

## EFFECTS OF A SYNTHETIC LYSOLECITHIN ANALOG ON THE PHASE TRANSITION OF MIXTURES OF PHOSPHATIDYLETHANOLAMINE AND PHOSPHATIDYLCHOLINE

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### 1. Introduction

Lysolecithin, i.e. 1-acyl-*sn*-glycero-3-phosphorylcholine, is a widely distributed surface-active and cytolytic phospholipid [1–3], which has previously been shown to induce cell fusion [4,5] as well as enhanced immune reactions [6]. Despite various efforts [1–3,7], however, the mechanism of action of lysolecithin on natural and model membrane is still poorly understood. So far, all attempts have failed to demonstrate an increased molecular mobility of membrane lipids upon addition of low amounts of lysolecithin, and Klopfenstein et al. [8] have shown that lysolecithin even at a molar ratio higher than 1 : 1 hardly affects the phase transition temperature of dipalmitoyllecithin.

The present study deals with the effects of a synthetic ether-desoxylysolecithin analog [1] on the miscibility of mixed lipid phases using phosphatidylethanolamine–phosphatidylcholine mixtures and differential scanning calorimetry.

### 2. Materials and methods

1,2-Dipalmitoyl-*sn*-glycero-3-phosphorylcholine (DPPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoryl-ethanolamine (DPPE), 1,2-distearoyl-*sn*-glycero-3-phosphorylcholine (DSPC), and 1,2-dimyristoyl-*sn*-glycero-3-phosphorylethanolamine (DMPE) were

purchased from Fluka, Neu-Ulm. Impurities detectable by thin-layer chromatography were below 2%. These lipids were used without further purification. 1-Hexadecyl-propanediol-3-phosphorylcholine (ether-desoxy-lysolecithin) was synthesized as described elsewhere [1].

The lipid mixtures were prepared by dissolving the desired amounts of the dry lipids (30–40 mg) in chloroform. After removal of the solvent by a stream of nitrogen, 25 ml of aqueous 0.05 M Tris–HCl buffer at pH 7.5 were added. The lipids were dispersed ultrasonically at a temperature above the transition temperature of the mixture. The lysolecithin was either mixed with the other lipids in the chloroform phase, or added to the dispersion as aqueous micellar solution. The calorimetric measurements were made using an adiabatic differential scanning calorimeter as described before [9,10]. The heating rate was 13.5°C/h. To test the reversibility of the system, three runs were made with each lipid mixture.

### 3. Results and discussion

Fig. 1a shows the calorigrams of an aqueous dispersion of DPPC before and after the addition of 5% (w/w) lysophosphatide. The maximum of the excess heat capacity curve is shifted to slightly lower temperatures, i.e., from 40.8°C to 40.2°C. The enthalpy of the transition is 7.7 kcal/mole lipid, which is only

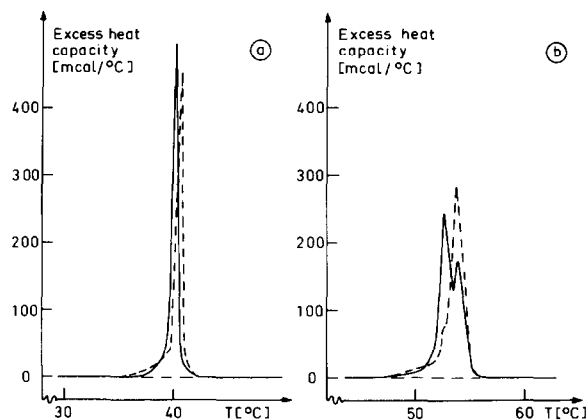


Fig.1. Calorimetric scans of an aqueous dispersion of (a) 38.5 mg DPPC and (b) 38.1 mg DSPC before (---) and after (—) the addition of 5% (w/w) lysolecithin.

slightly lower than the transition enthalpy of 8.1 kcal/mole obtained for pure DPPC and falls into the range of the reported values of 7.5–9.6 kcal/mole for the transition enthalpy of DPPC [8,11–14]. The width of the transition is unchanged after addition of the lysolecithin indicating that the co-operativity of the transition is not affected. These findings are in agreement with those obtained by Klopfenstein et al. [8] for L-palmitoyl-lysolecithin.

Mixtures of DSPC with lysolecithin exhibit a slightly different behaviour. Fig.1b shows the calorigrams of an aqueous dispersion of this compound before and after the addition of lysolecithin. The transition enthalpy for pure DSPC is 10.8 kcal/mole. After the addition of the lysophosphatide this value is unchanged. The single peak, however, found in the calorigram of DSPC, is split into two peaks, indicating the existence of two different lipid phases below the phase transition temperature.

Mixtures of DMPE and DPPE with the desoxy-lysolecithin reveal a quite different behaviour. Calorigrams of aqueous dispersions of DMPE with and without 5% (w/w) lysolecithin are shown in fig.2a. The transition temperature  $T_m$  is shifted to 47.5°C and the transition enthalpy is lowered to 5.0 kcal/mole, whereas pure DMPE has a  $T_m$ -value of 49.5°C and a transition enthalpy of 6.3 kcal/mole. The lysolecithin molecules, having a different polar group and a longer hydrocarbon chain, apparently disturb

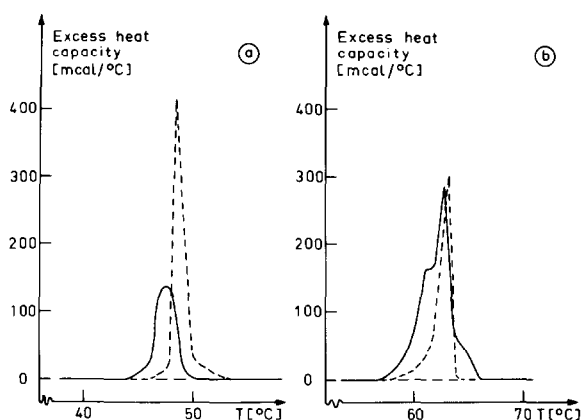


Fig.2. Calorimetric scans of an aqueous dispersion of (a) 39.5 mg DMPE and (b) 31 mg DPPE before (---) and after (—) the addition of 5% (w/w) lysolecithin.

the cooperative interactions of the phosphatidyl-ethanolamine molecules, which are linked by additional intermolecular interactions of the polar head groups [10,15]. This reduced co-operativity is the reason for the greater temperature interval and decreased transition enthalpy of the gel–liquid-crystal phase transition of this lipid mixture.

Fig.2b shows the calorigrams of a dispersion of DPPE with and without lysolecithin. The  $T_m$ -value is only slightly lower after the addition of the lysophosphatide. The transition enthalpy, though, is increased from 8.2 kcal/mole to 18.0 kcal/mole. This indicates, that in the crystalline state two different phases probably exist, which have to be mixed to give a homogeneous phase above the transition temperature. This would lead to a higher transition enthalpy, because the intermolecular bonds between the PE molecules would have to be broken [10].

As a consequence of this different mixing behaviour of lysolecithin with phosphatidylcholine and phosphatidylethanolamine one would expect a preferential association of the lysolecithin with lecithin in a mixture of DPPC and DPPE, for example.

The phase diagram for mixtures of DPPC and DPPE in the absence of lysolecithin is of the monotectic type with almost complete miscibility in the crystalline gel phase [10,16]. The phase transition temperature of an equimolar mixture of these compounds is 53°C, averaging almost exactly the transi-

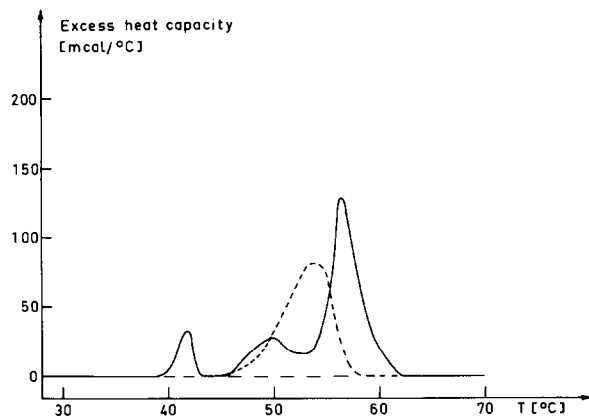


Fig.3. Calorimetric scans of an aqueous dispersion of 40.3 mg of an equimolar mixture of DPPE and DPPC before (—) and after (---) the addition of 5% (w/w) lysolecithin.

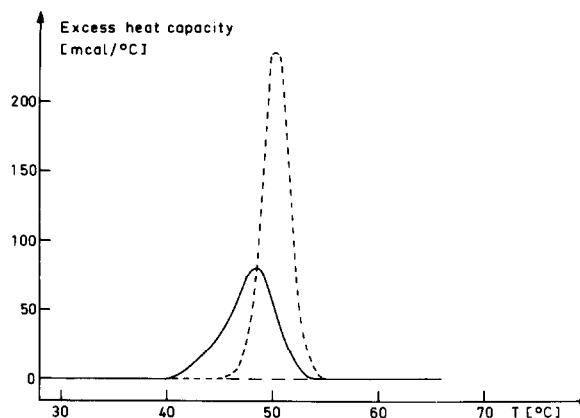


Fig.4. Calorimetric scans of an aqueous dispersion of 38.6 mg of an equimolar mixture of DMPE and DSPC before (—) and after (---) the addition of 5% (w/w) lysolecithin.

tion temperatures of the pure lipids DPPE and DPPC, which are 63°C and 41°C, respectively. The enthalpy of the transition of 7.7 kcal/mole lipid is only slightly lower than the mean of 8.15 kcal/mole lipid for the pure compounds [10]. The addition of 5% (w/w) of the desoxylysolecithin to this mixture results in a completely different mixing behaviour of this lipid system. Fig.3 shows the calorigrams of an aqueous dispersion of an equimolar mixture of DPPE and DPPC before and after the addition of lysophosphatide. The single peak found for the DPPE/DPPC mixture is split into three peaks with maxima at 42°C, 50°C, and 56.6°C. Obviously, a separation of three phases with different compositions has taken place. The enthalpy of the transition for all three peaks together is 10.0 kcal/mole lipid. This value is considerably higher than the enthalpy value before the addition of lysolecithin. The reason for this rise is most likely the same as for the extraordinary high transition enthalpy of DMPE/DSPC mixtures, which have limited miscibility in the gel phase [10]. Apparently, there is an additional energy-requirement to break intramolecular linkages of the polar groups of phosphatidylethanolamine when forming a homogeneous liquid crystalline phase with choline-lipids.

An equimolar mixture of DMPE and DSPC has a transition enthalpy of 13.5 kcal/mole lipid and a  $T_m$ -value of 50°C. As shown in fig.4, addition of 5% (w/w) of lysolecithin to this mixture leads to a dramatic

decrease of the transition enthalpy to 7.2 kcal/mole lipid, whereas the  $T_m$ -value is only slightly lowered to 48°C. Obviously, in this case the effect of lysolecithin is completely opposite to that observed with DPPE/DPPC mixtures: the former miscibility gap of DMPE and DSPC in the gel phase is abolished, so that homogeneous mixtures are formed not only above, but also below the phase transition temperature. One possible explanation for this effect of the lysophosphatide on DMPE/DSPC mixtures would be that the desoxy-lysolecithin molecule, having an intermediate chain length of 16 carbon atoms, facilitates the cocrystallization of DMPE, and DSPC, containing 14 and 18 carbon atoms per fatty acid chain, respectively.

The results of our calorimetric experiments show, that the effect of lysolecithin on lipid mixtures is highly complex and probably depends not only on the length of the fatty acid residues of phosphatidylethanolamine and phosphatidylcholine, but also on the length of the hydrophobic chain of the lysolecithin molecule. Nevertheless, the observed effects clearly prove, that relatively small amounts of lysolecithin have a pronounced influence on the phase transition of pure lipids and on the miscibility of different molecular species of phospholipids. Thus, it appears feasible to conclude, that sublytic amounts of lysolecithin might induce changes in the lateral distribution of lipids and perhaps proteins in a biomembrane.

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