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Effects of Hydrostatic Pressure on the Molecular Structure and Endothermic Phase Transitions of Phosphatidylcholine Bilayers: A Raman Scattering Study[†]

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ABSTRACT: The temperature dependences of the Raman spectra of aqueous dispersions of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) were monitored at different but constant pressures between 1 and 1210 bar. The changes observed in these Raman spectra are discussed in terms of the effects of high pressure on the phase state and molecular structure of lipid bilayers. It is demonstrated that the temperature of the endothermic gel to liquid-crystal phase transition, as well as the temperature of the pretransition, increases linearly with increasing hydrostatic pressure. The dT_m/dP values obtained from a wide range of pressures are 20.8 °C·kbar^{-1} for DPPC and 20.1 °C·kbar^{-1} for DMPC. The dT_p/dP value for DPPC is 16.2 °C·kbar^{-1}. It is also shown that the volume change that occurs at the gel to liquid-crystal transition is not constant; i.e., $d\Delta V_m/dP$ decreases by 6.2% (DPPC) or 6.3% (DMPC) per kilobar pressure. The volume change at the pretransition is also pressure dependent; the $d\Delta V_p/dP$ value of DPPC decreases by 4.7% per kilobar pressure.

Interest in the structure of biological membranes under elevated pressure has grown rapidly during the last decade (Macdonald, 1984; Wong, 1984; Yager & Peticolas, 1982; Heremans, 1982; Wann & Macdonald, 1980; Woodhouse, 1976; Trudell et al., 1974). While the experimental pressure range of interest, which also coincides with the pressure range experienced by marine organisms, is generally within 1–1000 bar (1 bar = 0.9869 atm = 101.3 kPa), studies have been performed on lipid membranes at pressures up to 32 000 bar (Wong & Mantsch, 1984).

The effect of pressure on lipid membranes is of particular interest to the study of anesthetics since the action of anesthetics can be antagonized by hydrostatic pressure (Johnson & Flagler, 1950; Lever et al., 1971; Smith et al., 1984). As anesthetics depress the transition temperature and "fluidize" the lipid bilayer, the antagonizing effect of pressure is generally considered to result from an elevation of the transition temperature and from changes induced in the dynamic structure of the lipid matrix (Trudell et al., 1975; Macdonald, 1978; Kamaya et al., 1979; Mountcastle et al., 1978).

The Raman spectroscopic technique is particularly sensitive to trans–gauche isomerizations in polymethylene chains and

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has been widely employed to monitor conformational changes in lipid bilayers [for reviews, see Wallach et al. (1979); Lord & Mendelsohn (1981), and Wong (1984)]. Thus, it was recently reported that clinical concentrations of anesthetics have no significant effect on the lipid chain conformation (Lieb et al., 1982). Furthermore, there are also Raman spectroscopic features sensitive to the dynamic properties and interchain interactions in lipid membranes. Therefore, in the present work, we use Raman spectroscopy to address the following question: "How does pressure affect the thermotropic phase transitions of aqueous phospholipid membranes and the changes in the dynamic and structural properties associated with these transitions?"

EXPERIMENTAL PROCEDURES

Aqueous dispersions of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC)[1] and DPPC (Sigma Chemical Co.), containing respectively 40 and 44 wt % double-distilled water, were prepared by heating the individual lipid–water mixture in a closed vial to about 70 °C, vortexing the heated sample, and allowing it to cool to room temperature. The heating–cooling cycle was repeated several times. The samples were placed in a quartz holder (Wong, 1981) which was mounted on a window plug fitted with a quartz window in a homemade hydrostatic pressure optical cell. The pressure transmitting fluid was naphtha. The pressure in the cell was generated directly with a 3-kbar Enerpac head pump and measured with a calibrated Heise–Bourdon gauge. An insulated jacket with "O" ring seals was mounted on the optical cell and a thermostated ethylene glycol–water mixture circulated between the jacket and the optical cell. The sample temperatures were measured with a thermocouple embedded in the optical cell near the sample holder.

The Raman spectra were excited with the 514.5-nm line from a CRL Model 12 argon ion laser with a power of 200 mW at the sample and collected with a Spex Model 14018 double monochromator using a cooled RCA C31034 photomultiplier. A backscattering geometry was employed. The data acquisition was performed with a Spex DM1 Datamate processor at a resolution of 2 cm^{-1}; the spectral data were then transferred onto magnetic tape, and frequency and spectral sensitivity calibrations were applied to each spectrum. Frequencies (as center of gravity), bandwidths, and peak heights were calculated by using software developed in this laboratory (Cameron et al., 1982; Wong et al., 1982).

RESULTS AND DISCUSSION

Pressure Dependence of the Endothermic Phase Transitions of DPPC and DMPC. At atmospheric pressure, the temperature of the gel to liquid-crystal phase transition, T_m, of fully hydrated DMPC dispersions is 24 °C, while that of DPPC is 41.5 °C (Mabrey & Sturtevant, 1976; Lenz et al., 1978; Nagle & Wilkinson, 1978; Silvius et al., 1979). As this phase transition exhibits first-order characteristics, T_m is expected to increase at higher pressure. Illustrated in Figure 1 are two Raman spectra of aqueous DPPC bilayers recorded at 64.2 and 78.6 °C at a hydrostatic pressure of 1210 bar. The spectrum at 78.6 °C is characteristic of the liquid-crystalline phase, while the spectrum at 64.2 °C is typical of the gel phase (Wong, 1984). Consequently, at a pressure of 1210 bar and a temperature of 64.2 °C (i.e., a temperature 22.7 °C above the critical temperature at atmospheric pressure), DPPC is still in the gel state. Pressure thus opposes the chain melting phase transition.

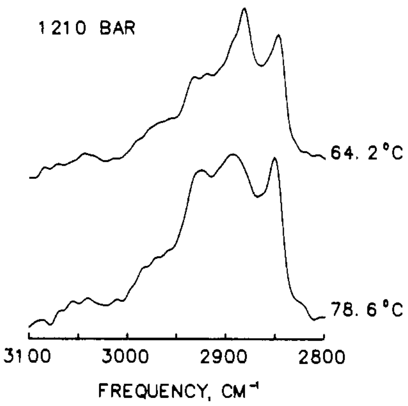

FIGURE 1: Representative Raman spectra of the C–H stretching region of aqueous DPPC dispersions recorded at a hydrostatic pressure of 1210 bar at 64.2 and 78.6 °C.

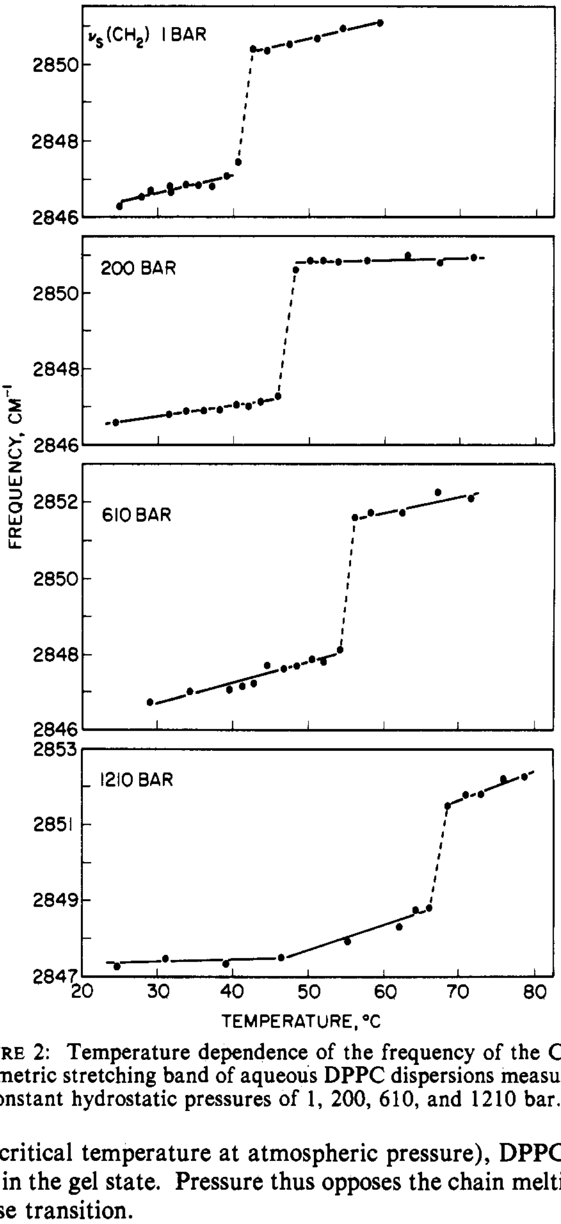

FIGURE 2: Temperature dependence of the frequency of the CH_2 symmetric stretching band of aqueous DPPC dispersions measured at constant hydrostatic pressures of 1, 200, 610, and 1210 bar.

In order to determine the detailed pressure dependence of T_m, we have monitored the thermotropic phase behavior of DMPC and DPPC at several pressures. Figure 2 illustrates the temperature profiles of the frequency of the CH_2 symmetric stretching band of DPPC at different pressures. This vibrational mode is very sensitive to conformational changes

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[1] Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; T_m, temperature of the gel to liquid-crystal phase transition; T_p, temperature of the pretransition.

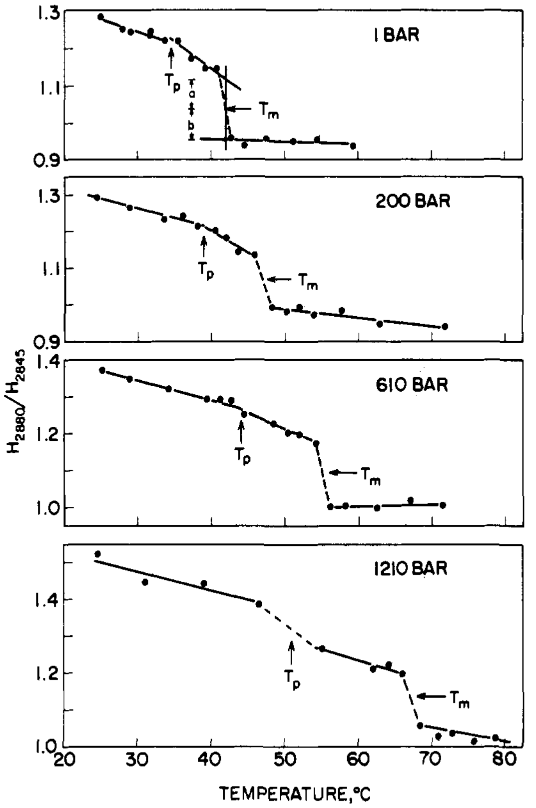

FIGURE 3: Temperature dependence of the peak height ratio between the CH_2 antisymmetric and symmetric stretching bands of DPPC measured at 1, 200, 610, and 1210 bar. T_m was obtained as the temperature at the midpoint of the discontinuities, i.e., $a = b$.

of the hydrocarbon chains and is conveniently used to characterize T_m in model membranes, though it is less sensitive to the structural changes at T_p (Wong, 1984; Casal & Mantsch, 1984). It is immediately evident from the data in Figure 2 that T_m, which is marked by a sharp increase in frequency, shifts to higher temperatures as the hydrostatic pressure is raised. Other Raman bands show similar discontinuities in the frequency vs. temperature plots recorded at different pressures. It is worth noting here that the absolute frequency values in Figure 2, measured at a reduced temperature (e.g., 5 °C below and 5 °C above T_m), increase slightly with pressure, both in the gel and in the liquid-crystalline phases.

A Raman spectral parameter often used to characterize T_m and T_p in aqueous lipid dispersions is the temperature dependence of the intensity (peak height) ratio between the CH_2 antisymmetric mode (2880 cm^{-1} band) and the corresponding CH_2 symmetric mode (2845 cm^{-1} band). Earlier studies (Snyder et al., 1978; Gaber & Peticolas, 1977; Huang et al., 1982; Mendelsohn & Koch, 1982; Wong et al., 1982; Wong & Mantsch, 1983, 1984) have demonstrated that this ratio represents a qualitative measure of the amount of interchain interactions in hydrocarbons, phospholipids, or aqueous surfactants. Figure 3 illustrates a plot of this ratio in DPPC, measured as a function of temperature at four different pressures. A clear discontinuity in the temperature dependence of this ratio is observed for each pressure at T_m, while T_p is marked by a change in slope.

The critical temperatures of the two endothermic transitions in DPPC obtained at various pressures are plotted against pressure in Figure 4 (bottom panel). A linear pressure dependence of both T_m and T_p is evident from these plots. The slopes of the transition temperatures vs. pressure yield a dT_m/dP value of 20.8 °C·$kbar^{-1}$ for the gel to liquid-crystal

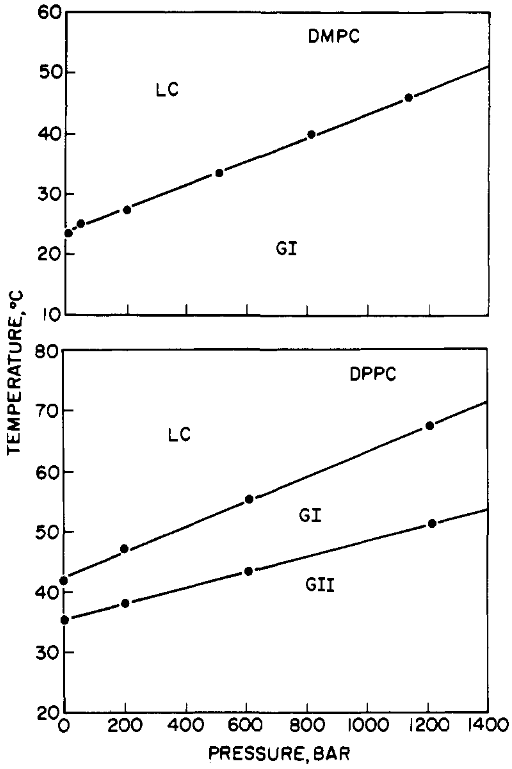

FIGURE 4: (Bottom panel) Pressure dependence of the critical temperatures T_m and T_p in DPPC: LC, liquid-crystalline phase; GI and GII, gel I and gel II phases [for nomenclature, see Wong (1984)]. (Top panel) Pressure dependence of the critical temperature at T_m in DMPC.

phase transition and a dT_p/dP value of 16.2 °C·$kbar^{-1}$ for the pretransition in DPPC.

The thermotropic phase behavior of fully hydrated DMPC bilayers at different pressures is similar to that of DPPC. As illustrated in Figure 4 (top panel), a linear pressure dependence of T_m is again observed in this system, and the dT_m/dP value for aqueous DMPC dispersions is 20.1 °C·$kbar^{-1}$, which is similar though slightly smaller than that of DPPC. We did not determine dT_p/dP for DMPC, but in view of the similar dT_m/dP values in DPPC and DMPC, we expect it to be close to that in DPPC. A dT_m/dP value of 40 °C·$kbar^{-1}$ had been reported previously for DMPC (Wong et al., 1982); however, this value was derived from the pressure-induced liquid-crystal to gel phase transition at 30 °C, whereas the present value of 20.1 °C·$kbar^{-1}$ was obtained by measuring the temperature-induced phase transition between the gel and liquid-crystalline phases at constant but different pressures.

Effect of Pressure on the Structural Changes at Temperatures near T_m. As seen from Figure 3, the interchain interactions in the gel phase decrease with increasing temperature, the rate of decrease being similar at various pressures; however, the slope is steeper in the gel phase between T_p and T_m. The decrease in the magnitude of interchain interactions in the gel phase is the result of an increase in the van der Waals radii of the chains and an increase in the rate of interchain reorientational fluctuations with an increase in temperature (Wong, 1984). It is also evident from Figure 3 that at constant temperature the interchain interactions in the gel phase are enhanced by pressure; this is the result of interchain compression and the damping of interchain reorientational fluctuations (Wong et al., 1982). The interchain interactions decrease sharply at T_m and become much weaker in the liquid-crystalline phase due to the drastic increase in the orientational mobility and the increase in the hard-core radii of the

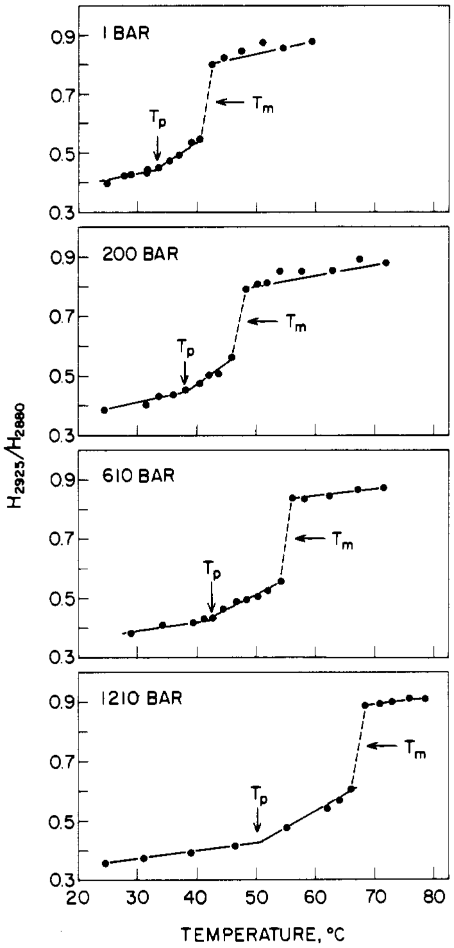

FIGURE 5: Temperature dependence of the peak height ratio between the disorder-allowed band at 2925 cm^{-1} and the CH_2 antisymmetric stretching band at 2880 cm^{-1} of DPPC measured at several pressures.

acyl chains in the liquid-crystalline phase as a result of the larger number of gauche rotamers present in this phase. The average cross-sectional area of the two acyl chains of each molecule in the liquid-crystalline phase is about 10 $Å^2$ larger than that in the gel phase (Ruocco & Shipley, 1982).

The interchain interactions, in both the gel and the liquid-crystalline phases at temperatures adjacent to T_m, are stronger at higher pressure. High pressure alone is expected to compress the interchain distance and to damp the reorientational fluctuations and thus enhance the interchain interactions. However, at higher pressure, the critical temperature T_m also shifts to higher temperatures; yet as mentioned above, the interchain interactions become weaker at higher temperature. Consequently, the present results indicate that at T_m the pressure-enhanced interchain interactions are not completely compensated by the reduction of interchain interactions arising from the increase in the transition temperature.

There are many factors that contribute to the temperature-induced shift of vibrational frequencies in condensed phase spectra. Among these, the contributions from the thermal strain, the volume-independent self-energy, and the intermolecular interactions are the most significant factors (Wong, 1975; Stinshov & Popova, 1961; Gurman, 1971). Strong intermolecular interactions will polarize and weaken the intramolecular interactions and thus decrease the intramolecular stretching frequencies. By comparing the temperature-dependent behavior of the $\nu_s(CH_2)$ frequency at different pressures (Figure 2) with that of the interchain interactions at identical pressures (Figue 3), it becomes apparent that at higher pressures the contribution from the interchain interactions plays the major role in the frequency shift at temperatures near the gel to liquid-crystal transition. At T_m, the interchain interactions decrease abruptly, and thus the frequency of the $\nu_s(CH_2)$ band increases dramatically.

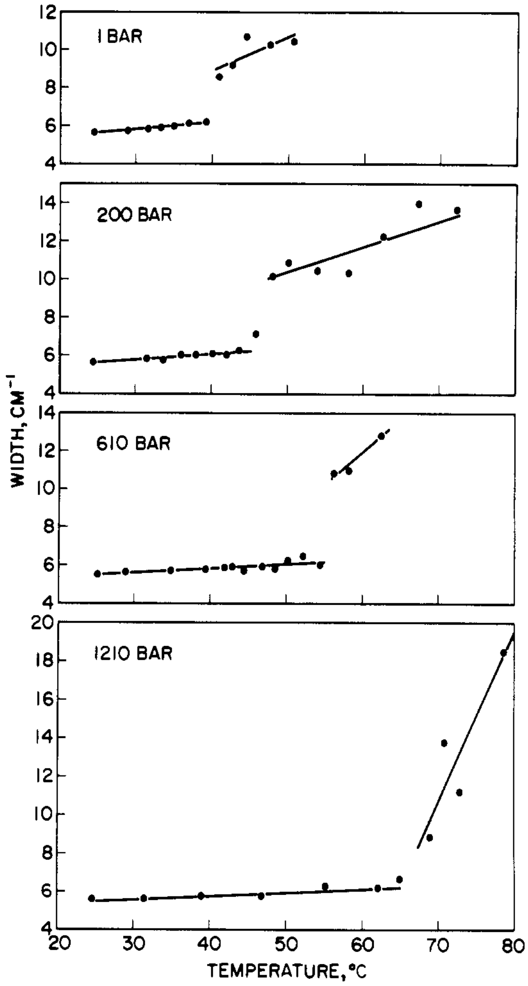

FIGURE 6: Temperature dependence of the full width at 0.8 peak height of the CH_2 twisting band of DPPC measured at several pressures.

A popular parameter for estimating the relative population of gauche conformers in lipid bilayers is the peak height ratio between the disorder-allowed CH_2 stretching band near 2925 cm^{-1} and the Raman-active CH_2 antisymmetric stretching band near 2880 cm^{-1} (Bunow & Levin, 1972; Gaber et al., 1978). The temperature dependence of this parameter at different pressures is illustrated in Figure 5 for DPPC. The plot shows that this ratio, and thus the number of gauche conformers, increases drastically at the transition from the gel phase to the liquid-crystalline phase. However, the number of gauche conformers in the liquid-crystalline phase near T_m is smaller at 1 and 200 bar compared to that at 610 and 1210 bar due to the lower T_m at lower pressures. Furthermore, at a pressure of 1210 bar, the number of gauche conformers in the gel phase before the transition to the liquid-crystalline phase is noticeably larger than that at lower pressure. On the other hand, at constant temperature, the number of gauche conformers in the gel phase decreases with increasing pressure.

Another spectral parameter, the width of depolarized Raman bands, has been widely used as a measure of the large-angle interchain reorientational mobility in lipid assemblies (Wong, 1984). Figure 6 shows the temperature dependence of the width of the depolarized CH_2 twisting band at four different pressures. In the gel phase, the width of this band, and thus the reorientational mobility of the acyl chains, increases slightly with increasing temperature at different pressures and reaches the same value just before the phase transition at T_m. In the liquid-crystalline phase, the magnitude of the reorientational mobility increases drastically and increases further with temperature, the rate of increase being faster at higher pressures.

Effect of Pressure on the Volume Change at the Critical Temperatures T_m and T_p. The main phase transition in lipid bilayers involves an increase in the molar volume, associated with the increase in intermolecular spacing and the positive enthalpy change at T_m [for a review, see Macdonald (1984)]. The source of the volume change is a loosening in the packing of the acyl chains along with a small contribution from head-group hydration and an increase in the hard-core radii of the chains (Nagle & Wilkinson, 1978).

From the Clausius–Clapeyron relationship, it follows that at T_m

$$dT_m/dP = \Delta V_m/\Delta S_m$$

where dT_m/dP is the change in the transition temperature T_m for a given increase in pressure P, ΔV_m is the molar volume change at T_m, and $\Delta S_m = \Delta H_m/T_m$. As already shown, DPPC and DMPC bilayers above their chain melting transition temperature become more ordered by an increase in pressure. In other words, an increase in pressure at constant temperature ($T \geq T_m$) will cause an isothermal transition from the liquid-crystalline to the gel phase. The susceptibility of a given lipid to this effect of pressure is determined by their $\Delta V_m/\Delta H_m$ values.

The enthalpy change of aqueous lipid dispersions at T_m at ambient pressure has been measured extensively [for a review, see Silvius (1982)]. The experimental ΔH_m values generally accepted for DPPC and DMPC are 8.74 and 5.44 kcal·mol^{-1}, respectively (Mabrey & Sturtevant, 1978). With these ΔH_m values, and the dT_m/dP and T_m values obtained in the present study, we have determined the volume change, ΔV_m, at this endothermic phase transition in DPPC and DMPC as a function of pressure.

Illustrated in Figure 7 is a plot of ΔV_m vs. pressure over a range of 1210 bar. The plots for DPPC and DMPC demonstrate a linear dependence of the volume change at T_m with pressure; $d\Delta V_m/dP$ decreases by 6.2% and 6.3% per kilobar pressure for DPPC and DMPC, respectively. The main transition in DPPC has been studied at medium–high pressures by differential scanning calorimetry and dilatometry measurements (Liu & Kay, 1977; Macdonald, 1978; Mountcastle et al., 1978) which indicated that ΔH_m was constant between 1 and 138 bar and ΔV_m was independent of pressure over the range 1–304 bar. Our observation of a small but finite decrease in the volume change at T_m over the pressure range 1–1210 bar (which assumes that ΔH_m is constant) could also be interpreted as indicating that ΔH_m increases with pressure (i.e., $d\Delta H_m/dP = 0.58$ kcal·mol^{-1}·kbar^{-1}) while ΔV_m is constant, or that both ΔH_m and ΔV_m change.

An analysis of the spectral changes at the gel to liquid-crystal transition at different pressures shows that at higher pressure the orientational mobility increases less and the decrease in interchain interactions is smaller. The smaller frequency shift of $\nu_s(CH_2)$ at the phase transition at higher pressure is consistent with these observations. Thus, both the changes in the orientational mobility and the interchain interactions indicate that at higher pressure the loosening of the chain packing at T_m is smaller. Moreover, the population of gauche conformers in the gel phase near T_m is considerably larger at 1210 bar, and thus the volume of the gel phase at T_m is larger at higher pressure. Consequently, it is expected that the volume change at the transition is smaller at higher pressure. Furthermore, the enthalpy change associated with these structural and dynamic changes at T_m should be smaller, not larger, at high pressure. Therefore, the present results strongly favor a decrease in the critical volume change at T_m, rather than an increase in ΔH_m at higher pressures.

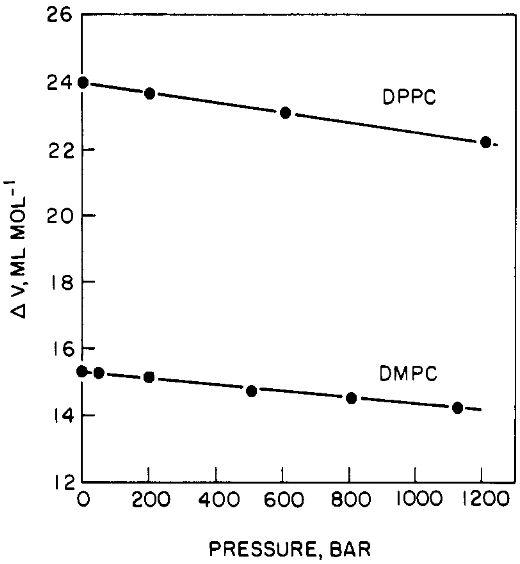

FIGURE 7: Pressure dependence of the critical volume changes at T_m in fully hydrated bilayers of DPPC and DMPC.

It should be noted that Chen & Sturtevant (1981) also reported unusually small ΔH_m values for "resynthesized" lipids, i.e., 6.43 kcal·mol^{-1} for DPPC and 5.04 kcal·mol^{-1} for DMPC. When these ΔH_m values are used in our calculation, the absolute values of ΔV_m change to 17.8 and 14.3 mL·mol^{-1} for DPPC and DMPC, respectively, at atmospheric pressure; nevertheless, the slopes in Figure 7 remain unaffected.

Finally, since in the case of DPPC we had also determined the pressure dependence of the pretransition, we were able to obtain a value for $d\Delta V_p/dP$. By using the relationship

$$dT_p/dP = \Delta V_p T_p/\Delta H_p$$

where dT_p/dP is the change in the pretransition temperature for a given increase in pressure, T_p is the pretransition temperature at a given pressure P, and $\Delta H_p = 1.8$ kcal·mol^{-1} (Mabrey & Sturtevant, 1976), one obtains a linear plot of ΔV_p vs. pressure, similar to that in Figure 7. The absolute value of ΔV_p decreases from 3.16 mL·mol^{-1} at atmospheric pressure to 3.01 mL·mol^{-1} at 1210 bar, which translates to a decrease of the volume change at T_p of 4.7% per kilobar pressure. As in the case of ΔV_m, independent calorimetric measurements at high pressures would have to establish whether ΔH_p is indeed constant or whether the change of ΔV_p really reflects a change of ΔH_p with pressure.

CONCLUSIONS

The present investigation illustrates the usefulness of the Raman spectroscopic technique for studying the effects of pressure on the critical phenomena in aqueous lipid systems. Besides being able to establish the pressure-induced shifts of the critical temperatures, it provides information regarding the effect of pressure on the conformational changes, the interchain interactions, the reorientational dynamics, and the volume changes at T_m and T_p as a function of temperature and pressure.

As higher pressure decreases, whereas higher temperature increases, the number of gauche conformers in the acyl chains, the present results suggest that the temperature effect dominates the pressure effect on the conformational changes near the gel to liquid-crystal phase transition. On the other hand, as pressure increases, whereas temperature decreases, the interchain interactions, the present results also suggest that the pressure effect dominates the temperature effect on the interchain interactions of both phases near T_m. Finally, the effects of pressure and temperature on the chain reorientational mobility are opposite but similar in magnitude and compensate each other in the gel phase; the mobility increases considerably in the liquid-crystalline phase and increases with a faster rate at higher pressure.

Since T_m increases with pressure while dT_m/dP is constant (20.8 °C·kbar^{-1} for DPPC and 20.1 °C·kbar^{-1} for DMPC), the ratio $\Delta V_m/\Delta H_m$ must decrease with pressure, meaning either a decrease in ΔV_m or an increase in ΔH_m, or both. The present Raman spectroscopic results suggest a decrease in the critical volume change at T_m, rather than an increase in ΔH_m at higher pressures.

Registry No. DMPC, 18194-24-6; DPPC, 63-89-8.

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