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A THERMODYNAMIC AND NMR INVESTIGATION OF 1-LYSO-PALMITOYLLECITHIN / 1,2-DIPALMITOYLPHOSPHATIDYLETHANOL-AMINE / WATER SYSTEM

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Abstract The effect of incorporation of 1-palmitoyl-sn-glycero-3-phosphocholine (PLPC) on the bilayer structures occurring in aqueous dispersions of 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) has been studied by phosphorus 31 nuclear magnetic resonance and calorimetric methods. The polymorphism of the system as a function of PLPC concentration has been defined. Experimental data allowed us to determine phase boundaries. Changes of molecular packing of the PLPC molecules in DPPE bilayers in the phase diagram are invoked to explain the experimental findings.

INTRODUCTION

Lysophospholipids, especially lysophosphatidylcholines, are known to occur as minor constituents of various cell membranes[1]. In particular lysophosphatidylcholine is a widely distributed surface-active and cytolytic phospholipid[2-4], which induces morphological changes in cells[5], facilitates cell fusion[6], causes hemolysis[7,8], affects the permeability properties of phosphatidylcholines liposomes[4,9] and enhances immune reactions[10].

Despite various efforts[2-4,11], the mechanism of lysolecithin action on natural and model membranes is poorly understood. The wedge shape of the lysophosphatidylcholine molecule is probably a factor resulting in the above biological properties. Intercalation of lysolecithin into planar bilayers consisting of approximately

cylindrically shaped phospholipid molecules, should induce curvature into the bilayer and cause changes in molecular packing and phase properties.

Several studies have been made of aqueous solutions of lysophospholipid-phospholipid systems. X-ray and light scattering studies[9] indicate that diacylphosphatidylcholines, containing up to 40-50 molar % of the lyso-compound, are still arranged in a lamellar phase. Klopfenstein et al.[12] have shown that lysolecithin hardly affects the phase transition temperature of dipalmitoyllecithin even at a molar ratio higher than 1:1 and suggested that lysophosphatidylcholine mixes homogeneously with 16:0/16:0 phosphatidylcholine. Van Echteld et al.[13] have demonstrated that up to 30 molar % of 1-palmitoyllysophosphatidylcholine, PLPC, can be incorporated into bilayers of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, DPPC.

Purified lysophospholipids dispersed in H_2O do not adopt a bilayer configuration but organize into micellar or cylindrical structures.[14] Lysophosphatidylcholine / phosphatidylethanolamine systems have also received some attention.[15-18]

In order to further explore the role of lysolecithin in membranes, we have examined more closely the effect of lysolecithin on the structure of bilayer membranes formed by phosphatidylethanolamines which have a strong tendency to form non lamellar mesophases even in excess of water.[14-16] We have studied 1-palmitoyl-sn-glycero-3-phosphocholine/1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine, PLPC / DPPE, mixtures in water, using ^{31}P nuclear magnetic resonance, differential scanning calorimetry, isothermal compressibility and scanning dilatometry. In this paper we have determined the phase boundaries in the phase diagram. The results suggest that the interaction of PLPC with DPPE dramatically affects its polymorphism and cause significant structural changes and lamellar disruption.

EXPERIMENTAL METHODS

DPPE and PLPC were obtained from Sigma Chemicals (Milan, Italy). Their purity was checked by TLC. Samples were prepared by dispersing the appropriate volumes of the dry lipids dissolved in chloroform/methanol (95/5 [v/v]) into glass tubes. Solvent was evaporated in vacuo and the residue dried under high vacuum for 24 h. The necessary amount of water to give a final concentration of 34 weight % was mixed with the lipids. The tube was flame sealed and the contents mixed by centrifugation back and forth at a

temperature slightly above 70°C, until homogeneity had been achieved. This process usually took about one day.

The samples were kept another day at the same temperature before recording NMR spectra. A second series of NMR spectra was recorded four weeks later. ^{31}P-NMR spectra were recorded on a MSL 300 Bruker spectrometer with a temperature control accuracy of ± 0.5°C. A Hahn echo sequence with a $\pi/2$ pulse width of 2 μs was used with proton decoupling. The delay between the $\pi/2$ pulse and the π pulse in the Hahn echo sequence was 40 μs and the repetition rate, 3 s. Spectra were recorded only after 30 minutes after a temperature change in order to allow samples to reach thermal equilibrium.

Scanning dilatometric measurements were performed with a Mettler TC10A processor equipped with a TMA40 thermomechanical analyzer. The scanning dilatometer is described in detail elsewhere.[19] Its sensitivity and reproducibility are $3.2x10^{-10}$ and 0.2 %, respectively. The temperature precision and stability were ± 0.2°C and ± 0.01°C. Each scan was recorded over the range 25 - 80°C, at a rate of 0.5°C/min.

Compressibility measurements were performed by using a force measuring probe ranging from 0.00 to 1.53 N. Since the area of the piston was 3.801 mm^2, the pressure exerted on the sample varies from 1 to 4 atmospheres.[20]

Calorimetric measurements were carried out with a micro differential scanning calorimeter (SETARAM, France). The sampling rate was 1 point/s and cells were scanned from 25 to 80°C. The precision and stability of the apparatus at a scanning rate of 0.5°C/min were ± 0.08°C and ± 0.01°C, respectively. The average noise level was about ± 0.4 mW and the reproducibility with refilling was about 10^{-7} $J°C^{-1}$.

RESULTS

^{31}P-NMR Measurements

Typical ^{31}P-NMR spectra, recorded at various temperatures, of the DPPE/PLPC/^2H_2O system, for different concentrations of PLPC, are shown in Figure 1. Samples containing up to 20 weight % of PLPC give spectra (not shown) with a line shape characteristic of a lamellar phase for the whole temperature range examined. The chemical shift anisotropy decreases at the main transition (around 62°C) from the gel phase to the

liquid crystalline state. For the sample containing 25 weight % PLPC (Figure 1a) the spectrum recorded at 35°C indicated the presence of a lamellar gel phase. Successive spectra are the superposition of two powder patterns: one arising from a lamellar phase consisting primarily of DPPE molecules, and a second from a cylindrically symmetric phase consisting predominantly of PLPC molecules.

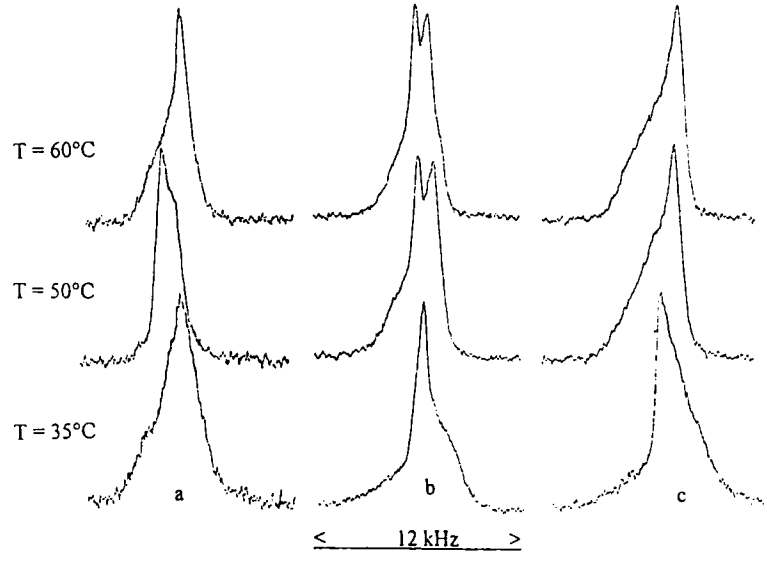

FIGURE 1 Typical ^{31}P-NMR spectra of DPPE/PLPC/$^{2}H_2O$ system recorded at 121.26 MHz as a function of temperature for different weight % of PLPC: (a) 25%; (b) 31%; (c) 33%.

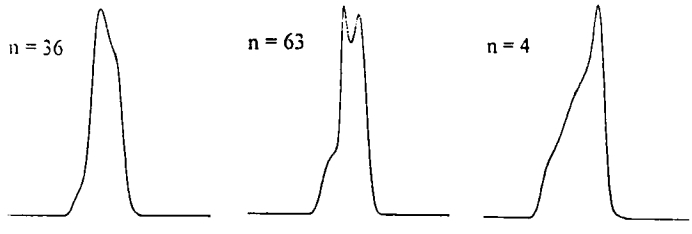

FIGURE 2 ^{31}P-NMR theoretical fits for DPPE/PLPC/$^{2}H_2O$ system at 50°C. n is the mole percentage of phosphorus segments with cylindrical symmetry used in the simulations.

The relative intensity of the lamellar phase increases with increasing temperature until 60°C when the powder pattern is mostly composed by a lamellar phase in the liquid crystalline state. In the case of samples containing more than 25 weight % PLPC the spectral lineshape is the superposition of a lamellar and a cylindrically symmetric powder pattern. The pattern assigned to the lamellar phase at lower temperatures is broader than that at higher temperatures and indicates the presence of a gel phase.

The ^{31}P-NMR results, together with other ^{2}H-NMR data[18], exclude the possibility of the coexistence of two phase-separated lamellar and cylindrically symmetric phases for the samples containing less than 28 weight % PLPC. A defective lamellar phase is the more likely structure for the sample containing 25 weight % PLPC. (vide infra)

The observed lineshape changes in the ^{31}P-NMR spectra were correlated with concentration changes in the mole fraction, n, of phospholipid molecules with cylindrical symmetry by fitting the experimental ^{31}P-NMR spectra to simulated lineshapes. ^{31}P-NMR spectra were generated by a linear combination of powder patterns of the cylindrical and lamellar phases.[21,22] Spectral lineshapes were found to depend on the concentration, n, of phosphate segments with cylindrical symmetry, two chemical shift anisotropic parameters and two broadening parameters. n is the major fitting parameter and could be determined with a precision of ±0.02. Our model of the transition is supported by the close similarity between simulated and experimental spectra (Figure 2).

Calorimetric measurements

Some typical differential scanning calorimetric traces for DPPE bilayers containing different lysolecithin concentrations are shown in Figure 3. The trace for the DPPE/water binary system (not shown) is characterized by a sharp highly-cooperative chain melting transition at 65.1°C, i.e. a transition from the L_β to the L_α phase. At lysolecithin concentrations of 5, 10, 20 weight %, the DSC traces are similar to that of the binary mixture, but there is a significant broadening and downward shift in the main transition. It drops to 47.3°C at 25 weight % PLPC. This sample also shows an anomalous broad peak with a width of about 15°C.[18]

At PLPC concentration of 28 weight %, the DSC run shows a single broad phase transition with a maximum at 61.8°C. At higher concentrations two peaks appear, i.e. the components demix.

Figure 4 shows the phase diagram of the DPPE/PLPC/^2H_2O mixture at fixed weight % ^2H_2O (34%) obtained by varying the weight % of PLPC from 0 to 33. The phase diagram was obtained by following the temperature evolution of ^{31}P-NMR lineshapes and DSC transition temperatures measured at the peak maximum.

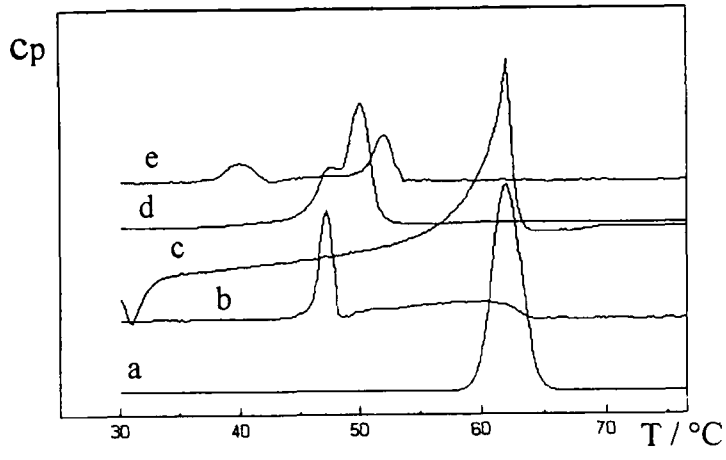

FIGURE 3 DSC traces for DPPE/PLPC/^2H_2O dispersions containing different weight % of PLPC: a) 20%; b) 25%; c) 28%; d) 31%; e) 33%.

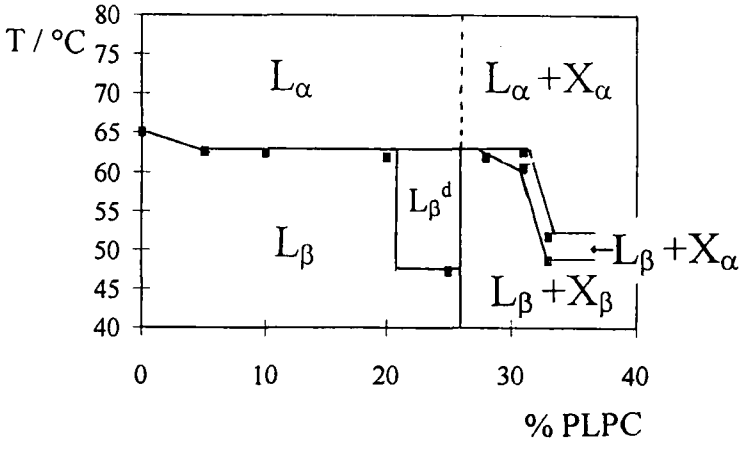

FIGURE 4 Phase diagram for DPPE/PLPC/^2H_2O system: L_β, gel phase; L_α, lamellar liquid crystalline state; L_β^d, defective gel phase; X_β, X_α macrodomains of PLPC molecules with rigid or melted hydrocarbon chains, respectively.

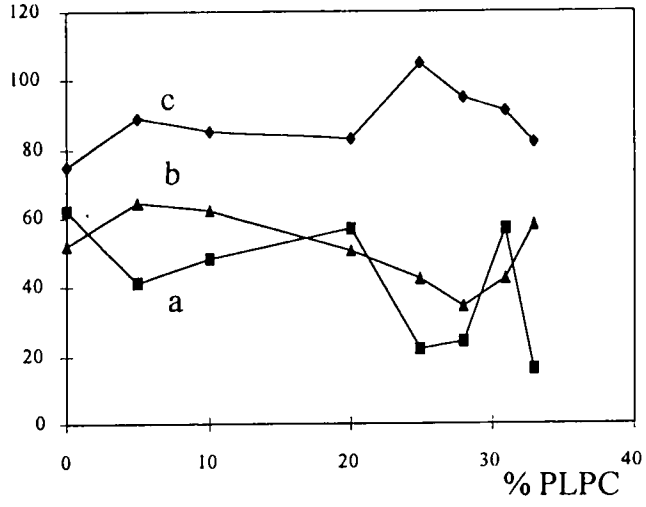

FIGURE 5 Thermodynamic functions at 40°C versus PLPC weight concentration: a) relative volume changes (x 10^3); b) isobaric expansion coefficient (x 10^7 $°C^{-1}$); c) isothermal compressibility coefficient (x 10^{11} m^2/N).

The thermal dependencies of relative volume changes at the L_β to L_α transition, $\Delta V/V$, isobaric coefficient of expansion, $\alpha = 1/V\ (\partial V/\partial T)_P$ and isothermal compressibility coefficient, $\beta = 1/V\ (\partial V/\partial P)_T$, in the gel phase are shown in Figure 5.

DISCUSSION

Volume changes arise from lateral expansion and have several contributing factors, the most important of which are the increase of gauche conformations in the lipid chains and repulsive interactions among the polar heads. A higher value of α means that molecules can move easily, i.e. intermolecular interactions are weaker. Figure 5 (curve b) shows that α increases in the gel phase when 5 weight % of PLPC is added and this means that intermolecular interactions among DPPE and PLPC molecules are energetically less favorable than those between DPPE molecules. In fact in a previous paper[17] it was stated that the presence in PLPC of the trimethylammonium group, which is incapable of H-bond formation, can have the effect of disrupting some intermolecular H-bonding between DPPE molecules when PLPC molecules are randomly incorporated into the

bilayer. This effect should reduce the phase transition temperature together with Van der Waals interaction between the hydrocarbon chains. The insertion of conically shaped molecules among a collection of cylindrically shaped DPPE molecules should produce an increased number of gauche conformers in the chains of PLPC for a given temperature.

Figure 5 (curve c) shows that β increases in the L_β phase as PLPC is inserted. A higher value of β implies that defects increase, i.e. PLPC molecules allow a poorer molecular packing in the bilayer structure.

Introduction of more PLPC (up to 20 weight %) into the DPPE bilayers does not affect the overall arrangement of the phospholipid molecules. ^{31}P-NMR spectra in both gel and liquid crystalline phases consist mostly of uniaxial powder patterns indicative of lamellar structures. However the $\Delta V/V$ value increases and becomes as large as the value for DPPE/^2H_2O binary system. Both α and β always have larger values than in pure DPPE bilayers, but they show a monotonic decrease. We suggest that microaggregates of PLPC randomly distributed in the DPPE bilayers could account for the more efficient packing of hydrocarbon chains and for the small decrease in the phase transition temperatures.

At 25 weight % PLPC the thermodynamic and NMR data are consistent with a lamellar phase containing cylindrically symmetric defects. Such defects increase in number up to 47.3°C where a sharp first order phase transition due to PLPC tail melting is observed. Such defects decrease during the anomalous phase transition to a constant value of 5 % at 65.1°C, above which DPPE molecules are in a liquid crystalline state and PLPC are distributed homogeneously in the DPPE bilayers.

Increasing the concentration of PLPC to 28 weight % induces behavior similar to that of the 25 weight % samples in the intensive thermodynamic functions. The broad peak is indicative of the formation of PLPC macrodomains with cylindrical symmetry and the value of β, which is still higher than pure DPPE, accounts for the lattice defects introduced by the PLPC molecules.

At higher concentrations we begin to observe phase separation. Increasing the weight concentration of PLPC to values larger than 25, i.e. molar ratios higher than 1, involves the increase of the dimensions of liquid crystalline clusters of PLPC arranged with cylindrical symmetry and their phase separation from lamellar bilayers.

CONCLUSIONS

We have determined the phase diagram of the DPPE/PLPC/^2H_2O system at a fixed ^2H_2O weight % (34 weight %). A complex polymorphism with increasing PLPC concentration up to 33 weight % PLPC was observed. Up to 20 weight % PLPC, the wedge like shape molecules of lysolecithin distributes randomly in the planar bilayers of DPPE, but they separate into a phase with cylindrical symmetry at higher concentrations. An intermediate structure made up of defective bilayers occurs at 25 weight % PLPC.

Complex formation, involving H-bond formation between PLPC and DPPE, may account for the observed structural changes and provide further insights into the behavior of lysolecithin in model membranes.

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