of two lipids in the gel and liquid-crystalline states. Our results demonstrate that dynamics in these lipid bilayers can also be described by a motional model involving fast internal motions and a slower axial motion of the whole molecule. The implications of a common molecular motion in glycerol-containing lipid systems is discussed, and the proposed motional description is compared with previous investigations of molecular dynamics in similar systems.

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[1] Abbreviations: β-DTGL, 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; DHAEPn, 1,2-di-*O*-hexadecyl-*sn*-glycerol 3-[(2-aminoethyl)phosphonate]; NPGS, *N*-palmitoylgalactosylsphingosine; DHPC, dihexadecylphosphatidylcholine; ^{2}H NMR, deuterium nuclear magnetic resonance.

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Experimental Procedures

1,2-Di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-[3,3-$^2H_2$]glycerol and 1,2-di-*O*-tetradecyl-3-*O*-(α-D-mannopyranosyl)-*sn*-[3,3-$^2H_2$]glycerol were prepared as described previously (Jarrell et al., 1986, 1987a,b; Ogawa & Beppu, 1982). 1,2-Di-*O*-hexadecyl-*sn*-[2-$^2H_1$]glycerol 3-[(2-aminoethyl)phosphonate] and 1,2-di-*O*-hexadecyl-*sn*-[3-$^2H_1$]glycerol 3-[(2-aminoethyl)phosphonate] were prepared as described elsewhere and are labeled to only 50% at the indicated positions (Van Calsteren, 1990). ^2H NMR samples consisted of multilamellar dispersions prepared by hydrating dry lipid with a 10-fold excess of deuterium-depleted water (Aldrich Chemical Co., Milwaukee, WI) in 5- or 10-mm (o.d.) sample tubes. The phosphonolipid samples were hydrated with a tris-acetate 25 mM, EDTA 2 mM buffer, pH 7.5, made with deuterium-depleted water; 75 mg were used for β-DTGL, 50 mg for α-DTML, and ≈10 mg for both phosphonolipid samples. Hydrated samples were heated cyclically to about 10 °C above the gel to liquid-crystalline phase transition temperature, with vortex mixing, and freeze-thawed to homogeneity (four to five cycles).

^2H NMR data were acquired to 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 (Davis et al., 1976) with full phase cycling (Griffin, 1981) and quadrature detection. The pulse spacing was typically 60 μs, with $\pi/2$ pulses of 3.8–4.0 (10-mm coil) or 2.3–2.5 μs (5-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 (Dufourc et al., 1984). Samples were enclosed in a glass jacket where the temperature was regulated to within ±0.5 °C. In some cases, for reasons of clarity, spectra symmetrized about the zero frequency are presented in the figures. However, it should be emphasized that the essential spectral features of interest are present before and after symmetrization.

Simulations were performed on a Sun 4-260 computer using a line-shape simulation program (Wittebort et al., 1987) based on the general formalism of Torchia and Szabo (1982). Simulated line shapes were corrected for the finite width of the experimental 90° pulses in the quadrupolar echo sequence (Bloom et al., 1980), and the partially recovered T_{1Z} spectra were further corrected for the finite width of the 180° pulse (Hiyama et al., 1986).

Results and Discussion

Glycolipid Bilayers. We first compare the line-shape and relaxation features of the glycolipid 1,2-di-*O*-tetradecyl-3-*O*-(α-D-mannopyranosyl)-*sn*-glycerol (α-DTML) with those previously observed for the glycolipid 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (β-DTGL) (Auger et al., 1990) in the gel and liquid-crystalline phases. The ^2H NMR spectra of β-DTGL and α-DTML labeled at the C3 position of the glycerol backbone are shown in Figure 1 at 55 and 25 °C, above and below the gel to liquid-crystalline phase transition temperature for both lipids (52 and 49 °C for β-DTGL and α-DTML, respectively) (Jarrell et al., 1986, 1987b). Above their phase transition temperatures, β-DTGL and α-DTML have been shown to exhibit lamellar and hexagonal structures, respectively (Jarrell et al., 1987b). Below the gel to liquid-crystalline phase transition temperature, the ^2H NMR spectra of both β-DTGL and α-DTML are strikingly similar (Figure 1B). They are broad (≈90 kHz) and characteristic of fast-limit axially asymmetric motion. In such cases, the

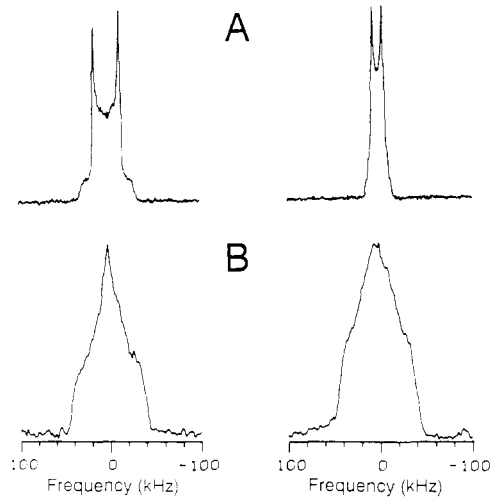

FIGURE 1: Experimental ^2H NMR spectra (30.7 MHz) of multilamellar dispersions of [3,3-$^2H_2$]β-DTGL (left) and [3,3-$^2H_2$]α-DTML (right) at (A) 55 °C and (B) 25 °C.

quadrupolar splitting depends on both Eulerian angles θ and ϕ defining the orientation of the motionally averaged electric field gradient tensor $\mathbf{V}_{ii}$ with respect to the magnetic field direction. The line shapes of the gel-phase spectra presented in Figure 1B are invariant to the spacing, τ, between $\pi/2$ pulses in the quadrupolar echo sequence for τ values between 40 and 120 μs, indicating that the axially asymmetric motion(s) giving rise to such spectra is(are) in the fast limit relative to the time scale of ^2H NMR line shapes ($>10^5$ s^{-1}). In the case of [3,3-$^2H_2$]β-DTGL, it has been demonstrated that such line shapes (powder and oriented sample) are best simulated by a three-site jump model with the C–^2H bonds oriented at 69° with respect to the axis of motional averaging and with relative site populations of 0.46, 0.34, and 0.20 (Auger et al., 1990). Thus, it is attractive to apply the same motional description to α-DTML. Since the gel-phase spectra of both β-DTGL and α-DTML (Figure 1B) are in the fast limit relative to the time scale of the ^2H NMR line shape, simulated line shapes were relatively insensitive to the jump rate k used in the simulations (for $k > 5 \times 10^6$ rad s^{-1}). As a result, a rate for this motion could not be obtained solely from the line-shape simulations. In the slow- or fast-limit motional regimes, other methods are necessary in order to obtain dynamic information. In the case of β-DTGL, spin–lattice relaxation (T_{1Z}) was shown to be anisotropic (dependent on the orientation of the bilayer normal relative to the static magnetic field direction) and was crucial to confirming the nature of the motion about the C2–C3 bond and to evaluating the motional rate (Auger et al., 1990).

In order to probe the correlation time of the motion giving rise to the experimental powder spectra of α-DTML in the gel phase and to compare the results with those of β-DTGL, we have performed inversion–recovery experiments on multilamellar dispersions of [3,3-$^2H_2$]α-DTML at 25 °C. The results shown for comparison in Figure 2A and B as a function of the delay T after the inverting pulse clearly demonstrate that there is a significant orientation dependence of the ^2H NMR spin–lattice relaxation times (T_{1Z}) in both lipid systems. From this figure, the similarity of the two systems is clear, with the outer edges of the powder spectrum recovering faster than does the central part. This effect is particularly visible near the null point, for T values of about 4 ms for β-DTGL and 5 ms for α-DTML. 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

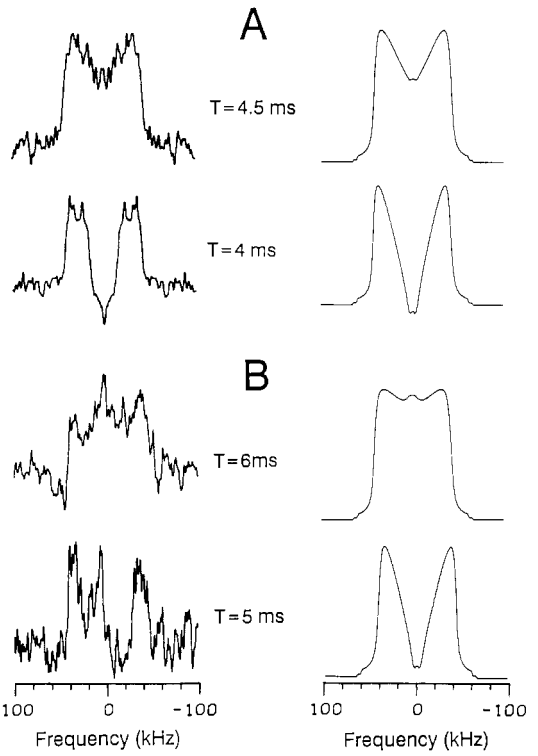

FIGURE 2: Experimental (left) and simulated (right) spectra of a multilamellar dispersion of (A) [3,3-$^2H_2$]β-DTGL and (B) [3,3-$^2H_2$]α-DTML in the gel phase (25 °C) as a function of the delay T in the inversion–recovery sequence 180_x–T–[90_x–τ–90_y]. Note that near the null point, a θ-dependence of the relaxation times T_{1Z} is observed. Number of accumulations for spectra in (B) was 90 000. Simulations were performed using a three-site jump model with relative site populations of 0.46, 0.34, and 0.20 and exchange rates of (A) 5×10^8 rad s^{-1} and (B) 6.2×10^8 rad s^{-1}. The C–^{2}H bonds of the 3-carbon of the glycerol backbone are oriented at 69° with respect to the axis of motional averaging.

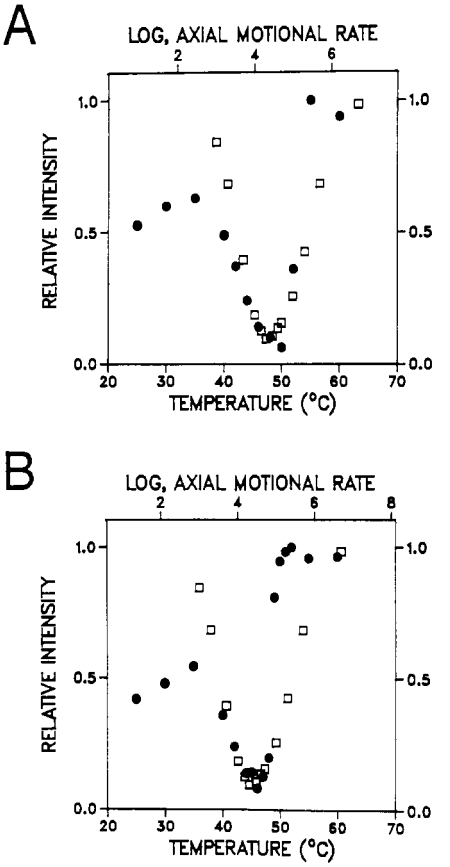

FIGURE 3: (●) Temperature dependence of the spectral intensity for multilamellar dispersions of (A) [3,3-$^2H_2$]β-DTGL and (B) [3,3-$^2H_2$]α-DTML. Note that just before the phase transition temperature, a dramatic decrease in intensity is observed. (□) Calculated spectral intensity as a function of the axial motional rate. Simulations were performed by using a nine-site model, in which the axial motion was modeled by an equally populated three-site jump.

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_{1Z} anisotropy. However, from inversion–recovery experiments on an oriented sample of β-DTGL (Auger et al., 1990), it has been determined that the spin–lattice relaxation times T_{1Z} have the following angular dependence:

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

Taking into account the powder and oriented spectral line shapes, as well as the θ- and ϕ-dependence (Auger et al., 1990) of the spin–lattice relaxation times T_{1Z}, the most satisfactory simulations of the line-shape and relaxation features of [3,3-$^2H_2$]β-DTGL in the gel phase were accomplished using a three-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 (Auger et al., 1990). Adapting this description to the mannolipid, the orientation dependence of spin–lattice relaxation times observed for [3,3-$^2H_2$]α-DTML could be equally well simulated by using the same motional model with only a slight increase of the exchange rate ($k = 6.2 \times 10^8$ rad s^{-1}), suggesting that similar motions are present in the gel phase of both lipids.

As the temperature of multilamellar dispersions of [3,3-$^2H_2$]α-DTML is varied from 25 (gel phase) to 60 °C (liquid-crystalline phase), a dramatic decrease in spectral intensity is observed just below the gel to liquid-crystalline phase transition temperature (Figure 3B, filled circles). This behavior is nearly identical with that of [3,3-$^2H_2$]β-DTGL as reproduced in Figure 3A for comparison. It was determined in the previous study of β-DTGL dynamics that a second motion, namely, rotation about the long axis of the molecule as a whole, was needed to account for the observed variation in quadrupolar echo amplitude and spectral line shape over the temperature range of 25–60 °C (Auger et al., 1990). This axial motion was first modeled simply by an equally populated three-site exchange model in which the three sites were coincident with the rotameric jump sites. Spectral simulations indicate that varying the rate of axial motion, while keeping the rotameric jump rate constant, can satisfactorily reproduce the experimental temperature dependence of spectral intensity of both β-DTGL and α-DTML and suggested that the axial motion for the two glycolipids in the gel phase at 25 °C is very slow on the ^{2}H NMR line-shape time scale ($\tau_c = 1/\Delta\nu_Q$) (Figure 3, open squares). This conclusion has recently been confirmed for β-DTGL (Auger & Jarrell, 1990) by two-dimensional ^{2}H NMR exchange techniques (Schmidt et al., 1986, 1987, 1988; Wefing & Spiess, 1988; Wefing et al., 1988), which are sensitive to the rate and mechanism of very slow motions. The results obtained by this technique indicate that a slow motion with a correlation time of the order of milliseconds is present in the gel phase of β-DTGL at 35 °C and that large-angle jumps rather than small-step Brownian diffusion best describes this motion (Auger & Jarrell, 1990).

Comparison of the line-shape and relaxation features of β-DTGL and α-DTML therefore suggests that the gel phases

of these two lipids can be described by very similar motional models. This result is somewhat surprising considering that these two lipids form two very different liquid-crystalline phases, lamellar and hexagonal for β-DTGL and α-DTML, respectively (Jarrell et al., 1987b). It is also interesting to note that the relative spectral intensities above the gel to liquid-crystalline phase transition temperature are not significantly affected by the nature (lamellar or hexagonal) of the liquid-crystalline phase.

The characteristic loss of intensity before the gel to liquid-crystalline phase transition has also been observed in two disaccharride glycolipid systems, 1,2-di-*O*-tetradecyl-3-*O*-(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-*sn*-glycerol (Carrier et al., 1989) and the lactose-containing glycolipid 1,2-di-*O*-tetradecyl-3-*O*-(4-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-*sn*-glycerol (Renou et al., 1989). Moreover, the spin–lattice relaxation times obtained for both lipids in the liquid-crystalline phase are very short (≈3–8 ms), which suggests that these systems exhibit motions similar to those observed for the two monosaccharide glycolipids investigated in the present study. Similar conclusions have also been drawn from line-shape and spin–lattice relaxation studies of another glycolipid, the acyl-chain-labeled *N*-palmitoylgalactosylsphingosine (NPGS) in the gel phase (Huang et al., 1980; Siminovitch et al., 1985, 1988). These studies indicated that axial diffusion in the gel phase is very slow on the ^{2}H NMR time scale ($\tau_c = 1/\Delta\nu_Q$) and that the gel-phase spectra are the result of a simple intermediate rate exchange process between gauche and trans conformers.

Fast internal motions, therefore, appear to be present in both the gel and liquid-crystalline phases of several glycolipid bilayers. If a similar motion occurs about the C2–C3 bond of the sphingosine residue, it may have considerable importance considering that glycosphingolipids can assume many biological roles (Hannun & Bell, 1989), such as the capacity to function as recognition sites [cell–cell recognition (Critchly et al., 1979), immune recognition (Hakamori, 1984), and toxin receptor (Fishman & Brady, 1976)] and as modulators of membrane structure (Sharom & Grant, 1977). In addition, this aspect must be considered when discussing the average orientation of glycolipid head groups and their conformations. In view of the important roles played by the glycolipid head group, it is clear that a knowledge of the dynamics of the carbohydrate moiety will be a prerequisite for a complete understanding of cell surface recognition at the molecular level.

Phospholipid Bilayers. Molecular dynamics of phosphatidylcholine and phosphatidylethanolamine bilayers in the gel and liquid-crystalline phases have been studied by ^{2}H and ^{13}C NMR (Wittebort et al., 1981; Blume et al., 1982a,b). The gel-phase line shapes of these lipids were interpreted in terms of whole molecule axial motion and internal jumps with rates ranging from $\approx 3 \times 10^4$ s^{-1} to 8×10^5 s^{-1}, which are intermediate rates on the ^{2}H NMR line-shape time scale. In addition, on heating, a loss of spectral intensity was observed just before the gel to liquid-crystalline phase transition, which was interpreted in terms of exchange on an intermediate time scale between gel and liquid-crystalline domains.

It is therefore of interest to investigate if the simple motional model used for the different glycolipid systems can be extrapolated to phospholipid systems. To do so, we have analyzed the line-shape and relaxation features in the gel and liquid-crystalline phases of a phosphonolipid analogue of the phosphatidylethanolamine, 1,2-di-*O*-hexadecyl-*sn*-glycerol 3-[(2-aminoethyl)phosphonate] (DHAEPn), labeled on both the C2 and C3 positions of the glycerol backbone. Like phosphatidylethanolamine, this lipid exhibits polymorphism, with a gel to lamellar liquid-crystalline phase transition temperature of 70.2 °C and a lamellar to hexagonal phase transition at 84.5 °C.

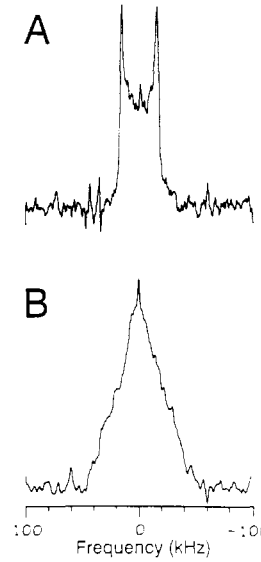

FIGURE 4: Experimental ^{2}H NMR spectra (30.7 MHz) of a multilamellar dispersion of [3-^{2}H$_1$]DHAEPn at (A) 75 °C and (B) 45 °C.

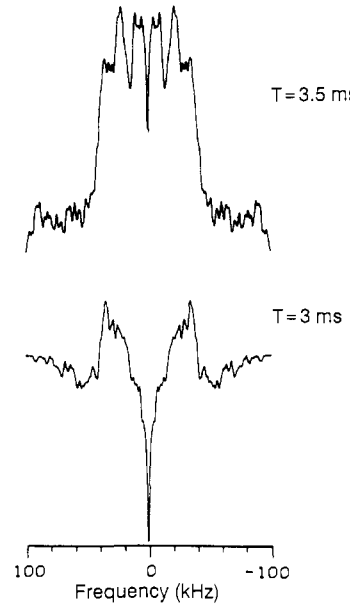

FIGURE 5: Partially recovered (T_{1Z}) spectra of a multilamellar dispersion of [3-^{2}H$_1$]DHAEPn at 45 °C as a function of the delay T in the inversion–recovery sequence. Number of accumulations for each spectrum was 300 000.

The ^{2}H NMR spectra of DHAEPn labeled at the C3 position of the glycerol backbone are shown in Figure 4 at 75 and 45 °C, above and below the gel to liquid-crystalline phase transition temperature for this lipid (70.2 °C). These spectra are very similar to those obtained for the two glycolipids β-DTGL and α-DTML in the gel phase at the same reduced temperature. Inversion–recovery experiments were also performed on multilamellar dispersions of DHAEPn, and the partially relaxed spectra shown in Figure 5 as a function of the delay T after the inverting pulse clearly demonstrate that (1) there is a significant orientation dependence of the ^{2}H NMR spin–lattice relaxation times (T_{1Z}) and (2) this anisotropic relaxation has an orientation dependence similar to that observed in glycolipid systems. The averaged spin–lattice

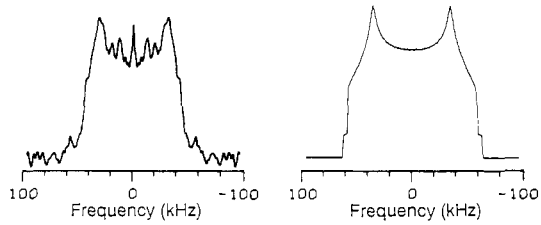

FIGURE 6: Experimental (left) and simulated (right) ^2H NMR spectra of a multilamellar dispersion of [2-$^2H_1$]DHAEPn at 45 °C. Number of accumulations was 250 000. Simulations were performed using a ±22° two-site jump with equal populations of the sites and a motional rate of 4×10^8 rad s^{-1}.

relaxation time value for [3-$^2H_1$]DHAEPn at 45 °C is 5.0 ms, which is again very close to that determined in glycolipid systems. These results suggest a common motion for DHAEPn, β-DTGL, and α-DTML.

The gel-phase ^2H NMR spectrum of DHAEPn labeled at the C2 position of the glycerol backbone is shown in Figure 6 (left spectrum) at 45 °C. As was observed for the C3-labeled phosphonolipid, this spectrum is very broad and characteristic of axially asymmetric motion and the corresponding partially relaxed spectra (Figure 7) also exhibit a significant orientation dependence of the spin–lattice relaxation times for DHAEPn labeled at the C2 position of the glycerol backbone. It should be noted that only 5 mg of the C2-labeled lipid was available. As a result the signal-to-noise (S/N) ratio for the partially relaxed spectra of gel-state lipid is poor (Figure 7, right). For this reason the symmetrized (leading to a $2^{1/2}$ increase in S/N) partially relaxed spectra are also presented (Figure 7, left) to facilitate visualization of the anisotropic relaxation that is manifest in the original spectra (Figure 7, right). Moreover, the average value of the spin–lattice relaxation time for this labeled position is again very short (8.2 ms), indicating that the motion dominating relaxation is fast on the ^2H NMR line-shape time scale.

It is interesting to note that the spectrum of C2-labeled DHAEPn in the gel phase is very similar to that obtained by Blume et al. (1982a) for dipalmitoylphosphatidylethanolamine (DPPE) labeled at the same position of the glycerol backbone. In the latter study, the DPPE spectral line shape was simulated by using a six-site jump model including an axial motion modeled by a three-site jump and a slow 20° torsional motion of the C–^2H bond with respect to the axial motional axis. The angle between the director axis and the C2–^2H bond vector was determined to be ≈24° at 45 °C. The line shape was well simulated by using an axial motional rate of 4.5×10^5 s^{-1} and a torsional motional rate of 3.9×10^4 s^{-1}. The population of C–^2H bonds oriented at 15° with respect to the diffusion axis was set to 0.6 in order to account for the experimental quadrupolar splitting. Although this motional model can satisfactorily reproduce the experimental spectral line shapes observed for both C2-labeled DPPE and DHAEPn at the same reduced temperature, it is important to notice that the rates of both motions involved are intermediate and therefore that neither of the motions in question could provide efficient spin–lattice relaxation. In fact, we have determined that such a motional model would result in spin–lattice relaxation times, T_{1Z}, of about 300 ms, which are 2 orders of magnitude longer than those observed experimentally for DHAEPn.

The dramatic loss of spectral intensity reported for both the C2- and C3-glycerol-labeled DPPEs (Blume et al., 1982a) has been explained by an exchange between gel and liquid-crystalline domains. We have also observed the variation in quadrupolar echo amplitude and spectral line shape for both C2- and C3-labeled DHAEPn (results shown in Figure 8 for [2-$^2H_1$]DHAEPn, filled circles). However, as for the glycolipid systems discussed previously, this decrease in spectral intensity was relatively well simulated simply by varying the axial motional rate while keeping the internal motional rate constant (Figure 8, open squares). These simulations reveal that the axial motional rate in the gel phase at 45 °C is about 1×10^3

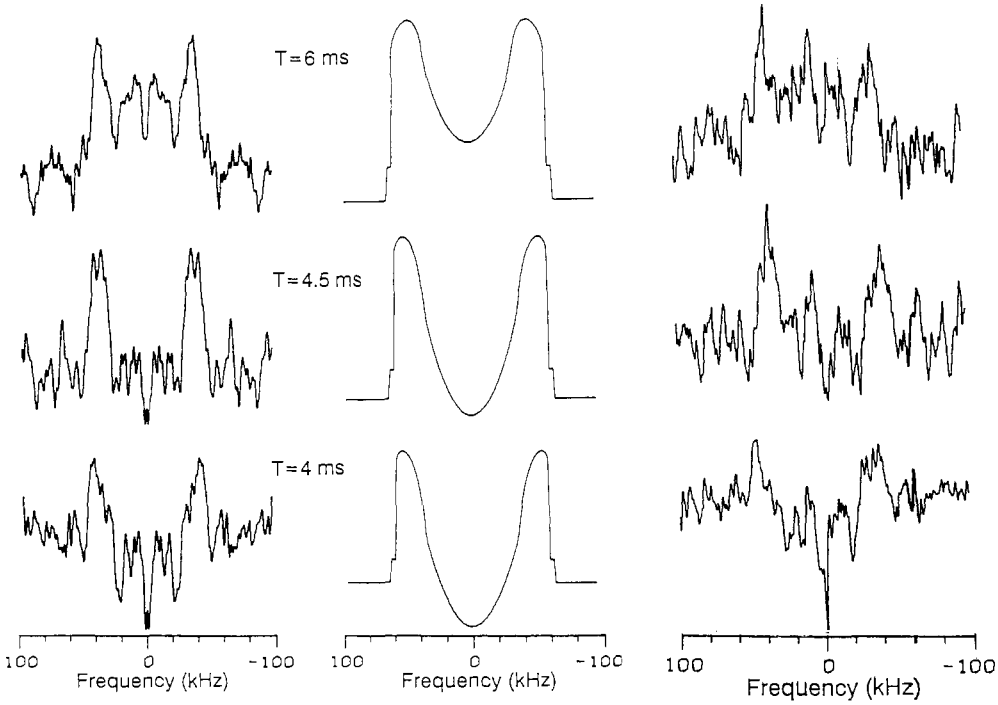

FIGURE 7: Experimental (right; left: symmetrization of corresponding spectra on right) and simulated (center) ^2H NMR spectra of multilamellar dispersions of [2-$^2H_1$]DHAEPn (5 mg) at 45 °C as a function of the delay T in the inversion–recovery sequence. Number of accumulations was 100 000 for $T = 4.0$ and 4.5 ms and 81 000 for $T = 6$ ms. Symmetrized spectra (left) are presented to facilitate visualization of the anisotropic relaxation effects evident in the spectra on the right. Simulations were performed using a ±22° two-site jump with equal populations of the sites and a motional rate of 4×10^8 rad s^{-1}.

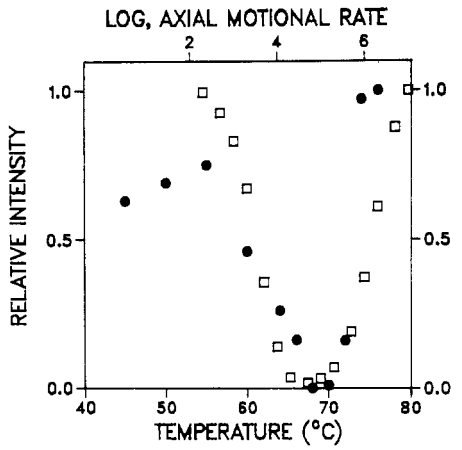

FIGURE 8: (●) Temperature dependence of the spectral intensity for a multilamellar dispersion of [2-$^2H_1$]DHAEPn. Note that just before the gel to liquid-crystalline phase transition temperature (70.2 °C), a dramatic decrease in intensity is observed. (□) Calculated spectral intensity as a function of the axial motional rate. Simulations were performed using a six-site model, including the two-site jump model described in Figures 6 and 7 and an axial motion modeled by an equally populated three-site jump.

s^{-1}, which is too slow to influence the ^2H NMR line shape at that temperature. The spectral line-shape and relaxation features of the C2-labeled DHAEPn were therefore simulated by using a two-site jump model inwhich the libration angle, the relative populations of the sites, and the motional rates were varied in order to fit the experimental line shape and spin–lattice relaxation time. These features were best simulated by use of a ±22° torsional motion with equal populations of the sites and a motional rate of 4×10^8 rad s^{-1} (Figures 6 and 7, right spectra). This results in a calculated averaged spin–lattice relaxation time of about 8 ms. It has been suggested that the motion at the C2 position is constrained by the fact that the *sn*-2 chain is attached to this carbon. However, if a fast motion exists at the C3 position of the glycerol, it is not unreasonable to expect some coupling to the 2-position. Thus, the line-shape and relaxation features of the phosphonolipid DHAEPn labeled at glycerol C2 and C3 can be described by two motions, a fast internal motion and a slower whole molecule axial motion.

It is interesting to note that for the fast internal motion at the C2–C3 bond of the glycerol backbone there is good agreement between the relative populations necessary to simulate the gel-phase line-shape and relaxation features of C3-labeled glycolipid and phospholipid bilayers (0.46, 0.34, 0.20) and those proposed for the gauche (+), gauche (−), and trans conformers about the glycerol C2–C3 bond in phospholipids [0.48 0.37, and 0.15 for dihexadecylphosphatidylcholine (DHPC) in D_2O] (Hauser et al., 1980). Moreover, a recent high-resolution NMR study has suggested that there is a rapid interconversion on the NMR line-shape time scale between two preferred conformations about the glycerol C1–C2 bond in the liquid-crystalline state of phospholipids (Hauser et al., 1988). The coexistence of these two rotamers was found to be consistent with the relatively small potential energy barriers calculated for the rotation about the C1–C2 and C2–C3 bonds of the glycerol backbone in phospholipids (McAlister et al., 1973). The results obtained in the present study are in agreement with the hypothesis of rapid equilibrium between different conformations of the glycerol backbone.

Conclusions

The present study demonstrates that the line-shape and relaxation features observed for two glycerol-labeled glycolipids β-DTGL and α-DTML, as well as for the phosphonolipid DHAEPn, can be well reproduced by a simple motional description in which there is fast internal jump at the glycerol backbone and an axial motion whose rate changes by at least a factor of 10^2 on going from the gel to the liquid-crystalline phase. The fast internal motion elucidated in this study suggests that a similar motion should be considered in the description of the head-group motion in both glycolipid and phospholipid bilayers. For example, it would be of interest to investigate ^{31}P spin–lattice relaxation in phospholipid bilayers (Milburn & Jeffrey, 1987, 1989) in terms of the motional model discussed in the present study.

The presence of fast internal motions in the glycerol backbone region in both the gel and liquid-crystalline phases of lipid bilayers can have important implications in understanding, for example, the interactions of exogenous molecules with lipid head groups or, in the case of glycolipid bilayers, phenomena such as lectin– or antibody–carbohydrate binding. Investigation of lipid dynamics in simple model systems can therefore be very useful for interpreting the physical properties of these lipids in the more complex environment of biological membranes, and for correlating changes in these physical properties with modulation of membrane function.

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Registry No. β-DTGL, 81281-23-4; DHAEPn, 1924-05-6; 1,2-di-*O*-tetradecyl-3-*O*-(α-D-mannopyranosyl)-*sn*-glycerol, 81281-29-0.

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H3 Cys-110 Is in Close Proximity to the C-Terminal Regions of H2B and H4 in a Nucleosome Core with an Altered Internal Arrangement of Histones†

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ABSTRACT: A particle obtained by nuclease digestion of nucleohistone complexes prepared by direct mixing of histones with DNA in 0.15 M NaCl was indistinguishable by composition and physical properties from nucleosome cores prepared under the same conditions from nucleohistone preannealed in 0.6 M NaCl. We show here that different photo-cross-links form when these particles are prepared from H3 labeled with photoaffinity reagents on the unique histone H3 cysteine. H3-H3 histone dimers were dominant when the particles were prepared by dilution of the nucleohistone from 0.6 M NaCl while H3-H2B and H3-H4 histone dimers were prominent if the nucleohistone complex was prepared directly in 0.15 M NaCl. Peptide mapping of the novel H3-H4 and H3-H2B dimers showed that Cys-110 of histone H3 is cross-linked to the 18 amino acid C-terminal end of H4 or to the 66 amino acid C-terminal half of H2B.

The nucleosome core is composed of 145 base pairs of DNA wrapped in a left-handed supercoil around a histone octamer containing two molecules each of H2A, H2B, H3, and H4 [for a review, see McGhee and Felsenfeld (1980) and van Holde (1989)]. Crystallographic studies have shown that the cysteine-110 residues of the H3 histones are close to each other across the dyad axis (Richmond et al., 1984; Uberbacher & Bunick, 1985). The histone pairs H2A-H2B and H3-H4 interact sufficiently strongly to form heterotypic dimeric structures in the absence of DNA. These can further associate to form $(H3-H4)_2$ tetramers and $2(H2A-H2B)/(H3-H4)_2$ octamers under conditions of high histone concentration or elevated ionic strength (D'Anna & Isenberg, 1974; Roark et al., 1974, 1976; Thomas & Kornberg, 1975; Eickbush & Moudrianakis, 1978; Ruiz-Carrillo & Jorcano, 1979; Godfrey et al., 1980; Benedict et al., 1984). A dyad axis within the

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