REORIENTATIONAL AND CONFORMATIONAL ORDERING PROCESSES AT ELEVATED PRESSURES IN 1,2-DIOLEOYL PHOSPHATIDYLCHOLINE

A Raman and Infrared Spectroscopic Study

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ABSTRACT Raman and infrared spectra of fully hydrated bilayers of 1,2-dioleoyl phosphatidylcholine (DOPC) were measured at increasing hydrostatic pressures up to −37 kbar. Under ambient conditions aqueous dispersions of DOPC are in the liquid crystalline state. The application of an external hydrostatic pressure induces conformational and dynamic ordering processes in DOPC, which trigger a first-order structural phase transition at 5 kbar from a disordered liquid crystalline state to a highly ordered gel state. In the gel phase the methylene chains of each molecule are fully extended and the two all-*trans* chain segments on both sides of the rigid *cis* double bond form a bent structure. The bent oleoyl chains in each molecule, as well as in neighboring molecules are packed parallel to each other. To achieve this parallel interchain packing, the double bonds of the *sn*-1 and *sn*-2 chains of each molecule must be aligned at the same position with respect to the bilayer interface which is achieved by a rotation of the C—C bonds in the glycerol moiety in the head group. The extremely strong interchain interactions in the gel phase of DOPC are unique for this lipid with *cis* dimono-unsaturated acyl chains. Our experimental results suggest that in the pressure-induced gel phase of DOPC the olefinic CH bonds are rotated out of the phase of the bent oleoyl chains and that the oleoyl chains of opposing bilayers bend towards opposite directions.

INTRODUCTION

The effect of hydrostatic pressure on the structural and dynamic properties of aqueous phospholipid dispersions has been studied extensively (Wong, 1984*a*, 1986, 1987*a*–*c*; Wong et al., 1988). In the liquid crystals of phospholipids with saturated alkyl chains intrachain conformational and interchain reorientational ordering processes are induced by external pressure, which in turn trigger a series of structural phase transitions from a disordered liquid crystalline state to a series of highly ordered gel states. However, very little is known about the structural and dynamic properties of lipids containing *cis* monounsaturated acyl chains and their relaxation under hydrostatic pressure, although they constitute a major part of the plasma membranes of most bacterial and mammalian cells.

We have therefore carried out a high-pressure infrared and Raman spectroscopic study on a *cis* dimonounsaturated lipid, namely 1,2-dioleoyl phosphatidylcholine (DOPC) dispersed in water (D_2O). When DOPC molecules are dispersed in water at room temperature and atmospheric pressure they form a liquid crystalline phase (Williams and Chapman, 1970).

Due to the presence of a rigid *cis* double bond in the middle of each hydrocarbon chain (Scheme I), the structural and dynamic properties of DOPC liquid crystals and their response to external hydrostatic pressure are expected to be different from those of lipids with saturated chains. Model calculations (Berde et al., 1980) suggest that the orientational and conformational ordering processes on going from the liquid-crystalline phase to the gel phase, which are observed in saturated lipids (Wong, 1986, 1987*a* and *b*), may not occur in DOPC and that a larger number of gauche bonds remain in the gel phase of DOPC so that the straight configuration of the hydrocarbon chains can be maintained. On the other hand, if conformational ordering does occur in the gel phase of DOPC at high pressure and thus the methylene chain segments on both sides of the *cis* double bond become highly extended, then the zig-zag planes of the two methylene chain segments must form a bent configuration at the *cis* double bond (Scheme I). In this case, a structural rearrangement in the head group region of DOPC is required to cope with the long-range correlation among the zig-zag planes of different chains in the bilayer lattice. The present high-pressure vibrational spectroscopic study is expected to provide new insight into

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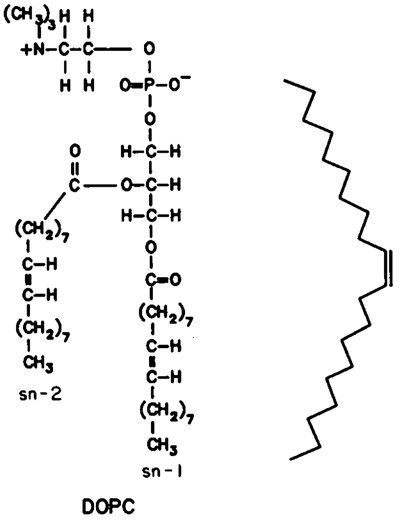

SCHEME I

these structural and dynamic properties of liquid crystals with *cis* dimonounsaturated chains.

EXPERIMENTAL

High-purity DOPC was obtained from Avanti Polar Lipids, Birmingham, AL. Fully hydrated DOPC dispersions in 50 wt% D_2O were prepared by heating the DOPC/D_2O mixture to ~40°C in a closed vial, vortexing while warm, and cooling to ~0°C. The heating-vortexing-cooling cycle was repeated three times, leading to a homogeneous sample. This sample was then placed at room temperature in the sample hole of a type Ia diamond anvil cell for the Raman spectroscopic investigation (Wong and Klug, 1983; Wong, 1984*b*) and in the sample hole of a type IIa diamond anvil cell for the infrared spectroscopic study (Wong and Klug, 1983; Wong et al., 1985). The pressures on the sample were determined from the ruby R line pressure scale in the Raman spectra (Wong, 1984*b*, 1987*b*) and from the α-quartz phonon bands in the infrared spectra (Wong, 1987*b*, Wong et al., 1985).

Raman spectra were excited by a Spectra-Physics model 164 argon ion laser (model 164, Spectra-Physics Inc., Mountain View, CA) operating at 514.5 nm with a power of 100 mW at the sample. The detection system included a Triplemate monochromator (model 1877, Spex Industries, Inc., Edison, NJ), an intensified multichannel detector (model TN-6122, Tracor Northern, Middleton, WI), and a multichannel analyzer (model TN-1710, Tracor Northern) interfaced with a Tandy 6000 HD microcomputer system.

Infrared spectra were measured at 28°C on a Fourier transform spectrophotometer (model DA3.02, Bomem, Vanier, Canada) with a liquid nitrogen cooled mercury cadmium telluride detector. For each spectrum, 512 interferograms were co-added and Fourier transformed to yield spectra with a resolution of 4 cm^{-1}. Details of the high pressure infrared and Raman spectroscopic measurements have been given previously (Wong, 1984*b*; Wong et al., 1985).

RESULTS AND DISCUSSION

Infrared and Raman spectra of DOPC liquid crystals in D_2O were measured as a function of pressure up to pressures of –37 kbar. Large changes in both the infrared and Raman spectra were induced by external pressure discussed below in terms of changes in the structural and dynamic properties of DOPC.

Structural Phase Transition

Fig. 1 shows Raman spectra of aqueous dispersions of DOPC in the region of the CH-stretching vibrations at increasing pressures. Below 4.2 kbar the spectra are typical of conformationally and orientationally disordered CH_2 chain systems, such as liquid *n*-alkanes (Wong et al., 1987), micellar solutions of surfactants (Wong and Mantsch, 1983a and b, 1986), or lipid bilayer dispersions in the liquid crystalline phase (Wong, 1984*a*). The strong band at 2,854 cm^{-1} is due to the CH_2 symmetric stretching mode $\nu_s CH_2$, while the weak and broad CH_2 asymmetric stretching band at 2,890 cm^{-1}, $\nu_{as}CH_2$, is buried under the strong Fermi resonance bands extending from 2,860 to 2,930 cm^{-1} (Wong, 1984*a*).

Abrupt changes in the Raman spectrum of aqueous DOPC take place between 4.2 and 5 kbar (see Fig. 1). Above 5 kbar, the $\nu_s CH_2$ band at 2,854 cm^{-1} becomes extremely weak and is hardly recognizable in the spectra. On the other hand, the $\nu_{as}CH_2$ band emerges as a very strong and narrow band on top of the broad Fermi resonance band. The olefinic CH stretching band at 3,010 cm^{-1}, ν=CH, also becomes narrower and stronger above 5 kbar and two new bands, at 2,826 and 2,870 cm^{-1}, appear in the spectra above 5 kbar. With increasing pressure the intensity of the 2,870 cm^{-1} band increases relative to that of the $\nu_{as}CH_2$ band and eventually it exceeds that of the $\nu_{as}CH_2$ band (Fig. 1); as the pressure increases a new band new 2,915 cm^{-1} appears gradually.

Other vibrational modes in the Raman spectrum of DOPC as well as in the infrared spectrum of the same sample (Siminovitch et al., 1987) also exhibit large

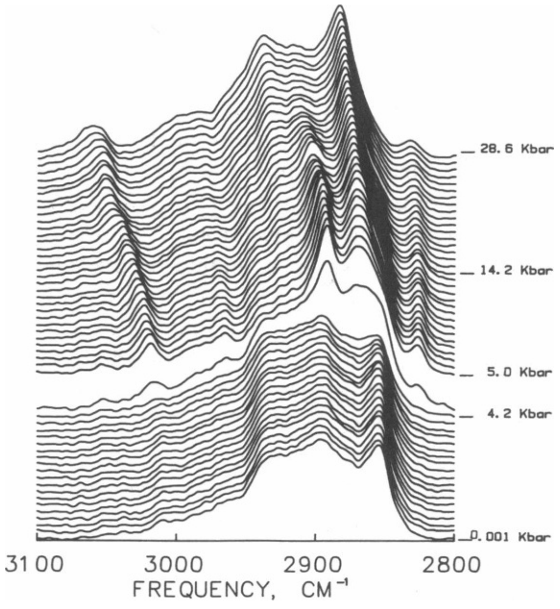

FIGURE 1 Raman spectra of aqueous dispersions (50:50 wt %) of DOPC in the region of the CH stretching vibrations recorded under increasing pressure (from 0.01 to 28.6 kbar).

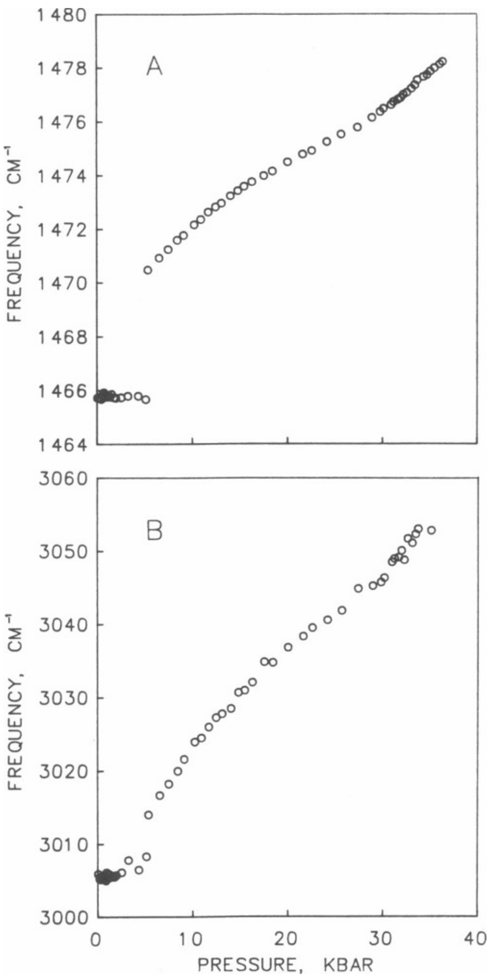

FIGURE 2 Pressure dependence of the infrared frequency of the δCH_2 (*A*) and $\nu{=}CH$ (*B*) bands of DOPC.

changes at 5 kbar (not shown). These changes are reversible, suggesting that a structural phase transition is induced by an external pressure of 5 kbar. The presence of a structural phase transition at 5 kbar is clearly evident from the discontinuities at 5 kbar in the pressure dependences of all the infrared and Raman spectral parameters as exemplified by the infrared frequencies of the CH_2 scissoring band, δCH_2, and the olefinic CH stretching band, $\nu{=}CH$ in Fig. 2, the CH_3 symmetric bending band, $\delta_s CH_3$, and the ester carbonyl stretching band, $\nu C{=}O$ in Fig. 3, or the Raman frequencies of the C=C stretching band, $\nu C{=}C$, and the symmetric CH_2 stretching band, $\nu_s CH_2$ in Fig. 4. The discontinuities in the pressure dependences of these frequencies reflect the volume change associated with this transition and are indicative of a first order phase transition.

Reorientational and Conformational Ordering

It is known that the broadening of the anisotropic component of condensed phase Raman bands is mainly due to an

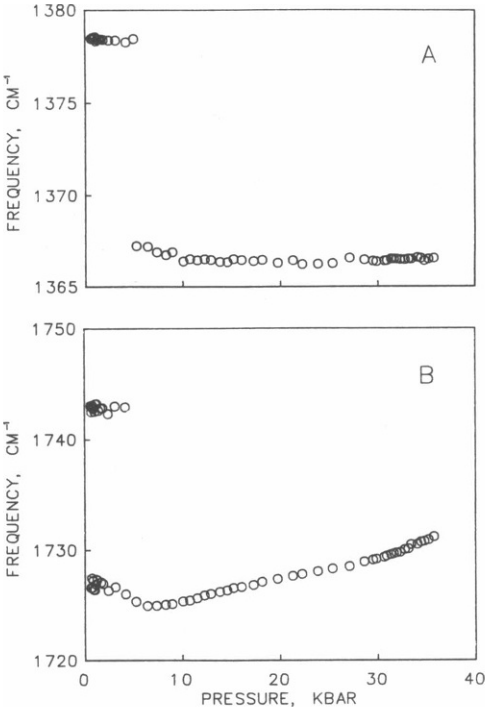

FIGURE 3 Pressure dependence of the infrared frequency of the $\delta_s CH_3$ (*A*) and $\nu C{=}O$ (*B*) bands of DOPC.

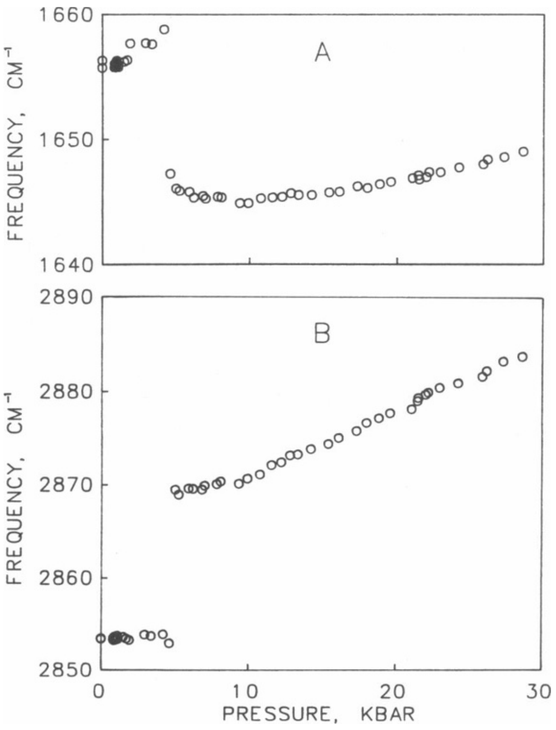

FIGURE 4 Pressure dependence of the Raman frequency of the $\nu C{=}C$ (*A*) and $\nu_s CH_2$ (*B*) bands of DOPC.

increase in the reorientational fluctuations of these molecules in the condensed phase (Wong, 1984*a*; Bartoli and Litovitz, 1972; Rakov, 1959; Cho et al., 1986*a*). Therefore, the widths of the depolarized and weakly polarized Raman bands of CH_2 chains, such as the CH_2 asymmetric stretching ($\nu_{as}CH_2$), the CH_2 bending (δCH_2), CH_2 twisting (τCH_2), and asymmetric (ν_{as}C—C) and symmetric (ν_sC—C) C—C stretching bands, all of which are dominated by the anisotropic component (Kobayashi and Tadokoro, 1980), are commonly used as a measure of the reorientational mobility of hydrocarbon chains in lipids (Wong, 1984*a*).

In the Raman spectrum of the liquid crystalline phase of DOPC (Fig. 1) the $\nu_{as}CH_2$ band is very broad and merges with the broad Fermi resonance band. This is characteristic of orientationally disordered chains in liquid *n*-alkanes, micellar solutions of surfactants, or aqueous lipid dispersions in the liquid crystalline phase (Wong, 1984*a*; Wong et al., 1987; Wong and Mantsch, 1983a and b, 1986). It indicates that the large-angle reorientational mobility of the DOPC molecules along the chain axis is extremely large in the liquid crystalline phase (Synder et al., 1980; Cho et al., 1986*b*). The highly disordered reorientation of the structure in the liquid crystalline phase of DOPC is consistent with the results obtained from fluorescence and NMR studies (Stubbs et al., 1981; Seelig et al., 1981).

Above 5 kbar, the $\nu_{as}CH_2$ band becomes much narrower and appears superimposed on the broad Fermi resonance band. Such narrow $\nu_{as}CH_2$ bands are found in solid *n*-alkanes (Wong et al., 1987), in the coagel phase of aqueous surfactants (Wong and Mantsch, 1983 a and b, 1986) and in the gel phase of aqueous lipid dispersions (Wong, 1984*a*), in which the reorientational fluctuations of the molecules are largely damped and the orientation of the CH_2 chains is highly ordered. The drastic changes in the Raman spectrum of aqueous DOPC at the critical pressure thus reflect the dynamic ordering of the hydrocarbon chains associated with a pressure-induced structural phase transition at 5 kbar. The Raman band shapes in the CH stretching region of DOPC in the liquid crystalline phase depict a conformationally highly disordered structure with a large number of gauche bonds. At the critical pressure, the number of gauche bonds decreases abruptly and the conformation of the chains becomes highly ordered.

The conformational ordering at the critical pressure is also evident from the Raman spectra in the C—C stretching region, as shown in Fig. 5. In the spectra of the liquid crystalline phase below 5 kbar there are two weak bands near 1,065 and 1,130 cm^{-1}, due to the symmetric-respective-to-asymmetric skeletal C—C stretching modes of alkyl chains with all-*trans* conformations; the skeletal C—C stretching band at a frequency around 1,080 cm^{-1} is characteristic of conformationally disordered CH_2 chains with gauche conformations (Wong, 1984*a*; Lippert and Peticolas, 1972, and references therein). Above 5 kbar, the 1,130 cm^{-1} band splits into two bands at 1,110 and 1,140 cm^{-1}, which are attributed to the asymmetric skeletal C—C stretching modes of the two all-*trans* CH_2 chain segments on the methyl side and on the head group side of the double bond (Lippert and Paticolas, 1972; Wong and Mantsch, 1983a).

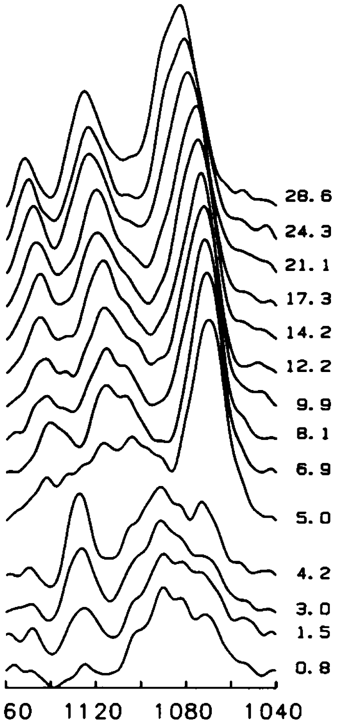

FIGURE 5 Raman spectra of aqueous dispersions of DOPC in the C—C stretching region at pressures between 0.8 and 28.6 kbar.

It is clear from Fig. 5 that in the liquid crystalline phase of DOPC the "gauche" band at 1,080 cm^{-1} is much stronger than the "all-*trans*" bands; yet, the relative intensity of the *trans* bands increase with increasing pressure in the liquid crystalline phase, which implies that external pressure induces some conformational ordering in the liquid crystalline phase, although the disordered gauche conformational dominates the structure of the hydrocarbon chains in this phase.

Above 5 kbar, the all-*trans* C—C stretching bands become very strong whereas the gauche C—C stretching band can hardly be recognized in the spectra, indicating that in the pressure-induced gel phase, the conformation of the chains is highly ordered and that both CH_2 chain segments are highly extended.

Because of the full extension of the two CH_2 chain segments in the gel phase of DOPC, and the rigid characteristics of the oleoyl *cis* double bond, the two zig-zag CH_2 chain segments on both sides of the *cis* double bond of each oleoyl chain form a bent structure as indicated in Scheme I. Because the reorientational fluctuations of a bent chain would require more space than those of a straight, disordered chain, reorientational fluctuations are forbidden at elevated pressures. Therefore, at the critical pressure the reorientational mobility of the oleoyl chains in DOPC is completely arrested and the conformationally disordered, straight chains of the liquid crystalline phase become conformationally ordered bent chains in the gel phase of DOPC.

The formation of such a bent configuration in the hydrocarbon chains of DOPC above 5 kbar is consistent with the dramatic decrease in the frequency of the double bond stretching mode, νC=C. Fig. 6 shows the Raman spectra of the νC=C band at three different pressures, i.e., at 4.23 kbar in the liquid crystalline state, at 4.66 kbar in the transition state, and at 5.04 kbar in the gel phase. It is evident from these spectra that the νC=C band undergoes a 12 cm^{-1} shift in frequency in going from the liquid crystalline phase to the gel phase. The decrease in the νC=C frequency at 5 kbar can only be interpreted in terms of an elongation of the C=C double bond in the gel phase, arising from the strong repulsive force between the hydrogen atoms of the adjacent CH_2 groups on both sides of the double bond. At a pressure as high as 5 kbar, compression along the direction normal to the bilayer interface causes a further bending of the two zig-zag chain segments at the level of the *cis* double bond. On the other hand, this pressure-induced deformation is opposed by the strong repulsion between the two hydrogen atoms of the CH_2 groups on both sides of the double bond, which are very close to each other in the bent configuration. The balance between the compression-induced chain bending and the van der Waals repulsion results in a slight elongation of the double bond.

The degree of chain bending increases between 5 and 10 kbar, as indicated by a further decrease in the νC=C frequency in this pressure range (Fig. 4 *A*). In the pressure range of 10 to 30 kbar, the νC=C frequency only increases slightly. In fact, such a high pressure induces a compression of the C=C double bond (Wong, 1987*b*), which causes an increase in the νC=C frequency. The balance between the elongation and compression of the C=C double bond explains the small increase in the νC=C frequency above 10 kbar. The pressure-induced elongation of the C=C double bond is supported by the anomalously large pressure shift of the ν=CH frequency, as discussed later.

Interchain Packing

In 1,2-diacyl phospholipids the ester group of the *sn*-1 and *sn*-2 chains have inequivalent conformations; the *sn*-2 chain is rotated about the C_1—C_2 bond as shown in Scheme I (Hauser et al., 1981). Consequently, the positions of the *cis* C=C double bonds of the *sn*-1 and *sn*-2 chains with respect to the head group are not the same. If this molecular configuration is to be maintained in the gel phase of DOPC above 5 kbar, then the bending in the *sn*-1 chain must occur at a position further away from the bilayer interface than that in the *sn*-2 chain. In this case, voids would be generated in the gel phase between the *sn*-1 and *sn*-2 chains and thus the overall volume of the molecule would increase, which is not allowed at high pressure. Therefore, to form a bent configuration in both the *sn*-1 and *sn*-2 chains, and at the same time maintain the closest packing, the C=C double bonds in the *sn*-1 and *sn*-2 chains must align with each other. The only way to achieve this is to move the *sn*-1 chain towards the interface and to align the C=C double bond of the *sn*-1 chain with that of the *sn*-2 chain by rotating the C—C bonds in the glycerol moiety in the head group. The fact that such a structural

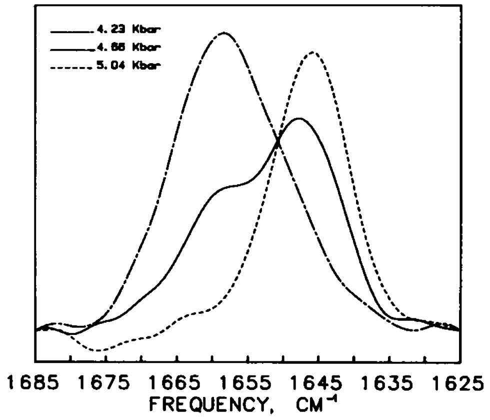

FIGURE 6 Representative Raman spectra of the νC=C band near the critical pressure.

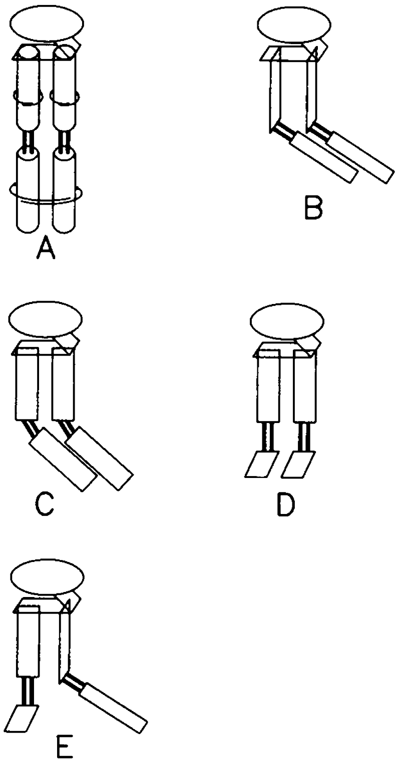

FIGURE 7 Schematic illustration of possible interchain packings in DOPC.

change indeed takes place at the transition pressure is evident from the changes in the infrared and Raman spectra in the ester C═O stretching mode, νC═O.

The infrared νC═O band is broad in the liquid crystalline phase. As in the case of other 1,2-diacyl phospholipids, this broad band can be resolved into two components at 1,743 and 1,727 cm^{-1} which are due to the νC═O modes of the nonhydrogen bonded *sn*-1 C═O group and that of the hydrogen bonded *sn*-2 C═O group, respectively (Wong and Mantsch, 1988). At 5 kbar, the νC═O band becomes narrower (5 cm^{-1} decrease in halfwidth) and shifts to lower frequency. The band cannot be resolved into two components by standard band narrowing techniques (Mantsch et al., 1986; Cameron and Moffatt, 1987). The frequency of this band in the gel phase above 5 kbar matches that of the hydrogen-bonded *sn*-2 νC═O band in the liquid crystalline phase, as shown in Fig. 3 *B*. These results indicate that above 5 kbar the environment of the *sn*-1 C═O group changes and becomes similar to that of the *sn*-2 C═O group, suggesting that the *sn*-1 C═O group moves closer to the bilayer interface so that it is now at the same level as the *sn*-2 C═O group. With this structural adjustment in the gel phase the chains in all DOPC molecules then are able to pack more closely.

Several possible packings of the bent chains in DOPC are shown schematically in Fig. 7. Fig. 7 *A* depicts the structure of the conformationally and reorientationally disordered DOPC molecules in the liquid crystalline phase, whereas Fig. 7, *B–E* represent four possible packings of the bent chains in the gel phase. The correlation field splitting of the CH_2 bending mode in the Raman and infrared spectra and that of the CH_2 rocking mode in the infrared spectrum are absent in the gel phase spectra of DOPC, which implies that the chains in each molecule and those of neighboring molecules are parallel to each other (Wong, 1984*a*, 1987*a*; Wong et al., 1988). Consequently, the packing with perpendicular chains shown in Fig. 7 *E* can be ruled out. In fact, the interchain packing illustrated in Fig. 7 *E* would occupy more space than that of the liquid crystalline phase (Fig. 7 *A*), and thus would be disallowed at elevated pressures.

A 120° rotation of the zig-zag chain segments on both sides of the *cis* C═C about the single C—C bonds adjacent to the double bond is required for the interchain packings shown in Fig. 7, *B* and *D*. The conformation of these chains has been referred to as skew/skew (Siminovitch et al., 1987; Koyama and Ikeda, 1980, and references therein). Thus, the double bonds in structures *B* and *D* are no longer *cis* double bonds and the HC═CH plane in each chain is 120° out of the plane of the bent chain.

The true *cis* double bond characteristics are found only in structure *C*. The spectral parameters observed in the present work and their pressure dependences indeed favor structure *C* for the pressure-induced gel phase of DOPC. First, the frequency of the olefinic stretching band, ν═CH, is extremely sensitive to pressure (Fig. 2 *B*). The frequency shift of this band is >2 cm^{-1} per kbar in the pressure range of 5 to 10 kbar, which is unusually large and very seldom observed for an internal mode (Wong, 1987*b*). This large pressure effect must result from the bent geometry of the oleoyl chain at the *cis* double bond, which leads to a very close contact of the ═C—H bond with the neighboring chain. Consequently, at high pressure the interchain interactions experienced by the olefinic bond become much stronger than those of any other functional group. If the *cis* characteristics of the olefinic bonds are removed and the ═C—H bonds are titled out of the way as in the skew/skew conformations of structures *B* and *D*, the pressure-induced interchain interactions experienced by the olefinic bonds would be considerably reduced and the pressure-induced shift of the ν═C—H frequency should be comparable with that of the CH_2 stretching modes.

The strong interchain interactions of the ═C—H groups in the gel phase imply that the interchain packing at this functional group is much tighter than that at other parts of the chains. This unique structural feature explains why the elongation of the C═C double bond is enhanced by external pressure in the range of 5 to 10 kbar (see Reorientational and Conformational Ordering). As elevated hydrostatic pressure promotes an overall tighter packing of the chains, one way to achieve this is to further bend the hydrocarbon chains at the *cis* double bound. Such a pressure-induced bending of the chains is opposed, however, by the repulsive force between the hydrogen atoms of the CH_2 groups adjacent to the double bond. Consequently, a further elongation of the double bond is required to compensate for this repulsion.

The CH out-of-plane wagging mode of the ═CH group, ω═CH, is softened by external pressure as indicated by the decrease in frequency of the ω═CH band with pressure (Fig. 8). The softening of the ω═CH mode by pressure strongly suggests that pressure induces a structural deformation of the olefinic groups in DOPC. In this pressure-induced deformation the ═C—H bonds rotate out of the plane of the bent chains, following the eigenvector of the out-of-plane wagging mode. As a consequence of this rotation, the strong interchain interactions at the olefinic groups are relaxed. In Fig. 7, *B* and *D*, the olefinic groups are already rotated out of the plane of the bent

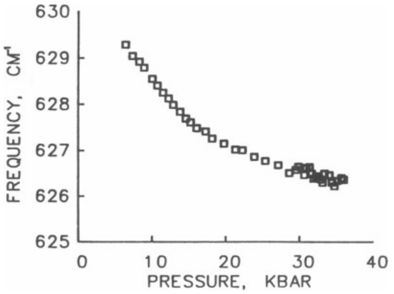

FIGURE 8 Pressure dependence of the infrared frequency of the ω═CH band.

chains by 120°. A further rotation of this functional group would result in a strengthening instead of a relaxation of the strong interchain interactions at the olefinic groups and thus would be disallowed at elevated pressures.

The present results suggest that the most plausible interchain packing in the pressure-induced gel phase of DOPC is that represented by Fig. 7 *C*. From a comparison of the Raman spectrum of the temperature-induced gel phase of DOPC with those of the L and H forms of oleic acid in a previous study (Koyama and Ikeda, 1980), it was concluded that the hydrocarbon chains in the temperature-induced gel phase of DOPC are in the skew/skew conformation, although the evidence given for the skew/skew structure was weak. On the other hand, the νC=C frequency of the temperature-induced gel phase increases by 6 cm^{-1} at the transition temperature, whereas that of the pressure-induced gel phase decreases by 12 cm^{-1} at the transition pressure (Fig. 4 *A*), a strong indication that the structure of the temperature-induced gel phase differs from that of the pressure-induced gel phase of DOPC.

In both the infrared and Raman spectra of the gel phase of DOPC, there is a narrow band at 2,826 cm^{-1} which has been assigned to the CH_2 stretching mode of the methylene groups adjacent to the olefinic group (Siminovitch et al., 1987). This band seems to be unique for 1,2-diacylphospholipids with two *cis* monounsaturated chains because it has not been observed in the vibrational spectra of 1,2-diacylphospholipids with saturated and single *cis* monounsaturated chains (Siminovitch et al., 1987), or in the spectra of fatty acids (or their salts) with saturated and *cis* monounsaturated chains (Wong et al., 1987; Wong and Mantsch, 1983a and b; Sinclair et al., 1952*a* and *b*; Cameron et al., 1982; Yang et al., 1985; Okabayashi, 1978; Davies et al., 1972). The fact that this band is not present in the vibrational spectra of oleic acid and its sodium salt, in which the CH_2 groups adjacent to the olefinic group exhibit the skew/skew conformation (Abrahamsson and Ryderstedt-Nahringbauer, 1962), strongly suggests that the interchain packing in the gel phase of DOPC resembles that shown in Fig. 7 *C*, rather than those in Fig. 7 *B* and *D*.

A dramatic decrease in the infrared frequency of the symmetric methyl bending mode of DOPC occurs at 5 kbar (Fig. 3 *A*); the frequency decreases further with increasing pressure in the range of 5 to 10 kbar and then remains more or less constant above 10 kbar. A similar softening of this mode with increasing pressure has been observed in solid *n*-hexadecane and was attributed to a structural deformation of the methyl groups (Wong et al., 1987). This suggests that the interactions between the methyl end groups of DOPC molecules are extremely strong and that the methyl groups must directly face each other in the bilayer lattice. Furthermore, it indicates that the packing of the oleoyl chains of opposing bilayers of DOPC is such that the chains bend towards opposite directions (Wong et al., 1987).

Fermi Resonance Interactions

It is well known that in the Raman spectra of systems with long methylene chains there are broad bands in the C—H stretching region that result from intrachain Fermi resonance interactions between the symmetric CH_2 stretching fundamental, $\nu_s CH_2$, and the binary combination of all the optically active CH_2 bending modes, $2\delta CH_2$, in the δCH_2 branch (Wong et al., 1987, and reference therein). The shape, peak height, and peak position of these Fermi bands are strongly dependent on the frequency dispersions of $2\delta CH_2$. Recently, it has been shown (Wong et al., 1987) that the frequency dispersion of $2\delta CH_2$ in solid *n*-hexadecane decreases with increasing pressure and that the frequency dispersion practically disappears at pressures of ~50 kbar when all the $2\delta CH_2$ bands concentrate within a very narrow frequency range. This means that as a result of the increase in density of the $2\delta CH_2$ states and the intensity mixing with all other totally symmetric bands the width of the broad Fermi band decreases and the peak height increases with increasing pressure. At ~50 kbar, this Fermi resonance band becomes a narrow band comparable with the fundamental $\nu_{as} CH_2$ band. In the case of DPPC bilayers (Wong and Mantsch, 1985*a*), external pressure also reduces the frequency dispersion of $2\delta CH_2$ mode, however, the $2\delta CH_2$ mode at high pressure is localized at a different frequency from that in *n*-hexadecane (Wong et al., 1987).

The intensity of the Fermi resonance band in aqueous DOPC also increases in the gel phase. However, at a pressure of 28.6 kbar the Fermi band is still very broad and overlaps increasingly with the $\nu_{as} CH_2$ band as the pressure increases (Fig. 1). This effect of pressure on the Fermi resonance interaction in DOPC is solely due to the different pressure effect on the $2\delta CH_2$ dispersion in DOPC. As seen in Fig. 9, the frequency dispersion of

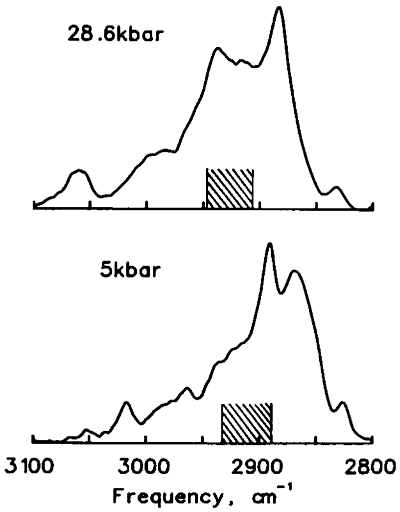

FIGURE 9 Raman spectra of aqueous DOPC in the CH stretching region at 5 and 28.6 kbar. The shaded area represents the frequency range of $2\delta CH_2$ at the indicated pressures.

$2\delta CH_2$ decreases only slightly between 5 and 28.6 kbar which results in maintaining a relatively broad band even at higher pressure. As pressure increases the frequency region of $2\delta CH_2$ shifts slightly towards higher frequencies which leads to an increased overlapp of this Fermi band with the $\nu_{as}CH_2$ band.

Interchain Interactions

The Raman peak height ratio between $\nu_{as}CH_2$ and ν_sCH_2 bands of lipids, $R = H_{2890}/H_{2850}$, has been widely used as a measure of the magnitude of interchain interactions (Wong, 1984*a*; Gaber and Peticolas, 1977; Mendelson and Koch, 1980; Snyder et al., 1978). The *R* values for aqueous DOPC dispersions are plotted against pressure in Fig. 10; since the ν_sCH_2 band is extremely weak at pressures above 7 kbar, only *R* values up to 7 kbar are shown in Fig. 10.

The *R* value in the liquid crystalline phase of DOPC is always >1; it increases with pressure and reaches 1.3 just before the critical pressure. Such large *R* values are observed for the first time in the liquid crystalline phase of a lipid indicating that the interchain interactions among the disordered oleoyl chains of DOPC are very large, much larger than those among disordered chains in the liquid crystalline phase of lipids with saturated chains.

At 5 kbar, the *R* parameter increases abruptly to 1.8. This *R* value is unusually large and indicates extremely strong interchain interactions in the gel phase of DOPC. In 1,2-diacyl phospholipids with saturated chains the *R* values of the pressure-induced gel phases range from 1.2 to 1.3 (Wong et al., 1982; Wong and Mantsch, 1984), however, in the GIV gel phase of DPPC it also reaches 1.8 (Wong and Mantsch, 1984). Thus, the large *R* value of DOPC is not surprising, because the gel phase of DOPC is formed only at a pressure as high as 5 kbar.

Recently, the reciprocal *R* values have been used to detect lipid bilayer structures with interdigitated hydrocarbon chains of opposing monolayers; it was shown that for such an interdigitated structure the $1/R$ value is lower than 0.75 (O'Leary and Levin, 1984, and references therein). The $1/R$ value of the gel phase of DOPC at 5 kbar is ~0.55; however, many lines of evidence indicate that the interdigitated bilayer structure does not exist in this phase. The interactions and correlations between the methyl groups on the hydrocarbon chains of opposing DOPC monolayers in the gel phase are extremely strong (see Interchain Packing). Such strong interactions and correlations are possible only if the methyl groups of the opposing monolayers face each other, which is not the case in an interdigitated structure. We also found that in the noninterdigitated gel phase of saturated diacyl lipids (Wong et al., 1982; Wong and Mantsch, 1984, 1985*b*) and in the noninterdigitated coagel phase of surfactants (Wong and Mantsch, 1983) the $1/R$ values are often much lower than 0.75.

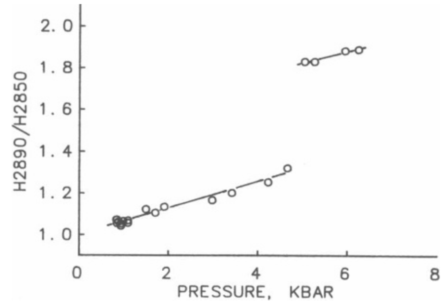

FIGURE 10 Pressure dependence of the peak height ratio between the $\nu_{as}CH_2$ and ν_sCH_2 Raman bands.

Strong interchain interactions in the gel phase of DOPC are also evident from the presence in the Raman spectrum of a strong interchain interaction band at 2,870 cm^{-1} (Fig. 1), originating from interchain Fermi interactions (Wong et al., 1987; Snyder et al., 1978). The intensity of this band is unusually high in the gel phase of DOPC and increases considerably with increasing pressure; at 9 kbar the peak height of this band exceeds that of the $\nu_{as}CH_2$ band. The strong interchain interactions in the gel phase of DOPC seem to be a unique feature of diacyl lipids with two *cis* monounsaturated chains.

CONCLUDING REMARKS

It is apparent from the present results that the effect of pressure on the liquid crystalline phase of DOPC is to induce intrachain conformational and interchain reorientational ordering processes which trigger a structural phase transition from a structurally and dynamically disordered liquid crystalline phase to a highly ordered gel phase in which the reorientational fluctuations are completely damped and the hydrocarbon chains are highly extended with a bent configuration at the *cis* double bond.

The packing of the oleoyl chains in the gel phase of DOPC is parallel to each other, with a molecular configuration resembling that represented in Fig. 7 *C*. To achieve such a close packing, the *cis* double bond on the *sn*-1 oleoyl chain has to move and align itself with the *cis* double bond on the *sn*-2 oleoyl chain, which is achieved by rotation of the C—C bonds in the glycerol moiety.

In the pressure-induced gel phase of DOPC there are extremely strong interactions between individual oleoyl chains and between the methyl end groups of opposing DOPC monolayers. From the pressure softening of the olefinic CH out-of-plane wagging mode and that of the symmetric CH_3 bending mode it is suggested that all olefinic CH bonds are rotated out of the plane of the bent oleoyl chains and that the oleoyl chains of opposing bilayers bend toward opposite directions. These pressure effects on the structural and dynamic properties of DOPC are expected to be unique for *cis* dimonounsaturated phospholipids.

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