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DIFFERENTIAL SCANNING CALORIMETRY ON MIXTURES OF LECITHIN, LYSOLECITHIN AND CHOLESTEROL

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The effect of increasing concentrations of lysolecithin (1-palmitoyl-*sn*-glycerol-3-phosphorylcholine) on the gel → liquid crystal thermal transition of lecithin (1,2-dipalmitoyl-*sn*-glycerol-3-phosphorylcholine) in the aqueous phase was studied by differential scanning calorimetry (DSC). Lysolecithin showed an endothermic transition at 3.4°C whereas the transition of the lecithin occurred at 42°C. No phase separation could be observed calorimetrically at lysolecithin concentrations up to 60 mol%. Freeze etch electron microscopy showed that mixtures containing as much as 50 mol% lysolecithin exist in a lamellar phase. The lysolecithin was found to cause an initial slight increase in the enthalpy of transition followed by a gradual decrease. The enthalpy increased again at very high lysolecithin concentrations. The lysolecithin also caused a non-linear decrease in the temperature at which the lecithin transition took place.

Cholesterol was found to decrease the enthalpy of transition of the lysolecithin, eliminating it at a concentration of 50 mol%. Cholesterol caused an increase in the temperature at which the lysolecithin transition took place.

I. Introduction

Lysolecithin has long been of interest because of its ability to interact with and disrupt membranes. However, the mechanism of lysolecithin's interaction with the membrane and the subsequent disruption is not understood. To gain additional information on this process there have been studies on lysis by lysolecithin analogs, synthetic lysolecithins of various hydrocarbon chain lengths, lytic lecithins [1] and modified lysolecithins [2]. The mere presence of lysolecithin in a membrane is not sufficient to cause lysis. Ibrahim and Thompson [3] showed that hydrolysis of nearly 20% of the phospholipids of intact erythrocytes by sea snake phospholipase A caused negligible lysis. Van Zutphen and van Deenen [4] found that stable black lipid membranes can be formed from egg lecithin containing up to 15% lysolecithin but not with 20% lysolecithin. The electrical resistance, however, was reduced

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100-fold by the presence of 2% lysolecithin in the membrane. Reman [5] found by X-ray diffraction that lysolecithin in egg lecithin up to concentrations of 40 mole% caused the lipid leaflet to thin. At higher lysolecithin concentrations a phase separation was apparent. On the other hand, a lytic lecithin (didecanoyl lecithin) up to concentrations of more than 70 mole%, caused a continued thinning of the leaflet with no apparent phase separation.

In this study the effect of a lysolecithin of known structure on the thermal transition of a lecithin of known structure, and the effect cholesterol on the thermal transition of lysolecithin were investigated.

II. Materials and methods

Dipalmitoyl phosphatidylcholine was synthesized by standard procedures [6]. The product contained more than 99% palmitic acid by gas–liquid chroma ɔgraphy and was approximately 99% phosphatidylcholine as determined by thin-layer chromatography. Lysolecithin was prepared by phospholipase A degradation of dipalmitoyl lecithin. Cholesterol, (cholest-5-en-3β-ol) was purchased from Fluka AG (Buchs, Switzerland).

The calorimetry was performed on a Perkin-Elmer DSC-1B instrument at range 1 unless otherwise noted. The usual heating rate was 8°C/min. The instrument was calibrated with distilled water, cyclohexane and naphthalene. An empty gold pan was used in the reference cell.

Liposomal lipid suspensions were prepared in distilled water or 50% ethylene glycol–water by agitation on a Vortex mixer while heating the mixture above the transition temperature of the lipid.

Quantities of lipids contained in the sample cells were determined by phosphorus analysis using the method of Fiske and Subbarow [7].

The samples for freeze-etch electron microscopy were quenched from 0°C and treated further as described by Ververgaert et al. [8, 9].

III. Results and discussion

The gel → liquid crystal transition observed for dipalmitoyl lecithin took place at 42.0°C and was quite sharp. This value falls within the range of 38°C reported by Lippert and Peticolas [10] as determined by laser Raman and 43.2°C reported by Giannoni et al. [11] as determined by DSC, but is 1°C above the generally accepted transition temperature of 41.0°C [12, 13]. No pre-transition was observed in the heat curve probably due to the presence of 50% ethylene glycol. Sample sizes and heating rates were kept constant so that the experiments were internally consistent. The heat of the gel → liquid crystal transition observed was 9.2 ± 0.4 kcal/mol. This value is intermediate between the values reported previously [12,13].

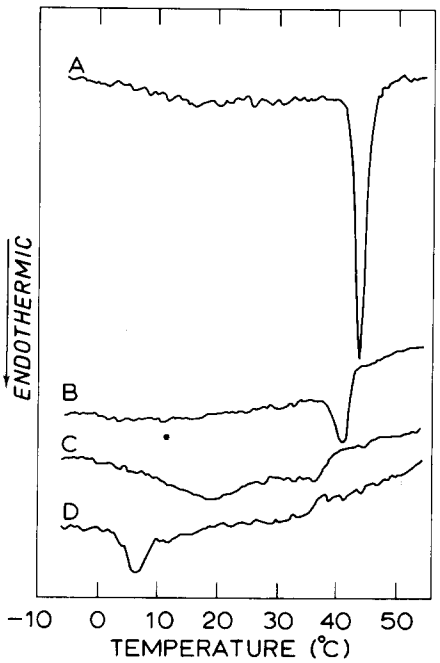

Fig. 1. Calorimetric scans showing the thermal transitions of lecithin–lysolecithin mixtures. Mixture A contained 10% lysolecithin; B, 61% lysolecithin; C, 89% lysolecithin, and D, 92% lysolecithin. Mixtures were prepared by mixing appropriate volumes of stock solutions of each component in a test tube, then drying them under a stream of N_2, followed by vacuum drying overnight. The resultant residues were suspended in 50% ethylene glycol.

At 3.4°C the palmitoyl lysolecithin underwent a thermal transition, the heat of which was 4.5 ± 0.8 kcal/mol. It is of interest to note that although the "melting" heat per mole of lysolecithin is only half of that for the diacyl compound, the heat per fatty acid residue is essentially identical in the two compounds. Since the same heat is required to convert one fatty acid residue of either the monoacyl or diacyl compound from the gel to the liquid crystalline state, the chains must exist in similar environments, i.e. the packing of the fatty acids in the micellar structure of lysolecithin [17, 18] must be similar to that found in the lamellar structure of lecithin.

The effect of increasing the concentration of lysolecithin on the thermal transition of lecithin is shown in figs. 1 through 3. The thermal transition of lecithin containing a small quantity of lysolecithin was quite sharp (fig. 1A). When the concentration of lysolecithin was increased to 61% (fig. 1B), the sharpness of the transition decreased markedly, accompanied by a decreased enthalpy of transition. A somewhat higher concentration of lysolecithin caused the appearance of a new broad transition at a temperature between those of lecithin and lysolecithin (fig. 1C). Because

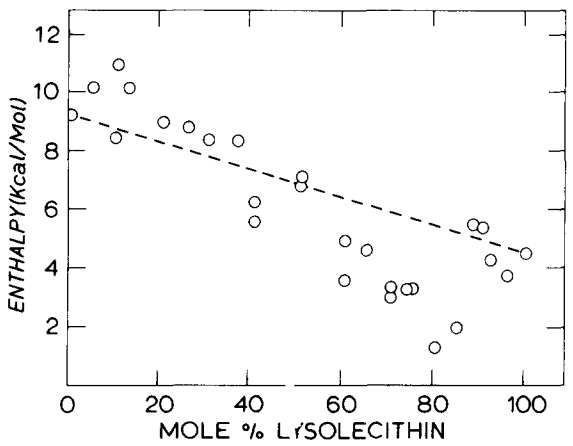

Fig. 2. Effect of lysolecithin on the enthalpy of transition of lecithin. The heat of transition for each reaction mixture was calculated. This, together with the knowledge of the moles of phospholipid in the reaction cell, made it possible to calculate the enthalpy of transition per mole of phospholipid present. The circles represent the observed values; the broken line, the values predicated on the basis of 4.6 kcal/mole of hydrocarbon chain.

this transition was rather broad the starting temperature was difficult to determine, with the model 1B calorimeter. When this sample was on the more sensitive Perkin-Elmer DSC-2B it appeared that this intermediate transition began at 13°C. The later transition which is the remnant of the lecithin transition peak, occurred at 30°C.

A further increase in lysolecithin concentration (fig. 1D) caused the appearance of the lysolecithin transition peak, although a trace of a transition was still visible at about 30°C (confirmed by analysis on the model 2B instrument). The intermediate peak was not pronounced but could have accounted for the tailing effect observed on the lysolecithin transition.

The effect of lysolecithin on the enthalpy of transition of lecithin is shown in fig. 2. The enthalpy of transition for the lecithin with two palmitic acid residues is 9.2 kcal and that for lysolecithin is 4.5 kcal. Apparently, 4.5–4.6 kcal is required to "melt" a palmitic acid residue in either of those compounds. The broken line in fig. 2 shows the enthalpies of transition expected on this basis for mixtures of the lecithin and lysolecithin. The observed values deviated from those expected. The slight initial rise in enthalpy values when lysolecithin was added was interpreted as an increased stability of the lecithin lamellar structure by the lysolecithin. Reman [5] observed that adding 10% lysolecithin to egg lecithin caused a marked thinning of the lipid leaflet and that further additions had less effect. He found that phase separation occurred above 40 mole% of lysolecithin, approximately the concentration at which the line for observed enthalpies in our study dropped below the line

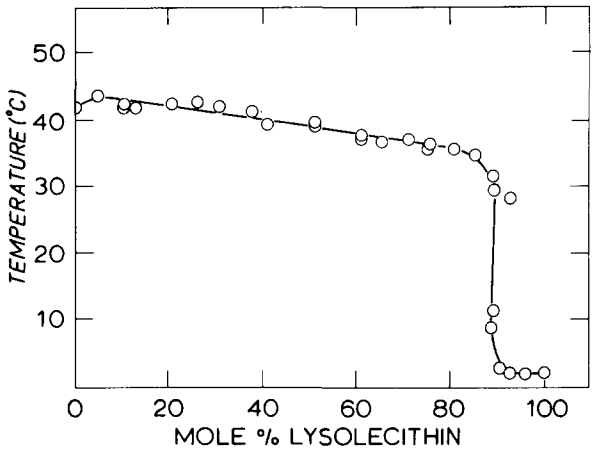

Fig. 3. Effect of lysolecithin on lecithin's transition temperature, taken as the temperature at which the first noticeable divergence from the base line occurred.

for the expected values (fig. 2). We believe that this drop was caused by the gradual formation of a new structure or phase of lecithin and lysolecithin which produced a low, broad transition (fig. 1C). This transition would not be distinguishable from the base line at lower concentrations of lysolecithin; thus, a portion of the enthalpy of transition was not observed. The rise in the observed values at higher lysolecithin concentrations coincided with the appearance of the lysolecithin thermal transition peak.

Increasing the concentration of lysolecithin caused a slow decrease in the transition temperature of the lecithin–lysolecithin mixture (fig. 3). The precipitous drop in the transition temperatures occurred in the region of the intermediate transition and levelled off to the transition temperature of pure lysolecithin.

Freeze etch electron microscopy reveals that with up to 50% lysolecithin the mixture forms a lamellar phase. However, while up to 30% lysolecithin normal liposomal structures are formed by the lamellae (fig. 4a), at 50% lysolecithin the lamellae are stacked bilayers (fig. 4b). At higher concentrations of lysolecithin a lamellar phase is still present, but particulate areas are also visible (fig. 4c). It is suggested that these particulate structures may reflect the hexagonal phase, as is found for a mixture of 60% lysolecithin (from egg lecithin) + 40% water by Deamer et al. [17]. So above 50 mol% there is a real phase separation*.

It has to be noted that at lower concentrations lysolecithin (below 20%) the mixture exhibits an undulated fracture face below the lipid phase transition. This phenomenon is found for several pure lecithins [9]. The homogeneity of the band-pattern indicates that there is no phase separation in the plane of bilayer, but that there is a homogeneous distribution of lysolecithin [9].

*Freeze etching shows that at 25 mol% of lysolecithin smooth areas next to areas with band-patterns are present indicating a phase segregation in the lateral plane of the bilayer.

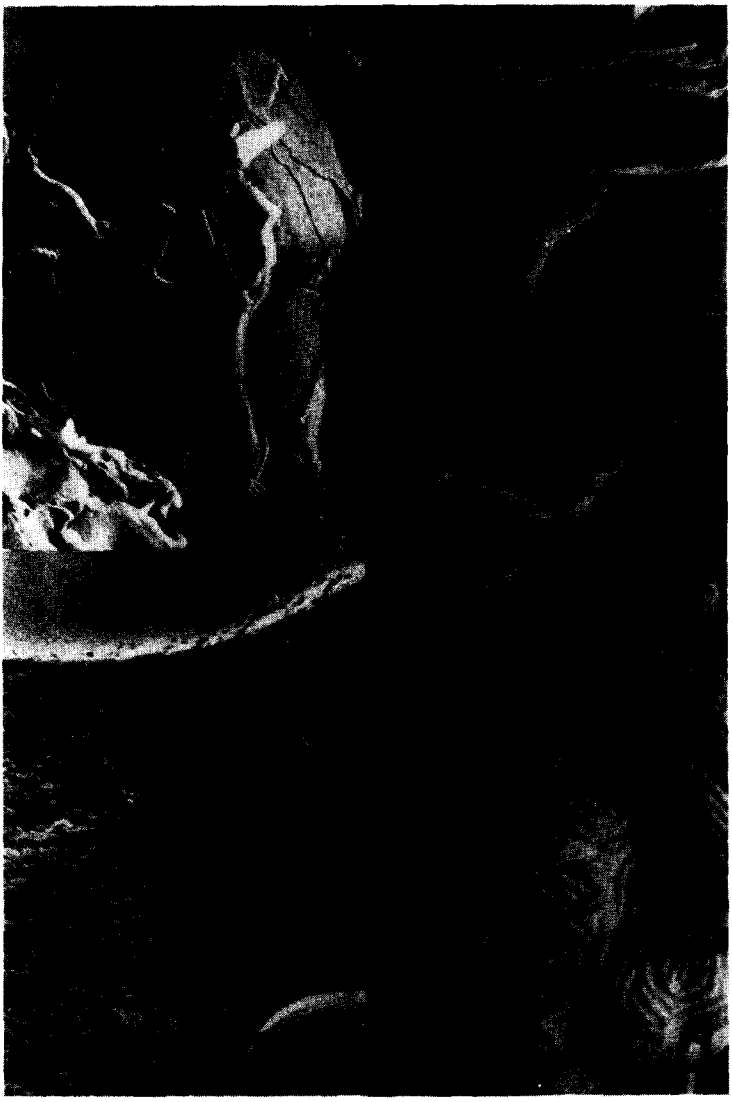

Fig. 4. Freeze etch electron micrographs of mixtures of lecithin and lysolecithin. A. 30% lysolecithin; B. 50% lysolecithin; C. 70% lysolecithin; D. 15% lysolecithin. The periodicity of the band pattern is about 400 Å. All samples were quenched from 23°C. A, B and D are fractured at −196°C and C was fractured and etched during 1 min at −100°C. Magnification about 60,000.

The effect of cholesterol on the thermal transition of the lysolecithin is shown in figs. 5 and 6. Initially the enthalpy of transition of lysolecithin increased at low concentrations of cholesterol (2.5%) and declined thereafter as concentrations were increased (fig.5). The enthalpy of transition became zero at 50 mole% of cholesterol.

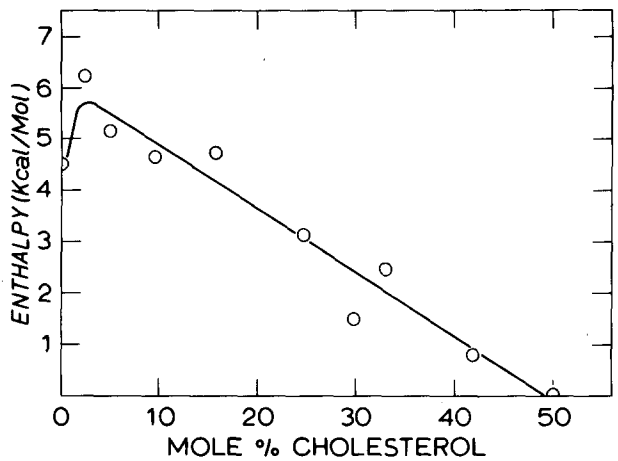

Fig. 5. Effect of cholesterol on the enthalpy of transition of lysolecithin. Stock solutions of cholesterol and lysolecithin were mixed and treated as described in the legend to fig. 1. Enthalpy per mole of phospholipid was calculated as described in fig. 2.

Cholesterol has been reported to eliminate the transition of lecithin when present at 50 mole% [12] or 33 mole% [13]. At 50 mole% of cholesterol, lecithin provides two acyl chains for interaction with the sterol; at 33 mole% there are four chains present for each sterol. Engleman and Rothman [14] proposed a model for lecithin–cholesterol based on the sterol's interaction with four hydrocarbon chains. However, in our case, interaction of lysolecithin at a ratio of 1 : 1 allowed one hydro-

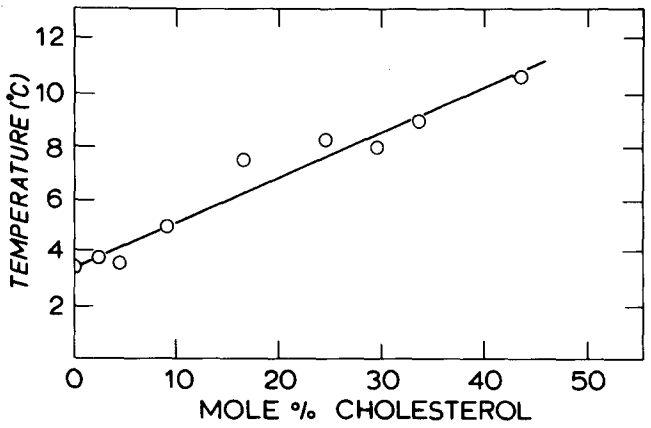

Fig. 6. Effect of cholesterol on the transition temperature of lysolecithin. The temperature of transitions were determined as described in fig. 3.

carbon chain for each sterol molecule. Thus, the model proposed for the lecithin–cholesterol system cannot be extended to the lysolecithin–cholesterol system.

Fig. 6 illustrates another difference between the lecithin–cholesterol and the lysolecithin–cholesterol systems: the transition temperature of lysolecithin increased uniformly with increasing cholesterol concentrations, whereas Phillips et al. [12] observed a linear decrease in the transition temperature of lecithin with increasing cholesterol concentration.

A possible explanation of the behavior of the lysolecithin–cholesterol system may be provided by the statement of Dervichian [15, 16] that an equimolar mixture of these compounds exists in a lamellar, liquid crystalline structure. Thus, the cholesterol causes the lysolecithin to adopt a structure similar to that of lecithin. Accompanying this change in structure, the transition temperature of the lysolecithin changes in the direction of that of the diacyl compound.

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