Biochemistry **1987**, *26*, 4045–4049

Thermotropic Phase Behavior of Model Membranes Composed of Phosphatidylcholines Containing *dl*-Methyl Anteisobranched Fatty Acids. 2. An Infrared Spectroscopy Study†

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Received November 26, 1986; Revised Manuscript Received February 17, 1987

ABSTRACT: The thermotropic phase behavior of four members of the homologous series of *dl*-methyl anteisobranched phosphatidylcholines was investigated by Fourier transform infrared spectroscopy. The odd-numbered phosphatidylcholines exhibit spectral changes in two distinct temperature ranges, while their even-numbered counterparts exhibit spectral changes within only a single temperature range. The high-temperature transition observed in the odd-numbered phosphatidylcholines and the single thermotropic event characteristic of the phase behavior of their even-numbered counterparts are both identified as gel/liquid-crystalline phase transitions. The low-temperature event exhibited only by the odd-numbered phospholipids is identified as a gel/gel phase transition that involves changes in the packing mode of the acyl chain methylene groups, as well as changes in the conformation of the glycerol ester interface. These infrared spectroscopic data thus suggest that at low temperatures the odd-numbered methyl anteisobranched phosphatidylcholines form a highly ordered condensed phase similar to the L_c phases of the linear saturated *n*-acylphosphatidylcholines. A comparable condensed phase was not formed by the even-numbered anteisobranched phosphatidylcholines under similar conditions. The properties of the gel states of the even-numbered anteisoacylphosphatidylcholines were generally similar to those of the high-temperature gel states of their odd-numbered counterparts. Those gel states exhibit spectral characteristics indicative of hexagonally packed but relatively mobile acyl chains. The temperature-dependent changes in the spectral characteristics of these gel states were continuous and were not resolved into the discrete but overlapping transitions observed by differential scanning calorimetry.

Fatty acyl chains containing a single methyl branch near their methyl termini predominate in the membrane lipids of a number of eubacteria, and this has prompted a number of studies on model membranes containing such fatty acyl structures (see the preceding paper by Lewis et al. for a full discussion and references). Of those, the methyl isobranched PC's[1,2] have been the object of considerable study [see Lewis et al. (1987) and references cited therein], but much less is known about the physical properties of methyl anteisobranched phospholipids. However, an understanding of the behavior of such lipids is of considerable interest since methyl anteisobranched fatty acids have been proposed to be the structural equivalents of unsaturated fatty acids [see Kaneda (1977) for a review]. Moreover, methyl anteisobranched fatty acids can support the growth of fatty acid auxotrophic microorganisms, thus indicating that such fatty acyl structures can support normal membrane function (Rodwell & Peterson, 1971; Silbert et al., 1973; Silvius & McElhaney, 1978). Thus, in order to further our understanding of these structures, we have synthesized a series of *dl*-methyl anteisobranched fatty acids and their respective diacyl-PC's, and the thermotropic phase behavior of the latter has been studied by DSC and ^{31}P NMR spectroscopy (Lewis et al., 1987). Those studies have shown that the phase behavior of the anteisoacyl-PC's is fairly complex and dependent upon whether their acyl chains contained an odd or even number of carbon atoms. In this paper, we describe additional studies using the noninvasive technique of FT-IR spectroscopy to further characterize the thermotropic phase transitions observed by DSC. Infrared spectroscopy is sensitive to the conformation and mobility of the acyl chains and the structure of the glycerol ester interface of the lipid molecules and can provide a snapshot of the entire lipid population at any given temperature (Mantsch, 1984; Casal & Mantsch, 1984). As a result, it provides valuable information about the structural basis of the phase changes described in the preceding paper (Lewis et al., 1987).

Materials and Methods

The 1,2-dianteisoacyl-PC's used in this and the accompanying study were synthesized and purified as described by Lewis et al. (1987). Samples for FT-IR spectroscopy were prepared at 60 wt % water or D_2O by using 0.5–1 mg of dry PC. The samples were then hydrated by two cycles of heating

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† Issued as NRCC Publication No. 26373. This work was supported in part by Operating and Major Equipment grants from the Medical Research Council of Canada and by Major Equipment grants from the Alberta Heritage Foundation for Medical Research (R.N.M.). C.M. was a recipient of a France–Canada exchange scholarship.

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[1] Abbreviations: PC, 1,2-diacyl-*sn*-glycero-3-phosphocholine; DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance; FT-IR, Fourier transform infrared; CH_2, methylene; C=O, carbonyl; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

[2] The phosphatidylcholines used in this study are described by the notation n_{ai} PC, where *n* denotes the number of carbon atoms per acyl chain and the subscript "ai" denotes methyl anteisobranching.

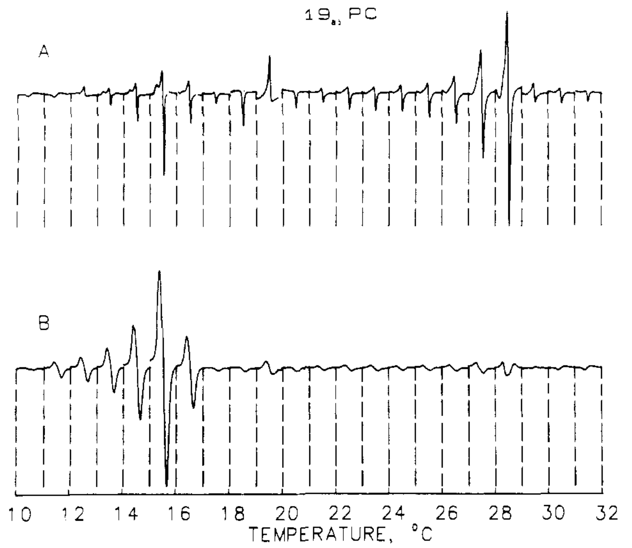

FIGURE 1: Infrared difference spectra (obtained in steps of 1 °C) of aqueous dispersions of 19_{ai} PC in the spectral region 2800–2900 cm^{-1} (acyl chain CH_2 symmetric stretching band) (A) and in the spectral region 1680–1780 cm^{-1} (ester C=O stretching band) (B). The difference spectra were generated by taking the difference between the higher temperature spectrum and the lower temperature spectrum and normalizing with respect to the temperature increment.

to 50 °C and slow cooling prior to the commencement of data acquisition. The infrared spectra were obtained on a Digilab FTS-15 FT-IR spectrometer fitted with a high-sensitivity, liquid nitrogen cooled, mercury–cadmium–telluride detector. The essential details pertinent to the acquisition of the interferograms and the subsequent data processing have been described in full detail elsewhere (Mantsch et al., 1985). Spectra were acquired at 1 °C intervals with a waiting period of 15 min between consequential spectra, which, if converted to a constant heating rate such as those used in the DSC experiments, would correspond to a heating rate of 3.75 °C h^{-1}.

Results and Discussion

In this study, FT-IR spectra of 17_{ai} PC, 18_{ai} PC, 19_{ai} PC, and 20_{ai} PC were acquired as a function of temperature. However, in this paper, the properties of only 18_{ai} PC and 19_{ai} PC will be described since their thermotropic phase behavior was found to be typical of the even- and odd-numbered members of this homologous series of compounds. Illustrated in Figure 1 are a series of infrared difference spectra obtained from 19_{ai} PC. The selected vibrational modes are the CH_2 symmetric stretching band, a typical acyl chain vibrational mode, and the ester C=O stretching band, a vibrational mode characteristic of the interfacial region of the bilayer. These 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 those two infrared bands exhibit changes in more than one temperature domain. The spectral parameter represented in Figure 1A shows the major change at temperatures near 29 °C, whereas the spectral parameter represented in Figure 1B shows major changes at temperatures near 15 °C. In addition, both spectral parameters exhibit minor changes at temperatures between 15 and 20 °C. The nature of the structural changes reflected by these difference spectra will be discussed later. The spectral parameters of the corresponding series of infrared difference spectra obtained from 18_{ai} PC (not shown here) exhibit only one major change at 18–19 °C and minor changes at lower temperatures. The temperature ranges over which major spectral changes occur are in good agreement with the DSC and ^{31}P NMR spectroscopic results [see Lewis et al. (1987)].

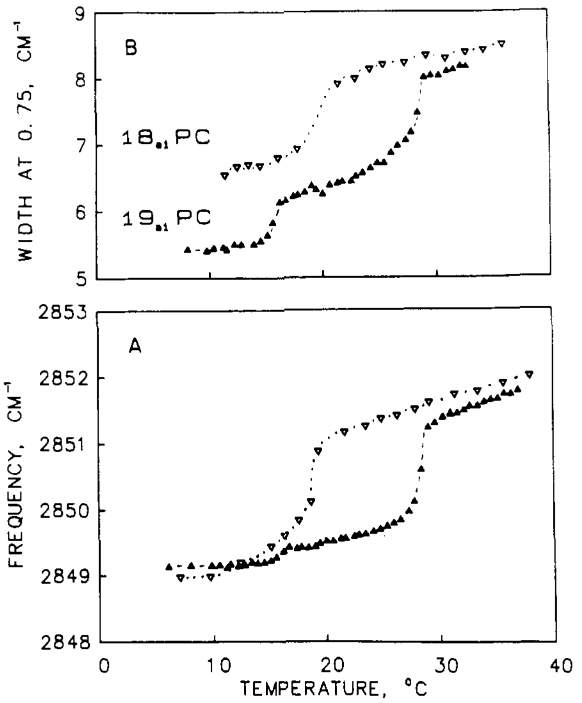

FIGURE 2: Temperature dependence of the frequency (A) and width (B) of the CH_2 symmetric stretching bands in the infrared spectra of 18_{ai} PC (open triangles) and 19_{ai} PC (solid triangles).

The assignment of the gel/liquid-crystalline phase transition is always an important consideration in studying the thermotropic phase behavior of lipids, and for these anteisoacyl-PC's it is especially important in view of the complexity of their phase behavior. The frequency of the C–H stretching modes is an infrared spectral parameter that is very sensitive to the conformational changes in polymethylene chains (Casal & Mantsch, 1984); in particular, conformational melting can be monitored via the strong infrared bands near 2850 and 2920 cm^{-1}. Those bands have been assigned to the symmetric and antisymmetric stretching vibrations of the methylene groups, respectively [for a review, see Mendelsohn and Mantsch (1986)], and the temperature-dependent variations in the position and width of those bands can be related to changes in the structure and dynamics of the polymethylene chains.

Figure 2A illustrates the temperature dependence of the frequency of the methylene stretching band near 2850 cm^{-1} for samples of 18_{ai} PC and 19_{ai} PC. Similar temperature profiles are obtained from the methylene antisymmetric stretching band near 2920 cm^{-1}. The large increase in the frequency of this vibrational mode at temperatures near 19 °C (18_{ai} PC) and 29 °C (19_{ai} PC) reflects the increase in conformational disorder of the acyl chains and clearly identifies the respective thermal events as the gel/liquid-crystalline phase transitions. The midpoints of the frequency vs. temperature plots represent the chain-melting phase transition temperatures of these PC's, and these were found to coincide with those determined by DSC [see Lewis et al. (1987)]. The data shown in Figure 2A also indicate that the thermotropic events that occur at temperatures below the onset of the gel/liquid-crystalline phase transitions do not significantly affect the intramolecular conformational state of the acyl chains. At temperatures below the onset of the gel/liquid-crystalline phase transitions of these lipids, the frequencies of the symmetric CH_2-stretching vibrational modes are in the range 2849–2849.5 cm^{-1}. Although the precise acyl chain confor-

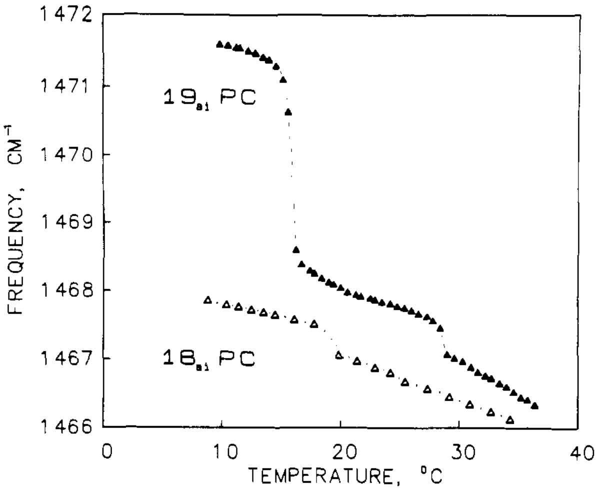

FIGURE 3: Temperature dependence of the frequency of the CH_2 scissoring bands in the infrared spectra of 18_{ai} PC (open triangles) and 19_{ai} PC (solid triangles).

mation cannot be obtained from the absolute frequency of this band, since absolute frequency may be slightly affected by vesicle size, refractive index, chain length, etc., methylene symmetric stretching frequency values below 2820 cm^{-1} are characteristic of the conformationally ordered polymethylene chains found in solid-like hydrocarbons (Snyder, 1967) or in gel-state lipids (Casal & Mantsch, 1984), while frequency values of greater than 2851 cm^{-1} are characterisitc of conformationally disordered polymethylene chains with a high content of gauche conformers, as found in liquid hydrocarbons or lipids in the liquid-crystalline state. Thus, all of the low-temperature thermotropic events observed in the phase behavior of these lipids, including the major low-temperature transition of suitably annealed samples of the odd-numbered homologues, are gel-state phenomena that do not involve any melting of the acyl chains.

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 hydrocarbon chains, the widths of the C–H stretching bands can be used to detect changes in the dynamics of the acyl chains; i.e., an increase in bandwidth reflects an increase in mobility. Illustrated in Figure 2B is the temperature dependence of the width of the CH_2 symmetric stretching band of 18_{ai} PC and 19_{ai} PC. These bandwidths vs. temperature profiles show major shifts at the same temperatures at which DSC endotherms of these phospholipids are observed. It is interesting, however, to note that in the case of the odd-numbered PC the low-temperature thermal event is associated with a considerable increase in the bandwidth of the CH_2 stretching band. It is thus clear that, like the acyl chain melting transition, the major gel-state transition characteristic only of the odd-numbered anteisobranched PC's is also associated with a considerable increase in the mobility of the acyl chains. At temperatures just below the onset of the main chain-melting phase transition of both of these lipids, there is a continuous, small but significant increase in the width of the CH_2 symmetric stretching band, particularly in the temperature range where the minor endothermic events were observed by DSC. Thus those minor thermotropic events observed by calorimetry probably involve small changes in the mobility of the acyl chains; i.e., there is a progression toward a more loosely packed gel state prior to the cooperative melting of the hydrocarbon chains.

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 near 1470 cm^{-1}. The number and frequency of such bands is dependent upon acyl chain packing and conformation. Figure 3 shows the temperature dependence of the frequency of the methylene scissoring bands of 18_{ai} PC and 19_{ai} PC. This plot illustrates the striking differences that exist between the low-temperature gel states of the odd- and even-numbered homologues of these anteisoacyl-PC's. A frequency value of $\cong$1468 cm^{-1} is observed in the gel phase of the even-numbered PC at all temperatures at which these gel phases are stable, while such a value is observed for the odd-numbered PC's only at temperatures between the major gel-state transition and the chain-melting phase transition. This frequency is characteristic of a loose hexagonal packing in which the acyl chains behave as independent rigid rotors (Cameron et al., 1980a,b, 1981). At the chain-melting phase transition of both the odd- and even-numbered anteisoacyl-PC's there is a small decrease in the frequency of the methylene scissoring mode to values near 1467 cm^{-1}. The latter value is characteristic of the disordered liquid-crystalline state of all the lipids that have been studied so far (unpublished observations from this laboratory).

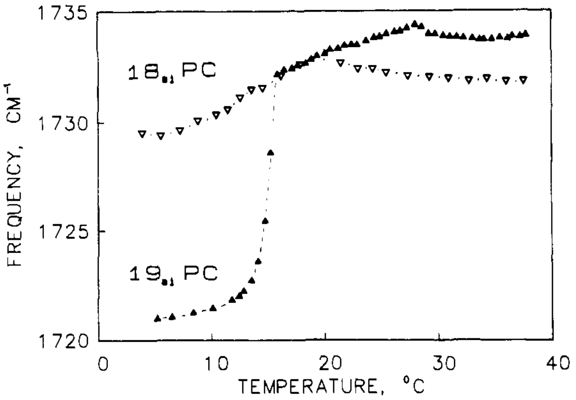

FIGURE 4: Temperature dependence of the position of the ester C=O stretching modes of 18_{ai} PC (open triangles) and 19_{ai} PC (solid triangles).

The low-temperature gel state formed by appropriate annealing of the odd-numbered anteisoacyl-PC's is characterized by a strong band at 1472 cm^{-1}. This methylene scissoring frequency has been observed in the highly condensed "subgel" phase of DPPC (Cameron & Mantsch, 1982) and the low-temperature gel states of the isoacyl-PC's (Mantsch et al., 1985). It has also been correlated with poorly hydrated lipids (Cameron & Mantsch, 1982) and with a triclinic packing mode in solid hydrocarbons (Snyder, 1961). It is thus clear that the low-temperature gel phase of these odd-numbered anteisoacyl-PC's is a highly ordered structure and that the major gel-state transition of these lipids involves a conversion to a more loosely packed gel state, which is characterized by a high degree of mobility in the hydrocarbon chains as well as the phosphate head group [see Lewis et al. (1987)].

Changes in the interaction between the acyl chains of phospholipid molecules are also expected to affect the molecular organization at the interfacial region of the bilayer. In saturated diacyl lipids the hydrophobic/hydrophilic interface of the bilayer can be conveniently monitored by observing the spectral properties of the ester carbonyl groups. That this is indeed also the case here is illustrated in Figures 4 and 5, which display the temperature-dependent changes in the frequency and bandwidth of the carbonyl stretching band contours. It is immediately apparent that all of the thermotropic events characteristic of this PC model membrane system affect the spectral parameter illustrated. In the case of the odd-numbered PC (solid symbols), the change from the condensed gel

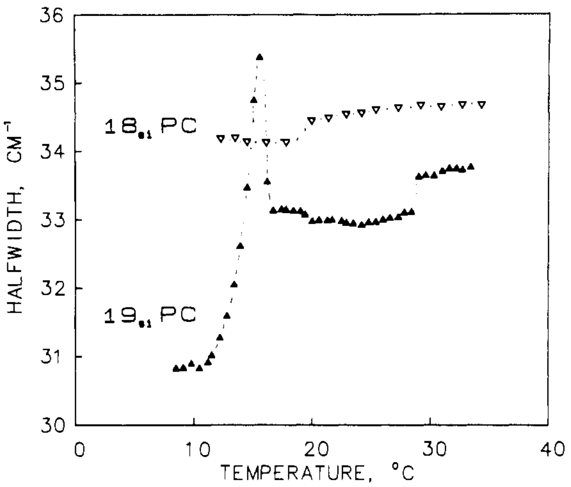

FIGURE 5: Temperature dependence of the width at half-height of the ester C=O stretching modes of 18_{ai} PC (open triangles) and 19_{ai} PC (solid triangles).

phase to the loosely packed gel state is marked by drastic changes in both the frequency and bandwidth of this vibrational mode, while the minor gel-state events and the gel/liquid-crystalline phase transition are marked only by small changes in these spectral parameters. In the case of the even-numbered compound (open symbols), only the latter spectral changes are evident. The above suggests that the conformational melting of the acyl chains and the other unassigned minor gel-state thermotropic events of the odd- and even-numbered numbers of this homologous series of PC's were not accompanied by major changes in the organization of the ester groups at the interfacial region of the bilayer.

The ester carbonyl stretching mode of these lipids gives rise to a broad band contour in the infrared spectrum (see Figure 6, bottom panel). The latter is known to be a composite of constituent bands arising in part from the different properties of the S_N1 and S_N2 ester groups, and this can obscure many of the fine details on the spectral changes that take place [see Mendelsohn and Mantsch (1986) and references cited therein]. The above can be resolved by the use of Fourier deconvolution techniques which reduce the intrinsic widths of the individual bands while retaining information pertinent to their frequencies and intensities (Kauppinen et al., 1981a,b). Illustrated in Figure 6 are the carbonyl stretching band contours of 18_{ai} PC and 19_{ai} PC (bottom panel) and the same spectra after deconvolution (top panel). The spectra shown were acquired at temperatures which bracket the major thermotropic events observed by DSC of the respective PC's. After deconvolution, the carbonyl stretching band contour of the condensed gel phase of the odd-numbered PC is shown to comprise three component bands. The major component (the strongest band) was observed at 1716 cm^{-1}, with a second strong band at 1725 cm^{-1} and a third band of lesser intensity at 1738 cm^{-1}. The strong band at 1716 cm^{-1} has been observed in the vibrational spectrum of crystalline DPPC dihydrate (Mushayakarara et al., 1982) and in the deconvoluted infrared spectra of the condensed gel phases of the even-numbered isoacyl-PC's (Mantsch et al., 1985). For the odd-numbered anteisoacyl-PC's described in this study, and in the case of the even-numbered isoacyl-PC's, the appearance of this band in the infrared spectrum seems to occur at the expense of the high-frequency component. This suggests that the formation of the condensed gel states of the odd-numbered anteisoacyl-PC's and those of the even-numbered isoacyl-PC's is accompanied by the adoption of a peculiar conformation of one of the ester carbonyl groups and that conformation may be similar to that found in crystalline hydrates of DPPC. The conversion to the liquid-crystalline as well as the other unassigned gel-state events is seen to involve an overall small shift in the frequency of the band at 1729 cm^{-1} (to 1730 cm^{-1}) and an increase in the relative intensity of the band at 1743 cm^{-1}. The two bands are similar to those observed in the liquid-crystalline states of all of the diacylphosphatidylcholines studies so far and have been assigned to the ester carbonyl vibrations of the S_N1 and S_N2 acyl chains of the lipid molecule (Bush et al., 1980; Levin et al., 1982).

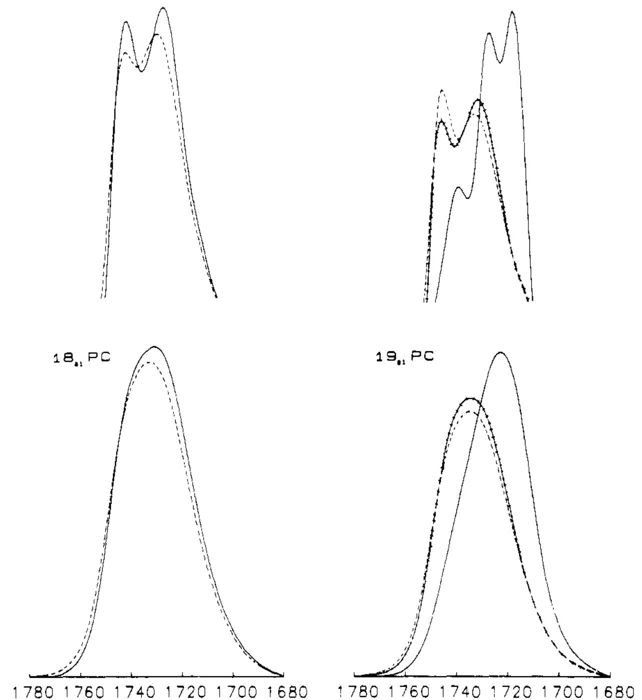

FIGURE 6: Characteristic infrared absorption band contours in the region of the C=O stretching modes of 18_{ai} PC (A) and 19_{ai} PC (B). In each case the bottom traces represent the experimentally observed band contours, whereas the top traces show the spectra after Fourier deconvolution leading to a reduction in the band widths by a factor of 2.5 [for details see Mantsch et al. (1986)]. Spectra were characterized for the low-temperature gel state (—), the intermediate gel state (++), and the liquid-crystalline state (--).

Unlike the odd-numbered anteisoacyl-PC's, the gel-state spectra of the even-numbered compounds (exemplified here by the 18_{ai} species) lack the strong band at 1716 cm^{-1}. At all temperatures the band contours of the stretching vibrational mode were found to be resolvable into two component bands at 1727 and 1742 cm^{-1} by deconvolution procedures. The observed thermotropic phase changes were accompanied by relatively small changes in the frequency of the low-frequency component but without any major change in the relative intensities of the two components. These changes were similar to but not identical with those characterized for the odd-numbered PC's at temperatures above that of their main gel-state transition. This suggests that there may be subtle differences in the organization of the interfacial ester groups in the loosely packed gel states of the odd- and even-numbered anteisoacyl-PC's.

CONCLUSIONS

It is clear from the data described here and in the preceding paper (Lewis et al., 1987) that the presence of a methyl group on the antepenultimate carbon atom of a saturated hydrocarbon chain has a major effect on the thermotropic phase behavior of the phospholipid molecule in which it is found and that many of those effects observed could not be predicted from studies on other model membrane systems. It should also be noted that the anteisobranched hydrocarbon chain can, in principle, adopt an all-trans conformation with the methyl branch aligned with the zigzag of the rest of the polymethylene chain, and another conformation with the methyl branch offset from the polymethylene zigzag. This could be a significant factor contributing to the complexity of the thermotropic phase properties of these lipids, along with the fact that these studies were done with a racemic fatty acid mixture. Studies are currently under way aimed at addressing these issues as well as the elucidation of the physical basis of all of the observations described in these studies.

ACKNOWLEDGMENTS

We are indebted to D. J. Moffatt for his invaluable assistance in the processing of the data.

Registry No. 17_{ai} PC, 73641-11-9; 18_{ai} PC, 73641-12-0; 19_{ai} PC, 73641-13-1; 20_{ai} PC, 108320-55-4.

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