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The effect of hydrostatic pressure on the bilayer structure of phosphatidylcholines containing ω-cyclohexyl fatty acyl chains *

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The barotropic behavior of aqueous dispersions of two representative ω-cyclohexyl phosphatidylcholines was investigated by pressure-tuning Fourier transform infrared spectroscopy. In the even-numbered homologue, 1,2-di-14-cyclohexyltetradecanoyl-*sn*-glycero-3-phosphocholine (14cyPC), the lipid molecules are orientationally disordered until the applied pressure reaches 2.1 kbar. This pressure marks the onset of correlation field splitting of the scissoring and rocking modes of the linear chain methylenes, as well as that of the cyclohexyl ring methylenes. It indicates immobilization of the entire acyl chains, whereby the zig-zag planes of the neighboring straight chain all-*trans* methylenes are oriented mainly perpendicular to each other. As judged from the magnitude of the correlation field splittings, the interchain interaction is weaker in 14cyPC than that in linear lipids (e.g., DMPC or DPPC). Upon an increase in pressure, up to 20 kbar, the zig-zag methylene planes in 14cyPC undergo a gradual transformation to a parallel orientation. In the odd-numbered homologue, 1,2-di-13-cyclohexyltridecanoyl-*sn*-glycero-3-phosphocholine (13cyPC), there is no correlation field splitting originating from the straight chain methylenes (up to 21 kbar). The linear, nonbranched segments of the ω-cyclohexyl chains in 13cyPC are closely packed with the all-*trans* methylene zig-zag planes oriented parallel to each other. There is, however, correlation field splitting of the ring methylenes, indicating interring interactions between the bulky cyclohexyl rings in opposing bilayer leaflets. There are major structural differences between the even- and odd-numbered homologues in the interfacial region, which remain even at high pressures. The ester carbonyl C=O stretching band in 14cyPC is a composite of two discrete bands which do not change considerably in intensity or frequency in the pressure range 2–20 kbar. In contrast, 13cyPC possesses an additional, low-frequency C=O stretching component at low pressures. As the pressure increases, the three component bands coalesce into a single C=O stretching band. Our results suggest equally oriented, fully hydrogen-bonded carbonyl groups in 13cyPC at pressures above approx. 10 kbar.

Introduction

Bacteria that live in extreme environments (e.g., at very high temperatures or low pH values), contain a number of unusual membrane lipids normally not found in other organisms. In the thermo-acidopohilic *Bacillus acidocaldarius*, saturated fatty acids with ω-cyclohexyl groups, as well as iso- and anteiso-branched groups occur as the major acyl chain components in these membrane lipids [1,2]. Studies on model systems have shown that membrane vesicles containing ω-cyclohexyl fatty acyl chains have a very low proton permeability and are generally quite resistant to permeation by small molecules [3–5]. The *Bacillus acidocaldarius*, which functions under such extreme environmental conditions as pH 1–3 and temperatures between 50 and 90 °C, has to maintain a pH of 5.8–6.3 in the cytoplasm for normal cell function [6].

Furthermore, in a fatty acid auxotrophic medium, some ω-cyclohexyl fatty acids are able to support normal growth of the mesophilic, cell wall-less micro-organism *Acholeplasma laidlawii* B. The only acyl groups found in these membrane lipids are derived from the ω-cyclohexyl fatty acids [7]. The experiments show that

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Abbreviations: 13cyPC, 1,2-cyclohexyltridecanoyl-*sn*-glycero-3-phosphocholine; 14cyPC, 1,2-cyclohexyltetradecanoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; PC, phosphatidylcholine.

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these fatty acids possess all the structural and physical properties to maintain normal membrane function, even under less extreme conditions than those preferred by the *Bacillus acidocaldarius*.

The thermotropic phase behavior of phosphatidylcholines containing ω-cyclohexyl fatty acids of different chain length, and the particular properties of such bilayer assemblies, have been investigated by several physical techniques, e.g., ^{31}P-NMR [7], DSC [7,9], ^{2}H-NMR [8], differential scanning densitometry [9] and FT-IR spectroscopy [10,11]. These experiments have shown that a strong odd-even effect exists with regard to such thermodynamic parameters as the gel to liquid-crystal phase transition temperature and enthalpy or the density in the gel state. The NMR and IR results also revealed differences in the mobility of the hydrophobic core and the polar interface of these lipids in the gel phase. The question remained, however, as to the nature of the structural differences between the odd and even-numbered representatives, and rather importantly, as to how the bulky end groups affect the lipid packing when compared to that of lipids with linear acyl chains such as DMPC or DPPC.

High pressure infrared spectroscopy has proven to be a valuable tool for studying the acyl chain packing and reorientational mobility of lipid assemblies [12–17]. We have selected two representative ω-cyclohexyl phosphatidylcholines, the odd-numbered 13cyPC and the even-numbered 14cyPC, to determine from their barotropic behavior the structural differences between their gel phases, and to show how the bulky cyclohexyl rings influence the dynamic behavior of the acyl chains.

Experimental

Materials. 13-Cyclohexyltridecanoic and 14-cyclohexyltetradecanoic acids were synthesized and purified according to the method described by Das Gupta et al. [18]. The starting materials for the copper-catalyzed Grignard reaction were 2-phenylethylbromide (respectively 3-phenylpropylbromide) and 11-bromoundecaneoic acid, all from Aldrich (Milwaukee). The synthesis of 1,2-di-13-cyclohexyltridecanoylphosphatidylcholine (13cyPC) and 1,2,-di-14-cyclohexyltetradecanoylphosphatidylcholine (14cyPC) was carried out by well established procedures [19]. Purity was checked by thin-layer chromatography using the solvent system $CHCl_3/CH_3OH/7$ M NH_3 (230 : 90 : 15, v/v/v). Differential scanning calorimetry, using a high sensitivity Privalov calorimeter (DASM-1M) yielded phase transition temperatures and enthalpies identical with those obtained for these two lipids by Lewis and McElhaney [7]. Further purification was achieved by dissolving the sample in a small volume of chloroform and precipitating the lipids with acetone. After centrifugation, the supernatant was removed and the samples dried by lyophilization. Fully hydrated lipid dispersions (> 50 wt% D_2O) were prepared by heating the lipid-D_2O mixtures in a closed vial above the gel to liquid-crystal phase transition temperatures, vortexing the heated samples and immediately freezing in solid CO_2. The heat-vortex-freeze cycle was repeated twice. The resulting homogeneous dispersions were then placed at room temperature, together with a small amount of powdered barium sulfate (used as internal pressure calibrant), in a 0.37 mm diameter hole on a 0.23 mm thick stainless steel gasket, mounted on a diamond anvil cell [13–16].

Methods. Infrared spectra were measured at 28 ° C on a Digilab Fourier transform spectrometer (Model FTS-60) equipped with a liquid nitrogen-cooled mercury cadmium telluride detector. The infrared beam was condensed by a sodium chloride lens system onto the sample in the diamond anvil cell. For each spectrum 512 scans were collected at a nominal resolution of 4 cm^{-1}, with an encoding interval of 2 cm^{-1}. Data reduction was performed with software developed in this laboratory. The pressure at the sample was determined from the frequency shift of the 983 cm^{-1} absorption band (at zero pressure) of barium sulfate [20]. To determine the position of instrumentally unresolvable infrared bands, Fourier-derivation or Fourier self-deconvolution techniques were used for band-narrowing [21,22]. Frequencies associated with particular vibrational modes were obtained from third order derivative spectra with breakpoints as indicated in the figure captions.

Results

Infrared spectra of aqueous dispersions of 13cyPC and 14cyPC were recorded as a function of pressure over the range 0.4 to 21 kbar. Since the temperature of the gel to liquid-crystal phase transition of these two lipids is between 34 and 35 ° C [7,8], only gel-state events are detected by an increase in pressure. The pressure-induced modifications of the CH_2 bending (δ_{CH_2}) and CH_2 rocking (γ_{CH_2}) modes, as well as that of the ester C=O stretching mode ($\nu_{C=O}$), are of major diagnostic value as they reflect the structural and dynamic properties of the acyl chains and that of the interfacial region of these molecules [16].

Illustrated in Fig. 1 are the infrared spectra the region 1400–1525 cm^{-1} of 13cyPC (Fig. 1A) and 14cyPC (Fig. 1B) at different hydrostatic pressures. The infrared bands in this region originate from the bending motions of CH_2 and CH_3 groups. At low pressure, the two most intense bands, which are at 1468 and 1450 cm^{-1} in both 13cyPC and 14cyPC, can be assigned to CH_2 scissoring vibrations of the straight-chain lipid methylene groups of the linear portion of the hydrocarbon chain and those of the cyclohexyl ring methylenes, respectively [10]. There are two other bands of lesser intensity in this spectral region, at 1490 cm^{-1} (the asymmetric CH_3 bending mode of the choline group) and at 1420 cm^{-1} (the CH_2 scissoring vibration of the hydrocarbon chain methylene next to the ester group).

As can be seen from Fig. 1, the barotropic behavior of the odd- and even-numbered phospholipids is quite different. The most noticeable difference is the appearance at elevated pressure of a splitting of the δ_{CH_2} mode in 14cyPC, but not in 13cyPC. This correlation field splitting is also observed for the CH_2 rocking mode of 14cyPC at 720 cm^{-1}. A strong shoulder on the high frequency side of this band emerges at pressures above 2.1 kbar that can be easily resolved into a separate band at approx. 730 cm^{-1} representing the γ_{CH_2} correlation field component (see Fig. 2, panel B, 14cyPC at 6 kbar). The splitting occurs when the acyl chains of phospholipid molecules within the bilayer become orientationally highly restricted and the planes of neighboring all-*trans* zig-zag methylene chains are oriented like in an orthorhombic or monoclinic subcell lattice [16]. The pressure-induced onset of the correlation field splitting of the δ_{CH_2} and γ_{CH_2} modes can be clearly seen from the frequency versus pressure plot shown in Fig. 3. The exact frequencies for this plot were obtained from spectra subjected to band narrowing techniques [21,22]. At low pressure, the single δ_{CH_2} scissoring band at 1468 cm^{-1} is typical of polymethylene chains with orientational disorder of neighboring zig-zag methylene chains, as in the rotator phase of solid paraffins [23] or in the L_β-type gel phase of lipid bilayers [24]. Raising the pressure to 2.1 kbar leads to a dampening of all reorientational motions of the straight-chain methylenes, which results in increased intermolecular interactions between neighboring chains. This manifests itself in the appearance of the correlation field splitting of the δ_{CH_2} and γ_{CH_2} modes.

The pressure-induced 'freezing out' of the molecular reorientations of the straight chain methylenes in 14cyPC can be compared with the behavior of linear-chain lipids like DMPC, where the onset of correlation field splitting occurs at 3.2 kbar. However, as illustrated in Fig. 3A, there are subtle differences between the pressure dependence of the correlation field splitting of the two lipids. The frequency difference between the two components of the δ_{CH_2} mode evolves more rapidly in the case of 14cyPC (between 2 and 4 kbar), whereas in DMPC this change is more gradual and occurs in the range between 3 and 8 kbar. On the other hand, once the correlation field splitting is fully established (above 8 kbar), the frequency difference is larger for DMPC (or

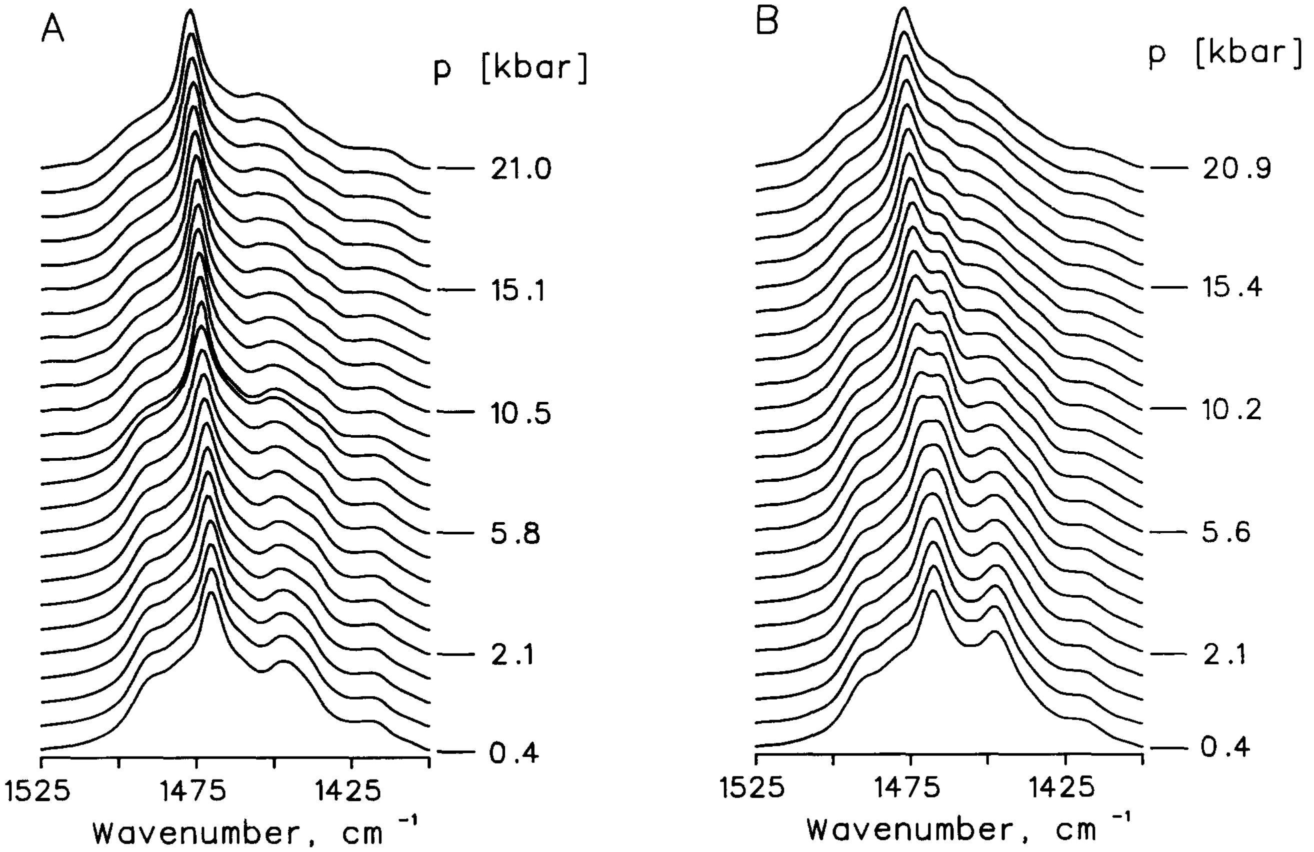

Fig. 1. Series of stacked contour plots of infrared spectra in the CH_2 scissoring region of aqueous dispersions of 13cyPC (A) and 14cyPC (B) acquired at increasing hydrostatic pressures.

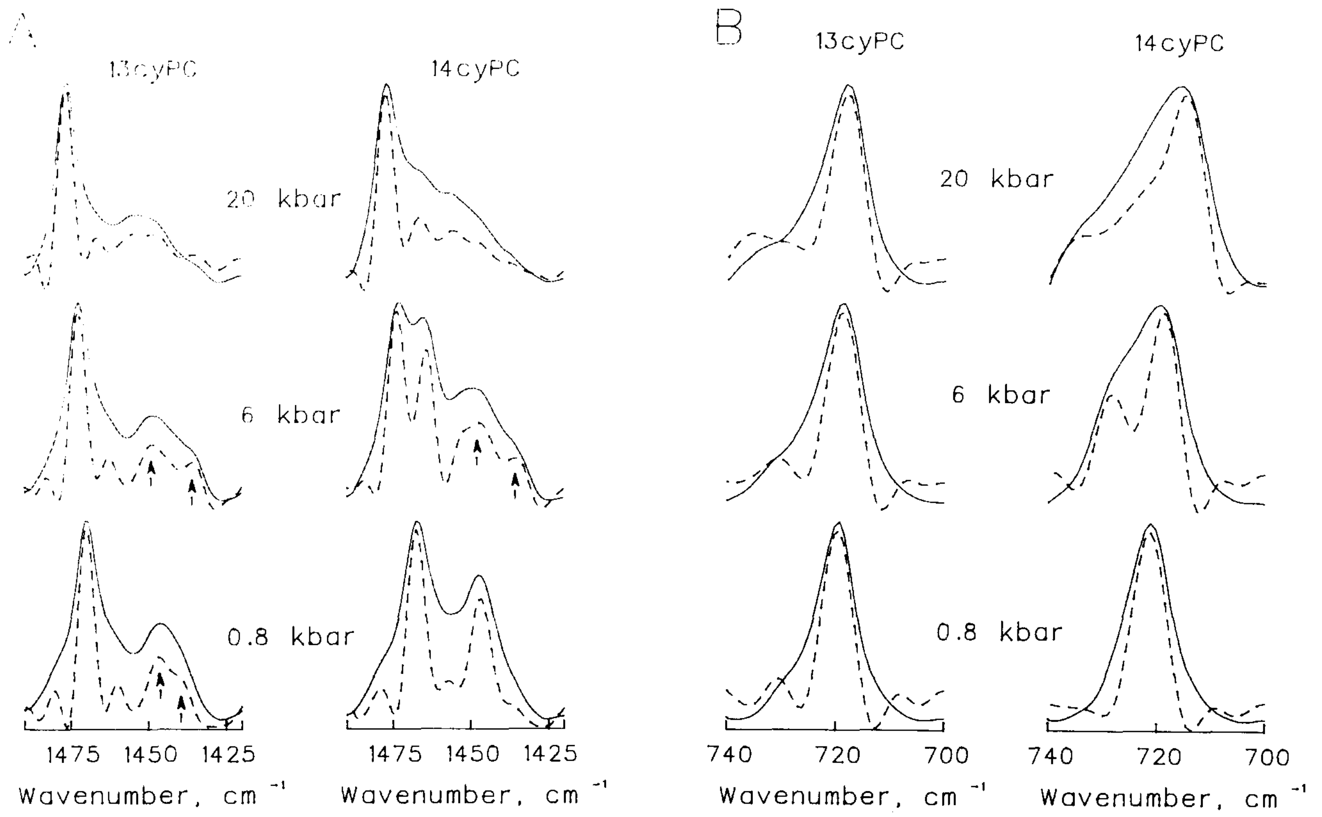

Fig. 2. Characteristic infrared absorption band contours of the δ_{CH_2} scissoring modes (panel A) and γ_{CH_2} rocking modes (panel B) of 13cyPC and 14cyPC at the indicated pressures. Broken lines represent spectra obtained after Fourier self-deconvolution with a band narrowing factor of two. Arrows in panel A indicate the δ_{CH_2} correlation field components of the ring methylenes.

DPPC) than for 14cyPC. The onset of a splitting in the methylene groups of the ω-cyclohexyl ring in 14cyPC at 2.1 kbar (see Fig. 1, band at 1450 cm^{-1}), reveals also a dampening of the reorientational motions in the ring system and an increase in 'inter-ring' interactions. The splitting of the δ_{CH_2} mode originating from the ring methylenes can be seen more clearly in Fig. 2 (panel A, 13cyPC and 14cyPC at 6 kbar, marked by arrows).

From such a comparison of 14cyPC with DMPC we conclude, that an exchange of the terminal methyl groups with the bulky ω-cyclohexyl rings has two effects on the pressure dependence of the straight chain meth-

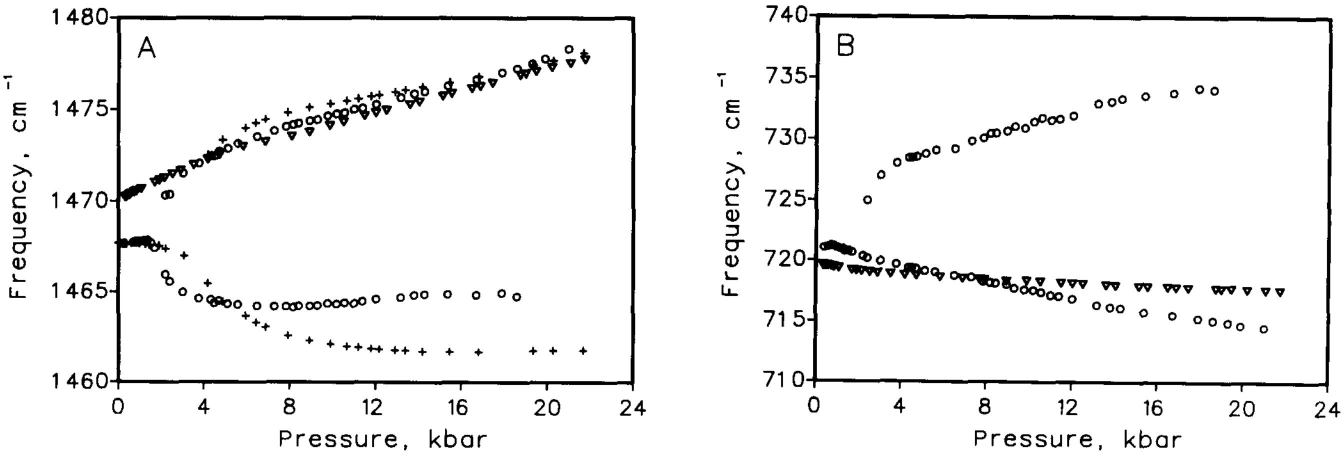

Fig. 3 Pressure dependence of the frequencies of the δ_{CH_2} modes (part A) and γ_{CH_2} modes (part B) of 13cyPC (▽), 14cyPC (○) and DMPC (+). The frequency positions were determined from third-order Fourier derivative spectra, using break points of 0.95 for the δ_{CH_2} modes and 0.7 for the γ_{CH_2} modes.

ylenes: (i) The 'freezing out' of the orientational motions in the linear chain methylenes of 14cyPC becomes an abrupt process. A likely reason for this is the simultaneous immobilization of the bulky cyclohexyl rings. The situation is different with DMPC, since the terminal methyl groups still possess a degree of reorientational mobility at the onset of the splitting. (ii) Because the frequency difference between the two correlation field components is a direct measure of the strength of interactions between neighboring chains, the methylene chains in DMPC above 8 kbar must be closer packed than those in 14cyPC.

The second conclusion can also serve for a more general discussion of the pressure-dependent behavior of the two correlation field components of the δ_{CH_2} scissoring and γ_{CH_2} rocking modes in these phospholipids. In DMPC, and in other linear-chain lipids, the two correlation field components are clearly resolved and the bands become narrower as the pressure increases [14–16]. In contradistinction to this, in 14cyPC the two correlation field bands are relatively broad and the intensity of the low-frequency δ_{CH_2} component decreases gradually with increasing pressure and almost disappears at 20 kbar. The same behavior is found for the high-frequency γ_{CH_2} component at 730 cm^{-1}, which gradually decreases in intensity as the pressure increases (see Fig. 2, panel B).

As was first demonstrated for linear hydrocarbons by Snyder [25], the ratio of the integrated intensities of the two infrared active correlation field components of the γ_{CH_2} rocking mode $I_{\gamma'}/I_{\gamma}$ can be related to the angle between neighboring *all-trans* zig-zag chain methylene planes. If these planes are oriented perpendicular to each other, this ratio is close to one, while it becomes zero, which means the absence of the γ' correlation field component, if neighboring zig-zag planes are oriented parallel to each other. Qualitatively, the same behavior is expected for the correlation field components of the CH_2 scissoring vibration (δ_{CH_2} at ≈ 1475 cm^{-1} and δ'_{CH_2} at ≈ 1465 cm^{-1}). By comparing the relative intensity ratios of the CH_2 scissoring modes $I_{\delta'}(1465\ cm^{-1})/I_{\delta}(1475\ cm^{-1})$ of 14cyPC in Fig. 2 panel A, with those of the CH_2 rocking modes $I_{\gamma'}(730\ cm^{-1})/I_{\gamma}(720\ cm^{-1})$ in Fig. 2 panel B, at 6 kbar and 20 kbar, the similar pressure dependence of this intensity ratio is obvious. At 20 kbar the correlation field components γ'_{CH_2} (≈ 730 cm^{-1}) and δ'_{CH_2} (1465 cm^{-1}) are difficult to detect, while both bands are relatively strong at 6 kbar.

With this in mind, the stacked contour plots of 14cyPC in Fig. 1 indicate a pressure-induced gradual reorientation of the *all-trans* methylene chains from predominantly perpendicular to almost parallel as the presssure is increased from 4 to 20 kbar. However, the relatively large bandwidth of the two correlation field components in comparison to that in phospholipids with linear, saturated and unsaturated acyl chains [12,16,26], reveals a residual orientational and/or motional disorder even at pressures above 4 kbar. This is corroborated by the smaller frequency separation between the correlation field components in 14cyPC, which reflects weaker interchain interactions compared to those in DMPC. In view of these observations we cannot assume perfect perpendicular orientation of neighboring chains in 14cyPC at the onset of the splitting. More likely is an orientational distribution of the interchain angle with a maximum probablity around 90°. Once the ring methylenes are 'locked in', there is enough space for the straight chain methylene zig-zag planes to change their orientation to parallel at increased hydrostatic pressures.

In contrast, in the gel phase of 13cyPC the correlation field splitting of the CH_2 bending and CH_2 rocking modes is absent over the whole pressure range examined in this study (see Fig. 1A and Fig. 2). This implies that the zig-zag planes of the straight chain methylenes in each molecule and those of neighboring molecules are oriented parallel to each other. Even at atmospheric pressure (≈ 0.001 kbar) the frequency of the single band of the CH_2 scissoring mode (at ≈ 1471 cm^{-1}) is indicative of a highly ordered phase with strong interchain interactions, as found in triclinically packed polymethylene chains of crystalline hydrocarbons [23,25]. Raising the pressure leads to a narrowing of this band with a concomitant broadening of the bands at 1450 cm^{-1} (ring methylenes) and 1490 cm^{-1} (choline methyl band). The pressure-induced change in the ring methylene band in 13cyPC is therefore not correlated with an associated change in the chain methylene band, indicating that the two parts in the hydrophobic core of the bilayer behave differently on increasing the pressure.

There are also clear differences between the methylene groups in the ω-cylohexyl rings of the two homologues as shown by the effect of pressure on the δ_{CH_2} band at 1450 cm^{-1}. In 13cyPC, at low pressure (0.4 kbar), the shape of this band is asymmetric and consists of two discrete bands (see Fig. 2, panel A). A raise in pressure leads to a well defined splitting of the band as evident from the spectra in Figs. 1 and 2. At higher pressures (above 10 kbar) the intensity of the low-frequency component decreases and both bands broaden considerably. In contrast, 14cyPC exhibits a single symmetric band (at 1449 cm^{-1}) up to a pressure of 2.1 kbar, which indicates weak molecular interactions between neighboring rings and a high degree of motional freedom. The splitting of the ring methylene band above 2.1 kbar reveals increased intermolecular interactions between neighboring rings, as in the case of 13cyPC. At pressures above approx. 10 kbar, due to the extreme broadening of these bands in 14cyPC, the spectral characteristics of the ω-cyclohexyl ring methylenes are difficult to interpret.

Although the splitting of the δ_{CH_2} modes of the ω-cyclohexyl ring methylenes in 13cyPC and 14cyPC must originate from intermolecular interactions of immobilized ring systems, the nature of these correlation field splittings can be interpreted in two ways: either as 'inter-ring' interactions inside each half of the bilayer, or as contact interactions between endgroup rings in opposing bilayer leaflets.

Fig. 4 shows stacked contour plots of the spectra of 13cyPC and 14cyPC in the C=O stretching region at increasing pressures. Resolution-enhanced spectra at three discrete pressures are depicted in Fig. 5. The different barotropic behavior of the odd- and even-numbered compounds is obvious from the pressure dependence of the shapes of the C = O stretching modes ($\nu_{C=O}$). In 14cyPC, the overall width of the C=O stretching band increases with increasing pressure (Fig. 4B), while it decreases in 13cyPC (Fig. 4A). Application of band narrowing procedures reveals that the broad C=O stretching band contour in 14cyPC consists of two discrete bands, centered at 1725 and 1742 cm^{-1} (Fig. 5), while in the odd-numbered 13cyPC the C=O stretching mode consists of three discrete bands at 1716, 1726 and 1740 cm^{-1}.

The effect of pressure on the two $\nu_{C=O}$ component bands in 14cyPC is illustrated in Fig. 6. It can be seen that the high frequency component band increases steadily in frequency with pressure (by about 5 cm^{-1} up to 20 kbar). This is the result of bond compression arising from an increase in intermolecular repulsion forces, and is a common feature of non-hydrogen bonded systems [27]. In contrast, the frequency of the other band decreases markedly on raising the pressure to 2.1 kbar. At this pressure, there is a change of slope in the pressure-frequency plot. Above 2.1 kbar the low-frequency band still decreases slightly until approx. 20 kbar. It is well known that hydrogen bonding causes a shift in the C=O stretching frequency by 10–20 cm^{-1} to lower wavenumbers [28]. Further evidence that the band at 1725 cm^{-1}, which had also been detected in the gel phase of 14cyPC at atmospheric pressure [8,10], is due to hydrogen bonded carbonyl groups, comes from its pressure dependence. The initial pressure-induced decrease in frequency is caused by the attractive forces of the water molecules, resulting in an elongation of the C=O bond. At higher pressures, the repulsive forces of hydrogen-bonded water molecules become dominant

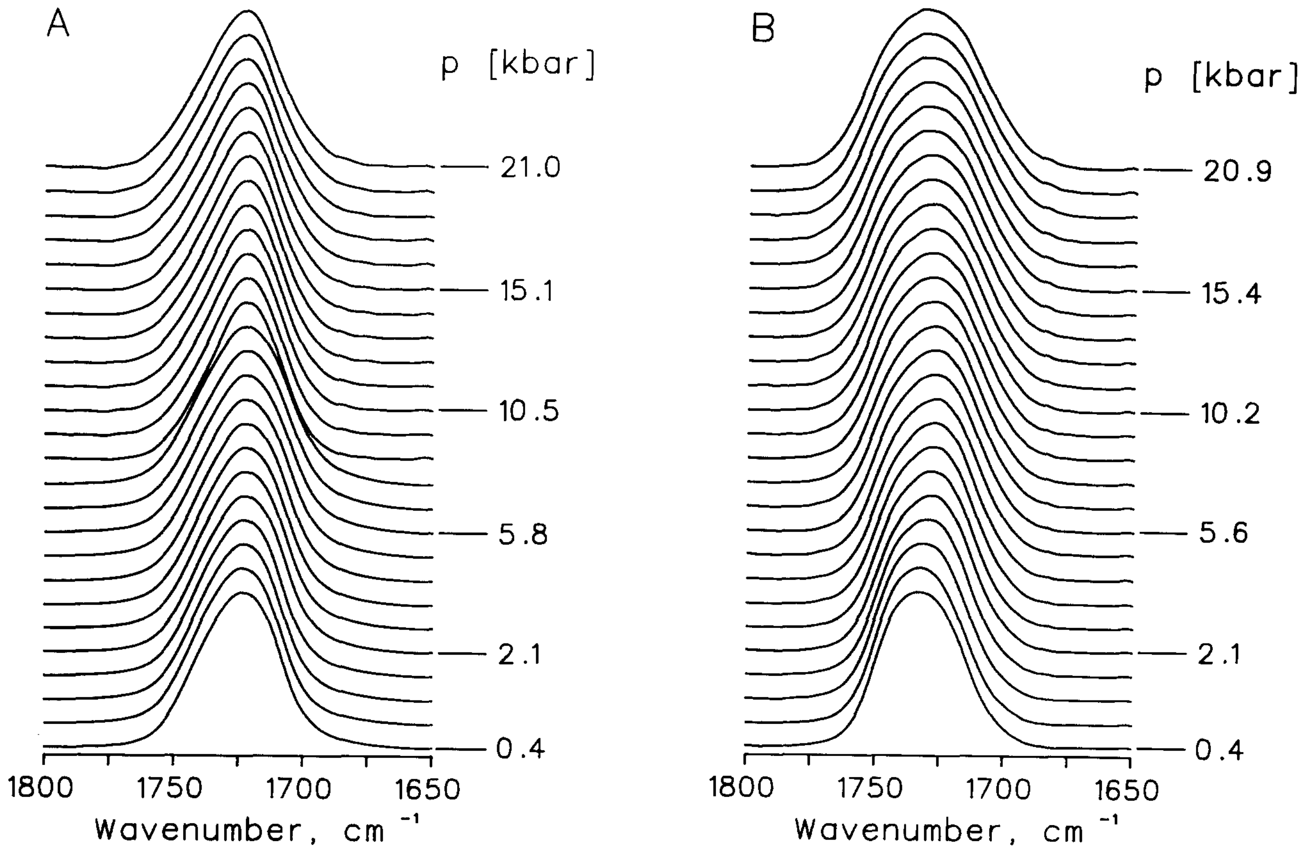

Fig. 4. Series of stacked contour plots of infrared spectra in the C=O stretching region of aqueous dispersions of 13cyPC (A) and 14cyPC (B) acquired at increasing hydrostatic pressures.

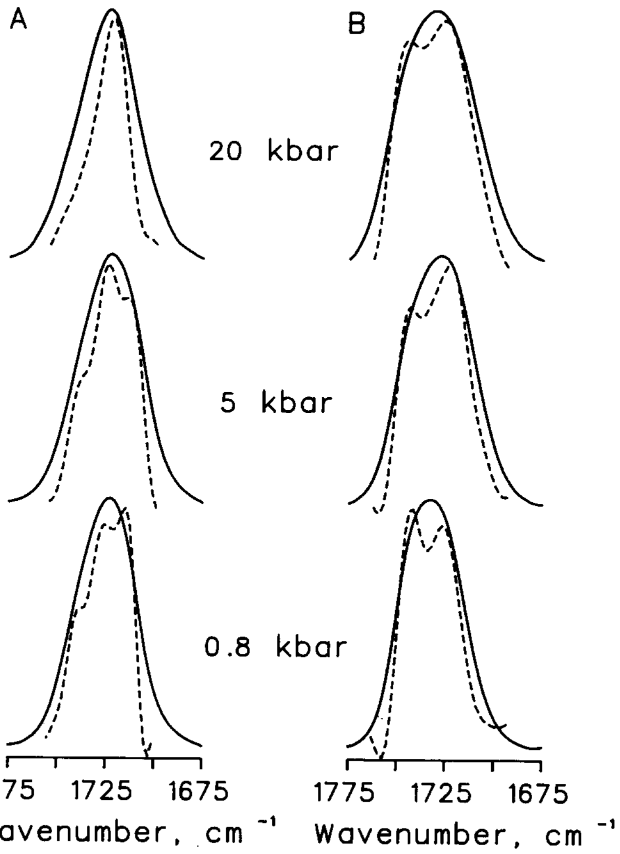

Fig. 5. Characteristic infrared absorption band contours of 13cyPC (A) and 14cyPC (B) in the region of the C=O stretching modes at the indicated pressures. Broken lines represent spectra obtained after Fourier self-deconvolution with a band narrowing factor of two.

and lead to a compression of the C=O bond, thus compensating for the elongation of the C=O bond. The observed pressure dependence of the low-frequency C=O band in 14cyPC is similar to that of other well-studied hydrogen-bonded systems [27].

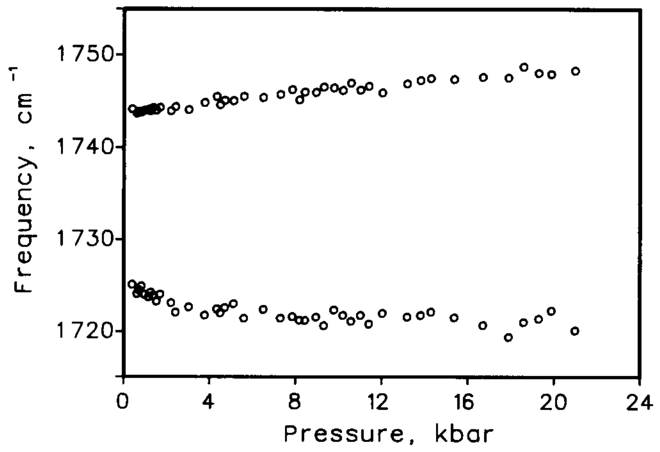

Fig. 6. Pressure dependence of the frequency of the two components of the C=O stretching mode of 14cyPC. The frequency positions were determined from third-order Fourier derivative spectra, using a break point of 0.4.

In the odd-numbered 13cyPC, the C=O stretching mode consists of three discrete bands at low pressure (Fig. 5). The same bands were also found in the gel phase of 13cyPC at atmospheric pressure [8,10]. The low-frequency band at 1716 cm^{-1} has been attributed to an ordered, immobilized gel phase with a different conformation of the glycerol backbone and/or a strongly hydrogen-bonded carbonyl oxygen [29]. The emergence of such a phase could result from a partial dehydration of the polar/apolar interface to form a crystalline phase containing tightly bound water(s) of crystallization (Ref. 30 and references therein). An increase in pressure leads to the loss of the band at 1740 cm^{-1} and the merging of the two remaining bands into a single band centered at 1720 cm^{-1}. From this, we conclude that an elevated pressure results in similarly oriented, fully hydrogen-bonded carbonyl groups in 13cyPC. The absence of a low-frequency C=O component at 1716 cm^{-1} in 14cyPC over the entire investigated pressure range indicates that such a 'crystalline phase' with tightly bound water molecules (as in 13cyPC) can not be formed by the even-numbered homologue.

Discussions

From this barotropic study of the gel phases of 13cyPC and 14cyPC, it is apparent that the behavior of the odd- and even-numbered ω-cyclohexyl phosphatidylcholines is very different. In the odd-numbered 13cyPCs, the zig-zag planes of the chain methylenes are aligned parallel to each other and this lipid forms a well ordered, tightly packed, gel phase with a high degree of interactions between neighboring chains. In the even-numbered 14cyPC, the onset of correlation field splitting occurs at 2.1 kbar, indicating the conversion from a loosely packed lattice with a high degree of motional freedom, to a crystal-like rigid lattice in which the zig-zag planes of the linear methylene groups are oriented mainly perpendicular to each other at pressures immediately above 2.1 kbar. At higher pressure, there is a gradual rearrangement of the zig-zag chain orientation from perpendicular to parallel to each other.

Previous ^{2}H-NMR experiments at atmospheric pressure of the gel state of specifically deuterated, fully hydrated 13cyPC and 14cyPC [8], have shown a nearly complete absence of motion in the odd-numbered 13cyPC, while in the even-numbered 14cyPC there were motional reorientations of the specifically deuterated methylene segments in the chains, as well as in the ring. This is consistent with our findings at pressures below 2.1 kbar. Recent X-ray studies of dry and hydrated samples of 13cyPC and 14cyPC have shown that the bilayer thickness of these systems, as estimated from the long spacings, can only be explained by either a tilt of the methylene chains by more than 40° against the bilayer normal, or by interdigitation of the two leaflets of the bilayer (Blume, personal communication).

High-pressure infrared spectroscopy has been used to differentiate between fully interdigitated and non-interdigitated lamellar phases [26]. It was demonstrated on structurally well-defined gel phases of phospholipids that full interdigitation is characterized by a narrowing of the correlation field component bands; qualitatively, interdigitation is apparent from the appearance of a deep valley between the two correlation field bands.

From an inspection of Fig. 1 one might rule out complete interdigitation in 14cyPC because in all spectra where the correlation field splitting is clearly discernible the component bands are broad and lack the deep valley between the correlation field components characteristic for interdigitated bilayers [16,26] over the entire pressure range up to 21 kbar. While inhomogeneous broadening may account for some of the increased band width in 14cyPC, we favor the alternative of non-interdigitated bilayers in the gel phase of 14cyPC, with a large tilt angle of the methylene chains relative to the bilayer normal. Ultimately, X-ray diffraction is required to definitively establish the absence or presence of interdigitation. In 13cyPC, the absence of a δ_{CH_2} correlation field splitting of the chain methylenes on one hand, and the δ_{CH_2} splitting of the ring methylenes on the other hand, also argues against fully interdigitated bilayers in the gel phase. The behavior of the δ_{CH_2} modes of the ω-cyclohexyl ring methylenes at increasing pressure is complex, and the broadening and loss of intensity of these bands relative to that of the strong δ_{CH_2} chain methylene band at 1472 cm^{-1} can only be explained by strong 'inter-ring' interactions. As the neighboring linear zig-zag chains are clearly packed parallel to each other, it seems reasonable to assume that the rings adopt the energetically most favorable chair conformation, which extends the all-*trans* conformation by at least three additional C–C bond lengths, leading to a good alignment of neighboring cyclohexyl rings within each monolayer of the membrane. In such a case the δ_{CH_2} correlation field splittting of the ring methylenes must be the result of interactions between rings from both leaflets of the bilayer. The question whether the bulky rings from both leaflets only touch each other, or whether partial interdigitation occurs, cannot be answered unequivocally. However, complete interdigitation can be ruled out, because with the parallel alignment of the zig-zag planes of the linear methylenes, there can not be strong intermolecular interactions with the ring methylenes.

X-ray measurements of multilayers of a homologuous series of dry, crystalline fatty acids bearing an ω-cyclohexyl ring at the end showed a tilt angle of about 60° against the normal of the basal plane, as deduced from an increase in the long spacings by subsequent elongation of the straight methylene chains by two CH_2 units, starting with 13-cyclohexyltridecanoic- or 14-cyclohexyltetradecanoic acid, respectively [31].

Structural differences between 13cyPC and 14cyPC remain even at high pressure. Though the pressure-induced reordering of the methylene planes in the linear part of the acyl chains in 14cyPC from mainly perpendicular to parallel to each other is a gradual process that leads to a similar packing arrangement as in 13cyPC, only minor changes are seen in the interfacial zone of the bilayer. Neither the frequency nor the intensity of the two components of the C=O stretching mode change significantly in 14cyPC over the pressure range 2–20 kbar. In contrast, in 13cyPC an increase in pressure leads to considerable changes in the frequency and intensity of the original three components of the C=O stretching mode, resulting in a single band at 1720 cm^{-1} at high pressure. A rationalization for this unusual behavior of 13cyPC under pressure could be that either a gradual decrease in the tilt angle and/or a reorientation of the glycerol moiety is needed to adopt a satisfactory packing of the ω-cyclohexyl rings. This would lead to a change in the orientation of the C=O groups in the interfacial region, which thus become alike and more exposed and therefore readily accessible to water molecules. It means, that pressure can cause a complete hydration of the carbonyl groups in the odd-numbered homologue, in contradistinction to the even-numbered homologue where the original proportion of hydrated and non-hydrated C=O groups remains nearly constant throughout the entire pressure range investigated. The latter behavior is typical of lipids with straight chains such as in DMPC or DPPC.

Recent pressure-dependent infrared experiments of pentadecane (which contains 13 linear CH_2 units) [32], and hexadecane (with 14 linear CH_2 units) [33], show remarkable similarities with 14cyPC (which also contains 13 linear CH_2 units) and 13cyPC (with 12 linear CH_2 units) with regard to the structure of the pressure-induced crystalline phases. At a pressure above 1.4 kbar pentadecane exists in an orthorhombic lattice with perpendicular orientation of neighboring all-*trans* methylene zig-zag planes. As the pressure increases further, this orientation changes gradually to almost parallel, which is similar to the behavior in 14cyPC. Hexadecane transforms at approx. 1.5 kbar from a liquid directly into a triclinically packed solid with a parallel alignment of the *trans* methylene zig-zag planes, which is analogous to the liquid-crystal to gel transition in 13cyPC.

Interestingly, the drastically different barotropic solid state behavior shown by pentadecane and hexadecane is fully retained in such complex compounds as 13cyPC and 14cyPC. Therefore, one must conclude that the packing arrangement of linear methylenes in the solid state is much less a function of the end groups (as an exchange of the terminal methyl groups with ester groups or with bulky cyclohexyl rings does not change the arrangement of the all-*trans* zig-zag planes), but that the structure-determining effect is the number of linear methylene groups.

While there are still unanswered questions regarding the origin of the odd-even effect in lipids, we hope that this barotropic study of the gel phases of two representatives of a homologuous series of ω-cyclohexylphosphatidylcholines will provide some insight into the structural and dynamic behavior of systems with a pronounced odd-even effect.

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