Effect of chain unsaturation on bilayer response to pressure

I. D. Skanes,[1] J. Stewart,[2] K. M. W. Keough,[3] and M. R. Morrow[1]
[1]*Department of Physics and Physical Oceanography, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3X7, Canada*
[2]*Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3X9, Canada*
[3]*Department of Biochemistry and Discipline of Pediatrics, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3X9, Canada*
(Received 21 August 2006; published 20 November 2006)

Using wide-line deuterium NMR, the effects of pressure on saturated-chain orientational order and gel–to–liquid-crystal phase transition temperature were observed in bilayers of 16:0-18:1 PC-d_{31} (POPC-d_{31}) and 16:0-18:2 PC-d_{31} (PLPC-d_{31}). Spectra were recorded for a range of pressures at selected temperatures and for a range of temperatures at selected pressures up to 193 MPa. For 16:0-18:1 PC-d_{31}, the main transition temperature increased by ~0.18 K/MPa, a rate that is similar to what is found for bilayers of disaturated PC's. For 16:0-18:2 PC-d_{31}, the increase in transition temperature with pressure was slightly smaller at ~0.13 K/MPa. To investigate the isothermal response of chain orientational order to pressure, spectra for each lipid were obtained for three pressures (ambient, 55 MPa, and 110 MPa) near room temperature (~25 °C) and for three pressures (ambient, 110 MPa, and 193 MPa) at higher temperature (~40 °C). These temperatures were chosen such that the difference between the higher observation temperature and the main transition of 16:0-18:1 PC-d_{31} would be similar to the difference between the lower observation temperature and the main transition of 16:0-18:2 PC-d_{31}. Application of a given pressure was found to raise the orientational order for all methylene groups on the saturated chain of a particular lipid by roughly similar amounts. For comparable pressure differences, the pressure-induced ordering of the 16:0-18:1 PC-d_{31} saturated chain at ~40 °C was greater than that of the corresponding chain in 16:0-18:2 PC-d_{31} at ~25 °C. These observations suggest that increasing levels of chain unsaturation may reduce the sensitivity of bilayer order to variations in pressure at corresponding temperatures relative to their ambient pressure transitions.

DOI: 10.1103/PhysRevE.74.051913 PACS number(s): 87.16.Dg, 82.56.Pp, 62.50.+p, 61.30.St

I. INTRODUCTION

The most common organizational motif for phospholipids dispersed in water is a bilayer. Such bilayers are the main structural element of cell membranes and also form an interesting class of anisotropic soft materials. The ways in which bilayers containing different components respond to changes in hydrostatic pressure reflect the balance between different interactions within the bilayer and can also provide insight into how membrane composition might enable marine organisms to accommodate changes in pressure.

The anisotropy of bilayer structure is reflected in the response of such materials to pressure. At fixed temperature, in the liquid-crystalline phase, increasing pressure raises average lipid chain orientational order and thus chain extension [1–5]. Raising the hydrostatic pressure applied to a bilayer dispersion generally raises the main liquid crystal to ordered phase transition temperature [3,4,6,7] and can stabilize ordered phases that are not accessible at ambient pressure [4,6–10].

Unsaturation of lipid acyl chains can have profound effects on lipid chain orientational order [11–14] and on bilayer phase behavior [15–18]. Unsaturation of lipid acyl chains also influences the sensitivity of bilayer phase transition temperatures to pressure [19,20].

Because of the effects of unsaturation on bilayer physical properties, the degree of lipid chain unsaturation has been a parameter of particular interest in studies of how membranes of marine organisms adapt to high pressure and/or variations in pressure. For example, the ratio of saturated to unsaturated fatty acids extracted from the liver mitochondrial membranes of fish was found to correlate negatively with the depth at which the fish were captured [21,22]. Bacteria that can adapt to different pressures also demonstrate a negative correlation between the ratio of saturated to unsaturated fatty acids in their membranes and the growth pressure [23,24]. The extent to which adaptation to high pressure involves fatty acids with differing degrees of unsaturation is unclear [22], but there is evidence that for some bacteria, monounsaturated fatty acids are a particular requirement for growth at high pressure [25].

Biological membranes contain lipids with a wide variety of chain lengths and degrees of unsaturation. While the overall ratio of saturated to unsaturated fatty acid generally decreases with growth pressure, adaptive changes in the relative abundance of individual fatty acids do not necessarily reflect a simple dependence on degree of unsaturation [21,24]. Efforts to understand the role of different fatty acids in adaptation to pressure would benefit from additional information regarding how the response of a bilayer to pressure depends on details of lipid structure.

In the work reported here, we have used ^{2}H-NMR to compare the effects of pressure on bilayer dispersions of 1-perdeuteropalmitoyl-2-oleoyl-*sn*-3-glycero-phosphocholine (POPC-d_{31} or 16:0-18:1 PC-d_{31}) and 1-perdeuteropalmitoyl-2-linoleoyl-*sn*-3-glycero-phosphocholine (PLPC-d_{31} or 16:0-18:2 PC-d_{31}) at 25 °C and 40 °C. Both lipids are fully deuterated on the saturated chain at the *sn*-1 position. They

differ only in the degree of unsaturation on the chain at the *sn*-2 position. The quadrupole splittings obtained from deuterons on the saturated chains of lipids in this dispersion are sensitive to the average amplitude of chain motions and thus provide information on the physical state of the bilayer and the local environment of the chains.

The transition from liquid crystal to ordered phase bilayers in PLPC-d_{31} at ambient pressure has been studied previously using ^{2}H-NMR [17]. It was found that the stable ordered phase below the transition was characterized by acyl chains that were nearly immobilized on the microsecond time scale of the ^{2}H-NMR experiment. This phase is similar to the L_C (subgel) phase observed in bilayers of some disaturated phospholipids [26–28]. Complete ordering of PLPC-d_{31} into the immobilized-chain-ordered phase required very slow cooling just above and through the transition temperature [17]. Formation of the ordered POPC-d_{31} bilayer phase does not display the same sensitivity to cooling rate.

Susceptibility to pressure-induced ordering decreases with increasing temperature [2,3,5]. For this reason, observations reported here have been made in such a way as to allow comparisons to be made between lipids at roughly corresponding temperatures relative to their ambient-pressure transition temperatures.

II. MATERIALS AND EXPERIMENTAL METHODS

POPC-d_{31} and PLPC-d_{31} were purchased from Avanti Polar Lipids (Alabaster, AL). Both lipids were used without further purification.

Before preparation of POPC-d_{31} samples, any residual solvent was removed from the dry lipid powders by overnight evacuation. POPC-d_{31} was hydrated by adding $\sim$400 μl of 0.05M phosphate buffer (pH=6.9–7) to a round bottom flask containing the dried lipid powder and then rotating the flask for $\sim$30 min. The sample was then centrifuged lightly in a benchtop centrifuge and excess buffer was removed by Pasteur pipette. The remaining dispersion was then sealed into a capsule formed by heat sealing the ends of a short segment of tube cut from a disposable polyethylene pipette.

In preparing the PLPC-d_{31} sample, additional measures were taken to minimize exposure of the sample to oxygen and light. The dry lipid powder was first dissolved in 0.5 ml of chloroform:methanol (1:1) from which oxygen had been partially removed by bubbling argon for $\sim$2 min. The solvent was then removed by evaporation under a stream of dry argon. Residual solvent was then removed by overnight evacuation in the presence of phosphorus pentoxide. Oxygen was also removed from phosphate buffer by bubbling with argon and 200 μl of this buffer was then added to a round bottom flask containing the dry lipid. Argon was bubbled through this dispersion for an additional 2 min after which the sample was immediately sealed into a polyethylene capsule as described above. In order to minimize the opportunity for sample degradation, the observations described below were spread over three separate PLPC-d_{31} preparations.

Deuterium NMR spectra were obtained using a locally-assembled spectrometer based on a 3.5-T superconducting magnet (Nalorac Cryogenics, CA) and a variable-pressure wide-line NMR probe [29]. The coil and capsule containing a given sample dispersion were inserted into a beryllium-copper cell that could be pressurized using hydraulic oil (AW ISO grade 32). Pressures were measured using a Bourdon tube gauge that had been calibrated against a dead weight gauge.

Spectra were acquired using the quadrupole echo sequence [30] with $\pi/2$ pulses of 2.5–4.0 μs in length and a pulse separation of 35 μs. Oversampling [31] was used to obtain effective dwell times of 4 μs for samples in the liquid-crystalline phase and 2 μs for gel-phase samples. The number of transients averaged to obtain a given spectrum ranged from 8000 to 32 000 with a repetition time of 0.5 s.

For multilamellar dispersions of chain-perdeuterated lipid in the liquid-crystalline phase, the ^{2}H-NMR spectra are superpositions of Pake doublets characteristic of fast, axially symmetric reorientation. The splitting for each doublet is proportional to the orientational order parameter,

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

for deuterons on the corresponding methylene segment of the lipid acyl chain. In this expression, θ_{CD} is the angle between the carbon-deuterium bond and the rotational axis of the molecule and the average is over conformations accessed by the reorienting chain.

To facilitate the extraction of quadrupole splittings for specific positions along an acyl chain, the spectrum corresponding to a sample with uniformly oriented bilayers can be obtained by transforming the observed spectrum in a process known as "de-Pake-ing" [32–35]. The "de-Pake-ing" transformation used in the current work was based on the fast-Fourier-transform approach described by McCabe and Wassall [36].

The dependence of S_{CD} on position along a lipid acyl chain is referred to as the orientational order parameter profile. The spectrum of a perdeuterated lipid chain, or the corresponding oriented-sample spectrum obtained by a "de-Pake-ing" transformation, can be analyzed to obtain the orientational order parameters present along the chain but not their assignments to specific positions, particularly in the plateau region of the profile corresponding to the portion of the chain closest to the head group. An orientational order parameter profile can be approximated from this information if orientational order is assumed to decrease monotonically with position along the chain from the head group end toward the methyl group end [37,38]. The resulting "smoothed" orientational order parameter profile is a good approximation to the real profile outside of the plateau region but approximates only the average behavior in the plateau region of the chain. For the work reported here, orientational order parameter profiles have been approximated by integrating "de-Paked" spectra over the spectral features corresponding to the methylene deuterons and then plotting frequency versus normalized, integrated intensity as described previously [5].

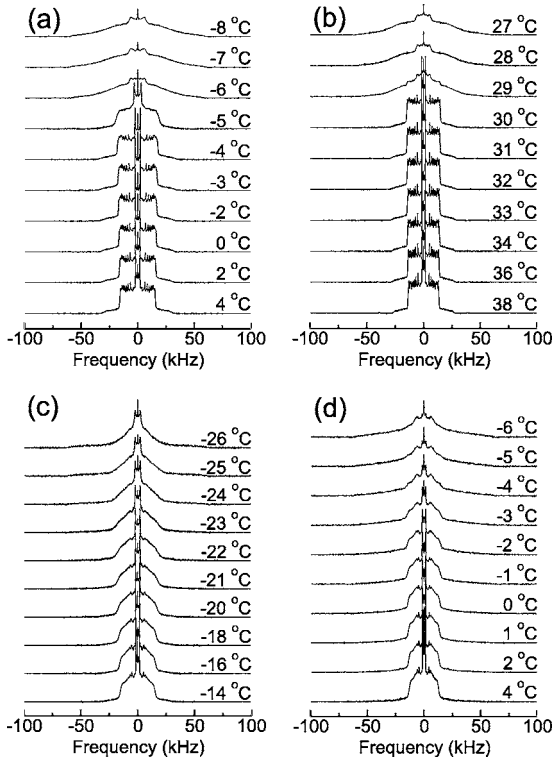

FIG. 1. Deuterium NMR spectra at selected temperatures from (a) POPC-d_{31} at ambient pressure, (b) POPC-d_{31} at 193 MPa, (c) PLPC-d_{31} at ambient pressure, and (d) PLPC-d_{31} at 193 MPa.

III. RESULTS

A. Isobaric spectral series

Figure 1 shows four series of ^{2}H-NMR spectra at selected temperatures obtained from the saturated chains of POPC-d_{31} at ambient pressure (0.1 MPa), POPC-d_{31} at 193 MPa, PLPC-d_{31} at ambient pressure, and PLPC-d_{31} at 193 MPa. At high temperature and ambient pressure, the ^{2}H-NMR spectra for these dispersions are superpositions of Pake doublets reflecting the fast, axially symmetric reorientation of each segment on the perdeuterated saturated chains.

The prominent edges in a given doublet arise from lipids reorienting about bilayer normals perpendicular to the applied magnetic field. These edges are split by

$$\Delta\nu = \frac{3}{4}\frac{e^2qQ}{h}S_{CD}, \tag{2}$$

where $\frac{e^2qQ}{h} = 167$ kHz, the quadrupole coupling constant for carbon-deuterium bonds, and S_{CD} is the orientational order parameter for deuterons on the corresponding chain segment. Splittings, are highest for deuterons at the head group end of the chain and decrease with increasing proximity to the bilayer center where the amplitude of reorientation is greater. The spectra for PLPC-d_{31} in the liquid-crystalline phase are characterized by generally smaller quadrupole splittings, suggesting that the average orientational order of the PLPC-d_{31}-saturated chain is smaller than that for POPC-d_{31} under corresponding conditions.

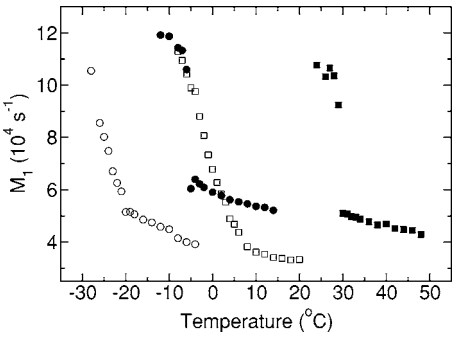

FIG. 2. Temperature dependence of first spectral moments from ^{2}H-NMR spectra for (●) POPC-d_{31} at ambient pressure, (■) POPC-d_{31} at 193 MPa, (○) PLPC-d_{31} at ambient pressure, and (□) PLPC-d_{31} at 193 MPa.

On cooling, the bilayers undergo a transition into a more ordered gel phase in which chain reorientation is slower and not axially symmetric over the characteristic time scale ($\sim 10^{-5}$ s) of the ^{2}H-NMR experiment. The resulting spectra are broader and features corresponding to specific deuterons, other than those on the rapidly rotating methyl group at the chain end, cannot be resolved.

At ambient pressure, the sharp transition displayed by POPC-d_{31} between −5 °C and −6 °C contrasts with the more gradual ordering of PLPC-d_{31} chains below −20 °C. It has previously been reported that the extent to which PLPC-d_{31} orders at the transition is sensitive to the rate at which the sample is cooled, particularly just at and above the transition temperature [17]. This likely reflects the slowness with which the unsaturated chains on this lipid assemble into a more ordered packing arrangement at temperatures near the transition.

The application of hydrostatic pressure generally raises the main transition temperature for bilayer dispersions [1,3,6,39,40]. For disaturated phosphatidylcholine lipids, the sensitivity of the transition temperature to pressure is ~0.2 K/MPa [3,4,6,7]. The change in the transition temperature for POPC-d_{31} on going from ambient to 193 MPa is of similar magnitude. While the more continuous nature of the transition for PLPC-d_{31} makes it more difficult to precisely determine the sensitivity of its transition to pressure, that sensitivity does appear to be less.

The average orientational order parameter for all deuterons on a perdeuterated saturated chain is proportional to the first moment,

$$M_1 = \frac{\int_0^\infty \omega f(\omega) d\omega}{\int_0^\infty f(\omega) d\omega} \tag{3}$$

of the ^{2}H-NMR spectrum where $f(\omega)$ is the spectrum

Figure 2 shows the temperature dependence of the first spectral moments obtained from the isobaric series of ^{2}H-NMR spectra displayed in Fig. 1 for POPC-d_{31} at ambient pressure, POPC-d_{31} at 193 MPa, PLPC-d_{31} at ambient pressure, and PLPC-d_{31} at 193 MPa.

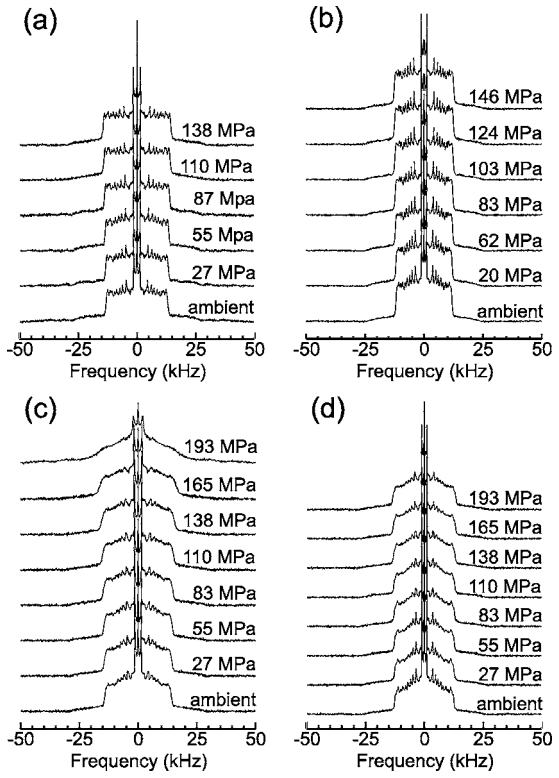

FIG. 3. Deuterium NMR spectra at selected pressures from (a) POPC-d_{31} at 25 °C, (b) POPC-d_{31} at 45 °C, (c) PLPC-d_{31} at 0 °C, and (d) PLPC-d_{31} at 25 °C.

The transition for POPC-d_{31} is sharp at both ambient pressure and at 193 MPa. The increase in transition temperature with pressure is ~0.18 K/MPa. The average saturated chain order just above the transition is higher at ambient pressure than at high pressure. This is consistent with previous observations of disaturated phospholipids for which it was found that the degree of ordering accommodated by the liquid-crystalline bilayer just above the transition decreased with increasing applied pressure [3,4].

The more continuous nature of the ambient-pressure transition for PLPC-d_{31} persists at 193 MPa despite an effective cooling rate of less than 1 K/h. This is consistent with previous reports that the PLPC-d_{31} transition became significantly less sharp when the cooling rate was increased to 1 K/h from 0.3 K/h. Nevertheless, comparison of the transitions for ambient pressure and 193 MPa suggests that the increase in transition temperature for PLPC-d_{31} is ~0.13 K/MPa and thus about 25% smaller than that for POPC-d_{31} or disaturated phospholipids like DPPC and DMPC.

B. Isothermal spectral series

Figure 3 shows four series of ^{2}H-NMR spectra at selected pressures from saturated chains of POPC-d_{31} at 25 °C, POPC-d_{31} at 45 °C, PLPC-d_{31} at 0 °C, and PLPC-d_{31} at 25 °C. Except for the case of PLPC-d_{31} at 0 °C and 193 MPa [panel (c)], both samples remain in the liquid-crystalline phase for all pressures and temperatures shown in Fig. 3. In all four series, the splittings across the entire liquid-crystal phase spectrum increase slightly with increasing pressure. Aside from this, the liquid-crystalline spectra in a given series change only slightly with pressure.

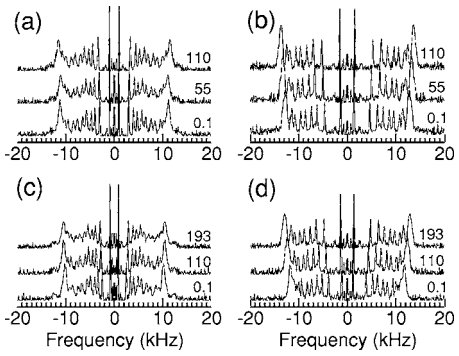

FIG. 4. Spectra corresponding to oriented samples obtained by "de-Pake-ing" of powder spectra obtained at selected pressures for (a) PLPC-d_{31} at 25 °C, (b) POPC-d_{31} at 25 °C, (c) PLPC-d_{31} at 40 °C, and (d) POPC-d_{31} at 40 °C. Spectra are labeled by pressure in MPa.

The increase in chain deuteron quadrupole splittings with pressure reflects a pressure-induced ordering of the saturated chain and thus an increase in overall extension of the chain with pressure. While hydrostatic pressure is transmitted uniformly through the liquid-crystalline bilayer structure, its effect is to increase interactions between neighboring chains and thus promote increased orientational order and closer packing of adjacent chains. Aspects of this response to pressure can be modeled using self-consistent mean field theory [41]. It has previously been reported that the extent to which saturated lipid chains order in response to pressure in the liquid-crystalline phase decreases with increasing temperature [5]. The same effect is seen here where, for each lipid, the quadrupole splittings are more sensitive to pressure at the lower observation temperature than at the higher observation temperature.

C. Pressure-induced chain ordering

In order to facilitate comparisons between the effects of pressure on chain order for the two lipids, spectra with enhanced signal to noise were collected at selected temperatures and pressures by averaging between 2 and 4 times the number of transients used for the spectra presented above. These powder spectra were then transformed using a "de-Pake-ing" process to obtain corresponding spectra that would be obtained by orienting the sample bilayers perpendicular to the magnetic field. Figure 4 shows the resulting "de-Paked" spectra at selected pressures for PLPC-d_{31} at 25 °C, POPC-d_{31} at 25 °C, PLPC-d_{31} at 40 °C, and POPC-d_{31} at 40 °C. In each spectrum, the prominent doublet with a splitting of less than 4 kHz arises from the rapidly rotating methyl group at end of the acyl chain closest to the bilayer center. The splittings of the resolved doublets, corresponding to methylene segments, increase with increasing proximity to the head group end of the chain. The superposition of unresolved doublets close to the largest splitting come from methylene seg-

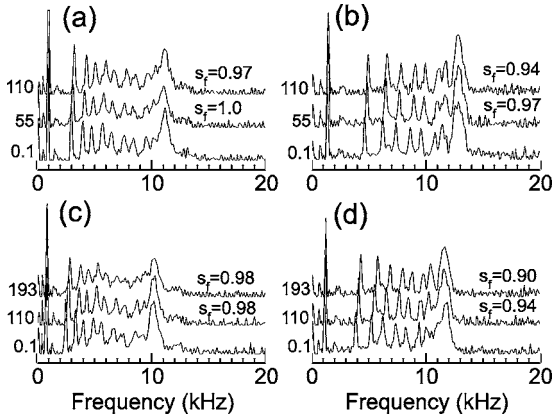

FIG. 5. Right halves of frequency-scaled "de-Paked" spectra for ambient pressure and elevated pressures for (a) PLPC-d_{31} at 25 °C, (b) POPC-d_{31} at 25 °C, (c) PLPC-d_{31} at 40 °C, and (d) POPC-d_{31} at 40 °C. The frequency axes of the high-pressure spectra have been scaled by the factor necessary to align the upper frequency edges of the spectral features arising from the saturated chain plateau deuterons with the corresponding edge in the ambient pressure spectra. Spectra are labeled by pressure and scaling factor s_f.

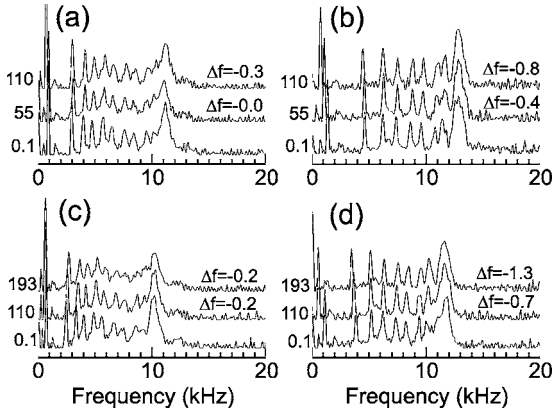

FIG. 6. Right halves of "de-Paked" spectra for ambient pressure and elevated pressures for (a) PLPC-d_{31} at 25 °C, (b) POPC-d_{31} at 25 °C, (c) PLPC-d_{31} at 40 °C, and (d) POPC-d_{31} at 40 °C. The high-pressure spectra have been shifted to the left by the frequency interval Δf necessary to align the upper frequency edges of the spectral features arising from the saturated chain plateau deuterons with the corresponding edge in the ambient-pressure spectra. Spectra are labeled by pressure and frequency interval by which spectra have been shifted.

ments near the head group end of the chain. These correspond to the plateau region of the orientational order parameter profile.

The observation temperatures 25 °C and 40 °C were selected to be at similar differences above the PLPC-d_{31} and POPC-d_{31} ambient-pressure transitions, respectively. Thus 25 °C and 40 °C constitute a roughly corresponding pair of temperatures, with respect to the ambient-pressure transitions, for PLPC-d_{31} and POPC-d_{31}, respectively.

For each lipid, the effect of pressure on quadrupole splittings across the spectrum is larger at the lower temperature. For each temperature, effect of pressure on quadrupole splittings is larger for POPC-d_{31} than for PLPC-d_{31}. The responses of the lipids at similar differences from their respective ambient-pressure transition temperatures can be compared by considering the series for PLPC-d_{31} at 25 °C and POPC-d_{31} at 40 °C. The difference in quadrupole splittings between ambient pressure and 110 MPa is significantly larger for POPC-d_{31} at 40 °C than for PLPC-d_{31} at 25 °C.

The way in which pressure-induced ordering is distributed along saturated acyl chains was previously examined for DMPC-d_{54}, POPC-d_{31}, and mixtures of these lipids [5]. In that work, it was found that the effect of pressure on qudrupole splittings in the liquid-crystalline phase could not be reproduced by a uniform scaling of orientational order for all positions along the chain. Similar comparisons have been applied to the spectra of Fig. 4.

Figure 5 shows right halves of frequency-scaled "de-Paked" spectra for ambient pressure and elevated pressures for PLPC-d_{31} at 25 °C, POPC-d_{31} at 25 °C, PLPC-d_{31} at 40 °C, and POPC-d_{31} at 40 °C. The high-pressure spectra have been scaled by the factor s_f required to align the upper frequency edges of spectral features arising from the saturated chain plateau deuterons with the corresponding edge in the ambient pressure spectra. If the quadrupole splittings for methylene deuterons along the saturated chain all scaled in the same way with pressure, the extent to which doublets in the high-pressure spectra aligned with the corresponding doublets at low pressure would be expected to be independent of position within the spectrum and, accordingly, independent of methylene position along the acyl chain. The sets of spectra in Fig. 5 do not, in fact, reflect uniform scaling of the doublet splittings with pressure. The departure from uniform scaling is most apparent in panels (b) and (d) corresponding to POPC-d_{31} at 25 °C and 40 °C, respectively.

Comparison of the frequency-scaled spectra each of the four series in Fig. 5 shows that for a given pressure difference, the ratios of the splittings for deuterons on the most ordered segments of the chain are smaller than the corresponding ratios for less ordered segments of the chain near the bilayer center. This difference is most apparent in the two series of POPC-d_{31} spectra but can be seen in all four sets of spectra. The nonuniformity of scaling along a saturated chain observed here is consistent with results from an earlier study of DMPC-d_{54} and POPC-d_{31} [5]. In that study, it was the absolute magnitude of the pressure-induced change in orientational order that was found to be roughly constant along the saturated acyl chain. In effect, pressure was found to shift the methylene region of the orientational order parameter profile vertically by an amount that was roughly constant across the spectrum. We have checked for this behavior in the current samples by shifting "de-Paked" spectra for different pressures along the frequency axis so as to compare the ranges of methylene splitting observable for a given sample at each pressure.

Figure 6 shows the resulting right halves of frequency-shifted "de-Paked" spectra for ambient pressure and elevated pressures for PLPC-d_{31} at 25 °C, POPC-d_{31} at 25 °C, PLPC-d_{31} at 40 °C, and POPC-d_{31} at 40 °C. The high-pressure spectra have been shifted to the left by the frequency difference Δf necessary for the upper frequency edges of the spectral features arising from the saturated chain plateau deuter-

ons to be aligned with the corresponding edge in the ambient-pressure spectra. Within each set of ambient-pressure and shifted spectra there is a rough alignment of corresponding methylene features. The methyl group features on the low-frequency side of each spectrum are not generally aligned by this process. The closest alignment, other than in the plateau region, occurs for the low-frequency features corresponding to the least ordered methylene segment on each chain. The approximate correspondence of maximum and minimum methylene splittings in each set of spectra implies that the range of orientational order along the saturated chain is approximately conserved as the average orientational order increases in response to applied pressure. This is also consistent with earlier observations of DMPC-d_{54} and POPC-d_{31} [5].

The frequency shifts required to align the plateau features in each set of spectra in Fig. 6 reflect the pressure-induced increase in quadrupole splitting, and thus chain orientational order, for deuterons in the plateau region of the chain. The relationship between the contribution to chain extension away from the bilayer surface by a given segment of saturated chain and the mean orientational order parameter for that segment is often assumed to be of the form $\langle l\rangle = a + b|S_{CD}|$ where a and b are constants [11,42–45]. If accessible acyl chain conformations are modeled using a diamondlike lattice, the segmental contribution to chain extension can be approximated as

$$\langle l\rangle \approx l_0\left[\frac{1}{2} + |S_{CD}|\right], \tag{4}$$

where $l_0 \approx 0.125$ nm [42,44].

The chain extensions per segment for methylene groups corresponding to the plateau region of the orientational order parameter profile will be roughly equal and, as shown by Nagle [44], the average cross-sectional area per saturated chain should be inversely proportional to the extension per segment for these plateau methylenes. The fractional reduction in area per saturated chain resulting from the application of hydrostatic pressure should thus equal the fractional increase in segmental extension for methylenes corresponding to the plateau in the orientational order parameter profile so that

$$\frac{\langle l\rangle_{\mathrm{pl,P}} - \langle l\rangle_{\mathrm{pl,amb}}}{\langle l\rangle_{\mathrm{pl,amb}}} \approx -\frac{\langle A\rangle_{\mathrm{P}} - \langle A\rangle_{\mathrm{amb}}}{\langle A\rangle_{\mathrm{amb}}}, \tag{5}$$

where $\langle A\rangle_{\mathrm{P}}$ and $\langle A\rangle_{\mathrm{amb}}$ are the average cross-sectional areas per saturated chain at high and ambient pressure, respectively, and $\langle l\rangle_{\mathrm{pl,P}}$ and $\langle l\rangle_{\mathrm{pl,amb}}$ are the corresponding extensions per segment in the plateau region of the chain.

For the purposes of comparison, the quadrupole splittings for the plateau deuterons can be taken from the peaks of the unresolved superposition of doublets having the largest splittings in each of the "de-Paked" spectra. These splittings can be converted to orientational order parameters and thus segmental extensions using Eqs. (2) and (4), respectively. Using Eq. (5) to relate changes in plateau segment extension to changes in saturated chain cross-sectional area provides a way to compare the responses of POPC-d_{31} and PLPC-d_{31} to pressure. When this is done, the shift in plateau splittings shown in Fig. 6 implies that the effect of applying 110 MPa to PLPC-d_{31} at 25 °C is to reduce saturated-chain cross-sectional area by ~0.7%. For POPC-d_{31} at the corresponding temperature of 40 °C, the same pressure reduces saturated-chain cross-sectional area by ~1.3%. At corresponding temperatures POPC-d_{31} is more susceptible to pressure-induced reduction of saturated chain cross-sectional area than PLPC-d_{31}.

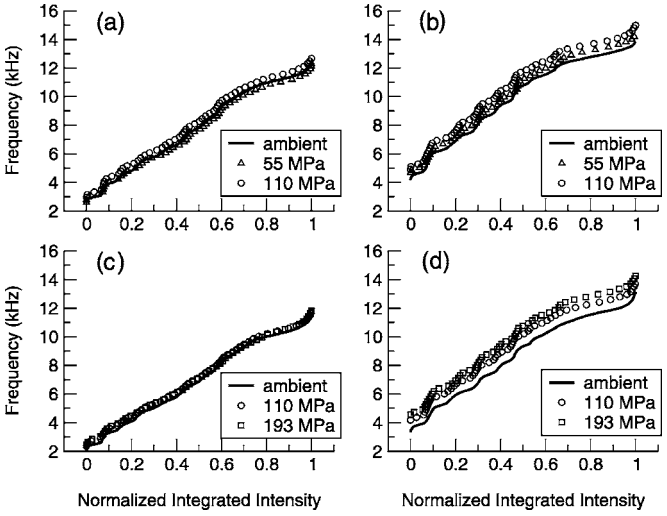

FIG. 7. Frequency versus normalized integrated intensity across the methylene region of the "de-Paked" spectra at ambient pressure (solid line) and elevated pressures (open symbols) for (a) PLPC-d_{31} at 25 °C, (b) POPC-d_{31} at 25 °C, (c) PLPC-d_{31} at 40 °C, and (d) POPC-d_{31} at 40 °C.

Orientational order parameter profiles provide another way to compare the degree of pressure-induced ordering displayed by the saturated chains of POPC-d_{31} and PLPC-d_{31}. Figure 7 shows frequency versus normalized integrated intensity across the methylene region of the "de-Paked" spectra at ambient pressure (solid line) and elevated pressures (open symbols) for PLPC-d_{31} at 25 °C, POPC-d_{31} at 25 °C, LPC-d_{31} at 40 °C, and POPC-d_{31} at 40 °C. As described previously [5], this transformation of the "de-Paked" spectrum provides a useful approximation to the corresponding "smoothed" orientational order parameter profile.

The two observation temperatures in Fig. 7, 25 °C and 40 °C, are about 45 °C above the ambient-pressure transition temperatures of PLPC-d_{31} and POPC-d_{31}, respectively. The effect of pressure on the orientational order parameter profile is greater for POPC-d_{31} than for PLPC-d_{31} at both temperatures. In particular, the shift in order parameter profile between ambient pressure and 110 MPa for POPC-d_{31} at 40 °C is larger than the corresponding shift for PLPC-d_{31} at the corresponding temperature of 25 °C. Comparison of the ambient-pressure and 193-MPa profiles for POPC-d_{31} at 40 °C illustrates the approximate uniformity of the absolute increase in orientational order along the chain with pressure.

IV. DISCUSSION

The observations reported here demonstrate some significant quantitative differences between the responses to pres-

sure of POPC-d_{31} and PLPC-d_{31} bilayer dispersions. At ambient pressure, the main liquid-crystal to ordered phase transition for PLPC-d_{31} occurs at a lower temperature and that transition is less sensitive to pressure than the main transition for POPC-d_{31}. Compared to POPC-d_{31}, liquid-crystalline PLPC-d_{31} is also more resistant to pressure-induced chain ordering both at a given absolute temperature and at corresponding temperatures relative to the ambient-pressure transition temperatures of the respective dispersions.

The ability of a marine organism to adapt to changes in hydrostatic pressure presumably depends, at least in part, on the extent to which the physical properties of its membranes can be maintained as pressure changes. Monounsaturation does not significantly change the pressure dependence of the main transition temperature relative to that for fully saturated lipids but it does lower the ambient-pressure transition temperature and thus the susceptibility to pressure-induced chain ordering at a given temperature. The presence of diunsaturated chains does reduce the sensitivity of the main transition temperature to pressure slightly but it also lowers the ambient-pressure transition temperature significantly. PLPC-d_{31} was found to be less susceptible to pressure-induced ordering than POPC-d_{31} at corresponding temperatures above the ambient-pressure transition temperature, and this difference is amplified, for a given absolute temperature, by the substantially lower ambient-pressure transition temperature of PLPC-d_{31}. While these simple considerations suggest how incremental changes in unsaturation affect the response of membranes to pressure, the situation in real organisms is much more complicated and any adaptive benefit of unsaturation is presumably balanced against metabolic cost.

As described above, the ambient-pressure properties of these two lipid dispersions differ and their responses to pressure also differ. Ultimately, all of these differences must be attributable to the incremental disordering effect of a diunsaturated *sn*-2 chain versus a monounsaturated *sn*-2 chain and it is interesting to consider how the observed differences might be related.

In this regard, one issue to be considered is whether comparison of pressure-induced chain ordering in the two lipid dispersions is complicated by the difference in the pressure dependences of the transition temperatures for the two systems. Susceptibility to pressure-induced chain ordering does increase with increasing proximity of the observation temperature to the transition temperature but the dependence on temperature is strongest just above the transition [3,5]. The comparisons in Figs. 4–7 were justified on the basis of 25 °C and 40 °C being roughly corresponding temperatures, relative to the ambient-pressure transition temperatures, for PLPC-d_{31} and POPC-d_{31}, respectively. However, the pressure dependences of the transition temperatures for the two dispersions are ~0.13 K/MPa and ~0.18 K/MPa for PLPC-d_{31} and POPC-d_{31}, respectively, so that application of 110 MPa raises the PLPC-d_{31} transition temperature by only ~14 °C compared to ~20 °C for POPC-d_{31}. In effect, at 110 MPa, POPC-d_{31} at 40 °C is about 6 °C closer to its transition at that pressure than is PLPC-d_{31} at 25 °C. To assess whether that difference might significantly affect the validity of comparing pressure-induced chain ordering of the two lipid dispersions at corresponding temperatures relative to the ambient-pressure transitions, it is helpful to compare the effect of pressure on a given lipid at 25 °C to the effect on the same lipid at 40 °C. This can conveniently be done by considering comparisons illustrated in Fig. 5 which shows that application of 110 MPa reduces the plateau deuteron splittings for PLPC-d_{31} by ~3% and ~2% at 25 °C and 40 °C, respectively and for POPC-d_{31} by ~6% at both temperatures. This suggests that the temperatures of 25 °C and 40 °C used in Figs. 4–7 are far enough above the relevant transition temperatures for the corresponding susceptibilities to pressure-induced chain ordering to be only weakly dependent on temperature. Accordingly, the observation that PLPC-d_{31} is more resistant to pressure-induced chain ordering than POPC-d_{31} under roughly similar conditions appears be a reflection of the mechanical properties of the two liquid-crystalline dispersions and not simply a consequence of the temperatures at which the observations were made.

The greater resistance of the PLPC-d_{31} liquid-crystalline phase to pressure-induced chain ordering, compared to POPC-d_{31}, suggests that the free energy of the PLPC-d_{31} liquid-crystalline phase is relatively less sensitive to pressure. This could contribute to the observed weaker pressure dependence of the PLPC-d_{31} transition temperature but it should also be noted that the stable PLPC-d_{31} phase below the transition is thought to be a subgel-like phase in which chains are less mobile than in the POPC-d_{31} gel phase [17]. Accordingly, the weaker dependence of the PLPC-d_{31} transition temperature on pressure may also indicate less incremental stabilization by pressure of the subgel-like PLPC-d_{31} ordered phase than of the POPC-d_{31} gel phase. Extending the comparisons described here to lipid dispersions containing more highly unsaturated chains, such as 16:0-20:4 PC-d_{31} (1-palmitoyl-2-arachidonoyl phosphatidylcholine), which also orders into a more subgel-like phase [18], might help to separate possible contributions to the pressure dependence of the main transition temperature.

V. CONCLUSIONS

Compared to POPC-d_{31}, liquid-crystalline PLPC-d_{31} is found to be more resistant to pressure-induced chain ordering both at a given absolute temperature and at corresponding temperatures relative to the ambient-pressure transition temperature of the respective dispersions. This likely indicates that the presence of two double bonds in the unsaturated chain increases the resistance of PLPC-d_{31} bilayers to pressure-induced ordering relative to that of POPC-d_{31} with a monounsaturated acyl chain.

ACKNOWLEDGMENTS

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (MRM) and the Canadian Institutes of Health Research (KMWK).

[1] L. F. Braganza and D. L. Worcester, Biochemistry **25**, 2591 (1986).
[2] D. A. Driscoll, S. Samarasinghe, S. Adamy, J. Jonas, and A. Jonas, Biochemistry **30**, 3322 (1991).
[3] B. B. Bonev and M. R. Morrow, Phys. Rev. E **55**, 5825 (1997).
[4] B. B. Bonev and M. R. Morrow, Can. J. Chem. **76**, 1512 (1998).
[5] A. Brown, I. Skanes, and M. R. Morrow, Phys. Rev. E **69**, 011913 (2004).
[6] D. A. Driscoll, J. Jonas, and A. Jonas, Chem. Phys. Lipids **58**, 97 (1991).
[7] H. Ichimori, T. Hata, H. Matsuki, and S. Kaneshina, Biochim. Biophys. Acta **1414**, 165 (1998).
[8] P. T. T. Wong and H. H. Mantsch, Biochim. Biophys. Acta **732**, 92 (1983).
[9] B. B. Bonev and M. R. Morrow, Biophys. J. **70**, 2727 (1996).
[10] C. Czeslik, O. Reis, R. Winter, and G. Rapp, Chem. Phys. Lipids **91**, 135 (1998).
[11] L. L. Holte, S. A. Peter, T. M. Sinnwell, and K. Gawrisch, Biophys. J. **68**, 2396 (1995).
[12] H. I. Petrache, A. Salmon, and M. F. Brown, J. Am. Chem. Soc. **123**, 12611 (2001).
[13] K. Gawrisch, N. V. Eldho, and L. L. Holte, Lipids **38**, 445 (2003).
[14] D. E. Warschawski and P. F. Devaux, Eur. Biophys. J. **34**, 987 (2005).
[15] K. P. Coolbear and K. M. W. Keough, Biochemistry **22**, 1466 (1983).
[16] L. L. Holte, S. A. Peter, T. M. Sinnwell, and K. Gawrisch, Biophys. J. **68**, 2396 (1995).
[17] M. R. Morrow, P. J. Davis, C. S. Jackman, and K. M. W. Keough, Biophys. J. **71**, 3207 (1996).
[18] C. S. Jackman, P. J. Davis, M. R. Morrow, and K. M. W. Keough, J. Phys. Chem. B **103**, 8830 (1999).
[19] S. Kaneshina, H. Ichimori, T. Hata, and H. Matsuki, Biochim. Biophys. Acta **1374**, 1 (1998).
[20] H. Ichimori, T. Hata, H. Matsuki, and S. Kaneshina, Chem. Phys. Lipids **100**, 151 (1999).
[21] A. R. Cossins and A. G. MacDonald, Biochim. Biophys. Acta **860**, 325 (1986).
[22] A. R. Cossins and A. G. MacDonald, J. Bioenerg. Biomembr. **21**, 115 (1989).
[23] E. F. DeLong and A. A. Yayanos, Science **228**, 1101 (1985).
[24] Y. Yano, A. Nakayama, K. Ishihara, and H. Saito, Appl. Environ. Microbiol. **64**, 479 (1998).
[25] E. E. Allen, D. Facciotti, and D. H. Bartlett, Appl. Environ. Microbiol. **65**, 1710 (1999).
[26] J. H. Davis, Biophys. J. **27**, 339 (1979).
[27] J. H. Davis, Biochim. Biophys. Acta **737**, 117 (1983).
[28] M. R. Morrow and J. H. Davis, Biochim. Biophys. Acta **904**, 61 (1987).
[29] B. B. Bonev and M. R. Morrow, Rev. Sci. Instrum. **68**, 1827 (1997).
[30] J. H. Davis, K. R. Jeffrey, M. Bloom, M. I. Valic, and T. P. Higgs, Chem. Phys. Lett. **42**, 390 (1976).
[31] R. S. Prosser, J. H. Davis, F. W. Dahlquist, and M. A. Lindorfer, Biochemistry **30**, 4687 (1991).
[32] M. Bloom, J. H. Davis, and A. L. MacKay, Chem. Phys. Lett. **80**, 198 (1981).
[33] E. Sternin, M. Bloom, and A. L. MacKay, J. Magn. Reson. (1969-1992) **55**, 274 (1983).
[34] H. Schäfer and E. Sternin, Phys. Canada **53**, 77 (1997).
[35] K. P. Whittall, E. Sternin, M. Bloom, and A. L. MacKay, J. Magn. Reson. (1969-1992) **84**, 64 (1989).
[36] M. A. McCabe and S. R. Wassall, J. Magn. Reson., Ser. B **106**, 80 (1995).
[37] M. Lafleur, B. Fine, E. Sternin, P. R. Cullis, and M. Bloom, Biophys. J. **56**, 1037 (1989).
[38] M. Lafleur, P. R. Cullis, and M. Bloom, Eur. Biophys. J. **19**, 55 (1990).
[39] S. Utoh and T. Takemura, Jpn. J. Appl. Phys., Part 1 **24**, 356 (1985).
[40] P. T. T. Wong, D. J. Siminovitch, and H. H. Mantsch, Biochim. Biophys. Acta **947**, 139 (1988).
[41] M. D. Whitmore and J. P. Whitehead, Can. J. Phys. **76**, 883 (1998).
[42] H. Schindler and J. Seelig, Biochemistry **14**, 2283 (1975).
[43] F. A. Nezil and M. Bloom, Biophys. J. **61**, 1176 (1992).
[44] J. F. Nagle, Biophys. J. **64**, 1476 (1993).
[45] B. D. Koenig, H. H. Strey, and K. Gawrisch, Biophys. J. **73**, 1954 (1997).