A CALORIMETRIC STUDY OF THE LIPID PHASE TRANSITIONS IN AQUEOUS DISPERSIONS OF PHOSPHORYLCHOLINE–PHOSPHORYLETHANOLAMINE MIXTURES

Alfred BLUME and Theodor ACKERMANN
Institut fuer Physikalische Chemie II, Universitaet Freiburg, 78 Freiburg, Hebelstrasse 38, W. Germany

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1. Introduction

Phospholipids are major components of biological membranes. Studies of the gel–liquid crystal transition of aqueous dispersions of synthetic phospholipids revealed that the transition temperature depends on the length and on the number of double bonds of the fatty acid chains [1]. Little is known, however, about the physiological significance of the different head groups of the phospholipids and their mixing behaviour. Recently, phase diagrams of mixtures of phosphorylcholines with different fatty acid chain lengths and of phosphorylcholines with phosphorylethanolamines have been reported [2–4].

We studied the thermal phase transition of mixtures of phosphorylcholines with phosphorylethanolamines in dilute aqueous dispersions. A newly developed adiabatic differential scanning calorimeter was employed to measure the heats of transition of the lipid mixtures.

2. Materials and methods

1,2-Dipalmitoyl-*sn*-glycero-3-phosphorylcholine (DPPC), 1,2-distearoyl-*sn*-glycero-3-phosphorylcholine (DSPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphorylethanolamine (DMPE), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylethanolamine (DPPE) were purchased from Fluka, Buchs. Traces of impurities were detected by thin layer chromatography on silica gel H plates (merck, Darmstadt). The lipids were used without further purification, though. For the preparation of the lipid dispersions 35–45 mg of the dry lipids were dissolved in chloroform, the solvent being removed later by a stream of nitrogen. After addition of 25 ml of aqueous 0.05 M Tris–HCl buffer at pH 7.5 the lipids were dispersed ultrasonically at temperatures above their respective transition temperatures. The calorimetric measurements were made using a new adiabatic differential scanning calorimeter [5]. Each compartment of the gold-plated double cell contained 25 ml of solution and solvent respectively. The lipid dispersions were heated with a rate of 14°C/hr. At least three runs were made with each lipid mixture. The values given in the figures are mean values, bars representing standard deviations from the mean.

3. Results and discussion

Fig. 1 shows a calorigram of an aqueous dispersion of an equimolar mixture of DPPE and DPPC. The excess heat capacity versus temperature curve shows one broad endothermic peak. The area limited by the registered curve and an interpolated base line corresponds to the heat of transition for the gel to liquid crystal phase transition. The phase diagram for mixtures of DPPE and DPPC obtained from the calorimetric measurements is shown in fig. 2. The 'solidus' and the 'liquidus' curve were determined from the temperatures of the beginning and the end of the phase transition respectively. The temperature interval of the phase transition becomes very broad even at low concentrations DPPE or DPPC. Assuming a

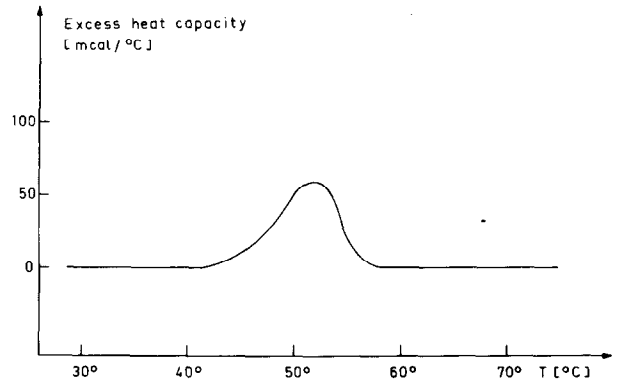

Fig. 1. Differential scanning calorimetry curve of an equimolar aquous dispersion of DPPE and DPPC.

two-state process for the transition, the Van 't Hoff equation can be put in the form: $(d\theta/dT)_{T_m} = \Delta H_{\text{Van 't Hoff}} / 4 \cdot R \cdot T_m^2$, where θ is the degree of conversion and T_m the temperature at $\theta = 1/2$ [6]. Thus $\Delta H_{\text{Van 't Hoff}}$ can be evaluated from the integrated excess heat capacity versus temperature curve. The value of the ratio $\Delta H_{\text{Van 't Hoff}} / \Delta H_{\text{cal}}$ (ΔH_{cal} is determined using the calorimetrically measured heat of transition and the molecular weights and mole fraction of the lipids present in the mixture), i.e., the size of the cooperative unit involved in the phase transition is only 15 for the equimolar mixture of DPPE and DPPC, compared to a value of 125 for the pure compound DPPC. The cooperativity of the phase transition of mixtures of different lipids is thus considerably lower. The phase diagram obtained from calorimetric measurements of the DPPE–DPPC system closely resembles the one reported by Shimshick and McConnell [4]. The heat of transition versus mole fraction of DPPE curve (fig. 2) shows only slight deviations from the mean value for the heat of transition of both lipids. This curve is similar to the one reported by Chapman [3]. Fig. 3 shows a calorigram of an aqueous dispersion of an equimolar mixture of DMPE and DSPC. There is a striking difference to the corresponding curve in fig. 1. The value of the heat of transition of 19 cal/g lipid is appreciably larger than the mean value of 11.9 cal/g lipid for the pure compounds. In contrast to the curve for the DPPE–DPPC system the endothermic peak is almost symmetric. The different behaviour of this system is also reflected by the corresponding curve for the heat of transition versus mole fraction of DMPE (fig. 4). The phase diagram in fig. 4 shows no discontinuity of the 'liquidus' curve, which would be an indication of the existence of an eutectic mixture. Mixtures of DMPE

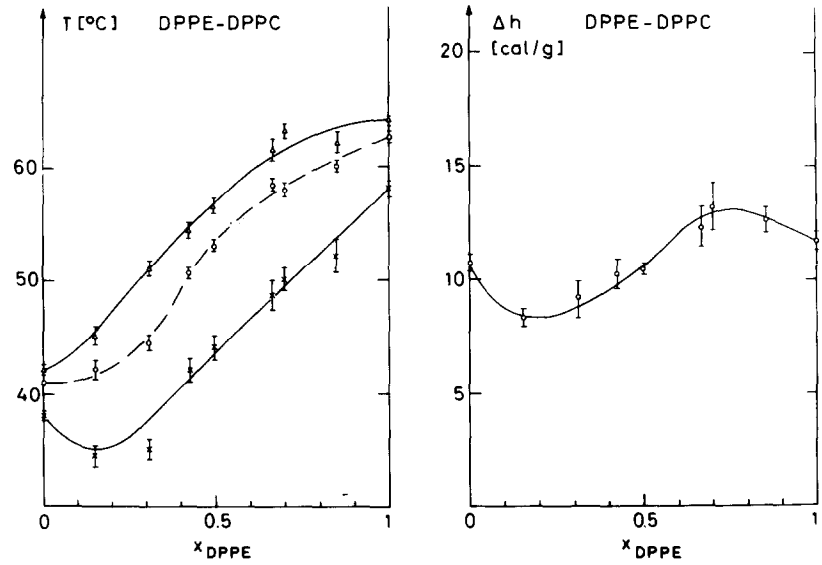

Fig. 2. Equilibrium phase diagram and variation of heat of transition Δh (in cal/g of lipid) for aqueous dispersions of binary mixtures of DPPE and DPPC. (x : beginning, Δ: end of the phase transition as determined from the differential scanning calorimetry curves, ○: temperatures of the maximum of the excess heat capacity).

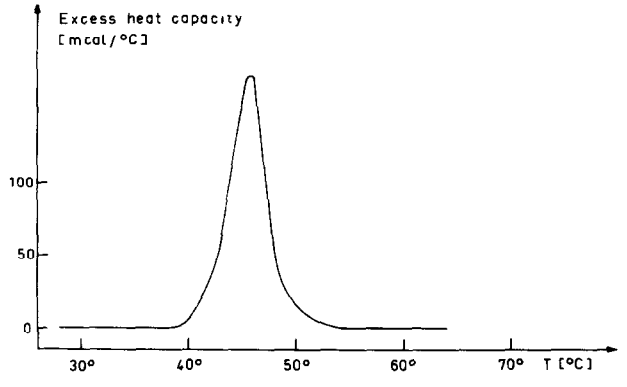

Fig. 3. Differential scanning calorimetry curve of an equimolar aqueous dispersion of DMPE and DSPC.

and DPPC show a similar phase behaviour (fig. 5). The largest value of the heat of transition is found for an aqueous dispersion of an equimolar mixture (fig. 5), as in the system DMPE–DSPC, where both compounds have different fatty acid chain lengths as well.

The results obtained indicate that for the DPPE–DPPC system complete miscibility occurs in the liquid crystalline phase. The 'solidus' curve, though, shows a minimum at 15–20 mole % of DPPE, indicating a limited miscibility in the gel phase for mixtures having this respective composition (fig. 2).

For the two systems, where the pure compounds have different fatty acid chain lengths, it seems reasonable to suppose a partial immiscibility in the crystalline gel phase. The almost horizontal parts of the 'solidus' curves in the phase diagrams of both systems are an indication for the existence of separate domains of the pure lipids in the same bilayer. The large value of the heat of transition of equimolar mixtures in these systems can be explained assuming different conformations of the polar head groups of phosphorylcholine and phosphorylethanolamine. Phillips et al. [7] have given an explanation for the different hydration behaviour of the two lipids. Based on X-ray investigations of the contribution of the polar group to the X-ray long spacing, they propose an arrangement of the phosphorylethanolamine head group tangential to the plane of the bilayer. The polar group of each molecule is bent up, because the ammonium group is attracted by electrostatic forces to the negatively charged phosphate group of the adjacent molecule. Due to the larger volume of the phosphorylcholine head group and the inability of the $\overset{+}{N}(CH_3)_3$-group to take part in hydrogen bonding, the polar groups of the phosphorylcholine mole-

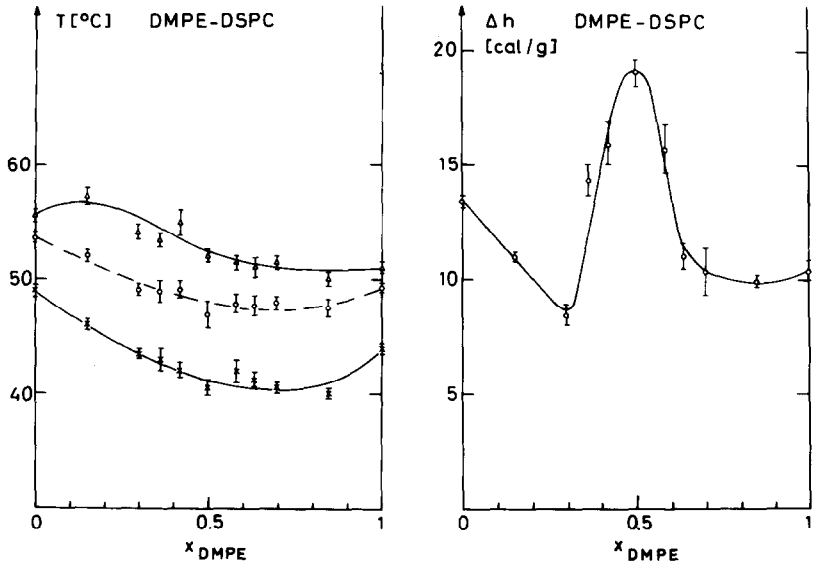

Fig. 4. Equilibrium phase diagram and variation of heat of transition for aqueous dispersions of binary mixtures of DMPE and DSPC (symbols see fig. 2).

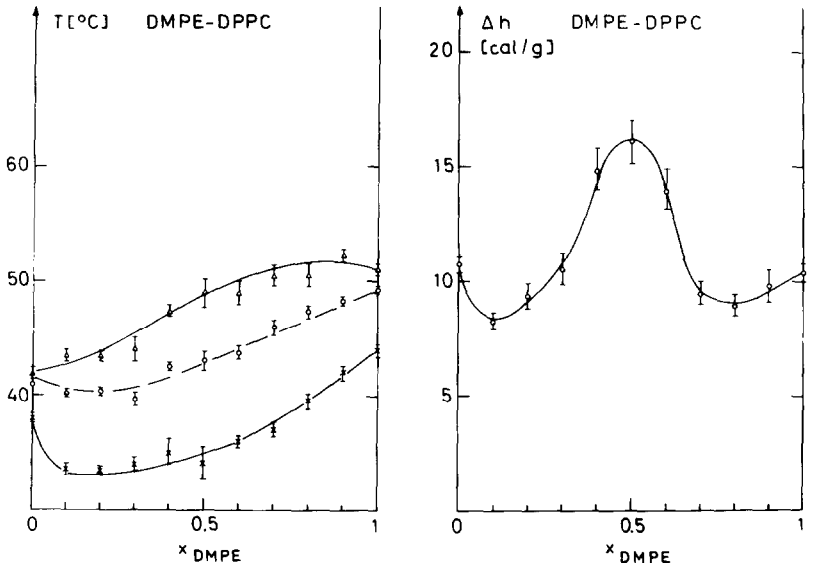

Fig. 5. Equilibrium phase diagram and variation of heat of transition for aqueous dispersions of binary mixtures of DMPE and DPPC (symbols see fig. 2).

cules are extended and their orientation is perpendicular to the plane of the bilayer. Within the temperature range of the phase transition for a mixture of DMPE and DSPC, for instance, where separate domains of DMPE and DSPC exist in the gel phase, the intermolecular attractive forces between the phosphorylethanolamine molecules have to be abolished in order to achieve a homogenous mixture of both components in the liquid crystalline phase. The additional energy required to remove these intermolecular attractive forces in the systems DMPE–DSPC and DMPE–DPPC is responsible for the increase of the heat of transition in mixtures of composition close to a mole fraction of 0.5. Despite the immiscibility in the crystalline gel phase the calorimetric heating curves showed only one endothermic peak. This is in contrast to systems described by other authors [2,8,9]. It might be due to the fact that the differences of the T_m values for the pure compounds in the systems DMPE–DSPC and DMPE–DPPC are only 4.2°C and 8.3°C respectively. The low values for the heat of transition of mixtures with a mole fraction lower than 0.2 and higher than 0.8 for phosphorylethanolamine can be explained by assuming complete miscibility in the gel phase for these mixtures.

Differential scanning calorimetry has the advantage over spectroscopic methods of giving an additional information, i.e., the heat of transition, which makes it possible to determine the size of the cooperative unit involved in the phase transition and to explain different molecular packing of lipids. Thus it becomes a powerful method to investigate the phase behaviour of aqueous dispersions of lipid mixtures.

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