Hydrostatic Pressure-Induced Conformational Changes in Phosphatidylcholine Headgroups: A ^{2}H NMR Study

B. B. Bonev and M. R. Morrow
Department of Physics, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, A1B 3X7

ABSTRACT The effects of pressure and temperature on 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine headgroup conformations were examined using deuterium nuclear magnetic resonance. Isothermal compression was found to produce a decrease in the choline α deuteron quadrupole splitting and increases in the choline β and γ deuteron quadrupole splittings. A similar counterdirectional change, seen in the presence of positive surface charge, has been attributed to tilting of the headgroup away from the bilayer surface in response to the torque exerted on the phosphocholine dipole by positive surface charges. The direction of the change in headgroup deuteron quadrupole splitting is consistent with the pressure-induced reduction in area per lipid in the liquid crystalline phase, which can be inferred from the ordering of phospholipid acyl chains under comparable conditions. The temperature dependences of the headgroup deuteron quadrupole splittings were also examined. It was found that at elevated pressure, the α splitting was insensitive to temperature, whereas the β and γ splittings decreased. The response of the β deuteron splitting to temperature was found to be weaker at elevated pressure than at ambient pressure.

INTRODUCTION

Interactions in the polar region of lipid bilayers are important determinants of membrane phase behavior and mechanical properties. Deuterium nuclear magnetic resonance (^{2}H NMR) has been used extensively to probe the conformational state of phospholipid headgroups under a variety of conditions (Akutsu and Seelig, 1981; Seelig et al., 1987; Macdonald and Seelig, 1988; Scherer and Seelig, 1989; Roux and Bloom, 1990; Macdonald et al., 1991; Bechinger and Seelig, 1991; Marassi and Macdonald, 1991, 1992). One of the most fascinating observations has been the response of headgroup conformation to changes in membrane surface charge density. The structure of the phosphatidylcholine headgroup is shown in Fig. 1. The α, β, and γ carbons are indicated. The electric dipole resulting from charge separation in the phosphocholine group is considered to lie roughly parallel to the bilayer surface under physiological conditions (Büldt et al., 1979; Seelig et al., 1987). Addition of positive charge to the bilayer surface is thought to result in a tilting of the P^--N^+ dipole away from the surface (Seelig et al., 1987). This effect has been observed through the quadrupole splittings of deuterons on the α and β carbons of the headgroup. In particular, counterdirectional change of the α and β deuteron quadrupole splittings has been taken as evidence that the response of the headgroup to changes in surface charge density involves a conformational change rather than ordering or disordering (Akutsu and Seelig, 1981; Macdonald and Seelig, 1988).

In this work, we have examined the response of headgroup deuteron quadrupole splitting to application of hydrostatic pressure. A number of high-pressure NMR studies of membranes have been carried out by Jonas and coworkers (Jonas et al., 1988; Driscoll et al., 1991a,b; Peng and Jonas, 1992). Control of pressure provides a means to separate the effects of volume and temperature on the mechanical properties of bilayers. The response of membrane properties to pressure can provide a rigorous test for theoretical membrane models in which pressure is a parameter. If the effect of an ambient pressure membrane perturbation can be mimicked by the application of hydrostatic pressure, the response to pressure may provide some insight into properties at ambient pressure. It will be shown below that the responses of the headgroup to changes in pressure and surface charge are somewhat analogous. Pressure effects are also relevant to the understanding of membrane properties and composition in marine organisms that experience high hydrostatic pressure or variations in hydrostatic pressure.

Tipping of the phosphatidylcholine electric dipole, in response to changes in surface charge density (Macdonald and Seelig, 1987; Roux et al., 1988; Dempsey et al., 1989; Roux et al., 1989; Rydall and Macdonald, 1992; Morrow et al., 1993; Pinheiro et al., 1994) or state of hydration (Bechinger and Seelig, 1991), provides a useful probe of changes in conditions near the bilayer surface. In the current work, we have used hydrostatic pressure to obtain effects that seem to be analogous to those observed when surface charge is varied. Peng and Jonas (1992) used ^{31}P NMR to study the response of the 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine headgroup to pressure in both gel and liquid crystalline phases. They considered the possibility that changes in available area might affect headgroup conformation. The phosphorus chemical shift, however, did not provide sufficient information to characterize headgroup orientation, and they attributed the observed change with

Received for publication 30 March 1995 and in final form 11 May 1995.
Address reprint requests to Dr. Michael R. Morrow, Department of Physics, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3X7, Canada. Tel.: 709-737-4361; Fax: 709-737-8739; E-mail: myke@kelvin.physics.mun.ca.

0006-3495/95/08/518/06 $2.00

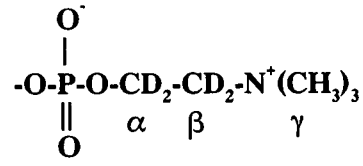

FIGURE 1 The phosphatidylcholine headgroup showing the α, β, and γ carbons.

pressure to slow motions. In the current work we have used headgroup deuteron quadrupole splittings to examine the effect of pressure on phospholipid headgroup conformation. These types of observation have the potential to provide considerable insight into the mechanical properties of the phospholipid headgroup and into the mechanism of surface-charge induced changes in headgroup conformation. Pressure provides a means to alter the headgroup conformation continuously in a single sample, without uncertainties associated with bilayer composition or charge location relative to the surface. We have also examined the effect of pressure on the sensitivity of the headgroup deuteron quadrupole splittings to temperature. These observations provide further insight into the mechanical properties of the bilayer.

MATERIALS AND METHODS

1,2-dimyristoyl-*sn*-glycero-3-(1,1',2,2'-*d*)-phosphocholine (DMPC-d_4) and 1,2-dipalmitoyl-*sn*-glycero-3-(d_{13})-phosphocholine (DPPC-d_{13}) were purchased from Avanti Polar Lipids (Birmingham, AL). When checked by thin-layer chromatography, the lipids were found to migrate as single spots. Before hydration in 0.1 M phosphate buffer (pH 7.2), the lipids were dried in vacuum for 5–8 h. All samples were in the form of multilamellar vesicles obtained by stirring the lipid with a fine glass rod in excess buffer above the main transition. After preparation, the multilamellar vesicle suspensions were transferred into a flexible polyethylene tube and heat-sealed.

The experiments were carried out over a range of pressures from ambient to 2.0 kbar and a range of temperatures from 20°C to 75°C, using a locally constructed high-pressure ^{2}H-NMR probe and spectrometer in conjunction with a 3.5-T superconducting magnet (Nalorac Cryogenics, Martinez, CA). Details of the spectrometer have been reported elsewhere (Morrow, 1990). Spectra were acquired using a phase-cycled quadrupole echo sequence (Davis et al., 1976) with a $\pi/2$ pulse length of between 2.2 and 2.4 μs and a pulse separation of 75 μs. The dwell time of the digitizer was 5 μs. Transients were oversampled by a factor of 4, as described previously (Prosser et al., 1991; Morrow et al. 1993), to give an effective dwell time of 20 μs. Depending on the sample size, between 8000 and 96,000 transients were averaged for each spectrum. Quadrupole splittings in the liquid crystalline phase were determined from the 90° edges of the axially symmetric Pake doublet.

Results and Discussion

For DMPC and DPPC, the main transition temperature is known to increase by about 22°C per 1 kbar increase in applied pressure. At 65°C, therefore, the liquid crystalline phase spans a range of ~2 kbar for DMPC and ~1 kbar for DPPC. Fig. 2 *a* shows ^{2}H-NMR spectra for DMPC-d_4 at 65°C for a range of pressures spanning the liquid crystalline phase. The spectra are superpositions of Pake doublet powder patterns characteristic of fast axially symmetric reorientation of the headgroup in the liquid crystalline phase. At ambient pressure, the outer and inner doublets arise from pairs of deuterons on the α and β carbons of the choline group, respectively. Fig. 2 *b* shows ^{2}H-NMR spectra for DPPC-d_{13} for a range of pressures, also at 65°C. The spectral features of DPPC-d_{13} are qualitatively similar to those of DMPC-d_4 except for an additional narrow doublet corresponding to the three γ deuteromethyl groups.

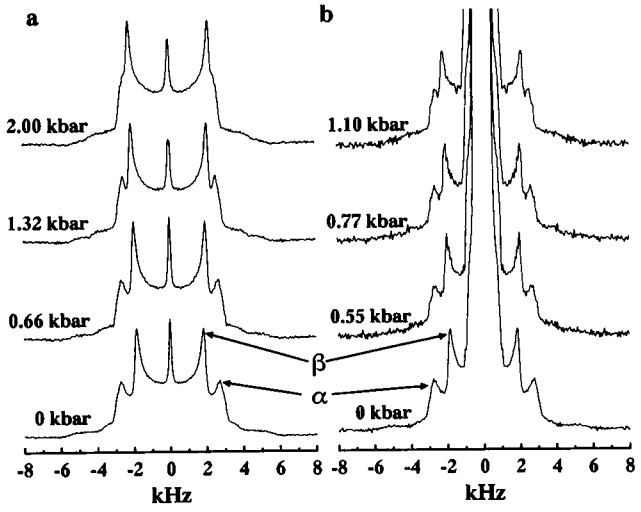

FIGURE 2 (a) DMPC-d_4 spectra at 65°C and selected hydrostatic pressures in bars above ambient; (b) DPPC-d_{13} spectra at 65°C and selected hydrostatic pressures in bars above ambient.

At ambient pressure, the DMPC-d_4 α deuteron splitting is 5.44 kHz. The proximity of the glycerol backbone seems to reduce the motional freedom of the α carbon resulting in a slight inequivalence of the α deuterons (Gally et al., 1975). Under decoupling conditions, Akutsu and Seelig (1981) reported a difference of about 300 Hz in the quadrupole splittings of the two α deuterons of DPPC. In the present work, there is one slightly broadened line, and we have used the average quadrupole splitting for the purposes of this analysis.

The pressure dependences of the DMPC-d_4 α and β deuteron quadrupole splittings at 65°C are shown in Fig. 3. With increasing pressure, the α splitting decreases in a roughly linear way with a slope of −270 Hz/kbar. The β deuteron quadrupole splitting increases with pressure with a slope of 350 Hz/kbar.

Fig. 3 also shows the pressure dependence of the α and β deuteron quadrupole splittings for DPPC-d_{13} at 65°C. Application of pressure reduces the α deuteron splitting with a slope of −580 Hz/kbar and increases the β deuteron splitting with a slope of 870 Hz/kbar. Deuterons on the three γ methyl groups are equivalent as a result of fast rotation about the C_β-N and N-C_γ bond axes. The quadrupole splittings of the γ deuterons increase by 130 Hz/kbar with pressure.

Counterdirectional changes in the magnitudes of the α and β deuteron quadrupole splittings have been observed

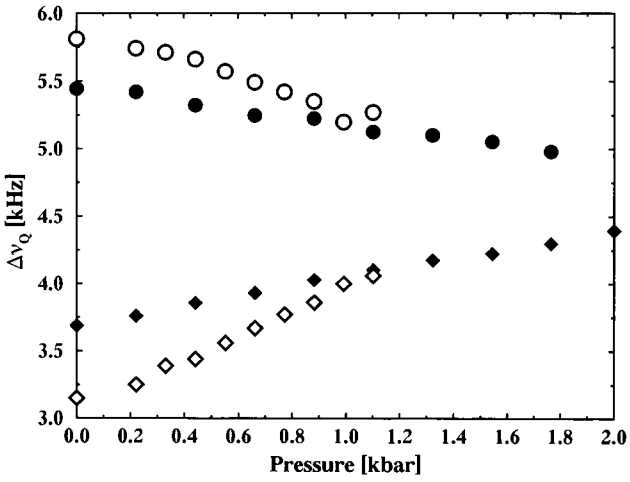

FIGURE 3 Pressure dependence of headgroup deuteron quadrupole splittings at 65°C for the DMPC-d_4 (●) α and (◆) β deuterons and for the DPPC-d_{13} (○)α and (◆) β deuterons.

previously in response to changes in membrane surface charge density (Seelig et al., 1987; Macdonald and Seelig, 1988; Dempsey et al., 1989; Kuchinka and Seelig, 1989; Scherer and Seelig, 1989; Roux et al., 1989; Macdonald et al., 1991; Marassi and Macdonald, 1992). Such behavior is generally taken to indicate a change in headgroup conformation rather than an increase or decrease in orientational order, which would be expected to simultaneously increase or decrease the magnitudes of the two splittings. An increase in the β deuteron quadrupole splitting accompanied by a decrease in the α deuteron quadrupole splitting has also been observed in response to the presence of metal ions in the aqueous phase (Gally et al., 1975; Akutsu and Seelig, 1981), positively charged amphiles (Macdonald and Seelig, 1988; Marassi and Macdonald, 1992; Scherer and Seelig, 1989), charged membrane intrinsic proteins (Kuchinka and Seelig, 1989), and polypeptides (Roux and Bloom, 1990). It is believed that such agents tilt the phosphocholine moiety toward a more upright position about the bond linking the glcerol backbone to the oxygen of the phosphate group (Macdonald et al., 1991) or about the C(1)-C(2) glycerol bond (Hauser et al., 1981). The torque applied to the headgroup is assumed to result from an interaction between positive surface charge, introduced by cation adsorption or present in charged membrane components, and the phosphocholine P^--N^+ dipole. The presence of negative surface charge is found to cause the opposite effect on the headgroup deuteron quadrupole splittings and is assumed to be associated with a tilting of the headgroup dipole toward the bilayer surface (Seelig et al., 1987). Bechinger and Seelig (1991) have reported that decreasing hydration also results in a tilting of the N^+ end of the dipole toward the surface.

Fluid lipid bilayers are very anisotropic. They are generally more compressible in the plane of the bilayer than along the bilayer normal. The consequence of this, as observed by neutron scattering (Braganza and Worcester, 1986; Winter and Pilgrim, 1989), is that the application of hydrostatic pressure increases bilayer thickness while reducing area per lipid. This effect can also be inferred from NMR results. Fig. 4 shows ^{2}H-NMR spectra of DMPC-d_{54} and DPPC-d_{62} for a series of applied pressures at 65°C. The sharp edges in the Pake doublet produced by deuterons at a specific site in the molecule arise from those molecules reorienting around axes perpendicular to the applied magnetic field. The splitting of these features is given by (Davis, 1983)

$$\Delta\nu_q = \frac{3}{4}\frac{e^2qQ}{h}S_{CD} \quad (1)$$

where e^2qQ/h is the quadrupole coupling, and the orientational order parameter S_{CD} is given by

$$S_{CD} = \frac{1}{2}\langle 3\cos^2\Theta_{CD} - 1\rangle. \quad (2)$$

In Eq. 2, Θ_{CD} is the angle between the carbon-deuterium bond and the bilayer normal, and the average is taken over orientations sampled by the carbon-deuterium bond. The orientational order parameter can be related to the contribution from a given methylene group to the mean extension of the chain in the direction of the bilayer normal (Schindler and Seelig, 1975). For a perdeuterated chain, the first spectral moment is proportional to the quadrupole splitting averaged over the deuterated sites in the chain and can thus be related to mean extension of the whole chain. Fig. 5 shows the pressure dependence of the first spectral moment (M_1) for DMPC-d_{54} and DPPC-d_{62} at 65°C. The increase in M_1 with pressure at fixed temperature indicates a pressure-induced ordering of the acyl chains, which presumably reflects a decrease, with pressure, of the fluid L_α phase area per lipid. Using a simple model (Schindler and Seelig, 1975; Morrow et al., 1992), the observed changes in M_1 are found to correspond to an increase in the mean extension by about 2.5% for DPPC over the 1 kbar pressure range examined

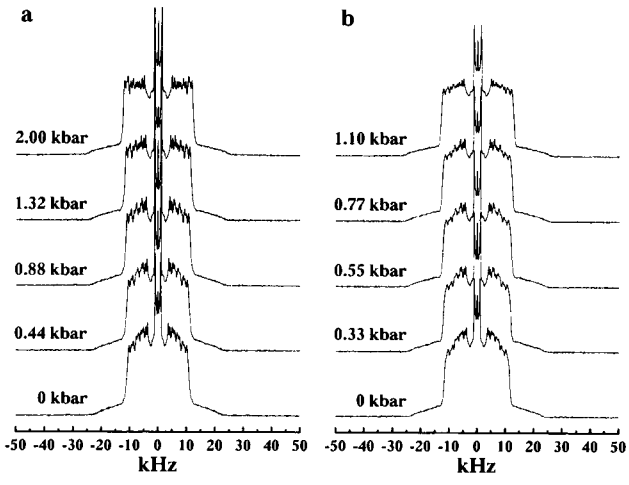

FIGURE 4 (a) ^{2}H-NMR spectra of DMPC-d_{54} for a series of applied hydrostatic pressures at 65°C; (b) ^{2}H-NMR spectra of DPPC-d_{62} for a series of applied hydrostatic pressures at 65°C.

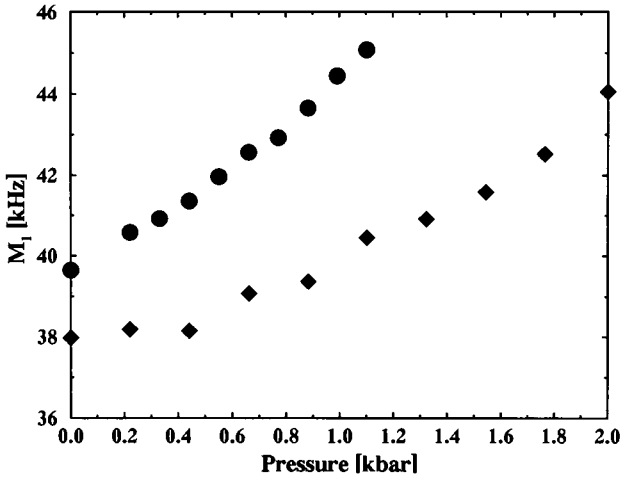

FIGURE 5 Pressure dependence of the first spectral moment (M_1) for spectra of (♦) DMPC-d_{54} and (●) DPPC-d_{62} obtained at T = 65°C.

and by about 3.5% for DMPC over a pressure range of 2 kbar. It is reasonable to assume that the pressure-induced change in fluid phase headgroup conformation and the increased chain ordering are both responses to steric constraints arising from a reduced area per lipid. It is interesting that the application of pressure changes the α and β deuteron splittings in directions opposite to those seen for decreasing hydration. This seems to argue against the possibility that the effects reported here might reflect a pressure-induced dehydration of the headgroup region.

It is significant that for both M_1 and the headgroup deuteron splittings the sensitivity to pressure is larger in DPPC than in DMPC. This presumably reflects a difference between the lateral response to hydrostatic pressure of the two bilayers at a common temperature. At ambient pressure, the main transition of DMPC-d_{54} is at about 19°C. For temperatures above 19°C, the transition from the fluid phase to the gel phase can be induced by increasing pressure. It is found that for higher temperatures, the magnitude of the jump in mean order at the pressure-induced transition increases slightly, and the sensitivity of M_1 to pressure in the fluid phase decreases slightly (B. Bonev and M. R. Morrow, unpublished observation). In effect, the lateral compression of the fluid bilayer in response to hydrostatic pressure decreases with increasing temperature above the ambient pressure transition temperature. Presumably, the weaker response to pressure of the DMPC headgroup deuteron quadrupole splittings reflects a smaller lateral response to hydrostatic pressure for that bilayer at 65°C.

The change in headgroup conformation resulting from alteration of membrane surface charge is often characterized by plotting the change in β deuteron splitting versus the change in α deuteron splitting (Akutsu and Seelig, 1981; Scherer and Seelig, 1989; Roux et al., 1989; Roux and Bloom, 1990; Bechinger and Seelig, 1991; Macdonald et al., 1991; Rydall and Macdonald, 1992; Pinheiro et al, 1994). The slopes of such α versus β correlation plots are found to cluster around −1 and −0.6 for negative and positive surface charge, respectively (Scherer and Seelig, 1989). For conformational changes driven by changes in the hydration state of the bilayer, the slope of the α versus β correlation plot is reported to be −0.76 (Bechinger and Seelig, 1991). The negative slope is taken as evidence of conformational change rather than ordering or disordering. The dependence of the slope magnitude on surface charge sign has been taken to indicate differences in the detailed nature of the interaction for positive and negative surface charge (Scherer and Seelig, 1989). Fig. 6 shows the correlation between changes in α and β deuteron splittings resulting from application of pressure at 65°C. Linear fits give slopes of −1.31 and −1.46 for DMPC and DPPC, respectively. The observed correlation supports the argument that pressure alters headgroup conformation rather than order. It is interesting that the magnitudes of the slopes are larger than those seen for either positive or negative surface charge or changes in hydration. This suggests that the nature of the conformational change induced by pressure is slightly different from that induced by either positive or negative surface charge or changes in hydration.

Another aspect of headgroup behavior that has been noted previously is the difference between the responses of the α and β deuteron quadrupole splittings to temperature. Akutsu and Seelig (1981) observed that the β deuteron splitting decreases with increasing temperature, and the α splitting is effectively unchanged. It has been suggested that increasing temperature increases torsional motion around the C_α-C_β bond. Fig. 7 *a* and *b* show DMPC-d_{54} headgroup deuteron spectra at ambient pressure and 1.5 kbar for selected temperatures. Fig. 8 displays the temperature dependence of the DMPC-d_4 headgroup deuteron splittings for ambient pressure and 1.5 kbar. The relative sensitivity of the headgroup deuteron splittings to temperature is only weakly affected by the application of pressure. Nevertheless, there is a slight decrease in the sensitivity of the β deuteron

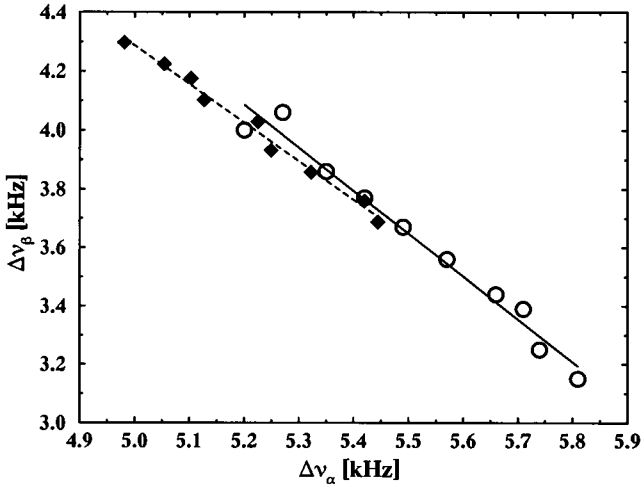

FIGURE 6 β deuteron quadrupole splitting versus α deuteron quadrupole splitting plot at 65°C for (♦) DMPC-d_{54} and (○) DPPC-d_{62}.

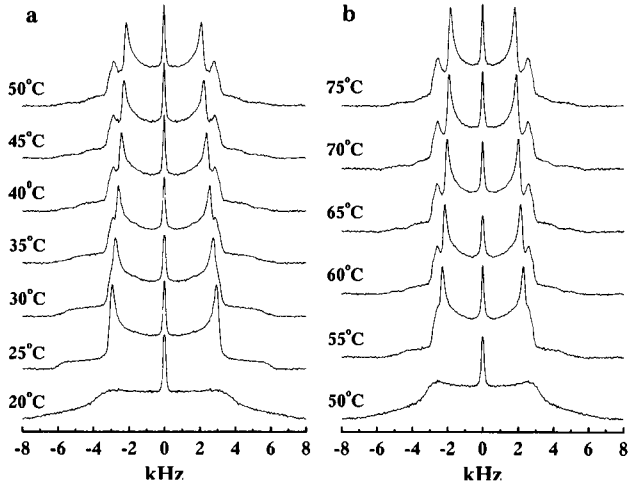

FIGURE 7 (a) ^{2}H-NMR spectra of DMPC-d_4 at ambient pressure for selected temperatures; (b) ^{2}H-NMR spectra of DMPC-d_4 at 1.5 kbar for selected temperatures.

splitting to temperature and a small but nonzero temperature dependence of the α deuteron splitting at elevated pressure.

The relatively weak response of the α deuteron splitting to temperature compared with the higher sensitivity of the β deuteron splitting may indicate that increasing temperature results in a headgroup conformational change that either reinforces or diminishes the effect of temperature-induced headgroup disorder. Because area per lipid molecule increases with temperature, one conceivable conformational change is a tilt of the headgroup toward the bilayer surface. The resulting increase in α deuteron splitting could then be offset by an increase in headgroup disorder, whereas the decrease in β deuteron splitting could be reinforced by increased headgroup disorder. If this is the case at ambient pressure, then a decrease in the lateral thermal expansion of the bilayer with increasing pressure could account for the slight difference in the temperature dependence of the headgroup deuteron splittings at higher pressure. Unpublished results from this laboratory suggest that elevated pressure does indeed slightly reduce the effect of temperature on area per lipid, but clarification of this point would require a more thorough investigation of the temperature dependence of headgroup deuteron splittings at elevated pressure.

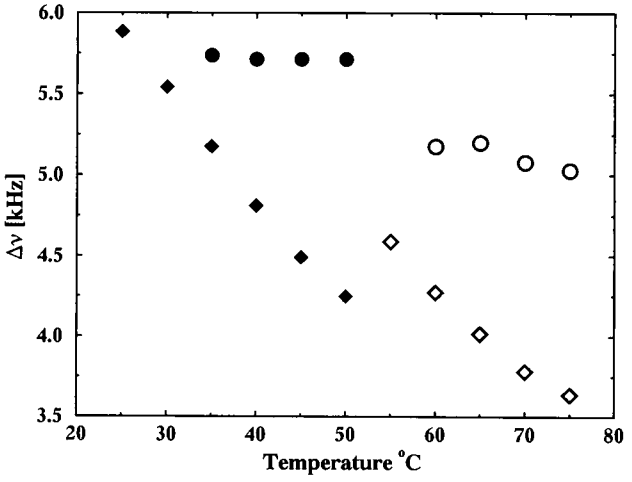

FIGURE 8 The temperature dependence of headgroup deuteron quadrupole splittings at ambient pressure (solid symbols) and 1.5 kbar (open symbols) for the α (diamonds) and β (circles) headgroup deuterons.

CONCLUSIONS

In DMPC and DPPC bilayers, application of hydrostatic pressure reduces the quadrupole splitting of α headgroup deuterons and increases the quadrupole splitting of β headgroup deuterons. These counterdirectional changes in α and β splittings suggest that the headgroup response to pressure is primarily a change in conformation rather than a change in headgroup orientational order. On the basis of a comparison with the effect of membrane surface charge on headgroup orientation, it seems that an increase in hydrostatic pressure results in a tipping of the headgroup away from the bilayer surface. This is consistent with the reduction in area per lipid with increasing pressure implied by the isothermal pressure dependence of mean order in chain perdeuterated DMPC and DPPC. The effect of pressure on the headgroup conformation, as indicated by the headgroup deuterons, is analogous to the effect of membrane surface charge. By using hydrostatic pressure to continuously vary headgroup orientation in a single sample, it may be possible to obtain useful insights into the mechanical properties of the headgroup region of the bilayer and into the mechanism for surface charge-induced changes in phospholipid headgroup conformation.

This work was supported by the Natural Sciences and Engineering Research Council of Canada. The authors are indebted to William Kieley for his technical assistance in the construction of the high pressure probe and to Juli Atherton for her help in data collection and analysis.

REFERENCES

Akutsu, H., and J. Seelig. 1981. Interaction of metal ions with phosphatidylcholine bilayer membranes. *Biochemistry.* 20:7366–7373.

Bechinger, B., and J. Seelig. 1991. Conformational changes of the phosphatidylcholine headgroup due to membrane dehydration. A ^{2}H-NMR study. *Chem. Phys. Lipids.* 58:1–5.

Braganza, L. F., and D. L. Worcester. 1986. Hydrostatic pressure induces hydrocarbon chain interdigitation in single-component phospholipid bilayers. *Biochemistry.* 25:2591–2595.

Büldt, G, H. U. Gally, J. Seelig, and G. Zaccai. 1979. Neutron diffraction studies on phosphatidylcholine model membranes. I. Head group conformation. *J. Mol. Biol.* 134:673–691.

Davis, J. H. 1983. The description of membrane lipid confromation, order, and dynamics by ^{2}H-NMR. *Biochim. Biophys. Acta.* 737:117–171.

Davis, J. H., K. R. Jeffrey, M. Bloom, M. I. Valic, and T. P. Higgs. 1976. *Chem. Phys. Lett.* 42:390–394.

Dempsey, C., M. Bitbol, and A. Watts. 1989. Interaction of melittin with mixed phospholipid membranes composed of dimyristoylphosphatidylcholine and dimyristoylphosphatidylserine studied by deuterium NMR. *Biochemistry.* 28:6590–6596.

Driscoll, D. A., S. Samarasinghe, S. Adamy, J. Jonas, and A. Jonas. 1991a. Pressure effects on dipalmitoylphosphatidylcholine bilayers measured by ^{2}H nuclear magnetic resonance. *Biochemistry.* 30:3322–3327.

Driscoll, D. A., J. Jonas, and A. Jonas. 1991b. High pressure ^{2}H nuclear magnetic resonance study of the gel phases of dipalmitoylphosphatidylcholine. *Chem. Phys. Lipids.* 58:97–104.

Gally, H.-U., W. Niederberger, and J. Seeling. 1975. Conformation and motion of the choline head group in bilayers of dipalmitoyl-3-*sn*-phosphatidylcholine. *Biochemistry.* 14:3647–3652.

Hauser, H., I. Pascher, R. H. Pearson, and S. Sundell. 1981. Preferred conformation and molecular packing of phosphatidylethanolamine and phosphatidylcholine. *Biochim. Biophys. Acta.* 650:21–51.

Jonas, J., C.-L. Xie, A. Jonas, P. J. Grandinetti, D. Campbell, and D. Driscoll. 1988. High-resolution ^{13}C NMR study of pressure effects on the main phase transition in L-α-dipalmitoyl phosphatidylcholine vesicles. *Proc. Natl. Acad. Sci. USA.* 85:4115–4117.

Kuchinka, E., and J. Seelig. 1989. Interaction of melittin with phosphatidylcholine membranes. Binding isotherm and lipid head-group conformation. *Biochemistry.* 28:4216–4221.

Macdonald, P. M., and J. Seelig. 1987. Calcium binding to mixed cardiolipin-phosphatidylcholine bilayers as studied by deuterium nuclear magnetic resonance. *Biochemistry.* 26:6292–6298.

Macdonald, P. M., and J. Seelig. 1988. Anion binding to neutral and positively charged lipid membranes. *Biochemistry.* 27:6769–6775.

Macdonald, P. M., J. Leisen, and F. M. Marassi. 1991. Response of phosphatidylcholine in the gel and liquid-crystalline states to membrane surface charges. *Biochemistry.* 30:3558–3566.

Marassi, F. M., and P. M. Macdonald. 1991. Response of the headgroup of phosphatidylglycerol to membrane surface charge as studied by deuterium and phosphorus-31 nuclear magnetic resonance. *Biochemistry.* 30:10558–10566.

Marassi, F. M., and P. M. Macdonald. 1992. Response of the phosphatidylcholine headgroup to membrane surface charge in ternary mixtures of neutral, cationic, and anionic lipids: a deuterium NMR study. *Biochemistry.* 31:10031–10036.

Morrow, M. R. 1990. Transverse nuclear spin relaxation in phosphatidylcholine bilayers containing gramicidin. *Biochim. Biophys. Acta.* 1023: 197–205.

Morrow, M. R., J. P. Whitehead, and D. Lu. 1992. Chain-length dependence of lipid properties near the liquid crystal to gel phase transition. *Biophys. J.* 63:18–27.

Morrow, M. R., S. Taneva, G. A. Simatos, L. A. Allwood, and K. M. W. Keough. 1993. ^{2}H NMR studies of the effect of pulmonary surfactant SP-C on 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine headgroup: a model for transbilayer peptides in surfactant and biological membranes. *Biochemistry.* 32:11338–11344.

Peng, X., and J. Jonas. 1992. High-pressure ^{31}P NMR study of dipalmitoylphosphatidylcholine bilayers. *Biochemistry.* 31:6383–6390.

Pinheiro, T. J. T., A. A. Duralski, and A. Watts. 1994. Phospholipid headgroup-headgroup electrostatic interactions in mixed bilayers of cardiolipin with phosphatidylcholines studied by ^{2}H NMR. *Biochemistry.* 33:4896–4902.

Prosser, R. S., J. H. Davis, F. W. Dahlquist, and M. A. Lindorfer. 1991. ^{2}H nuclear magnetic resonance of the gramicidin A backbone in a phospholipid bilayer. *Biochemistry.* 30:4687–4696.

Roux, M., J.-M. Neumann, M. Bloom, and P. F. Devaux. 1988. ^{2}H and ^{31}P NMR study of pentalysine interaction with headgroup deuterated phosphatidylcholine and phosphatidylserine. *Eur. Biophys. J.* 16:267–273.

Roux, M., J.-M. Neumann, R. S. Hodges, P. F. Devaux, and M. Bloom. 1989. Conformational changes of phospholipid headgroups induced by a cationic integral membrane peptide as seen by deuterium magnetic resonance. *Biochemistry.* 28:2313–2321.

Roux, M., and M. Bloom. 1990. Ca^{2+}, Mg^{2+}, Li^+, Na^+, and K^+ distributions in the headgroup region of binary membranes of phosphatidylcholine and phosphatidylserine as seen by deuterium NMR. *Biochemistry.* 29:7077–7089.

Rydall, J. R., and P. M. Macdonald. 1992. Influence of staphylococcal δ-toxin on the phosphatidylcholine headgroup as observed using ^{2}H-NMR. *Biochim. Biophys. Acta.* 1111:211–220.

Scherer, P. G., and J. Seelig. 1989. Electric charge effects on phospholipid headgroups. Phosphatidylcholine in mixtures with cationic and anionic amphiphiles. *Biochemistry.* 28:7720–7728.

Schindler, H., and J. Seelig. 1975. Deuterium order parameters in relation to thermodynamic properties of a phospholipid bilayer. A statistical mechanical interpretation. *Biochemistry.* 14:2238–2287.

Seelig, J., P. M. Macdonald, and P. G. Scherer. 1987. Phospholipid head groups as sensors of electric charge in membranes. *Biochemistry.* 26: 7535–7541.

Winter, R., and W.-C. Pilgrim. 1989. A SANS study of high pressure phase transitions in model membranes. *Ber. Bunsen-ges. Phys. Chem.* 93: 708–717.