Thermotropic Phase Behavior of Model Membranes Composed of Phosphatidylcholines Containing Iso-Branched Fatty Acids. 2. Infrared and ^{31}P NMR Spectroscopic Studies†

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Received July 30, 1984; Revised Manuscript Received November 26, 1984

ABSTRACT: The polymorphic phase behavior of aqueous dispersions of a number of representative phosphatidylcholines with methyl iso-branched fatty acyl chains was investigated by Fourier transform infrared (FT-IR) and phosphorus-31 nuclear magnetic resonance (^{31}P NMR) spectroscopy. For the longer chain phosphatidylcholines, where two transitions are resolved on the temperature scale, the higher temperature event can unequivocally be assigned to the melting of the acyl chains (i.e., a gel/liquid-crystalline phase transition), whereas the lower temperature event is shown to involve a change in the packing mode of the methylene and carbonyl groups of the hydrocarbon chains in the gel state (i.e., a gel/gel transition). The infrared spectroscopic data suggest that the methyl iso-branched phosphatidylcholines assume a partially dehydrated, highly ordered state at low temperatures, resembling the L_c phase recently described for the long-chain *n*-saturated phosphatidylcholines. At higher temperatures, some branched-chain phosphatidylcholines appear to assume a fully hydrated, loosely packed gel phase similar to but not identical with the $P_{\beta'}$ phase of their linear saturated analogues. Thus, the iso-branched phosphatidylcholine gel/gel transition corresponds, at least approximately, to a summation of the structural changes accompanying both the subtransition and the pretransition characteristic of the longer chain *n*-saturated phosphatidylcholines. The infrared spectroscopic data also show that, in the low-temperature gel state, there are significant differences between the odd- and even-numbered isoacylphosphatidylcholines with respect to their hydrocarbon chain packing modes as well as to their head group and interfacial hydration states. The three phases resolved for the longer chain isoacylphosphatidylcholines were also characterized by different ^{31}P NMR line shapes, which are indicative of differences in the mobilities of the phosphorus head group in the three states. In particular, the motion of the phosphate head group is quite restricted in the low-temperature gel state but increases considerably at temperatures above the gel/gel transition, approaching the relatively fast motion characteristic of these iso-branched phosphatidylcholines in the liquid-crystalline state. In cases where only a single thermotropic event was resolved, both spectroscopic techniques showed that the structural changes characteristic of that event were equivalent to those of both the gel/gel transition and the gel/liquid-crystalline phase transition, which either occurred concomitantly or occurred in rapid succession within a narrow temperature range.

Methyl iso- and anteiso-branched saturated fatty acids are fairly common constituents of membrane lipids of eubacteria. In addition, these branched-chain fatty acids are able to support the growth of several fatty acid auxotropic procaryotic microorganisms in which these fatty acid classes do not naturally occur [see Kaneda (1977) and Kannenberg et al. (1983)]. In the preceding paper, the results of a differential scanning calorimetry (DSC)[1] study of the thermotropic phase behavior of aqueous dispersions of a series of methyl iso-branched PC's were reported (Lewis & McElhaney, 1985). In general, these iso-branched PC model membranes were found to exhibit two endothermic events in the temperature ranges studied: a relatively slow, less energetic, lower temperature transition and a fast, higher energy, higher temperature transition. In this paper, the results of FT–IR and ^{31}P NMR spectroscopic studies of the thermotropic phase behavior of representative methyl iso-branched PC's are presented. These spectroscopic studies were designed to complement the previous DSC investigation, which provided reliable thermodynamic data but no information about the structural basis of the phenomena observed. In contrast, infrared spectroscopy directly monitors acyl chain and carbonyl head-group conformation without the use of probe molecules and provides a snapshot of the entire lipid population at a given temperature (Mantsch, 1984; Casal & Mantsch, 1984), while ^{31}P NMR spectroscopy provides a nonperturbing monitor of the mobility

† This work was supported in part by an operating grant from the Medical Research Council of Canada (R.N.M.). Issued as N.R.C.C. Publication No. 23473.
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[1] Abbreviations: NMR, nuclear magnetic resonance; DSC, differential scanning calorimetry; PC, 1,2-diacyl-*sn*-glycero-3-phosphocholine; FT-IR, Fourier transform infrared; C_{ri}, shorthand notation describing the iso-branched fatty acids (*n* denotes the total number of carbon atoms with the subscript i to denote iso branching); CH_2, methylene; C=O, carbonyl; T_{gl}, gel/liquid-crystalline phase transition temperature; T_{gg}, gel/gel transition temperature; C–H, carbon–hydrogen bond; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

0006-2960/85/0424-2440$01.50/0

of the phosphate head group. Thus, in conjunction these techniques provide valuable information about the structural basis of the thermotropic phase behavior described by the preceding DSC study (Lewis & McElhaney, 1985).

Experimental Procedures

Materials and Sample Preparation for Infrared Spectroscopy. The 1,2-diisoacyl-PC's (C_{ni} = 16–20) were synthesized and purified as previously described (Lewis & McElhaney, 1985). Samples for infrared spectroscopy were prepared at 60 wt % water or D_2O by using about 0.5 mg of the dry phosphatidylcholine. To ensure complete hydration, all lipid/water mixtures were heated to about 70 °C and then allowed to cool prior to the commencement of the infrared measurements. Temperatures were increased in steps of 1 °C with a waiting period of 15 min between consecutive spectra, which, if converted to a constant heating rate such as those used in DSC measurements, would correspond to a heating rate of 3.75 °C h^{-1}.

Acquisition and Processing of Infrared Data. Infrared spectra were obtained on a Digilab FTS-15 Fourier transform infrared spectrometer fitted with a high-sensitivity mercury/cadmium telluride detector. Interferograms were obtained with a maximum optical retardation of 0.5 cm. Typically, 250 scans were coadded, triangularly apodized, and zero-filled once to yield a final resolution of 2 cm^{-1} and an encoding interval of 1 cm^{-1}. For temperature regulation, a cell mount was used with thermostatically controlled water, and temperatures were stable to within 0.05 °C. The operations of regulating the temperature and recording individual spectra were completely under the control of the spectrometer computer.

Infrared frequencies were determined with an uncertainty of less than ±0.05 cm^{-1} by using a center of gravity algorithm (Cameron et al., 1982). In order to visualize overlapping infrared bands, particularly in the region of the broad carbonyl stretching bands, these spectral regions were subjected to Fourier self-deconvolution, which removes the inherent line width from the bands while preserving frequency position and integrated intensities (Kauppinen et al., 1981a,b). Bandwidths were determined relative to linearly interpolated base lines, the widths being computed at various fractions of their peak height. Infrared difference spectra were generated by taking the absorbance of the ratio of a higher temperature single-beam spectrum to that of a lower temperature and normalizing with respect to the temperature increment. This procedure leads to positive deviations from the base line where the absorbance has increased with increasing temperature (Mantsch et al., 1981).

^{31}P NMR: Sample Preparation and Data Acquisition. ^{31}P NMR spectra were recorded on a Bruker HXS 270 NMR spectrometer operating in the Fourier transform mode with quadrature detection. Typical spectra were obtained with a spectral width of ±25 kHz, and acquisition time of 40.9 ms, a pulse delay of 2.0 s, and a pulse width of 20 μs (72° pulse angle). The proton broad-band decoupling power (5 W) was switched on only during the data acquisition, and this resulted in minimal heating effects. Exponential multiplication of the free induction decay was used to reduce spectral noise, resulting in a 100-Hz line broadening. A 30–40-mg aliquot of the phospholipid sampe was dispersed in 1.5 cm^3 of water containing enough deuterium oxide (20% by volume) to provide a usable deuterium lock signal while ensuring that the freezing point of the bulk aqueous phase remained below 2 °C. The samples were run in 10-mm NMR tubes equipped with a Teflon vortex plug. Chemical shifts are reported in parts per million upfield from phosphoric acid (85%).

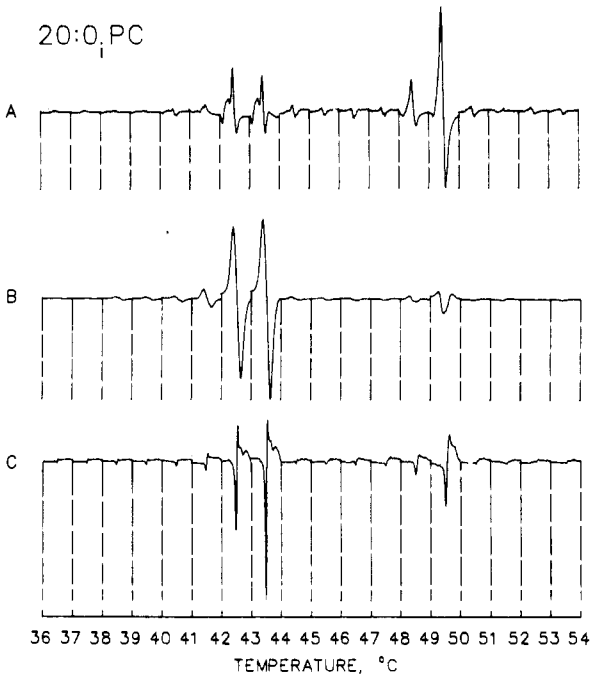

FIGURE 1: Infrared difference spectra, obtained in 1 °C intervals, of aqueous dispersions of 20i PC (A) in the region 2900–2800 cm^{-1} (CH_2 symmetric stretching band), (B) in the region 1780–1680 cm^{-1} (C=O stretching band), and (C) in the region 1500–1400 cm^{-1} (CH_2 scissoring band).

Results and Discussion

Infrared Spectroscopy. (A) Thermotropic Phase Behavior. Infrared spectra of each of the five methyl iso-branched diacyl-PC's (C_{ni} = 16–20) were recorded at various temperatures between 10 and 60 °C. The DSC heating endotherms of the C_{17i} C_{19i}, and C_{20i} compounds showed two distinct thermal events (Lewis & McElhaney, 1985), and the corresponding IR difference spectra also show two transitions occurring at the same temperatures. The DSC heating scans of the C_{16i} and C_{18i} compounds, on the other hand, each show only one resolvable thermal event, as do the corresponding infrared difference spectra. It is thus clear that both techniques are monitoring the same thermotropic transitions.

Infrared bands of major diagnostic value are the carbon–hydrogen stretching and bending modes, which monitor the structural and conformational changes in the acyl chain region, and the carbon–oxygen stretching bands, which monitor the temperature-induced changes of the carbonyl moiety in the interfacial region (Mantsch et al., 1982; Cameron et al., 1983; Dluhy et al., 1983; Casal & Mantsch, 1984). Illustrated in Figure 1 are three series of infrared difference spectra obtained from 20i PC; the selected vibrational modes are the CH_2 stretching bands (Figure 1A), the C=O stretching bands (Figure 1B), and the CH_2 bending (scissoring) bands (Figure 1C). These infrared difference spectra were obtained in steps of 1 °C and reflect the changes in the nature of the vibrational modes as a function of temperature. It is clear that each of the three infrared bands monitored exhibits two changes with temperature, one between 42 and 44 °C and another between 48 and 50 °C. The spectral parameter represented in Figure 1A shows the major change at the higher temperature, whereas the spectral parameters represented in Figure 1B,C show the major change at the lower temperature. The nature of the structural changes reflected by these difference spectra will be discussed later. The three spectral parameters of the corresponding series of infrared difference spectra obtained from 18i PC (not shown here) exhibit only one change over

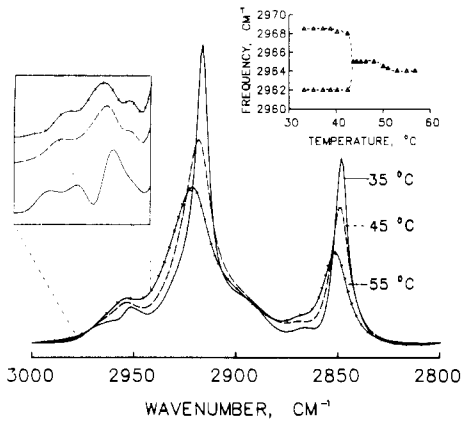

FIGURE 2: Infrared absorption bands of aqueous dispersions of 20i PC in the region of the acyl chain carbon–hydrogen stretching vibrations at 35 (—), 45 (---), and 55 °C (+). The left-side inset shows a blowup of the spectral region 2980–2940 cm^{-1}, while the right-side inset shows the frequency/temperature relationship of the asymmetric methyl carbon–hydrogen stretching vibrations.

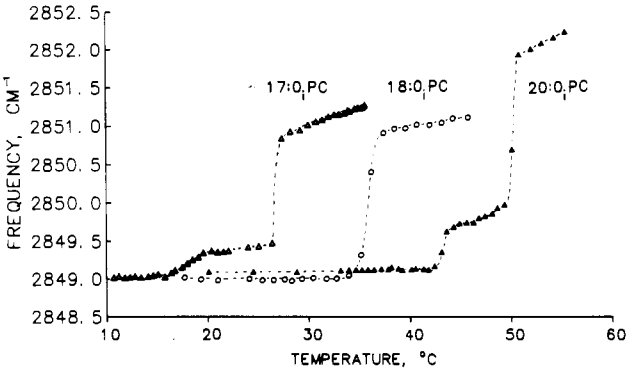

FIGURE 3: Temperature dependence of the frequency of the CH_2 symmetric stretching bands in the infrared spectra of 17i PC, 18i PC, and 20i PC.

the temperature range examined, with that change occurring between 36 and 38 °C. The infrared difference spectra of 16i PC also exhibit only one thermal event, but centered near 23 °C, while those of the 17i and 19i species each show two distinct thermal events (data not presented), in good agreement with the DSC results.

(B) Carbon–Hydrogen Stretching Mode. An important consideration in these studies is the assignment of the gel/liquid-crystalline phase transition, especially for those compounds for which two distinct thermal events have been identified. The infrared spectral parameter most sensitive to conformational changes in lipid acyl chains is the frequency of the C–H stretching modes (Mantsch, 1984). Illustrated in Figure 2 is the region of the carbon–hydrogen stretching vibrations of 20i PC at three temperatures which bracket the two thermal events. The strong bands at 2920 and 2850 cm^{-1} are the CH_2 asymmetric and symmetric stretching modes, respectively, and the weaker bands near 2960 and 2870 cm^{-1} are the CH_3 asymmetric and symmetric stretching bands, respectively, of the terminal methyl groups. The choline methyl bands at 3040 cm^{-1} are very broad and are not displayed in this figure. All of these infrared bands exhibit temperature-dependent variations in their position and width which can be related to changes in the structure and dynamics of the vibrating groups (Casal & Mantsch, 1984).

The changes in band position are detailed in Figure 3, which shows the temperature dependence of the frequencies of the methylene stretching band near 2850 cm^{-1} for three representative isoacyl-PC's. Similar temperature profiles are obtained from the methylene asymmetric stretching band near 2920 cm^{-1}. The frequency profile described by 20i PC in Figure 3 is invariant at temperatures below 42 °C and shows discontinuous increases in frequencies at 43 and 50 °C, with a small gradual increase in frequency at temperatures between 43 and 50 °C. At temperatures below 42 °C, the frequency of the band is constant at about 2849 cm^{-1} and increases by 0.6 and 2 cm^{-1} at 43 and 50 °C, respectively. Methylene symmetric stretching frequency values below 2850 cm^{-1} are characteristic of conformationally ordered polymethylene chains found in solidlike hydrocarbons (Snyder, 1967) or in gel phase lipids (Casal & Mantsch, 1984); therefore, the transition at 43 °C cannot be assigned to the acyl chain melting transition of that phospholipid. The major change in frequency of this band is observed at 50 °C, and the frequency value of 2852 cm^{-1} attained above this temperature is characteristic of conformationally disordered polymethylene chains with a high content of gauche conformers, as found in liquid hydrocarbons or in phospholipids which are in the liquid-crystalline phase. The larger increase in the frequency of this vibrational mode reflects the increase in conformational disorder of the acyl chains and clearly identifies the thermal event at 50 °C as the gel/liquid-crystalline phase transition of 20i PC. The midpoint of the sigmoidal frequency vs. temperature plot is generally taken to represent the gel/liquid-crystalline phase transition temperature, T_{gl}, and coincides with the reversal temperature of the higher temperature endotherm resolved by DSC.

Figure 3 also shows the temperature profiles of the methylene symmetric stretching vibrations of two other isoacyl-PC's. The 17i species shows a pattern similar to that of the 20i species, with a small increase in frequency at the low-temperature event and the major shift in frequency at the high-temperature event near 28 °C, an observation which identifies the latter thermal event as the gel/liquid-crystalline phase transition. For the 18i PC, there is only one discontinuity in the frequency/temperature plot at 37 °C, and the change in frequency characterizes the associated thermal event as the acyl chain melting phase transition. In order to avoid an overcrowded figure, the temperature profiles for the 16i and 19i PC's were not included in Figure 3. However, the former shows only one major increase in frequency characteristic of the acyl chain melting transition at 23 °C, while the latter shows a minor increase in frequency at 25 °C and the major change characteristic of the acyl chain melting transition at 43 °C.

While the frequency of the C–H stretching bands can be used to detect conformational changes, i.e., changes in the trans/gauche ratio of the lipid acyl chains, the widths of the C–H stretching bands can be used to detect changes in the mobility of the acyl chains; i.e., an increase in bandwidth reflects an increase in mobility. Illustrated in Figure 4 is the temperature dependence of the bandwidth of the C–H stretching modes of the even-numbered isoacyl-PC's studied (C_{n_i} = 16, 18, and 20). The temperature profiles shown in Figure 4, and those of the odd-numbered members (C_{n_i} = 17, 19, ...; not shown here), show discontinuities at the same temperatures as those characterized by the DSC endotherms of those phospholipids. Similar spectral changes have been observed at the melting of solid hydrocarbons (Sheppard, 1959; Snyder, 1967), thus confirming the nature of the thermal event assigned to the acyl chain melting transition of these isoacyl-PC's. It is, however, interesting to note that the low-temperature thermal event resolved for some of the PC's studied (C_{n_i} = 17, 19, and 20) is associated with a considerable

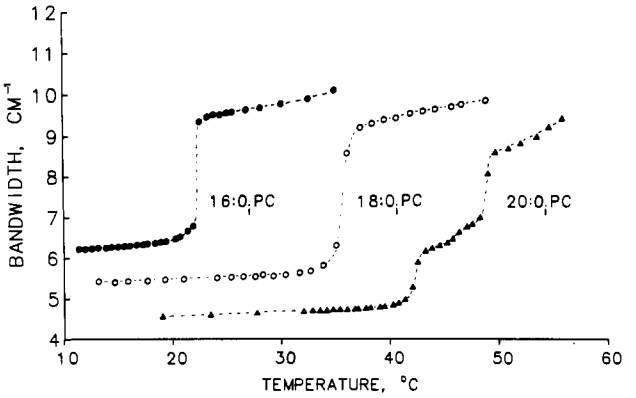

FIGURE 4: Temperature dependence of the width of the CH_2 symmetric stretching bands in the infrared spectra of 16i PC, 18i PC, and 20i PC. Bandwidths were computed at 75% of their peak height.

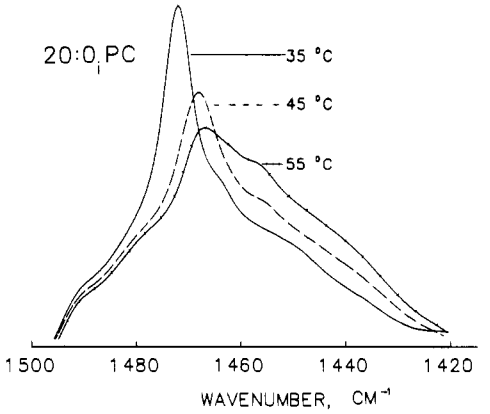

FIGURE 5: Infrared spectra of aqueous dispersions of 20i PC in the region of the methylene deformation modes at three different temperatures which bracket the two phase transitions at T_{gg} and T_{gl}.

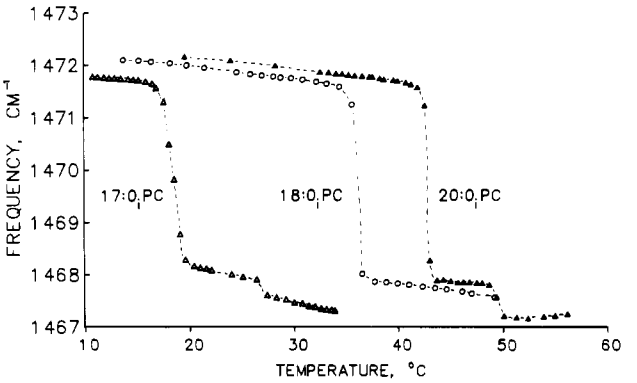

FIGURE 6: Temperature dependence of the frequency of the CH_2 scissoring bands in the infrared spectra of 17i PC, 18i PC, and 20i PC.

increase in the bandwidth of the C–H stretching vibrational modes. This indicates that the low-temperature event and the acyl chain melting transition are both associated with considerable increases in the mobility of the acyl chains.

Another of the issues under consideration in these studies is the nature of the thermal event other than the acyl chain melting transition. The frequency vs. temperature profiles for 17i PC and 20i PC shown in Figure 3 clearly identify the low-temperature thermal event as a gel/gel phase change. The frequencies of the CH_2 symmetric and asymmetric stretching vibrations of the isoacyl-PC's investigated here are diagnostic of gel phase infrared spectra at all temperatures below T_{gl}; however, the small increase in frequency at T_{gg} (the gel–gel phase transition temperature) is indicative of an increase in the population of gauche conformers at that temperature. Similar changes have been observed in studies on the gel-state behavior of 1,2-diacylphosphatidylcholines containing identical saturated *n*-acyl chains (Mantsch et al., 1982; Cameron & Mantsch, 1982); these changes were associated with the pretransition and to a greater extent the subtransition characteristic of these lipids. The increase in the bandwidth at T_{gg} shown in Figure 4 corroborates this observation; it indicates an increase in the mobility of the acyl chains at temperature above T_{gg}, suggesting a loose hexagonal packing pattern between T_{gg} and T_{gl}, a packing pattern in which the methyl-branched acyl chains can be viewed as rigid rotors behaving independently of each other (Cameron et al., 1980a).

(C) C–H Bending and Deformation Modes. The most useful information regarding the chain packing and interchain interactions in the gel phase of lipids can be obtained from the CH_2 bending or scissoring mode, which gives rise to infrared bands around 1470 cm^{-1}. The number and frequency of these bands are dependent on acyl chain packing and conformation. Figure 5 shows the infrared spectra of 20i PC between 1420 and 1500 cm^{-1} at three temperatures which bracket the two transitions resolved by DSC. In this region, the low-temperature gel phase ($T < T_{gg}$) is characterized by a strong band at 1472 cm^{-1} which, at temperatures between T_{gg} and T_{gl}, broadens and is shifted to 1468 cm^{-1}. The transition to the liquid-crystalline phase is characterized by further broadening of the band and a very small shift to lower frequencies. Figure 6 shows the temperature dependence of the frequency of the methylene scissoring bands of three representative isoacyl-PC's (C_{ni} = 17, 18, and 20). For the 17i and 20i compounds, as well as for the 19i species (not shown here), a decrease in the frequency of this band from 1472 to 1468 cm^{-1} is observed at T_{gg}, while only a minor further decrease is observed at T_{gl}. In the case of 18i PC (and 16i PC, not shown here), there is only one discontinuity in the plot at the acyl chain melting temperature. However, the gel phase frequency of 1472 cm^{-1} is identical with that of the low-temperature gel phases of the other PC's studied (C_{ni} = 17, 19, and 20), an observation which suggests that the single transition characteristic of the 16i and 18i compounds is the result of either a concomitant occurrence of the two phase transitions (gel/gel and gel/liquid-crystalline) or a sequential occurrence within a very narrow temperature range.

Methylene scissoring frequencies between 1467 and 1468 cm^{-1} have been found to be characteristic of the disordered liquid-crystalline state of all the lipids studied so far (unpublished observations from this laboratory). In the gel state, a methylene scissoring frequency of 1468 cm^{-1} has been found to be characteristic of a hexagonal packing of the acyl chains (Cameron et al., 1980a,b; 1981). Thus, the data shown in Figure 6 reinforce the contention of a loose hexagonal chain packing in the gel phase at temperatures between T_{gg} and T_{gl}. In addition, the methylene scissoring frequency of 1472 cm^{-1} has been correlated with poorly hydrated lipids (Cameron & Mantsch, 1982) as well as a triclinic chain packing in solid hydrocarbons (Snyder, 1961). However, that is not definitive since other chain packing modes cannot be ruled out (e.g., distorted orthorhombic or monoclinic). In any event, X-ray diffraction studies will be required to establish the exact nature of the chain packing of these isoacyl-PC's.

Other characteristic bands due to acyl chain vibrations are the methyl modes. As already demonstrated in the inserts on Figure 2, the methyl asymmetric stretching modes show site-symmetric splitting in the gel phase below T_{gg}. This is indicative of tightly packed acyl chains with strong nearest-neighbor interactions at the isopropyl end (McPhail et al.,

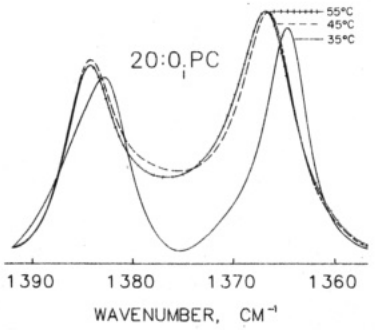

FIGURE 7: Infrared spectra of aqueous dispersions of 20i PC in the region of the symmetric methyl deformation bands of the isopropyl groups at three different temperatures which bracket the two phase changes at T_{gg} and T_{gl}.

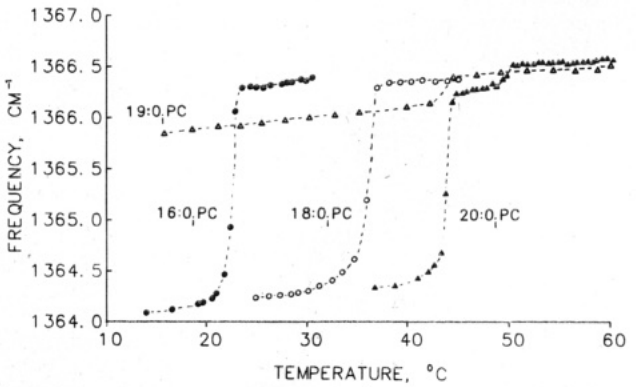

FIGURE 8: Temperature dependence of the low-frequency component of the umbrella-type vibration of the isopropyl groups in 16i PC, 18i PC, 19i PC, and 20i PC.

1984). The two band components become degenerate in the less rigidly packed gel phase between T_{gg} and T_{gl}, and the frequency of this gel phase mode hardly changes at T_{gl}. Of equal interest are the methyl deformation modes. While the methyl asymmetric deformation bands appear only as shoulders on the high-frequency side of the methylene scissoring band (see Figure 5), the methyl symmetric deformation mode, also known as the umbrella mode, appears as a well-defined band around 1375 cm^{-1}. A characteristic of isopropyl groups is that this umbrella mode is split into two component bands centered around 1365 and 1385 cm^{-1} (Jones & Sandorfy, 1956). Figure 7 shows these two bands at three different temperatures which bracket the two phase transitions of 20i PC. It is evident from this figure that the major change, i.e., a shift in frequency, occurs at T_{gg}, with only a minor change at T_{gl}. Details of the change in the frequency of one of the band components as a function of temperature are shown in Figure 8 for the 16i, 18i, 19i, and 20i PC's. A close inspection of those temperature profiles shows that the frequency changes characteristic of the 16i and 18i PC's were equal to the total frequency change characteristic of both the gel/gel and gel/liquid-crystalline phase transitions of 20i PC. This supports a previous suggestion that the only observable phase change characterstic of the 16i and 18i compounds is equivalent to both transitions which are separated on the temperature scale for the longer chain compounds. In addition, it is also apparent from Figure 8 that there is a pronounced difference in the behavior of the odd- and even-numbered compounds with respect to the temperature profiles of the infrared parameter under observation. Unlike the 20i compound, both 19i PC and 17i PC (not shown here) show no frequency change at T_{gg} and display only the minor shift in frequency at T_{gl} ($\sim$0.5 cm^{-1}), as was observed for the gel/

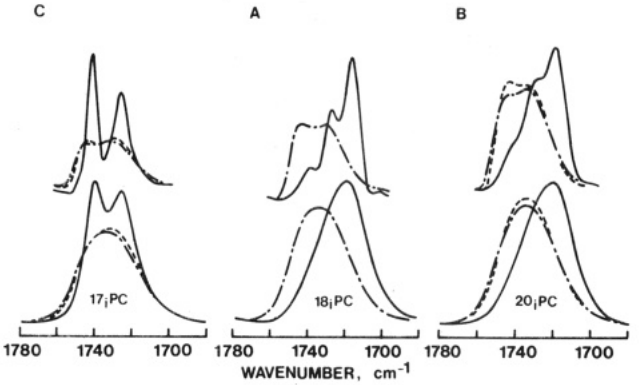

FIGURE 9: Characteristic infrared absorption band contours in the region of the C=O stretching modes of (A) 18i PC, (B) 20i PC, and (C) 17i PC. In each case, the bottom traces represent the experimentally observed band contours while the top traces show the same spectra after Fourier self-deconvolution which leads to a reduction in the bandwidths by a factor of 2.5. Spectra were characterized for the low-temperature gel state (—), the intermediate gel state (- - -), and the liquid-crystalline state (-·-).

liquid-crystalline phase transition of 20i PC. This suggests that there are significant differences in the acyl chain packing modes characteristic of the low-temperature gel phases of the odd- and even-numbered isoacyl-PC's.

(*D*) *Carbonyl Stretching Mode.* The region of the infrared spectrum between 1680 and 1780 cm^{-1} is particularly interesting in these studies, since, given the absence of carbon–carbon double bonds in these phospholipids, the only infrared-active bands in that region are the stretching vibrations of the carbonyl groups at the interfacial region of the lipid bilayer. Illustrated in Figure 9 are the carbonyl stretching band contours of three representative isoacyl-PC's and the spectra after Fourier self-deconvolution to reduce the intrinsic bandwidths. The spectra shown in Figure 9 were acquired at temperatures which bracket the transition(s) characterized by DSC for the respective PC's. After deconvolution, the carbonyl stretching band contour in the gel phase of 18i PC is shown to be comprised of three components bands (see Figure 9A): a major component (the strongest band) at 1716 cm^{-1} and two other bands of lesser intensity at 1725 and 1740 cm^{-1}. In the liquid-crystalline state, the strong band at 1716 cm^{-1} disappears, while the 1740-cm^{-1} band increases in intensity and shifts to 1742 cm^{-1}. It is also clear that the gel phase spectra of 20i PC at temperatures below T_{gg} (see Figure 9B) are similar to the gel phase spectra of 18i PC (and of 16i PC, which is not shown here). For the 20i compound, the major spectral changes occur at its gel/gel transition, which is associated with the disappearance of the strong band at 1716 cm^{-1} and an increase in the intensity of the band near 1740 cm^{-1}. A close inspection of the deconvoluted spectra of 20i PC shows that the gel-state spectra of 20i PC at temperatures above T_{gg} are similar to, though not identical with, the spectra acquired at temperatures above T_{gl}, since the gel/liquid-crystalline phase transition is associated with a slight decrease in the intensity of the band at 1742 cm^{-1} relative to the band at 1725 cm^{-1}. The above observations clearly support the view that the single transition characteristic of the 16i and 18i species is equivalent to both the gel/gel and gel/liquid-crystalline phase transitions, which are resolved for the longer chain compounds.

Unlike the even-numbered isoacyl-PC's, the low-temperature gel-state spectra of the odd-numbered compounds, as exemplified by the 17i species (see Figure 9C), lack the strong band at 1716 cm^{-1}. At all temperatures, the band contours of the carbonyl stretching vibrational mode were found to consist of

two component bands at 1726 and 1740 cm^{-1} after deconvolution (see the deconvoluted spectra in Figure 9C). These bands exhibit relatively small changes with respect to their characteristic frequencies, bandwidths, and relative intensities at both the gel/gel and gel/liquid-crystalline phase transitions. These observations suggest that, at temperatures below T_{gg}, there are considerable differences in the gel phase structure of the interfacial regions of the odd- and even-numbered isoacyl-PC's. The strong band at 1716 cm^{-1}, characteristic of the low-temperature, gel-state spectra of the even-numbered isoacyl-PC's, has been observed in the spectra of solid or poorly hydrated diacyl lipids (Mushayakarara et al., 1984; Cameron & Mantsch, 1982). Thus, the gel/gel transition of the even-numbered compounds may involve significant changes in the hydration of the interfacial glycerol moiety, in addition to the acyl chain packing changes inferred earlier. The absence of the strong band at 1716 cm^{-1} in the low-temperature gel-state spectra of the odd-numbered compounds probably indicates that hydration changes are not a significant component of the gel/gel transitions of these PC's and may provide a structural basis for the different ranges of enthalpy values characteristic of the gel/gel transitions of the odd- and even-numbered isoacyl-PC's (Lewis & McElhaney, 1985).

In the liquid-crystalline state, the carbonyl stretching bands for all the isoacyl-PC's studied were found to be very similar (see Figure 9) and to resemble those characteristic of the liquid-crystalline states of diacyl-PC's with saturated *n*-acyl chains (Casal & Mantsch, 1984). In all cases, the carbonyl stretching mode is observed at a frequency near 1737 cm^{-1}, and the Fourier band resolution technique reveals that the broad-band contour consists of two component bands at frequencies near 1743 and 1728 cm^{-1}. The latter band has been assigned to the ester carbonyl vibrations of the *sn*-1 and *sn*-2 acyl chains of phosphatidylcholines containing saturated *n*-acyl hydrocarbon chains (Mushayakarara & Levin, 1982). This indicates that, unlike the low-temperature gel states, there are few or no differences in the liquid-crystalline states of the odd- and even-numbered isoacyl-PC's with respect to the structure of the interfacial region. The spectra shown in Figure 9 also suggest that the same may apply to the gel states of these lipids at temperatures above T_{gg}.

^{31}P Nuclear Magnetic Resonance Spectroscopy. The DSC study (Lewis & McElhaney, 1985), as well as the infrared spectroscopic data described above, clearly shows that the thermotropic phase behavior of these methyl iso-branched PC's involves three distinct phases: the liquid-crystalline state, a low-temperature gel state, and an intermediate gel state, which we suggested might be necessary for interconversion between the two other states (Lewis & McElhaney, 1985). Since such lipid conformational changes are likely to affect the motion of the phosphate head group, ^{31}P NMR spectroscopy seemed a feasible supplementary spectroscopic tool for the characterization of the above phases. The ^{31}P NMR spectra shown in Figure 10 are of an aqueous dispersion of 20i PC and were acquired at three temperatures which bracket the two phase transitions described for that compound. The low-temperature gel-state spectrum (Figure 10A) has a line shape typical of slow motion on the ^{31}P NMR time scale such that motional averaging of the ^{31}P shift tensor is incomplete (Seelig, 1978; Smith & Deslauries, 1982). The gel-state spectra acquired at temperatures above T_{gg} (Figure 10B) are indicative of faster motion of the phosphate group which, at temperatures above T_{gl}, approaches the line shape typical of the liquid-crystalline state of a phospholipid undergoing fast axially symmetric motion in a bilayer structure (Figure 10C). However, it is clear from the broader line shape in Figure 10B that the limiting condition of fast axially asymmetric motion in a lipid bilayer is not attained in the gel-state conformation at temperatures above T_{gg}.

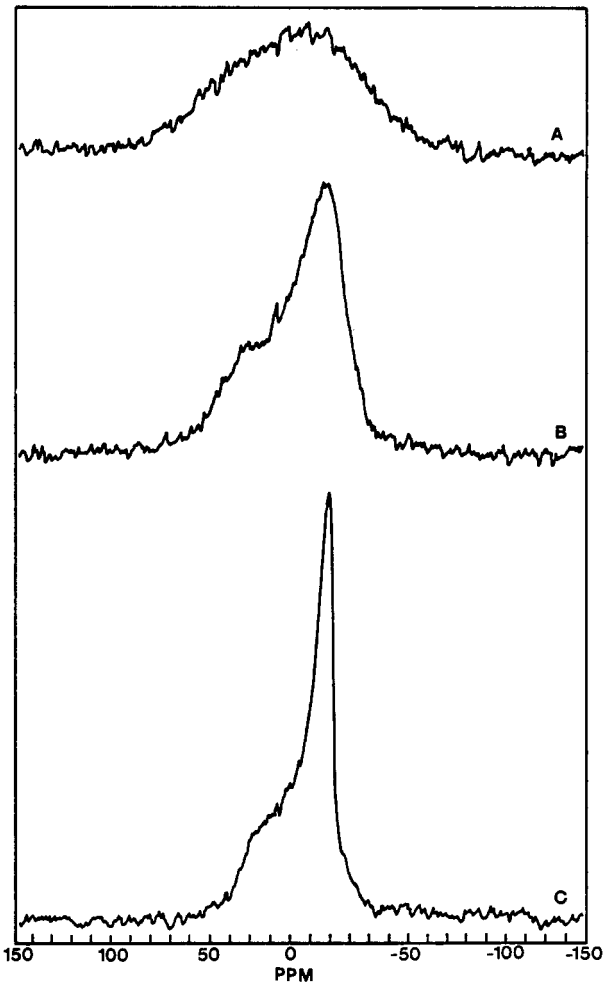

FIGURE 10: ^{31}P NMR spectra of aqueous dispersions of 20i PC at three different temperatures which bracket the two phase transitions at T_{gg} (43.7 °C) and T_{gl} (50.3 °C). The spectra were acquired at (A) 30, (B) 46, and (C) 55 °C.

The line shapes described above are characteristic of all the longer chain isoacyl-PC's for which the gel/gel transition and the gel/liquid-crystalline phase transitions are completely separated on the temperature scale. Interestingly, there were no discernible differences in the gel-state ^{31}P NMR spectra of the odd- and even-numbered compounds at temperatures below T_{gg}, despite the significant differences in the hydrocarbon chain packing mode and interfacial hydration of these two series of PC's shown by the infrared data. Similar spectral line shapes have been reported for DPPC at temperatures which bracket the subtransition and the gel/liquid-crystalline phase transition (Fuldner, 1981). That study did not report any discernible differences in the ^{31}P NMR line shapes which would characterize the pretransition of DPPC. The spectral changes reported here suggest that the structural changes characteristic of the gel/gel transitions of the isoacyl-PC's may have some similarities with those of the subtransition of DPPC (and other saturated *n*-acyl-PC's), at least with respect to the motional properties of the phosphate head group, and provide additional support for one of the inferences drawn from the infrared spectroscopic data described above.

Given the above observations, an attempt was made to characterize the behavior of the shorter chain isoacyl-PC's by ^{31}P NMR spectroscopy, especially in the cases for which the

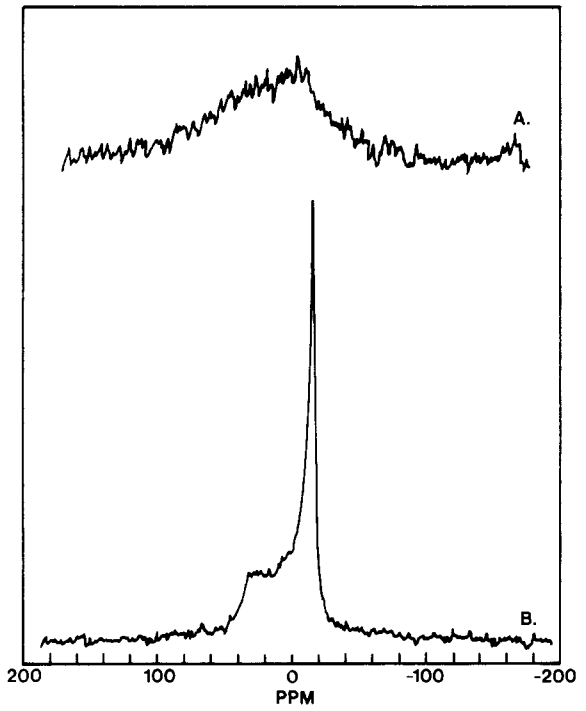

FIGURE 11: ^{31}P NMR spectra of an aqueous dispersion of 14i PC with different thermal histories. Spectrum A was acquired at 2 °C after the sample was warmed from −20 °C. Spectrum B was acquired at 2 °C after the sample was cooled from 20 °C.

gel/liquid-crystalline phase transition temperature is not demonstrably higher than the gel/gel transition temperature [see Lewis & McElhaney (1985)]. The spectra shown in Figure 11 are of 14i PC, which is typical of those compounds, as characterized by DSC. The top spectrum (spectrum A) shows the broad line shape indicative of slow motion on the ^{31}P NMR time scale, while the bottom spectrum (spectrum B) is typical of a phospholipid undergoing fast axially symmetric motion in a phospholipid bilayer at temperatures above T_{gl}. In fact, both spectra were acquired at 2 °C on the same sample with different thermal histories. Spectrum A was acquired after the sample was frozen at −20 °C and then held at 2 °C until the ice melted, while spectrum B was acquired after the sample was cooled to 2 °C from room temperature. These observations are very interesting since they provide additional evidence that the structural changes concomitant with the single endotherm (or exotherm) resolved for these compounds are the equivalent of both the gel/gel and gel/liquid-crystalline phase transitions. In addition, they also show that the conformation of these shorter chain isoacyl-PC's at temperatures between those of their heating endotherms and cooling exotherms is dependent upon their thermal histories. Once the sample is cooled below T_f (the characteristic temperature of the cooling exotherm), it attains the spectral characteristics of the stable low-temperature conformation and retains those properties until heated to temperatures above T_m (the characteristic temperature of the heating endotherm), at which temperature it attains the spectral characteristics of the liquid-crystalline state and retains those properties until cooled to temperatures below T_f [see the preceding paper (Lewis & McElhaney, 1985) for a fuller discussion of the thermotropic behavior of the these shorter chain isoacyl-PC's].

ACKNOWLEDGMENTS

We are indebted to Drs. Brian D. Sykes and Manfred Brauer for the availability of time on the NMR spectrometer and for assistance in the acquisition of the ^{31}P NMR spectra.

Registry No. 14iPC, 71368-24-6; 16iPC, 71368-22-4; 17iPC, 71368-21-3; 18iPC, 60683-79-6; 19iPC, 95799-74-9; 20iPC, 95799-75-0.

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