Effect of pressure on the dimyristoylphosphatidylcholine bilayer main transition

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Deuterium nuclear magnetic resonance was used to study the effect of pressure on the liquid crystal to gel phase transition in multilamellar vesicles of chain perdeuterated dimyristoylphosphatidylcholine (DMPC-d_{54}). The first spectral moment (M_1), which is proportional to the mean orientational order parameter for the DMPC-d_{54} acyl chain, was measured as a function of temperature at ambient pressure and at 160 MPa and as a function of pressure at 25 °C, 35 °C, and 45 °C. Application of hydrostatic pressure was found to increase the magnitude of the jump in M_1 at the transition and to reduce the sensitivity of M_1 to temperature just above the transition. These observations suggest that the separation between the observed first-order transition and the critical point for phospholipid bilayers increases with increasing pressure. [S1063-651X(97)02005-9]

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I. INTRODUCTION

When dispersed in excess water, diacylphosphatidylcholines form bilayers that are related to biological membranes. The high-temperature state of most bilayers is a liquid crystalline phase in which acyl chain segments undergo fast trans-gauche isomerization and the lipid molecules diffuse laterally in the plane of the bilayer. Upon cooling or application of pressure, the bilayer undergoes a transition from the chain-melted liquid crystalline phase into a gel phase in which the chains become more ordered into a nearly all-trans state. This transition, referred to as the main transition, involves changes in both area per lipid and bilayer thickness. The change in mean orientational order of acyl methylene groups at the main transition can be examined by using deuterium nuclear magnetic resonance to observe chain perdeuterated lipid bilayers. Deuterium NMR is less sensitive to changes in gel phase molecular tilt which occur at the pretransition temperature.

The phospholipid bilayer main transition has been studied extensively using a variety of techniques. Observations of a finite latent heat and a discontinuous change in area per lipid at the transition provide strong evidence that this is a first order transition [1–5]. However, the behavior of properties like the excess heat capacity [6] and the ultrasonic velocity [7,8] suggest that phase fluctuations become significant in the neighborhood of the transition. Discussions of the weak first order nature of the main transition have referred to a critical point on the liquid crystal to gel coexistence curve [9–12] or, alternatively, to the pseudocritical (or spinodal) point on the curve determined by the equation of state [13–21]. It has been found that, within a family of saturated diacyl phosphatidylcholines, the discontinuity in the acyl chain mean orientational order at the transition and the separation between the spinodal points and the transition temperature both decrease with decreasing acyl chain length [12]. This is consistent with an earlier suggestion that the separation between the transition temperature and the critical point might increase with increasing chain length [22].

Control of pressure provides a way to separate effects of volume and temperature on bilayer properties. NMR studies of bilayers at high pressure have been used to examine properties including phase behavior [23–25] and headgroup orientation [26,27]. One important effect of applied pressure on bilayers is an increase in transition temperatures [25,28–30]. For example, the main transition in dimyristoylphosphatidylcholine (DMPC) rises by about 20 °C per 100 MPa of applied pressure [27]. Part of the interest in pressure as a variable for bilayer studies arises from the observation that the response of a phospholipid bilayer to the application of hydrostatic pressure is anisotropic. One consequence of this anisotropy is that, in the liquid crystalline phase, the application of pressure increases bilayer thickness and reduces area per lipid [28,31]. This provides a way in which to isothermally vary area per lipid [27]. The response of the bilayer to applied pressure provides an additional rigorous test for statistical mechanical models of bilayer phase behavior. As such, knowledge of this response may also contribute to our understanding of ambient pressure properties.

In the present work, the effect of pressure and temperature on the mean orientational order parameter for chain perdeuterated dimyristoylphosphatidylcholine (DMPC-d_{54}), particularly near the transition, has been examined by deuterium nuclear magnetic resonance. The observations are discussed in terms of the possible effect of pressure on the separation of the observed transition from the critical point for the system. The choice of DMPC-d_{54} for this study allows moderate pressures to be used without having to expose the sample to excessively high temperatures at which sample stability might be a limiting factor.

II. EXPERIMENT

Perdeuterated myristic acid was prepared using the procedure presented by Hsiao, Ottaway, and Wetlaufer [32]. DMPC-d_{54} was then synthesized by acylation of glycerophosphocholine using the method of Gupta, Rudhakrishnian, and Khorana [33]. The final product was purified on a 1.5 m Sephadex LH-20 (Pharmacia Biotech, Baie d'Urfe, P.Q.) liquid chromatography column and eluted with 100% ethanol which was redistilled before use. The lipid was found to migrate as a single spot under thin layer chromatography. Lipids were dried under vaccuum for 5–8 h before being hydrated in a 100 mM phosphate buffer (pH 7.2) to approxi-

mately 100 water molecules per lipid. Multilamellar vesicles (MLV) were prepared by stirring thoroughly with a fine glass rod 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 270 MPa and temperatures ranging from $-20\,^{\circ}\mathrm{C}$ to $80\,^{\circ}\mathrm{C}$. The spectrometer was also constructed locally. 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 guage calibrated against a dead weight guage 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 quadrupole echo sequence [34]. The $\pi/2$ pulse was 2.9 μs long. The separation between $\pi/2$ pulses in the quadrupole echo sequence was typically 40 μs. Oversampling [35] was used to obtain effective dwell times of 4 μs and 2 μs for acquisition of spectra in the liquid crystal and gel phases, respectively. Between 4000 and 6000 transients were normally averaged to obtain the free induction decay.

The orientational order parameter for a given acyl chain deuteron in the liquid crystalline phase is given by

$$S_{CD}=\tfrac{1}{2}\langle 3\cos^2\theta_{CD}-1\rangle, \tag{1}$$

where θ_{CD} is the angle between the carbon-deuterium bond and the rotational axis of the molecule. The average is over the accessible chain conformations. The first spectral moment, calculated over half of the spectrum, is given by

$$M_1=\frac{\int_0^\infty \omega\, f(\omega)\, d\omega}{\int_0^\infty f(\omega)\, d\omega}, \tag{2}$$

where $f(\omega)$ is the spectrum. For a chain-perdeuterated spectrum, M_1 within the liquid crystalline phase is related to the mean orientational order parameter of the chain deuterons, $\overline{S_{CD}}$, by

$$M_1=-\frac{\pi}{\sqrt{3}}\frac{e^2qQ}{h}\overline{S_{CD}}, \tag{3}$$

where $e^2qQ/h=167$ kHz is the quadrupole coupling constant for a deuterium nucleus in a carbon-deuterium bond.

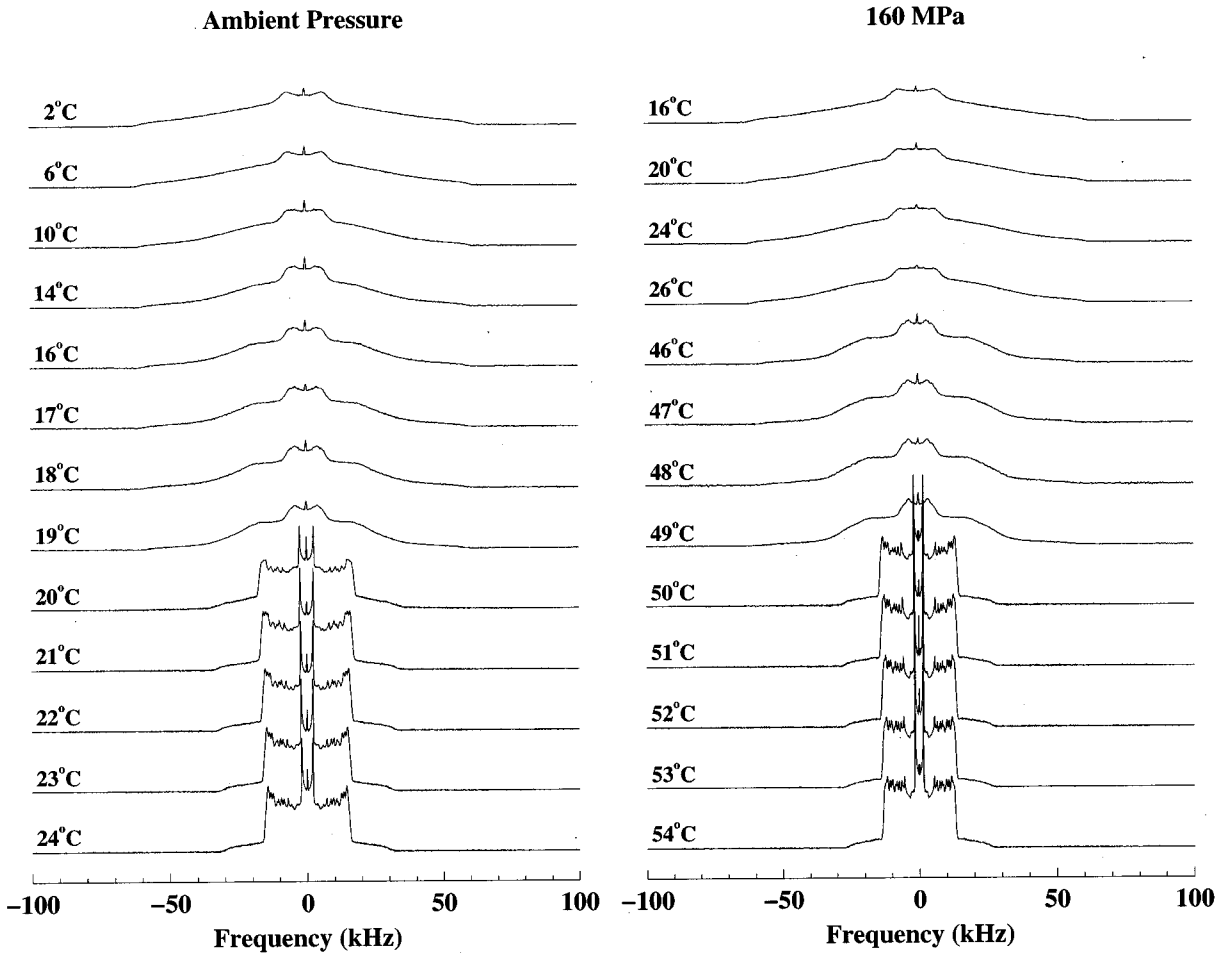

FIG. 1. ^{2}H NMR spectra of DMPC-d_{54} at selected temperatures for ambient pressure and for 160 MPa.

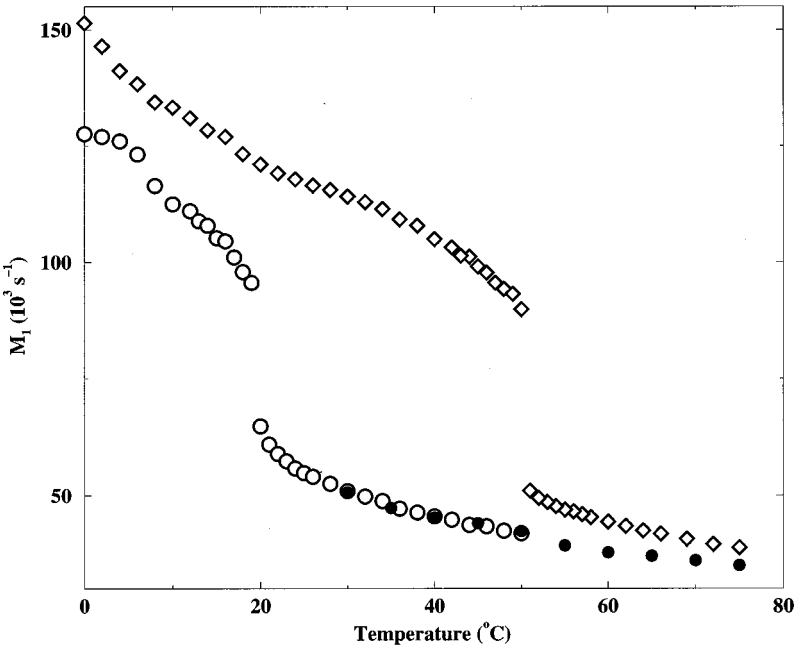

FIG. 2. Temperature dependence of M_1 for DMPC-d_{54} at ambient pressure (circles) and 160 MPa (diamonds). The open and filled circles distinguish two separate sequences of spectra.

III. RESULTS

Figure 1 shows the temperature dependence of ^{2}H NMR spectra for DMPC-d_{54} at ambient pressure and at an applied hydrostatic pressure of 160 MPa. Isobaric conditions during the high pressure temperature scan were maintained by separately controlling the temperature of the connected high-pressure reservoir outside of the magnet. Spectra at both pressures display a sharp transition from the liquid crystalline phase at higher temperature to the gel phase at lower temperature. The liquid crystalline phase spectra are superpositions of Pake doublets characteristic of fast axially symmetric reorientation. The distribution of doublet quadrupole splittings reflects the dependence of the orientational order parameter on the position along the chain in the liquid crystalline phase [1,36–38]. Spectra at temperatures below the transition reflect the slower, axially asymmetric chain motion characteristic of the gel phase. The spectrum of a deuteron in a static carbon-deuterium bond is an axially symmetric Pake doublet with prominent edges at ± 63 kHz which are associated with bonds oriented perpendicular to the applied magnetic field [38]. The appearance of intensity at this splitting in the lowest temperature spectra indicates the onset of the transition into the highly ordered L_C phase. Formation of the L_C phase is slow and the cooling rates used in the present work did not allow complete transformation of the sample into this phase.

Comparison of the ambient pressure and 160 MPa liquid crystal spectra shown in Fig. 1 illustrates two interesting effects of pressure on the behavior of DMPC-d_{54}. At corresponding temperatures above the transition, the ambient pressure spectra display larger quadrupole splittings than the higher-pressure spectra. It can also be seen that, just above the transitions, the splittings for the ambient pressure spectra increase more rapidly with decreasing temperature than those of the corresponding 160 MPa spectra. These observations indicate that increased pressure reduces the degree of acyl chain orientational ordering that can be accommodated by the liquid crystalline phase before the gel phase becomes more stable. They also indicate that, just above the transition, the sensitivity of the acyl chain orientational order to temperature decreases with increasing applied pressure. The similarity of the high-pressure and ambient pressure gel phase spectra, at corresponding temperatures just below the transition, suggests that the gel phase is somewhat less sensitive to pressure.

The first spectral moment (M_1) provides a way in which to compare quadrupole splittings and chain order more quantitatively. Figure 2 shows the temperature dependence of M_1 for DMPC-d_{54} spectra at ambient pressure and 160 MPa. For all temperatures, M_1 is higher at 160 MPa than at ambient pressure. At ambient pressure, the sensitivity of M_1 to temperature increases substantially as the temperature approaches that of the transition. This sensitivity is reduced at the higher pressure. The liquid crystalline phase, just above the respective transitions, is significantly more ordered at ambient pressure than at 160 MPa. While the gel phase order, just below the transition, is also reduced at the higher pressure, the effect is less pronounced. As a result, the magnitude of the jump in M_1 at the transition is greater at the higher pressure.

Spectra were also collected while changing pressure at three fixed temperatures. Figure 3 shows the pressure dependence of spectra collected at 25 °C, 35 °C, and 45 °C. Again, characteristic liquid crystal and gel phase spectra are observed. Figure 4 shows corresponding M_1 values as a function of pressure for the three temperatures. These isothermal experiments show that the effect of pressure on the liquid crystalline phase orientational order decreases with increasing temperature. This indicates that the lateral compressibility of the bilayer decreases with increasing temperature. Figure 5 shows the isobaric and isothermal M_1 results together in order to illustrate the combined effects of temperature and pressure on the DMPC-d_{54} chain order.

The observation that applied pressure reduces the sensi-

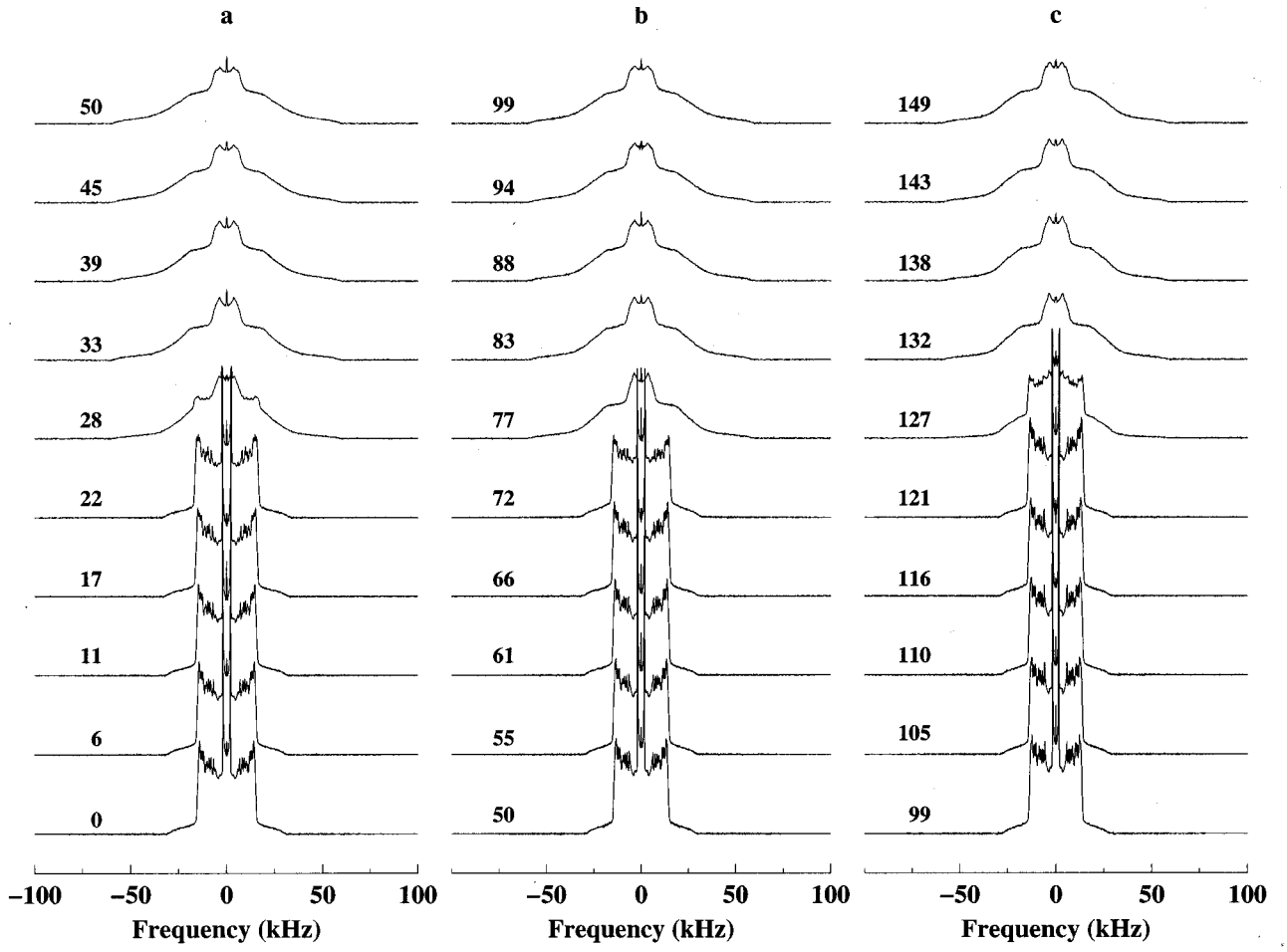

FIG. 3. ^{2}H NMR spectra of DMPC-d_{54} at selected pressures for (a) 25 °C, (b) 35 °C, and (c) 45 °C. Individual spectra are labeled by pressure in MPa.

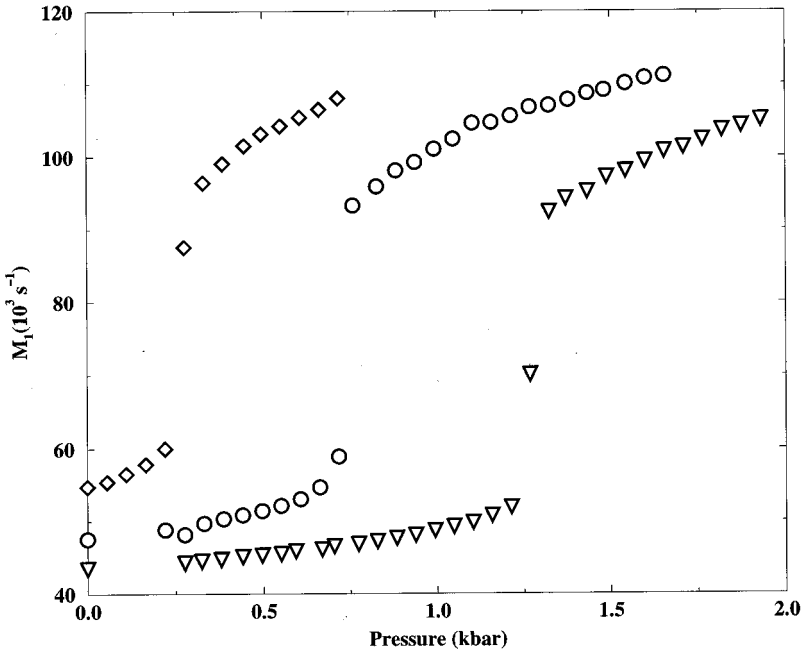

FIG. 4. Pressure dependence of M_1 for DMPC-d_{54} at 25 °C (diamonds), 35 °C (circles), and 45 °C (triangles).

tivity of M_1 to temperature, just above the transition, and increases the jump in M_1 at the transition, is consistent with an increase in the first order character of the transition with increasing applied pressure. One way in which to compare the ambient pressure and 160 MPa observations is to fit each set of observations to the equation of state corresponding to a particular phenomenological model and compare the separations of the transition temperature and spinodal points implied by the two fits.

In an earlier study, the main transitions of DMPC-d_{54}, chain perdeuterated dipalmitoylphosphatidylcholine (DPPC-d_{62}), and chain perdeuterated distearoylphosphatidylcholine (DSPC-d_{70}) were compared in this way by fitting ^{2}H NMR observations to an equation of state obtained by expanding the free energy in terms of an order parameter given by

$$s=\frac{\langle l\rangle^{-1}-\langle l\rangle_c^{-1}}{\langle l\rangle_c^{-1}}, \tag{4}$$

where $\langle l\rangle$ is the mean extension per segment of the acyl chain and $\langle l\rangle_c$ is its value at the critical point [12]. Mean extension per segment was estimated from first moments of the perdeuterated chain ^{2}H NMR spectra as discussed below. The initial motivation for using an order parameter based on an inverse chain extension was the expectation that its temperature dependence should approximate that of area per lipid [12]. For the purpose of characterizing the temperature dependence of M_1 near the transition, this identification is not essential.

The mean chain extension per segment $\langle l\rangle$ was obtained from M_1 by assuming that the chain in the liquid crystalline phase disorders primarily by axially symmetric gauche-trans isomerization. The extension of the acyl chain can then be approximated by a linear function of the orientational order parameter [36,37,39,40]. The simple expression of Schindler and Seelig [39], averaged over the chain deuterons, gives [12]

$$\langle l\rangle=l_0\left[\frac{1}{2}+\sqrt{\frac{3}{\pi}}\frac{hM_1}{e^2qQ}\right], \tag{5}$$

where $l_0=0.125$ nm is the component of a carbon-carbon bond perpendicular to the methylene CD_2 plane and $e^2qQ/h\approx 167$ kHz is the quadrupole coupling constant for an acyl chain CD bond. While calculation of the chain extension in this way does not take account of conformations in which a chain bends back upon itself near the methyl end [41–43], small angle neutron scattering measurements have provided some support for this form of relationship [21].

The calculation of chain extension from M_1 can be formally extended across the transition into the gel phase. The resulting discontinuity in apparent extension is found to be consistent with reported changes in bilayer thickness at the transition [12] despite the fact that the gel phase spectra are characteristic of axially asymmetric motion. This may reflect a relatively weak dependence of M_1 on asymmetry parameter (Kilfoil [53]).

Figure 6 shows the temperature dependence of $\langle l\rangle^{-1}$ from spectra obtained in the liquid crystalline phase and slightly below the transition at ambient pressure and at 160 MPa. To convert $\langle l\rangle^{-1}$ to the thermodynamic order parameter s, a value of $\langle l\rangle_c$ is chosen so that $s=0$ falls close to the center of the jump at the transition.

The temperature dependence of the inverse chain extension is fit using a phenomenological free energy given by [12]

$$G=G_0\left[\frac{s^4}{4}+\alpha(T-T_c)\frac{s^2}{2}+\beta(T_m-T)s\right], \tag{6}$$

where $\alpha>0$, $\beta>0$, T_c is the critical temperature and T_m is the transition temperature. The parameter G_0 is a scaling factor that cannot be determined from these experiments.

The equation of state, found by minimizing G with respect to s is

$$s^3+\alpha(T-T_c)s+\beta(T_m-T)=0. \tag{7}$$

At the spinodal points, $(s_\pm, T_\pm)$, of the model equation of state, the second derivative of G with respect to s is zero. This condition yields

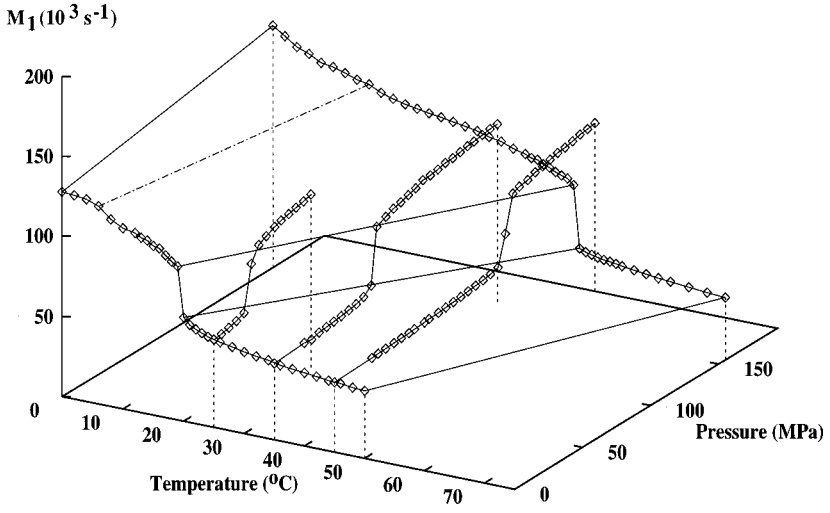

FIG. 5. Pressure and temperature dependence of M_1 for DMPC-d_{54}.

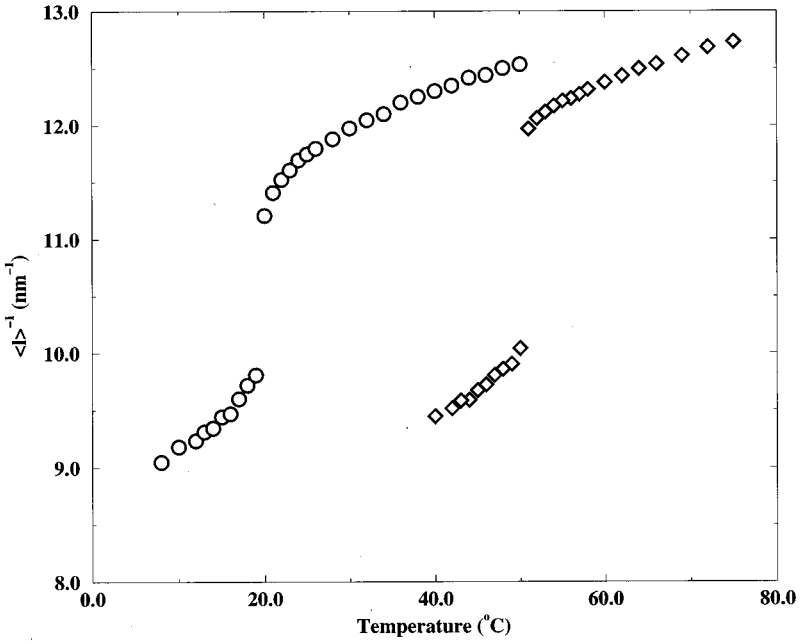

FIG. 6. Temperature dependence of the inverse mean chain extension per segment $\langle l\rangle^{-1}$, obtained from ^{2}H NMR spectra for ambient pressure (circles) and 160 MPa (diamonds).

$$\alpha=\frac{3s_+^2}{T_c-T_+} \tag{8}$$

and

$$\beta=\frac{2s_+^3}{T_m-T_+}, \tag{9}$$

where (s_+,T_+) is the spinodal at the stability limit of the liquid crystalline phase. Corresponding relationships involving (s_-,T_-), the stability limit for the gel phase, can also be derived.

Substituting for α and β in the equation of state and making the assumption that

$$(T_m-T_+)\ll(T_c-T_m)\leqslant(T_c-T_+) \tag{10}$$

yields

$$T=T_+ +\frac{(T_m-T_+)}{2}\left[3\left(\frac{s-s_+}{s_+}\right)^2+\left(\frac{s-s_+}{s_+}\right)^3\right]. \tag{11}$$

Equation (11) provides a way in which to obtain the spinodal points for the model equation of state from the temperature dependence of the order parameter, s. This can be done by plotting T as a function of $3([s-s_+]/s_+)^2+([s-s_+]/s_+)^3$ while varying s_+ until the values of T_+ obtained from the slope and intercept are consistent.

Figure 7 shows T versus $3([s-s_+]/s_+)^2+([s-s_+]/s_+)^3$ for liquid crystal phase data at ambient

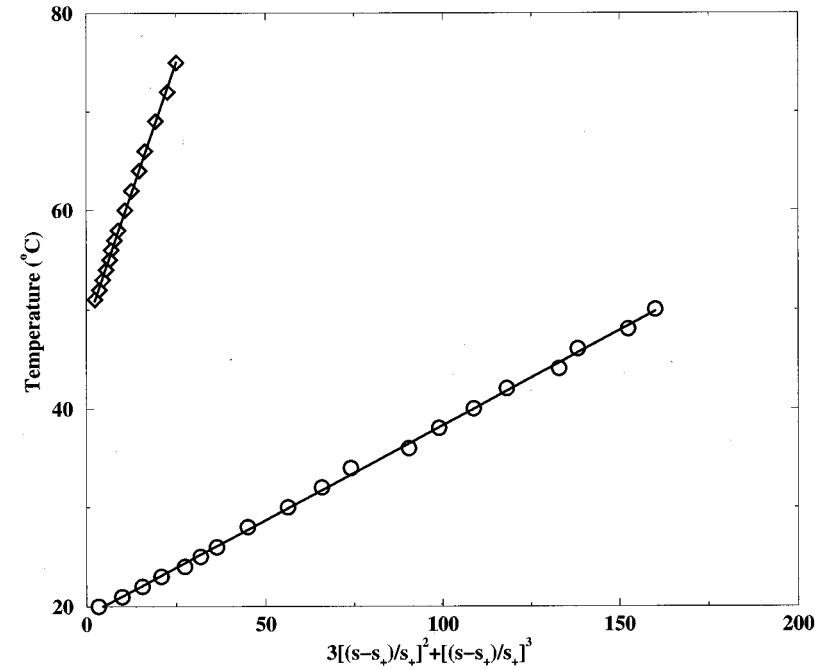

FIG. 7. Temperature vs $\{3([s-s_+]/s_+)^2+([s-s_+]/s_+)\}$ for DMPC-d_{54} at ambient pressure using $s_+=0.0321$ (circles) and at 160 MPa using $s_+=0.0510$ (diamonds). At ambient pressure, the order parameter is calculated from $\langle l\rangle^{-1}$ using $\langle l\rangle_c^{-1}=10.53$ nm^{-1}. At 160 MPa, the order parameter is calculated from $\langle l\rangle^{-1}$ using $\langle l\rangle_c^{-1}=10.98$ nm^{-1}.

TABLE I. Parameters used to simulate temperature dependence of $\langle l \rangle^{-1}$ in Fig. 8.

Parameter	Ambient	160 MPa
T_m (°C)	19.5	50.5
$\langle l \rangle_c^{-1}$ (nm^{-1})	10.53	10.98
$\langle l \rangle_+^{-1}$ (nm^{-1})	10.87	11.54
T_+ (°C)	19.2	48.3
$(T_m - T_+)$ (°C)	0.3	2.2
$10^{-4}\beta$ ($°C^{-1}$)	2.07	1.17

pressure and 160 MPa. For this plot $\langle l \rangle_c^{-1}$ has been fixed and s_+ has been adjusted such that the intercept and slope yield the same value for T_+ as per Eq. (11).

For a fixed value of $\langle l \rangle_c^{-1}$, the requirement that values for T_+ determined from the slope and intercept agree to within about 0.5% fixes s_+ to within about 0.1%. The parameters obtained in this way are summarized in Table I. The ambient pressure values for $\langle l \rangle_c^{-1}$, $\langle l \rangle_+^{-1}$, and $(T_m - T_+)$ are all within 1% of the values reported in Ref. [12]. The solid lines in Fig. 8 show model values of $\langle l \rangle^{-1}$ corresponding to the fits shown in Fig. 7. Comparison of the model equations of state obtained by fitting the ambient pressure and 160 MPa observations to the same phenomenological model suggests that the separation between the transition temperature and the spinodal points, and thus the first order nature of the transition, increases with increasing applied pressure.

IV. DISCUSSION

The effects of applied hydrostatic pressure on the main transition temperatures of DMPC [28,31,44,45] and DPPC [25,28,31,46–48] have been studied extensively. The work reported here primarily concerns the effect of pressure on the temperature dependence of the chain order near the DMPC bilayer main transition. As more detailed theoretical models of bilayer behavior are developed, the response of bilayer properties to applied pressure should be useful for testing the assumptions underlying such models.

Comparison of the temperature dependence of M_1 at ambient pressure and 160 MPa shows that pressure reduces the amount of chain ordering that can be accommodated by the liquid crystalline phase before it becomes unstable with respect to the gel phase. The effect of pressure on the isobaric temperature dependence of M_1, shown in Fig. 2, also implies that the application of pressure increases the abruptness of the transition and the magnitude of the jump in chain order at the transition. The spinodal points, obtained by fitting to the phenomenological equation of state, are separated from the transition temperatures by about 0.3 °C at ambient pressure and by 2.2 °C at 160 MPa. While the critical temperature cannot be extracted from the current results without a more precisely defined onset of the transition [12], these observations suggest that increased pressure increases the separation between the transition temperature and the critical point.

In this work, the phenomenological model has merely provided a means to compare the temperature dependences of mean orientational order for two pressures near the respective transitions. No attempt has been made to relate parameters of the phenomenological model to observable bilayer properties. It is interesting to note, however, that β appears to be sensitive to applied pressure while earlier work indicated that it was not sensitive to the acyl chain length [12]. Because the effects of applied pressure and increased acyl chain length on the transition are otherwise similar, this difference suggests it could be useful to pursue a more direct relationship between β and observable bilayer properties.

In this work and in Ref. [12], inverse chain extension plays the role of an order parameter. Area per lipid, however, would seem to be a more natural variable and some comment on the relationship between these quantities is warranted. It has been pointed out that there can exist bilayer states in which the mean chain extension is not equal to half the hydrophobic thickness of the bilayer and that area per lipid is

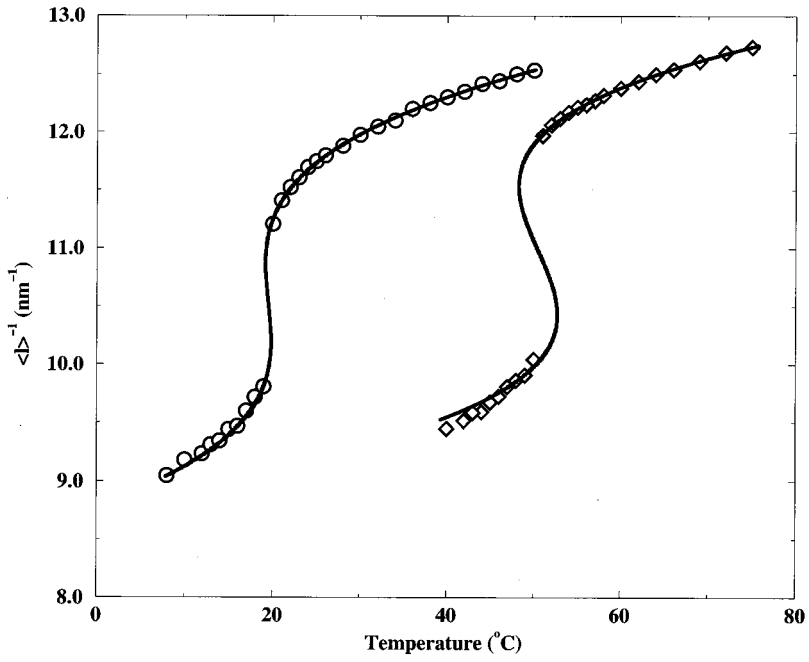

FIG. 8. Temperature dependence of the inverse mean chain extension per segment $\langle l \rangle^{-1}$, obtained from ^{2}H NMR spectra for ambient pressure (circles) and 160 MPa (diamonds). The solid lines show $\langle l \rangle^{-1}$ calculated from the model using the spinodal points corresponding to the lines shown in Fig. 7.

more appropriately associated with the value of S_{CD} in the plateau region of the orientational order parameter profile [41]. There is, however, a close correlation between the shape of the smoothed orientational order parameter profile and the plateau values of the orientational order parameter [49,50] and there may be, in effect, little practical difference between the two ways of characterizing mean chain order in the liquid crystalline phase. Indeed, it was recently shown that chain extension estimates derived from data in Ref. [12] agree reasonably well with small angle neutron scattering measurments in the liquid crystalline phase [21]. The absence of substantial disagreement between direct measurements of bilayer thickness and estimates from ^{2}H NMR may indicate that the counterexample states cited in Ref. [41] do not significantly skew the ensemble averaging, which is inherent in the estimation of bilayer thickness from NMR-derived chain extensions. The fact that the plateau value for the orientational order parameter is not well defined in the gel state provides some motivation to consider whether M_1 can be used to characterize the state of the bilayer across the transition. Some encouragement, in this regard, comes from the observation, as was pointed out in Ref. [12], that the change in the bilayer extension across the transition, estimated using Eq. (5), is consistent with some neutron and x-ray diffraction results.

If the change in volume of the bilayer through the transition is neglected, the variation of $\langle l \rangle^{-1}$ with temperature, might be expected to approximate that of area per lipid, the thermodynamic variable conjugate to lateral pressure. The pressure and temperature dependence of lipid bilayer specific volume has been examined in a number of studies [44,46,51,52]. Recently, Böttner *et al.* [44] reported data showing that the specific volume of DMPC changes by about 2.5% at the main transition and, in total, by about 6% between 10 °C and 50 °C. Under the same conditions, the inverse mean chain extension, shown in Fig. 6, changes by about 15% at the transition and by about 30% between 10 °C and 50 °C. The results of Böttner *et al.* [44] also show that when pressure is varied at 30 °C, the change in specific volume is about 2% at the main transition and, in total, about 8% between ambient pressure and 100 MPa [44]. At 35 °C, the inverse mean chain extension corresponding to the data of Fig. 2 changes by about 15% at the transition, and by about 27% between ambient pressure and 100 MPa. Thus changes in area per lipid account for between 70% and 80% of the observed change in inverse chain extension with temperature and pressure. The proportionality is not precise, though, and inverse mean chain extension can only be taken as a rough indication of how area per lipid varies.

V. CONCLUSIONS

The main transition of DMPC-d_{54} at 160 MPa has been studied by isobaric variation of the temperature. The application of pressure increases the magnitude of the jump in chain order at the transition and appears to increase the first order character of the transition. At ambient pressure, the behavior of the spectral first moment can be fit in the liquid crystal and gel phases using an expansion of free energy in terms of an order parameter based on inverse mean chain extension. The temperature dependence of chain order at 160 MPa can be fit in the same way although the ability of the model to reproduce the gel phase behavior appears to be reduced at high pressure. The spinodal points of the model equations of state are farther from the transition temperature at high pressure than at ambient pressure. This observation is consistent with a pressure-induced enhancement of the first order nature of the transition. Experiments in which pressure is varied isothermally indicate that the first order character of the pressure-induced transition increases with increasing temperature. No evidence of an interdigitated gel phase is observed under the conditions of the experiments reported here.

ACKNOWLEDGMENTS

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