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An infrared spectroscopic study of the thermotropic phase behavior of phosphatidylcholines containing ω-cyclohexyl fatty acyl chains *

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The thermotropic phase behavior of an odd- and an even-numbered member of the homologous series of 1,2-di-ω-cyclohexylphosphatidylcholines was studied using Fourier transform infrared spectroscopy. The results obtained indicate that the pronounced discontinuities in the behavior of the odd- and even-numbered homologues observed by differential scanning calorimetry can be attributed to differences in the organization of their respective gel states. The single phase transition exhibited by the odd-numbered compounds upon heating is shown by infrared spectroscopy to be a direct transition from a condensed, subgel-like phase (L_c phase) to the liquid-crystalline state (L_α phase). In contrast, the multiple transitions exhibited by the even-numbered homologues are shown to be due to the initial conversion of an L_β-like phase to a more loosely packed gel phase, followed by the acyl chain-melting transition. Moreover, the major changes in the interaction between the acyl chains, and in the organization of the interfacial region of the bilayers formed by the even-numbered homologues, occur at temperatures below that of the onset of the chain-melting phase transition. The infrared spectroscopic changes observed also suggest that above the chain-melting transition, the odd- and even-numbered homologues form similar liquid-crystalline phases that are more 'ordered' than those of normal saturated straight-chain phosphatidylcholines. Most likely this is because the large size and the intrinsic rigidity of the ω-cyclohexyl group reduces the conformational disorder of the liquid-crystalline state by 'dampening' all acyl chain motions. The formation of a relatively ordered liquid-crystalline state may be the critical property exploited by the thermoacidophylic organisms in which ω-cyclohexyl fatty acids naturally occur.

Introduction

The thermotropic phase properties of lipids containing bulky groups at or near to their nonpolar terminii are of considerable interest from both the physical and biological viewpoints. One group of such lipids, the ω-cyclohexyl fatty acids, are of particular interest since they are the predominant hydrocarbon chain structures in the membrane lipids of the thermoacidophile *Bacillus acidocaldarius* and the bacteriophage ϕNS11 which infects it [1–5]. However, this class of fatty acids can also support normal membrane function under conditions less extreme than those favoring *B. acidocaldarius*, since they occur as significant components of the membrane lipids of the mesophylic bacterium *Curtobacterium pusillum* [6] and they can also support normal growth and function of the mesophylic mycoplasma *Acholeplasma laidlawii* B under conditions where the cell membrane lipids are essentially fatty acyl chain homogeneous (unpublished experiments from this laboratory). From the physical perspective, the presence of the bulky and fairly rigid cyclohexyl rings in the middle of a lipid

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Abbreviations: 13cyPC, 1,2-di-13-cyclohexyltridecanoyl-*sn*-glycero-3-phosphocholine; 14cyPC, 1,2-di-14-cyclohexyltetradecanoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2 di-palmitoyl-*sn*-glycero-3-phosphocholine; PC, phosphatidylcholine; DSC, differential scanning calorimetry; ^{31}P-NMR, Phosphorus-31 nuclear magnetic resonance; CH_2, methylene; CO, carbonyl.

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bilayer should profoundly affect the fatty acyl chain packing modes and dynamics of those bilayers.

Despite the obvious physical and biological interest of the ω-cyclohexyl fatty acids, there have been relatively few studies of the physical properties of these molecules and their derivatives. Recent DSC studies on 1,2-di-ω-cyclohexyl PCs [7–9] have shown that lipid bilayers composed of such molecules exhibit lower gel to liquid-crystalline phase transition temperatures than do their linear saturated chain counterparts of comparable acyl chain length or carbon number, and one of those studies [8] has also demonstrated that the physical properties of those lipids, as seen by both DSC and ^{31}P-NMR spectroscopy, are strongly dependent upon whether the acyl chains contain an odd- or an even-number of carbon atoms. Monolayer film studies [10] have shown that at temperatures below and above the chain-melting phase transition, the ω-cyclohexyl PCs occupy a larger and a smaller area per molecule, respectively, than do the saturated straight-chain PCs, and that the molecular interactions in the liquid-expanded state are stronger than those of all other PCs studied so far. This study also demonstrated that the ω-cyclohexyl PCs form liquid-expanded monolayers that are stable to higher temperatures than those formed by many other PCs [10]. Moreover, other studies have shown that lipid vesicles composed of lipids containing ω-cyclohexyl fatty acyl chains are considerably more resistant to permeation by small molecules than are vesicles containing other classes of fatty acids [9,11,12]. Indeed, this property may be critical to the viability of the *B. acidocaldarius* under the extreme conditions favoring its normal growth. However, although such physical studies have revealed some interesting and somewhat unexpected properties of lipids containing ω-cyclohexyl fatty acyl chains, the molecular basis of the physical behavior of these lipids is largely unknown. Thus we have synthesized a homologous series of ω-cyclohexyl fatty acids and their accompanying PCs [8] and begun a thorough study of the properties of both model and natural membranes containing such fatty acyl chain structures. This paper describes the use of Fourier transform infrared spectroscopy, a non-invasive spectroscopic technique, to study the structural basis of the thermotropic phase behavior of two representative 1,2-di-ω-cyclohexyl PCs.

Materials and Methods

1,2-Di-13-cyclohexyltridecanoyl PC and 1,2-di-14-cyclohexyltetradecanoyl PC were synthesized from their respective fatty acids and purified by silicic acid chromatography by methods previously used in this laboratory [8,13]. The infrared spectra were recorded on either a Digilab FTS-15 or a Digilab FTS-60 Fourier transform infrared spectrometer equipped with high-sensitivity mercury-cadmium-telluride detectors. The sample preparation methodology, data acquisition and data processing procedures have also been described in full detail elsewhere [14,25].

Results

The thermotropic phase properties of 1,2-di-13-cyclohexyltridecanoyl PC and 1,2-di-14-cyclohexyltetradecanoyl PC were studied as representative members of the odd- and even-numbered members of this homologous series of lipids, respectively. Typical DSC heating endotherms illustrating the differences in the thermotropic phase behavior of these two lipids are shown in Fig. 1 (for a detailed description of our DSC studies on these lipids, see Ref. 8). In this study, infrared spectra of ^2H_2O dispersions of 13cyPC and 14cyPC were acquired as a function of temperature between 8 °C and 40 °C. For both lipids, temperature-dependent changes in band contours were observed in the regions of the spectrum which includes the methylene stretching modes (2800–3000 cm^{-1}), the carbonyl stretching modes (1700–1800 cm^{-1}), and the methylene deformation modes (1400–1500 cm^{-1}), at the calorimetrically determined phase transition temperatures. Thus it is clear that DSC and infrared spectroscopy are both monitoring the same thermotropic process. The spectral changes associated with the CH_2 stretching, CH_2 bending and CO stretching vibrational modes are of major diagnostic value, since they can provide valuable structural and conformational information about the thermotropic changes which occur in the acyl chain and interfacial regions of the lipid molecules (see Ref. 15 and references cited therein). The nature of the structural

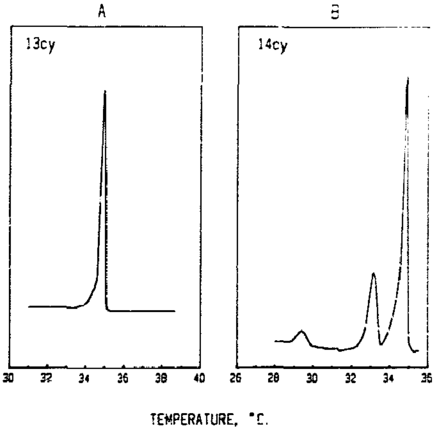

Fig. 1. DSC heating endotherms of aqueous dispersions of (A) 1,2-di-13-cyclohexyltridecanoylphosphatidylcholine and (B) 1,2-di-14-cyclohexyltetradecanoylphosphatidylcholine.

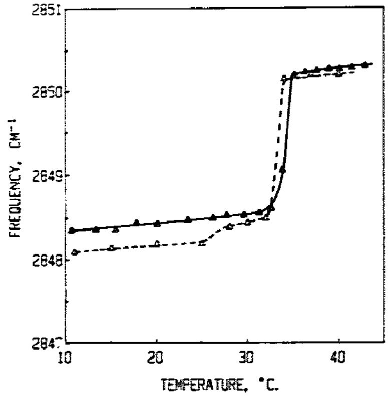

Fig. 2. Temperature dependence of the frequency of the CH_2 symmetric stretching vibrational modes of 13cyPC (closed symbols) and 14cyPC (open symbols).

changes reflected by the changes in the infrared spectra is described below.

(A) The methylene stretching modes

The CH_2 stretching vibration gives rise to bands at frequencies near 2850 cm^{-1} and 2920 cm^{-1}, attributable to the symmetric and asymmetric stretching modes, respectively, and are very sensitive to the conformational changes in the acyl chains of lipid bilayers [16]. Illustrated in Fig. 2 are temperature-dependent changes in the frequency of the CH_2 symmetric stretching band of the two ω-cyclohexyl PCs studied. The odd-numbered compound shows a single discontinuous increase in frequency (1.8 cm^{-1}) at temperatures near 34°C. Increases in the CH_2 stretching frequency of this magnitude are characteristic of the increase in the conformational disorder which occurs upon the melting of polymethylene chains, and have previously been observed at the gel to liquid-crystalline phase transitions of a number of different phospholipids [17]. Thus it is clear that the structural changes which occur at the single resolvable thermotropic event exhibited by the odd-numbered compound encompass the chain-melting event. In the case of the even-numbered compound, however, a small discontinuous increase in frequency (≈ 0.15 cm^{-1}) is observed at temperatures below 30°C and a large discontinuous increase in frequency (1.8 cm^{-1}) is observed at temperatures between 32°C and 35°C. This indicates that the melting of the acyl chains occurs at temperatures above 30°C and that the thermotropic events occurring at temperatures below 30°C must be assigned to gel-state transitions.

Since there are two distinct classes of methylene groups on the acyl chains of these lipid molecules (chain methylenes and ring methylenes), the use of Fourier self-deconvolution procedures (see Ref. 18) to resolve the CH_2 symmetric stretching band contour into its component bands makes it possible to extract additional information from the raw data obtained. Illustrated in Fig. 3 are the normal and deconvolved CH_2 symmetric stretching band contours of both lipids at temperatures below and above their respective thermotropic phase transitions. The data show that at all temperatures the band contours of both lipids can be resolved into signals attributable to the presence of at least two subpopulations of vibrating methylene groups. For both lipids the increase in frequency which occurs upon the melting of the acyl chains is directly attributable to the changes in a low-frequency component of the CH_2 symmetric stretching band contour, since this is the only component which exhibits a sharp increase in frequency (from 2844 to 2847 cm^{-1}) upon heating through the chain-melting phase transition temperature. The frequency range covered by this band is considerably lower than that normally observed at the gel to liquid-crystalline phase transition of saturated straight chain PCs (from 2849 to 2853 cm^{-1}; see Ref. 17). The deconvolved band contour also reveals another component arising from a subpopulation which gives rise to the high-frequency component resolved at 2855 cm^{-1}. This component, which is absent from the infrared spectra of the saturated straight-chain lipids, is insensitive to changes in temperature and arises from the vibrations of the methylene groups on the cyclohexyl ring [19,20]. Its insensitivity to temperature indicates that there are no major structural or conformational

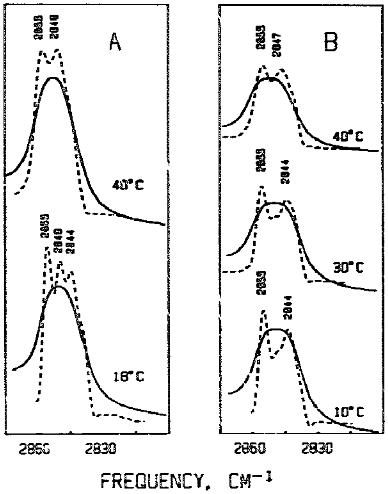

Fig. 3. Characteristic infrared absorption band contours of the CH_2 symmetric-stretching modes of (A) 13cyPC and (B) 14cyPC. The spectra were acquired at the temperatures indicated. In each panel the solid line represents the observed band contour while the dashed line represents the same spectra after Fourier self deconvolution leading to a reduction of bandwidth by a factor of 2.

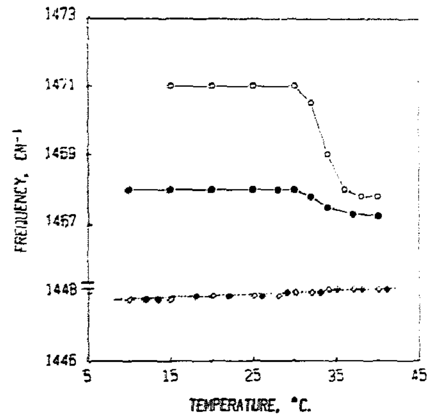

Fig. 4. Temperature dependence of the frequency of the CH_2 scissoring modes of the chain methylenes (solid lines) and ring methylenes (dashed lines) of 13cyPC (open symbols) and 14cyPC (filled symbols).

changes in the terminal cyclohexyl ring when either the odd- or the even-numbered lipids undergo any of the thermotropic phase transitions observed. It is also clear that the deconvolution of the CH_2 symmetric stretching band contour of the gel phase of the odd-numbered compound contains an additional band at 2849 cm^{-1} that is either very weak or absent in the deconvolved band contour of the even-numbered homologue. This additional band indicates that there are significant differences in the gel-state packing of the acyl chains of the odd- and even-numbered homologues.

(B) The methylene bending modes

The methylene scissoring vibrational mode which gives rise to infrared absorptions near 1460 cm^{-1} is very sensitive to changes in hydrocarbon chain packing and interchain interactions [17,18]. The band contours of the spectral region incorporating the CH_2 scissoring vibrational mode exhibit two major bands at frequencies near 1470 cm^{-1} and 1448 cm^{-1}, and these have been assigned to the scissoring vibrations of the chain methylenes and cyclohexyl ring methylenes, respectively [19,20]. The temperature dependence of the frequencies of these two bands (Fig. 4) indicates that the CH_2 scissoring band of the cyclohexyl ring methylenes of both 13cyPC and 14cyPC is insensitive to the thermotropic phase changes which are occurring. This provides further evidence that structural and/or conformational changes in the cyclohexyl ring are not significant contributors to the thermotropic phase changes exhibited by these lipids.

Fig. 4 also shows that the temperature-dependent spectral changes in the chain CH_2 scissoring modes of the odd- and even-numbered homologues are different. At low temperatures the odd-numbered compound shows a single sharp band at 1471 cm^{-1} which exhibits a large decrease in frequency (to 1468 cm^{-1}) when the lipid undergoes its single thermotropic phase transition. The single sharp band at 1471 cm^{-1} is indicative of ordered solid-like phases with strong interchain interactions, as in triclinically packed polymethylene chains of crystalline hydrocarbons [21,22]. In contrast, methylene scissoring frequencies near 1468 cm^{-1} are typical of crystalline polymethylene chains with a loose hexagonal packing, as may be found in the rotator phase of solid paraffins [23] or the L_β type gel phases of lipid bilayers [17]. Melted polymethylene chains exhibit CH_2 scissoring frequencies between 1467 and 1468 cm^{-1} [15]. Spectral changes similar to those exhibited by 13cyPC have also been observed at the subgel to liquid-crystalline (L_c/L_α) phase transition exhibited by some isoacylPCs [14] and at the subgel to gel (L_c/L_β) phase transitions of DPPC [24], isoacyl- and anteisoacylPCs [14,25]. It is thus clear that the gel phase formed by the odd-numbered ω-cyclohexylPCs is a subgel-like phase and that the single thermotropic phase transition exhibited by the odd-numbered homologues are in fact direct transitions from L_c-like phases to the liquid-crystalline state (i.e., L_c/L_α phase transitions).

In the case of the even-numbered ω-cyclohexylPC, the temperature dependence of the frequency of the CH_2 scissoring vibrational mode illustrated in Fig. 4 is somewhat misleading, since the observed band contour is in fact the summation of two component bands, as becomes obvious when Fourier self deconvolution procedures are used to resolve the band contour into its components (see Fig. 5A). At low temperatures the CH_2 scissoring band contour of the even-numbered homologue is resolvable into two bands with maxima around 1472 cm^{-1} and 1466 cm^{-1}. These bands arise as a result of factor-group splitting of the methylene scissoring band and are typical of orthorhombic packing of ordered polymethylene chains in which there is interchain coupling [21]. The temperature dependence of these component bands (see Fig. 5B) shows that the factor-group splitting is present at all temperatures below 25 °C and that the onset of the low-temperature, solid-phase transition observed at temperatures between 25 and 30 °C results in the collapse of these two bands into one band centered at 1468 cm^{-1}. This band is indicative of a hexagonally packed solid phase and shows little change in frequency at the onset of the chain-melting phase transition. The above data thus indicate that with the even-numbered compound, the low-temperature thermal event detected by DSC is a gel/gel phase transition in which the major structural changes involves a conversion from orthorhombically packed to hexagonally packed acyl chains.

(C) The carbonyl stretching modes

The region of the infrared spectrum between 1700 and 1800 cm^{-1} includes the ester carbonyl vibrational

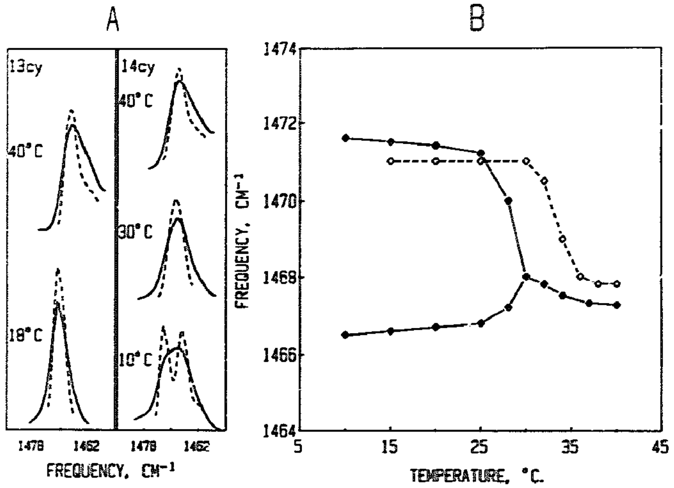

Fig. 5. Panel A. Characteristic infrared absorption band contours in the region of the CH_2 scissoring mode of the chain methylenes of 13cyPC and 14cyPC. The spectra were acquired at the temperatures indicated. In each panel the solid line represents the observed band contour while the dashed line represents the same spectra after Fourier self deconvolution leading to a reduction of band width by a factor of 2. Panel B. Comparison of the temperature dependence of the frequencies of the CH_2 scissoring modes of the chain methylenes of 13cyPC (dashed line) and 14cyPC (solid line).

modes and these can be used to characterize the temperature-dependent changes in the polar/polar interfacial region of lipid bilayers [17,18]. In this region of the infrared spectrum, hydrated diacyl lipids usually exhibit a broad band contour which has been shown to be a composite of at least two bands by resolution-enhancement techniques (see Ref. 18; a review). Two bands with maxima near 1743 cm^{-1} and 1728 cm^{-1} have been assigned to the stretching vibrations of the *sn*-1 and *sn*-2 ester carbonyl groups, respectively [26,27)] In the case of 13cyPC the band center of the broad CO stretching band contour shifts to higher frequencies (from 1718 to 1735 cm^{-1}; see Fig. 6) at the single phase transition observed at 34°C, while in contrast, the sum of all the thermotropic phase changes exhibited by the even-numbered homologue is associated with a considerably smaller shift (from 1728 to 1735 cm^{-1}; see Fig. 6). The reasons for the marked difference in the behavior of these two lipids become more obvious when Fourier self-deconvolution is used to resolve the broad band contour into its component bands (see Fig. 6). At low temperatures, the CO stretching band contour of 13cyPC is resolvable into three component bands (1713, 1728 and 1741 cm^{-1}), of which the low frequency band near 1713 cm^{-1} is the sharpest and most intense. This band disappears upon conversion to the liquid-crystalline state with a concomitant small upward shift in the frequency and an increase in the relative intensity of the high-frequency component near 1743 cm^{-1}. A similar low-frequency band has also been observed in the deconvolved CO stretching band contours of the L_c phases formed by the even-numbered isoacylPCs [14], the odd-numbered anteisoacylPCs [25], a thio analogue of DPPC [28] and in some crystalline hydrates of DPPC [29]. Thus, the appearance of this band in the infrared spec-

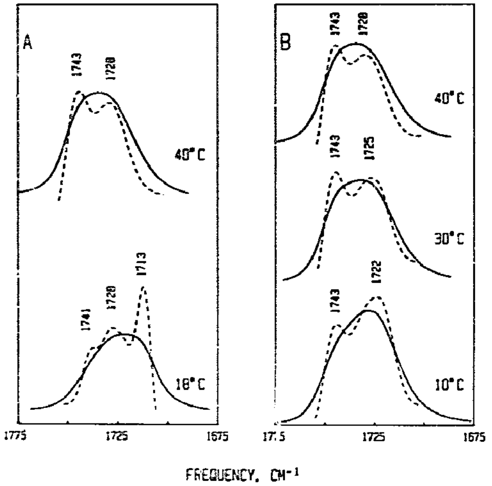

Fig. 6. Characteristic infrared absorption band contours in the region of the CO stretching modes of (A) 13cyPC and (B) 14cyPC. The spectra were acquired at the temperatures indicated. In each panel the solid line represents the observed band contour while the dashed line represents the same spectra Fourier self deconvolution leading to a reduction of bandwidth by a factor of 2.5. The high peak height of the low-frequency band after deconvolution indicates that this band is considerably narrower than the other two bands.

trum of 13cyPC provides further evidence for the crystal-like nature of the gel phases formed by the odd-numbered ω-cyclohexylPCs, and reinforces the conclusion that the single heating endothermic transition exhibited by these lipids is a L_c/L_α transition. The appearance of such a low-frequency ester carbonyl stretching band may be the result of the formation of an ordered phase with a different conformation at the glycerol backbone [30], and/or a strongly hydrogen-bonded carbonyl oxygen [30]. This could arise as a result of the partial dehydration of the polar/apolar interface to form a crystal-like phase containing tightly bound water(s) of crystallization [31].

In the case of the even-numbered homologue, however, deconvolution of the CO stretching band contour resolves two component bands at all temperatures (see Fig. 6B). The data show that at low temperatures the low-frequency component is the more intense and that the relative intensity of the high-frequency component increases with increasing temperature. Furthermore, in the liquid-crystalline state, the relative intensity of the high-frequency component is comparable to or possibly greater than that of the low-frequency component. Such shifts in relative intensity are typical of the temperature-dependent changes in the CO band contours of most lipids as they undergo a transition from a L_β-type gel phase to the liquid-crystalline state [15] and are generally ascribed to the relatively small changes in hydration of the bilayer polar/apolar interface, and/or to small changes in the conformation of the glycerol backbone, which occur at the normal gel/liquid crystalline phase transition. However, with the even-numbered compound, infrared difference spectra (not shown here) indicate that structural changes occur at the polar/apolar interface of this particular lipid bilayer at temperatures which coincide with the low-temperature gel-state phase transition as well as at the chain-melting phase transition. This indicates that despite the fact that the sum of the thermotropic changes at the polar/apolar interface of the even numbered ω-cyclohexylPC bilayer is equivalent to those occurring at a typical gel to liquid-crystalline phase transition, these bilayers differ in that the structural changes at the polar/apolar interface and the onset of conformational disorder in the acyl chains are not coincident. This aspect of the gel-phase behavior of these even-numbered ω-cyclohexylPCs is remarkably similar to that exhibited by the 'minor' gel state event which precede the onset of the chain-melting transition of both the odd- and even-numbered anteisoacylPCs [25]. Evidently, major structural changes, which probably disorder the polar/apolar interfaces of these bilayers, occur at temperatures below those of the onset of their respective chain-melting phase transitions. This probably accounts for the fact that at those temperatures, the mobilities of their phosphate headgroups are indistinguishable by ^{31}P-NMR spectroscopy from those of liquid-crystalline phospholipid bilayers [25].

Discussion

One of the dominant features of previously reported DSC, ^{31}P-NMR spectroscopic [8] and monolayer film studies [10] of the ω-cyclohexyl PCs is the marked difference in the behavior of the odd- and even-numbered members of this homologous series of lipids. This odd/even effect is also manifest in these infrared spectroscopic studies. It is clear that the radically different structures of the gel states formed by the odd- and even-numbered ω-cyclohexylPCs form the basis for the pronounced odd-even effects observed. The odd-numbered homologues form more condensed, crystal-like gel phases in which there are strong interchain interactions and possibly a closely packed polar/apolar interface containing tightly bound water(s) of crystallization. In contrast, their even-numbered counterparts form a more loosely organized gel phase with considerably weaker interchain interactions and with a more hydrated and possibly more disordered polar/polar interface. Such structural differences between the odd- and even-numbered homologues would certainly account for the larger total transition enthalpies of the odd-numbered homologues [8], and for the fact that in the gel state their phosphate headgroups are considerably less mobile than those of their even-numbered counterparts [8]. The odd/even effect that is so pronounced in the behavior of these ω-cyclohexylPCs is generally ascribed to the formation of a solid or solid-like phase by long-chain paraffinic compounds in which either the odd- or even-numbered homologues (or both) have their long chains tilted to the end group planes [32]. Thus, one might expect that a gel phase(s) with tilted acyl chains should be formed by one or both of the lipids studied. Such a conclusion, however, is outside the scope of the type of spectroscopic data presented here and can only be proven by X-ray diffraction studies. Nevertheless, the fact that there is evidence for highly ordered, triclinically packed acyl chains in the gel state of the odd-numbered homologues, coupled with the evidence for loosely packed gel state with the even-numbered homologues, suggests that the formation of a gel states with tilted acyl chains would be more probably with the odd-numbered compounds.

The data presented here also provide some insight into the effect of the ω-cyclohexyl ring on the properties of the liquid-crystalline states formed by these lipids. The major finding here is that the frequencies of the CH_2 stretching vibrational mode upon melting of the acyl chains are considerably lower than those of the saturated, straight-chain PCs. This suggests that the degree of conformational disorder in the liquid-crystalline state of the ω-cyclohexylPC bilayer is less than that of the L_α phase of bilayers composed of saturated, straight-chain PCs. In principle this should not be too surprising, especially since evidence is also presented here that there are no changes in the conformation of the cyclohexyl ring coincident with any of the thermotropic events detected in these lipid bilayers. Given that the cyclohexyl ring is an intrinsically rigid and bulky structure which does not participate in the co-operative processes occuring in the hydrophobic domain of these bilayers, it is reasonable to expect that the presence of such a bulky and rigid structure at the end of a flexible polymethylene chain would have a 'dampening' effect on all motion of the chain segments. The net effect of this would be a reduction in the conformational disorder of the liquid-crystalline state. The formation of liquid-crystalline states which are somewhat more 'ordered' than those normally found in bilayers containing saturated acyl chains have also been inferred from ^{19}F-NMR spectroscopic studies of *Acholeplasma laidlawii* B membranes containing predominantly methyl iso- and anteiso-branched fatty acyl chains [33,34]. A comparison of the infrared spectroscopic data presented here and comparable data on the methyl iso- and anteiso-branched PCs [14,25], also suggests that the ω-cyclohexyl ring induces a greater 'ordering effect' on the liquid-crystalline state of lipid bilayers than do the smaller terminal isopropyl and isobutyl groups, respectively. The 'ordering' of the liquid-crystalline state induced by the ω-cyclohexyl ring may account for the greater stability of liquid-expanded monolayer films composed of these lipids [10], and the fact that liposomes of ω-cyclohexyl lipids are highly resistant to permeation by small molecules [9,11,12]. These properties are probably exploited by the microorganism *Bacillus acidocaldarius* under the extreme conditions favoring its growth.

Finally, it is also of interest to compare some of the gel-state characteristics of these ω-cyclohexylPCs with those of the saturated, straight-chain PCs and the branched-chain isoacyl and anteisoacylPCs that have been studied (see Refs. 14, 15, 24, 25). A particularly noteworthy feature of the infrared spectroscopic data presented here is that the deconvolved CO stretching band contours of the subgel-like phase formed by the odd-numbered ω-cyclohexylPCs contains a strong low-frequency band. Interestingly, this low-frequency band is also present in the infrared spectra of the L_c phases formed by even-numbered isoacylPCs and odd-numbered anteisoacylPCs, but is absent from those of the L_c phases of the odd-numbered isoacylPCs and DPPC (see Refs. 25, 14, 24). Although the exact structural basis for the appearance of this low-frequency CO band is not yet determined, one can suggest that the L_c phases of the PCs which exhibit this feature also have some common structural properties that are different from those of the L_c phases of DPPC and odd-numbered isoacylPCs. Moreover, it is also reasonable to suggest that the acyl chains of the two groups of lipids also share some common structural features which promote the formation of L_c phases with such common features. At this stage it is not clear what the critical common features may be and the picture is further complicated by the fact that the even-numbered ω-cyclohexylPCs form L_β-type gel phases which are very similar to the L_β-type gel phases that are formed by both the odd- and even-numbered anteisoacylPCs but dissimilar from those of all the other PCs so far studied. Further studies are required to provide a more complete molecular explanation of the above phenomena, and of the way(s) in which acyl chain structure affects the physical properties of lipid bilayers in general.

Acknowledgements

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