Biochimica et Biophysica Acta, 946 (1988) 178–184
Elsevier

BBA 70585

BBA Report

Eutectic phase behavior of 1-stearoyl-2-caprylphosphatidylcholine and dimyristoylphosphatidylcholine mixtures *

Hai-nan Lin and Ching-hsien Huang

Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, VA 22908 (U.S.A.)

(Received 25 July 1988)

Key words: Phospholipid mixture; Phase behavior; Eutectic system; Stearoylcaprylphosphatidylcholine; Dimyristoylphosphatidylcholine; Differential scanning calorimetry

The thermotropic behavior of aqueous dispersions of C(18):C(10)PC/diC(14)PC mixtures with different molar ratios has been investigated by high-resolution differential scanning calorimetry. C(18):C(10)PC is a highly asymmetric lipid molecule, whereas diC(14)PC is a symmetric species with the same molecular weight. Their packing properties in the bilayer are known to be similar at $T > T_m$, but very dissimilar at $T < T_m$. Calorimetric results indicate that C(18):C(10)PC and diC(14)PC are completely miscible in the liquid-crystalline state. In the gel state, however, C(18):C(10)PC and diC(14)PC are only partially miscible. The temperature-composition phase diagram for C(18):C(10)PC/diC(14)PC mixtures has the shape characteristic of a typical eutectic system.

Phospholipids are well known to be a major class of lipid components in biological membranes. In fact, phospholipids isolated from most biological membranes are typical of the mixed acyl chain variety, meaning that the two acyl chains attached to carbons 1 and 2 of the glycerol backbone are different. Detailed information concerning the mixing behavior of various mixed-chain phospholipids in the two-dimensional spatial regions of membranes is scanty, although it is expected that mixtures of two phospholipids with very similar structure can exhibit isomorphous behavior of mixing in the same plane of the bilayer [1]. An example of the isomorphous system involving a binary mixture of highly asymmetric mixed-chain phospholipids is the C(10):C(22)PC/C(22):C(12)PC mixture. A simple temperature-composition phase diagram of cigar-shape is observed for this binary mixture, indicating that the two component lipids with very similar structure are indeed completely miscible in both the gel and liquid-crystalline phases over the entire composition range [2].

In this communication, we report the mixing behavior of C(18):C(10)PC with diC(14)PC, using high-resolution differential scanning calorimetry (DSC). C(18):C(10)PC is a highly asymmetric phospholipid molecule with the *sn*-1 acyl chain in the fully extended conformation twice as long as the zig-zag *sn*-2 acyl chain. DiC(14)PC, however,

* It is with gratitude and admiration that we dedicate this paper to Lee Shih-Ching on the occasion of his 80th birthday.

Abbreviations: C(18):C(10)PC, 1-stearoyl-2-caprylphosphatidylcholine; 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; diC(14)PC, dimyristoylphosphatidylcholine; DSC, differential scanning calorimetry; T_m, the main phase transition temperature; $\Delta T_{1/2}$, transition peak width at half-height.

Correspondence: C. Huang, Department of Biochemistry, Box 440, University of Virginia School of Medicine, Charlottesville, VA 22908, U.S.A.

is a nearly symmetric phospholipid molecule. These two phospholipid species have a common structural feature; namely, they have identical numbers of total methylene units in their two acyl chains. In this study, we have examined the phase equilibrium of C(18):C(10)PC/diC(14)PC mixtures with different compositions at various temperatures but at constant ambient pressure. Our calorimetric data demonstrate that C(18):C(10)PC and diC(14)PC are only partially miscible in the gel state and are completely miscible in the liquid-crystalline state. The temperature-composition phase diagram for C(18):C(10)PC/diC(14)PC mixtures is thus typical of an eutectic system.

Isomerically pure (> 98 mol%) C(18):C(10)PC was first prepared by acylation of 1-stearoyllysophosphatidylcholine with capric anhydride at room temperature, and then purified by silicic acid column chromatography as described elsewhere [3,4]. DiC(14)PC with purity greater than 99 mol% was obtained from Avanti Polar Lipids, Inc. (Birmingham, AL).

The high-resolution DSC studies were performed on a Microcal MC-2 microcalorimeter equipped with the DA-2 digital interface and data acquisition utility for automatic collection (Microcal Inc., Amhurst, MA). A constant heating scan rate of 15 C°/h was generally used.

In order to ascertain that the two lipids under study were mixed in the same liposome, dispersions of equimolar mixtures of colyophilized C(18):C(10)PC and diC(14)PC were prepared using different methods, and the DSC scans from these samples were then used to compare with the heating thermogram obtained with the mixture of pure C(18):C(10)PC and diC(14)PC dispersions at 1:1 molar ratio. The 1:1 mixture of pure C(18):C(10)PC and diC(14)PC dispersion was prepared as described elsewhere [2]. Briefly, pure C(18):C(10)PC and diC(14)PC were dispersed separately in NaCl (50 mM) aqueous solution containing 5 mM phosphate buffer and 1 mM EDTA (pH 7.4) to give each lipid dispersion a concentration of 3 mM. Equal volumes of the pure lipid dispersion were mixed and then subjected to the heating/cooling cycle between 40 and 10°C three times, followed by incubating in the calorimeter at 0°C for 2 days prior to the DSC heating

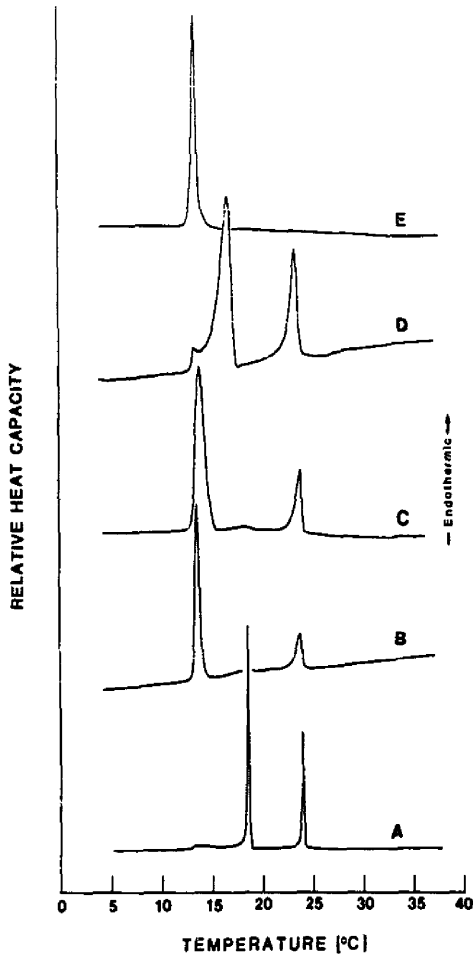

Fig. 1. DSC heating thermograms of samples prepared from (A) pure C(18):C(10)PC and diC(14)PC dispersions at 1:1 mixture. (B–E) equimolar aqueous mixture of colyophilized C(18):C(10)PC and diC(14)PC using four different methods discussed in the text.

run. The resulting heating thermogram of the mixture is shown in Fig. 1A. The thermogram is characterized by two sharp endothermic transitions peaked at 18.6°C ($\Delta T_{1/2} = 0.33$ C°) and 24.0°C ($\Delta T_{1/2} = 0.20$ C°). These transition temperatures correspond well with those recorded for pure component lipids (vide post). Also, a minor broad peak is observed at 14.2°C in Fig. 1A; it corresponds to the pretransition of the diC(14)PC dispersion. This thermogram presented in Fig. 1A

is used as a control showing that the two lipids in the 1:1 mixture are not mixed in the same liposome.

The equimolar mixtures of colyophilized C(18):C(10)PC and diC(14)PC were prepared by four different methods. Initially, equal amounts of dried samples of C(18):C(10)PC and diC(14)PC were weighed out individually. They were mixed and dissolved in benzene followed by colyophilization. After colyophilization, the mixture was 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 of 4.6–6.8 mM. The resulting suspension was then treated differently [1]. In the first method, the suspension was vortexed vigorously at 0°C for three minutes, and then stored at 0°C for two days followed by DSC scan. A typical DSC scan of an equimolar mixture of colyophilized C(18):C(10)PC/diC(14)PC prepared according to the first method is shown in Fig. 1B. This thermogram is characterized by two prominent endothermic transitions occurring at 13.5°C ($\Delta T_{1/2}$ = 0.65 C°) and 23.7°C ($\Delta T_{1/2}$ = 0.70 C°), respectively, and a minor broad transition at 17.8°C. Repeated scans of the same sample gave virtually identical heating thermograms, except that the minor transition at 17.8°C was not observable. This irreversible broad peak at 17.8°C appears to correspond to the $L_c \rightarrow P_{\beta'}$ transition for diC(14)PC dispersions [5]. (2) In the second method, the lipid suspension was heated to 40°C, and the sample was vortexed for 1 min at the elevated temperature. The sample was cooled immediately to 0°C and maintained at 0°C for 3 min. Then, the heating/vortexing/cooling cycle was repeated twice. Prior to DSC experiments, the sample was incubated at 0°C for two days. A DSC scan of the 1:1 mixture of benzene-colyophilized C(18):C(10)PC/diC(14)PC which had been subjected to repeated heating/vortexing/cooling cycles is shown in Fig. 1C. The thermogram exhibits two prominent thermal transitions at 13.8°C ($\Delta T_{1/2}$ = 1.2 C°) and 23.8°C ($\Delta T_{1/2}$ = 0.75 C°), and a broad transition at 17.8°C. Clearly, this DSC curve is similar to that of Fig. 1B, except that the transition peaks are more broadened. (3) The third method is a modification of the second one just described. The lipid sample was subjected to brief sonication, instead of vortexing, at 40°C, using a bath-type sonicator (Branson, model B-220, 100 watts). An aqueous dispersion of 1:1 mixture of benzene-colyophilized C(18):C(10)PC/diC(14)PC which had been subjected to heating/sonication/cooling cycles exhibits calorimetrically two prominent endotherms with maxima occurring at 16.9 and 23.5°C (Fig. 1D). The thermal transition at 16.9°C shown in Fig. 1D is rather 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 and appears to correspond to the pretransition of diC(14)PC lamellae. Comparing Figs. 1A–1D, it is evident that a good mixing of C(18):C(10)PC with diC(14)PC in liposomes depends on the experimental method employed for preparation of the binary mixture. Judging from the value of $\Delta T_{1/2}$, it appears that the third method invoiving brief intermittent sonications of the colyophilized mixture gave a better mixing between the two lipid components in the liposome. (4) Finally, we chose to add an additional step of chemical mixing in the procedure. In this fourth method, dried powders of preweighed C(18):C(10)PC and diC(14)PC were dissolved in $CHCl_3$. After thoroughly vortexing, the solvent $CHCl_3$ was removed by a stream of N_2 gas. The dried film of C(18):C(10)PC/diC(14)PC was suspended in benzene and then colyophilized. The lyophilized mixture was suspended in buffered NaCl solution, and the suspension was subjected to the heating/sonication/cooling cycle three times. The temperature dependence of excess heat capacity of an equimolar mixture of C(18):C(10)PC/diC(14)PC prepared from this method is shown in Fig. 1E. This thermogram is remarkably different from those shown in Figs. 1 A–1D. The single sharp endotherm ($\Delta T_{1/2}$ = 0.5 C°) peaked at 13.4°C as shown in Fig. 1E can thus be taken as strong evidence to suggest that the two component lipids in the C(18):C(10)PC/diC(14)PC binary mixture prepared according to the fourth method have mixed well in the same liposome. Based on the DSC results shown in Fig. 1, we were convinced to adopt the fourth method as a general method to prepare binary mixtures of C(18):C(10)PC/diC(14)PC with different compositions in all subsequent experiments.

Fig. 2 shows some representative DSC scans for

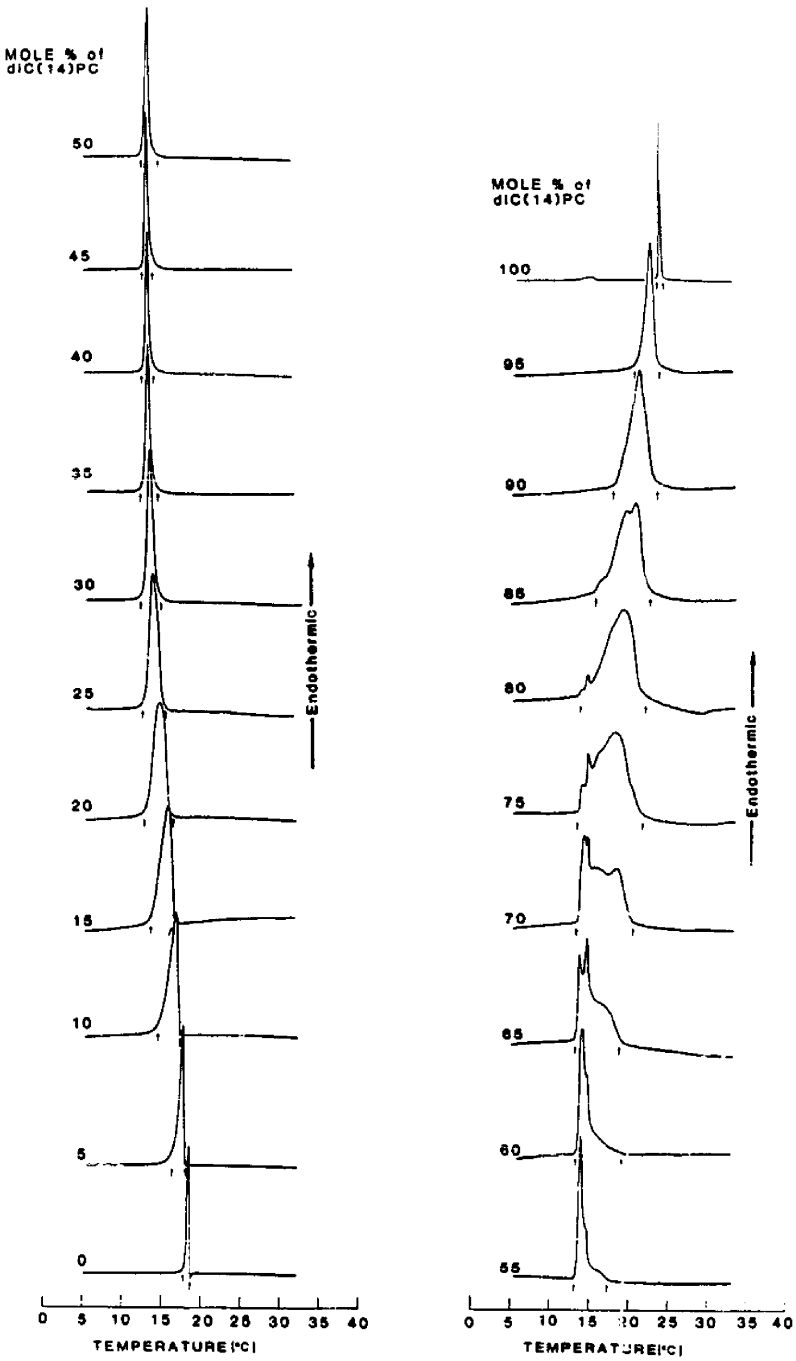

Fig. 2. DSC hating thermograms for samples of C(18) : C(10)PC containing various contents of diC(14)PC.

a series of C(18):C(10)PC/diC(14)PC, the DSC scans of the mixtures are characterized by a highly cooperative endotherm. The transition temperature decreases continually from 18.6°C to 13.3°C as the relative content of diC(14)PC in the mixture increases from 0 to 40%. It is important to note that the single transition curve shown in Fig. 2 is gradually broadened by the incorporation of 5–20% of diC(14)PC; however, the transition curve is progressively narrowed upon further increasing the diC(14)PC content up to about 45%. In fact, the phase transition curve of the 40% mixture is almost as sharp as the one obtained with pure C(18):C(10)PC dispersion (Fig. 2). Above 50% of diC(14)PC, all transition curves shown in Fig. 2 exhibit complex patterns, indicating that the melting of lipid acyl chains in the mixtures with higher content of diC(14)PC proceeds in a complicated pathway.

The onset and completion temperatures of the various transition curves as indicated by the arrows in Fig. 2 have been used to construct the solidus and liquidus, respectively, of the temperature-composition phase diagram for C(18):C(10)PC/diC(14)PC mixtures, after correcting for the finite widths of the transitions of pure C(18):C(10)PC and diC(14)PC species by using the procedure of Mabrey and Sturtevant [6]. The resulting phase diagram, shown in Fig. 3, has a shape which is the hallmark of a eutectic system [7]. In Fig. 3, the solidus, defined by the corrected onset temperatures, is observed to decrease continually with increasing diC(14)PC content up to 20%; it becomes horizontal over the composition range of 25–75% of diC(14)PC, and then increases continually over the remaining range of 80–100% of diC(14)PC. The liquidus is observed to decrease continually with increasing content of diC(14)PC up to 40%. At 40%, the corrected completion temperature reaches a minimum of 13.4°C; this is the eutectic point. As the relative content of diC(14)PC is increased further, the liquidus in-

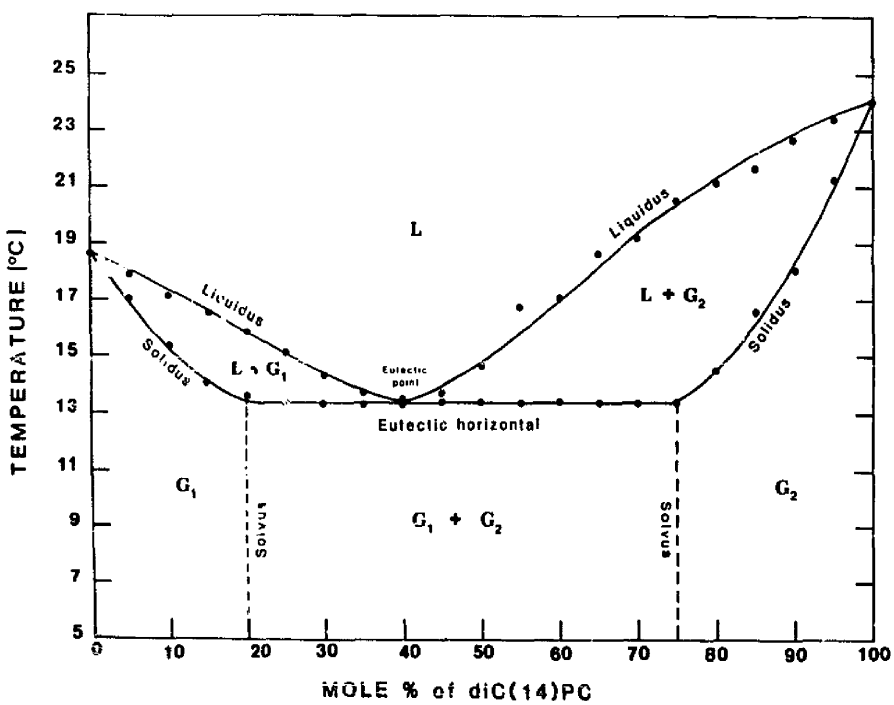

Fig. 3. Phase diagram for C(18):C(10)PC/diC(14)PC system. L, liquid-crystalline phase; G_1, C(18):C(10)PC-enriched gel phase; G_2, diC(14)PC-enriched gel phase.

creases continually up to 100% of diC(14)PC.

According to the theory of phase diagrams [7], the mixing behavior of the binary mixture of C(18) : C(10)PC/diC(14)PC can be delineated based on Fig. 3. In this system, there are three one-phase regions (the miscible liquid-crystalline phase of the C(18) : C(10)PC/diC(14)PC mixture (L), the C(18) : C(10)PC-enriched gel phase (G_1), and the diC(14)PC-enriched gel phase (G_2)), three two phase regions (($G_1 + G_2$), ($L + G_1$), and ($L + G_2$)), and one degenerated three-phase region, the eutectic point. These regions are designated in Fig. 3. For instance, below the eutectic horizontal and surrounded by the dotted solvus lines, the two gel phases G_1 and G_2 are immiscible but co-existent.

Since the bilayer structure of pure C(18) : C(10)PC and diC(14)PC lamellae at temperatures both above and below T_m are known [8–10], one can ask whether the eutectic reaction

$$L \underset{\text{heating}}{\overset{\text{cooling}}{\rightleftharpoons}} G_1 + G_2$$

underlying the observed phase diagram of C(18) : C(10)PC/diC(14)PC mixtures is compatible with the known structural information. At temperatures below T_m, C(18) : C(10)PC molecules in excess water are known to self-assemble into mixed interdigitated lamellae [8,9]. In contrast, diC(14)PC molecules are packed into non-interdigitated gel lamellae [10]. Because of their different packing modes at $T < T_m$, the bilayer thickness of diC(14)PC lamellae, for instance, is about 30.3% larger than that of C(18) : C(10)PC lamellae at 10 °C [8]. Consequently, the van der Waals' contact surface between diC(14)PC molecules in the plane of the bilayer is considerably bigger than that between C(18) : C(10)PC molecules, resulting in stronger lipid–lipid interactions among pure diC(14)PC molecules. These stronger lipid–lipid interactions will promote the lateral phase separation of diC(14)PCs to form non-interdigitated gel-domains in the bilayer. If the C(18) : C(10)PC-enriched phase (G_1) and the diC(14)PC-enriched phase (G_2) are assumed structurally to be similar to the pure gel phases of C(18) : C(10)PC and diC(14)PC, respectively, then the two component lipids are expected to assemble into separate interdigitated G_1 and non-interdigitated G_2 gel-domains in the same plane of the bilayer at $T < T_m$ due to their dissimilarity in packings. This expectation is indeed born out by the phase diagram in which a two-phase ($G_1 + G_2$) region is shown to be present at temperatures below the eutectic horizontal. At temperature above T_m, C(18) : C(10)PC and diC(14)PC molecules are packed into liquid-crystalline state bilayers with nearly identical bilayer thickness [8]. Because of the similar packing properties, C(18) : C(10)PC and diC(14)PC molecules are expected to mix homogeneously over the entire composition range at $T > T_m$. This is indeed one of the mixing characteristics exhibited by the eutectic phase diagram shown in Fig. 3.

In summary, results of DSC experiments on binary mixtures of C(18) : C(10)PC/diC(14)PC indicate that these two lipids are completely miscible in the liquid-crystalline phase and mostly immiscible in the gel state. A eutectic point is detected at 13.4 °C and 40% of diC(14)PC. The observed eutectic behavior is indeed expected based on the known packing similarity between the two component lipids at $T > T_m$ and the known packing dissimilarity at $T < T_m$. To our best knowledge, this is the first example of a eutectic system observed for binary mixtures of diacyl phosphoglycerides in which the mixing behavior of a binary system derived from DSC data can be correlated directly with the packing and structural properties of the component lipids obtained from X-ray diffraction studies.

This work was supported by U.S. Public Health Service Grant GM-17452 from National Institutes of Health, Department of Health and Human Services. We thank Professor T.E. Thompson for stimulating discussions.

References

1 Cevc, G. and Marsh, D. (1987) in Phospholipid Bilayers: Physical Principles and Models, p. 377, John Wiley and Sons, New York.
2 Xu, H., Stephenson, F.A. and Huang, C. (1987) Biochemistry 26, 5148–5453.
3 Mason, J.T., Broccoli, A.V. and Huang, C. (1981) Anal. Biochem. 113, 96–101.
4 Xu, H. and Huang, C. (1987) Biochemistry 26, 1036–1043.

5 Lewis, R.N.A.H., Mak, N. and McElhaney, R.N. (1987) Biochemistry 26, 6118–6126.
6 Mabrey, S. and Sturtevant, J.M. (1976) Proc. Natl. Acad. Sci. USA 73, 3862–3866.
7 Yeh, H.C. (1970) in Phase Diagrams: Materials Science and Technology (Alper, A.M., ed.), Vol. 1, pp. 167–197, Academic Press, New York.
8 Hui, S.W., Mason, J.T. and Huang, C. (1984) Biochemistry 23, 5570–5577.
9 McIntosh, J.T., Simon, S.A., Ellington, J.C. and Porter, N.A. (1984) Biochemistry 23, 4038–4044.
10 Janiak, M.J., Small, D.M. and Shipley, G.G. (1976) Biochemistry 15, 4575–4580.