HIGH-PRESSURE INFRARED SPECTROSCOPY OF ETHER- AND ESTER-LINKED PHOSPHATIDYLCHOLINE AQUEOUS DISPERSIONS

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ABSTRACT Infrared spectra of aqueous dispersions of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), and its ether-linked analogue, 1,2-dihexadecyl-*sn*-glycero-3-phosphocholine (DHPC), were measured in a diamond anvil cell at 28°C as a function of pressure up to 20 kbar. Although these two lipids differ only in the linkages to the saturated hydrocarbon chains, significant differences were observed in their barotropic behavior. Most notable were the magnitudes of the pressure-induced correlation field splittings of the methylene scissoring and rocking modes, and the relative intensities of the corresponding component bands. In the case of the scissoring mode, not only can the correlation field component band be resolved at a lower pressure in DHPC (1.2 kbar, as compared with 2.2 kbar in DPPC), but the initial magnitude of the correlation field splitting in DHPC, particularly <9 kbar, is significantly greater than that observed in DPPC. These differences are attributed to the presence of an interdigitated lamellar gel phase in DHPC. At all pressures where the correlation field component band $\delta'CH_2$ can be resolved, the relative peak height/intensity ratio $R = I\delta'/I\delta$ is greater in DPPC than in DHPC, suggesting that this parameter may be useful as a test of interdigitation.

INTRODUCTION

Ether-linked glycerophospholipids containing long alkyl or 1-alkenyl chains are found in a wide variety of biological membranes. The diether analogue of phosphatidylglycerophosphate is the major component of polar lipids in extremely halophilic bacteria (Kates, 1972), while alkyl and 1-alkenyl moieties are found in phospholipids of normal mammalian tissues (Horrocks, 1972; Pugh et al., 1977) and, at higher levels, in cancer cells (Snyder, 1972; Friedberg and Halpert, 1978). Although the wide distribution of ether lipids in biological tissues is now well established, and the biochemical pathways leading to ether lipid synthesis have been elucidated (Snyder et al., 1985), very little is known about the biological significance of most ether lipid species. In this regard, a better understanding of the physical properties of ether lipid bilayers should be useful, and towards that end, techniques such as surface balance (Paltauf et al., 1971), differential scanning calorimetry (Vaughan and Keough, 1974; Lee and Fitzgerald, 1980; Boggs et al., 1981; Seddon et al., 1983), x-ray diffraction (Schwarz et al., 1976), fluorescence spectroscopy (Bittman et al., 1981), vibrational spectroscopy (Mushayakarara and Mantsch, 1985; Huang et al., 1986; Levin et al., 1985*a*, Lewis et al., 1986) and NMR spectroscopy (Hauser, 1981; Hauser et al., 1981; Siminovitch et al., 1983; Ruocco et al., 1985*a,b;* Jarrell et al., 1986) have been used to study the physical properties of ether lipids in bilayers. Despite the diversity of physical techniques that have been used in these studies, the precise role that the ether linkage plays in modulating the physical properties of the lipid bilayer remains unclear. However, it is clear from this work that the change in linkage may, under certain circumstances, have a profound effect on the thermal behavior and molecular packing of ether phospholipids. For example, in the case of the phosphatidylethanolamines (PE), the change in linkage (from ester in 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine [DPPE] to ether in 1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine [DHPE]) results in a fivefold increase in both the entropy and enthalpy of the lamellar-to-inverted hexagonal (H_{II}) phase transition, as well as a 40°C reduction in the transition temperature itself (Seddon et al., 1983). This lowering of the lamellar-to-inverted hexagonal (H_{II}) phase-transition temperature, first observed by Boggs et al. (1981) in a differential scanning calorimetry (DSC) study of synthetic ether-linked analogues of PE, reflects a destabilization of the lamellar phase by the ether linkage. In the case of the phosphatidylcholines, early investigations of DHPC using physical techniques such as DSC (Vaughan and Keough, 1974), x-ray diffraction (Schwarz et al., 1976), and high-resolution ^{1}H-NMR (Hauser, 1981; Hauser et al., 1981) suggested that the replacement of ester linkages by ether bonds had little effect on thermal behavior, bilayer structure, or conformation of the phosphatidylcholine (PC) headgroup. However, more recent studies using DSC, x-ray diffraction, and solid-state ^{14}N-, ^{31}P-, and ^{2}H-NMR (Siminovitch et al., 1983; Ruocco et al.,

1985*a,b*) clearly indicate that the change in linkage does have a significant effect on structure and dynamics in the low temperature gel phases.

Vibrational spectroscopy is a technique eminently suited to studies of the structural and dynamic properties of phospholipid bilayers. Many of the spectral parameters of interest that can be used to characterize the Raman and infrared bands of various normal modes are quite sensitive to the conformation of certain functional groups, the interchain packing, the inter- and intrachain interactions and the chain mobility. In particular, the correlation field splitting of the CH_2 scissoring mode, δCH_2, in Raman and infrared spectra is very useful for the characterization of interchain packing (Wong, 1984; Boerio and Koenig, 1970). 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 lipid bilayers have been gained from studies of their barotropic behavior (Wong, 1984; Wong, 1986). For example, under hydrostatic pressure, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) bilayers have been shown to exhibit a very rich phase behavior, with a total of five pressure-induced structural phase transitions well identified (Wong and Mantsch, 1984; Wong and Mantsch, 1985*a,b*). Because these pressure-induced structural phase transitions involve modifications in acyl chain packing as a result of intrachain conformational and interchain reorientational ordering processes (Wong, 1986), studies using high-pressure vibrational spectroscopy should be especially fruitful in defining the nature of the changes in chain packing induced by ether linkages. Moreover, because previous studies pointed to a change in molecular packing in DHPC as the explanation of observed differences between ester- and ether-linked PCs (Ruocco et al., 1985*a,b*), we would expect any corresponding changes in the dynamic structure of the hydrocarbon chains of 1,2-dihexadecyl-*sn*-glycero-3-phosphocholine (DHPC) to be clearly reflected in a comparison of the barotropic behavior of DHPC and DPPC, as monitored by vibrational spectroscopy. There are a number of reasons for expecting that infrared spectroscopy, in particular, should be advantageous in providing information on interchain structure in these systems. First, the correlation field component of δCH_2 is relatively weak in the Raman spectra of DPPC (Wong and Mantsch, 1984, 1985*a;* Wong et al., 1982), whereas it is strong in the corresponding infrared spectra (Cameron et al., 1980*a, b*). Secondly, the rocking mode, γCH_2, of polymethylene chains and its associated correlation field component band, $\gamma' CH_2$, are infrared active, and the relative intensity of these components can be related to the relative orientation of the chains (Snyder, 1961; Wong and Mantsch, 1985*b*). The advantages of infrared spectroscopy for the determination of interchain structure have been demonstrated in a recent high-pressure study of DPPC (Wong and Mantsch, 1985*b*). Accordingly, in this work, we use high-pressure infrared spectroscopy to address the following question: "Given that there are differences in the packing of the hydrocarbon chains in the gel phases of DPPC and DHPC, what does a comparison of the barotropic behavior of these two lipids reveal about accompanying changes in dynamical structure?"

MATERIALS AND METHODS

DPPC was obtained from Sigma Chemical Co. (St. Louis, MO), while DHPC (puriss.) was obtained from Fluka Chemical Corp. (Hauppauge, NY). DHPC was shown to be >99% pure by thin-layer chromatography and by DSC. Fully hydrated (≥40 wt% D_2O, Merck, Sharp & Dohme, Montreal, Quebec) lipid dispersions were prepared for the infrared experiments by heating lipid/D_2O mixtures in a closed vial to ~60°C, followed by vortexing. After immediate freezing of the sample in dry ice, the heat/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 diam 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 on a Bomem model DA3.02 Fourier transform spectrophotometer (Bomem, Vanier, Quebec) 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., 1982/83). For each spectrum, typically 1,000 scans were co-added, at a spectral resolution of 4 cm^{-1}. Data reduction was performed using software developed in this laboratory. To eliminate the possibility of spurious differences in spectral parameters between DHPC and DPPC 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 using a breakpoint of 0.7 in the Fourier domain, and pressures calculated from these frequencies according to the expression $P = a_0 + a_1 \Delta\nu + a_2 \Delta\nu^2$, where $a_1 = 1.2062$, $a_2 = 0.015154$, and $\Delta\nu$ is the measured frequency shift. Arbitrarily setting zero pressure to correspond to the lowest pressure measured for the quartz band ($\Delta\nu = 0$), we set $a_0 \equiv 0$. Frequencies associated with particular modes were obtained from third-order derivative spectra, using breakpoints as indicated in the Figure captions (for details, see Moffatt et al., 1986).

RESULTS

A trivial comparison that can be made between the infrared spectra of DHPC and DPPC is to note the absence of the C=O stretching band in the spectra of DHPC. In thermotropic studies of model membranes, this band has served as a useful monitor of thermal-phase transitions (Casal and Mantsch, 1984) and lipid–protein interactions (Mendelsohn and Mantsch, 1986). In this study, which compares the barotropic behavior of DHPC and DPPC, the lack of the C=O functional group in DHPC does not represent a significant loss of information because, as previously noted (Wong and Mantsch, 1985*b*), neither the intensity nor the frequency of the ester C=O stretch mode in DPPC are significantly affected by external pressure.

A more interesting consequence of the change in linkage to the hydrocarbon chains is the marked reduction in the intensity of the CH_2 wagging band progression in DHPC observed in the region 1,380–1,190 cm^{-1}. On the one hand,

although this region is dominated in phospholipids by the relatively intense O═P═O antisymmetric stretching band of the phosphate group ~1,220 cm^{-1} and the D-O-D bending band at 1,215 cm^{-1}, the overlapping bands of the wagging progression are clearly visible in the spectra of DPPC shown in Fig. 1 *A*. On the other hand, the corresponding region in DHPC provides little evidence for this progression, as shown in the spectra of Fig. 1 *B*, and it is only by resolution enhancement (derivation or deconvolution) (Mantsch et al., 1986; Moffatt et al., 1986) of these spectra or by calculation of the difference spectrum (Cameron et al., 1980*a*) of liquid crystalline and gel-phase lipid that these components can be resolved at all.

The attenuated progression in DHPC can be attributed to the absence of the C═O group, because it is well known that the intensity of this progression depends strongly on the nature of the end groups of the corresponding hydrocarbon chain (Fischmeister, 1975; Fringeli and Günthard, 1981). For example, in *n*-alkanes, the CH_2 wagging bands have low intensity, whereas in fatty acids and esters, the coupling with vibrations of polar endgroups such as —COOH or —CO—OR results in a considerable enhancement in the intensity of this band progression. In DPPC, the band progression is sufficiently intense at all pressures >3 kbar to allow the resolution of the first eight components. The peak positions at 10 kbar of these components are tabulated in Table I, and agree well with frequencies identified previously (Cameron et al., 1980*b*) in the gel phase of DPPC. The frequencies of the wagging band components do not show a significant pressure dependence, increasing by <0.2 cm^{-1}/kbar over the pressure range investigated. A small jump in the peak frequency of the second and third components of the progression in DPPC at ~4.9 kbar shows that these components are sensitive to the structural-phase transition previously identified at 4.8 kbar (Wong and Mantsch, 1985*b*). The corresponding band progression in DHPC is very much weaker, reflecting the absence of the C═O group, as noted above. Of those components which can be resolved in DHPC, Table I shows that there is a downward shift in frequency, the same as that observed in the band progression of fatty acids when the number of coupled methylene oscillators is increased.

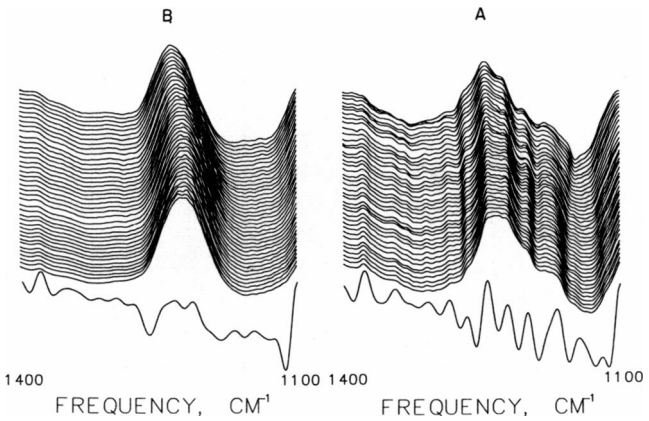

FIGURE 1. Stacked contour plots of the infrared spectra of fully hydrated (*A*) DPPC and (*B*) DHPC in the spectral region containing the CH_2 wagging band progression. In this and the following figure, displayed spectra have been interpolated in the frequency domain. Below each contour plot, the lowest-pressure spectrum has been resolution enhanced by Fourier derivation to illustrate better the components of the wagging band progression.

TABLE I
FREQUENCIES* AT 10 KBAR OF THE FIRST SIX COMPONENTS‡ OF THE CH_2 WAGGING BAND PROGRESSION IN DHPC AND DPPC

	Frequencies	
	cm^{-1}	
k	DPPC	DHPC
1	1,201.0	1,197.2
2	1,223.5	1,216.8
3	1,245.8	1,236.2
4	1,267.1	—
5	1,287.6	1,278.3
6	1,311.0	1,297.4

*Frequencies were determined from interpolated third-order derivative spectra, calculated using a breakpoint of 0.3 in the Fourier domain. Error in these frequencies is ±0.1 cm^{-1} for DPPC and ±1.0 cm^{-1} for DHPC.
‡Components are labeled by index *k*, where *k* is an integer counting bands in the progression according to the phase difference ϕ between adjacent oscillators in the methylene chain: $\phi/\pi = k/m$, where *m* is related to the number of oscillators.

Bands that arise from functional groups in the interfacial region are also affected by the change in linkage. For example, a C—O single bond stretch gives rise to characteristic asymmetric C—O—C stretching bands with distinctly different intensities and frequencies in each of these lipids. In DHPC at pressures <1 kbar, there is a single, relatively weak band around 1,125 cm^{-1} which can only be resolved from a derivative spectrum; the fact that this band is absent in spectra of DPPC, and that it is close to the absorption band of aliphatic ethers near 1,120–1,125 cm^{-1} (Bellamy, 1975; Colthup et al., 1975) leads us to tentatively assign this band to the asymmetric C—O—C stretch. In DPPC, the presence of the polar C═O group leads to an enhancement of the C—O stretch intensity and an increase in frequency, giving rise to a well-resolved asymmetric C—O—C stretching band around 1,170 cm^{-1}. Derivative spectra at low pressures show that this band is actually composed of two bands at ~1,167 and ~1,182 cm^{-1}, as observed previously in solid DPPC at room temperature and hydrated DPPC at low temperature (0°C) (Casal, 1981). These two bands have been attributed to different conformations for the fatty acid ester groups of the *sn*-1 and *sn*-2 chain (Fringeli, 1977). The presence of the polar C═O group in DPPC also leads to a well-resolved scissoring band $\delta_\alpha CH_2$ at 1,418 cm^{-1} for the α-methylene group of the hydrocarbon chain; the corresponding band in DHPC is too weak to be resolved.

The pressure dependence of the infrared spectra of DHPC has in common with DPPC the feature in which increases in pressure are accompanied by drastic changes in the infrared spectra. Because our high-pressure investigation of DHPC was motivated by questions concerning structure and interchain packing in the gel phase, we focus initially on the two spectral regions that are most diagnostic for these purposes, regions whose bands correspond to the methylene scissoring and rocking modes.

Fig. 2 compares the pressure dependence of the methylene scissoring mode δCH_2, and the methylene rocking mode γCH_2, in DHPC and DPPC. As the pressure is increased in both lipids, we observe in the spectra of Fig. 2 *A* a pressure-induced correlation field splitting of the δCH_2 mode, which at low pressures (<0.01 kbar), gives rise to a single band ~1,470 cm^{-1}. In each lipid, the first manifestation of this splitting as the pressure increases is a shoulder on the high-frequency side of δCH_2, which steadily gains intensity until a well-defined correlation field component band $\delta' CH_2$ becomes apparent at ~1.2 kbar in DHPC and ~2.2 kbar in DPPC. The pressure dependences of the frequencies of the CH_2 scissoring mode components in DHPC and DPPC are compared in Fig. 3. Further increases in pressure then result in a relatively rapid, nonlinear increase in the magnitude of this splitting (see also Fig. 5), until a characteristic pressure P_L, different in each lipid, is reached. The pressure dependences of the correlation field splitting in DHPC and DPPC, scaled to the frequency ν_0 of the δCH_2 mode at atmospheric pressure (nominally zero pressure), $\Delta\nu/\nu_0$, are compared in Fig. 5. Above P_L, the splitting increases in a linear fashion with pressure. We note that $P_L \simeq 4$ kbar in DHPC, whereas in DPPC, $P_L \simeq 8$ kbar. An inspection of

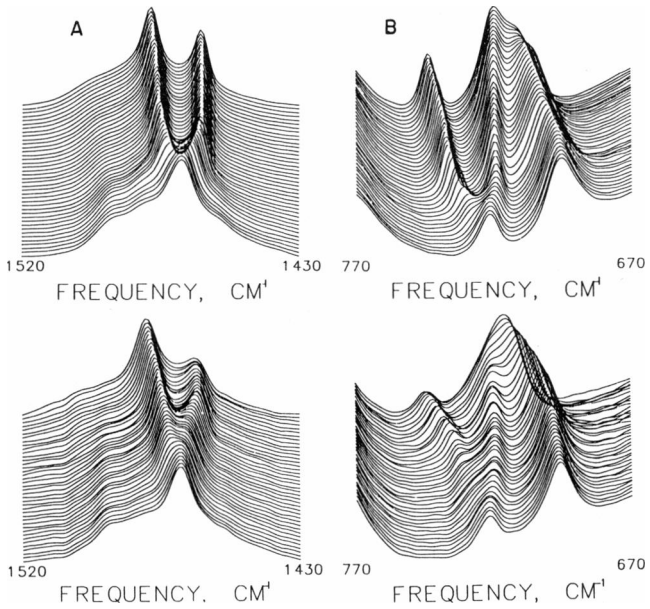

FIGURE 2. Stacked contour plots of the infrared spectra of aqueous DHPC (*top half*) and DPPC (*bottom half*) in (*A*) the CH_2 scissoring region and in (*B*) the CH_2 rocking region.

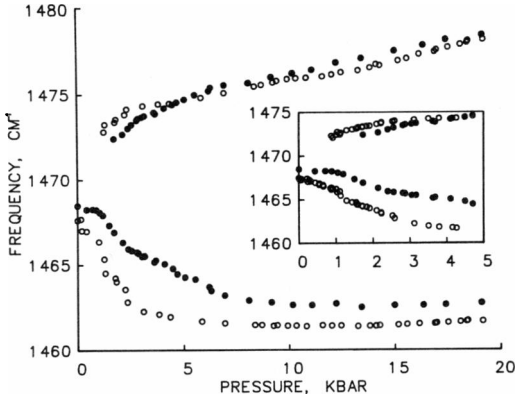

FIGURE 3. Pressure dependence of the frequency of the δCH_2 mode in DHPC (*open circles*) and DPPC (*filled circles*). Frequencies were determined from interpolated third-order derivative spectra, calculated using a breakpoint of 0.95 in the Fourier domain (see Moffatt et al., 1986). (*Inset*) Pressure dependence for both lipids of the same spectral parameter, but only for pressures up to 5 kbar. DPPC frequencies plotted are the same as those in the corresponding pressure range of the larger plot, whereas DHPC frequencies in this region <5 kbar represent the results of a separate pressure run. In every case, pressure dependences were found to be completely reversible and reproducible.

Figs. 3 and 5 reveals that not only does the correlation field component band $\delta' CH_2$ first become apparent at a lower pressure in DHPC, as noted above, but also that below ~9 kbar, the correlation field splitting in DHPC increases more rapidly with increasing pressure. Not until ~9 kbar do the magnitude and pressure dependence of the correlation field splitting become very similar. The DPPC spectra of Fig. 2 *A* show that when the correlation field component band $\delta' CH_2$ first becomes apparent, its relative intensity is noticeably greater than that of the δCH_2 band, and this trend continues to higher pressure. In contrast, the initial intensity of the correlation field component band $\delta' CH_2$ in

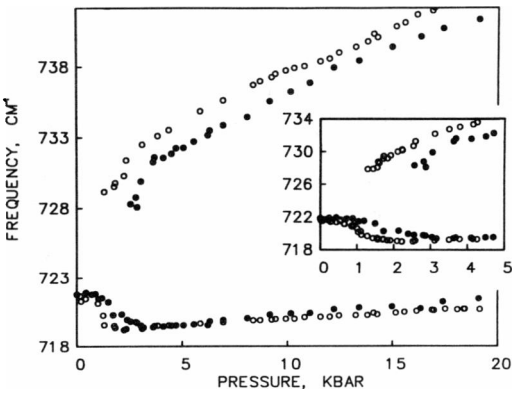

FIGURE 4. Pressure dependence of the frequency of the γCH_2 mode in DHPC (*open circles*) and DPPC (*filled circles*). Frequencies were determined from interpolated third-order derivative spectra, calculated using a breakpoint of 0.95 in the Fourier domain. (*Inset*) Pressure dependence of the same spectral parameter, but only for pressures up to 5 kbar. As with Fig. 3, DPPC frequencies plotted are the same as those in the corresponding pressure range of the larger plot, whereas DHPC frequencies represent the results of a separate pressure run.

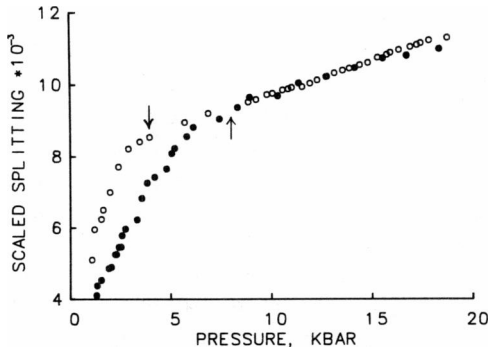

FIGURE 5. Pressure dependence of the scaled splitting, $\Delta\nu/\nu_0$, in DHPC (*open circles*) and DPPC (*filled circles*), where $\Delta\nu$ is the derived correlation field splitting of the δCH_2 mode, and ν_0 is the frequency of the δCH_2 mode at atmospheric pressure. Arrows mark pressure P_L (see text) in each lipid.

DHPC is not that much greater than that of the δCH_2 band, and this trend also continues to higher pressure. Because the integrated intensities of the $\delta'CH_2$ and δCH_2 component bands would be difficult to obtain owing to interference from nearby overlapping bands, we compare the $\delta'CH_2/\delta CH_2$ intensity ratio in DHPC and DPPC in terms of their peak height ratio, whose pressure dependence is shown in Fig. 6 for both lipids. It is clear from this figure that at all pressures where the correlation field band $\delta'CH_2$ is clearly resolvable, the peak height ratio in DPPC is always greater than the corresponding ratio in DHPC. The significance of this result will be discussed later.

As with the CH_2 scissoring mode, the spectra of Fig. 2 *B* show that increased pressure in both lipids induces a correlation field splitting of the CH_2 rocking mode, γCH_2, which at low pressures (<0.01 kbar) gives rise to a single band at ~721 cm^{-1}. (The band at 695 cm^{-1} in this figure is due to the phonon mode of the pressure calibrant quartz.) In a similar fashion to the correlation field splitting of the δCH_2 mode, the first manifestation of the γCH_2 correlation field splitting is a shoulder on the high frequency side of the rocking band, which steadily gains intensity until a well-defined correlation field component band $\gamma'CH_2$ is resolvable at ~1.2 kbar in DHPC and ~2.5 kbar in DPPC. The pressure dependences of the frequencies of the CH_2 rocking mode components in DHPC and DPPC are compared in Fig. 4. It is evident from this figure that the frequency of the correlation field component band, $\gamma'CH_2$, steadily increases with pressure in both lipids, whereas that of the γCH_2 band first decreases, but then remains essentially constant at >2 kbar in DHPC and at >3 kbar in DPPC. We also note from this figure that not only does the correlation field component band $\gamma'CH_2$ first become apparent at a lower pressure in DHPC, as noted above, but also that at all pressures, the correlation field splitting in DHPC is larger than its counterpart in DPPC. From the first appearance of the correlation field component band in the DHPC spectra of Fig. 2 *B*, the intensity ratio $I\gamma'/I\gamma$ is greater than the corresponding ratio in DPPC, and this trend continues to higher pressures.

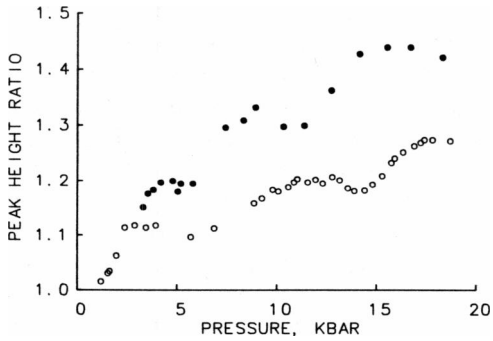

FIGURE 6. Pressure dependence of the peak height/intensity ratio $R = I\delta'/I\delta$ in DHPC (*open circles*) and DPPC (*filled circles*).

DISCUSSION

In the first high-pressure studies of DPPC by Raman spectroscopy (Wong and Mantsch, 1984; Wong and Mantsch, 1985*a*), structural-phase transitions were identified by discontinuities in the pressure dependence of various Raman spectral parameters. Although a subsequent high-pressure infrared study (Wong and Mantsch, 1985*b*) was able to confirm the critical pressures of the earlier Raman work, critical pressures were only manifested as subtle changes in slope of the pressure dependences of various spectral parameters. Accordingly, without supporting Raman spectroscopic evidence, we make no attempt to definitively identify the critical pressures of structural-phase transitions in DHPC. However, for the purpose of comparing the barotropic behavior of DHPC with DPPC, we can at least make a tentative identification of these critical pressures in DHPC by examining the pressure dependence of certain infrared spectral parameters, principally that of the frequency of the ν_s CH_2 band at 2,850 cm^{-1} (see Fig. 7). In DHPC, we find evidence for structural-phase transitions at critical pressures (at 28°C) of 1.2, 2.6, and 11.9 kbar, which should be compared with

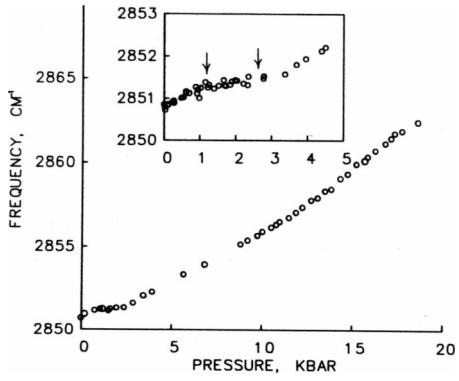

FIGURE 7. Pressure dependence of the frequency of the ν_s CH_2 mode in DHPC. (*Inset*) Pressure dependence of the same spectral parameter, but only for pressures up to 5 kbar. Frequencies plotted represent the results of a separate pressure run. Arrows mark putative structural phase transitions (see text).

the three critical pressures (at 28°C) in DPPC of 1.7 (±0.2), 4.8 (±0.2), and 15 (±1.0) kbar, corresponding (in DPPC), to the GII/GIII, GIII/GIV, and GIV/GV phase transitions, respectively (Wong and Mantsch, 1984; Wong and Mantsch, 1985*a*). We suspect, but cannot confirm without additional evidence from Raman spectroscopy experiments, that the three critical pressures in DHPC correspond to analogous structural-phase transitions. The fact that the critical pressures in DHPC are, without exception, lower than those in DPPC is most likely the result of stronger intermolecular interactions in DHPC (vide infra).

Aside from the more obvious differences between DHPC and DPPC in the infrared spectra described in the Results section, it is in the frequencies and intensities of the pressure-induced correlation field component bands of the rocking $\gamma'CH_2$ and scissoring $\delta'CH_2$ modes that we find the most dramatic differences between DHPC and DPPC. Most notable are the relative magnitudes of the correlation field splittings of the methylene scissoring and rocking modes, and the relative intensities of their respective components. Because the frequencies of the component bands of these modes can be determined with high accuracy (±0.1 cm^{-1}), a reliable, quantitative comparison of the derived correlation field splittings can be made. The results of this comparison are shown in Figs. 3–5. On the other hand, there is at present, no method for quantitatively determining the relative intensities of the CH_2 rocking and scissoring mode components. In the absence of the quartz bands, which necessarily introduce an additional complication in extracting quantitative information through curve fitting, the relative integrated intensities of the CH_2 rocking band and its associated correlation field component band could be used to determine the relative orientation of the chains (Snyder, 1961). Although qualitative information on the ratio $I\gamma'/I\gamma$ can be obtained by curve fitting, even in the presence of the quartz bands (Wong and Mantsch, 1985*b*), we have found this procedure to be unreliable for quantitative work, because the fit is extremely sensitive to the method of baseline correction, a task rendered even more difficult than usual by the shoulder of the intense quartz 801-cm^{-1} band. Consequently, we defer a quantitative analysis of the ratio of the CH_2 rocking mode components until pressure runs can be analyzed without the interference of the quartz bands. Methods are currently being developed in this laboratory to do just that, taking advantage of the H-OD stretching band at 3,380 cm^{-1} as an internal pressure calibrant. Even without the results of a quantitative analysis of the rocking mode components, a preliminary curve-fitting analysis of the DHPC and DPPC data in this spectral region does corroborate an observation noted in Results, that $I\gamma'/I\gamma$ (DHPC) > $I\gamma'/I\gamma$(DPPC) at all pressures where the correlation field component band can be resolved. At the present time, we can only interpret this result as yet another manifestation of the differences in chain packing induced by the change in linkage. This spectral region is now being reexamined in some detail in these lipids, and in a number of other related systems, by high-pressure infrared spectroscopy, in the expectation that a quantitative analysis of this region, free of the interfering effects of the quartz bands, will yield new information on chain orientation.

In and of itself, the existence of a correlation field splitting of δCH_2 in the infrared spectra of DHPC cannot be used to deduce the interchain packing, although the absence of the splitting is characteristic of hexagonal or triclinic packing. Conversely, the correlation field splitting is only observed when the hydrocarbon chains are packed in a monoclinic or orthorhombic crystal lattice (Snyder, 1961). From an investigation of the correlation field splitting in both infrared and Raman spectra of the high-pressure phases of DPPC, the interchain packing in the GII, GIII, GIV, and GV phases was determined to be distorted hexagonal, monoclinic, and perpendicular and parallel orthorhombic, respectively (Wong, 1984; Wong, 1986). Lacking additional evidence from the correlation field splitting of the Raman spectra, we cannot determine the precise nature of the interchain packing in DHPC, nor can we assume that there are analogous high-pressure phases present. Even in assigning the number of chains per unit cell, the problem in DHPC is further complicated by x-ray diffraction measurements which strongly suggest the existence of a fully interdigitated lamellar gel phase at all temperatures below the pretransition at 35°C (Ruocco et al., 1985*a*). In that our experiments are carried out in a diamond anvil cell, which equilibrates at a temperature of 28°C, the lowest-pressure gel phase in DHPC will be interdigitated, and this interdigitated structure will undoubtedly persist to higher pressures. Therefore, to the extent that the correlation field splittings of the rocking and scissoring modes are interchain in origin (Snyder, 1961), we conclude that the greater magnitude of the splittings in all of the high-pressure phases of DHPC is a direct reflection of an interdigitated lamellar structure. Contrary to crystalline paraffin chains, where the interchain interaction is entirely intermolecular (Snyder, 1961), the interchain interaction between hydrocarbon chains of 1,2-diacylphospholipids could be intramolecular or intermolecular in origin. At least in DPPC, for example, at pressures <5 kbar, the correlation field splittings of the methylene rocking and scissoring modes are largely intramolecular in origin, arising from correlations between two acyl chains within each DPPC molecule (Wong and Mantsch, 1985*b*). It is likely that the same is true in DHPC, where correlations between two alkyl chains within each molecule would represent the dominant contribution to the interchain interactions. This view is supported by solid-state NMR studies (Ruocco et al., 1985*a, b*), which indicate that axial diffusion, a reorientational motion

which would eliminate intermolecular interactions between neighboring molecules, persists to low temperatures in DHPC interdigitated bilayers.

Recently, there has been considerable interest in phospholipids which, either spontaneously (Hauser et al., 1980; Serrallach et al., 1983; Jain et al., 1985; Levin et al., 1985*b*; Ruocco et al., 1985*a*; Hui and Huang, 1986; Mattai et al., 1986; Pascher and Sundell, 1986; Pascher et al., 1986; Sheridan, 1986), or in the presence of inducing agents such as glycerol (McDaniel et al., 1983; McIntosh et al., 1983; O'Leary and Levin, 1984), assemble into interdigitated bilayers. The degree of interdigitation, which may range from complete in octadecyl-2-methylphosphatidylcholine (Pascher et al., 1986), involving both head groups and hydrocarbon chains, to partial in mixed-chain phospholipids with substantially different chain lengths, such as sphingomyelin (Levin et al., 1985*b*), will depend on the packing geometry of the interdigitating lipids, a property easily modulated by conditions of temperature and/or hydration. In as much as many of the well-characterized interdigitators, particularly the lysophospholipids and some of their 2-acetyl analogues, play important roles in membrane-associated events such as lysis, fusion, and cell activation, some effort has been devoted to characterizing their physical properties, as well as detecting the presence and degree of interdigitation, when it exists. Until very recently, the only method of inferring the presence of interdigitating bilayers has been x-ray diffraction (McDaniel et al., 1983; McIntosh et al., 1983; Serrallach et al., 1983; Ruocco et al., 1985*a*; Hui and Huang, 1986), although the effects of interdigitation on solid-state ^{14}N-(Siminovitch et al., 1983), ^{31}P-(Ruocco et al., 1985*a*), and ^{2}H-(Ruocco et al., 1985*b*) NMR lineshapes of DHPC in the gel phase are dramatic, indicating that in this lipid, interdigitation has a significant effect on the dynamic properties of the lipid molecules.

A recent Raman spectroscopic study of chain interdigitation demonstrated that the peak height intensity ratio I_{2850}/I_{2880} for the symmetric and asymmetric methylene C-H stretching modes is lower for interdigitated DPPC/perdeuterated glycerol dispersions than for noninterdigitated DPPC/water dispersions (O'Leary and Levin, 1984). On this basis, this ratio has been proposed as a test of chain interdigitation. Unfortunately, the reliability of this parameter for determining chain interdigitation may depend upon the chains being packed in an hexagonal lattice (O'Leary and Levin, 1984), although a subsequent study of perovskite-type layer compounds suggests that this ratio may also be used as a test of interdigitation when the chains are packed in a monoclinic lattice (Casal et al., 1985). Furthermore, it fails to diagnose interdigitation reliably in the gel phase of DHPC (Levin et al., 1985; Lewis et al., 1986). As described in Results, we have introduced in this study a new parameter, the peak height intensity ratio $R = I\delta'/I\delta$ of the CH_2 scissoring mode components, which may be useful as an alternative method of determining chain interdigitation. The pressure dependence of this parameter for both DHPC and DPPC is shown in Fig. 6, and it clearly distinguishes between these lipids. We predict, and have verified (unpublished results from this laboratory) in the case of known interdigitators such as lyso PC (Hui and Huang, 1986) and 1,3-DPPC (Serrallach et al., 1983), that in fully interdigitated bilayers, the R parameter will exhibit a pressure dependence very similar to that of DHPC. We are presently developing the potential of this parameter as a diagnostic tool for both the presence and degree of interdigitation, and will report on this work in future publications.

CONCLUSIONS

In the present infrared study comparing DHPC and DPPC, we have identified a number of important spectroscopic differences between these two lipids, which differ only in their linkages to the saturated hydrocarbon chains. As a direct consequence of the change from ester to ether linkages, there is a marked reduction in the intensity of the CH_2 wagging band progression of DHPC owing to the absence of the polar C=O group. The differences between DHPC and DPPC in the barotropic behavior of the CH_2 scissoring and rocking mode bands can be attributed to interdigitated bilayers in DHPC, a packing structure directly induced by the change in linkage. Thus, in DHPC, which is one example of an interdigitated system, the barotropic behavior of the CH_2 scissoring and rocking modes, and what they reveal of the effect of interdigitation on the dynamical structure of the hydrocarbon chains, should be applicable to similar interdigitated systems. Although the presence of interdigitated bilayers is reliably and easily detected by x-ray or neutron diffraction, such techniques can only provide static geometrical information on bilayer thickness or molecular surface area per lipid molecule. Only through the use of vibrational or NMR spectroscopic techniques can information on hydrocarbon chain dynamics and interactions in the interdigitated bilayer be provided.

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