Effects of Cis and Trans Unsaturation on the Structure of Phospholipid Bilayers: A High-Pressure Infrared Spectroscopic Study†

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ABSTRACT: In order to compare the effects of cis and trans unsaturation on the structure and packing of phospholipid bilayers, infrared spectra of aqueous dispersions of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine (DEPC) were measured in a diamond anvil cell at 28 °C as a function of pressure up to 36 kbar. The infrared spectra indicate that DEPC and DOPC undergo pressure-induced liquid-crystalline to gel phase transitions at critical pressures of 0.7 and 5.2 kbar, respectively. Below their respective critical pressures, the infrared spectra of DOPC and DEPC are essentially indistinguishable, whereas above these pressures, there are very pronounced differences in the barotropic behavior of these two lipids. Specifically, at the 5.2-kbar transition in DOPC, there are significant changes in the frequencies, intensities, and widths of bands associated with the interfacial C=O groups, the olefinic CH=CH groups, and the terminal CH_3 groups, whereas the corresponding bands of DEPC are, by contrast, relatively insensitive to the pressure-induced phase transition. The unusual band shape changes in DOPC are attributed to a unique packing arrangement of the oleoyl acyl chains required to accommodate the bent geometries of adjacent cis double bonds. Moreover, above 5 kbar in DEPC, well-defined correlation field splittings of the CH_2 scissoring and rocking modes are observed, with magnitudes very similar to those observed at comparable pressures in saturated lipid systems. The absence of correlation field splittings of the corresponding bands of DOPC up to 36 kbar suggests that the bent oleoyl acyl chains are closely packed with all chains oriented parallel to each other.

The phospholipids of most bacterial and mammalian cell membranes contain a high percentage of cis unsaturated fatty acyl chains, a fact that has challenged biophysicists to study and interpret the physical implications of the cis olefin group in the hydrocarbon chains of biological and model membrane systems. Aside from the unique and rigid configuration of the cis double bond, which may be critical in certain enzymatic recognition processes, cis unsaturation in biological membranes appears to be a mechanism for modulating the "fluidity" of the membrane in response to changes in the external environment. This modulation is but one example of homeoviscous adaptation of membranes to changes in thermodynamic-intensive variables such as temperature or pressure (Macdonald & Cossins, 1985; DeLong & Yayanos, 1985; Cossins & Macdonald, 1986). By regulating the composition of the cell membrane through changes in fatty acyl chain length or unsaturation, the organism is thereby able to maintain the fluidity of the lipid bilayer within tolerable limits. The molecular mechanism underlying membrane adaptation to the stresses of extreme temperatures or pressures is poorly understood, especially since very few of the physical techniques that have been used to examine the effects of acyl chain unsaturation in model membrane systems have been able to provide detailed structural information on the conformations of the hydrocarbon chains in the region of the double bond. Obtaining such information in both the liquid-crystalline *and* gel phases of model systems will be a prerequisite to developing a molecular explanation of how increases in acyl chain unsaturation translate into the well-characterized increase in fluidity and depression of the gel to liquid-crystalline phase transition temperature. Thus, although the *effects* of unsaturation in model systems have been well characterized by film balance (Chapman et al., 1966), Raman spectroscopic (Lippert & Peticolas, 1972), fluorescence spectroscopic (Stubbs et al., 1981; Vincent & Gallay, 1984), and differential scanning calorimetric (Silvius & McElhaney, 1979; Silvius, 1986) techniques, these studies have not been able to resolve the fundamental question of the relationship between the dynamical structure of the unsaturated hydrocarbon chains and the observed bulk properties of the lipid bilayer. ^2H NMR[1] studies of selectively deuteriated liquid-crystalline model membranes represent the first step in this direction, and they have provided detailed information at a molecular level on the orientation of the cis double bond and on the influence of cis and trans double bonds on molecular order in the immediate vicinity (Seelig & Seelig, 1977; Seelig & Waespe-Sarcevic, 1978; Seelig et al., 1981). However, until more sophisticated methods of interpreting ^2H NMR *gel*-phase line shapes have been developed, conformational studies of unsaturated lipids by ^2H NMR will be most useful in the liquid-crystalline phase. Recently, Raman spectroscopy has shown great promise for the elucidation of cis unsaturated fatty acid chain conformation in the crystalline state of fatty acids and in the gel and liquid-crystalline phases of lipid bilayers (Koyama & Ikeda, 1980). Ultimately, the conformations of the cis and trans unsaturated hydrocarbon chains of phospholipids, and their packing arrangements in lipid bilayers, can best be determined

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[1] Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DEPC, 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine; HOPC, 1-hexadecyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; OPPC, 1-oleoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine; PEPC, 1-palmitoyl-2-elaidoyl-*sn*-glycero-3-phosphocholine; 1,3-DPPC, 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine; NMR, nuclear magnetic resonance.

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by X-ray diffraction. It is therefore unfortunate that very few X-ray crystallographic studies have been performed on unsaturated hydrocarbon chains, either as fatty acids or as fatty acyl derivatives (Small, 1984, 1986). As a consequence, the structural arrangement of unsaturated hydrocarbon chains in the gel phase of phospholipid dispersions, or in the crystalline state of fatty acids, is largely unknown.

Among the more intriguing questions raised by the bent geometry of the cis double bond is what modifications take place in the packing of gel-phase 1,2-diacyl phospholipids to accommodate cis unsaturated chains? In the case of mixed chain phospholipids, for example, interactions between an unsaturated chain in the 2-position and a saturated chain in the 1-position present steric problems that may only be overcome by new conformations of the chains, possibly leading to unique and unusual packing arrangements in the solid state. In the absence of detailed X-ray crystallographic studies of these systems, such arrangements must remain the subject of speculation (Small, 1984). A closely related, but distinct problem, is the packing arrangement of 1,2-diacyl phospholipids containing *two* unsaturated chains. In this study, we examine the structural and dynamic consequences of unsaturation in such systems by using high-pressure infrared spectroscopy to compare the barotropic behavior of cis and trans unsaturated phosphatidylcholine bilayers. Until recently, most investigations of phospholipid bilayers using vibrational spectroscopy have concentrated on the thermotropic behavior of these systems, but with the addition of high pressure as a variable parameter, new insights into the dynamical structure of *saturated* lipid bilayers have been gained from studies of their barotropic behavior (Wong, 1984, 1986). Although confined to saturated systems such as DMPC or DPPC, these studies have yielded a wealth of information on the pressure effects on interchain interactions, conformational changes, and reorientational dynamics, as well as the pressure and temperature dependence of the volume change at the main phase transition (T_m) and pretransition (T_p) temperatures. Similar information in *unsaturated* lipid systems will be essential in interpreting the role of cis unsaturation in homeoviscous adaptation of membranes to changes in the thermodynamic variables of temperature *or* pressure. Since the DOPC gel to liquid-crystalline phase transition temperature −16 °C is so low (Silvius & McElhaney, 1979), information on structure and dynamics in the gel phase of this lipid has been difficult to obtain by conventional techniques, but as the results of this investigation demonstrate, such information is readily accessible in the corresponding pressure-induced gel phase.

Materials and Methods

High-purity DOPC and DEPC were obtained from Avanti Polar Lipids (Birmingham, AL). Fully hydrated (≥40 wt % D_2O; Merck Sharp & Dohme, Montreal, Quebec) lipid dispersions were prepared for the infrared experiments by vortexing lipid/D_2O mixtures in a closed vial at room temperature. After immediate freezing of the samples in dry ice, the vortex/freeze cycle was then repeated twice again. Homogeneous dispersions resulting from this freeze/thaw cycle were then placed at room temperature, together with powdered α-quartz and KRS-5, in a 0.34-mm diameter hole on a 0.23-mm-thick stainless steel gasket mounted on a diamond anvil cell, as described previously (Wong et al., 1985). Infrared spectra of the samples were measured at 28 °C on a Bomem Model DA3.02 Fourier transform spectrophotometer with a liquid nitrogen cooled mercury cadmium telluride detector. The infrared beam was condensed by a sodium chloride lens system onto the pinhole of the diamond anvil cell (Mao et al., 1983). For each spectrum, typically 1000 scans were coadded, at a spectral resolution of 4 cm^{-1}. Data reduction was performed with software developed in this laboratory. To eliminate the possibility of spurious differences in spectral parameters between DOPC and DEPC introduced by differences in the treatment of data, the analysis of data was performed in *exactly* the same fashion for both lipids. Pressures were determined from the 695-cm^{-1} infrared absorption band of quartz (Wong et al., 1985). Frequencies of this band were obtained from third-order derivative spectra, calculated with a breakpoint of 0.7 in the Fourier domain, and pressures were calculated from these frequencies according to the expression $P = a_0 + a_1\Delta\nu + a_2\Delta\nu^2$, where $a_1 = 1.2062$, $a_2 = 0.015054$, and $\Delta\nu$ is the measured frequency shift. Setting zero pressure to correspond to the frequency of the quartz band at atmospheric pressure ($\Delta\nu = 0$), we set $a_0 \equiv 0$. In order to separate instrumentally unresolvable infrared band contours, Fourier derivation techniques (Moffatt et al., 1986) were applied. As an alternative to Fourier deconvolution (Mantsch et al. 1986), Fourier derivation is a method to enhance spectral resolution based on the generation of pth-order derivative band profiles (p real, $p \geq 1$). Frequencies associated with particular modes were obtained from third or higher order derivative spectra, with breakpoints as indicated in the figure captions.

Results and Discussion

Pressure Dependence of Infrared Spectra of DOPC and DEPC: CH_2 Stretching Mode Region (2800–3100 cm^{-1}). Figure 1 compares the pressure dependence of the infrared spectra of DOPC and DEPC bilayers in the CH_2 stretching region at pressures between 10 and 36 000 bar. Inasmuch as the pressure dependence of the DEPC infrared spectra is very similar to that observed in DPPC bilayers (Wong & Mantsch, 1985a), we briefly review the principal spectral features to be found in this region, common to both DPPC and DEPC bilayers. The dominant infrared bands (at 2850 and 2920 cm^{-1} at 10 bar) arise from the CH_2 symmetric [$\nu_s(CH_2)$] and antisymmetric [$\nu_{as}(CH_2)$] stretching modes, respectively, of the acyl chain methylene groups, while the weaker bands at 2873 and 2956 cm^{-1} are due to the symmetric [$\nu_s(CH_3)$] and asymmetric [$\nu_{as}(CH_3)$] stretching modes, respectively, of the terminal methyl group. A broad spectral feature, evident at higher pressures as a shoulder on the low-frequency side of the $\nu_{as}(CH_2)$ band, is the result of Fermi resonant coupling between the $\nu_s(CH_2)$ mode and the binary combination of the CH_2 bending modes with B_{3u} symmetry (Snyder et al., 1978). With these basic spectral features in mind, the most prominent and distinctive spectral features of the DOPC infrared spectra can be discussed.

The pressure dependences of various infrared spectral parameters can be used to determine the critical pressures of structural phase transitions (Wong & Mantsch, 1985a; Wong et al., 1986; Siminovitch et al., 1987) in lipid bilayers. In this infrared study, we made no attempt to enumerate all of the pressure-induced phase transitions in either of these unsaturated lipid systems. Instead, we find it sufficient to identify, in each lipid, the critical pressure of the gel to liquid-crystalline phase transition. Assuming that this transition in either of the unsaturated lipids is a first-order transition, as it is in DPPC, then from the Clausius–Clapeyron relationship, the critical temperature T_m can be raised by applying external pressure. Since both of these lipids are in the liquid-crystalline phase at ambient temperature (28 °C) in the diamond anvil cell, elevation of pressure should induce the transition to the gel phase. This pressure-induced increase in T_m has been described and studied in detail in saturated lipid systems

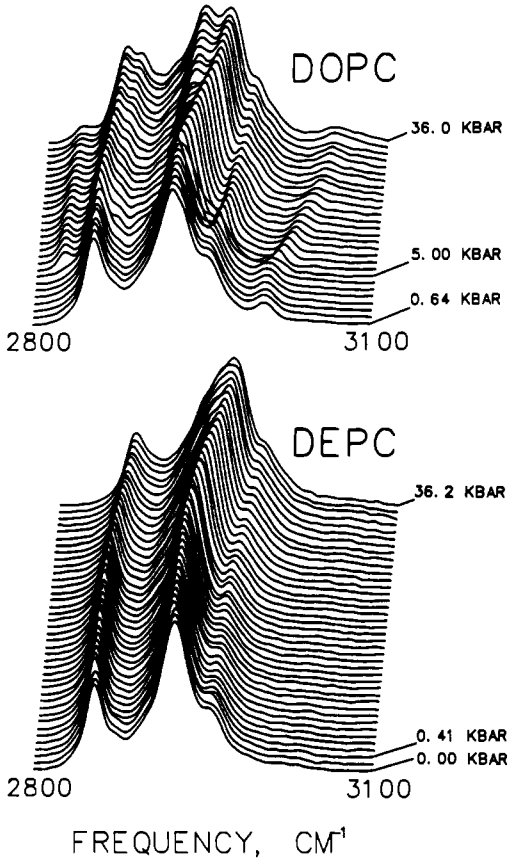

FIGURE 1: Stacked contour plots of infrared spectra of aqueous DOPC (top) and DEPC (bottom) in the CH_2 stretching region. In this and in figures to follow, displayed spectra have been interpolated in the frequency domain. To the right of each contour plot, the lowest and highest pressures measured are indicated, as well as the pressure of the contour just below the pressure-induced liquid-crystalline to gel phase transition. For each lipid, the same pressure ranges apply to all of the figures, with the exception of Figures 3 and 5, where the stacked contour plots of DOPC begin below 0.01 kbar.

(Wong et al., 1982; Wong & Mantsch, 1985c) but not as yet in unsaturated lipid bilayers. Monitoring the frequency of the $\nu_s(CH_2)$ mode at 2850 cm^{-1}, we observe an abrupt drop in frequency at 0.7 kbar in DEPC and at 5.2 kbar in DOPC, as shown in Figure 2, indicating that these are the critical pressures for T_m = 28 °C. The pressure dependence of T_m in saturated lipid bilayers is linear, yielding values for dT_m/dP of 20.1 °C·$kbar^{-1}$ in DMPC and 20.8 °C·$kbar^{-1}$ in DPPC (Wong & Mantsch, 1985c). These values can only be obtained by measuring the *temperature*-induced phase transition between the gel and liquid-crystalline phases at *constant* but different pressures. Thus, since the critical pressures for DOPC and DEPC in this study were derived from the *pressure*-induced liquid-crystalline to gel phase transition at 28 °C, they cannot be used to estimate dT_m/dP. However, they at least provide an upper bound for dT_m/dP, just as the critical pressure of 0.6 kbar at 30 °C in DMPC bilayers provided an upper bound for dT_m/dP of 40 °C·$kbar^{-1}$ (Wong et al., 1982). In the same way, using a value of −16 °C for T_m in DOPC bilayers at atmospheric pressure (Silvius & McElhaney, 1979) and a critical pressure of 5.2 kbar at 28 °C, we determine an upper bound for dT_m/dP of 8.3 °C·$kbar^{-1}$, which is anomalously low in comparison to the canonical value of ~20 °C·$kbar^{-1}$ usually observed in either model or biological membrane systems (Macdonald & Cossins, 1985). The value that we estimate as an upper bound for dT_m/dP in DOPC bilayers is also low in comparison to previous estimates of 21 (Chong & Weber, 1983) and 22.8 °C·$kbar^{-1}$ (Chong & Cossins, 1984), both deduced from fluorescence polarization measurements of liquid-crystalline DOPC bilayers under pressure. No isothermal pressure-induced transition was directly observed in either study, since the upper limit of the pressure range was 2 kbar. We are led to the conclusion that, in DOPC bilayers, either dT_m/dP is pressure-dependent, a behavior noted in a previous fluorescence polarization study of DOPC (Chong & Weber, 1983) but attributed to experimental error, or the value of dT_m/dP is, in fact, anomalously low. Experiments are presently under way in our laboratory to address this problem.

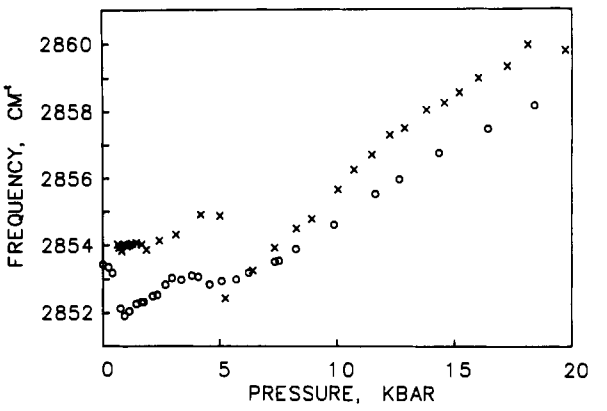

FIGURE 2: Pressure dependence of the frequency of the $\nu_s(CH_2)$ mode in DOPC (×) and DEPC (○). Frequencies were determined from interpolated third-order derivative spectra, calculated with a breakpoint of 0.3 in the Fourier domain. [For details, see Moffatt et al. (1986).]

Below their respective critical pressures, the infrared spectra of DOPC and DEPC shown in Figure 1 are essentially indistinguishable, except for the olefinic C–H stretching band at 3007 cm^{-1} in the spectra of DOPC. The corresponding band is of course absent in the spectra of DEPC, since the C–H dipole moments of the trans double bond cancel. Above their respective critical pressure, however, there are a number of striking differences between the infrared spectra of these two unsaturated lipids. Most of these differences are the result of profound changes in the infrared spectra of DOPC that occur at the pressure-induced liquid-crystalline to gel phase transition. Almost without exception, the DOPC stretching bands shift in frequency, increase in intensity and, in some cases, become significantly narrower. Certainly these changes are not unique to the infrared spectra of DOPC, nor are they confined to the C–H stretching region (vide infra), but in no other lipid (unpublished results from this laboratory) are they so marked. The barotropic behavior of these bands is also revealing. For example, the olefinic C–H stretching frequency is extremely sensitive to pressure, so that in the 5-kbar pressure range immediately above the transition at 5.2 kbar, $d\nu/dP \approx$ 1.9 cm^{-1}·$kbar^{-1}$, a rate exceeded only by the pressure dependence of the $\nu_{as}(CH_2)$ band frequency. The pressure sensitivity of the olefinic C–H stretching frequency appears to be a characteristic feature of the pressure-induced gel phase of cis unsaturated lipids containing one *or* two unsaturated hydrocarbon chains, since the same barotropic behavior is observed in the infrared spectra of HOPC, POPC, and OPPC bilayers (unpublished results from this laboratory).

At pressures above the transition in DOPC bilayers, there is a significant and abrupt enhancement in the intensity of both the symmetric [$\nu_s(CH_3)$] and asymmetric [$\nu_{as}(CH_3)$] stretching mode bands of the terminal methyl group, at 2961 and 2873 cm^{-1}, respectively. The increased intensity of the $\nu_{as}(CH_3)$ band is particularly noticeable immediately above the transition pressure (see also Figure 8, and discussion below), but as the pressure increases, the intensity decreases as a shoulder de-

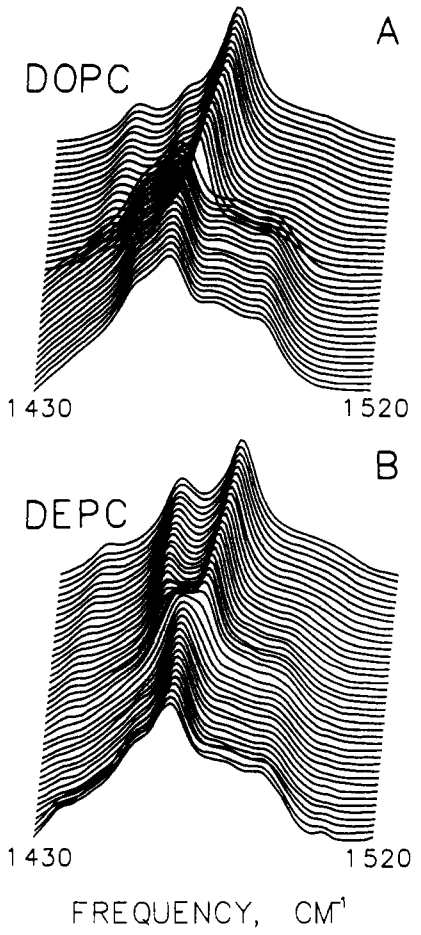

FIGURE 3: Stacked contour plots of infrared spectra of aqueous DOPC (A) and DEPC (B) in the CH_2 scissoring region.

velops on the high-frequency side of $\nu_{as}(CH_3)$, and the band eventually splits. We note that similar barotropic behavior is not observed in other lipids so far investigated, except for bilayers of 1,3-DPPC (unpublished results from this laboratory), which interdigitate in the gel phase (Serrallach et al., 1983). In the pressure-induced gel phase of DEPC bilayers, the barotropic behavior of the corresponding bands, monitored by the pressure dependence of the respective band frequencies, is very similar, although there is no detectable splitting of the $\nu_{as}(CH_3)$ band, perhaps due to the fact that the intensity of this band does not increase at the phase transition.

In the low-pressure infrared spectra of the unsaturated lipids shown in Figure 1, as well as in the corresponding spectra of saturated lipids such as DPPC (Wong & Mantsch, 1985a), the Fermi resonance band appears only as a weak shoulder on the low-frequency side of the prominent $\nu_{as}(CH_2)$ band. At high pressures ($\geq$12 kbar), this band both intensifies and broadens, giving rise to a very similar broad but distinct shoulder on the low-frequency side of the $\nu_{as}(CH_2)$ band in the spectra of DEPC and DPPC. The corresponding spectra of DOPC at these pressures are distinguished by a particularly intense Fermi resonance band, whose intensity is unusual in comparison to that observed in the spectra of any other saturated or unsaturated lipid bilayer. Thus, in agreement with a previous high-pressure infrared study of DPPC (Wong & Mantsch, 1985a), we can conclude that, in both saturated *and* unsaturated lipid bilayers, high pressure leads to a narrowing of the Fermi resonance band, manifested as an increase in peak height intensity.

Finally, in the pressure-induced gel phase of DOPC bilayers, an entirely new band in the C–H stretching region appears

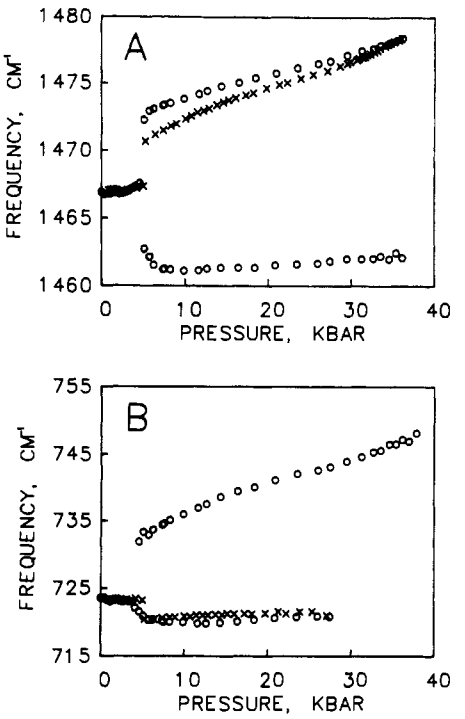

FIGURE 4: Pressure dependence of the frequency of the $\delta(CH_2)$ mode in DOPC (×) and DEPC (○) (A) and of the $\gamma(CH_2)$ mode in DOPC (×) and DEPC (○) (B). Frequencies of the $\delta(CH_2)$ mode were determined from fifth-order derivative spectra, calculated with a breakpoint of 0.5 in the Fourier domain. Frequencies of the $\gamma(CH_2)$ mode were determined from third-order derivative spectra, calculated with a breakpoint of 0.7 in the Fourier domain.

at 2823 cm^{-1} immediately above the critical pressure at 5.2 kbar (see Figure 8). It appears that this band is characteristic of bilayers composed only of cis unsaturated hydrocarbon chains, since the same band is also observed in fatty acid/soap complexes (e.g., oleic acid/sodium oleate) containing oleoyl hydrocarbon chains but is not observed in the corresponding complexes with elaidoyl chains, nor is it observed in any of the lipids with a *single* oleoyl chain (e.g., POPC, OPPC, or HOPC) (unpublished results from this laboratory). Since there can be little doubt that this band arises from the C–H stretching mode of a CH_2 group, we tentatively assign this band to the CH_2 groups adjacent to the olefinic group, since these are the only methylene groups that are likely to be unusual in DOPC.

CH_2 Deformation (1350–1500 cm^{-1}) and Rocking (600–770 cm^{-1}) Regions. A comparison of the pressure dependence of the methylene scissoring mode band $\delta(CH_2)$ in the spectra of DOPC and DEPC shown in Figure 3 reveals that the barotropic behavior of this band is very different in these two lipids. Most noteworthy is the absence of a correlation field splitting of either the $\delta(CH_2)$ mode (Figure 3A) or the $\gamma(CH_2)$ mode (data not shown) in the spectra of DOPC, although the bands of both modes shift and intensify at the critical pressure of 5.2 kbar (see also Figure 8). On the other hand, in the spectra of DEPC above ~5 kbar, we clearly observed a pressure-induced correlation field splitting of both the $\delta(CH_2)$ mode (Figure 3B) and the $\gamma(CH_2)$ mode (data not shown). The pressure dependences of the frequencies of the CH_2 scissoring and rocking mode components in DOPC (×) and DEPC (○) are compared in parts A and B of Figure 4, respectively. It is significant that in DEPC each of the modes in question do not split until ~5 kbar (close to the critical pressure in DOPC), and when they do split, the magnitudes of the respective correlation field splittings are very similar to those observed *at the same pressure* in DPPC (Wong & Mantsch,

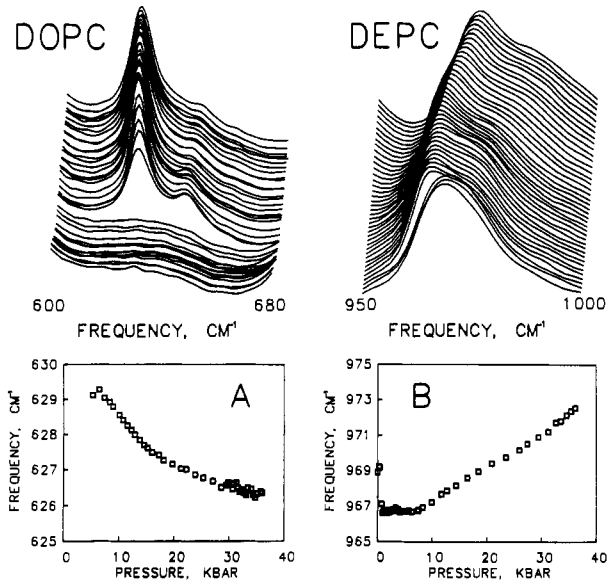

FIGURE 5: Stacked contour plots of infrared spectra of aqueous DOPC (top left) and DEPC (top right) in the out-of-plane CH wagging regions. Below each contour plot, the pressure dependence of the frequency of the out-of-plane CH wagging mode for DOPC (A) and DEPC (B) is shown. Frequencies were determined from interpolated third-order derivative spectra, calculated with a breakpoint of 0.3 in the Fourier domain.

1985a). Thus, even up to pressures of 5 kbar, the presence of double bonds (cis or trans) apparently introduces enough disorder in the form of gauche conformers to render any correlation field splittings of the $\delta(CH_2)$ or $\gamma(CH_2)$ modes unobservable. Only at relatively high pressures are the acyl chains sufficiently ordered to allow the interchain interactions that give rise to correlation field splittings. Although this pressure-induced ordering is delayed to much higher pressures in the unsaturated lipid systems, the transition to an ordered system is much more abrupt but, once completed in the case of DEPC, gives rise to a correlation field splitting whose magnitude is similar to that observed from the saturated DPPC bilayer at the same pressure. Since the restricted orientational mobility of the bent hydrocarbon chains of DOPC could not permit an orientationally disordered structure at high pressures, the absence of a correlation field splitting of the $\delta(CH_2)$ mode band in the spectra of DOPC can only be consistent with an equivalent, parallel orientation of the acyl chains, packed in a highly ordered and rigid lattice. In the pressure-induced gel phase of DOPE bilayers, a similar parallel orientation of the acyl chains has been deduced from the absence of a correlation field splitting (Wong et al., 1986). We note that the intensity of the scissoring mode band $\delta(CH_2)$ in DOPC above the critical pressure of 5.2 kbar (shifted to higher frequency, but not split) is significantly greater than the correlation field component band $\delta(CH_2)$ of DEPC at the same pressure, since the components of the $\delta(CH_2)$ mode overlap in DOPC, leading to an enhanced intensity.

A comparison of the spectral region between 600 and 690 cm^{-1} in Figure 5 shows that just above the 5.2-kbar transition in DOPC, a new band, relatively intense in comparison to the CH_2 rocking band, appears at 630 cm^{-1}. Barotropic studies carried out in this laboratory on other saturated and unsaturated lipid systems, including POPC, OPPC, and HOPC bilayers, suggest that the spectral feature around 630 cm^{-1} is unique to cis unsaturated lipids containing one double bond on *each* of the two hydrocarbon chains. Not only is this band relatively intense, but with the sole exception of some of the bands of the CH_2 wagging band progression, it is the only band whose frequency has a negative pressure dependence (see Figure 5A). It is well-known that some of the strongest bands in hydrocarbon olefins are the out-of-plane hydrogen wag vibrations (Colthup et al., 1975). In particular, the in-phase, out-of-plane CH wagging of the cis disubstituted hydrocarbon olefins gives rise to a band near 650–730 cm^{-1}, whose frequency variability is distinctive in comparison to the corresponding band of the trans form (Colthup et al., 1975; Bellamy, 1975). Although a frequency of 630 cm^{-1} is outside the spectral range usually associated with the CH out-of-plane deformation mode, taking into account the well-characterized frequency variability of this band, we assign the 630-cm^{-1} spectral feature to the out-of-plane CH wag. The corresponding band for the out-of-plane CH wagging mode of the trans disubstituted olefinic groups of DEPC is contained in the spectral region 950–1000 cm^{-1} (Bellamy, 1975; Colthup et al., 1975), whose pressure dependence is also shown in Figure 5. Although this region is complicated by the presence of the C–N–C stretching modes of the choline head group near 970 cm^{-1}, the only unique signature of the trans double bond in DEPC bilayers is provided by the CH wagging band around 969 cm^{-1}. The pressure dependence of the frequency of this band, determined by resolution enhancement techniques, is shown in Figure 5B. At the 0.7-kbar phase transition, the band frequency drops by $\sim$2 cm^{-1} and then remains invariant to increases in pressure up to $\sim$7 kbar, above which the band frequency displays the usual pressure dependence, increasing monotonically up to the highest pressure studied. Note that this behavior contrasts sharply with that observed in DOPC, where the CH wagging band frequency *decreases* monotonically at all pressures above the 5.2-kbar phase transition. Since the change in the DEPC band shape in the 950–1000 cm^{-1} spectral region at the 0.7-kbar transition is identical with that observed at the temperature-induced transition (Jaworsky & Mendelsohn, 1986), we anticipate that the temperature-induced transition in DOPC will also be accompanied by significant changes in the band shape of the out-of-plane CH wagging mode.

Carbonyl Stretching Region (1650–1800 cm^{-1}). The pressure dependence of the ester carbonyl stretching mode band in DOPC and DEPC bilayers is compared in Figure 6. Due to the well-established multicomponent nature of this band contour in diacyl phospholipids (Casal & Mantsch, 1984), in each case, the pressure dependence of the corresponding resolution-enhanced spectra (Mantsch et al., 1986) is also displayed. These resolution-enhanced spectra show that at all pressures there are at least two components in the C=O band contour of both lipids. The pressure dependences of the frequencies of these components in DOPC and DEPC are shown in Figure 7. Also shown for comparison are the pressure dependences of the frequency of the C=O band for both lipids, determined from the center of gravity of the composite band contour. Focusing for the moment on the pressure dependence of the C=O band contour (Figure 6, top) and their associated "band" frequencies (Figure 7, bottom), we note that in DOPC (×) there is an abrupt drop in frequency at the 5.2-kbar transition ($\Delta\nu \approx -10$ cm^{-1}) and then the frequency steadily increases with pressure, whereas in DEPC (○), there is a small increase in frequency ($\Delta\nu \approx 2$ cm^{-1}) at the 0.7-kbar transition, followed by a steady increase in frequency; up to 30 kbar, this shift amounts to approximately 6 cm^{-1}, very similar in magnitude to the shift observed in the pressure range between 9 and 30 kbar in DOPE bilayers (Wong et al., 1986). What are the molecular mechanisms underlying these observed shifts? Certainly, as noted previously in a high-pressure in-

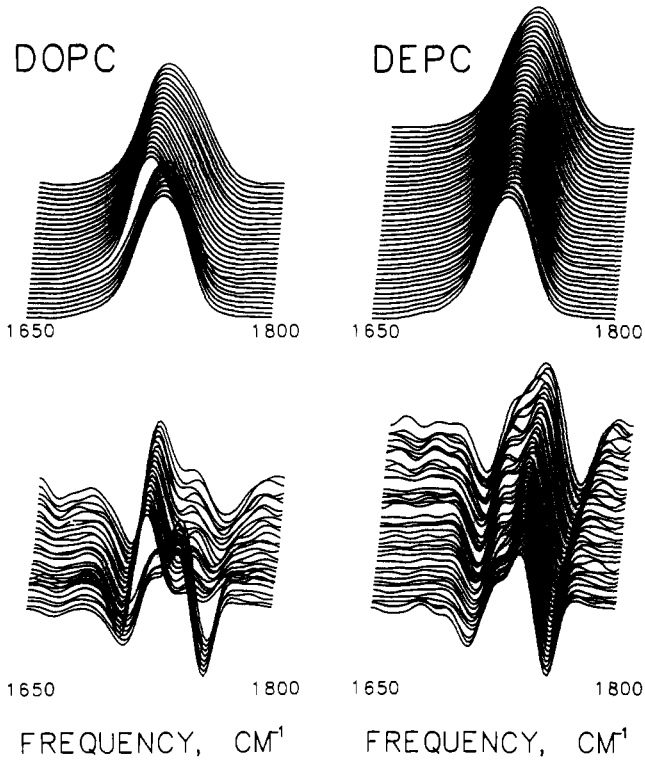

FIGURE 6: Stacked contour plots of the infrared spectra of aqueous DOPC (top left) and DEPC (top right) in the C=O stretching region. Below each contour plot, stacked plots of the corresponding resolution-enhanced spectra are shown for DOPC (bottom left) and DEPC (bottom right). Resolution enhancement was carried out by using a third-order derivative, calculated with a breakpoint of 0.3 in the Fourier domain.

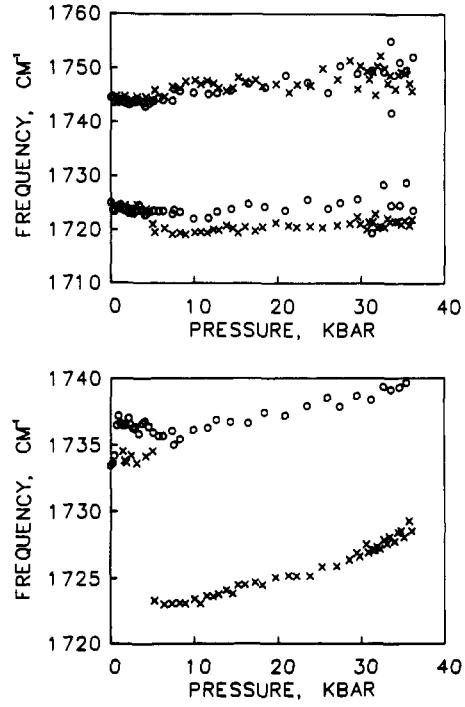

FIGURE 7: Top: Pressure dependence of the frequency of the C=O stretching mode components in DOPC (×) and DEPC (○). Frequencies were determined from fifth-order derivative spectra, calculated with a breakpoint of 0.3 in the Fourier domain. (Bottom) Pressure dependence of the frequency of the C=O stretching mode bands in DOPC (×) and DEPC (○). Frequencies (center of gravity) were determined from spectra smoothed in the Fourier domain.

frared study of DOPE bilayers (Wong et al., 1986), compression of the C=O bond and the concomitant increase in intermolecular interactions with increasing pressure will increase the C=O stretching frequency. However, Figures 6 and 7 show that the C=O band is composed of at least two components, separated by at least 20 cm^{-1}. Changes in the relative intensities of these components alone, without *any* frequency shifts of the individual components, could, in principle, generate shifts (positive or negative) as large as 15 cm^{-1}. Indeed, the large 10-cm^{-1} shift in the C=O band frequency at the transition in DOPC is in large part due to the drastic change in the relative intensities of the two band components, as shown in the resolution-enhanced spectra of Figure 6. Furthermore, the frequency data plotted in Figure 7 show that for any component the magnitude of the shift is less than 5 cm^{-1}, and only for the lower frequency component is there a negative shift of this magnitude. Therefore, we suggest that interpretations of shifts in the center of gravity or peak maximum of a composite band contour may be misleading. The relative intensity change in the two components of the C=O band of DOPC at the transition is probably due to a conformational change at the interface that affects the environment of both C=O groups. This change must occur to accommodate an unusual packing of the bent hydrocarbon chains (vide infra).

Nature of the 5.2-kbar Phase Transition and Associated Structural Changes in DOPC. In comparing the barotropic behavior of the trans unsaturated DEPC lipid bilayer with that of its cis unsaturated counterpart DOPC, we have documented and described in the previous sections a number of dramatic changes in the infrared spectra of DOPC that take place at the 5.2-kbar transition. Such changes include significant increases in band intensities, decreases in bandwidths, and shifts in band frequencies. Unlike similar changes that occur at pressure- or temperature-induced transitions in the infrared spectra of related systems, the magnitude of these changes at the transition in DOPC bilayers is so large that they are easily discernible without band shape analysis. A graphic illustration of this point is provided by Figure 8, in which we have displayed infrared spectra in several spectral regions, including those discussed previously, at pressures just below and above the 5.2-kbar transition. In at least two cases, for example, new bands at 2820 and 630 cm^{-1} appear above this transition, which have been tentatively assigned to the C—H stretching mode of methylene groups adjacent to the cis double bond and to the out-of-plane =CH deformation mode, respectively.

The absence of a correlation field splitting indicates the interchain packing in the gel phase of DOPC bilayers must be very rigid and highly ordered, with a parallel orientation of all acyl chains in the lattice. In view of the packing constraints imposed by the bent geometry of the cis double bond (Sundaralingam, 1972), the packing arrangement must be very unusual, possibly forcing changes in conformation to accommodate adjacent cis double bonds. A global view of these packing accommodations is provided by the barotropic behavior of three functional groups that are particularly sensitive to the structural transition at 5.2 kbar, the ester carbonyl (C=O) group at the bilayer interface, the double bond (C-H=CH) group in the middle of the hydrocarbon chain, and the terminal methyl group (CH_3). Ordering of the methyl groups is suggested by the behavior of the stretching [$\nu(CH_3)$] and deformation [$\delta(CH_3)$] modes. The $\nu_{as}(CH_3)$ band shifts and intensifies dramatically at the transition and then splits as the pressure increases. The intensity of the $\nu_s(CH_3)$ band increases steadily in the pressure-induced gel phase. The corresponding deformation mode bands are also revealing. The $\delta_s(CH_3)$ band at 1378 cm^{-1} shifts down to 1367 cm^{-1} at the

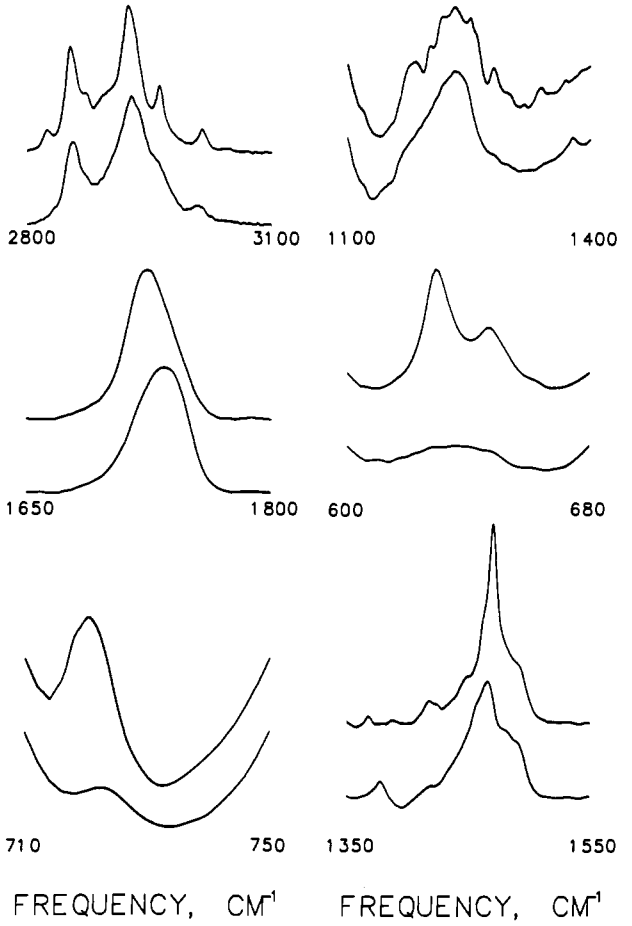

FIGURE 8: Infrared spectra of aqueous DOPC in several spectral regions, at pressures just below and above the 5.2-kbar transition. (Left panel) CH_2 stretching region (top), C=O stretching region (middle), and CH_2 rocking region (bottom). (Right panel) CH_2 wagging band progression region (top), out-of-plane CH wagging region (middle), and CH_2 scissoring region (bottom).

transition and then appears to split at higher pressure, while the $\delta_{as}(CH_3)$ band, at 1456 cm^{-1}, also appears to split at the transition. By contrast, the corresponding stretching and deformation mode bands in DEPC do not intensify or shift in frequency above the transition at 0.7 kbar, and only the $\delta_s(CH_3)$ band appears to split at higher pressure.

As we have seen earlier, some of the most dramatic band shape changes at the transition in DOPC are associated with the olefinic stretching [ν(=CH)] and deformation (=CH wag) modes. The enhanced pressure sensitivity of the olefinic C–H stretch frequency at pressures above the transition can be rationalized by the bent geometry of the cis double bond, which exposes the two C–H groups and lends the C–H bonds more susceptible to the effects of compression and increased intermolecular interactions as the pressure increases. Either of these pressure-induced effects would result in the observed increase in frequency of this band. The abrupt enhancement in the intensity of the =CH deformation mode band shown in Figure 8 is particularly distinctive and suggests that, at the 5.2-kbar transition from a disordered liquid-crystalline phase to an ordered gel phase, the single C—C bonds adjacent to the double bond assume a fixed conformation. Support for this suggestion can be provided by an examination of the possible conformations around the sp^2, C–C axes at each end of the cis double bond (Shimanouchi et al., 1971; Sundaralingam, 1972; Schurink & de Jong, 1977; Koyama & Ikeda, 1980; Applegate & Glomset, 1986). From an analysis of rotational isomerism about the sp^2, C–C axes in model compounds of *cis*-1,4-polybutadienes (Shimanouchi et al., 1971; Schurink & de Jong, 1977; Applegate & Glomset, 1986), it has been shown that the most stable conformations are skew, skew and skew, skew′, where "skew" and "skew′" denote torsion angles of ~120° and ~−120°, respectively, about the single C–C bonds adjacent to the double bond. There is a preference for these torsion angles to have opposite signs, so in crystalline oleic acid, for example, the exact torsion angles determined by X-ray diffraction are 132°, −128° (Abrahamsson & Ryderstedt-Nahringbauer, 1962), while in crystalline linoleic acid, the corresponding angles are −119°, 123° and 124°, −121° (Ernst et al., 1979). A notable exception to the skew, skew and skew, skew′ conformations can be found in the case of the oleate chain in crystalline cholesteryl oleate (Craven & Guerina, 1979), where the torsion angles for the C–C bonds adjacent to the double bond are (113°, 70°). Also unusual in this conformation is the torsion angle of 36° about the cis double bond, an angle that is typically 0 ± 3° (Sundaralingam, 1972). Due to a unique conformation of the cholesteryl oleate chain in the immediate vicinity of the cis double bond, including two gauche conformers and eclipsing of C–C and C–H bonds, the chain sections above and below the double bond are almost parallel but with a 0.5-Å relative displacement. The result is an almost straight oleate chain but with a kink at the cis double bond. The existence of such an unusual conformation for the cis double bond region is not anticipated on the basis of either theoretical or experimental studies of model compounds, nor is it observed in any of the crystalline (poly)unsaturated fatty acids so far investigated by X-ray diffraction. It is more likely that such an unusual conformation of the oleate chain would be found in mixed chain phospholipids, which require close packing of saturated and unsaturated chains. We note that, in the crystal structure of cholesteryl oleate, the oleate chains from adjacent monolayers are deeply interdigitated, with an antiparallel arrangement of the chains in which the kinks of the double bond regions are adjacent to the saturated regions of other chains. The inefficiency of this packing arrangement, with no indication of a regular subcell structure, may be similar to that found in biomembranes, whose 1,2-diacyl phospholipids usually contain both a saturated and an unsaturated chain. The high-pressure gel phase of DOPC, on the other hand, appears to require a very efficient close packing, with the cis double bonds of each oleoyl chain adjacent to each other. In the following discussion, we limit ourselves to a consideration of the skew, skew and skew, skew′ conformations only.

Although both of the stable conformations, skew, skew and skew, skew′, produce a bend at the middle of the hydrocarbon chain, the preferred conformation skew, skew′ produces a larger bend. In a comparison of the Raman spectra of low (form L, mp = 13 °C) and high (form H, mp = 16 °C) melting point crystals of oleic acid, Koyama and Ikeda (1980) observed pronounced spectral differences between forms L and H, which they ascribed to rotational isomerism around the sp^2, C–C axes attached to the C=C bond. Specifically, downward shifts in the ν(C=C) band (1662 cm^{-1} in form L to 1643 cm^{-1} in form H) and in the out-of-plane CH wagging band (700 cm^{-1} in form L to 646 cm^{-1} in form H) were attributed to a change from the skew, skew′ conformation in form L to the skew, skew conformation in form H. Similar changes observed in the Raman spectra of other cis unsaturated fatty acids were likewise attributed to a conformational change from skew, skew′ to skew, skew, although the magnitudes of the frequency and intensity shifts observed in each case were influenced by the position of the double bond (e.g., petroselinic vs. oleic acid)

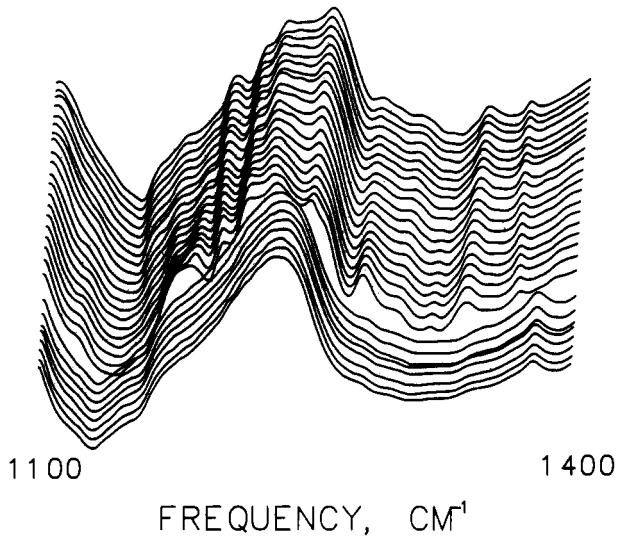

FIGURE 9: Stacked contour plots of infrared spectra of aqueous DOPC in the spectral region containing the CH_2 wagging band progression.

and by the presence of other cis double bonds (e.g., linoleic vs. oleic acid). The two vibrational modes of oleic acid most influenced by the change in conformation from skew, skew′ in form L to skew, skew in form H are the C=C stretching mode and the C—H out-of-plane deformation mode, the latter of which undergoes the largest change in intensity at the pressure-induced phase transition in DOPC bilayers. Therefore, we suggest that, in the pressure-induced gel phase of DOPC, the adjacent C—C bonds adopt one of these conformations, most likely the skew, skew conformation. It is not clear whether the skew, skew conformation is favored by pressure due to a volume effect or whether it is the result of increased intermolecular interactions in the rigid and highly ordered hydrocarbon chain lattice of the pressure-induced gel phase. In this regard, it is worth noting that, in the temperature-induced gel phase of DOPC, the conformation is also skew, skew (Koyama & Ikeda, 1980), and thus just the smaller bend of the skew, skew conformation (Sundaralingam, 1972) may be sufficient to favor this conformation in the gel phase. As an example of how strong intermolecular interactions in the pressure-induced gel phase may be the dominant influence on conformational changes, we note the intense CH_2 wagging band progression in the infrared spectra of DOPC in the region 1190–1380 cm^{-1} at pressures immediately above the 5.2-kbar transition (see Figure 9), indicating that at least some of the polymethylene chain segments of the oleoyl chain are in their trans conformation, despite the fact that, under pressure, gauche conformers should be favored by the volume change (Taniguchi et al., 1981; Wong et al., 1983). Although this region is dominated in phospholipids by the relatively intense O=P=O antisymmetric stretching band of the phosphate group around 1220 cm^{-1} and, more importantly, by the D—O—D bending band at 1215 cm^{-1}, the overlapping bands of the CH_2 wagging progression are clearly visible in the spectra of DOPC shown in Figure 9. A conformational change from skew, skew′ to skew, skew when the polymethylene chain segments are all-trans should result in a significant increase in the end-to-end distance of the hydrocarbon chain (Koyama & Ikeda, 1980), leading to a corresponding increase in the thickness of the DOPC bilayer. Whether or not the bilayer will remain stable at these pressures will depend on the magnitude of the increase and the lateral compressibility of the bilayer, which should compensate for the thickness increase by reducing the cross-sectional area occupied per chain and lead to a net decrease in the total volume of the system. Barotropic studies of DPPC using X-ray diffraction show that, in the liquid-crystalline phase, the lateral compressibility exceeds the transverse compressibility, actually resulting in an increase in bilayer thickness with increasing pressure, whereas in the gel phase, the lamellar periodicity decreases with increasing pressure (Stamatoff et al., 1978). Barotropic studies of saturated lipid bilayers using neutron diffraction demonstrate that, at high enough pressures and temperatures, the pressure-induced gel phase of DPPC eventually converts to an interdigitated phase due to the resultant decrease in cross-sectional area per chain in the interdigitated phase (Braganza & Worcester, 1986). In principle, under appropriate conditions of temperature and pressure, an equivalent pressure-induced interdigitation should occur in unsaturated lipid bilayers, although the bent geometry of the cis double bond and the effects of rotational isomerism around the adjacent C–C bonds (vide supra) may result in considerable differences between the barotropic behavior of the saturated and unsaturated lipid systems. In fact, there is evidence to suggest (vide infra) that the liquid-crystalline phase of DOPC converts *directly* to a pressure-induced interdigitated gel phase, unlike any of the saturated systems, all of which first convert to a noninterdigitated gel phase under pressure.

Resolution enhancement of the DOPC C=O band contour in the liquid-crystalline phase reveals two components, whose relative intensities change significantly at the 5.2-kbar phase transition. If this intensity change is due to a conformational change involving both carbonyl groups, what is this change, and why does it occur in DOPC bilayers and not in DEPC bilayers? Definitive answers to these questions could be provided by X-ray or neutron diffraction studies on the gel phase of these unsaturated systems and by X-ray crystallographic studies on crystalline forms of DOPC and DEPC. With the lack of such structural information on these lipids in the solid state, a consideration of the ^2H NMR data in the disordered liquid-crystalline phases of these lipids can provide clues on the gel phase structure adopted in each case. The results of these experiments on lipids specifically deuteriated at the 9,10-positions of the oleic and elaidic acyl chains demonstrate that even in the liquid crystalline phase, the average conformation of the hydrocarbon chains in the double bond region is quite different in these two lipids. Specifically, for 1,2-[9,10-$^2H_2$]dielaidoyl-*sn*-glycero-3-phosphocholine, the *sn*-1 and *sn*-2 chains give rise to the *same* quadrupole splitting at the 9′,10′-positions, whereas *three* quadrupole splittings are observed in the ^2H NMR spectrum of 1,2-[9,10-$^2H_2$]-dioleoyl-*sn*-glycero-3-phosphocholine (Seelig & Waespe-Sarcevic, 1978; Seelig et al., 1981). These results clearly indicate that in DOPC the *sn*-1 and the *sn*-2 fatty acyl chains assume *different* average conformations in the region of the double bond, whereas in DEPC, the corresponding conformations are *identical*. The molecular origin of this difference between DOPC and DEPC is easily understood from a consideration of the glycerol backbone conformation. In this conformation, which is common to all naturally occurring phospholipids and is independent of the nature of the polar head group (Browning & Seelig, 1980) or the degree of unsaturation (Büldt et al., 1978; Seelig & Waespe-Sarcevic, 1978), the glycerol backbone is oriented almost perpendicular to the bilayer surface, with the *sn*-1 chain continuing in this direction, whereas the *sn*-2 chain begins parallel to the membrane surface and then is bent perpendicular to it after the C-2′ segment (Hitchcock et al., 1974; Seelig & Seelig, 1975; Büldt et al., 1978; Pearson & Pascher, 1979). Due to the bent configuration of the *sn*-2 chain, all segments of the *sn*-2 chain

are shifted toward the bilayer surface and are therefore closer to the lipid/water interface than the corresponding segments of the *sn*-1 chain. Despite this staggering of the chains, in bilayers whose lipids contain two saturated or two trans unsaturated chains of equal length, the effects of the physical inequivalence near the interface are lost in the plateau region, so that specifically deuteriated acyl chains of these lipids, each labeled at the *same* segment position, give rise to the *same* quadrupole splitting. The equivalence of the *sn*-1 and *sn*-2 chains in saturated and trans unsaturated lipid bilayers reflects the similar behavior of the saturated and trans unsaturated chains. Even in mixed chain lipids, the results of ^{2}H NMR studies on POPC and PEPC bilayers indicate that the behavior of the *sn*-2-elaidic acyl chain resembles more that of a fully saturated chain than that of a cis unsaturated chain (Seelig & Waespe-Sarcevic, 1978).

In cis unsaturated DOPC bilayers, staggering of the chains means that the double bond of the *sn*-2 chain will be closer to the membrane surface than the corresponding double bond of the *sn*-1 chain, presenting steric problems due to the rigid and bent geometry of the cis double bond. Adopting different average conformations in each chain in the region of the double bond, including a different orientation of each double bond with respect to the bilayer normal, alleviates these steric problems and permits efficient packing of the hydrocarbon chains. Such rearrangements are easily achieved in the liquid-crystalline phase due to the disordered polymethylene chain segments above and below the double bond. In the pressure- or temperature-induced gel phase of DOPC, however, both polymethylene chain segments are in an extended all-trans configuration, suggesting that a staggered arrangement of the chains is very unlikely. We suggest that, at the 5.2-kbar transition in DOPC, there is a conformational change of the glycerol backbone that permits adjacent packing of the double bonds of the *sn*-1 and *sn*-2 chains, placing them at the same distance from the bilayer interface. Such a change will of course alter the environment of both carbonyl groups and explains the dramatic change in the relative intensities of the carbonyl bands of the *sn*-1 and *sn*-2 chains. Similar packing of the cis double bonds of oleoyl chains in the solid state is also observed in crystalline forms of oleic acid (Abrahamsson & Ryderstedt-Nahringbauer, 1962) and 1,2-dioleoyl-3-acyl-*sn*-glycerols (Fahey et al., 1985). The glycerol backbone conformational change may lead to an interdigitated bilayer similar to that observed in 1,3-DPPC (Serrallach et al., 1983), since the barotropic behavior of the $\nu_{as}(CH_3)$ band of the terminal methyl groups is very similar in these lipids (vide supra). Confirmation of this interdigitated structure will require X-ray diffraction experiments.

Nature of the 0.7-kbar Phase Transition and Associated Structural Changes in DEPC. In contrast to the pressure-induced transition at 5.2 kbar in DOPC bilayers, the corresponding event at 0.7 kbar in DEPC bilayers is in some respects very similar to that observed in previous infrared thermotropic studies of saturated lipid systems. For example, the band shape changes in the spectral region 1430–1520 cm^{-1} are identical with those observed in saturated systems. In addition, the decrease in frequency of the $\nu_s(CH_2)$ band at 0.7 kbar, while somewhat smaller in absolute magnitude than that observed in saturated systems, nevertheless suggests a corresponding decrease in the number of gauche conformers. However, the absence of a correlation field splitting of the CH_2 scissoring or rocking modes until much higher pressure ($\geq$5 kbar) indicates that the gel phase of DEPC remains more disordered than the corresponding phase of a saturated system such as DPPC, which has a well-defined correlation field splitting above 2 kbar (Wong & Mantsch, 1985a). This unusual barotropic behavior of the CH_2 scissoring and rocking modes in DEPC brings out an important distinction between DEPC and DOPC, namely, that in DEPC there are two transitions, one at low pressure (0.7 kbar), which we have identified as the pressure-induced liquid-crystalline to gel phase transition, and the other at $\sim$5 kbar, which is reflected in an abrupt and large splitting of the CH_2 scissoring and rocking bands. (For the sake of argument, we exclude structural phase transitions at pressures above 10 kbar in either lipid system.) In fact, the existence of two events as a function of pressure in DEPC is also reflected in the barotropic behavior of the =CH deformation mode band. At the 0.7-kbar transition, there is a 2-cm^{-1} decrease, qualitatively similar to that observed at 11 °C in a thermotropic study of DEPC bilayers (Jaworsky & Mendelsohn, 1986), above which the band frequency does not change until just above the higher pressure event at 5 kbar, at which point there is a noticeable change in $d\nu/dP$ for this band (see Figure 5B). On the other hand, in DOPC, there is basically *one* event at 5.2 kbar, which appears to encompass not only the pressure-induced transition from the disordered liquid-crystalline phase to the ordered gel phase but also major conformational and packing changes (vide supra). That this is so only serves to emphasize the profound effect that the bent geometry of the cis double bond has on the structure and packing of unsaturated lipids in bilayers.

Conclusions

In this study, we have used pressure as a variable parameter to demonstrate that there are profound differences in the barotropic behavior of cis and trans unsaturated phospholipids monitored by infrared spectroscopy. In particular, not only are the critical pressures of the liquid-crystalline to gel phase transitions very different in DOPC and DEPC but the nature of this event is also distinctly different in these two lipids. These differences can be directly attributed to the different geometrical configurations of the cis and trans double bonds, which lead to distinctly different dynamical structures in the pressure-induced gel phases of DOPC and DEPC. In particular, it appears that the bent geometry of the cis double bond can only be accommodated in the gel phase by a unique packing arrangement in which the bent oleoyl acyl chains are closely packed with all chains oriented parallel to each other, and the cis double bonds of each chain are adjacent and at the same distance from the bilayer surface.

It is interesting to note that, in a previous statistical mechanical description of the effects of acyl chain unsaturation on the chain melting phase transition (Berde et al., 1980), the only model that could explain the dependence of transition temperature on cis double bond position incorporated *no* long-range orientational correlations between the chains on different molecules. We have not found evidence for such a "disordered" solid phase model in our infrared experiments, and in fact, in the context of the models considered by Berde et al. (1980), our results support an "ordered" solid phase model. In both the "ordered" and "disordered" models for the low-temperature gel phase of cis unsaturated phospholipids considered by Berde et al. (1980), it is assumed that all of the single C–C bonds of the oleoyl acyl chain are in their trans configurations. Although the intense wagging band progression in the pressure-induced gel phase of DOPC implies a significant portion of the polymethylene chain segments of the oleoyl acyl chain are in their trans conformations, on the basis of the infrared results alone we cannot rule out the presence of gauche conformers, particularly between the double

bond and the terminal methyl group. Whether or not there are gauche conformers could be established by Raman spectroscopy of these systems. The outcome of these experiments and their interpretation will be discussed in a future publication. Since the canonical packing arrangement of both saturated *and* unsaturated 1,2-diacyl phospholipids in the liquid crystalline or the gel phase incorporates a staggering of the two acyl chains, adjacent cis double bonds in the pressure-induced gel phase of DOPC must include a change in conformation of the glycerol backbone. We suggest that it is this latter change which accounts for the striking change in the relative intensities of the *sn*-1 and *sn*-2 C=O bands at the 5.2-kbar transition.

We note that, with the exception of the olefinic =CH stretching band, the infrared spectra of DOPC and DEPC in the liquid-crystalline phase are essentially indistinguishable, and it is only in the pressure-induced gel phase of these systems that distinct differences are observed. On the other hand, ^{2}H NMR spectroscopy of DOPC and DEPC labeled at the 9′,10′-positions reveal substantial differences between these two lipids in the liquid-crystalline phase. Thus, in their ability to detect conformational and structural differences between cis and trans unsaturated phospholipids, infrared spectroscopy of unsaturated lipids in the gel phase complements ^{2}H NMR spectroscopy of these systems in the liquid-crystalline phase.

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Mixed-Chain Phosphatidylcholine Bilayers: Structure and Properties†

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ABSTRACT: Calorimetric and X-ray diffraction data are reported for two series of saturated mixed-chain phosphatidylcholines (PCs), 18:0/*n*:0-PC and *n*:0/18:0-PC, where the *sn*-1 and *sn*-2 fatty acyl chains on the glycerol backbone are systematically varied by two methylene groups from 18:0 to 10:0 (n = 18, 16, 14, 12, or 10). Fully hydrated PCs were annealed at −4 °C and their multilamellar dispersions characterized by differential scanning calorimetry and X-ray diffraction. All mixed-chain PCs form low-temperature "crystalline" bilayer phases following low-temperature incubation, except 18:0/10:0-PC. The subtransition temperature (T_s) shifts toward the main (chain melting) transition temperature (T_m) as the *sn*-1 or *sn*-2 fatty acyl chain is reduced in length; for the shorter chain PCs (18:0/12:0-PC, 12:0/18:0-PC, and 10:0/18:0-PC), T_s is 1–2 °C greater than T_m, and the subtransition enthalpy (ΔH_s) is much greater than for the longer acyl chain PCs. T_m decreases with acyl chain length for both series of PCs except 18:0/10:0-PC, while for the positional isomers, *n*:0/18:0-PC and 18:0/*n*:0-PC, T_m is higher for the isomer with the longer acyl chain in the *sn*-2 position of the glycerol backbone. The conversion from the crystalline bilayer L_c phase to the liquid-crystalline L_α phase with melted hydrocarbon chains occurs through a series of phase changes which are chain length dependent. For example, 18:0/18:0-PC undergoes the phase changes $L_c \rightarrow L_\beta' \rightarrow P_\beta' \rightarrow L_\alpha$, while the shorter chain PC, 10:0/18:0-PC, is directly transformed from the L_c phase to the L_α phase. However, normalized enthalpy and entropy data suggest that the overall thermodynamic change, $L_c \rightarrow L_\alpha$, is essentially chain length independent. On cooling, the conversion to the L_c phases occurs via bilayer gel phases, L_β', for the longer chain PCs or through triple-chain interdigitated bilayer gel phases, L_β*, for the shorter chain PC 18:0/12:0-PC and possibly 10:0/18:0-PC. Molecular models indicate that the bilayer gel phases for the more asymmetric PC series, 18:0/*n*:0-PC, must undergo progressive interdigitation with chain length reduction to maintain maximum chain–chain interaction. The L_β* phase of 18:0/10:0-PC is the most stable structure for this PC below T_m. The formation and stability of the triple-chain structures can be rationalized from molecular models.

The lipid composition of biological membranes is generally complex, with individual lipids differing from each other in a number of ways, viz., variation in the functional groups in the head-group region and variation in chain lengths at the *sn*-1 and *sn*-2 positions of the glycerol backbone, as well the degree of unsaturation of the acyl chains. With respect to the acyl chains, the tendency is for the *sn*-1 position to consist of saturated fatty acyl chains while the *sn*-2 position consists of unsaturated, branched, or short saturated chains.

Earlier investigations of model phospholipid systems have focused on phosphatidylcholines (PCs) containing identical acyl chains in the *sn*-1 and *sn*-2 positions of the glycerol backbone, and a number of studies have been reported using differential scanning calorimetry (DSC), X-ray diffraction, and spectroscopic and dilatometric methods (Chapman et al., 1967; Levine et al., 1968; Tardieu et al., 1973; Mabrey & Sturtevant, 1976; Janiak et al., 1976, 1979; Chen et al., 1980; Füldner, 1981; Ruocco & Shipley, 1982a,b; Nagle & Wilkinson, 1982; Cameron & Mantsch, 1982). With hydrated 1,2-dipalmitoyl-L-phosphatidylcholine (DPPC or 16:0/16:0-PC) as a classic example of a PC containing identical acyl chains, the following picture emerges for the structural changes accompanying the cooling of 16:0/16:0-PC from the liquid-crystalline bilayer phase (L_α) (Chen et al., 1980; Füldner, 1981; Ruocco & Shipley, 1982a,b):

$$L_\alpha \leftrightarrow P_\beta' \leftrightarrow L_\beta' \underset{\text{fast}}{\overset{\text{slow}}{\rightleftharpoons}} L_c$$

The liquid-crystalline bilayer phase (L_α) undergoes a transition to a "rippled" gel phase (P_β') and then a further transition to a tilted chain, bilayer gel phase (L_β'). The L_β' phase is metastable at low temperatures and undergoes a slow conversion to a "crystalline" bilayer phase (L_c) characterized by increased chain packing and dehydration (Chen et al., 1980; Füldner, 1981; Ruocco & Shipley, 1982a,b; Nagle & Wilkinson, 1982).

Recognizing the heterogeneity with respect to fatty acyl composition in biological membranes, more recent studies have concentrated on the physical properties of saturated mixed-chain PCs where the fatty acids attached at the *sn*-1 and *sn*-2 positions differ in chain length (Keough & Davis, 1979; Chen & Sturtevant, 1981; Stümpel et al., 1981, 1983; Mason et al., 1981a,b, 1983; Huang et al., 1983; Huang & Levin, 1983;

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