Effect of pressure on dimyristoylphosphatidylcholine headgroup dynamics

Darius C. Fiech, Boyan B. Bonev, and Michael R. Morrow
Department of Physics and Physical Oceanography, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X7
(Received 15 May 1997)

^{2}H NMR has been used to examine the effect of applied hydrostatic pressure on the dynamics of headgroup-deuterated dimyristoylphosphatidylcholine (DMPC-d_4). Quadrupole splittings, spin-lattice relaxation time (T_1), and quadrupole echo decay times (T_2^{qe}) have been measured at 65 °C for a series of pressures up to 2 kbar and for a series of temperatures at ambient pressure and at 1.5 kbar. Quadrupole Carr-Purcell-Meiboom-Gill (q-CPMG) decays have been studied under similar conditions. Within the liquid crystalline phase, application of hydrostatic pressure is observed to alter quadrupole splittings of the α and β choline deuterons in a way that is consistent with a tilt of the headgroup away from the bilayer surface. Pressure also increases T_2^{qe} in the liquid crystalline phase. q-CPMG decays in the liquid crystalline phase are consistent with a distribution of slow motions in the bilayer. The application of pressure appears to extend the range of this distribution to longer correlation times. In the gel phase, the spin-lattice relaxation and quadrupole echo decay rate are both found to be less sensitive to the application of pressure. [S1063-651X(98)10003-X]

PACS number(s): 87.22.Bt, 77.84.Nh, 87.64.Hd

I. INTRODUCTION

Physical properties of biological membranes can be modeled by self-assembled bilayers that form upon hydration of phospholipids. Interactions in the polar headgroup region of such bilayers play an important role in determining such properties.

Phospholipids having a phosphatidylcholine headgroup are important constituents of biological membranes and are widely utilized in model membranes. It has been shown that the average orientation of the phosphatidylcholine headgroup dipole can be altered by changes in charge density at the bilayer surface [1–3]. This change in average orientation gives rise to characteristic shifts in the quadrupole splitting of deuterons specifically substituted for hydrogen atoms on the choline group. In particular, deuterons attached to the α and β methylene groups of the headgroup choline moiety display counterdirectional changes in quadrupole splitting as effective surface charge is varied by, for example, addition of charged amphiphiles to the bilayer. Analogous shifts, in response to the application of hydrostatic pressure, have been interpreted as indicating a change in headgroup orientation in response to pressure-induced reduction in bilayer area per lipid [4].

While quadrupole splittings can give some information about the effects of pressure or surface charge on average headgroup orientation, information on how these factors influence time scales or amplitudes of headgroup reorientation must be obtained from relaxation measurements. The dynamics of headgroup motions have been examined through studies of spin-lattice relaxation of the ^{2}H-NMR signal in labeled headgroups [5–7] and of the ^{31}P-NMR signal in unlabeled samples [8,9]. A comprehensive analysis of headgroup dynamics was also carried out using a variety of ^{31}P-NMR relaxation measurements [10]. Peng and Jonas [11] used ^{31}P-NMR spin-lattice relaxation measurements to obtain information regarding the effect of applied hydrostatic pressure on headgroup dynamics.

Motions in a liquid crystalline phospholipid bilayer can span a range of characteristic times from shorter than 10^{-8} s for internal segment rotations [8,10] and long axis rotation and reorientation [10] to times longer than 10^{-4} s for collective motions of the bilayer surface or reorientation due to diffusion of molecules through regions in which the direction of the bilayer normal changes significantly [10,12]. Motions with characteristic times close to the inverse of the Larmor frequency are effective in relaxing the spin system toward thermal equilibrium. The characteristic time for this relaxation is the spin-lattice relaxation time or T_1. The deuteron quadrupole Hamiltonian is orientation dependent and motions that alter orientation of the electric field gradient tensor principal axis system with characteristic times around 10^{-4}–10^{-3} s reduce the extent to which it is possible to refocus the nuclear magnetization into an echo using the deuteron quadrupole echo pulse sequence. If an initial $\pi/2$ pulse at $t=0$ is followed by a second $\pi/2$ pulse, shifted in phase by 90°, at time $t=\tau$, the quadrupole echo forms at $t=2\tau$ for systems in which the quadrupole interaction is the dominant perturbation to the Zeeman interaction. The characteristic time for decay of the quadrupole echo with increasing 2τ is T_2^{qe}. Bloom and Sternin [12] demonstrated that there were motions whose correlation times were too long for them to contribute to motional narrowing but which still contributed to decay of the quadrupole echo. They used quadrupole Carr-Purcell-Meiboom-Gill (q-CPMG) multiple pulse experiments to separate contributions to the echo decay rate of such adiabatic motions from those of motions that are effective in motionally narrowing the deuteron spectrum. Effects of slow motions on q-CPMG echo decay rates have been discussed by Vega [13], Blicharski [14], and Müller *et al.* [15]. Adiabatic bilayer motions may include diffusion of molecules around curved vesicle surfaces [12] and collective modes of the bilayer surface [16]. A better understanding of the slow motions, in particular, would be useful since these

are sometimes the spectroscopically accessible aspects of bilayer behavior that are most sensitive to perturbation by biologically relevant factors such as the presence of intrinsic or extrinsic proteins.

NMR has been used to examine the effect of pressure on a variety of bilayer properties [17–19] including headgroup orientation [4,11]. The response of a bilayer system to applied pressure can provide insights into how bilayer properties are determined by interactions within the bilayer at ambient pressure. In particular, because of the anisotropy of bilayer elastic properties, application of pressure provides an isothermal way in which to vary area per lipid. The effect of pressure on deuteron relaxation times may thus provide some insights into how different motions depend on the physical state of the bilayer. Because pressure also shifts the main bilayer transition temperature, it may also be useful to examine the effect of pressure on the temperature dependence of bilayer properties with respect to the transition temperature.

Peng and Jonas [11] measured the ^{31}P spin-lattice relaxation time (T_1), for dipalmitoylphosphatidylcholine (DPPC) as a function of pressure at a fixed temperature of 50 °C. In the liquid crystalline phase, T_1 was found to decrease with increasing pressure. It decreased discontinuously at the main transition and then increased with decreasing pressure in the gel phases. This was interpreted in terms of an increase in motional correlation time at the pressure-induced transition. This group also measured the ^{2}H spin-lattice relaxation times and transverse relaxation times for selectively chain-deuterated DPPC, again as a function of pressure at a fixed temperature of 50 °C [20]. For each of the chain positions examined at their ^{2}H spectrometer frequency of 27.6 MHz, T_1 decreased with increasing pressure in the liquid crystalline phase, increased discontinuously at the lowest pressure-induced transition, and then continued to decrease with increasing pressure in the lowest pressure gel phase. The transverse relaxation times decreased with increasing pressure in the liquid crystalline phase, decreased discontinuously at the lowest pressure-induced transition, and then increased with increasing pressure in the lowest pressure gel phase.

The complicated relaxation behavior reported by Jonas and co-workers [11,20] reflects the wide range of motions present in the bilayer. Their measurements were all performed isothermally. The information available from such measurements can be complemented by isobaric measurements, in which correlation times can be presumed to vary monotonically with temperature, and by echo-train decay measurements, which can separate the effects, on quadrupole echo decay, of adiabatic and faster motions. In the present work, ^{2}H NMR has been used to examine the effect of applied hydrostatic pressure on the dynamics of headgroup-deuterated dimyristoylphosphatidylcholine (DMPC-d_4). Quadrupole splittings, spin-lattice relaxation time (T_1), and quadrupole echo decay times (T_2^{qe}) have been measured at 65 °C for a series of pressures up to 2 kbar and for a series of temperatures at ambient pressure and at 1.5 kbar. Quadrupole Carr-Purcell-Meiboom-Gill (q-CPMG) decays have been studied under similar conditions.

II. MATERIALS AND METHODS

1,2-dimyristoyl-*sn*-glycero-3-(1,1′,2,2′-d)-phosphocholine was purchased from Avanti Polar Lipids (Birmingham, AL) and used without further purification. Samples in the form of multilamellar vesicles (MLV) were prepared by stirring thoroughly with a fine glass rod in excess 0.1*M* phosphate buffer (*p*H 7.2) at a temperature above the main transition. The resulting MLV suspensions were transferred into flexible polyethylene tubes, which were then heat sealed.

Deuterium NMR was carried out in a 3.5 T superconducting magnet (Nalorac Cryogenics, Martinez, CA) using a locally built probe capable of operating at applied hydrostatic pressures up to 2.7 kbar over a temperature range from −20 °C to 80 °C [21]. The coil and tube containing the MLV suspension were enclosed within a beryllium-copper cell, which was pressurized with hydraulic oil (AW ISO grade 32). A Bourdon tube gauge calibrated against a dead weight gauge was used to measure pressure in the cell.

The pressure cell within the NMR probe was connected to an external vessel containing approximately twice the volume. By controlling the temperature of the external vessel, it was possible to compensate for changes in probe cell temperature and thus to maintain isobaric conditions over the course of an experiment in which sample temperature was varied.

Spectra were acquired using a phase-cycled quadrupole echo sequence [22]. The $\pi/2$ pulse was between 2.5 and 2.9 μs long. The separation between $\pi/2$ pulses in the quadrupole echo sequence ranged from 40 μs to 1 ms. Oversampling [23,24] by a factor of 4 was used to obtain effective dwell times of 20 μs for transients, which were collected with a digitizer dwell time of 5 μs. Between 4000 and 6000 transients were normally averaged to obtain quadrupole echos used in the determination of T_2^{qe}. The equilibration time between applications of the pulse sequence was 0.45 s.

The quadrupole Carr-Purcell-Meiboom-Gill (q-CPMG) pulse sequence,

$$[\pi/2_x - \tau - (\pi/2_y - 2\tau)_n],$$

was first applied to lipid bilayer systems by Bloom and Sternin [12]. Echoes are recorded at multiples of 2τ. In the present work, the number of echoes collected and the range of pulse separations employed depended on the state of the sample. For liquid crystalline phase samples, measurements ranged from the collection of 40 echoes with $\tau = 75$ μs to 8 echoes with $\tau = 500$ μs. In the gel phase, the range was from 40 echoes with $\tau = 30$ μs to 16 echoes with $\tau = 105$ μs. In both cases, 8000 transients were averaged for each experiment.

Spin-lattice relaxation times were measured by applying a π pulse to invert the deuteron magnetization and then, after an interval τ_1, sampling the recovered magnetization using a quadrupole echo pulse sequence. In the liquid crystalline phase, τ_1 was varied between 0.01 and 50 ms. In the gel phase, τ_1 was varied between 0.01 and 20 ms. For both phases, 12 000 transients were averaged for each experiment.

III. RESULTS AND DISCUSSION

A. Quadrupole splittings

Figure 1 shows the effect of pressure on the quadrupole splittings of the α and β choline deuterons of DMPC-d_4 at

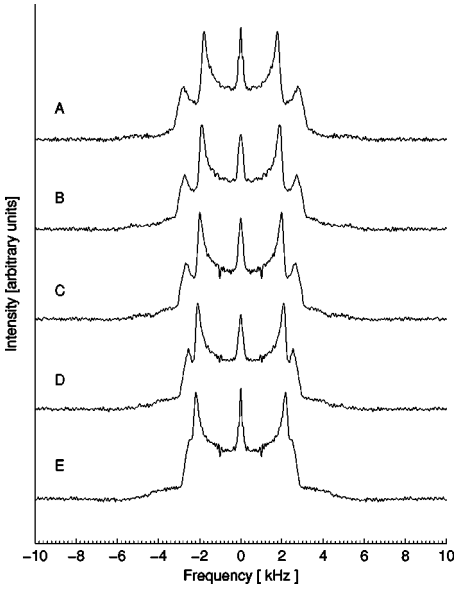

FIG. 1. ^{2}H-NMR spectra of DMPC-d_4 at 65 °C for (a) ambient pressure, (b) 0.5 kbar, (c) 1.0 kbar, (d) 1.5 kbar, and (e) 2.0 kbar. The doublet with the larger (smaller) splitting is assigned to the α (β) choline deuteron. The observed counterdirectional change in splitting with applied pressure is consistent with a pressure induced tilt of the headgroup away from the bilayer surface.

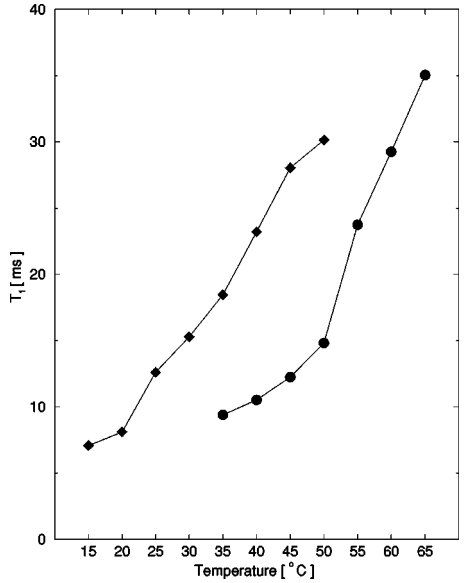

FIG. 2. The temperature dependence of the spin-lattice relaxation time (T_1) for DMPC-d_4 at ambient pressure (solid diamonds) and at 1.5 kbar (solid circles).

65 °C. Using the dePaking algorithm developed by Bloom and co-workers [25,26], powder pattern spectra can be transformed to yield the spectra (not shown) that would be expected for an oriented bilayer sample. The doublets with the larger and smaller splittings are assigned to the α and β choline deuterons, respectively. The spectra shown in Fig. 1 indicate that applied pressure decreases the α splitting and increases the β splitting. This is consistent with earlier results [4] and has been interpreted as being indicative of a pressure-induced tilt of the headgroup away from the bilayer as might be expected for a reduction of area per lipid with increasing pressure.

B. Spin-lattice relaxation

Figure 2 shows the temperature dependence of the spin-lattice relaxation time, T_1, obtained by inversion recovery measurements at ambient pressure and at 1.5 kbar. The bilayer main phase transitions are at roughly 23 °C and 53 °C respectively. Pressure increases the magnitude of the step in T_1 at the transition. The increase of T_1 with temperature indicates that the motions responsible for spin-lattice relaxation are in the short correlation time regime for both phases. Increasing temperature shortens the correlation times for these motions and reduces the resulting spectral density at the Larmor frequency. The magnitudes and temperature dependence of T_1 observed in the liquid crystalline phase are comparable to those reported for choline deuterons in DPPC [5,6] and DOPC [7].

Increased pressure extends the temperature range over which the gel phase is stable. Comparison of the ambient pressure and 1.5 kbar T_1 values in the respective gel phases suggests that pressure may not directly influence the spectral density of motions that are responsible for spin-lattice relaxation in the gel phase. In contrast, the liquid crystal T_1 values do display a marked isothermal pressure dependence. Figure 3 shows the pressure dependence of T_1 at 65 °C.

The spin-lattice relaxation time T_1 is sensitive to motions that modulate the quadrupole interaction with correlation times close to the inverse of the Larmor frequency, 23.215 MHz in these experiments. At room temperature and above, these motions, which are typically fast conformational changes or rotational diffusion, have correlation times that are shorter than the inverse of the Larmor frequency so that T_1 is expected to increase with increasing temperature. The bilayer main transition at ambient pressure has only a small effect on the correlation times for these motions in the headgroup.

Based on the use of a comprehensive dynamical model to analyze ^{31}P-NMR relaxation results, Dufourc *et al.* [10] re-

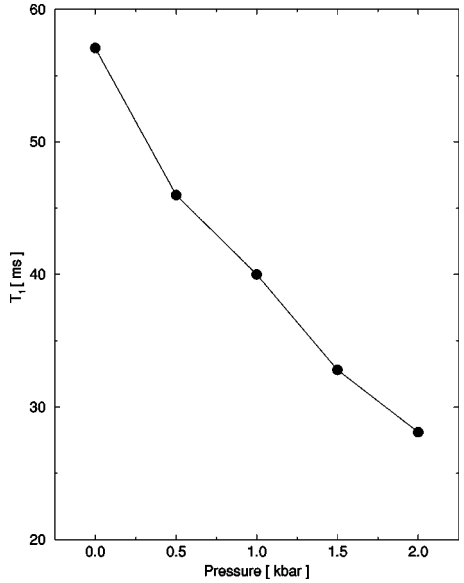

FIG. 3. The pressure dependence of the spin-lattice relaxation time (T_1) for DMPC-d_4 at 65 °C.

ported that fast headgroup motions present in the L_α phase of DMPC include free rotation of the phosphate group, with a correlation time between 4×10^{-10} and 7×10^{-10} s, and rotational diffusion and fluctuation of the long molecular axis with correlation times between 10^{-11} and 10^{-8} s.

In liquid crystalline DPPC bilayers, T_1 decreases with increasing pressure for both the ^{31}P site [11] and selectively deuterated chain sites [20]. The present results indicate that T_1 values for DMPC headgroup deuteron in the liquid crystalline phase also decrease with increasing pressure at fixed temperature. The isobaric results reported here confirm that relevant liquid crystalline headgroup motions are in the short correlation time limit. The observation that T_1 decreases with increasing pressure at fixed temperature in this phase thus suggests that pressure increases the correlation times of fast headgroup motions, thereby shifting T_1 toward its value at the T_1 minimum. The application of hydrostatic pressure also raises the bilayer main transition and reduces area per lipid, particularly in the liquid crystalline phase. One way in which applied pressure might increase correlation time for such motions would be if the resulting reduction in area per lipid gave rise to an increased coupling of headgroup conformational states between neighboring lipid molecules.

For DPPC at 50 °C, ^{31}P spin lattice relaxation times are reported to increase with pressure in the lowest pressure gel phase [11] whereas the spin-lattice relaxation times for chain deuterons decrease with increasing pressure in the same phase [20]. Both observations imply DPPC gel phase motions for which the correlation times are sensitive to pressure. For DMPC-d_4, as noted above, the motions responsible for spin-lattice relaxation appear to remain in the short correlation time limit for both phases over the range of pressures covered here and the present results suggest, albeit indirectly, that pressure has less effect on T_1 in the gel phase than in the liquid crystalline phase. This raises the interesting possibility that chain length might influence the extent to which gel phase correlation times respond to pressure.

C. Quadrupole echo decays

Figure 4 shows quadrupole echo decays for a series of temperatures at ambient pressure and at 1.5 kbar and for a series of pressures at 65 °C. Figure 5 shows corresponding T_2^{qe} values as a function of temperature for ambient pressure and 1.5 kbar and as a function of pressure at 65 °C. The nonexponential decays observed in the liquid crystalline phase for short values of τ suggest that slow motions contribute significantly to the echo decays [14,15]. This observation is consistent with the results of quadrupole Carr-Purcell-Meiboom-Gill (q-CPMG) experiments described below. The departure from exponential decay increases with increasing pressure for fixed temperature but does not change significantly with temperature for fixed pressure. The values for T_2^{qe} shown in Fig. 5 for the liquid crystalline phase were calculated from data for larger τ values for which the decays are observed to be effectively exponential.

A motion that modulates the quadrupole Hamiltonian can be characterized by the second moment, ΔM_2, of that modulation. For motions that give rise to motional narrowing,

$$\Delta M_2 = M_2 - M_{2r}, \tag{1}$$

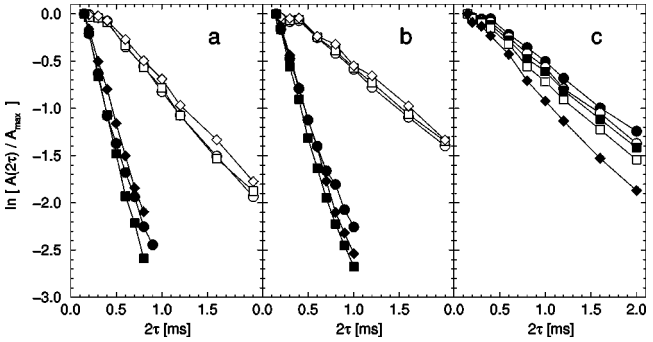

FIG. 4. (a) DMPC-d_4 quadrupole echo decays at ambient pressure for 35 °C (open circle), 30 °C (open square), 25 °C (open diamond), 20 °C (solid circle), 15 °C (solid square), and 10 °C (solid diamond). (b) DMPC-d_4 quadrupole echo decays at 1.5 kbar for 65 °C (open circle), 60 °C (open square), 55 °C (open diamond), 50 °C (solid circle), 45 °C (solid square), and 40 °C (solid diamond). (c) DMPC-d_4 quadrupole echo decays at 65 °C for 2.0 kbar (solid circle), 1.5 kbar (open circle), 1.0 kbar (solid square), 0.5 kbar (open square), and ambient pressure (solid diamond). Echo amplitudes are normalized to the maximum observed value.

where M_2 is the full second moment of the interaction and M_{2r} is the residual second moment of the motionally narrowed spectrum [27]. A given motion i with a correlation time τ_{ci} shorter that $(\Delta M_{2i})^{-1}$ makes a contribution to the echo decay rate of

$$\left(\frac{1}{T_2^{qe}}\right)_i = \Delta M_{2i}\tau_{ci}. \tag{2}$$

Because correlation times generally decrease with increasing temperature, the observation that T_2^{qe} decreases with increasing temperature suggests that motions that are too slow to satisfy $\tau_{ci} \ll (\Delta M_{2i})^{-1}$ may contribute significantly to echo decay in the liquid crystalline phase. If transverse relaxation in the liquid crystalline phase is dominated by such slow motions, the observed increase in T_2^{qe} with pressure in the liquid crystalline phase could reflect either an increase in correlation time τ_{ci} or a reduction in ΔM_{2i} for such motions.

In the liquid crystalline phase, slow motions that contribute to decay of the echo may include lateral diffusion around curved vesicle surfaces or collective bilayer modes. While

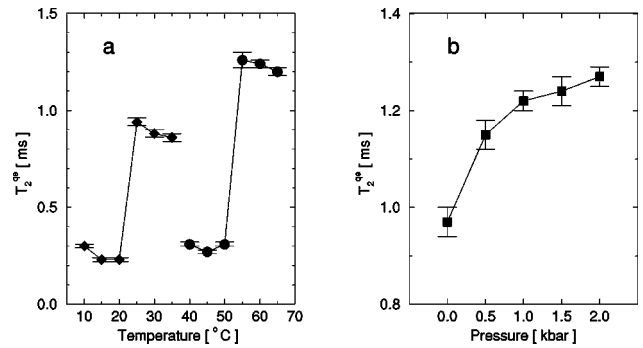

FIG. 5. (a) The temperature dependence of the quadrupole echo decay time (T_2^{qe}) for DMPC-d_4 at ambient pressure (solid diamonds) and 1.5 kbar (solid circles). (b) The pressure dependence of the quadrupole echo decay time (T_2^{qe}) at 65 °C for DMPC-d_4.

these motions are expected to freeze out in the gel phase, more localized motions are slowed into the range where they contribute significantly to echo decay so that the echo decay rate increases sharply at the liquid crystal to gel transition. Within the gel phase, the motions that contribute to echo decay generally have correlation times that are long enough that the contribution to the echo decay rate decreases as the correlation times increase. The result is that T_2^{qe} increases with decreasing temperature in the gel phase. Figure 5 shows that T_2^{qe} just below the transition is roughly the same at ambient pressure and 1.5 kbar. This suggests that the increase in temperature required to maintain a given separation from the transition temperature partially offsets the effect of pressure on motions contributing to echo decay in the gel phase.

Dufourc *et al.* [10] found that the ^{31}P Hahn echo decay time (T_{2E}) in the liquid crystalline phase of DMPC was sensitive to slow motions, that they identified as collective modes, and to rotation and fluctuations which they identified as intermolecular motions. The ^{31}P echo decay times reported by Dufourc *et al.* displayed a minimum about 45 °C below the main transition temperature and a discontinuity at the pretransition temperature. A minimum was also observed in the $P_{\beta'}$ phase just below the main transition temperature. Above the transition, T_{2E} was observed to decrease with increasing temperature. This was interpreted as indicating the presence of very slow motions in the liquid crystalline phase.

The ambient pressure values of T_2^{qe} reported here do not display a noticeable discontinuity at the pretransition. Like the ^{31}P echo decay times, though, they do decrease with increasing temperature in the liquid crystalline phase. This is taken to indicate that headgroup deuteron transverse relaxation in the liquid crystalline phase is dominated by slow motions.

It is interesting to note that for the DPPC chain deuterons examined by Peng *et al.* [20], the transverse relaxation times decrease with increasing pressure in the liquid crystalline phase and increase with increasing pressure in the lowest pressure gel phase. This suggests that the relative sensitivity of transverse relaxation to adiabatic and faster motions for DMPC headgroup deuterons and DPPC chain deuterons is different. Because the relevant slow motions are not yet fully characterized, it is not clear whether this is due to the difference between chain and headgroup dynamics or whether chain-length effects might also contribute. Evidence that chain length can significantly affect slow motions has been reported by Fenske and Jarrell [28] whose ^{31}P two-dimensional solid-state exchange experiments indicate that the lateral diffusion rate for DPPC is greater than that for DMPC at comparable reduced temperatures.

In order to obtain some insight into the distribution of motions contributing to quadrupole echo decay, particularly in the liquid crystalline phase, and the possible effect of pressure on such a distribution, q-CPMG decays were examined under various conditions of temperature and pressure.

D. Quadrupole Carr-Purcell-Meiboom-Gill decays

Quadrupole echo decay is sensitive to motions covering a wide range of time scales. Bloom and Sternin [12] used the q-CPMG multiple pulse sequence $[90_x-\tau-(90_y-2\tau)_n]$ to demonstrate the sensitivity of transverse relaxation to motions that were too slow to cause motional narrowing (adiabatic motions). For a given motion that is modulating a portion of the quadrupole Hamiltonian with a second moment ΔM_{2i} and a correlation time τ_{ci}, the contribution to the decay of the echo at $2n\tau$ is [14]

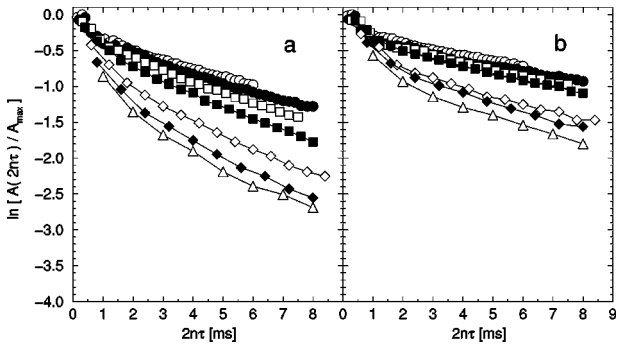

FIG. 6. (a) Quadrupole Carr-Purcell-Meiboom-Gill decays for DMPC-d_4 at ambient pressure and 30 °C for pulse separations (τ) of 75 μs (open circle), 100 μs (solid circle), 150 μs (open square), 200 μs (solid square), 300 μs (open diamond), 400 μs (solid diamond), and 500 μs (open triangle). (b) Quadrupole Carr-Purcell-Meiboom-Gill decays for DMPC-d_4 at 1.5 kbar and 60 °C for pulse separations (τ) of 75 μs (open circle), 100 μs (solid circle), 150 μs (open square), 200 μs (solid square), 300 μs (open diamond), 400 μs (solid diamond), and 500 μs (open triangle). Echo amplitudes are normalized to the maximum observed value.

$$\left(\frac{1}{T_2^{\text{q-CPMG}}}\right)_i = \Delta M_{2i}\tau_{ci}\left[1-\frac{\tau_{ci}}{\tau}\tanh\left(\frac{\tau}{\tau_{ci}}\right)\right]. \tag{3}$$

Motions for which $\tau_{ci} \ll (\Delta M_{2i})^{-1/2}$ is satisfied can contribute to motional narrowing. Such motions will generally also satisfy $\tau_{ci} \ll \tau$, so that their contribution to the q-CPMG echo decay rate will be $(T_2^{\text{q-CPMG}})_i^{-1} \approx \Delta M_{2i}\tau_{ci}$ independent of the pulse separation. Motions for which $\tau_{ci} \gg (\Delta M_{2i})^{-1/2}$ is satisfied do not contribute to motional narrowing and are labeled adiabatic. For these motions, it may be possible to select q-CPMG pulse separations such that $\tau_{ci} \gg \tau$, so that the q-CPMG decay rate is given by $(T_2^{\text{q-CPMG}})_i^{-1} \approx \Delta M_{2i}\tau^2/(3\tau_{ci})$, which depends on pulse separation. The first echo in each q-CPMG decay is equivalent to the quadrupole echo corresponding to that pulse separation τ.

Quadrupole-CPMG experiments were done on DMPC-d_4 for a series of temperatures at ambient pressure and at 1.5 kbar and for a series of pressures at 65 °C. Figure 6 shows typical q-CPMG decays in the liquid crystal phase at 30 °C for ambient pressure and at 60 °C for 1.5 kbar. In both cases, the temperature is about 7 °C above the main transition. The decays are clearly dependent on the pulse spacing τ, which indicates that adiabatic motions contribute significantly to decay of the quadrupole echo in the liquid crystalline phase. The decays for larger τ values are nonexponential but approach a common decay rate for large values of $2n\tau$. This suggests that each molecule is subject to one common motion, for which τ_{ci} is short on the experimental time scale, and at least one slow (adiabatic) motion and that there is a distribution of slow motions.

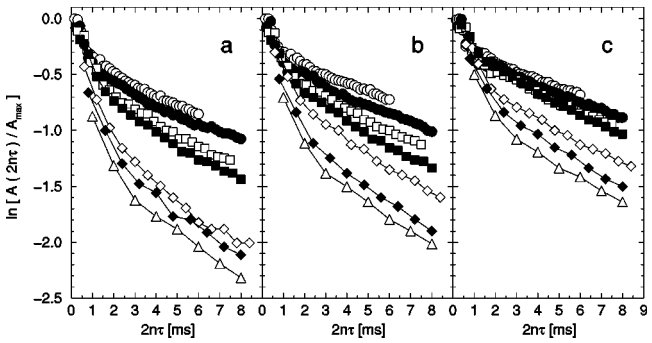

FIG. 7. Quadrupole Carr-Purcell-Meiboom-Gill decays for DMPC-d_4 for (a) ambient pressure, (b) 1.0 kbar, and (c) 2.0 kbar at 65 °C using pulse separations, τ, of 75 μs (open circle), 100 μs (solid circle), 150 μs (open square), 200 μs (solid square), 300 μs (open diamond), 400 μs (solid diamond), and 500 μs (open triangle). Echo amplitudes are normalized to the maximum observed value.

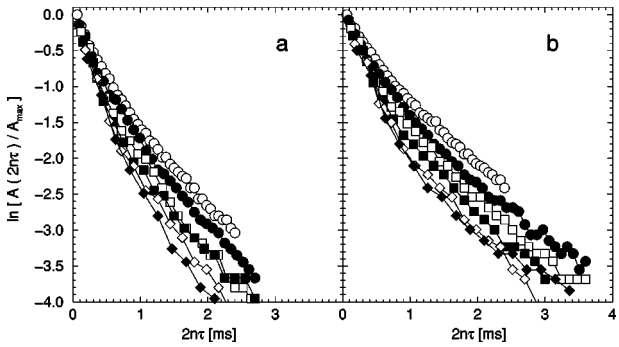

FIG. 8. (a) Quadrupole Carr-Purcell-Meiboom-Gill decays for DMPC-d_4 at ambient pressure and 20 °C for pulse separations (τ) of 30 μs (open circle), 45 μs (solid circle), 60 μs (open square), 75 μs (solid square), 90 μs (open diamond), and 105 μs (solid diamond). (b) Quadrupole Carr-Purcell-Meiboom-Gill decays for DMPC-d_4 at 1.5 kbar and 50 °C for pulse separations (τ) of 30 μs (open circle), 45 μs (solid circle), 60 μs (open square), 75 μs (solid square), 90 μs (open diamond), and 105 μs (solid diamond). Echo amplitudes are normalized to the maximum observed value.

The limiting decay at large $2n\tau$ for small pulse separation is presumed to reflect a fast motion that is common to all lipids in the sample. If all lipids also shared a common slow motion, the expected q-CPMG decays would be exponential with a rate given by $(T_2^{\text{q-CPMG}})^{-1} \propto \tau^2$. The observed decays are nonexponential. The initial slopes do not display a clear τ^2 dependence. The separations between nearly exponential limiting decay curves obtained for different pulse separations, τ, suggests a broad distribution of slow motions.

For a molecule undergoing a particular set of motions, the observed q-CPMG decay rate is a sum of contributions from each motion. The contributions to the q-CPMG decay rate from motions with correlation times that are long compared to τ are proportional to $(\tau_{ci})^{-1}$. For a given pulse separation, the signal from those molecules whose slow motions have the shortest correlation times decay most quickly. For molecules having slow motions with longer correlation times, the contribution to the echo decay rate from the common fast motion is relatively more important. It is the signal from these molecules that persists for large values of $2n\tau$ and gives rise to the roughly exponential limiting decay. The way in which the q-CPMG echo decays vary with τ should, in principle, contain information about the distribution of slow motions.

For the shortest τ values, the contribution to the q-CPMG echo decay rate from the slowest adiabatic motions is small and the observed decay is determined largely by the common fast internal motion. For $\tau = 0.075$ ms, $T_2^{\text{q-CPMG}}$ is about 13 ms for 1.5 kbar at 60 °C and 9 ms for ambient pressure at 30 °C. For larger values of τ, the limiting decay rates at large $2n\tau$ for high and low pressure are separated by similar differences. As suggested below, this difference in the limiting decay rates may reflect the fact that temperature was increased in order to maintain a constant separation from the transition as pressure was raised.

Figure 7 shows q-CPMG decays for ambient pressure, 1 kbar, and 2 kbar at 65 °C. While the quadrupole echo decay times do change with pressure at this temperature, the limiting slopes of the q-CPMG decays for large $2n\tau$ are not very sensitive to pressure. The result is that the spread with τ of the limiting decays for large values of $2n\tau$ decreases with increasing pressure. Since these limiting decays are dominated by the contribution to the relaxation rate from common fast motions, this suggests that the correlation times for such motions at fixed temperature are only weakly dependent on applied hydrostatic pressure. Observation that the application of pressure reduces the effect of τ on q-CPMG echo decays at large $2n\tau$ suggests that pressure either reduces the ΔM_{2i} values for the adiabatic motions or increases the corresponding correlation times τ_{ci}.

Figure 8 shows typical q-CPMG decays in the gel phase for ambient pressure at 20 °C and for 1.5 kbar at 50 °C. In each case, the temperature is about 3 °C below the main transition. Even for very short τ values, $T_2^{\text{q-CPMG}}$ is only about twice T_2^{qe} at these temperatures. This suggests that adiabatic motions account for only about half of the quadrupole echo decay rate just below the transition. While the quadrupole echo decay rates are similar for the two experiments shown in Fig. 8, the limiting q-CPMG echo decay rates for large $2n\tau$ are lower at 1.5 kbar and 50 °C than at ambient pressure and 20 °C. The behavior of the limiting decays reflects the effect of temperature on the correlation time of faster motions whose contribution to the echo decay is not entirely removed by the q-CPMG sequence. The observation that the quadrupole echo decay time is similar for the two experiments may indicate that the contribution to the decay rate from the slower motions in the gel phase increases on going from ambient pressure at 20 °C to 1.5 kbar at 50 °C.

The q-CPMG sequence refocuses decay due to motions with correlation times much longer than the pulse separation. The observation of τ-dependent decays indicates the presence of such slow motions and is consistent with the observation that T_2^{qe} in the liquid crystalline phase decreases with increasing temperature. It has been suggested that diffusion around the vesicle surface [12] or collective motions [16] might be responsible for the slow modulation of the orientation-dependent quadrupole interaction. For vesicle samples, both mechanisms may contribute.

The observation of nonexponential q-CPMG decays implies that different portions of the sample are affected by

different slow motions. One possible explanation for the existence of different slow motions is the distribution of vesicle radii expected for a typical dispersion of multilamellar vesicles. The distribution of vesicle radii in a dispersion has recently been characterized using excitation transfer ^{31}P NMR by Heaton *et al.* [29]. Effects due to the distribution of vesicle radii have also been reported for ^{2}H NMR two-dimensional exchange spectroscopy studies of specifically deuterated phospholipid [30].

E. Simulation of quadrupole and q-CPMG echo decays

For molecules affected by a dominant motion, which may be a slow motion specific to a given population, and by faster motions that have a common effect on all molecules, q-CPMG echo amplitudes are obtained by summing signals that decay according to Eq. (3) to give

$$A(2n\tau)=\sum_{i=1}^{N} A_i \exp\left(-2n\tau\left\{\Delta M_{2i}\tau_{ci}\left[1-\frac{\tau_{ci}}{\tau}\times\tanh\left(\frac{\tau}{\tau_{ci}}\right)\right]+\frac{1}{T_2'}\right\}\right), \tag{4}$$

where A_i is amplitude of the signal from a given population, ΔM_{2i} and τ_{ci} are characteristic of the motion specific to that population, and $(T_2')^{-1}$ is the contribution to the relaxation rate from fast motions common to all lipids in the sample. This expression, evaluated for $n=1$, also describes the τ dependence of the quadrupole echo amplitudes.

In order to obtain some insight into the motions reflected by the observed decays, q-CPMG and quadrupole echo decays were simulated using a simple discrete distribution of slow motions. Resulting simulations were compared to observed decays at 30 °C for ambient pressure and 60 °C for 1.5 kbar.

The simplest distributions for which general features of the observed decays could be reproduced consisted of three populations having different correlation times for slower motions and a common relaxation rate, $1/T_2'$, due to fast motions. If the dominant slow motion is diffusion of molecules around a curved vesicle surface, ΔM_{2i} should correspond to the residual second moment, M_{2r}, and the correlation times should depend on the diffusion constant D and vesicle radius R through [12]

$$\tau_c=\frac{R^2}{6D}. \tag{5}$$

In order to consider whether such motion might plausibly account for the observed decays, the simulations were performed with ΔM_2 set to the M_{2r} values obtained from corresponding spectra. At 30 °C for ambient pressure and at 60 °C for 1.5 kbar, the observed M_{2r} values were 263×10^6 and 167×10^6 s^{-2}, respectively.

The value of $1/T_2'$ was chosen to yield the q-CPMG decay observed at large n in echo trains collected using small values of the pulse separation τ. With M_{2r} and $1/T_2'$ held constant, the correlation times and relative sizes of the three

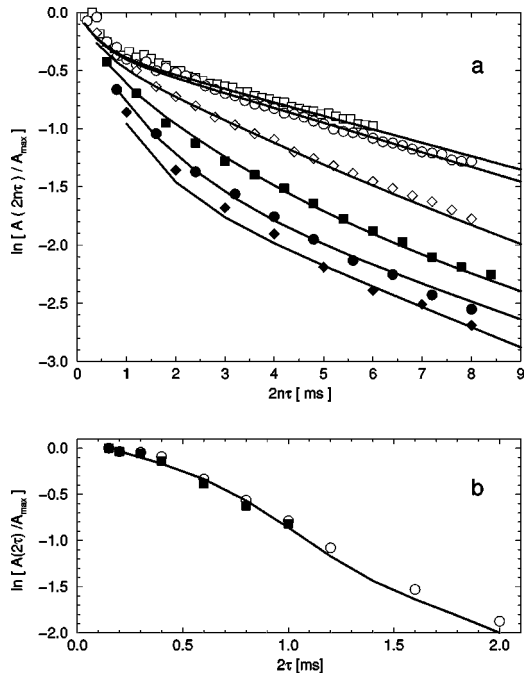

FIG. 9. Comparison of (a) q-Carr-Purcell-Meiboom-Gill echo and (b) quadrupole echo decays for DMPC-d_4 observed at ambient pressure and 30 °C with decays simulated using the parameters listed in Table I. In panel (a), observed q-CPMG decays are shown for as symbols for τ values of 0.075 ms (open square), 0.1 ms (open circle), 0.2 ms (open diamond), 0.3 ms (solid square), 0.4 ms (solid circle), and 0.5 ms (solid diamond). Corresponding simulated decays are shown as solid lines. Panel (b) shows the τ dependence of the first echo of each q-CPMG experiment (solid square) along with an extended set of echoes obtained from a separate quadrupole echo experiment (open circle). The τ dependence of the simulated quadrupole echo amplitudes is shown as a solid line.

populations were adjusted until the agreement of the simulation with the observed decays, as judged by inspection, was satisfactory. As is discussed below, it was possible to obtain similar simulations for different sets of parameters. In effect, the different sets of parameters corresponded to choosing different ways in which to approximate the actual distribution of slow motions by two discrete populations. Figure 9 compares observations at ambient pressure and 30 °C with q-CPMG and quadrupole echo decays simulated using the particular set of parameters shown in Table I. Figure 10 compares observations at 1.5 kbar and 60 °C with decays simulated using the parameters shown in Table II.

In the sets of simulation parameters shown in Table I and in Table II, the two longest correlation times of each set are long compared both to τ for all of the experiments performed and to $(M_{2r})^{-1/2}$. These motions are thus adiabatic and in

TABLE I. Parameters for the simulation of q-CPMG echo decays at ambient pressure and 30 °C shown in Fig. 9.

A_i	τ_{ci} (ms)	ΔM_{2i} (10^6 s^{-2})	$(T_2')^{-1}$ (s^{-1})
0.27	0.01	263	100
0.46	20	263	100
0.27	300	263	100

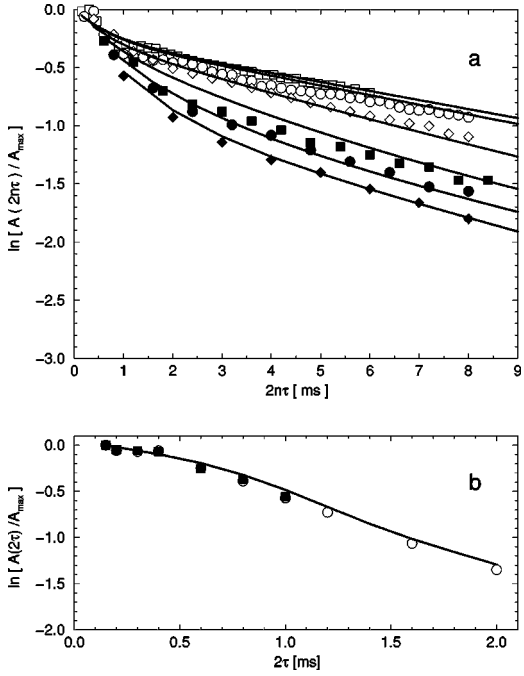

FIG. 10. Comparison of (a) q-Carr-Purcell-Meiboom-Gill echo and (b) quadrupole echo decays for DMPC-d_4 observed at 1.5 kbar and 60 °C with decays simulated using the parameters listed in Table II. In panel (a), observed q-CPMG decays are shown as symbols for τ values of 0.075 ms (open square), 0.1 ms (open circle), 0.2 ms (open diamond), 0.3 ms (solid square), 0.4 ms (solid circle), and 0.5 ms (solid diamond). Corresponding simulated decays are shown as solid lines. Panel (b) shows the τ dependence of the first echo of each q-CPMG experiment (solid square) along with an extended set of echoes obtained from a separate quadrupole echo experiment (open circle). The τ dependence of the simulated quadrupole echo amplitudes is shown as a solid line.

the regime where their contributions to the echo decay rate are inversely proportional to τ_{ci} and dependent on the pulse separation τ. The shortest correlation time in each set of simulation parameters is less than $(M_{2r})^{-1/2}$ but of the same order of magnitude. The corresponding motion is in the range between adiabatic motions and those contributing to motional narrowing. The shortest correlation times in Tables I and II are, however, much smaller than the pulse separations used in the experiment and the contributions of these motions to the echo decay rates are proportional to τ_{ci} and independent of pulse separation.

Specific aspects of the simulated decays are affected by particular simulation parameters. The limiting behavior of the simulated q-CPMG decays for large $2n\tau$ is sensitive to $(T_2')^{-1}$. For simulated echo trains corresponding to a small pulse separation, the nonexponential q-CPMG decays at small values of $2n\tau$ reflect the presence of a population having a short correlation time and a fast echo decay. The presence of such a population is also necessary to reproduce the small τ behavior of the quadrupole echo decay. At large values of $2n\tau$, the separation between adjacent q-CPMG decay curves does not vary monotonically with τ but reflects details of the slow motion correlation time distribution. For the two populations with larger values of τ_{ci}, the simulated decays are sensitive the ratio $\Delta M_{2i}/\tau_{ci}$ so that separation of ΔM_{2i} and τ_{ci} must be based on other considerations. While

TABLE II. Parameters for the simulation of q-CPMG echo decays at 1.5 kbar and 60 °C shown in Fig. 10.

A_i	τ_{ci} (ms)	ΔM_{2i} (10^6 s^{-2})	$(T_2')^{-1}$ (s^{-1})
0.21	0.01	167	70
0.37	20	167	70
0.42	300	167	70

a distribution consisting of three populations is too coarse to fully account for the observed decays at intermediate values of τ, it does reproduce the decays observed for higher and lower values of τ.

It was possible to generate similar simulations by raising (lowering) the two longest correlation times while reducing (increasing) the fraction of molecules having the largest correlation time. This reflects the fact that when the continuous distribution of slow motions is approximated by two discrete populations, the result is not sensitive to the precise way in which the distribution is divided. Better definition of the distribution would presumably require comparison with decays obtained using a larger number of more closely spaced τ values.

The two longest correlation times listed in Table I are long enough that the corresponding motions can be considered adiabatic. If adiabatic motions dominating echo decay are related to diffusion of lipids around curved vesicle surfaces, then the identification of M_{2r} with ΔM_{2i} is appropriate and the correlation times should depend on vesicle radii through Eq. (5). For a diffusion constant $D \approx 4\times10^{-12}$ m^2 s^{-1} [12,27], the two longest correlation times in Table I, 20 and 300 ms, would correspond to radii of curvature of 0.7 and 2.7 μm. These fall within the range obtained by Heaton *et al.* [29] using excitation transfer ^{31}P NMR to directly measure molecular rotation due to translational diffusion in vesicles. They found that approximately 20% of the molecules exist in vesicles with radii greater than 2 μm.

The shortest of the correlation times falls into the fast motion regime where its contribution to the echo decay rate is $(1/T_2^{\text{q-CPMG}})_i \approx \Delta M_{2i}\tau_{ci}$ independent of the pulse separation τ. The signal from this population displays the largest echo decay rate in the sample. This correlation time also falls into the intermediate range between being adiabatic and contributing to motional narrowing. The identification of ΔM_{2i} with M_{2r} is thus not fully justified. The simulation is only slightly altered, however, if this population is assigned $\tau_{ci} = 0.05$ ms and $\Delta M_{2i} = 60\times10^6$ s^{-2}. For correlation times longer than this, the simulation can no longer reproduce both the quadrupole echo decay and the short τ q-CPMG decays simultaneously. This provides, at least, an indication of the order of magnitude for the correlation time associated with this motion. If this motion involves diffusion over a curved region of the bilayer surface, the corresponding radius of curvature would be less than about 30 nm. This is clearly too small to associate with the radius of curvature of a vesicle.

Two-dimensional exchange spectroscopy, which measures the distribution of angles through which molecules reorient during the mixing time in the pulse sequence of the two-dimensional experiment [31], has been used to obtain information about slow reorientation. Fenske and Jarrell [28], using ^{31}P two-dimensional exchange spectroscopy, re-

ported correlation times for orientational exchange due to lateral diffusion of 44 ms in liquid crystalline DMPC bilayers and 8 ms in liquid crystalline DPPC bilayers. Auger *et al.* [32] found a similar correlation time for DPPC using ^{2}H two-dimensional exchange spectroscopy on selectively chain-deuterated DPPC. Macquaire and Bloom [30] included the possible effects of a distribution of vesicle radii on slow motion correlation times in their analysis of ^{2}H NMR two-dimensional exchange spectroscopy on DPPC deuterated at the α position of choline. They interpreted their observations in terms of diffusion over vesicles with radii of curvature falling predominantly in the 1–2 μm range. In addition, about 15% of the molecules were associated with radii of curvature much greater than 1 μm. In order to account for their observations, however, it was also necessary to consider small angle reorientation due to surface roughness on scales much less than 1 μm.

If surface roughness also accounts for the results attributed here to the population with the short correlation time, it is important to recognize that the molecules in this population could simultaneously be experiencing other slow motions. The contribution to the echo decay rate from the motion with the shortest correlation time is much larger than the contributions from the adiabatic motions. The echo decay for a molecule experiencing both motions would be dominated by the effects of the short correlation time motion and would thus be associated with that population.

When hydrostatic pressure is applied and the temperature is raised to maintain a particular separation from the transition temperature, the quadrupole echo decay rate and the separations between q-CPMG decay curves for different values of τ are reduced. The simulation shown in Fig. 10 was carried out with ΔM_{2i} fixed at the value of M_{2r} observed for 60 °C and 1.5 kbar. The other differences from the parameters used for the simulation shown in Fig. 9 are a reduction in $(T_2')^{-1}$ and an increase in the fraction of the signal associated with the longest correlation time. The difference in $(T_2')^{-1}$ between the two simulations presumably reflects a reduction with increasing temperature of the correlation times for common fast motions contributing to echo decay. As shown in Fig. 7, isothermal application of pressure has little effect on the q-CPMG decay for small values of the pulse separation τ where the decay is dominated by $(T_2')^{-1}$. The primary change in the slow motions between the two simulations is the apparent extension of the distribution to longer correlation times. Similar simulations (not shown) of the q-CPMG decays displayed in Fig. 7 for ambient pressure and 2.0 kbar at 65 °C confirmed that the effect of pressure on the q-CPMG decay at fixed temperature could be largely accounted for by increasing the largest correlation time and the fraction of the population corresponding to that correlation time. Limited pressure cycling did not suggest significant changes in echo decay behavior that might be expected for irreversible changes in vesicle radius. The change in the slow motion distribution with pressure may thus indicate a decrease in the translational diffusion constant with increased pressure.

The possibility that the translational diffusion constant might decrease on going from ambient pressure at 30 ° C to 1.5 kbar at 60 °C is of some interest. The ^{2}H NMR first spectral moments for perdeuterated DMPC show that the chain orientational order decreases slightly with increased pressure if the separation between the sample temperature and the transition temperature is held constant [33]. If hydrostatic pressure is applied isothermally, the same study showed that the effect of pressure on the first spectral moment in the liquid crystalline phase decreases with increasing temperature. The first spectral moment is proportional to the mean orientational order of the chain. For the current study, this implies that raising the pressure and temperature from 30 °C and ambient pressure to 60 °C and 1.5 kbar should involve a slight decrease in chain order but also a decrease in lateral compressibility of the bilayer. Any reduction in the translational diffusion constant under these circumstances presumably reflects the latter effect rather than the former.

IV. CONCLUDING REMARKS

This study provides some general insights into how bilayer motions sensed by a phospholipid headgroup deuteron label are affected by the application of hydrostatic pressure. The isothermal application of pressure in the liquid crystalline phase reduced the spin-lattice relaxation time T_1. This was taken to indicate a pressure induced increase of the correlation times for fast motions contributing to spin-lattice relaxation.

Simulation of the quadrupole and q-CPMG decays was used to obtain an approximation of the distribution of relevant slow motions. The results suggest that diffusion of lipid molecules around curved vesicle surfaces with a distribution of radii is a plausible identification of some slow motions contributing to the observed decays but does not rule out other possible adiabatic motions. Because other slow motions, such as local bilayer undulations, are also expected to contribute to echo decay, any distribution of vesicle radii extracted from such observations should be approached with caution. The simulation does, however, provide some insight into the breadth of the distribution required to account for the observed decays and into how the general distribution of slow motions is affected by the application of pressure.

Simulation of the quadrupole and q-CPMG decay observations also suggested that the application of pressure affects the distribution of slow motions contributing to these processes. If diffusion of lipid molecules around curved vesicle surfaces is an important adiabatic motion, the effect of pressure on the distribution of slow motions might reflect a pressure-induced reduction in the translational diffusion constant.

In general, the effect of pressure on relaxation and echo decay rates in the gel phase was found to be somewhat weaker.

ACKNOWLEDGMENTS

The authors thank William Kieley for his contributions to the construction of the high pressure NMR probe. This work was supported by the Natural Sciences and Engineering Research Council of Canada. The authors are grateful to Professors M. Bloom and E. Sternin for supplying the dePakeing software used in this work and to Professor Franz Fujara for helpful comments. D.F. was a visiting graduate student from the University of Dortmund, Germany.

[1] J. Seelig, P. M. Macdonald, and P. G. Scherer, Biochemistry **26**, 7535 (1987).
[2] P. G. Scherer and J. Seelig, Biochemistry **28**, 7720 (1989).
[3] P. M. Macdonald, J. Leisen, and F. M. Marassi, Biochemistry **30**, 10 558 (1991).
[4] B. B. Bonev and M. R. Morrow, Biophys. J. **69**, 518 (1995).
[5] H.-U. Gally, W. Niederberger, and J. Seelig, Biochemistry **14**, 3647 (1975).
[6] J. L. Browning, Biochemistry **20**, 7144 (1981).
[7] A. S. Ulrich and A. Watts, Biophys. J. **66**, 1441 (1994).
[8] M. P. Milburn and K. R. Jeffrey, Biophys. J. **52**, 791 (1987).
[9] M. P. Milburn and K. R. Jeffrey, Biophys. J. **56**, 543 (1989).
[10] E. J. Dufourc, C. Mayer, J. Stohrer, G. Altoff, and G. Kothe, Biophys. J. **61**, 42 (1992).
[11] X. Peng and J. Jonas, Biochemistry **31**, 6383 (1992).
[12] M. Bloom and E. Sternin, Biochemistry **26**, 2101 (1987).
[13] A. J. Vega, J. Magn. Reson. **65**, 252 (1985).
[14] J. S. Blicharski, Can. J. Phys. **64**, 733 (1986).
[15] K. Müller, R. Poupko, and Z. Luz, J. Magn. Reson. **90**, 19 (1990).
[16] J. Stohrer, G. Gröbner, D. Reimer, K. Weisz, C. Mayer, and G. Kothe, J. Chem. Phys. **95**, 672 (1991).
[17] J. Jonas, C.-L. Xie, A. Jonas, P. J. Grandinetti, D. Campbell, and D. Driscoll, Proc. Natl. Acad. Sci. USA **85**, 4115 (1988).
[18] D. A. Driscoll, S. Samarasinghe, S. Adamy, J. Jonas, and A. Jonas, Biochemistry **30**, 3322 (1991).
[19] D. A. Driscoll, J. Jonas, and A. Jonas, Chem. Phys. Lipids **58**, 97 (1991).
[20] X. Peng, A. Jonas, and J. Jonas, Biophys. J. **68**, 1137 (1995).
[21] B. B. Bonev and M. R. Morrow, Rev. Sci. Instrum. **68**, 1827 (1997).
[22] J. H. Davis, K. R. Jeffrey, M. Bloom, M. I. Valic, and T. P. Higgs, Chem. Phys. Lett. **42**, 390 (1976).
[23] R. S. Prosser, J. H. Davis, F. W. Dahlquist, and M. A. Lindorfer, Biochemistry **30**, 4687 (1991).
[24] M. R. Morrow, S. Taneva, G. A. Simatos, L. A. Allwood, and K. M. W. Keough, Biochemistry **32**, 11 338 (1993).
[25] M. Bloom, J. H. Davis, and A. L. MacKay, Chem. Phys. Lett. **80**, 198 (1981).
[26] E. Sternin, M. Bloom, and A. L. MacKay, J. Magn. Reson. **55**, 274 (1983).
[27] M. Bloom, E. E. Burnell, A. L. MacKay, C. P. Nichol, M. I. Valic, and G. Weeks, Biochemistry **17**, 5750 (1978).
[28] D. B. Fenske and H. C. Jerrell, Biophys. J. **59**, 55 (1991).
[29] N. J. Heaton, G. Althoff, and, and G. Kothe, J. Phys. Chem. **100**, 4944 (1996).
[30] F. Macquaire and M. Bloom, Phys. Rev. E **51**, 4735 (1995).
[31] C. Schmidt, B. Blümlich, and H. W. Spiess, J. Magn. Reson. **79**, 269 (1988).
[32] M. Auger, I. C. P. Smith, and H. C. Jarrell, Biophys. J. **59**, 31 (1991).
[33] B. B. Bonev and M. R. Morrow, Phys. Rev. E **55**, 5825 (1997).