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STRUCTURE OF 1-ACYL LYSOPHOSPHATIDYLCHOLINE AND FATTY ACID COMPLEX IN BILAYERS

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Summary

The phase transition characteristics of bilayers formed in a codispersion of 1-acyl lysophosphatidylcholine and a fatty acid depend on the chain length of both the components and on the pH of the aqueous medium. Incorporation of cholesterol as a third component abolishes the transition. It is suggested that acyl chain interactions between fatty acid and 1-acyl lysophosphatidylcholine molecules in their aqueous codispersions are maximized by close-packing such that the acyl chains of both molecules are aligned parallel to each other and the carboxyl group is located in the vicinity of the 2-hydroxyl group of lysophosphatidylcholine. The shape and size of a functional dimer thus formed are similar but not identical to those of 1,2-diacyl phosphatidylcholine. Several predictions arising from this suggestion, including phase separation in codispersions of fatty acid + 1-acyl lysophosphatidylcholine + diacyl phosphatidylcholine, are experimentally confirmed.

Aqueous dispersions of equimolar mixtures of 1-acyl lysophosphatidylcholine and long-chain fatty acids from bilayers [1]. The phase transition characteristics of these bilayer dispersions depend upon the acyl chain length in both components. This suggests that the phase transition characteristics of such a system depend upon acyl chain interactions. In order to understand better such chain-chain interactions, we have studied the thermotropic phase transition properties of codispersions of 1-acyl lysophosphatidylcholine with various fatty acids in the aqueous phase. The results suggest that the acyl chains of

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lysophosphatidylcholine and fatty acid associate to form a dimer, and that the shape of the dimer is similar but not identical to the shape of diacyl phosphatidylcholine.

Materials and Methods

All compounds used in this study were analytical grade or greater than 99% pure.

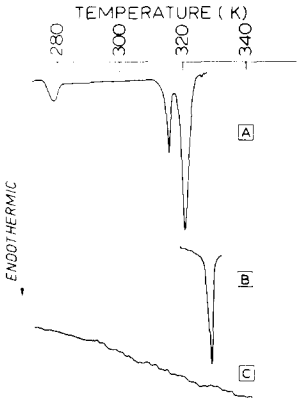
1-Acyl lysophosphatidylcholines were prepared by the action of phospholipase A_2 (*Crotalus adamanteus* venom) on appropriate diacyl phosphatidylcholines in moist diethyl ether. The codispersions were prepared by drying $CHCl_3/CH_3OH$ (3 : 2, v/v) solutions, and the dry lipid film was dispersed in 100 mM KCl, 200 mM Tris-HCl (pH 7.5) unless stated otherwise. All dispersions were mixed at 60—80°C for 60—90 s, and then incubated for 2—3 h at 50°C. For differential scanning calorimetry, 15 μl of dispersion containing 1—2 μmol lipids were transferred into aluminium pans. The sealed pans were scanned in the 260—340 K range on a Perkin-Elmer DSC-2 instrument at 5 K/min at a sensitivity of 1 mcal.

Results and Discussion

Aqueous dispersions of 1-acyl lysophosphatidylcholines form micelles that exhibit a broad endothermic transition; this occurs at 278 K for the 1-palmitoyl analog [2]. When fatty acids are codispersed with lysophosphatidylcholine a twin transition appears at higher temperature [1]. As shown in Fig. 1 and discussed below, the shape and position of these transitions depend upon the nature of the two components in codispersions. These transitions are endothermic, exhibit a high cooperativity, show little hysteresis in transition temperature and total enthalpy when one compares a cooling with a heating scan, and the transition disappears when cholesterol is incorporated as a third component in the codispersions (Fig. 1c). Thus, dispersions of 1-palmitoyl lysophosphatidylcholine with varying mole fractions of palmitic acid exhibit a twin transition at 316 and 322 K [1]. The enthalpic contribution of the twin transition depends upon the mole fraction of palmitic acid in the mixture (Fig. 2).

The transition due to 1-palmitoyl lysophosphatidylcholine is not seen when the mole fraction of palmitic acid exceeds about 0.3, however, the enthalpy of the twin transition at 316 and 322 K increases at least until the mole fractions of palmitic acid is 0.5. The position of either of these peaks does not change over the whole concentration range, however, a higher mole fraction of palmitic acid in codispersions significantly broadens the twin transition. These observations suggest that the twin transition is due to aggregated structures formed by an interaction of the fatty acid with the lysophosphatidylcholine.

As shown elsewhere [1] the equimolar codispersions of palmitic acid and 1-palmitoyl lysophosphatidylcholine are in a lamellar bilayer vesicle form, and this form is also seen when the proportions of fatty acid to lysophosphatidylcholine is 1 : 2 (Fig. 3). The existence of the bilayer form in codispersions, in contrast to micellar dispersions formed by either of the components under the same conditions, raises interesting questions about the state of the two molec-



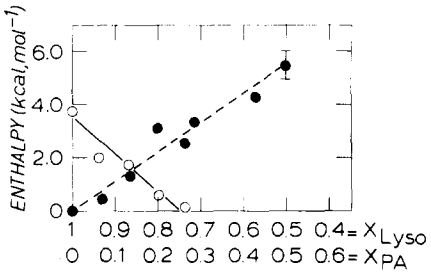


Fig. 1. Phase transition profiles of aqueous codispersions of 1-palmitoyl lysophosphatidylcholine with: (A) 10 mol% palmitic acid; (B) 50 mol% *n*-hexadecan-1-ol; and (C) 33 mol% palmitic acid and 33 mol% cholesterol.

Fig. 2. Enthalpy of transition at 278 K (○) and at 316 + 322 K (●) for codispersions of 1-palmitoyl lysophosphatidylcholine with varying mole fractions of palmitic acid (X_{PA}).

ular species in the codispersion. The phase transition temperature (T_m) for the equimolar palmitic acid and 1-palmitoyl lyosphosphatidylcholine dispersions is very close to the main gel-to-liquid crystalline endothermic phase transition temperature for dipalmitoyl phosphatidylcholine. However, the enthalpy of transition of the diacyl phosphatidylcholine is somewhat higher than that of the codispersion containing lysophosphatidylcholine + fatty acid [1]. These

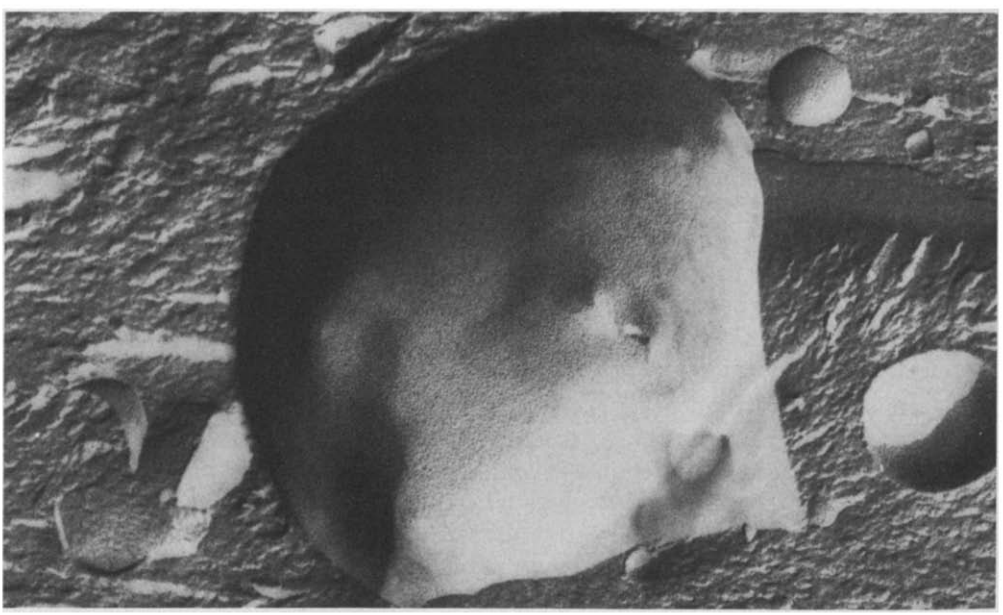


Fig. 3. Electron micrograph of the freeze-fracture replica of codispersions of 1-palmitoyl lysophosphatidylcholine with palmitic acid at a 2 : 1 mole ratio. Magnification, ×77 000.

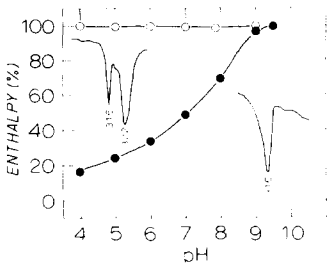


Fig. 4. Relative enthalpies of transition at various pH values for equimolar codispersions of 1-palmitoyl lysophosphatidylcholine with *n*-hexadecanol (○———○, T_m at 330 K) and with palmitic acid (●———●, for the peak at 316 K). The transition profiles in the inset are for the mixture containing palmitic acid codispersed at pH 4.0 (left) and pH 9.0 (right).

observations imply that the acyl chain interactions in these two systems may be similar. The twin transition exhibited by a codispersion suggests the existence of two different species that undergo transition. Since palmitoyl lysophosphatidylcholine mixed with 1-hexadecanol exhibits only a single transition at 330 K (Fig. 1b), it appears that the twin transition is a characteristic of dispersions containing fatty acids. As shown in Fig. 4, the relative enthalpic contribution of the two peaks in a twin transition is pH dependent, and the apparent pK_a of the group responsible for the change is 7.5. Therefore, the peak at lower temperature could arise from ionized fatty acid and the higher melting peak in a twin transition could be due to un-ionized acid. This is consistent with the observation that the 330 K peak for *n*-hexadecanol containing codispersions does not change in area as a function of pH from 5 to 9 (Fig. 4). While this suggests a contribution of the fatty acid head group to the transition characteristics of codispersions, it is impossible to ascertain at this stage whether domains or patches containing ionized and un-ionized fatty acid coexist in the same bilayer, or if the structures containing ionized fatty acid are produced during the phase transition. However, the two forms of fatty acids giving rise to two different populations of bilayer-enclosed structures are unlikely to exist. The width of the pH profile (Fig. 4) is also considerably greater than what one would expect from a simple dissociation of a fatty acid. An explanation for such observations must await a better understanding of acid-base equilibria in interface regions of a bilayer. However, for the following discussion that focuses on the role of acyl chains in determining the phase transition characteristics, we have compared only the temperatures at the midpoint of the first transition (T_m), while the enthalpy values refer to the whole twin transition for the heating scan.

An insight into the nature of interactions between 1-acyl lysophosphatidylcholine and fatty acid molecules in the bilayer of equimolar codispersions can be obtained by comparing T_m values when the acyl chain length is varied. For such comparisons, it is assumed that an increased overlap or interaction between acyl chains is manifested in a higher T_m and enthalpy of transition. The values presented in Table I show that for dispersions containing the same acyl chain in both components, the T_m values are almost equal to those for the corresponding symmetrical phosphatidylcholines. However, T_m values for the

TABLE I

TRANSITION TEMPERATURES (T_m) OF AQUEOUS DISPERSIONS OF DIACYL PHOSPHATIDYLCHOLINES AND OF THE CODISPERSIONS OF 1-ACYL LYSOPHOSPHATIDYLCHOLINE + FATTY ACID

Position		T_m (K)	
1	2	Phosphatidylcholine	Lysophosphatidylcholine + fatty acid (1 : 1) (first peak)
18 : 0	18 : 0	328	328
16 : 0	16 : 0	315	316
14 : 0	14 : 0	296	296
16	14	300 *	308
14	16	308 *	298
18	16	317 *	323
16	18	322 *	317

* Data from Ref. 3.

pairs of mixed-chain phosphatidylcholines are significantly different from those of the corresponding mixtures of components with different acyl chains. Curiously enough, T_m values for such asymmetrical systems exhibit opposite patterns in codispersions and in phosphatidylcholine bilayers. Thus, 1-long, 2-short phosphatidylcholine has a lower T_m value compared to 1-short,2-long phosphatidylcholine [3]. However, codispersions of short-1-acyl lysophosphatidylcholine and long fatty acid show a lower T_m value compared to codispersions of a long-1-acyl lysophosphatidylcholine and a short fatty acid. This suggests the non-equivalence of acyl chains in positions 1 and 2 in both systems. However, an opposite dependence on chain lengths is observed in the corresponding diacyl phosphatidylcholine [3]. As discussed below such differences can be rationalized if one postulates that the 1-acyl lysophosphatidylcholine molecule to form a dimer in the bilayer.

The molecular arrangement that explains best the behaviour of fatty acid and 1-acyl lysophosphatidylcholine is compared with diacyl phosphatidylcholine in Fig. 5. From gross thermodynamic considerations, it is assumed that the acyl chains of fatty acid and 1-acyl lysophosphatidylcholine are aligned parallel to each other, and that their polar groups are oriented towards the aqueous phase. The conformation of the glycerophosphorylcholine moiety in 1-acyl lysophosphatidylcholine is assumed to be the same as that for diacyl phosphatidylcholines [4—6]. Thus, the fatty acyl chain is aligned parallel to the 1-acyl chain of lysophosphatidylcholine. In an all-*trans* conformation these two acyl chains can be as close-packed as they presumably are in the gel phase of diacyl phosphatidylcholine bilayers. While this accounts for a coincidence of T_m values for the two bilayer systems under disscusion, some subtle yet important differences are revealed by an examination of the molecular models. For example, the relative positions of the methylene residues on the neighbouring chains are not the same, and the overall size and relative positioning of polar groups are not identical in the two systems. Due to addition of a hydroxy group, the size and polarity of the $-COO^- \cdot HO-C2$(glycerol) moiety is increased, and therefore the relative positions of the methylene residues in the two acyl

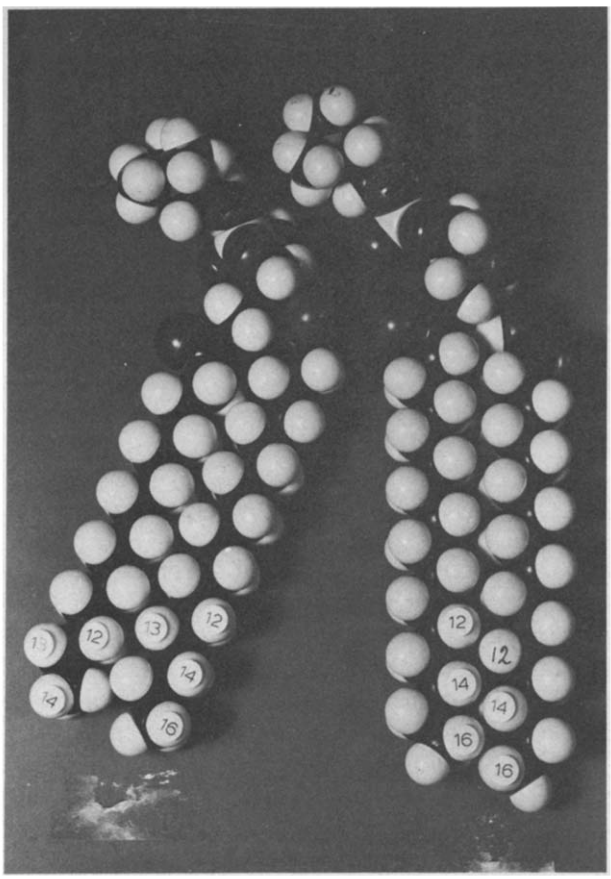


Fig. 5. Molecular models of 1-myristoyl-2-palmitoyl phosphatidylcholine (left, chain 1 and 2), and close packed 1-palmitoyl lysophosphatidylcholine with palmitic acid (right, chain 1 and 2).

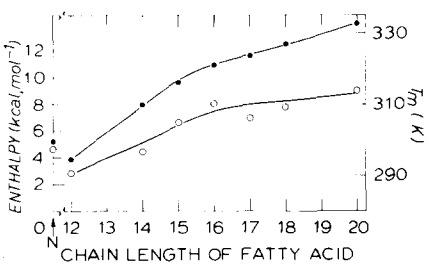
chains in codispersions are shifted away from the polar region compared to the relative positions of methylene residues in the two chains of a diacyl phosphatidylcholine molecule. Thus, in diacyl phosphatidylcholines, the C14 of the 1-acyl chain is close to C15-C16 of the 2-acyl chain in the same molecule. However, in the dimer the C14 of 1-acyl lysophosphatidylcholine is closer to C13-C14 of the fatty acid chain. Such an alignment of the two chains predicts several interesting properties of a bilayer formed from the dimers, and as discused below these are consistent with experimental observations.

(a) The transition temperatures of diacyl phosphatidylcholine bilayers and the corresponding codispersions of fatty acid and 1-acyl lysophosphatidylcholine are very close to each other when the two acyl chains are identical. An examination of their models suggests that in both cases the same number of methylene residues interact with each other. For example, only 14 or 15 residues overlap in dipalmitoyl phosphatidylcholine. In close-packed 1-palmitoyl lysophosphatidylcholine and palmitic acid in un-ionized form, 16 residues overlap and in the ionized form 14 or 15 residues overlap. This difference is accentuated in phosphatidylcholines and the corresponding close-packed

dimers when the two chains are not identical. Thus, in a 1-palmitoyl lysophosphatidylcholine and myristic acid mixture, 14 methylene residues interact, and in a 1-myristoyl lysophosphatidylcholine and palmitic acid mixture only about 12 residues overlap. The situation in the corresponding diacyl phosphatidylcholine [3] is quite the opposite where 1-palmitoyl-2-myristoyl phosphatidylcholine has an overlap of 12 residues, and in 1-myristoyl-2-palmitoyl phosphatidylcholine 14 residues interact. Such considerations could therefore account for opposite patterns of T_m values for 1-short,2-long and 1-long,2-short pairs in the two types of bilayer.

(b) The ionized fatty acids give a lower transition temperature for codispersions. This implies a loss of acyl chain interaction on ionization of fatty acid. Such a loss may be achieved by a shift of the fatty acid chain towards the aqueous phase due to a change in the polarity of the carboxl group, and/or by lateral repulsion of carboxylate from phosphate ions, by maximization of the interaction of the carboxylate ion with the ammonium group of choline, or simply by accommodation of negative surface charge density at the interface. Apparently, overlap of one methylene residue is effectively lost by ionization which corresponds to a decrease of about 6 K in T_m on ionization. This is based on the observation that an increase in the chain length by two residues changes T_m by 12—15 K. Similarly, codispersions containing 1-palmitoyl lysophosphatidylcholine and *n*-hexadecanol show a transition at 330 K which is about 8 K higher than the transition for codispersions containing un-ionized fatty acid. This is consistent with the suggestion that in the acyl chain of the alcohol, an extra methylene group interacts with the acyl chain of lysophosphatidylcholine.

(c) The extent of acyl chain interactions manifested in T_m and in the enthalpy of transition also depends upon the relative size of the two chains in codispersions. As shown in Fig. 6, both of these quantities increase monotonically with the chain length of a fatty acid codispersed with 1-stearyl phosphatidylcholine. However, on a per methylene residue basis, an increase in chain length beyond C16 does not contribute as much as the methylene residues up to C16. This would imply that the stearoyl chain of 1-acyl lysophosphatidylcholine



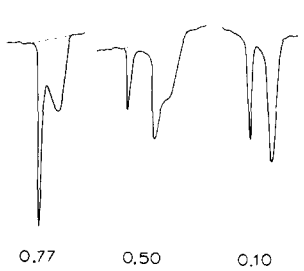


Fig. 6. Dependence of transition temperature (T_m) (•) and enthalpy of transition (○) of 1-stearoyl lysophosphatidylcholine codispersed without and with fatty acids of various chain lengths in equimolar proportions.

Fig. 7. Phase transition profiles of codispersions of dipalmitoyl phosphatidylcholine containing varying mole fractions of 1-palmitoyl lysophosphatidylcholine + palmitic acid (1 : 1). The values shown are mole fractions (X_{DPPC}) of dipalmitoyl phosphatidylcholine.

interacts more effectively on a per methylene residues basis with up to 16 methylene residues of a chain than with the subsequent methylene residues of a larger chain. Also, lauric acid codispersed with 1-stearoyl lysophosphatidylcholine does not significantly change T_m. This suggests that the last four methylene residues of the stearoyl chain are left without a methylene chain in their vicinity which can increase its state of disorder.

(d) The acyl chains in diacyl phosphatidylcholine bilayers in the gel phase are tilted at an angle of about 30° to the plane of the bilayer [7]. Such a tilt would not be expected in binary codispersions where acyl chains could be essentially perpendicular to the plane of the bilayer, since an increased size of the polar group in the vicinity of C2 glycerol aligns the chains essentially perpendicular to the glycerol-COOH (fatty acid) axis. This is consistent with the phosphatidylcholine codispersed with dipalmitoyl phosphatidylcholine exhibiting a phase separation as shown in Fig. 7. This would be expected if tilted acyl chains of dipalmitoyl phosphatidylcholine cannot be close-packed with the perpendicular chains of lysophosphatidylcholine and fatty acid bilayers, even when all the acyl chains have the same number of methylene residues. Interestingly, codispersions of dipalmitoyl phosphatidylcholine with either palmitic acid [8,9] or with 1-palmitoyl lysophpsphatidylcholine [10,11] do not exhibit any phase separation. The composition of the phases giving rise to the various transitions in the ternary system of Fig. 7 is under investigation. It should, however, be noted that the diacyl phosphatidylcholine bilayers containing more than 10 mol% fatty acids [12—14] or lysophosphatidylcholine [10] do not exhibit ideal miscibility, and the ionization of fatty acids in a bilayer is a complex function of both the pH of the aqueous phase and of the composition of the bilayer [11].

To recapitulate, considerations of polarity and molecular geometry suggest that 1-acyl phosphatidylcholine can close-pack with fatty acids to form a conformationally and orientationally restricted analog of phosphatidylcholine. Such a concept has been useful in understanding the behaviour of diacyl phospholipid and cholesterol mixtures [15]. The properties of fatty acid + lysophosphatidylcholine codispersions suggest that the dimeric analog is similar to diacyl phosphatidylcholine in the sense that it forms bilayer-enclosed structures when dispersed in water, and that the two acyl chains are non-equivalent. However, the packing characteristics of the dimer analog in a bilayer are sufficiently different that a phase separation is observed in dispersions of mixtures palmitic acid. Such similarities and differences can be rationalized from examination of molecular models in which the acyl chains of the close-packed dimer are aligned perpendicular to the bilayer plane, whereas the chains in diacyl phosphatidylcholine bilayers are tilted. It can hardly be overemphasized that the creation of phase boundaries in diacyl phosphatidylcholine bilayers by the products of hydrolysis by phospholipase A_2 could have an as yet unappreciated regulatory role of the enzyme in processes ranging from prostaglandin synthesis to incorporation and modulation of membrane proteins.

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