Effects of Hydrostatic Pressure on Bilayer Phase Behavior and Dynamics of Dilauroylphosphatidylcholine

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ABSTRACT Deuterium nuclear magnetic resonance spectroscopy was used to study the thermotropic phase behavior of dilauroylphosphatidylcholine (DLPC) bilayers at pressures up to 221 MPa. Pressure was found to separate the liquid crystal to gel transition from the gel to ordered crystalline phase transition. The jump in chain order observed on cooling through the transition into the gel phase was found to be small and thus consistent with the trend in longer chain saturated diacyl phosphatidylcholines. On cooling, DLPC was observed to enter an unusual state above the transition into the gel phase. This unusual state displayed fluid-like conformational order but short transverse relaxation times. It was found to be much better pronounced and to span a broader temperature range at elevated pressure than at lower pressures. Transverse relaxation measurements of deuterons on the chain α-carbons revealed a substantial slowing of molecular motions within the temperature range of the unusual fluid phase. The observation of such a phase at high pressure appears to be consistent with recent reports of an unusual fluid phase, L_x, in DLPC at ambient pressure.

INTRODUCTION

Saturated diacylphosphatidylcholines (diacyl-PCs) in excess water form bilayers with a rich thermotropic behavior. The members of this family with 14, 16, and 18 carbon acyl chains have been studied extensively. For these lipids, various bilayer properties, including the transition temperature, the transition enthalpy, and the jump in mean chain order at the chain melting transition, are found to depend systematically on acyl chain length (Morrow et al., 1992). Dilauroylphosphatidylcholine (DLPC) has acyl chains of 12 carbons and is one of the shorter chain diacyl-PCs able to form stable bilayers in excess water. DLPC has attracted significant interest, largely because of the ways in which its properties appear to deviate from trends set by the longer chain homologs (Finegold and Singer, 1986; Morrow and Davis, 1987). For example, extrapolation from observations on longer chain diacyl-PCs (Morrow et al., 1992) would suggest that the jump in mean chain order at the gel to liquid crystal transition of DLPC should be relatively small or nonexistent. DLPC is actually observed to undergo a strong phase change, near 0°C, which differs significantly from the normal "main" transition seen with the longer chain homologs. There is strong evidence that the ordered phase involved in this transition is a crystalline (L_C) phase rather than a gel phase (Finegold and Singer, 1986; Morrow and Davis, 1987). Thermal analysis shows the transition to be relatively broad and, under some circumstances, resolvable into two excess heat capacity maxima (Mabrey and Sturtevant, 1976; Finegold and Singer, 1986; Morrow and Davis, 1987; Finegold et al., 1990). When studied by deuterium nuclear magnetic resonance (^{2}H NMR), first spectral moments (M_1) display a large discontinuity near 0°C. Just above this transition, the behavior of M_1 departs from the temperature dependence normally associated with the liquid crystalline phase just above the main transition of longer saturated diacyl-PCs. One possible explanation of the observed behavior around the transition is that as DLPC is cooled from the liquid crystalline phase, its approach to the gel phase is interrupted by a nearly coincident transition to the L_C or subgel phase.

It has recently been suggested that the transition from the ordered phase to the liquid crystalline phase proceeds via a partially disordered intermediate phase, which has been labeled L_x (Finegold et al., 1990). The existence of an intermediate phase has received support from x-ray diffraction results (Hatta et al., 1994), which show a faster increase in bilayer thickness with cooling, just above the main transition, than is seen in the ordinary liquid crystalline phase of longer chain diacyl-PCs. The same study reports no difference in the local symmetry between this intermediate phase and the regular liquid crystal. An important issue with some bearing on the interpretation of DLPC phase behavior is the stability or metastability of ordered DLPC bilayer phases (Finegold and Singer, 1986; Lewis and McElhaney, 1990).

The application of hydrostatic pressure to a highly anisotropic system, such as a lipid bilayer, provides a useful way to gain further insights into the interactions that underly observed bilayer behavior. NMR studies at high pressure have been used to study bilayer phase behavior (Jonas et al., 1988; Driscoll et al., 1991a,b) and headgroup properties (Peng and Jonas, 1992; Bonev and Morrow, 1995). The Clausius-Clapeyron equation relates the pressure dependence of a transition temperature to the volume change and latent heat associated with that transition. An interesting consequence of the Clausius-Clapeyron equation is that transition temperatures for various bilayer phase transitions display different rates of change with applied hydrostatic

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pressure. In particular, transitions that are unobservable at ambient pressure can become observable at elevated pressure. Similarly, transitions that are effectively coincident at ambient pressure may become separate at higher pressure.

Pressure can also influence the magnitude of the change in bilayer properties at a transition. Application of hydrostatic pressure is found to increase phospholipid bilayer thickness and reduce the area per molecule (Braganza and Worcester, 1986; Winter and Pilgrim, 1989; Bonev and Morrow, 1995). The temperature of the main bilayer transition increases with applied pressure. There is some evidence from ^{2}H NMR measurements in this laboratory that the magnitude of the jump in chain order at the transition also increases with applied hydrostatic pressure (B. Bonev and M. R. Morrow, unpublished observations). In a limited sense, then, application of hydrostatic pressure mimics some of the effect of increased acyl chain length on transition temperature and the jump in chain order at the transition (Morrow et al., 1992) in a given family of diacyl-PCs.

In this work we have used ^{2}H NMR to study the phase behavior and dynamics of chain perdeuterated and selectively deuterated DLPC in bilayers at elevated pressure. Of particular interest is the question of whether it is possible to use applied pressure to separate the liquid crystal to gel transition from the gel to crystal (L_C) transition in DLPC. Through quadrupole splittings, ^{2}H NMR provides information on the orientational order of deuterated molecular segments averaged over the time scale of the solid-state NMR experiment ($\sim 10^{-5}$ s). The average orientational order of chain perdeuterated lipids can be obtained from the first spectral moment. The transverse relaxation of a deuteron on a lipid molecule is sensitive to slow molecular motions that modulate the quadrupole interaction. All of these measurements can be used to characterize the phase behavior of bilayers. By observing phases that become accessible only under high pressure and by examining the extent to which the observed behavior is sensitive to sample thermal history, it is possible to obtain some insight into the observed phase behavior and phase metastability at ambient pressure.

MATERIALS AND METHODS

DLPC was synthesized by acylation of glycerophosphocholine with deuterated lauric anhydride, using the method of Gupta et al. (1977). Perdeuterated lauric acid was prepared by the method of Hsiao et al. (Hsiao, 1974), and specific α-labeling was obtained by exchange with KOD at 200°C in excess D_2O. The final product was purified on a 1.5-m Sephadex LH-20 (Pharmacia Biotech, Baie d'Urfé, P.Q.) liquid chromatography column and eluted with 100% ethanol redistilled before use. The purity of the lipids was checked by thin-layer chromatography. All were found to migrate as single spots. Before hydration, the lipids were vacuum-dried for 5 to 8 h. Lipid samples were hydrated in 100 mM phosphate buffer (pH 7.2) to approximately 110 water molecules per lipid. To obtain multilamellar vesicles (MLVs) the samples were stirred thoroughly with a fine glass rod above the main transition. After preparation the MLV suspensions in excess water were transferred into flexible polyethylene tubes and heat-sealed.

Deuterium NMR was performed in a 3.5-T superconducting magnet (Nalorac Cryogenics, Martinez, CA), using a locally constructed spectrometer (Morrow, 1990). Experiments were carried out using a ^{2}H NMR probe designed and built to operate at applied hydrostatic pressures up to 270 MPa with temperature regulation between −20°C and 80°C. A manuscript describing this probe is in preparation. The coil and the sealed flexible tube, containing the MLV suspension, were placed in a beryllium-copper cell, which was pressurized with oil (AW ISO grade 32). Pressure within the cell was measured with a resolution of ±1.4 MPa using a Bourdon tube gauge (model 47-18340; American Instrument Co., Silver Spring, MD) calibrated against a dead-weight gauge to an accuracy of ±1%. Pressures quoted are gauge pressures and were thus measured relative to ambient pressure.

A quadrupole echo sequence ($90°-\tau-90°-\tau-$echo) was used for acquisition of spectra (Davis et al., 1976). The duration of the $\pi/2$ pulse was between 2.5 and 3.1 μs. Except where otherwise noted, the pulse separation was 35 μs. For transverse relaxation measurements, pulse separation was varied between 20 μs and 600 μs in the fluid phase and between 20 μs and 160 μs for phases with higher relaxation rates. Oversampling (Prosser et al., 1991) was used to obtain an effective data acquisition dwell time of 4 μs in the liquid crystalline phase and 2 μs in the lower temperature gel and crystalline phases. For the perdeuterated samples 2000 transients were averaged in the liquid crystalline phase, and up to 6000 were averaged in the crystalline phase. With the specifically labeled lipid, between 16,000 and 32,000 transients were averaged for each spectrum.

Mean transverse relaxation rates $\langle 1/T_{2e}\rangle$ of chain deuterons were obtained from the initial decay of the quadrupole echo amplitudes using

$$A_0(2\tau) = A_0(0)e^{-2\tau\langle 1/T_{2e}\rangle}, \qquad (1)$$

where A_0 is the amplitude of the echo. The decrease in spectral area with increasing pulse separation gave similar results. For convenience, the inverse of the mean transverse relaxation rate, $\langle 1/T_{2e}\rangle^{-1}$, will be referred to as the effective transverse relaxation time $\overline{T}_{2e}$.

RESULTS

DLPC-d_{46} chain order

Spectra of perdeuterated DLPC (1,2-bis(perdeuteriolauroyl)-*sn*-glycero-3-phosphocholine; DLPC-d_{46}) were obtained while cooling from 30°C to −13°C at ambient pressure and from 50°C to −20°C at 150 MPa (1.5 kbar). Representative spectra for DLPC-d_{46} are shown in Fig. 1. The high temperature spectra consist of superpositions of powder patterns (Pake doublets) that are typical of axially symmetric reorientation, with a correlation time that is short compared to the characteristic ^{2}H NMR time scale ($\tau_c \ll 10^{-5}$ s). They indicate a liquid crystalline or fluid bilayer phase.

The ambient pressure spectra at 0°C, −1°C, and −2°C show a relative reduction in the intensity of those doublets with larger splittings due, presumably, to a faster echo decay for deuterons on more motionally restricted methylene groups close to the glycerol backbone. For these deuterons, the correlation times (τ_c) for motions contributing to transverse relaxation are presumably approaching $(\Delta M_2)^{-1/2}$, where ΔM_2 is that part of the second moment modulated by the motions concerned. Between −2°C and −3°C, the sample undergoes a sharp transition to a highly ordered phase. The presence of intensity at ±63 kHz indicates chains that are nearly immobilized on the NMR time scale and suggests a crystalline or L_C phase. The spectral feature with shoulders at about a third of the maximum

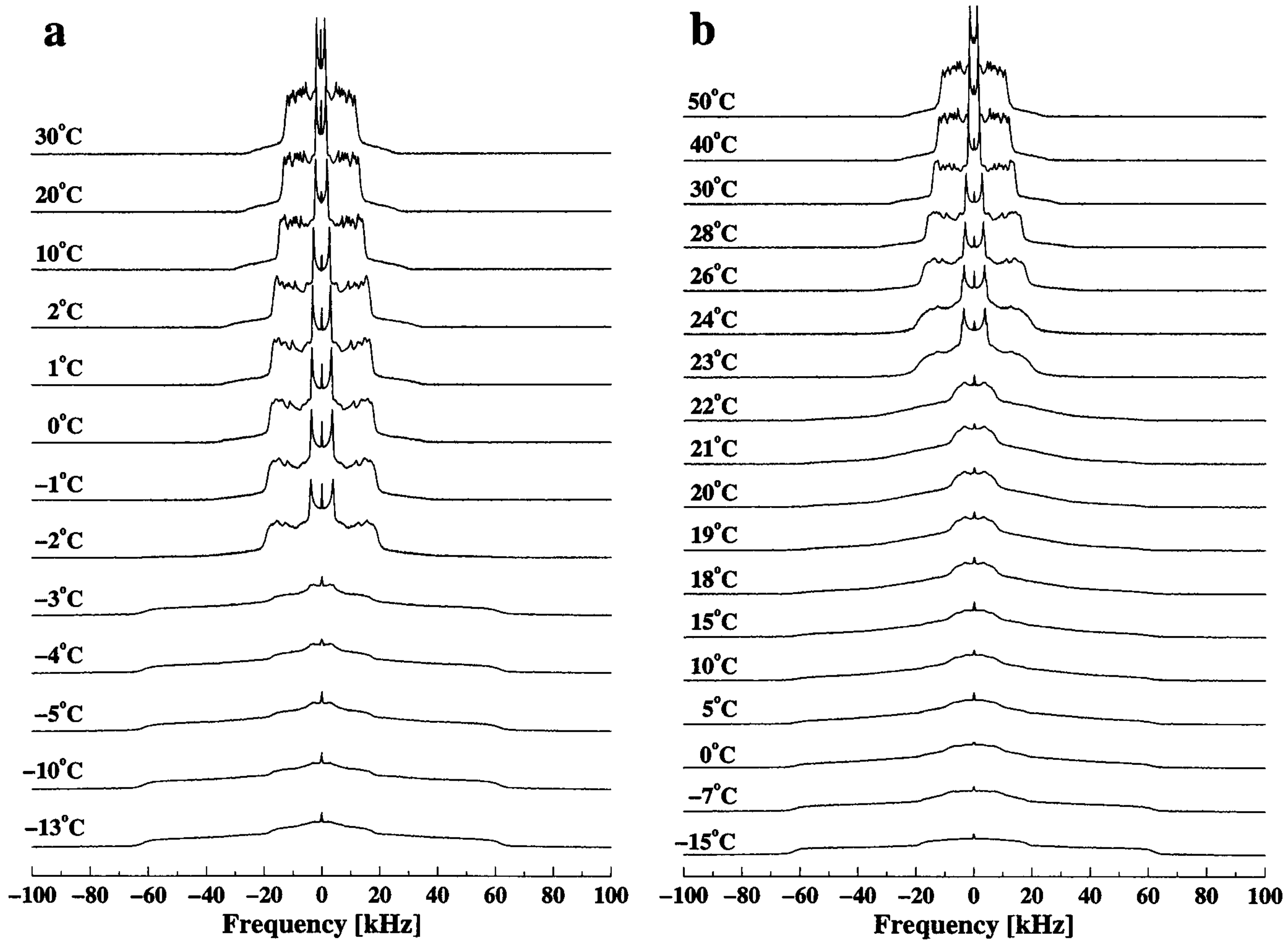

FIGURE 1 (*a*) ^{2}H NMR spectra of DLPC-d_{46} at ambient pressure and selected temperatures. (*b*) ^{2}H NMR of DLPC-d_{46} at 150 MPa and selected temperatures.

splitting corresponds to one of the methyl groups undergoing fast reorientation about an effectively immobilized threefold rotation axis. The spectral feature in the ordered phase spectra with the smallest splitting may indicate that the threefold axes of some chain methyls continue to undergo some reorientation.

At 150 MPa, the spectra between 28°C and 23°C are typical of axially symmetric motion, but the increase in quadrupole splitting with decreasing temperature is accompanied by a strong reduction in the intensity of those doublets with the largest splittings. At 22°C, DLPC-d$_{46}$ undergoes a transition to a more ordered phase characterized by slower reorientations that lack axial symmetry. The spectrum in this phase is closer to the gel phase spectrum of longer chain diacyl-PCs than to the L$_C$ phase spectrum. Below 19°C there is a growth of intensity near ±63 kHz, indicating that the sample is undergoing a continuous transformation into a more rigid, presumably L$_C$, phase. The spectral features in the -15°C spectrum at 150 MPa are characteristic of immobilized chains and methyl groups reorienting about an immobilized threefold axis.

With the sample held at 150 MPa, spectra were collected in a contiguous series while cooling from 50°C to -20°C, while warming back to 40°C, and while cooling again to -20°C. Within the liquid crystalline phase of perdeuterated lipid samples, the first spectral moment (M_1) is proportional to the mean orientational order parameter of the carbon-deuterium bonds (Davis, 1983). In the more ordered lower temperature phases, the first spectral moment remains a useful indicator of orientational order. Fig. 2 *a* shows M_1 as a function of temperature at ambient pressure and 150 MPa for DLPC-d_{46} spectra collected during the first cooling series. At ambient pressure, the gradual increase of M_1 with decreasing temperature is interrupted by a sharp jump from 63 kHz to over 150 kHz. Gel phase values of M_1 in 1,2-bis(perdeuteriopalmitoyl)-*sn*-glycero-3-phosphocholine (DPPC-d_{62}) typically range from about 100 kHz to 130 kHz (Morrow et al., 1992). The large M_1 observed below the ambient pressure transition temperature in DLPC-d_{46} is comparable to M_1 values for nearly rigid lattice spectra observed in the crystalline L$_C$ phase of other symmetric saturated diacyl-PCs.

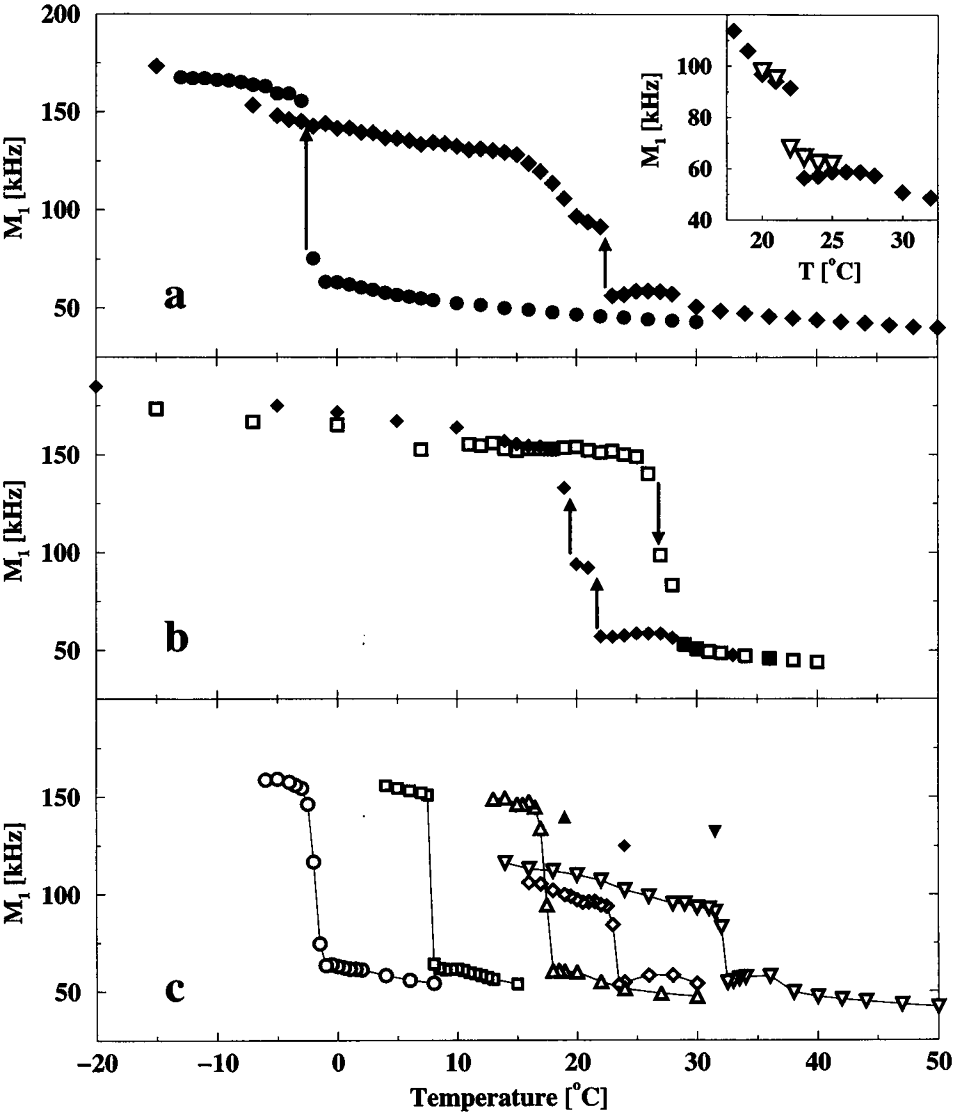

FIGURE 2 Temperature dependence of first spectral moments (M_1) for DLPC-d_{46} (*a*) obtained with quadrupole echo pulse separation of 35 μs while cooling (●) at ambient pressure and (◆) at 150 MPa. The arrows indicate the time ordering of data collection for each series. The inset shows M_1 values for spectra collected with a quadrupole echo pulse separation of (▽) 20 μs while cooling at 150 MPa from 25°C to 20°C. M_1 values for spectra collected with a quadrupole echo pulse separation of (◆) 35 μs are shown for comparison. (*b*) First spectral moments for DLPC-d_{46} at 150 MPa obtained (□) while heating immediately after the 150 MPa cooling series shown in *a* and (◆) while re-cooling immediately after the preceding heating series. The arrows indicate the time ordering of data collection for each series. (*c*) Temperature dependence of first spectral moments (M_1) for a second sample of DLPC-d_{46} obtained while cooling at (○) ambient pressure, (□) 55 MPa, (△) 110 MPa, (◇) 165 MPa, and (▽) 221 MPa. The effective cooling rate close to the transition at each pressure was approximately 0.6°/h. After the cooling series at 110 MPa, 165 MPa, and 221 MPa, the sample was warmed and held at a temperature close to the transition. M_1 values for the resulting L$_C$ phase spectra are shown as corresponding filled symbols for (▲) 110 MPa, (◆) 165 MPa, and (▼) 221 MPa.

Just above the ambient pressure transition shown in Fig. 2 *a*, there is a flattening of M_1 versus temperature that is not seen above the transition for longer chain saturated diacyl-PCs (Morrow et al., 1992). This reflects the enhanced echo decay rate for deuterons with larger splittings seen at 0°C, −1°C, and −2°C in the ambient pressure spectra of Fig. 1. This behavior is in general agreement with previous ^{2}H NMR observations of DLPC-d_{46} at ambient pressure (Morrow and Davis, 1987), where M_1 was recorded at smaller temperature intervals near the transition. At ambient pressure, this relative reduction in the intensity of the most highly split doublets is not apparent above 1°C, and it is difficult to assign an upper temperature boundary to the unusual behavior. At 150 MPa, the relative reduction in the intensity from the most ordered deuterons is more apparent and covers a range of at least 6°. It would thus appear that at 150 MPa, the anomalous behavior of M_1 above the transition covers a broader temperature range than at ambient pressure. At 150 MPa, M_1 actually decreases with decreasing temperature below 26°C.

In quadrupole echo experiments, a significant dependence of transverse relaxation rate on the quadrupole splitting of deuterons along a perdeuterated chain gives rise to a dependence of M_1 on quadrupole echo pulse separation (Morrow, 1990). The inset in Fig. 2 *a* shows that, from 25°C to 23°C, reducing the pulse separation from 35 μs to 20 μs raises the observed M_1. This indicates that differential transverse relaxation along the chain is largely responsible for the apparent decrease in M_1 with decreasing temperature below 26°C. The spectra above 30°C do not appear to be distorted by the effects of differential transverse relaxation along the chain. Because such effects are only expected to reduce the observed M_1 from its intrinsic value, the change in slope of M_1 versus temperature at 30°C is presumably significant.

Between 23°C and 22°C, M_1 at 150 MPa jumps from 56 to 91 kHz. The DLPC-d_{46} spectra at 22°C, 21°C, and 20°C are observed to be most similar to those seen in the gel phase of other symmetric saturated diacyl-PCs (Morrow et al., 1992). The effective cooling rate through this range was about 0.4°/h. Between 20°C and 15°C, M_1 rises as the spectra become increasingly characteristic of a highly ordered, nearly rigid lattice phase. As *T* is reduced further, M_1 continues to increase, eventually approaching the values observed at ambient pressure in the highly ordered L_C phase.

At −15°C, the cooling series described above was stopped and a second 150 MPa series of spectra, collected as the sample was warmed back through the transition, started. M_1 values for this warming series are shown as open squares in Fig. 2 *b*. A comparison of this heating series with the initial cooling series (filled diamonds in Fig. 2 *a*) indicates pronounced hysteresis in the 150 MPa phase behavior. There is little reduction in M_1 as the sample is warmed from −13°C to 25°C. Between 26°C and 29°C, M_1 drops to 52 kHz and merges with the M_1 values for the liquid crystalline region of the initial cooling series at 150 MPa. In light of recent reports of an unusual fluid phase in DLPC at ambient pressure (Finegold et al., 1990; Hatta et al., 1994), it is interesting that the transition on heating occurs above the range from 22°C to 25°C, in which the fluid phase spectra show evidence of a strong dependence of transverse relaxation on position along the chain.

At 40°C, the warming series was stopped and a second cooling series started. M_1 values collected during this cooling series are shown as solid diamonds in Fig. 2 *b*. These closely reproduce the first 150 MPa cooling series down to 19°C. Again, spectra and M_1 values characteristic of a gel phase are observed in a small temperature range close to 20°C. At 18°C, M_1 jumps to 152 kHz and increases slowly to 185 kHz as the temperature is lowered to −20°C. The spectral features in the region below 18°C are characteristic of the L_C phase. The primary difference between the first cooling series and second cooling series is the more abrupt transition into the L_C phase during the second cooling experiment.

The behavior of DLPC-d_{46} as it is cooled from 29°C to 23°C at 150 MPa is clearly unusual. The spectra (Fig. 1) display splittings characteristic of liquid crystalline chain disorder but are distorted by differential transverse relaxation along the chain. The resulting M_1 values (Fig. 2, *a* and *b*) actually decrease with decreasing temperature below 26°C. The leveling of M_1 just above the transition at ambient pressure, although less pronounced, is also unusual for a saturated diacyl-PC system. These unusual behaviors at ambient and elevated pressure are presumably related. To obtain additional information about the pressure dependence of the phase behavior, spectra were collected near the transition for a series of pressures using a second DLPC-d_{46} sample. Fig. 2 *c* shows M_1 values at ambient pressure, 55 MPa, 110 MPa, 165 MPa, and 221 MPa for this sample. For each pressure, spectra were collected while cooling at either 1° intervals (2° intervals for 221 MPa) or, closer to the transition, at 0.5° intervals. The effective cooling rate, for the latter situation, was about 0.6°/h.

The results displayed in Fig. 2 *c* show that, with increasing pressure, there is a slight increase in the temperature range over which the unusual fluid phase occurs. This pressure series provides some confidence that the pronounced region of unusual fluid phase observed at high pressure can be identified with the leveling of M_1 at ambient pressure and thus with the L_x phase reported by Finegold et al. (1990) and Hatta et al. (1994). Inspection of Fig. 2 *c* indicates that the distinction between this phase and the more normal liquid crystalline phase becomes more pronounced with increasing pressure.

The measurements shown in Fig. 2 *c* were also used to address the issue of metastability near the transition. For pressures of 110 MPa and below, the transition proceeds directly from L_x into the highly ordered crystalline phase. For 165 MPa and 221 MPa, a transition from the L_x phase to the gel phase is observed. For both 165 MPa and 221 MPa, the gel phase persists to a lower temperature than would be expected from a comparison with Fig. 2 *a*. This suggests that the molecular rearrangements necessary to order the chains into the crystalline L_C phase may be hindered if the temperature is lowered too quickly into a range in which such motions are significantly slowed.

In an attempt to identify stable phases near the transition, limited temperature cycling experiments were carried out in conjunction with the cooling series at 110 MPa, 165 MPa, and 221 MPa. In each case it was possible to obtain the L_C phase from either the L_x or gel phase by cooling the sample well below the L_x to ordered phase transition temperature and then warming back to within a few degrees of this temperature.

At the end of the 110 MPa cooling series, the sample was warmed back to 19°C, a temperature at which the L_x phase was observed during cooling. The resulting crystalline L_C phase spectrum, represented by a filled triangle in Fig. 2 *c*, did not change over a period of 3 h, suggesting that the crystalline phase is stable at this pressure and temperature.

At the end of the 165 MPa cooling series, the sample was warmed from 15°C to 24°C, a temperature at which the L_x phase was previously observed on cooling. The sample was held at this temperature for 3 h. Over this period, the spectrum went from being characteristic of a gel phase to being characteristic of a crystalline L_C phase (filled diamond in Fig. 2 *c*), suggesting that it is the crystalline phase that is stable at this pressure and temperature.

The conditions under which the L_C phase could be obtained appear to be more restricted at higher pressures. When the cooling series at 221 MPa reached 24°C, the sample was warmed back to 30°C, a temperature just below the observed L_x to gel transition at this pressure. The M_1 value obtained was close to its previous value at 30°C, suggesting that, at this pressure, cooling in the gel phase is reversible down to 24°C. Cooling was then continued to 14°C. After this, the sample was warmed back to 35°C, a temperature at which the L_x phase was observed on cooling, and then cooled to back to 30°C, where the sample was held for about 10 h. During this period the spectrum went from being characteristic of the gel phase to being characteristic of the L_C phase (filled inverted triangle in Fig. 2 *c*). Upon further warming at 221 MPa, a transition from L_C to liquid crystal was observed between 33°C and 38°C.

It would thus appear that the L_C phase is stable over at least part of the temperature range in which the L_x and gel phases are observed on initial cooling. However, it also appears that increasing pressure increasingly hinders nucleation of the L_C phase and that once nucleation occurs, growth of the L_C phase is slow if the temperature is more than a few degrees below the range in which the L_x phase is observed during the initial cooling.

Specific label observations

To gain a better understanding of the bilayer state near the transition, spectra and mean transverse relaxation rates were obtained at ambient pressure and at 150 MPa using DLPC selectively deuterated on the α-carbons of both chains (1,2-di[2,2-$^2H_2$]lauroyl-*sn*-glycero-3-phosphocholine; DLPC-α-d_4). Methyl deuteron splittings at 150 MPa were also extracted from the fluid phase spectra of DLPC-d_{46}. Fig. 3 shows spectra of DLPC-α-d_4 obtained at selected temperatures while cooling the sample at ambient pressure and at 150 MPa. The spectra at ambient pressure show the sharp transition from a liquid crystalline phase to the highly ordered L_C phase characterized by a spectrum with intensity out to ±63 kHz. The slight difference between the DLPC-d_{46} and DLPC-α-d_4 transition temperatures is a known isotope effect (Morrow and Davis, 1987).

At 150 MPa, the width of the DLPC-α-d_4 spectrum at 25°C is similar to that of the liquid crystalline phase spectrum at 30°C. The shape of the spectrum, though, is distorted by the variation in transverse relaxation rate among the α-deuterons. The spectra at 23°C and 20°C have some of the character of the gel phase. The 17°C spectrum is beginning to display intensity at ±63 kHz, indicating the onset of the highly ordered phase.

Fig. 4 shows the temperature dependence of the effective transverse relaxation time for DLPC-α-d_4 at ambient pressure and at 150 MPa. For comparison, the temperature dependence of M_1 for DLPC-d_{46} at these pressures is reproduced from Fig. 2 *a*. At ambient pressure, $\overline{T_{2e}}$ undergoes a rapid decrease from approximately 590 μs at 8°C to about 190 μs at 0°C. Below 0°C the relaxation times increase slightly to 260 μs as the temperature is lowered to −10°C. At 150 MPa, the transverse relaxation rates are higher in the liquid crystalline phase and correspond to relaxation times

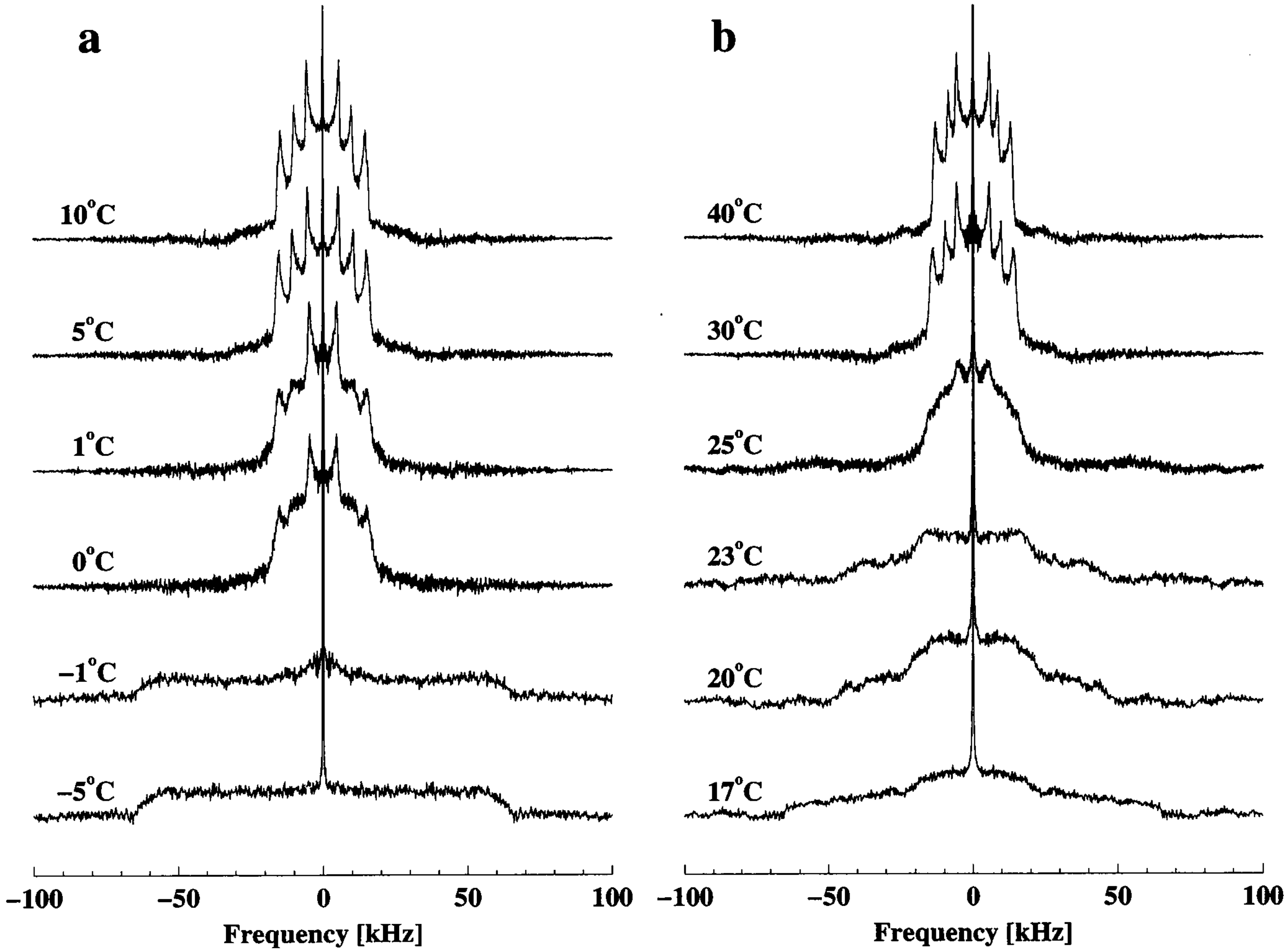

FIGURE 3 (*a*) ^2H NMR spectra of DLPC-α-d_4 at ambient pressure and selected temperatures. (*b*) ^2H NMR spectra of DLPC-α-d_4 at 150 MPa and selected temperatures.

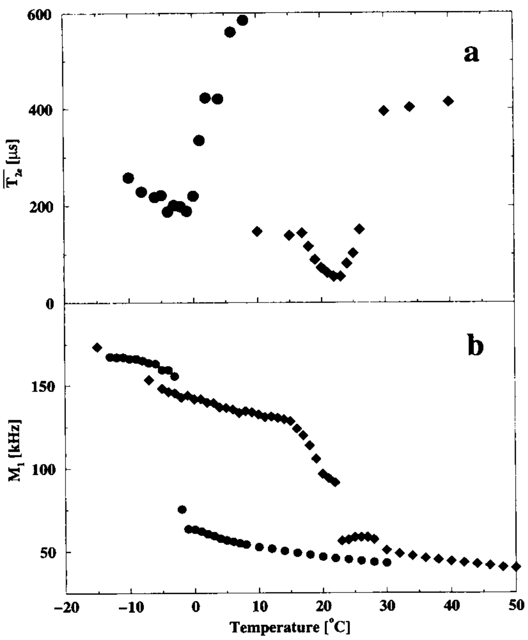

FIGURE 4 (*a*) Effective transverse relaxation times of DLPC-α-d_4 obtained while cooling at (●) ambient pressure and at (◆) 150 MPa. For comparison, *b* shows M_1 values for DLPC-d_{46} reproduced from Fig. 2 *a* for cooling series at (●) ambient pressure and (◆) 150 MPa.

on the order of 400 μs between 40°C and 30°C. Application of pressure thus appears to increase the correlation times of those motions responsible for transverse relaxation in the liquid crystalline phase. Between 30°C and 24°C, the temperature range over which the L_x phase is observed, T_{2e} decreases from 400 μs to 54 μs. The minimum in T_{2e} occurs at the L_x to gel transition temperature. From 24°C to 17°C, T_{2e} gradually increases to about 140 μs as the temperature is lowered. At 150 MPa, no significant increase in T_{2e} is observed below 17°C, the temperature at which the gel to L_C conversion appears to be complete.

The methyl deuteron quadrupole splittings of DLPC-d_{46} can also be used to examine chain orientational order in a way that is insensitive to the dependence of transverse relaxation on position along the chain. Fig. 5 shows methyl deuteron quadrupole splittings obtained as DLPC-d_{46} at 150 MPa was cooled through the liquid crystal and L_x temperature ranges. These splittings are extracted from the spectra used for the 150 MPa results in Fig. 2 *a*. The L_x phase is present below 30°C. The discontinuity in the methyl deuteron splittings at this point is small relative to the total change in methyl splitting over the temperature range observed. There is, however, a striking change in the slope at this point. The phenomenon occurring at this point is clearly not typical of bilayer phase transitions normally observed by ^{2}H NMR.

DISCUSSION

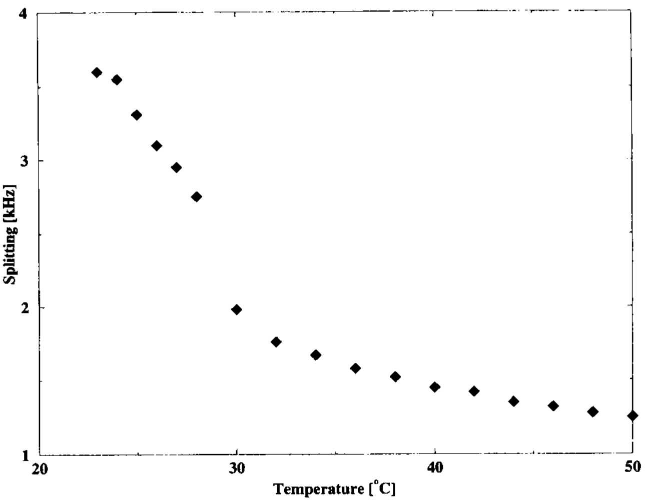

FIGURE 5 DLPC-d_{46} chain methyl deuteron quadrupole splittings obtained while cooling the sample at 150 MPa.

The unusual properties of DLPC (Mabrey and Sturtevant, 1976; Finegold and Singer, 1986; Morrow and Davis, 1987; Finegold et al., 1990; Hatta et al., 1994) may reflect a different balance between interactions in the headgroup and hydrophobic regions of the bilayer than is seen in longer chain homologs. The high-pressure observations presented here provide some useful insights into the unusual physical properties of DLPC bilayers at ambient pressure and thus into the question of how bilayer properties are determined by chain length.

The unusual fluid phase region

The transition from liquid crystal to ordered phase in DLPC proceeds in two steps (Mabrey and Sturtevant, 1976; Finegold and Singer, 1986; Morrow and Davis, 1987; Finegold et al., 1990). The shape of the excess heat capacity function through this transition has been observed to be sensitive to sample hydration (Morrow and Davis, 1987) and thermal history (Finegold and Singer, 1986). Within the narrow temperature range bracketed by the two calorimetrically observed features, DLPC exists in an unusual fluid phase, which has been labeled L_x (Finegold et al., 1990; Hatta et al., 1994). The present work reports a region of unusual fluid phase behavior that grows more pronounced with increasing applied pressure. Spectra in this region are characterized by fluid-like quadrupole splittings but short transverse relaxation times that vary significantly with position along the chain. The pressure series depicted in Fig. 2 *c* provides strong evidence for identification of the high-pressure anomalous fluid region with the L_x phase reported by Finegold and Singer (Finegold et al., 1990) and by Hatta et al. (1994) at ambient pressure.

As can be seen from Fig. 2, there is no evidence of a significant discontinuity in M_1 between the L_x phase and the liquid crystalline phase. This implies that the amplitudes of reorientation experienced by the DLPC acyl chains are continuous across the boundary between the two phases.

This is consistent with the report by Hatta et al. (1994) that the properties of the L_x phase merge gradually with those of the liquid crystalline phase. As a result, the liquid crystal to L_x transition temperature can only be approximated from M_1 data. There is, however, a relatively sharp change in transverse relaxation rate on passing from liquid crystal to L_x. This suggests that chain motions do slow significantly as the sample passes into this temperature region from the fluid phase.

It is interesting to speculate that two aspects of the normal diacyl-PC main transition may be displayed separately in DLPC at high pressure. The liquid crystal to gel transition involves a discontinuous increase in chain conformational order and an increase in the orientational and positional correlation of neighboring chains. These aspects of the transition are strongly coupled in longer chain systems. Observation of the L_x phase in DLPC suggests that there may be a partial decoupling of interchain and intrachain order for short chain systems. The liquid crystal to L_x transition does not appear to involve a significant increase in chain conformational order. Behavior of the chain dynamics, however, may reflect an increasingly correlated motion of neighboring molecules in the L_x phase. Particularly at high pressure, the temperature at which the DLPC chains are orientationally ordered appears to be suppressed relative to the onset of interchain correlations, which presumably slow the motions responsible for transverse relaxation and lead to shorter transverse relaxation times.

Metastability at elevated pressure

Although more extensive temperature cycling studies would be required to fully characterize the behavior of this system, high-pressure observations presented here suggest metastability of the gel phase, when present, and of the L_x phase, over at least part of the temperature range in which it is observed. It also appears that until the L_C phase begins to nucleate, formation of the L_x and gel phase is largely reversible. High pressure appears to hinder nucleation of L_C, allowing the gel phase to be observed over a temperature range that depends on the cooling rate. During the initial cooling series for each pressure, samples spent several hours in the L_x phase with no evidence of L_C nucleation. Once the L_C phase begins to nucleate at lower temperature, however, the chain ordering appears to be slow unless the temperature is close to the lower boundary of the L_x temperature range.

Given the metastability reported here, some caution should be exercised when comparing observations of DLPC phase behavior. From results presented by Hatta et al. (1994), it can be inferred that the L_x phase, at ambient pressure, exists over a temperature range of at least 7°. In the present work, boundaries of the L_x phase are well defined at high pressure. At lower pressures, however, the width of the temperature range in which unusual spectral behavior is evident is only a few degrees. The difference between this width and the one inferred from the results of Hatta et al. (1994) may, in part, reflect different sensitivities of the two approaches to L_x properties. It should also be noted, though, that the behavior of DLPC near the transition may be very sensitive to sample history and experimental protocol. The L_x phase of DLPC is reported to exist in a narrow temperature range between two calorimetrically observed transitions (Finegold et al., 1990; Hatta et al., 1994). The higher transition in this pair, which can presumably be identified as the L_x to liquid crystal transition, is sensitive to sample hydration (Morrow and Davis, 1987). Sample hydration might thus affect the extent to which the L_x to liquid crystal boundary can be defined. It should also be noted that Hatta et al. (1994) chose a protocol in which formation of the L_C phase was avoided. In the present work, formation of the L_C phase could be controlled by the cooling rate at high pressure. At lower pressures, however, the cooling rate was such that formation of the L_C phase was always observed. Given the hysteresis displayed by the data in Fig. 2 *b*, it is apparent that details of the DLPC behavior near the transition will be sensitive to experimental conditions and thus difficult to uniquely characterize.

The fluid to gel transition

The weakly first-order nature of the liquid crystal to gel transition in diacyl phosphatidylcholines is thought to reflect the influence of a nearby critical point on the gel/liquid crystal coexistence curve. Earlier studies exploring this issue have been reviewed in conjunction with recent work on the chain-length dependence of pseudocritical behavior near this transition (Ipsen et al., 1990; Morrow et al., 1992; Lemmich et al., 1995). The present study was initially motivated by the question of whether application of pressure would permit observation of the change in mean orientational order parameter at the liquid crystal to gel transition of DLPC. In an earlier study involving 1,2-bis(perdeuteriostearoyl)-*sn*-glycero-3-phosphocholine (DSPC-d_{70}), DPPC-d_{62}, and 1,2-bis(perdeuteriomyristoyl)-*sn*-glycero-3-phosphocholine (DMPC-d_{54}), the chain length dependence of the jump in chain order at the liquid crystal to gel transition was related to a possible decrease, with decreasing chain length, of the separation between the observed transition temperature and a critical temperature on the area-temperature coexistence curve for each lipid (Morrow et al., 1992). Because DLPC-d_{46} at ambient pressure undergoes a transition from the fluid to L_C phase, it was not possible to test the extrapolation of the observed trend to shorter chain length. The present work shows that the gel phase can be obtained for applied hydrostatic pressures above about 150 MPa. Interpretation of the jump in chain order at the fluid to gel transition, however, is now complicated by the observation that it is the L_x phase that undergoes a transition into the gel phase on cooling at these pressures and by the observation that both the L_x and gel phases appear to be metastable near that transition. Nevertheless, as is suggested by the results of Hatta et al. (1994), the L_x phase does share some of the orientational order properties of the normal liquid crystalline phase. Because of the relationship between bilayer thickness and chain orientational order in the fluid phase, the comparison with longer chain diacyl-PCs remains valid to some extent.

When the DLPC-d_{46} values of M_1 are obtained with a short quadrupole echo pulse separation, the jump in M_1 at the transition is observed to be small, indicating that the liquid crystal to gel phase transition is, indeed, weak. The relationship between chain length and the jump in chain order at the gel to liquid crystal transition can, qualitatively, be extended to DLPC. This provides additional support to the suggestion that, for saturated symmetric diacyl-PCs, the separation between the transition temperature and the critical temperature for the liquid crystal to gel phase change decreases with decreasing chain length (Ipsen et al., 1990; Morrow et al., 1992).

CONCLUSIONS

Comparison within the family of diacyl-PCs provides a means of examining the effect of interactions within the hydrophobic region of the bilayer on its properties. The unusual ambient pressure behavior of DLPC, in comparison with that of longer chain diacyl-PCs, is a reflection of the shortness of the DLPC acyl chains. High pressure, by separating some of the phase transitions from each other, provides a clearer view of ambient pressure properties, including the existence of an unusual fluid phase, the proximity of the gel to liquid crystal transition to a critical point, and the intervention of the liquid crystal to crystal transition before the liquid crystal to gel transition occurs. At the same time, however, the response of DLPC to pressure, in itself, provides some interesting insights into the effect of chain length on bilayer properties.

The ambient pressure phase behavior of dilauroylphosphatidylcholine differs in a number of ways from that of longer chain saturated diacylphosphatidylcholines. Because it can alter relationships between various bilayer phase transition temperatures, elevated pressure gives insights into the unusual ambient pressure behavior of dilauroylphosphatidylcholine. At elevated pressure, a region of unusual fluid phase becomes significantly more pronounced than at ambient pressure, and a region of gel phase appears between the fluid and crystalline phases. This can be identified with the L_x phase reported by Finegold et al. (1990) and by Hatta et al. (1994). On warming the sample at elevated pressure, only the crystalline to liquid crystalline transition is observed. The small jump in chain orientational order observed at the fluid to gel transition on cooling is consistent with the chain-length dependence of this transition observed with longer chain diacylphsophatidylcholines at ambient pressure.

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