Slow motions in lipid bilayers

Direct detection by two-dimensional solid-state deuterium nuclear magnetic resonance

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ABSTRACT Two-dimensional solid-state ^{2}H NMR spectroscopy of specifically deuteriated lipids is used to detect and to characterize the rate *and* mode of slow motions in two lipid bilayer systems. Lateral diffusion of lipid molecules over the curved surface of dipalmitoylphosphatidylcholine liposomes can be detected by two-dimensional exchange ^{2}H NMR and it is shown that molecular orientational exchange is complete on the timescale of 100 ms. In contrast, it is shown that for the glycolipid 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (β-DTGL), there is no evidence of a corresponding orientational exchange in the liquid-crystalline phase suggesting that this lipid forms relatively flat bilayers. In the gel phase of hydrated multibilayers of β-DTGL, a slow (10^3 s^{-1}) whole molecule axial motion is demonstrated at 40°C. Comparison of the experimental and simulated 2D-NMR ridge patterns suggests that large angle jumps about the long molecular axis, rather than small step Brownian diffusion, can best account for the 2D-exchange spectra of β-DTGL in the gel phase. The significance of this technique for the study of dynamics in other biological systems is discussed.

INTRODUCTION

Determining the rate and nature of molecular motions in membrane systems is of considerable importance in understanding their physicochemical properties and how these properties are affected by membrane composition or temperature. Because membrane systems often exhibit complex motions, it is important to characterize the rate of molecular motion over a broad range to achieve as complete a description of membrane dynamics as possible. Solid-state NMR and in particular ^{2}H NMR[1] spectroscopy is well suited to studies of membrane systems (Seelig, 1977; Griffin, 1981; Davis, 1983, 1986; Bloom and Smith, 1985; Smith, 1989), because it provides observation windows in the range where the frequencies of most molecular motions occur ($1–10^{10}$ s^{-1}, for lipid bilayers) (Kimmich et al., 1983). Motions occurring on the time scale of the Larmor frequency ($10^7–10^{10}$ s^{-1}) may be probed by spin-lattice relaxation rates whereas lineshape studies can elucidate motions on the $10^3–10^6$ s^{-1} time scale. These techniques can also provide discrimination between possible motional modes, but are not applicable for slower motions ($< 10^3$ s^{-1}).

The quadrupolar echo amplitude decays with a time constant, T_{2e}, which has been shown to be sensitive to slow motions (Jeffrey, 1981). Measurements of transverse relaxation times T_{2e} in lipid bilayers have been used to obtain correlation times of slow motions, in particular those of slow lateral diffusion over curved membrane surfaces (Bloom and Sternin, 1987; Perly et al., 1985). However, in general, such measurements do not necessarily provide information about the nature of the slow motions. An alternative source of information about the rates and nature of slow molecular motions is the two-dimensional exchange technique, which has been used extensively for structural elucidation in solution (Bodenhausen et al., 1987). More recently, the 2D-exchange experiment has also been applied to amorphous solids and powders. For example, ^{13}C spectra have been obtained in static (Edzes and Bernards, 1984) and in rotating samples (Harbison et al., 1985). In 2D-NMR spectra of static powders, the correlation of two tensorial interactions gives rise to ridge patterns exhibiting characteristic geometrical figures. Two different interactions, such as, for example, anisotropic shielding and dipolar coupling, may be correlated (Linder et al., 1980). On the other hand, one interaction may be correlated with itself, providing information on dynamic exchange processes such as molecular reorientation. The 2D exchange technique has recently been applied to deuterium NMR, extending the frequency range over which motions may be probed to the millisecond or longer time scales in simple molecular systems (Schmidt et al., 1986, 1987, 1988; Wefing and Spiess, 1988; Wefing et al., 1988), and in more complex glycolipid bilayer systems (Auger and Jarrell, 1990). Moreover, ^{2}H NMR exchange techniques have been shown to be very powerful in

[1] *Abbreviations used in this paper:* DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; β-DTGL, 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol; ^{2}H NMR, deuterium nuclear magnetic resonance; NPGS, *N*-palmitoylgalactosyl sphingosine.

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distinguishing between motions characterized by either large angle jumps or free diffusion (Schmidt et al., 1986, 1987; Wefing et al., 1988; Auger and Jarrell, 1990). Because the quadrupole coupling tensor of chemically bound deuterons is frequently axially symmetric, the singularities of the 2D-exchange signals give rise to simple geometric ridge patterns from which the angles through which the molecules rotate can be read directly. Two-dimensional ^2H exchange spectra can therefore yield information about molecular dynamics in a model-independent fashion.

In the present study we illustrate the use of ^2H exchange NMR to approach the characterization of slow molecular motion in lipid bilayers such as lateral diffusion of lipid molecules. In addition, we extend a previous study (Auger and Jarrell, 1990) in which this approach was used to detect and characterize a slow motion about the molecular long axis in the gel phase of the glycolipid 1,2-di-*O*-tetradecyl-3-*O*-glucopyranosyl-*sn*-glycerol. The implications of this technique for the study of other biological systems are discussed.

EXPERIMENTAL SECTION

1,2-Di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-[3,3-$^2H_2$]-glycerol (β-DTGL) was prepared as described previously (Jarrell et al., 1987) and perdeuteriated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine ([$^2H_{62}$]DPPC) was purchased from Avanti Polar Lipids, Inc., (Birmingham, AL). The ^2H NMR samples consisted of multilamellar dispersions prepared by hydrating 75 mg (0.11 mmol) of β-DTGL or 30 mg (0.04 mmol) of perdeuteriated DPPC with a 10-fold excess of deuterium-depleted water (Aldrich Chemical Co., Milwaukee, WI) in a 10-mm (o.d.) sample tube. The hydrated samples were heated cyclically to 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 at 30.7 MHz on a "home-built" solid-state NMR spectrometer operated by a model 1280 computer (Nicolet Computer Graphics Div., Martinez, CA). Two-dimensional data were obtained by the pulse sequence: 90_y-t_1-54_ϕ-t_{mix}-54_ϕ-Δ-90_x-Δ-t_2 presented by Schmidt et al. (1988), and data processed as described previously (Auger and Jarrell, 1990). Quadrature detection and phase cycling (Schmidt et al., 1988) were used to record the spectra. The π/2 pulse length was typically 4.0–4.2 μs (10-mm coil), and the refocusing time Δ was 60 μs. The recycle time was always $> 5T_{1Z}$ and $5T_{1Q}$ and the mixing times t_{mix} are indicated in the figure captions. The data sets were 128 points in the F_2 dimension and 16 (β-DTGL experiments in the gel phase) and 32 points (β-DTGL and DPPC experiments in the liquid-crystalline phase) in the F_1 dimension. For each serial file in a given experiment, 50,000–100,000 and 2,000–3,000 transients were accumulated for β-DTGL and DPPC, respectively. The spectral width in both dimensions was 500 kHz for β-DTGL in the gel phase, and 200 kHz for DPPC and the glycolipid in the liquid-crystalline phase. Zero filling to 256 values, as well as a Gaussian apodization function, were applied in both dimensions during data processing. Samples were enclosed in a glass jacket where the temperature was regulated to within ±0.5°C. Spectral simulations were performed on a model 4-260 computer (Sun Microsystems, Inc., Mountain View, CA) using a modification of a deuterium 2D-exchange simulation program (H. C. Jarrell, unpublished results). Spectral simulations as a function of the mixing time, t_{mix}, for the DPPC acyl chain terminal CD_3 groups were based on the isotropic diffusional formalism of Wefing and co-workers (Wefing et al., 1988) as outlined elsewhere (Fenske, D. B., and H. C. Jarrell, manuscript submitted for publication).

RESULTS AND DISCUSSION

Compared with the rapid molecular reorientations about the bilayer normal of membranes, translational motion of lipid molecules along the bilayer surface can lead to much slower reorientation. The latter motion has been investigated by several one-dimensional NMR studies. Brown and Davis (1981) have shown that in the liquid-crystalline phase of DPPC, lateral diffusion is sufficiently rapid that each lipid molecule samples all orientations during times of the order of T_1, thus, apparently preventing the observation of the orientation dependence of spin-lattice relaxation. They have estimated from selective inversion recovery experiments that the phospholipid molecules will diffuse through an angular displacement >1 rad in 5 ms. A similar study involving the selective excitation of ^{31}P NMR lineshapes has suggested a correlation time of 17 ms for lateral diffusion of phospholipids over the curved liposomal surface (Larsen et al., 1987). On the other hand, using a quadrupolar CPMG pulse train, Bloom and Sternin (1987) have shown that the most plausible mechanism for slow transverse relaxation (T_2) in phospholipid bilayers is lateral diffusion of the phospholipid molecules along curved membrane surfaces and have estimated a correlation time of ≈ 100 ms for that motion. Angular-dependent ^2H transverse relaxation, T_{2e}, has been observed with lipids from the membranes of *A. laidlawii* (Rance, 1981) and with phosphatidylethanolamine bilayers (Perly et al., 1985) which has been attributed to lateral diffusion effects. Whereas such studies have established the presence of a slow motion in the liquid-crystalline phase of lipid bilayers, with the motion reasonably assumed to be diffusive in nature, the correlation times agree less well. It is of interest, therefore, to investigate if such a motion can be detected and characterized directly by two-dimensional solid-state deuterium NMR. To do so, we have measured the 2D-exchange spectra of a multilamellar dispersion of perdeuteriated DPPC ([$^2H_{62}$]DPPC) in the liquid-crystalline phase at 50°C, as a function of the mixing time t_{mix}. The spin-lattice relaxation times (T_1) obtained for the acyl chain-labeled phospholipids (from ≈ 30 to 250 ms for the plateau and the tail regions, respectively) allow the use of mixing times as long as T_1 and therefore permit the investigation of slow motions occurring on this time scale.

The 2D-exchange spectra and the corresponding con-

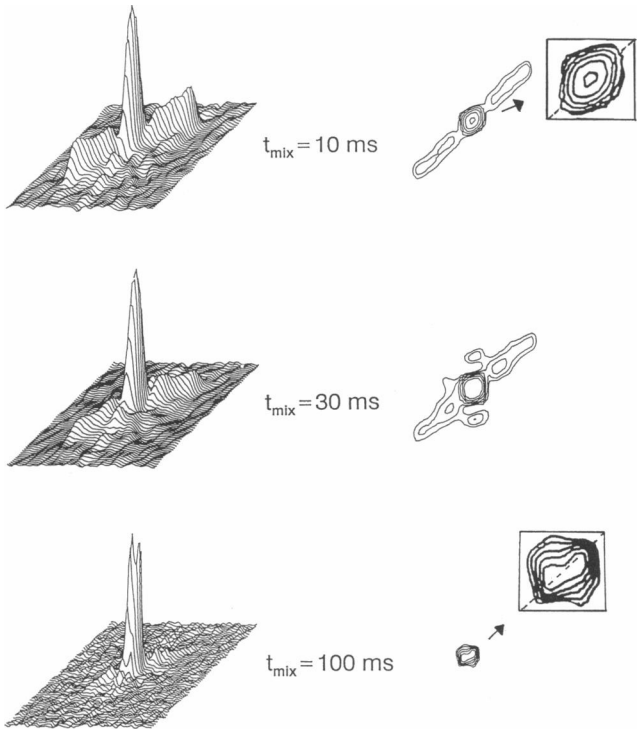

FIGURE 1. 2D absorption mode exchange spectra and corresponding contour plots of a multilamellar dispersion of perdeuteriated DPPC at 51°C for different mixing times. For t_{mix} = 100 ms, dashed line of the inset represents where the diagonal would occur. The total spectral width in both dimensions is ±25 kHz.

tour plots of perdeuteriated DPPC are shown in Fig. 1 for mixing times t_{mix} of 10, 30, and 100 ms. It is interesting to note that at a t_{mix} of 10 ms, ridge patterns are not manifest clearly for this multiply labeled system. This indicates that motions occurring in the liquid-crystalline phase of DPPC bilayers are either too fast or too slow to be readily detected on a time scale of ≈ 10 ms. However, as the mixing time is increased to 30 ms, off-diagonal intensity become clearly distinguishable. For a mixing time of 100 ms, a dramatic loss in signal intensity occurs, due to spin-lattice relaxation, the remaining spectrum being due mostly to the terminal methyl groups of the acyl chains. Close inspection (see Fig. 1 insets which expand the contours associated with the CD_3 group) of the 2D spectrum of the methyl groups and the corresponding contour plot obtained at t_{mix} = 100 ms indicates little intensity along the diagonal, which suggests that the slow motion giving rise to the 2D-ridge patterns involves orientational exchange amongst a large number of sites and, thus, is most likely diffusive in nature. 2D-spectral simulations of the methyl group spectra in the absence and in the presence of complete isotropic reorientation (data not shown) suggested that complete orientational exchange had occurred on a time scale of < 100 ms. It is reasonable to expect that the slow motion detected is lateral diffusion of the lipid molecules over the curved surface of the liposomes. A more quantitative measure of the correlation time for lateral diffusion, t_d, can be obtained by analysis of the spectra at several mixing times (Wefing et al., 1988*a*, Fenske, D. B., and H. C. Jarrell, manuscript submitted). A series of spectra were simulated for ratios of t_{mix}/t_d ranging in value from 0 to 50. Reasonable fits to the experimental spectra for t_{mix} values of 10 and 100 ms (Fig. 1) were obtained using t_{mix}/t_d ratios of 1 and 10, respectively (Fig. 2), giving a t_d value of ~10 ms. It is interesting to note that this value is close to the value of 8 ms obtained for DPPC by ^{31}P two-dimensional solid-state NMR (Fenske, D. B., and H. C. Jarrell, manuscript submitted). In contrast to one dimensional magnetization exchange experiments, the 2D experiments permit a direct visualization of the exchange process.

Although the agreement between the experimental (Fig. 1) and simulated (Fig. 2) spectra for DPPC acyl CD_3 groups is good, the use of lipid labeled only at the acyl chain terminus is preferred for a more quantitative analysis for the following reasons. There is a gradient in spin-lattice relaxation times along the acyl chain (Davis, 1983) which leads to a complex variation in spectral

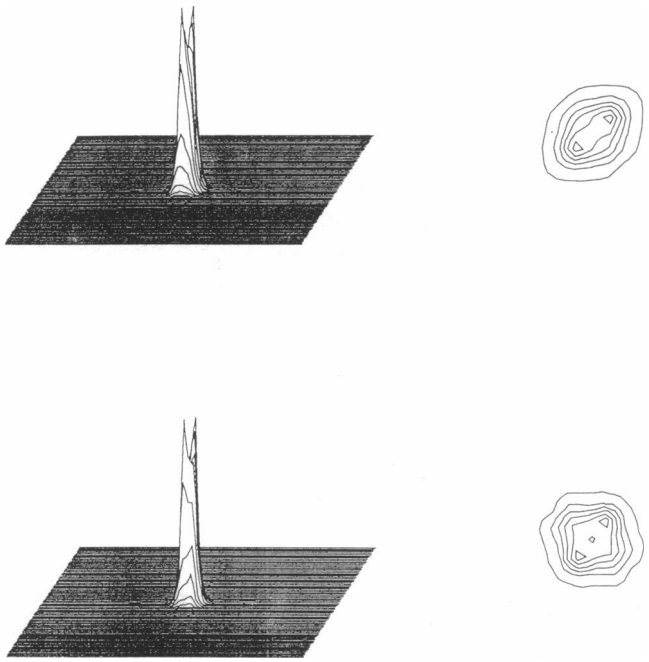

FIGURE 2 Simulated 2D-exchange spectra of the terminal methyl groups of the DPPC acyl chains. Simulations were performed as described in the text using t_{mix}/t_d = 1 (*top*) and 10 (*bottom*). The total spectral width in both dimensions is ±12.5 kHz.

intensity as a function of mixing time which is not related to the slow motional process of interest. In addition, the digital resolution of the spectra for the methyl group is too poor under the present experimental conditions. Because of the presence of the much larger quadrupolar splittings associated with other acyl chain positions, large spectral widths must be employed, making difficult monitoring of the detailed dependence of the CD_3 group spectra on t_{mix}.

Whereas lateral diffusion of phospholipids has been detected in both one- and two-dimensional NMR spectroscopy, including its leading to orientationally averaged spin-lattice relaxation (Brown and Davis, 1981), some glycolipids do not appear to exhibit similar behavior. Aqueous multilamellar dispersions of glycolipids in the liquid-crystalline phase, exhibit anisotropic spin-lattice relaxation (Auger et al., 1990*a*; Renou et al., 1989; Speyer et al., 1989), suggesting smaller rates of lateral diffusion than occurs in phospholipid systems, or smaller curvature of the liposomes. To explore the degree, if any, to which lateral diffusion leads to molecular orientational exchange for the glycolipid 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (β-DTGL), we have measured the 2D-exchange spectra of this lipid labeled at the glycerol C3 position in the lamellar (55°C) and hexagonal (60°C) liquid-crystalline phases (Fig. 3). In the hexagonal phase the lipid molecules diffuse about the cylinder long axis at a rate which is rapid on the time scale of the quadrupolar interaction. Consequently, this phase is expected to exhibit no effects due to lateral diffusion and serves as a control in the present study. In both cases, only the diagonal is observed for $t_{mix} = 2$ ms, confirming that diffusion of the lipid molecules along the curved membrane surfaces does not occur on the millisecond-time scale for this lipid in the liquid-crystalline phase. Because the spin-lattice relaxation times for deuterons at the C3 position are 4–6 ms, an increase of the mixing time > 4 ms would result in a large decrease in signal intensity, due to spin-lattice relaxation effects during the mixing period. Therefore, obtaining a quantitative measure of the correlation time in this glycolipid system is difficult. However, Spiess and co-workers (Wefing et al., 1988) have shown, and we have confirmed in test simulations (Jarrell, H. C., unpublished results) that when the ratio t_{mix}/t_d is ≥ 0.1 the molecular reorientation is manifest in the ^2H 2D-exchange spectra. Because in the β-DTGL system no exchange is evident at t_{mix} of 2 ms, the correlation time can be concluded conservatively to be > 10 ms. Based on the results with DPPC the use of β-DTGL labeled at the chain methyl positions would allow the system to be probed for mixing times up to ~ 100 ms and permit a more quantitative determination of the correlation time. Investigations of closely related

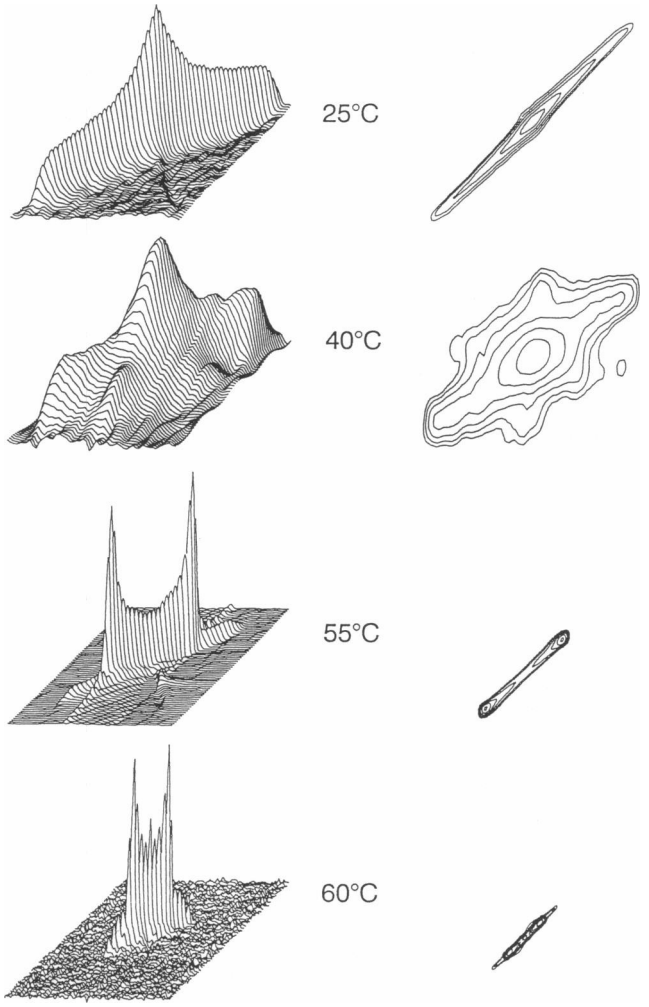

FIGURE 3 Temperature dependence of the 2D–absorption mode exchange spectra and corresponding contour plots of an aqueous multilamellar dispersion of [3,3-$^2H_2$]β-DTGL, t_{mix} = 2 ms. The total spectral width in both dimensions is ±50 kHz.

diacylglyceroglycolipid systems have shown that the lateral-diffusion rates for these systems are greater than those of the corresponding phospholipid systems by an order of magnitude (Wieslander et al., 1981). Thus, the present result for even the short mixing time indicates that β-DTGL forms bilayers which exhibit less curvature than DPPC, that is they are relatively flat. This conclusion is in accord with electron microscopy studies of a number of diacylglyceroglycolipids (Mannock et al., 1985; Sen et al., 1982) which concluded that the monoglycosyl lipids form bilayer sheet structures in water.

Because the experiments presented so far are limited by the relatively short ^2H spin-lattice relaxation times, investigation of the solid-state two-dimensional spectra of other nuclei, such as ^{31}P or ^{13}C, for which much longer

spin-lattice relaxation times are obtained would extend the time scale (≈ 5 s) during which slow molecular motions could be probed. Such studies are now underway in our laboratory to probe motions on time scales of a second or longer (Fenske, D. C., and H. C. Jarrell, unpublished results).

We have demonstrated recently that in the gel phase β-DTGL exhibits two dominant motions in the glycerol backbone region (Auger et al., 1990*a*) which are characterized by an internal three-site jump about the glycerol C2–C3 bond with relative site populations of 0.46, 0.34, and 0.20 and a correlation time of 6.7×10^{-10} s, and by a slow rotation about the molecular long axis. The rate of the latter motion was shown to change by at least three orders of magnitude over the temperature range 25–60°C. Similar conclusions have been reached from lineshape and spin-lattice relaxation studies of other glycolipids and phospholipids (Auger et al., 1990*b*) and of acyl chain-labeled *N*-palmitoylgalactosyl sphingosine (NPGS) in the gel phase (Huang et al., 1980; Siminovitch et al., 1985, 1988). Whereas these studies indicated that axial rotation is very slow on the ^{2}H NMR time scale ($\tau_c = 1/\Delta\nu_Q$), they could not provide detailed information about the nature of the slow axial motion. In an attempt to characterize this slow motion better, we examined the 2D-^{2}H exchange spectra of β-DTGL (Auger and Jarrell, 1990) at temperatures below that of the gel to liquid-crystalline phase transition (52°C for β-DTGL [Jarrell et al., 1986]). It should be mentioned that because lateral diffusion is not detectable in the 2D spectra of the liquid-crystalline phase of β-DTGL, under the present experimental conditions, this motion may also be safely neglected in the gel phase. For convenience of discussion, we reproduce some of the results presented previously (Auger and Jarrell, 1990) in Fig. 3 (*top two panels*), the 2D-exchange powder spectra of glycerol-labeled β-DTGL in the gel phase at 25 and at 40°C (where orientational exchange is maximal) for $t_{mix} = 2$ ms. At 25°C, the lineshape of the diagonal spectrum is very close to that obtained for the normal 1D spectrum of β-DTGL in the gel phase, and is characteristic of fast-limit axially asymmetric motion (associated with rapid isomerization about the glycerol (C2–C3 bond). However, the absence of cross peaks or ridge patterns in the 2D spectrum indicates that additional motions, if present at this temperature are either too slow or too fast on the millisecond time scale to be detected by this technique. The 2D exchange spectrum of β-DTGL at 40°C for $t_{mix} = 2$ ms (Fig. 3) indicates that at this temperature, ridge patterns become distinguishable. The latter result has been interpreted (Auger and Jarrell, 1990) as reflecting the presence of reorientation about the molecular long axis on the ms time scale, and is consistent with earlier ^{2}H NMR lineshape results (Auger et al., 1990*a*). It is important to note that the choice of mixing time is limited by the spin-lattice relaxation times, which are 4–6 ms for β-DTGL in the gel phase. An increase of the mixing time above 4 ms would result in a large decrease in signal intensity, due to spin-lattice relaxation effects during the mixing period.

In addition to providing an estimate of the correlation time, the 2D spectra observed for β-DTGL also provide information about the nature of the slow motion. The results at 40°C shown in Fig. 3 have been analyzed by spectral simulations in which an axial reorientation was modeled by jumps about the molecular long axis (Auger and Jarrell, 1990) amongst three or twelve sites. Simulations using the three-site axial jump model gave the better fit. However, while the fits between simulations and the experimental spectrum were adequate, they were not entirely satisfactory. In the latter study the digital resolution in the ω_1 frequency domain (defined by the increments in t_1) was greater than that of the experiment. Simulations in which the experimental resolution was more closely approximated are presented in Fig. 4 for both the 3- and 12-site models. Whereas the three-site model still better fits the experiment, the distinction between the two jump models is not as evident as preliminary results suggested (Auger and Jarrell, 1990). Additional simulations in which the axial motion was modeled by a jump amongst an increasingly larger number of sites (<50) gave results similar to those shown for the 12-site model (Fig. 4 *A*). Whereas the use of a discrete jump model to describe the axial reorientation of β-DTGL may be questioned, the present results suggest that the axial motion is not diffusive. One might consider replacing the discrete motion with one involving diffusion in a threefold rotational potential as has been considered for acyl chain *trans*-gauche isomerization (Edholm and Blomberg, 1979). In the present case this description is not considered as probable for the following reasons. It has been shown that both the ^{2}H quadrupolar-echo intensity and powder lineshape are significantly affected when a discrete jump motion is replaced with diffusion in rotational potential (Wittebort et al., 1987). Because it has been demonstrated previously for β-DTGL dynamics that the quadrupolar-echo amplitude and lineshapes at 40°C were well simulated using an axial jump model involving three or more sites (Auger et al., 1990*a*), the use of potential wells does not appear necessary. In the latter study an activation energy, E_a, for motion about the molecular long axis was found to be ~ 100 kJ/mol. It has been shown for motion between potential wells where the barrier height exceeds 10 RT (where R is the ideal gas constant and T is temperature), that a jump model is an adequate description (Edholm and Blomberg, 1979; Wittebort et al.,

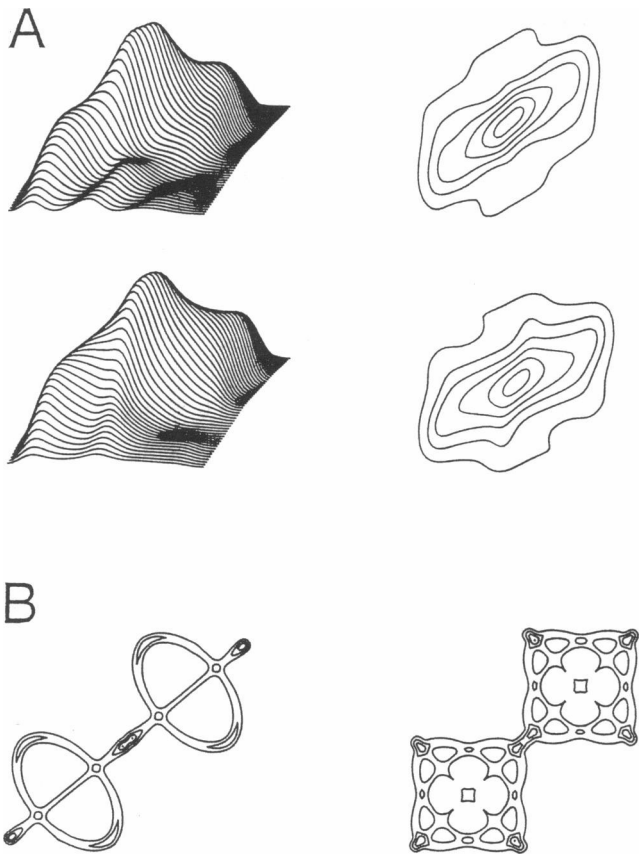

FIGURE 4 (*A*) 2D-spectral simulations performed using a composite motional model including both the fast rotameric three-site jump model (internal mode) needed to account for the gel phase lineshape and relaxation features of [3,3-$^2H_2$] β-DTGL (Auger et al., 1989*b*) and a slow axial motion (whole molecule mode), modeled by a three-site (*top*) or a 12-site (*bottom*) equally populated jump, with the jump axis being coincident with the rotameric jump axis (*A*) Ridge patterns. (*B*) Simulated 90° oriented-sample ^2H 2D-exchange spectra (for complete orientational exchange) for the jump models used in *A*: three-site jump (*left*); 12-site jump (right). The total spectral width in both dimensions is ±50 kHz.

1978). For β-DTGL where E_a is $\gg 10\,RT$ (assuming that this reflects barrier heights for rotation) it is reasonable to use a jump model for the long axis motion. Interestingly, a similar conclusion was reached in a recent study of cholesterol dynamics in which it has been shown that the anisotropic spin-lattice relaxation (T_{1Z} and T_{1Q}) behavior of ^2H nuclei on cholesterol in a phospholipid matrix were best simulated using a large angle (three-site) jump model in comparison to small step Brownian diffusion (Bonmatin et al., 1990). Other studies have also suggested that large angle jumps may be favored in highly ordered systems (Vold and Vold, 1988; Dammers et al., 1988).

In the case of reorientation of DPPC molecules over the curved liposome surface, it was shown that because of the diffusive character of the motion, the 2D-exchange spectra showed essentially no diagonal with all the spectral intensity distributed in the ridge patterns (Fig. 1, *inset,* and Fig. 2, *inset*). For axial reorientation of β-DTGL, some orientations of the jump axis with respect to the magnetic field direction (such as 0°) lead to no frequency change and hence no cross peaks in the 2D spectrum. As a result even for a motion amongst a large number of sites (Fig. 4, 12 sites) the diagonal contains considerable intensity. Additionally, unlike the situation with DPPC where the maximum frequency change is from −0.5 A1 to 1.0 A1 (where A1 is the residual quadrupolar interaction) corresponding to exchange of the 90 and 0° bilayer normal orientation, the axial motion gives a maximum frequency change of 0.0–1.0 A1. This accounts in part for the less striking changes in the 2D spectrum for β-DTGL in the gel phase as compared with that of DPPC. It has been shown that for the spin-lattice relaxation studies, the use of samples which have been macroscopically oriented between glass plates can be particularly effective in lineshape studies (Auger et al., 1990*a*). Given the relatively subtle changes which arise in the simulated 2D spectra of Fig. 4 *A* when a small and large angle reorientation are compared, it is of interest to explore the possible utility of oriented samples in these studies. Fig. 4 *B* shows simulated 2D contour plots for the same motional models used for generating Fig. 4 *A* but with the motional axis oriented at 90° with respect to the magnetic field direction; the 90° orientation was selected because the expected frequency changes are greatest for this orientation. It is readily seen that in contrast with the differences seen with 2D powder lineshapes, the differences between the 3-site and 12-site axial jump model are enhanced. The simulations suggest that oriented-sample 2D-NMR studies may in some cases offer a more sensitive approach to the discrimination between closely related modes of molecular reorientation.

CONCLUSIONS

We have demonstrated that two-dimensional solid-state NMR techniques can characterize the time scale and nature of slow motions in lipid bilayer systems by providing an observation window in a frequency range ($< 1 \times 10^3\ s^{-1}$) not accessible from lineshape and spin-lattice relaxation studies. Two cases have been discussed, the slow whole-molecule axial motion in the gel phase of glycolipid bilayers, and lateral diffusion over the curved surface of phospholipid multibilayers in the liquid-crystalline phase. This demonstration of the validity of the technique for the study of lipid systems opens

additional avenues in the study of dynamics in biological systems. For example, this technique may be useful in probing lipid-protein interactions. In such systems, slow motions of lipid molecules have been shown to be more sensitive to such perturbations (Meier et al., 1987). The technique may also be useful in the study of surface distortions, such as rippling, which has been suggested to occur before the transition from lamellar to nonlamellar liquid-crystalline phase. Moreover, the ability to monitor both the rate and nature of slow molecular motion suggests that two-dimensional NMR techniques could be very useful in probing changes in the properties of membrane surfaces upon binding to exogenous molecules such as lectins or antibodies.

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