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Binary Mixtures of Saturated and Unsaturated Mixed-Chain Phosphatidylcholines. A Differential Scanning Calorimetry Study[†]

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ABSTRACT: High-resolution differential scanning calorimetry (DSC) has been used to study the aqueous dispersions of mixed-chain phosphatidylcholines prepared from colyophilized mixtures of C(18):C(11:1Δ^{10})PC/C(18):C(10)PC and C(18):C(11:1Δ^{10})PC/C(18):C(11)PC of various molar ratios. These mixed-chain phospholipids are characterized by a marked disparity in their acyl-chain lengths; however, the *sn*-1 acyl chain in the fully extended conformation is about twice as long as the *sn*-2 acyl chain. Their thermotropic behavior was determined, and the phase diagrams of these two mixtures were constructed from the calorimetric data. Results indicate that C(18):C(11:1Δ^{10})PC/C(18):C(10)PC and C(18):C(11:1Δ^{10})PC/C(18):C(11)PC are miscible in all proportions with a near-ideal behavior of mixing in the gel and liquid-crystalline phases. Equimolar mixtures of diC(14)PC/C(18):C(11:1Δ^{10})PC, diC(14)PC/C(18):C(10)PC, and diC(14)PC/C(18):C(11)PC have also been studied by DSC. These phosphatidylcholines in the 1:1 mixture differ in T_m by less than 11 °C; however, they exhibit gel-phase immiscibility in the plane of the bilayer. Taken together, these studies suggest that C(18):C(11)PC and C(18):C(11:1Δ^{10})PC are packed similarly to C(18):C(10)PC in excess water as mixed interdigitated bilayers, at $T < T_m$, which transform into partially interdigitated bilayers when heated above T_m.

In recent years, high-sensitivity differential scanning calorimetry (DSC)[1] has been widely used to characterize the thermodynamic behavior of lipid bilayers composed of binary mixtures in excess water. Most of the studies, however, have focused on mixtures of one type of saturated symmetric diacylphosphatidylcholines such as diC(14)PC or diC(16)PC with other lipids. In general, the observed phase behavior of the binary lipid mixture is rather complex, since diC(14)PC or diC(16)PC bilayers exhibit multiple phases such as L_c, L_{β}', and P_{β}' at temperatures below the gel to liquid-crystalline phase transition. We have recently reported on the phase behavior of a number of highly asymmetric phosphatidylcholines in which one acyl chain is approximately twice as long as the other (Xu & Huang, 1987). Fully hydrated bilayers of these purely asymmetric PCs exhibit only a single highly cooperative phase transition over a wide temperature range, indicating that only a single type of gel phase exists below the phase transition temperature. On the basis of data obtained with X-ray diffraction and other techniques, this gel phase is designated the mixed interdigitated phase, in which the long acyl chains from opposing monolayers interpenetrate fully across the entire hydrocarbon width of the bilayer, while the short acyl chain of one lipid molecule packs end-to-end with the short acyl chain of another lipid molecule in the opposing bilayer leaflet [for a review, see Huang and Mason (1986)]. We have also studied the binary mixtures of C(10):C(22)PC/C(22):C(12)PC. These two lipid species are distinctively different in their acyl-chain lengths esterified at the *sn*-1 and *sn*-2 positions of the glycerol backbone; however, one of the fully extended acyl chains in each of the lipid molecules is almost twice as long as the other. A simple phase diagram has been constructed for C(10):C(22)PC/C(22):C(12)PC mixtures on the basis of DSC data. This cigar-shaped phase diagram together with ^{31}P NMR results indicates that C(10):C(22)PC and C(22):C(12)PC can mix nearly ideally in the bilayer in both the gel and liquid-crystalline phases (Xu et al., 1987). Such ideal mixing implies that the lipid–lipid lateral interactions for C(10):C(22)PC/C(22):C(12)PC mixtures in the plane of the membrane are virtually identical with those for the pure components. This phase behavior can thus be used to support the conjectures that mixtures of C(10):C(22)PC/C(22):C(12)PC can form a mixed interdigitated phase at $T < T_m$ and that the total bilayer thickness for the C(10):C(22)PC/C(22):C(12)PC binary system at various molar ratios is most likely invariant.

In this paper, we have extended our previous DSC studies on binary mixtures of highly asymmetric phosphatidylcholines to include a species of monounsaturated phosphatidylcholine. These phosphatidylcholines, C(18):C(11)PC, C(18):C(10)PC, and C(18):C(11:1Δ^{10})PC, are characterized by a marked disparity in their acyl-chain lengths. However, if a conformational bend at an α-methylene segment near the interface region is assumed to be present in the *sn*-2 acyl chain (Huang & Mason, 1986), the *sn*-1 acyl chain in the fully extended conformation is about twice as long as the zigzag *sn*-2 acyl chain for all these asymmetric PCs.

MATERIALS AND METHODS

1-Stearoyllysophosphatidylcholine and diC(14)PC, each with 99 mol % purity, were obtained from Avanti Polar Lipids, Inc. (Birmingham, AL). Capric and undecanoic anhydrides with purity greater than 99% were obtained from Nu Chek Prep, Inc. (Elysian, MN). C(18):C(10)PC and C(18):C-

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[1] Abbreviations: diC(X)PC, saturated symmetric diacylphosphatidylcholine having X carbons in each of the acyl chains; DSC, differential scanning calorimetry; PC, phosphatidylcholine; C(18):C(11:1Δ^{10})PC, 1-stearoyl-2-undecenoyl(Δ^{10})-*sn*-glycero-3-phosphocholine; C(X):C(Y)PC, saturated L-α-phosphatidylcholine having X carbons in the *sn*-1 acyl chain and Y carbons in the *sn*-2 acyl chain.

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(11)PC were prepared by acylation of 1-stearoyllysophosphatidylcholine with capric anhydride and undecanoic anhydride, respectively, at room temperature according to the modified semisynthetic method of Mason et al. (1981) as described elsewhere (Xu & Huang, 1987).

Synthesis of $C(18)$:$C(11$:$1\Delta^{10})PC$. 1-StearoyllysoPC (Avanti Polar Lipids, Inc.) was acylated at room temperature with a 10-fold molar excess of 10-undecenylic anhydride in hydrocarbon-stabilized chloroform under a nitrogen atmosphere. The reaction mixture was stirred magnetically for 6 h in the presence of 1.2 equiv of 4-pyrrolidinopyridine as a catalyst. The fatty acid anhydride was prepared from 10-undecenoic acid (Sigma) and dicyclohexylcarbodiimide in carbon tetrachloride (Selinger & Lapidot, 1966). The product, C(18):C(11:1Δ^{10})PC, was purified by flash chromatography on silica gel 60 by elution with chloroform, chloroform–methanol (9:1), and then chloroform–methanol (3:2). After suspended silica gel was removed by passing a chloroform solution of the product through a 0.45-μm Metricel filter, the product was further purified by preparative thin-layer chromatography followed by lyophilization with benzene, giving a white solid (62% yield). Elemental analysis (Desert Analytics, Tucson, AZ) Calcd for $C_{37}H_{72}O_8NP\cdot 1.5H_2O$ (716.97): C, 61.98; H, 10.54; N, 1.95. Found: C, 61.83; H, 10.67; N, 2.07. We have found that mixed-chain phosphatidylcholines synthesized by this acylation procedure are isomerically pure, with the extent of acyl migration less than 1% (Ali & Bittman, 1989).

Sample Preparation. Binary mixtures of C(18):C(11:1Δ^{10})PC/C(18):C(10)PC and C(18):C(11:1Δ^{10})PC/C(18):C(11)PC of known molar ratios were prepared from the corresponding colyophilized lipid mixtures and suspended in 50 mM NaCl aqueous solution containing 5 mM phosphate buffer and 1 mM EDTA at pH 7.4 to give a total lipid concentration in the range 2.6–4.1 mM. Aqueous dispersions of 1:1 mixtures of diC(14)PC/C(18):C(11:1Δ^{10})PC, diC(14)PC/C(18):C(10)PC, and diC(14)PC/C(18):C(11)PC were also prepared similarly from the corresponding colyophilized lipid mixtures. The exact lipid concentrations were determined by phosphorus analysis (Gomori, 1942). Detailed procedures for preparing binary mixtures have been given elsewhere (Xu et al., 1987). The binary lipid dispersion prepared from two different asymmetric PCs was first warmed to 35 °C, which is about 14 °C above the gel to liquid-crystalline phase transition temperature of the higher melting component of the lipid sample [C(18):C(11)PC], followed by brief sonication under a N_2 atmosphere using a bath-type sonicator (Branson, Model B-220, 100 W). The sample was then immediately cooled to 10 °C. The warming/sonication/cooling cycle was then repeated two more times. Finally, the dispersion was incubated at 0 °C for a minimum of 2 days to allow thermal equilibration of the lipid components in the gel phase prior to calorimetry. The equimolar mixtures of diC(14)PC with C(18):C(11:1Δ^{10})PC, C(18):C(10)PC, and C(18):C(11)PC were treated the same way, except that the sample was warmed to 40 °C, which is 16 °C above the T_m of diC(14)PC.

DSC. The thermograms of the samples were recorded on a high-resolution MC-2 differential scanning microcalorimeter equipped with the DA-2 digital interface and data acquisition utility for automatic data collection (Microcal Inc., Amherst, MA). The lipid dispersions were thermally equilibrated in the microcalorimeter at 0 °C for 1.5 h before heat was applied. Samples were heated at a constant rate of 15 °C/h in an ascending temperature mode, starting from a common temperature of 0 °C and ending at 35 or 40 °C. All DSC experiments were repeated at least once to ensure reproducible results. After cooling, the sample was allowed to equilibrate thermally in the calorimeter for 90 min at 0 °C prior to the second heating scan. The onset and completion temperatures of the transition were determined by the extrapolations of the peak slopes with the base line. Phase diagrams were constructed on the basis of the onset and completion temperatures of the thermograms recorded for a series of the binary mixtures, as previously described (Xu et al., 1987). The transition temperatures and transition enthalpies were also determined as described previously (Xu & Huang, 1987). The experimental errors in ΔH were introduced primarily by the uncertainty in lipid concentrations, which were the average value of three determinations for each lipid sample.

RESULTS

$C(18)$:$C(11$:$1\Delta^{10})PC/C(18)$:$C(10)PC$ Mixtures. The heating thermogram for C(18):C(11:1Δ^{10})PC dispersions, as illustrated in the bottommost curve of Figure 1A, exhibits a single endothermic transition with a transition peak width at half-height ($\Delta T_{1/2}$) of 0.50 ± 0.01 °C; this transition is peaked at a T_m of 13.3 ± 0.1 °C with a well-defined calorimetric enthalpy (ΔH = 6.1 ± 0.5 kcal/mol). The DSC scan of C(18):C(10)PC dispersions, shown as the uppermost curve in Figure 1A, is characterized by a sharper endothermic transition with $\Delta T_{1/2}$ = 0.23 ± 0.01 °C, ΔH = 9.0 ± 0.6 kcal/mol, and T_m = 18.6 ± 0.1 °C. This single transition has been reported previously; it corresponds to the gel to liquid-crystalline phase transition (Huang & Mason, 1986; Xu & Huang, 1987).

Samples of C(18):C(11:1Δ^{10})PC containing various molar ratios of C(18):C(10)PC, which have been thermally equilibrated for at least 48 h at 0 °C prior to DSC scans, exhibit single endothermic phase transitions (Figure 1A). Within experimental errors, the values of T_m appear to increase linearly with increasing C(18):C(10)PC concentration (Figure 2). In fact, the values of T_m for the mixtures can be approximated to the sum of the partial molar fraction of the corresponding parameters for the pure components; namely, $T_m(\text{mixture}) = T_m{}^1X^1 + T_m{}^2X^2$, where x stands for the mole fraction and the superscripts 1 and 2 stand for the pure components of the binary mixture. Also shown in Figure 2 is the plot of transition enthalpy (ΔH) versus the mole fraction of C(18):C(10)PC. Despite the large scatter of the data points, the values of ΔH for the binary mixtures are found to increase progressively with increasing mole fraction of C(18):C(10)PC; moreover, the experimental values are all within 11% of the computed values obtained by assuming an ideal sum of the partial molar enthalpies. Thus, the calorimetric data of C(18):C(11:1Δ^{10})PC/C(18):C(10)PC mixtures shown in Figure 2 appear to be consistent with a complete miscibility of the two component lipids over the entire composition range.

The temperature–composition phase diagram for C(18):C(11:1Δ^{10})PC/C(18):C(10)PC mixtures is shown in Figure 3A. The solidus and liquidus lines of this phase diagram are constructed on the basis of the onset and completion temperatures, respectively, of a series of transition curves (Figure 1) after correction for the finite width of the transition curves of the pure components (Mabrey & Sturtevant, 1976). It is evident that the solidus and liquidus lines do show smooth and continuous changes in the phase diagram; moreover, they do not display either a point of sharp inflection or a region of horizontal line. Also shown are the calculated solidus and liquidus lines (dotted curves) of the theoretical phase diagram expected for an ideal mixing of the lipid species in both the gel and liquid-crystalline phases using the procedure given by Mabrey and Sturtevant (1976). It can be seen that the experimental

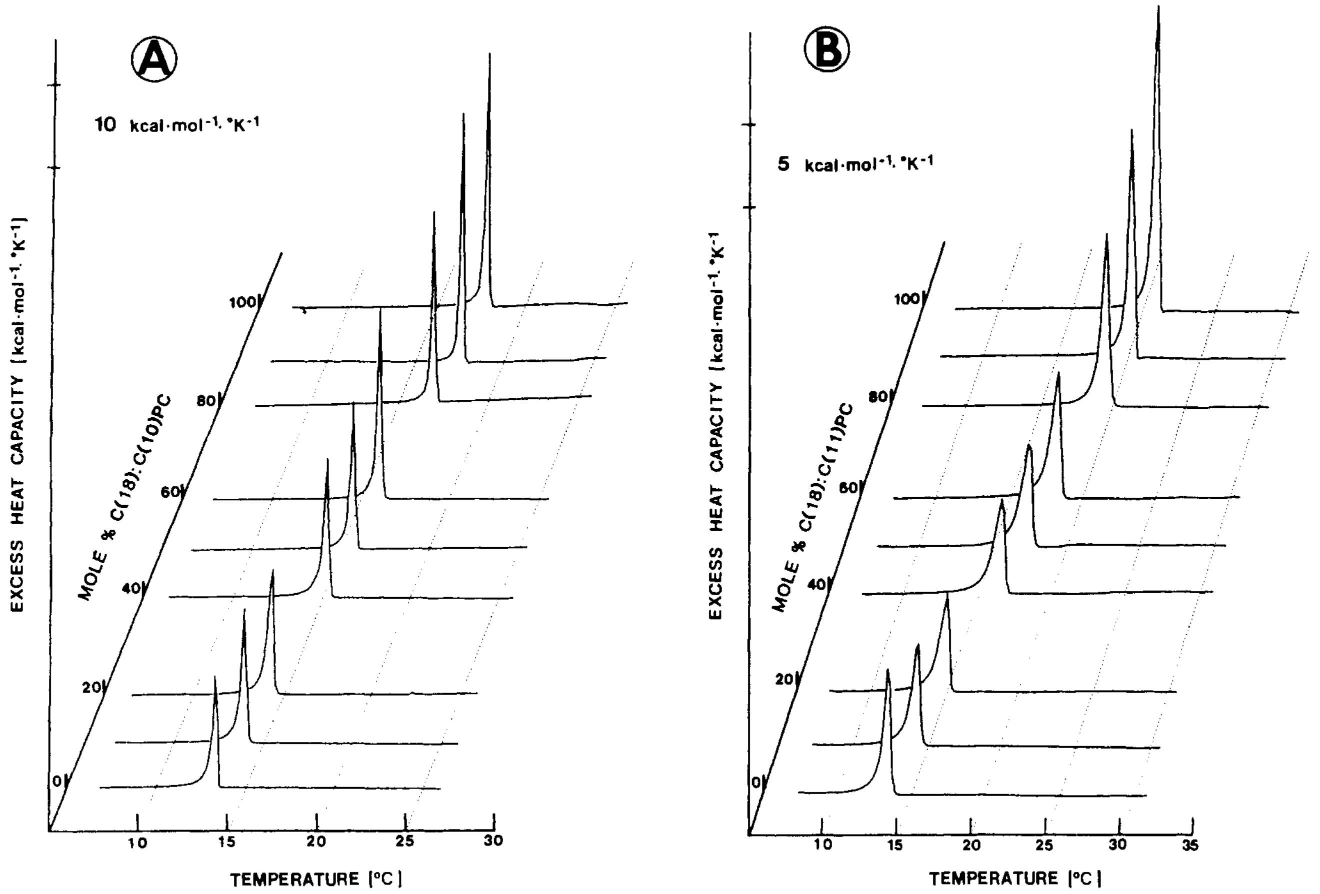

FIGURE 1: Representative DSC heating thermograms for fully hydrated samples of C(18):C(11:1Δ^{10})PC containing various concentrations of (A) C(18):C(10)PC and (B) C(18):C(11)PC. Samples of binary mixtures were preequilibrated at 0 °C for a minimum of 2 days prior to calorimetric scans. The curves are plotted relative to each other according to their mole percentages of C(18):C(10)PC and C(18):C(11)PC as indicated on the slanted axis in (A) and (B), respectively.

lines show slightly negative deviation from those theoretical lines. The cigar-shaped phase diagram for C(18):C(11:1Δ^{10})PC/C(18):C(10)PC shown in Figure 3A, therefore, reflects a complete miscibility of the component lipids with homogeneous, near-ideal mixing in both phases over the entire composition range.

C(18):C(11:1Δ^{10})PC/C(18):C(11)PC Mixtures. The uppermost DSC curve shown in Figure 1B illustrates a representative heating thermogram obtained with samples of pure C(18):C(11)PC. The values of T_m, $\Delta T_{1/2}$, and ΔH for C(18):C(11)PC are 21.4 °C, 0.4 °C, and 9.9 kcal/mol, respectively; these values agree well with previously reported ones (Xu & Huang, 1987). Figure 1B also shows heating thermograms for a series of C(18):C(11:1Δ^{10})PC/C(18):C(11)PC binary mixtures. The transition curves for the mixtures are slightly broadened in comparison with those of the pure component lipids. The largest values of $\Delta T_{1/2}$, 0.8 °C, is found for the sample of an equimolar mixture. The values of T_m for the binary mixtures are seen, within experimental errors, to increase linearly with increasing mole fraction of C(18):C(11)PC between the pure C(18):C(11:1Δ^{10})PC and C(18):C(11)PC values (Figure 2). The values of ΔH for the mixtures presented in Figure 2 show less than 22% deviations from the sum of the partial molar enthalpies of the pure components.

In Figure 3B, the experimentally determined phase diagram (solid lines), derived from the transition curves shown in Figure 1B, and the theoretically calculated phase diagram (dotted lines), obtained on the basis of an ideal mixing of the component lipids in both the gel and liquid-crystalline phases, are presented for C(18):C(11:1Δ^{10})PC/C(18):C(11)PC mixtures. It is evident that the experimental lines of the equilibrium phase diagram exhibit slightly negative deviation from the theoretical phase boundaries. These results thus indicate that C(18):C(11:1Δ^{10})PC and C(18):C(11)PC are miscible in the bilayer over the entire composition range and that the behavior of mixing is near ideal in both the gel and liquid-crystalline phases.

Equimolar Mixtures of DiC(14)PC with C(18):C(11:1Δ^{10})PC, C(18):C(10)PC, and C(18):C(11)PC. Figure 4 represents the temperature dependence of excess heat capacity of equimolar mixtures of diC(14)PC/C(18):C(11:1Δ^{10})PC, diC(14)PC/C(18):C(10)PC, and diC(14)PC/C(18):C(11)PC. In contrast to 1:1 mixtures of C(18):C(11:1Δ^{10})PC/C(18):C(10)PC and C(18):C(11:1Δ^{10})PC/C(18):C(11)PC that exhibit single, sharp phase transitions as shown in Figure 1, the calorimetric scans of equimolar mixtures of diC(14)PC with the highly asymmetric PCs are characterized by multiple well-defined peaks as shown in Figure 4. In one case, the thermotropic behavior of the equimolar mixture depends on the thermal history of the sample.

The initial heating thermogram for an equimolar mixture of diC(14)PC/C(18):C(11:1Δ^{10})PC, which has been thermally equilibrated at 0 °C for 5 days prior to calorimetry, is presented in Figure 4a. Two highly cooperative transitions and a broad overlapped endothermic transition are discernible in the temperature range 0–40 °C. The low-temperature endotherm is sharp and symmetric; it occurs over a narrow temperature range ($\Delta T_{1/2}$ = 0.75 °C) over which the maximal excess heat capacity is observed at a T_m of 10.8 °C. This transition corresponds closely to that recorded for dispersions of pure C(18):C(11:1Δ^{10})PC, one of the components of the

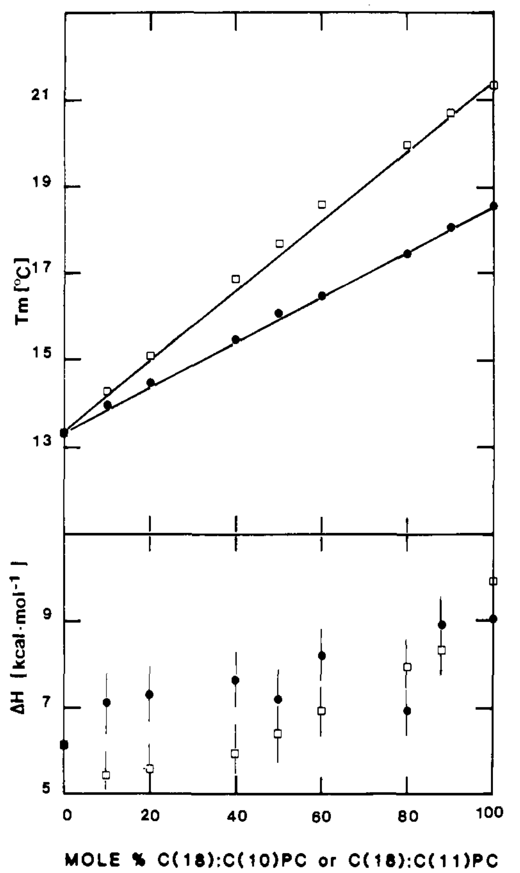

FIGURE 2: Plots of transition temperature (T_m) and transition enthalpy (ΔH) of the phase transition versus the mole fraction of C(18):C(10)PC (solid circles) or C(18):C(11)PC (open squares) in C(18):C(11:1Δ^{10})PC/C(18):C(10)PC or C(18):C(11:1Δ^{10})PC/C(18):C(10)PC binary mixtures.

Table I: Thermodynamic Parameters Associated with the Phase Transition of Highly Asymmetric Phosphatidylcholine Dispersions

phospholipid dispersions	T_m (°C)	$\Delta T_{1/2}$ (°C)	ΔH (kcal/mol)	ΔS (eu/mol)
C(18):C(11:1Δ^{10})PC	13.3[a]	0.50[a]	6.1[a]	21.3[a]
C(18):C(11)PC	21.4[a]	0.40[a]	9.9[a]	33.6[a]
	21.3[b]	0.34[b]	9.2[b]	31.3[b]
C(18):C(10)PC	18.6[a]	0.23[a]	9.0[a]	31.0[a]
	19.2[b]	0.63[b]	8.9[b]	30.3[b]

[a] Data from the present work. The experimental errors in T_m, $\Delta T_{1/2}$, and ΔH are within 1%, 5%, and 15%, respectively. The value of ΔS, the transition entropy, is estimated from the Clausius equality as $\Delta S = \Delta H/T_m$, assuming a first-order equilibrium transition. [b] Taken from Xu and Huang (1987).

binary mixture; however, the value of T_m has dropped 2.5 °C in comparison with the pure component (Table I). The second broad endotherm, seen near the middle of the temperature profile shown in Figure 4a, appears to be a superposition of several minor transitions. This overlapping feature peaked at ~17 °C resembles the $L_c \rightarrow P_{\beta}'$ phase transition recorded for pure diC(14)PC dispersions at 16.2 °C (Lewis et al., 1987). Also shown in Figure 4a is a third endothermic transition occurring at a higher temperature of 23.7 °C. This highly cooperative transition ($\Delta T_{1/2}$ = 0.58 °C) corresponds closely to the main $P_{\beta}' \rightarrow L_{\alpha}$ phase transition exhibited by dispersions of pure diC(14)PC (Lewis et al., 1987; Xu & Huang, 1987); the value of T_m is only slighly downshifted (0.2 °C) in comparison with that of the pure component.

The two well-resolved major peaks observed in Figure 4a can be readily interpreted as indicating the coexistence of separate gel domains of nearly pure lipid components in a 1:1 mixture of diC(14)PC/C(18):C(11:1Δ^{10})PC at temperatures below 10.8 °C and also that each of the calorimetrically observed sharp transitions represents the chain melting of phospholipids in separate gel domains composed primarily of one kind of lipid components in the plane of the bilayer. The calorimetric results thus demonstrate that diC(14)PC and C(18):C(11:1Δ^{10})PC at a 1:1 molar ratio are immiscible in the gel state, and in this respect the nonmixing behavior of diC(14)PC with C(18):C(11:1Δ^{10})PC is completely different from that of C(18):C(10)PC or C(18):C(11)PC with the same second component lipid. It should be noted that separated gel domains in the same bilayer have been detected recently by DSC for a 1:1 mixture of diC(14)PC/diC(20)PC (Melchior, 1986).

After the initial heating scan, the sample was cooled from 40 to 0 °C in the calorimeter. Prior to the second heating scan, the sample was allowed to equilibrate at 0 °C for 90 min in the calorimeter. Figure 4b illustrates the second heating DSC curve of the 1:1 mixture of diC(14)PC/C(18):C(11:1Δ^{10})PC that has displayed the initial heating DSC curve shown in Figure 4a. This thermogram is characterized by two sharp well-defined endotherms with maxima occurring at 10.9 and 23.7 °C, respectively. These two prominent endotherms correspond to the low- and high-temperature transitions detected in the initial heating scan. The absence of a broad overlapping transition centered at ~17 °C in the second heating scan suggests that this broad endotherm between the two major endotherms observed in the initial scan is irreversible under the experimental conditions.

In Figure 4c, two prominent endothermic transitions with maxima occurring at 16.9 and 23.5 °C are observed in the initial heating scan of an equimolar mixture of diC(14)PC/C(18):C(10)PC. This sample has been preincubated at 0 °C for 3 days. Lowering the temperature of the sample in the calorimeter to 0 °C followed by incubating at 0 °C for 90 min and then rescanning results in an identical thermogram. The thermal transition peaked at 16.9 °C is broad ($\Delta T_{1/2}$ = 1.1 °C), with a low-temperature shoulder at 13.5 °C. This shoulder persists in the second heating scan. Therefore, the broad thermal transition peaked at 16.9 °C is likely a composite, overlapping feature composed of a pretransition of lamellar diC(14)PCs and a gel to liquid-crystalline phase transition of lamellar C(18):C(10)PCs. It is noted that the observed T_m of 16.9 °C is only 1.7 °C lower than the T_m value observed for the gel to liquid-crystalline phase transition exhibited by dispersions of pure C(18):C(10)PC (Table I). The second prominent endotherm shown in Figure 4c is peaked at 23.5 °C with a $\Delta T_{1/2}$ of 0.8 °C. The T_m value is only 0.4 °C smaller than the typical value recorded for the $P_{\beta}' \rightarrow L_{\alpha}$ phase transition exhibited by dispersions of pure diC(14)PC (Lewis et al., 1987; Xu & Huang, 1987). The thermogram with distinct multiple transitions, shown in Figure 4c, can thus be taken as evidence to indicate that in the gel state of the 1:1 mixture of diC(14)PC/C(18):C(10)PC the component lipids are mostly immiscible in the plane of the bilayer and that most of the component lipids exist as separate phases at equilibrium in the gel state.

A representative heating thermogram for aqueous dispersions of an equimolar mixture of diC(14)PC/C(18):C(11)PC is presented in Figure 4d. This sample has been incubated at 0 °C for 3 days prior to DSC scan; however, repeated scans of the sample from 0 °C result in identical thermograms. Three endothermic transitions with maxima occurring at 13.4, 19.4, and 23.6 °C are observed in Figure 4d. The small endotherm at 13.4 °C appears to be the pretransition of diC-

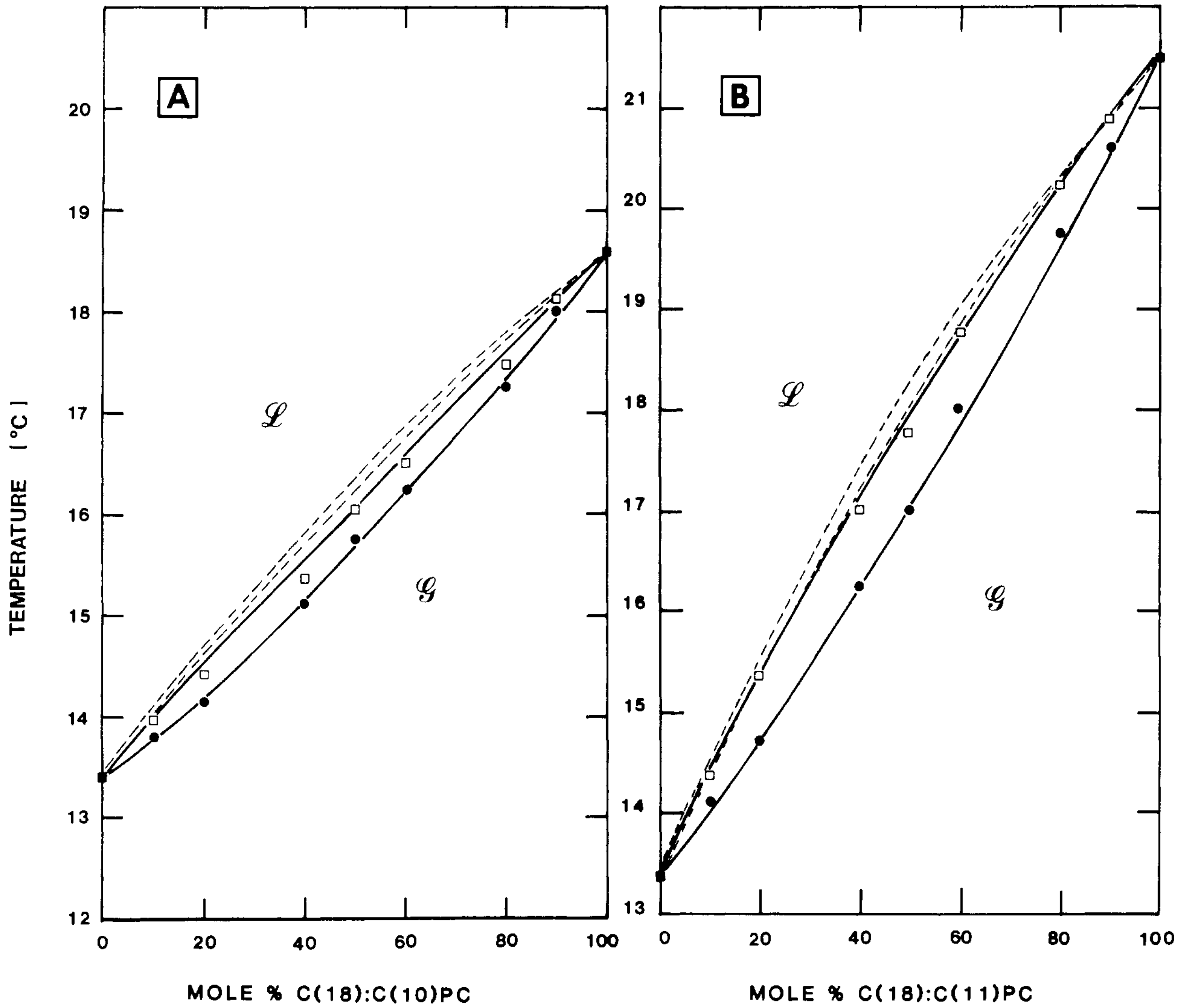

FIGURE 3: Phase diagrams for (A) the C(18):C(11:1Δ^{10})PC/C(18):C(10)PC system and (B) the C(18):C(11:1Δ^{10})PC/C(18):C(11)PC system. The phase boundaries (solid lines) were constructed from the calorimetric data by connecting the onset temperatures (solid circles) and completion temperatures (open circles) of the phase transitions. The dotted lines are the phase boundaries of the calculated phase diagrams expected for ideal mixing of the component lipids. G and L denote the gel and liquid-crystalline phases, respectively.

(14)PC. The two major endotherms at 19.4 and 23.6 °C correspond closely to the gel to liquid-crystalline phase transition of each of the two components of the mixture, although there is clearly a small downshift in T_m and a broadening of the transition curve in comparison with the endotherm of each of the pure components. This is presumably due to the melting of the C(18):C(11)PC [or diC(14)PC] enriched bilayers with relatively small amounts of diC(14)PC [or C(18):C(11)PC] incorporated in separated gel domains. An additional noteworthy observation is the base line, which shifts upward continuously. It is not clear what causes this deviation from a horizontal base line.

In any case, the distinct transition peaks shown in Figure 4d again suggest that in the gel state there is immiscibility between the two component phospholipids and that almost all phospholipids of each component are self-assembled into separate gel domains.

DISCUSSION

In this study, the thermotropic phase behavior of C(18):C(11:1Δ^{10})PC dispersions is reported for the first time. A single endothermic phase transition is detected calorimetrically for C(18):C(11:1Δ^{10})PC dispersions between 0 and 35 °C. The thermodynamic parameters (T_m, $\Delta T_{1/2}$, ΔH, and ΔS) associated with the phase transition are listed in Table I together with those of the corresponding saturated asymmetric PCs. It is most interesting to note that the incorporation of a double bond into the C(18):C(11)PC molecule between carbon atoms 10 and 11 in the *sn*-2 acyl chains lowers the value of T_m by only 8 °C. By contrast, the values of T_m for C(18):C(18:1Δ^9)PC and C(16):C(18:1Δ^9)PC lamellae are about 48 and 52 °C lower than the corresponding values for C(18):C(18)PC and C(16):C(18)PC lamellae, respectively (Keough, 1986; Xu & Huang, 1987). On the basis of calorimetric studies of a series of symmetric phosphatidylcholines with a cis double bond at different positions along each acyl chain, Barton and Gunstone (1975) have shown that the value of T_m for the unsaturated PC lamella is influenced by the location of the cis double bond. The value of T_m is minimal, that is, the shift relative to the corresponding saturated PC is maximal, when each cis double bond is located near the center of the acyl chain; in addition, the value of T_m increases progressively as each double bond moves toward either end of the fatty acyl chain (Barton & Gunstone, 1975). The observed 8 °C shift in T_m for C(18):C(11:1Δ^{10})PC relative to C(18):C(11)PC may, therefore, be attributed to the location of the double bond, which is placed at the chain end between carbon atoms 10 and 11 in the *sn*-2 acyl chain.

Since the molecular conformation and packing property of C(18):C(10)PC in the bilayer at temperatures above and below

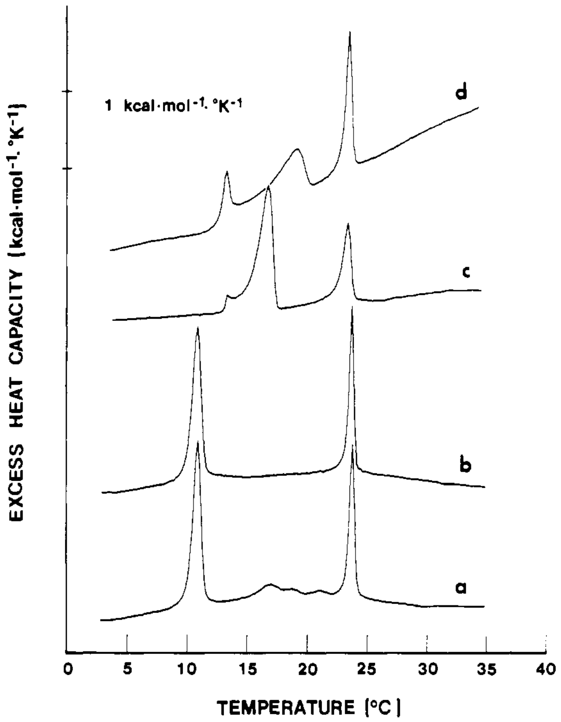

FIGURE 4: Excess heat capacity versus temperature for aqueous equimolar mixtures of diC(14)PC with C(18):C(11:1Δ^{10})PC (curves a and b), C(18):C(10)PC (curve c), and C(18):C(11)PC (curve d). Curve b is the second DSC heating curve following the initial heating scan (curve a). The preincubation times at 0 °C for samples prior to the initial heating run are (a) 5, (c) 3, and (d) 3 days.

T_m are well characterized (McIntosh et al., 1984; Hui et al., 1984; Mattai et al., 1987), our DSC results with C(18):C(11:1Δ^{10})PC/C(18):C(10)PC binary mixtures can be used to postulate the molecular packing of C(18):C(11:1Δ^{10})PC in bilayers. At $T < T_m$, C(18):C(10)PC molecules with acyl chains in a nearly all-trans conformation are packed in a mixed interdigitated bilayer in the presence of excess water; this packing mode is characterized by having the area per phospholipid molecule at the lipid/water interface encompass three acyl chains. At $T > T_m$, C(18):C(10)PC molecules in the bilayer are known to pack into a partially interdigitated mode. In this state, the short chain of one lipid in one leaflet packs end-to-end with the long chain of another lipid in the opposing leaflet of the bilayer. Furthermore, the acyl chains are no longer in a nearly all-trans conformation, but they contain several gauche rotamers. In the present work, C(18):C(11:1Δ^{10})PC in the bilayer is shown to be homogeneously miscible with C(18):C(10)PC in both the gel and liquid-crystalline phases. In order to achieve a complete miscibility of both lipid species (say, A and B) with near-ideal mixing in the gel and liquid-crystalline phases, the lipid–lipid lateral interactions between the two component lipids (E_{AB}) must be similar to those between the pure components (E_{AA} and E_{BB}) at temperatures both above and below the T_m. These similar lipid–lipid lateral interactions can, in turn, be interpreted to imply that these two kinds of lipid molecules are most likely to be very similar in their packing properties in bilayers. Our DSC results (Figure 2) and the equilibrium phase diagram (Figure 3A) can thus be taken to suggest that C(18):C(11:1Δ^{10})PC molecules, in excess water, also adopt a mixed-interdigitated packing mode at $T < T_m$ and a partially interdigitated packing mode at $T > T_m$ in the bilayer.

Our DSC studies also indicate that C(18):C(11:1Δ^{10})PC and C(18):C(11)PC are completely miscible in all proportions in both the gel and liquid-crystalline phases, and the mixing behavior is near ideal. This near-ideal behavior of complete miscibility is undoubtedly due to the similar lipid–lipid lateral interactions between the two components in comparison with those between pure components. Again, we can attribute these similar lipid–lipid lateral interactions to packing similarities of these two lipid species in the bilayer at temperatures above and below T_m. Although we know of no direct experimental data that describe the packing properties of C(18):C(11)PC in the bilayer, two lines of evidence strongly suggest that C(18):C(11)PC molecules adopt the mixed and partially interdigitated modes at temperatures below and above T_m, respectively. (1) Previous X-ray diffraction studies indicate that both C(18):C(10)PC and C(18):C(12)PC molecules are packed nearly identically in the gel and liquid-crystalline states (Hui et al., 1984). In line with the data obtained with C(18):C(10)PC and C(18):C(12)PC, the C(18):C(11)PC molecules are most likely to pack into the mixed-interdigitated and partially interdigitated modes at temperatures below and above T_m, respectively. (2) Experimental data from a series of comparative DSC studies on interdigitated and noninterdigitated bilayer systems show that the thermodynamic parameters associated with the main phase transition are distinctively different for the two systems. Thermodynamic parameters obtained with C(18):C(11)PC lamellae are consistent with the packing model whereby the C(18):C(11)PC molecules are self-assembled into a mixed-interdigitated bilayer at $T < T_m$ (Xu & Huang, 1987). On the basis of these two lines of indirect evidence, lamellar C(18):C(11)PC can be reasonably assumed to have packing properties similar to C(18):C(10)PC in the bilayer; hence, a complete miscibility between C(18):C(11:1Δ^{10})PC and C(18):C(11)PC with near-ideal mixing behavior in both phases can be taken as evidence to argue in favor that C(18):C(11:1Δ^{10})PC molecules adopt a mixed-interdigitated packing mode in the bilayer at $T < T_m$ and a partially interdigitated packing mode at $T > T_m$.

The excess heat capacity profiles for equimolar mixtures of diC(14)PC with C(18):C(11:1Δ^{10})PC, C(18):C(10)PC, and C(18):C(11)PC, shown in Figure 4, can be used to further substantiate the foregoing suggestion that C(18):C(11:1Δ^{10})PC molecules are most likely packed into a mixed-interdigitated bilayer at $T < T_m$. Before discussing the significance of the thermograms, it should be noted that Curatolo et al. (1985) concluded, on the basis of calorimetric data obtained with 21 pairs of binary PC mixtures, that binary mixtures of PCs exhibit gel-state miscibility if the difference in the transition temperature between the component PCs (ΔT_m) is less than 33 °C. DiC(14)PC and diC(16)PC, for instance, differ in T_m by 17.5 °C; they mix nearly ideally in the bilayer in both the gel and liquid-crystalline states (Mabrey & Sturtevant, 1976). When the value of ΔT_m is greater than 33 °C, binary mixtures of PCs exhibit gel-state immiscibility (Curatolo et al., 1985). The value of ΔT_m for diC(14)PC and diC(20)PC, for instance, is 41 °C, and the 1:1 mixture of diC(14)PC/diC(20)PC exhibits calorimetrically two major endotherms that correspond to the melting of separate gel domains of each lipid species (Melchior, 1986). The values of ΔT_m between the two component PCs in diC(14)PC/C(18):C(11:1Δ^{10})PC, diC(14)-PC/C(18):C(10)PC, and diC(14)PC/C(18):C(11)PC mixtures are 10.6, 5.3, and 2.5 °C, respectively. These mixtures would be expected to exhibit gel-state miscibility on the basis of ΔT_m according to the general conclusion of Curatolo et al. (1985). The thermograms shown in Figure 4, however, demonstrate convincingly that diC(14)PC molecules are not completely miscible in the gel state with C(18):C(11:1Δ^{10})PC, C(18):C(10)PC, and C(18):C(11)PC, respectively, in the 1:1 mixtures. An interesting question therefore arises as to why the three binary mixtures shown in Figure 4 exist as two separate phases at equilibrium in the gel state.

It is well-known that diC(14)PC molecules are self-assembled into noninterdigitated lamellae in excess water. In contrast, C(18):C(10)PC or C(18):C(12)PC molecules in the presence of excess water are packed into a mixed-interdigitated bilayer at $T < T_m$. Because of their different packing modes, the bilayer thickness for noninterdigitated diC(14)PC lamellae at $T < T_m$ is substantially greater than that for mixed-interdigitated C(18):C(10)PC lamellae. At 10 °C, for instance, the bilayer thickness for diC(14)PC lamellae is 43 Å (Janiak et al., 1976), whereas a bilayer thickness of 33 Å is known for C(18):C(10)PC lamellae (Hui et al., 1984). This 10-Å difference in the bilayer thickness contributes to a considerably larger area of van der Waals contact surface between diC(14)PC molecules in the plane of the bilayer. The lipid–lipid lateral interactions between diC(14)PC molecules at 10 °C must, therefore, be much stronger than those between C(18):C(10)PC molecules. Consequently, for diC(14)PC/C(18):C(10)PC mixtures the equilibrium state at $T < T_m$ is expected to be the one in which there are separate gel phases in the same plane of the bilayer. This expectation is indeed borne out by the thermogram shown in Figure 4c. The present study thus suggests that in order to have a gel-state miscibility for binary PC mixtures, a ΔT_m values of less than 33 °C is not sufficient. It is necessary that the lipid components must also have similar packing modes in the bilayer.

The observed gel-state immiscibility of diC(14)PC with C(18):C(11)PC and C(18):C(11:1Δ^{10})PC can be argued similarly as due to the relatively stronger attractive forces between diC(14)PC molecules in the gel-state bilayer. The strong lipid–lipid lateral interactions between diC(14)PCs in the plane of the gel-state bilayer have become possible because C(18):C(11)PC and C(18):C(11:1Δ^{10})PC, similar to C(18):C(10)PC, are most likely packed in a totally different mode in the mixtures. We believe that these highly asymmetric PCs are all packed in a mixed-interdigitated bilayer, at $T < T_m$, in the presence of excess water. However, we should emphasize that X-ray diffraction or other spectroscopic studies are ultimately required to prove definitively the packing models of C(18):C(11)PC and C(18):C(11:1Δ^{10})PC bilayers as suggested by the present work.

In summary, results of DSC experiments on binary lipid mixtures can provide information about the lipid–lipid lateral interactions. The present study reveals that C(18):C(11:1Δ^{10})PC/C(18):C(10)PC and C(18):C(11:1Δ^{10})PC/C(18):C(11)PC mixtures are miscible in all proportions, with a near-ideal behavior of mixing in the gel and liquid-crystalline phases. This behavior is taken to reflect that the incorporation of a double bond at the end of the *sn*-2 acyl chain does not exert a pronounced disruptive effect on the overall lipid–lipid lateral interactions for highly asymmetric PCs in the bilayer. Consequently, the overall packing mode of the highly asymmetric phosphatidylcholine molecule with a terminal double bond in the binary mixtures used in Figure 1 can be viewed to resemble closely that of the bilayer composed of single, saturated lipid component. Thus, the C(18):C(11:1Δ^{10})PC molecules, in either the pure one-component system or the two-component system with C(18):C(10)PC or C(18):C(11)PC, are most likely to assemble at $T < T_m$ into a mixed-interdigitated bilayer that, when heated above the T_m, converts to a partially interdigitated phase. We have also demonstrated for the first time that equimolar mixtures of diC(14)PC/C(18):C(11:1Δ^{10})PC, diC(14)PC/C(18):C(10)PC, and diC(14)PC/C(18):C(11)PC exhibit gel-state immiscibility in the plane of the bilayer. This is extremely interesting, since the component PCs in each of the three binary mixtures differ in T_m by less than 11 °C. This immiscibility is attributed to the very strong attractive forces between diC(14)PC molecules in the plane of the bilayer at $T < T_m$. The strong lipid–lipid lateral interactions between diC(14)PC molecules in the 1:1 mixtures reflect that the packing of the highly asymmetric PCs differs markedly from that of diC(14)PCs in the gel-state bilayer. On the basis of the known packing mode of C(18):C(10)PC in the bilayer at $T < T_m$ and the similar nonmixing behavior of C(18):C(10)PC, C(18):C(11:1Δ^{10})PC, and C(18):C(11)PC with diC(14)PC at $T < T_m$, we suggest that C(18):C(11:1Δ^{10})PC and C(18):C(11)PC in the equimolar mixtures with diC(14)PC are assembled at $T < T_m$ into separate gel domains in which these highly asymmetric PCs are most likely to adopt a mixed-interdigitated packing mode. At $T > T_m$, however, these binary mixtures are expected to be miscible due to their similar packing properties. Future structural studies are required to verify the packing models proposed by this work.

Registry No. C(18):C(11:1Δ^{10})PC, 117439-50-6; C(18):C(10)PC, 78119-50-3; C(18):C(11)PC, 106268-88-6; diC(14)PC, 18194-24-6; 1-stearoyllysoPC, 5655-17-4; 10-undecenylic anhydride, 17393-29-2; 10-undecenoic acid, 112-38-9.

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