Thermotropic Behavior of Bilayers Formed from Mixed-Chain Phosphatidylcholines†

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ABSTRACT: The six possible phosphatidylcholines containing two different chains derived from myristic, palmitic, and stearic acids were synthesized, and their bilayer structures were investigated by high-sensitivity differential scanning microcalorimetry. Chain migration during the syntheses caused each of the lipids to contain about 10% of the corresponding positional isomer. A phase diagram for each pair of isomers was constructed to permit estimation of the transition properties of the pure mixed-length phospholipids. The phase transitions of these lipids were found to be similar to those of saturated like-chain phosphatidylcholines. The main transition temperatures and enthalpies fall within the range of those for the like-chain lipids. In each pair of positional isomers, the isomer having the longer chain at position 2 on the glycerol backbone has the higher transition temperature and enthalpy. The transition curves of the pure mixed-chain lipids with myristic acid at position 2 and either palmitic or stearic acid at position 1 exhibited two partially separated peaks for the main transition. No satisfactory interpretation of this unexpected phenomenon has been developed.

The lipids occurring in biological membranes are generally complex mixtures with respect both to head groups and to inter- and intramolecular mixing of acyl chains. Despite extensive studies on model systems containing phospholipids with two identical acyl chains, relatively little attention has been paid to the effect of intramolecular mixing of different fatty acid chains on the properties of model membranes. Here, we extend recent high-sensitivity differential scanning calorimetry (DSC)[1] investigations (Mabrey & Sturtevant, 1976, 1978) of lipid phase behavior to include phosphatidylcholines with two acyl chains of different lengths in the same molecule.

X-ray studies (Tardieu et al., 1973) on lipid systems of low hydration indicated that some mixed-chain phospholipids may have different phase behavior in bilayers than lipids with the same chain length. However, except for the X-ray data and a few monolayer studies (Ghosh et al., 1973; De Kruyff et al., 1973), multilamellar suspensions of this type of mixed-chain lipids have not been systematically investigated. A year after the work reported here was initiated, the synthesis of the four mixed-chain phosphatidylcholines containing myristoyl and palmitoyl chains and their study by low-sensitivity DSC were published (Keough & Davis, 1979). It was found that the transition temperatures and enthalpies of the mixed-length lipid bilayers fall between those of the two corresponding diacyl lipids. For each pair of positional isomers, the lipid with the longer chain at position 2 of the glycerol backbone was found to have the higher transition temperature and enthalpy.

In the present study, the six possible mixed-chain phosphatidylcholines containing acyl chains derived from myristic (C_{14}), palmitic (C_{16}), and stearic (C_{18}) acids were synthesized, and the phase behavior of their aqueous suspensions was investigated by high-sensitivity differential scanning calorimetry. In contrast to the data obtained in the low-sensitivity DSC study (Keough & Davis, 1979), the use of high-sensitivity DSC not only provides more accurate measurements of transition properties but also reveals some interesting fine structure in the phase transitions of mixed-length lipid bilayers which has not been previously observed in studies of lipid membranes. The phase transitions of these mixed-chain lipid bilayers are dependent on the difference in chain length, the chain distribution on the glycerol backbone, and the absolute length of the fatty acid chain at position 2 on the glycerol backbone. Differences in the chain lengths also influence the "pretransition" behavior of these lipid bilayers.

We have recently discovered that some saturated-chain phosphatidylcholines show a "subtransition" at a temperature lower than that of the pretransition after prolonged cooling (Chen et al., 1980). The subtransition behavior of the mixed-chain lipids has not as yet been investigated. In preliminary experiments, we have found (Chen & Sturtevant, 1979) that 1-stearoyl-2-oleoylphosphatidylcholine shows a very broad main transition centered at about 7 °C.

Materials and Methods

Diacyl Lipids and Fatty Acids. Dimyristoyl-, dipalmitoyl-, and distearoylphosphatidylcholines were purchased from both Sigma and Calbiochem. These commercial lipids were found to be quite pure as judged by their phase transition behavior and were used without further purification. Fatty acids were obtained from several different commercial sources and were purified by recrystallization 3 times from ethanol–water.

Synthesis of Mixed-Chain Phosphatidylcholines. Mixed-chain phospholipids with positional specificity for different fatty acids were synthesized according to established procedures (Cubero-Robles & van den Berg, 1960) with minor modification.

Commercial diacylphosphatidylcholines were hydrolyzed with phospholipase A (*Crotalus atrox* snake venom obtained from Sigma) to give the lysolecithin with the first desired specific fatty acid at position 1. The acylation of the lysolecithins with the second desired fatty acid at position 2 was achieved by reaction with a mixture of the anhydride and the sodium salt of the appropriate purified fatty acid. For better

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[1] Abbreviations used: DSC, differential scanning calorimetry; DMPC, 1,2-dimyristoylphosphatidylcholine; DPPC, 1,2-dipalmitoylphosphatidylcholine; DSPC, 1,2-distearoylphosphatidylcholine; DMPE, 1,2-dimyristoylphosphatidylethanolamine; SMPC, 1-stearoyl-2-myristoylphosphatidylcholine; MSPC, 1-myristoyl-2-stearoylphosphatidylcholine; PMPC, 1-palmitoyl-2-myristoylphosphatidylcholine; MPPC, 1-myristoyl-2-palmitoylphosphatidylcholine; SPPC, 1-stearoyl-2-palmitoylphosphatidylcholine; PSPC, 1-palmitoyl-2-stearoylphosphatidylcholine; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid).

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Table I: Fatty Acid Compositions of Synthetic Mixed-Chain Phosphatidylcholines

lipid R_1R_2PC	batch no.[a]	total mol % in synthesis product R_1	total mol % in synthesis product R_2	mol % of corresponding isomer[b]
SMPC	I	50.1	49.9	10.6
	II[c]	50.6	49.4	9.3
	III	50.7	49.3	11.2
MSPC	I[c]	50.6	49.4	10.0
	II	49.8	50.2	12.7
PMPC	I	49.4	50.6	8.2
	II[c]	49.5	50.5	8.4
	III	50.6	49.4	10.7
MPPC	I	49.6	50.4	26.6
	II[c]	49.2	50.8	16.3
SPPC	I[c]	50.2	49.8	12.3
	II	51.2	48.8	18.2
PSPC	I	49.1	50.9	11.2
	II[c]	49.2	50.8	11.0

[a] Starting materials from different sources were used in the synthesis of the different batches. [b] Obtained from the analysis of fatty acid content of lysolecithin (position 1) and free fatty acid (position 2) released by enzymatic hydrolysis (see text). [c] Data for this batch were used in Figure 1.

mixing of the reactants, the previously employed sodium oxide was replaced by the sodium salt of the acid, and the reaction mixtures were briefly stirred in carbon tetrachloride before being evaporated to dryness. The solid reaction mixture was then heated under vacuum for 1–2 days at (but not above) the melting temperature of the acid anhydride to ensure sufficient yield and minimum chain migration.[2] The product lipids were purified by silica gel column chromatography and precipitation from acetone–chloroform.[3]

Two completely different sources of starting materials were used in the synthesis of different batches of mixed-length lipids to minimize the problem of impurity from a single source. No significant differences in phase behavior were observed between bilayers of lipids from different batches.

For purposes of comparison, exactly the same synthetic and purification procedures were applied to the repreparation of the parent diacylphosphatidylcholines from the same starting materials. Since the nature of the phase transition of diacyllipids has been thoroughly investigated and is well understood (Albon & Sturtevant, 1978), these resynthesized diacyllipids were useful as reference materials in our study of the mixed-chain lipids.

Analysis of the Synthetic Lipids. The synthetic mixed-chain phosphatidylcholines were analyzed for fatty acid composition and positional specificity by treatment with phospholipase A followed by thin-layer and gas–liquid chromatography of the hydrolysis products. The total fatty acid composition and the percentage of positional isomer present in each mixed-length lipid preparation are shown in Table I. The extent of chain migration during synthesis is similar to that reported previously (Keough & Davis, 1979).

Differential Scanning Calorimetry. Differential scanning calorimetry was performed with the high-sensitivity instrument described by Privalov et al. (1975). All calorimetric data for the main transitions were calculated from 0.1 K min^{-1} scans. Occasionally, and for all pretransition data, a scan rate of 0.5 K min^{-1} was used.

[2] A recently developed method of lipid acylation with (dimethylamino)pyridine as catalyst at room temperature is repoted to involve chain migration to the extent of only 5% (Gupta et al., 1977).

[3] This purification does not remove the contaminating positional isomers.

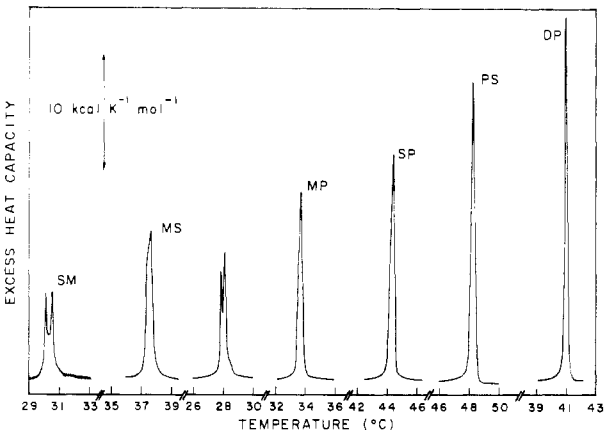

FIGURE 1: Tracings of the calorimetrically observed main transitions of the six synthetic mixed-chain phosphatidylcholines and of DPPC, taken at a scan rate of 0.1 K min^{-1}. Each mixed-chain lipid was contaminated by its positional isomer in the amounts given in Table I. The actual noise level is indicated in the first tracing in the figure.

In the preparation of samples for DSC study, a lipid or lipid mixture was dissolved in a small amount of chloroform and dried in a vacuum oven at 50 °C for 1–2 h. An appropriate amount of 0.01 M Pipes buffer, pH 7.4, was then added to give a lipid concentration of 1–2 mg mL^{-1}. The mixture was incubated at a temperature a few degrees above the phase transition temperature for 1 h, and the lipid suspension was then shaken on a vortex mixer. This procedure gives multilamellar suspensions.

Results and Discussion

Phase Transition of Mixed-Chain Lipids. The DSC traces for the gel to liquid crystal transitions of the six mixed-length phosphatidylcholines are shown in Figure 1 together with that for DPPC, all taken at a scan rate of 0.1 K min^{-1}. The transitions of MPPC, SPPC, and PSPC are very similar to that of DPPC. The transitions of SMPC and PMPC show two partially resolved peaks. It is interesting that neither MSPC nor MPPC show double peaks, although the trace for MSPC is unusually broad. The resolution of the double peaks does not increase with further reduction of the scan rate. If we note that a difference of 2 carbon atoms in a total length of 14 or 16 carbon atoms (as in PMPC) is relatively greater than that of 2 in 16 or 18 (as in SPPC), it appears that as the relative difference in chain length decreases the two peaks move closer to each other and finally merge.

Since the purity of the resynthesized diacylphosphatidylcholines was as good or better than that of the commercial lipids, as indicated by narrower transition widths and larger enthalpies, we may conclude that apart from the problem of chain interchange the synthetic method employed did not introduce any significant contamination into the mixed-chain lipids.

Pretransition of Mixed-Chain Lipids. All the mixed-chain lipids show the so-called pretransition seen with diacylphosphatidylcholines with the one exception of MSPC. Scans of these transitions, run at 0.5 K min^{-1}, are shown in Figure 2. It is interesting that the pretransition of SMPC is especially large although its isomer appears to show none.

Phase Diagrams of Mixtures of Positional Isomers. As indicated by the results of the fatty acid analyses shown in Table I, the synthetic mixed-chain lipids are actually mixtures with about 10% of the corresponding positional isomer. For estimation of the transition properties of the pure lipids, the properties observed for the synthesized lipids and for various mixtures of positional isomers were plotted as functions of the

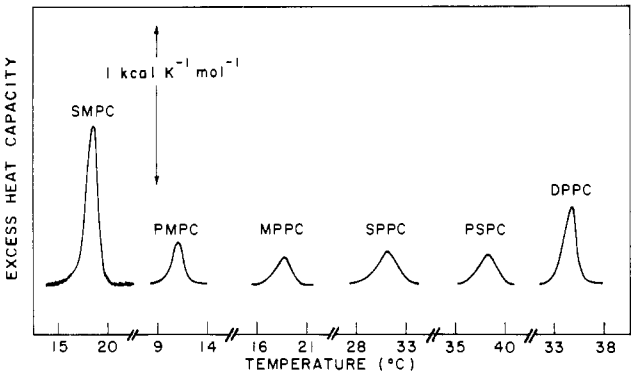

FIGURE 2: Tracings of the DSC curves for the pretransitions of SMPC, PMPC, MPPC, SPPC, PSPC (same samples as used for Figure 1), and DPPC, obtained at a scan rate of 0.5 K min^{-1}. No pretransition was detected in the case of MSPC. The actual noise level is indicated in the first tracing in the figure.

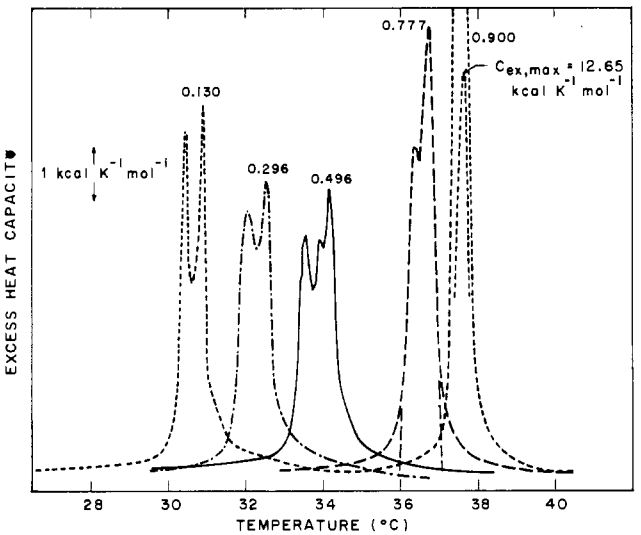

FIGURE 3: Transition curves observed at 0.1 K min^{-1} for various mixtures of SMPC and MSPC. The mole fraction of MSPC, corrected for the known extent of chain migration during synthesis, is given next to each curve. The estimation of the initiation (T_i) and completion (T_f) temperatures plotted in Figure 4 is indicated for the curve for 0.777 mol % MSPC.

composition, the latter being calculated by including the known compositions of the synthetic materials. Extrapolation then yielded values for the pure lipids. Measurements made with different batches of synthesized lipids were in good agreement. Some representative DSC curves for mixtures of SMPC and MSPC are shown in Figure 3.

Phase diagrams for the three isomer pairs, showing initiation temperature, T_i (solidus curves), and completion temperatures, T_f (liquidus curves), are given in Figure 4. The fact that the liquidus and solidus curves are only slightly curved and are nearly parallel shows that the positional isomers form very nearly ideal mixtures. The initiation and completion temperatures for the pure lipids given in Table II were estimated by least squaring the data to second-degree polynomials.

The variation of transition enthalpy with composition in each pair of positional isomers is shown in Figure 5. The fact that these enthalpies are well fit by linear equations is another indication of very nearly ideal mixing. The extrapolated values for the pure lipids are given in Table II.

Phase Transition Behavior of Pure Mixed-Chain Phosphatidylcholines. A number of interesting points are exhibited by the transition data for pure mixed-chain and resynthesized like-chain phosphatidylcholines assembled in Table II. For each pair of positional isomers, the main transition tempera-

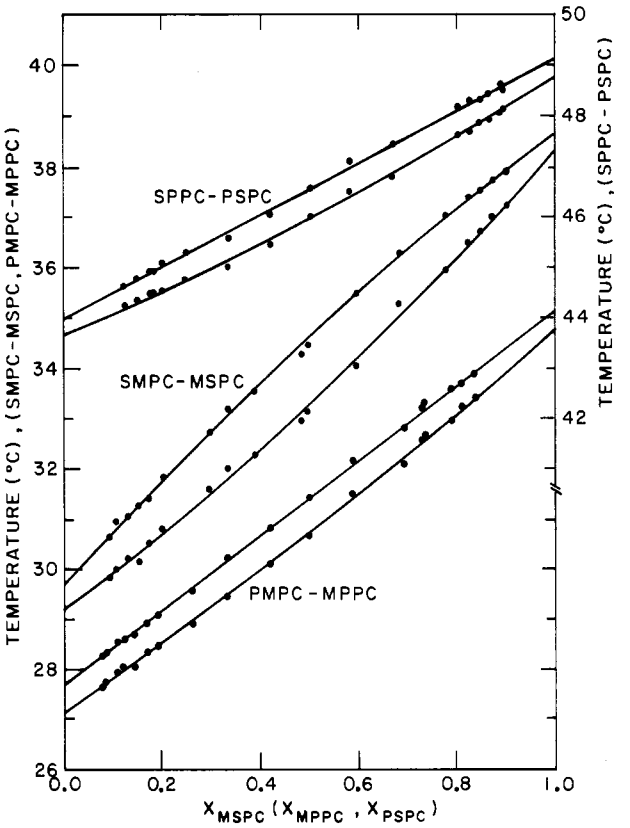

FIGURE 4: Phase digrams for mixtures of positional isomers constructed from initiation (T_i) and completion (T_f) temperatures read from the observed DSC transition curves (cf. Figure 3). No correction for the finite transition widths of the pure components (Mabrey & Sturtevant, 1976) has been applied. The solid curves are quadratic least-squares fits to the experimental points.

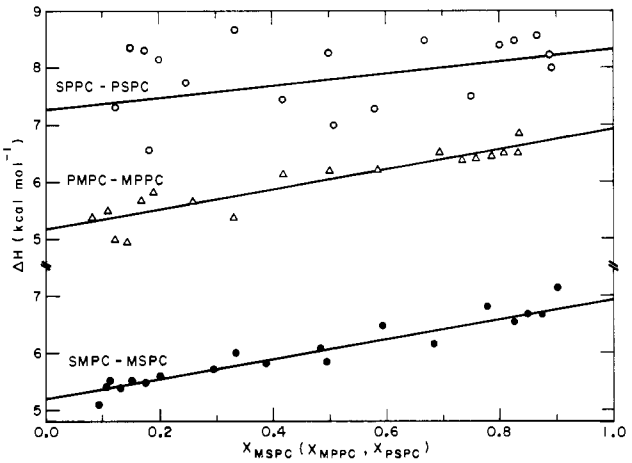

FIGURE 5: Variation of transition enthalpy with composition for the three pairs of positional isomers. Mole fractions were corrected for the known contaminations by the isomeric species. The lines are linear least-squares fits to the experimental data.

tures lie between those of the corresponding like-chain lipids, and that isomer having the longer chain in the 2 position has the higher transition temperature. This latter regularity is also observed with the transition enthalpies; furthermore, in the cases where the difference in the chain length is only two methylene groups, the isomer having the longer chain in the 2 position has an enthalpy even larger than that of the higher corresponding like-chain compound. These observations strongly suggest that the two chains in a phosphatidylcholine bilayer are not identically packed, even when the two chains are of the same length. The conformational nonequivalence of chains 1 and 2 has been reported on the basis of deuterium

Table II: Calorimetric Parameters for the Phase Transitions of Pure Mixed-Chain Phosphatidylcholines[a]

	main transitions							pretransitions	
lipid	$T_{m1,a}$ (°C)	$T_{m1,b}$ (°C)	ΔT_{m1} (K)	$\Delta T_{1/2}$ (K)	ΔH_1 (kcal mol^{-1})	T_i^d (°C)	T_f^d (°C)	T_{m2} (°C)	ΔH_2 (kcal mol^{-1})
SMPC	29.37 ± 0.08	29.62 ± 0.07	0.26 ± 0.03	0.36 ± 0.04[c]	5.20 ± 0.23	29.19 ± 0.11	29.70 ± 0.09	20.03 ± 0.16	1.95 ± 0.12
MSPC	38.63 ± 0.08	38.55 ± 0.07	−0.09 ± 0.03	0.16 ± 0.04	6.93 ± 0.23	38.28 ± 0.11	38.68 ± 0.09		
PMPC	27.28 ± 0.07	27.54 ± 0.07	0.24 ± 0.01	0.32 ± 0.03[c]	5.16 ± 0.25	27.14 ± 0.08	27.65 ± 0.05	10.77 ± 0.17	0.28 ± 0.04
MPPC	35.08 ± 0.07	35.05 ± 0.07		0.20 ± 0.03	6.92 ± 0.25	34.77 ± 0.08	35.10 ± 0.05	22.76 ± 0.17	0.22 ± 0.04
SPPC	43.87 ± 0.07			0.17 ± 0.03	7.26 ± 0.63	43.68 ± 0.09	43.97 ± 0.07	30.78 ± 0.18	0.52 ± 0.08
PSPC	48.98 ± 0.07			0.14 ± 0.03	8.33 ± 0.63	48.77 ± 0.09	49.12 ± 0.07	39.91 ± 0.18	0.36 ± 0.08
DMPC[b]	23.59			0.13	5.03	23.40	23.70	14.4	0.83
DPPC[b]	41.05			0.14	6.43	40.90	41.20	34.8	0.92
DSPC[b]	54.15			0.15	7.89	54.00	54.30	50.4	0.89

[a] The values listed were obtained by extrapolation of data observed with various mixtures of each pair of positional isomers. A scan rate of 0.1 K min^{-1} was used for the main transitions and 0.5 K min^{-1} for the pretransitions. The uncertainties given are the standard deviations from least-squares-fitted curves. [b] DSC measurements on resynthesized lipids. The enthalpy values given here are significantly lower than those reported earlier (Mabrey & Sturtevant, 1976). In numerous intervening experiments focused on the main transition of DPPC, we have found that the transition enthalpy is strongly dependent on the details of preparation of the multilamellar suspension employed. As an extreme example, a sample of DPPC was suspended in water by being heated to 60 °C and vigorously vortexted for several minutes at this temperature in a vial containing several glass beads. After being cooled to room temperature, this sample was scanned at 0.06 K min^{-1} and gave a transition enthalpy of 5.48 kcal mol^{-1}. On being cooled in the calorimeter and rescanned, the enthalpy increased to 7.02 kcal mol^{-1}. (This was a very pure sample of DPPC as evidenced by the fact that the transition width at half-maximal excess specific heat was only 0.13 °C.) It seems likely that the vigorous vortexing of this sample in the presence of glass beads produced a significant fraction of small vesicles, a form of suspension which is known to have a smaller enthalpy than that of the multilamellar form. A uniform procedure for preparing suspensions was used throughout the present work, so that the reported enthalpies should be mutually consistent. An additional source of variability in the enthalpies observed for lipid phase transitions may be the difficulty of accurately sampling heterogeneous suspensions. We have no explanation for the fact that in recent years we have never observed a main transition enthalpy for DPPC in excess of 7.5 kcal mol^{-1}, although in our 1976 paper we reported 8.7 kcal mol^{-1}. It should be noted that in every experiment the heat absorption is directly compared with the heat evolution produced by a known current flowing under scanning conditions through the calorimeter heater of known resistance for a known period of time. [c] Total widths of the double-peaked transition curves at half-maximal height of the higher temperature peak. [d] Initiation (T_i) and completion (T_f) temperatures.

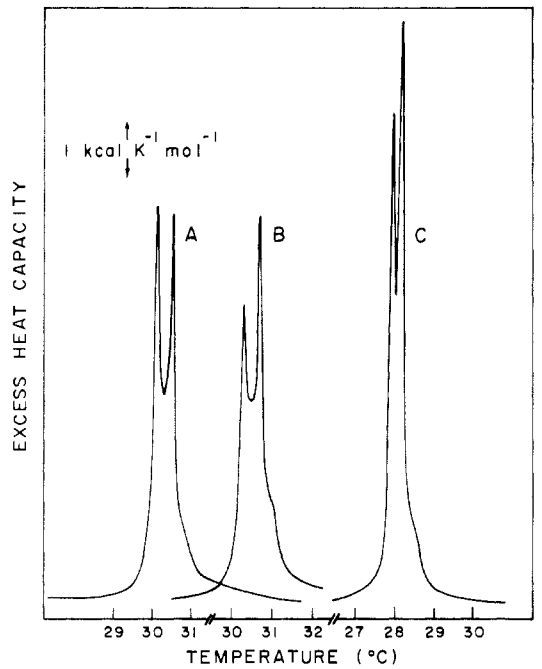

FIGURE 6: Tracings of the main transitions of (A) SMPC (with 9.3 mol % MSPC) and (C) PMPC (with 8.2 mol % MPPC) plotted on expanded temperature scales. Scan B shows the effect on the transition of SMPC of holding the suspension precisely at the temperature of the lower peak for 10 h in the calorimeter after completion of scan A.

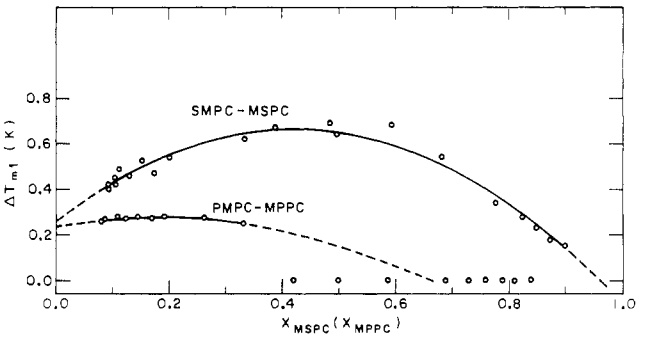

FIGURE 7: Variation with composition of the double-peak separations, ΔT_{m1}, for the SMPC–MSPC and PMPC–MPPC systems. For the latter system, no peak separation could be detected for $X_{MPPC} > 0.33$. The solid curves are quadratic least-squares fits to the observed data (for $X_{MPPC} \leq 0.33$ in the case of PMPC–MPPC).

magnetic resonance (Seelig & Seelig, 1975; Haberkorn et al., 1977; Oldfield et al., 1978), neutron diffraction (Büldt et al., 1978), and Raman studies of selectively deuterated DMPC and DPPC (Gaber et al., 1978). X-ray data for crystalline DMPE (Hitchcock et al., 1974) and DMPC (Pearson & Pascher, 1979) have led to the proposal that the acyl chain at the 2 position is bent at C2 so that the 1 chain penetrates deeper into the center of the bilayer. This difference in chain penetration results in the compounds with longer 2 chains having their chains penetrating to less different depths than in their isomers, with resulting stronger chain interactions and better packing in the bilayer. It is interesting that the transition temperature of MPPC is higher than that of SMPC, although the latter lipid has a higher molecular weight. This observation implies that the relation between bilayer thickness and transition temperature is more complex than previously suggested (Keough & Davis, 1979).

Double-Peak Transitions. Both SMPC and PMPC show double-peaked transitions (Figure 6). The extrapolated peak separations for the pure lipids are both close to 0.25 K (Figure 7), so that this fine structure can only be observed by means of high-sensitivity DSC at very low scan rates. In the case of the MSPC–SMPC pair, the least-squared curve for ΔT_{m1}, the peak separation, as a function of composition indicates $\Delta T_{m1} = 0$ at $X_{MSPC} = 0.96$, where X_{MSPC} is the mole fraction of MSPC, and −0.09 K for pure MSPC. This figure may not be significantly different from zero. The least-squared curve for the MPPC–PMPC pair indicates that $\Delta T_{m1} = 0$ when

X_{MPPC} = 0.67 although experimentally two peaks are not observed for $X_{MPPC} > 0.33$. It is estimated that $\Delta T_{m1} = 0$ also for pure MPPC.

The heights of each of the peaks in the doublets in Figure 6 increase as the mole fraction of PMPC or SMPC approaches unity. The transition shown by the synthesis product containing 90.7 mol % SMPC and 9.3 mol % MSPC can be arbitrarily resolved into two transition curves, each corresponding to a van't Hoff enthalpy (Mabrey & Sturtevant, 1978) of about 3.6×10^3 kcal mol^{-1}, or an average cooperative unit containing 700 lipid molecules. It may thus be assumed that the two peaks for pure SMPC would have sharpnesses indicating cooperative units of at least 1000 lipid molecules.

As seen in Figure 3, an equimolar mixture of SMPC and MSPC exhibits a triple-peaked transition curve. This observation is consistent with one of the pure components in this mixture having a double-peaked transition, since it has previously been observed (Mabrey & Sturtevant, 1976) that a nearly ideal mixture of two components, each of which exhibits a normally sharp single peak, can show a double-peaked transition curve. The shoulder seen in Figure 6 on the high-temperature side of each of the transitions is presumably due to the presence of a significant amount of the corresponding positional isomer.

As discussed above, differences in the depth of chain penetration play an important role in the phase transition behavior of lipid bilayers. If, as indicated by the recent X-ray study of crystalline DMPC (Pearson & Pascher, 1979), the chain at position 1 in the lipid containing two equal chains extends into the bilayer about three methylene groups farther than the chain at position 2, then we should expect the difference in penetration of the two chains in SMPC to be about *seven* methylene groups. With such a large difference, it would seem that interdigitation of the chains on opposite sides of the bilayer would be energetically favored over a random packing of the chains and that segregation of the bilayer into regions of interdigitation and regions of random packing might be the cause of the observed double-peaked transition. Such segregation, despite the indicated energy difference of only $1/4kT$, might be rationalized in terms of the highly cooperative character of the chain packing in these bilayers. The randomly oriented regions would necessarily involve introduction of considerable *gauche* bonding to accommodate the longer chains within the bilayer thickness defined by the interdigitated domains. If the two different types of regions have to be considered as two distinct phases in the thermodynamic sense, the phase rule would lead us to expect a constant initiation temperature for the transition independent of the fraction of material in interdigitated form. Since we have no way of varying this quantity, we have no way of checking for invariance in this system. On the other hand, it may be suggested (Rubenstein et al., 1980) that the regions of interdigitated chains and random chains constitute "microphases" not subject to classical thermodynamic restrictions and that the gel phase should really be considered to be homogeneous. A serious weakness in this interpretation of the double-peaked transition is that with a difference in interchain contacts between interdigitated and noninterdigitated forms of several methylene groups a difference in T_m of 20 °C or more would be expected.

Another interpretation of the double-peaked transition, which is also not fully convincing, is to consider that we have a homogeneous gel phase made up of two components, the interdigitated lipid domains and the domains of randomly packed chains. It can be shown that a double-peaked transition similar to that observed is predicted if the system is assumed to deviate markedly from ideal behavior. A difference in T_m of about 1 °C is required in this interpretation.

If the double-peaked transition curves are indeed due to the presence of two different types of chain packing in the gel phase, it would be expected that the distribution between the two packing types might be affected by annealing the suspension. As shown in Figure 6, we found that a change in the shape but not the total area of the transition curve results from prolonged heating in the calorimeter at precisely the temperature of the lower peak. However, the significance of this change is not obvious, since although the maximal excess heat capacity of the lower peak decreased, that of the upper peak remained unchanged, indicating that the loss in area of the lower peak was balanced primarily by an increase in the area of the shoulder on the upper peak. Furthermore, when the suspension after scan B in Figure 6 was cooled in the calorimeter and rescanned, the trace was found to be identical with scan B. In view of the fact that scan A was obtained with material which had been rapidly scanned to 35 °C and cooled in the calorimeter, one must conclude that the change shown in Figure 6 is not readily reversible even in the liquid crystal phase.

It is interesting that neither pure SPPC nor 1-stearoyl-2-pentadecanoylphosphatidylcholine containing 11 mol % of positional isomer shows a double-peaked main transition. It has been reported (Büldt et al., 1978) that in 5% hydrated DPPC the 1 chain penetrates into the bilayer farther than the 2 chain by an amount corresponding to only 1.5 carbon–carbon bonds, whereas in the cases of DMPC (Pearson & Pascher, 1979) and DMPE (Hitchcock et al., 1974) the difference in penetration is 3 carbon–carbon bonds. It thus appears that so far as the mixed-chain lipids considered in this paper are concerned the difference in penetration is large enough to lead to a double-peaked transition only when a myristoyl chain occupies the 2 position.

Whatever the difficulties of interpretation, the data presented in Figure 7 leave little room for doubt that *pure* SMPC and PMPC both show double-peaked transitions.

Pretransitions. The data given in Table II for the pretransitions shown by the mixed-chain lipids are not as reliable as those for the main transitions primarily because considerably higher lipid concentrations than were used would be needed for these relatively weak transitions. The variation of enthalpy and transition temperature with composition in both the PMPC–MPPC and SPPC–PSPC systems indicates approximately ideal mixing. The enthalpies for all four of these lipids are considerably smaller than those for the corresponding like-chain lipids. For each pair, the isomer having the longer chain in the 1 position has T_m lower than either of the corresponding like-chain compounds.

The SMPC–MSPC pair shows some unexpected properties. Whereas the enthalpy for SMPC is over twice as large as that for DMPC or DSPC, no pretransition is detectable for MSPC. Furthermore, as MSPC is added to SMPC, both the enthalpy and the T_m decrease linearly until the enthalpy becomes zero at 40 mol % MSPC, at which composition the T_m has dropped to about 13.5 °C.

All the pretransitions were observed at a scan rate of 0.5 K min^{-1}. In view of the fact that the pretransition of DPPC has been shown to be relatively slow (Lentz et al., 1978), the pretransition properties given in this report may be to some extent dependent on scan rate.

Acknowledgments

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Novel Surface Phase Containing Cholesteryl Esters. 1. Structural Characteristics Determined from Surface Pressure-Area Measurements†

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ABSTRACT: The behavior of cholesteryl myristoleate in mixtures with dioleoylphosphatidylcholine was investigated at the air-water interface. In addition to the previously described monolayer phase [Smaby, J. M., Baumann, W. J., & Brockman, H. L. (1979) *J. Lipid Res. 20*, 789-795], a second surface phase has been identified. Analysis of surface pressure and molecular area data as a function of composition shows that the molecules in the second phase can exist in two miscible, double-layer states or packing arrangements, only one of which contains lecithin. The mixed double-layer state is preferentially formed and has stoichiometry ranging between 2.0 and 9.5 molecules of cholesteryl ester for each lecithin molecule. The structure of this state resembles a mixed monolayer of pressure-dependent composition and area which is covered by a second layer of cholesteryl ester at 38.2 Å^2/molecule. The cholesteryl myristoleate/lecithin ratio of the layer in contact with the aqueous phase ranges from 0 to 2.8 between 39 and 0 mN/m. The second double-layer state is equivalent to a monolayer of cholesteryl ester at the lipid-water interface, covered by a layer of cholesteryl ester molecules at 38.2 Å^2. Overall, our data show that the presence of lecithin at a lipid-water interface has a definite ordering effect on cholesteryl ester molecules at least 30-50 Å from the interface.

Models of lipoproteins and arterial lipid deposits normally depict all of the cholesteryl ester in a bulk lipid phase, surrounded by a monolayer of more polar lipids [e.g., Shen et al. (1977)]. Such models are based on known bulk properties of cholesteryl esters, in particular their insolubility in water and low solubility in lamellar phospholipid phases in the presence of excess water (Janiak et al., 1974). It is important biologically to know if cholesteryl esters are present in finite amounts in the surface phase surrounding the cores of lipoproteins or arterial lipid deposits and in what state(s) they exist. We have previously studied the properties of cholesteryl esters in mixtures with other lipids (colipids) at the air-water interface. A mixed monolayer phase was formed provided the colipid had fluid acyl chains and the cholesteryl esters contained 9-cis unsaturation in the acyl moiety. The stability of this phase depended upon its composition; as the mole fraction of cholesteryl ester approached 0.5, the collapse pressure of the monolayer approached 0 (Smaby et al., 1979). Such low collapse pressures were of interest because it has been reported that in pure form the cholesteryl esters employed exhibit small, but finite, collapse pressures (Kwong et al., 1971; Lundberg & Bergstrom, 1974; Cadenhead & Phillips, 1967). To clarify this apparent anomaly, we have studied the surface behavior of mixtures of cholesteryl myristoleate and dioleoyl-

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