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STUDIES ON THE THERMOTROPIC BEHAVIOR OF AQUEOUS PHOSPHATIDYLETHANOLAMINES *

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The thermal response of aqueous dispersions of a series of synthetic saturated phosphatidylethanolamines was studied by differential scanning calorimetry and by infrared spectroscopy. Dispersions which had not been previously heated above t_m, the temperature of the gel to liquid crystalline transition, showed transitions at a higher temperature, t_{m+h}, having a considerably greater enthalpy change. It is demonstrated that the higher temperature transition is due to the simultaneous hydration and acyl chain melting of these saturated phosphatidylethanolamines. This transition has not been observed in the corresponding phosphatidylcholines.

Introduction

Fully hydrated DLPE, DMPE and DPPE bilayers exhibit gel to liquid crystalline phase transitions, at neutral pH, at 30.5, 49.1 and 63.1°C, respectively [1–3]. In a recent calorimetric study [3] a second transition, at 44°C, was reported for DLPE, which was tentatively assigned to a liquid crystalline to inverted hexagonal transition. This is at variance with the observation that DLPE [4] and DMPE [5] remain in the bilayer phase at temperatures as high as 90°C, although a bilayer to inverted hexagonal transition has been observed for DPPE and DSPE under extreme conditions [6], i.e., at high salt concentration (1 M NaCl) and at elevated temperatures (above 100°C).

In this paper we have reproduced the second, higher temperature transition of DLPE at 44°C and assign it to the simultaneous hydration and acyl chain melting of a poorly hydrated polycrystalline sample. We also demonstrate the occurrence of the same type of transition in DMPE and DPPE, and show that the precise nature of this transition is dependent on the particular polymorph used in the sample preparation.

Experimental procedure

Materials

DLPE was obtained from Calbiochem (La Jolla, CA), Sigma (St. Louis, MO) and Serdary (London, Ontario), DMPE from Calbiochem. DPPE from Calbiochem and from Sigma. All samples were pure by thin-layer chromatography. Different crystalline polymorphs were obtained by recrystallization or slow evaporation from ethanol, chloroform, *n*-butanol, glacial acetic acid, cyclohexane or chloroform/methanol (2 : 1, v/v). Aqueous dispersions of DLPE, DMPE and DPPE for DSC were prepared from 2 mg of the solid phosphatidylethanolamine in 3 ml 0.1 M sodium

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Abbreviations: DLPE, DMPE and DPPE; 1,2-dilauroyl-, 1,2-dimyristoyl- and 1,2-dipalmitoyl-3-*sn*-phosphatidylethanolamine, respectively; t_m, temperature of the gel to liquid crystalline phase transition (m for acyl chain melting); t_{m+h}, temperature of the hydration and chain melting phase transition (h for hydration); DSC, differential scanning calorimetry.

phosphate buffer (pH 7.0) (or in 3 ml 0.03 M sodium borax buffer (pH 9.4)), by vortexing for several minutes at room temperature. The lipid dispersion was immersed for several minutes in an acetone/solid CO_2 bath and then allowed to thaw; the freeze-thawing cycle was repeated three times. The temperature of the sample never exceeded room temperature (22°C) during its preparation. Essentially identical results were obtained with lipid dispersions which were sonicated in such a way that the temperature of the sample never exceeded 22°C.

Methods

Calorimetric data were obtained with a Microcal MC-1 differential scanning calorimeter using a scanning rate of 1 K/min. The sample was equilibrated in the calorimeter at 5°C and a first calorimetric trace was recorded between 5 and 70°C. The sample was cooled in the calorimeter to 5°C and allowed to equilibrate for 1 h, and a second calorimetric trace was recorded over the same temperature interval. Enthalpies were calculated from the peak areas, which were determined by weight. Transition temperatures were obtained from the midpoint of the corresponding endotherm. Infrared spectra of KBr pellets were recorded at a resolution of 1 cm^{-1} on a BOMEM DA3-02 Fourier transform infrared spectrometer equipped with a liquid nitrogen cooled mercury cadmium telluride detector, a high speed vector processor and a PDP-11-03 computer for data processing. Infrared spectra of aqueous dispersions of DLPE, DMPE and DPPE were obtained with a Digilab FTS-11 system. Details of the methods used for sample handling and data processing have been described elsewhere [7].

Results

Microcalorimetry: Phase transitions

It is common practice that phospholipid dispersions are first heated above t_m, the temperature of the corresponding gel to liquid crystalline phase transition, before commencing the actual measurements. Our DSC measurements of DLPE, DMPE and DPPE dispersions which previously had been heated to 70°C showed endothermic transitions (in phosphate buffer at neutral pH) at 30.5, 49.0 and 63.2°C, respectively, in good agreement with the temperatures reported in the literature for the corresponding gel to liquid crystalline phase transitions [1–3].

However, different results were obtained when the DSC measurements were performed on samples which had been dispersed by vortexing and freeze-thawing (or sonicating), without allowing the temperature to reach t_m. As demonstrated by trace A in Fig. 1, DLPE dispersions prepared in this way show no transition around 30°C; instead, one observes a very strong endothermic peak at 44.0°C. At temperatures below 44°C the sample had the appearance of a flocculant suspension, above 44°C it became an opalescent suspension, typical of a well dispersed liquid crystalline phase. After the sample was cooled to 5°C it had the milky appearance of a typical gel phase. When the DSC run was repeated, giving trace B in Fig. 1, the transition at 44°C was completely abolished, and the only endothermic peak at 30.5°C corresponds to the previously reported gel to liquid crystalline phase transition. This peak, however, is less endothermic than the peak at 44°C by a factor of 3.8.

Similar results were obtained with DLPE dispersions at pH 9.4; the high-temperature transition was now shifted to 41.1°C, (Fig. 1 trace C). When the DSC run was repeated the transition occurred at 28.5°C (Fig. 1, trace D). The ratio of the area of the high-temperature peak to that of

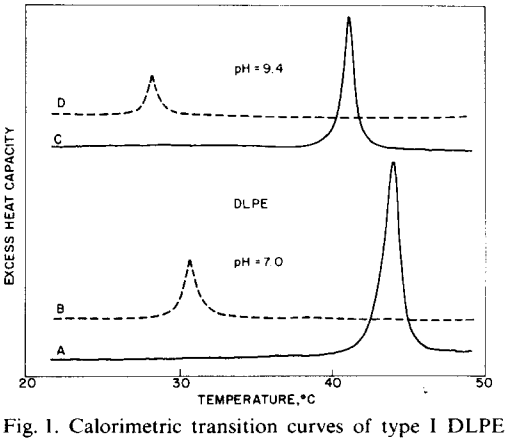

Fig. 1. Calorimetric transition curves of type I DLPE dispersions at two pH values. The solid traces show scans obtained from samples prepared by vortexing at 22°C and freeze-thawing. The corresponding broken traces were obtained after heating the samples to 70°C, then cooling to 5°C.

the low-temperature peak was also 3.8. The reduction in the temperature of the gel to liquid crystalline transition with increasing pH had been previously reported [3]; a similar reduction in the temperature of the more endothermic high temperature peak is observed here.

In Figs. 2C and E we show the DSC traces obtained from DMPE and DPPE dispersions prepared at room temperature, and in Figs. 2D and F the traces obtained after cooling the samples from 70 to 5°C. The behavior in both cases is similar to that observed with DLPE dispersions. The initial flocculant suspensions give highly endothermic transitions (solid traces) at temperatures higher than those previously reported for the gel to liquid crystalline transitions, whereas the peaks obtained after cooling (broken traces) correspond to the reported t_m values. As with DLPE dispersions, the higher temperature transition at t_{m+h} is much more endothermic than the lower temperature transition at t_m. The corresponding transition temperatures and enthalpies are summarized in Table I. However, the temperature difference between the two transitions is clearly reduced by increasing the chain length, and one might expect that at a length of C_{18} or C_{20} the two transitions would occur virtually at the same temperature. ΔH_{m+h} does not appear to vary significantly with pH, in agreement with what was found for the chain melting transition enthalpy, ΔH_m, of saturated phosphatidylethanolamines [3].

During the course of this investigation we found that certain commercial phosphatidylethanolamines gave two high-temperature endothermic peaks. For DLPE dispersions at pH 7.0 these were observed at 43.7 and 42.0°C, as illustrate in Fig. 2A. This was shown to be the case when the solid phosphatidylethanolamine was in a certain polymorphic form, which we designated as type II (see below). With type II DMPE and DPPE the high temperature peak is asymmetric, a clear splitting was only observed in type II DLPE. The DSC traces A to D in Fig. 1 and C to F in Fig. 2 were all obtained from type I crystals.

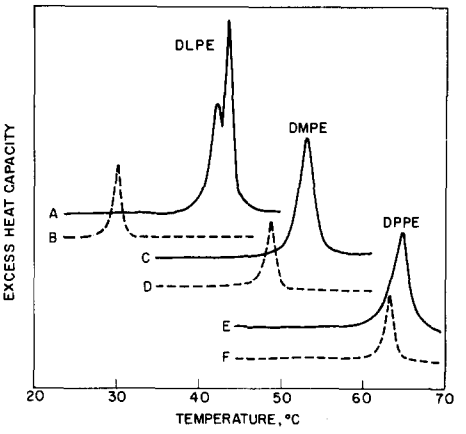

Fig. 2. Calorimetric transition curves of type II DLPE (A and B), of type I DMPE (C and D), and of type I DPPE dispersions (E and F), all at pH 7.0. See captions to Fig. 1 for explanation of solid and broken traces.

Infrared spectroscopy: Polymorphism

The occurrence of different polymorphs of anhydrous phosphatidylethanolamines has been known since 1966 [8]. The crystal form is dependent on the solvent used for crystallization and to date three polymorphs have been identified [9–11]. Two of these are highly crystalline with the acyl chains rigidly packed in an orthorhombic ($O_\perp$ or

TABLE I

TRANSITION TEMPERATURES AND TRANSITION ENTHALPIES OF SATURATED PHOSPHATIDYLETHANOLAMINES

Lipid	t_m (°C)	t_{m+h} (°C)	A_{m+h}/A_m	ΔH_m (kcal/mol)	ΔH_{m+h} (kcal/mol)
DLPE	30.5	44.0	3.8 [a]	3.5 [b]	13.3
DMPE	49.0	53.2	2.6 [a]	5.7 [b]	14.8
DPPE	63.2	64.9	2.1 [a]	8.8 [b]	18.5

[a] Ratio of the area (as weight) of the two peaks.
[b] Data from Ref. 3.

HS1) subcell. In the third form the acyl chains are packed in a hexagonal subcell and have considerable mobility about their long axes.

The two classes of polymorphs give highly distinctive infrared spectra [8]. Fig. 3 shows the infrared spectrum of DPPE recrystallized from ethanol (bottom) and from chloroform (top). We designate the crystalline polymorph obtained from ethanol as type I and that obtained from chloroform as type II. Individual frequencies with assignments are listed in Table II. Virtually identical spectra were obtained from the corresponding DMPE and DLPE polymorphs. The type I spectra exhibit crystal field splitting of the CH_2 scissoring and CH_2 rocking bands which is characteristic for acyl chains packed in an orthorhombic methylene subcell [14,15]; such splitting is absent in the type II spectra which exhibit single CH_2 scissoring and rocking bands at 1469 and 720 cm^{-1} characteristic of the more loosely packed hexagonal methylene subcell [14,15]. From a comparison of all the features of type I and type II spectra and particularly of the modes sensitive to the chain packing, it can be concluded that type I is the more rigidly packed polymorph. Many of the differences observed between type I and type II spectra are due to headgroup vibrations and indicate that not only the acyl chain packing, but also the structure and conformation of the phosphoethanolamine moiety are different. Recrystallization from chloroform/methanol (2 : 1, v/v) also gave type I crystals, as did precipitation from chloroform with acetone. However, the use of other solvents, such as glacial acetic acid, *n*-butanol and cyclohexane, led to mixed crystals, as judged from the corresponding infrared spectra. Similar variations in crystal form were found in commercial samples, and are clearly a function of the final purification step used by the various manufacturers.

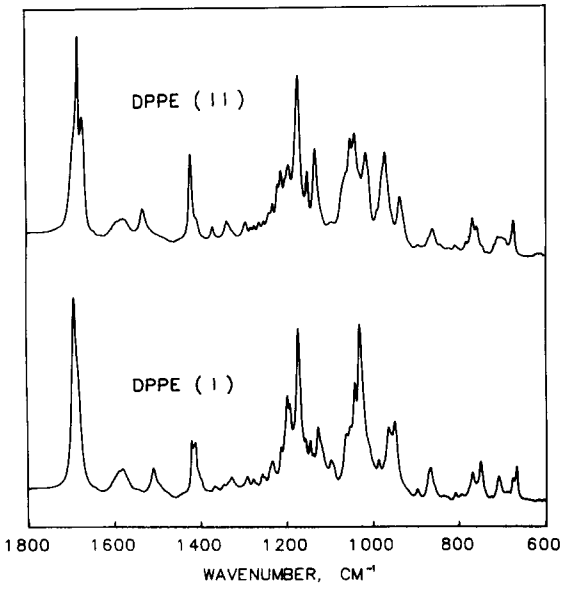

Fig. 3. Fourier transform infrared spectra of type I and type II DPPE crystals.

TABLE II

SELECTED INFRARED ABSORPTION BANDS OF TWO CRYSTALLINE POLYMORPHS OF DPPE

Frequencies (in cm^{-1}) are rounded off to the nearest integer; w, m, s and v stand for weak, medium, strong and very.

Type I	Type II	Description of vibration [a]
2957 m	2957 m	CH_3 asym. stretch
2918 vs	2917 vs	CH_2 antisym. stretch
2873 m	2873 m	CH_3 sym. stretch
2850 vs	2849 vs	CH_2 sym. stretch
1743 vs	1733 vs 1722 s	C = O stretch
1638 w	1625 w	NH_3^+ asym. bend
1561 m	1581 m	NH_3^+ sym. bend
1472 s 1463 s	1469 s	CH_2 scissoring
1420 w	1418 w	αCH_2 scissoring
1380 w	1384 w	CH_3 sym. bend
1225 vs	1220 vs	PO_2^- antisym. stretch
1082 vs	1088 s	PO_2^- sym. stretch
1016 m 1002 s	1018 s	CCN^+ antisym. stretch
919 m	908 m	CCN^+ sym. stretch
822 m 802 s	815 m 805 m	POC antisym. stretch
762 m	750 m	POC sym. stretch
729 m 719 m	720 m	CH_2 rock
559 m	549 m	PO_2^- rock

[a] See Refs. 12 and 13.

Discussion

The object of this study was to clarify the nature of the high temperature transition of DLPE,

and to establish whether DMPE and DPPE undergo a similar transition. Our calorimetric data show clearly that all three exhibit the high-temperature transition at t_{m+h}. However, once the sample has been taken to a temperature above that of this transition, further cooling and heating result only in the observation of the previously reported gel to liquid crystalline phase transition at t_m.

Infrared spectra of aqueous DLPE, DMPE and DPPE dispersions support the postulate that in all cases the lower temperature transition is from the gel phase to the liquid crystalline phase. The major structural change at this transition, as detected by the temperature dependence of the infrared spectra, is the introduction of a large number of *gauche* rotamers into the acyl chains which is characteristic for the chain melting phenomenon [16].

Samples which exhibit only the high temperature transition have infrared spectra which at temperatures below t_{m+h} are almost identical with those of the anhydrous solid. This shows that sonication or freeze-thawing does not result in hydration of the sample. The infrared spectra show that at the high-temperature transition there is both hydration of the headgroup and the appearance of a large number of *gauche* rotamers in the acyl chains. Therefore we assign this transition to the concomitant hydration and chain melting of the solid, polycrystalline, phosphatidylethanolamine. Such an assignment is supported by the considerably higher enthalpy change of this transition compared to that observed at t_m. In fact, this transition is analogous to the coagel to micelle transition of poorly hydrated surfactants [17,18]. The occurrence of this transition is independent of whether the dispersion is prepared from type I or type II crystals; however, in the latter case we observed a twin transition. As yet we have no explanation for the splitting of this transition when the sample is prepared from type II crystals. A conclusion of practical value is that the heating of dispersions of saturated phosphatidylethanolamines to temperatures above t_m but below t_{m+h} does not result in hydration. Complete hydration occurs only when the aqueous dispersions are taken to temperatures above t_{m+h}.

Furthermore, the hydration phenomenon of the phosphoethanolamine headgroup is expected to be less sensitive to the increase in chain length than the chain melting phenomenon. This is confirmed by the calorimetric measurements which show that as the acyl chain length is increased, the temperature difference between the two transitions decreases. In these terms the total enthalpy change may, to a first approximation, be written as $\Delta H_{total} = \Delta H_{head} + \Delta H_{chain}$. Taking ΔH_{chain} as being that of the lower temperature transition, $\Delta H_{head} = 9.8$, 9.1 and 9.7 kcal/mol for DLPE, DMPE and DPPE, respectively. This relatively constant figure further supports our approach to this transition.

Finally, the fact that this hydration-melting phase transition is observed in phosphatidylethanolamines but not in phosphatidylcholines is most likely due to the different nature of the water-headgroup interactions in these phospholipids. Phosphatidylcholines are highly hygroscopic molecules, and the phosphate groups of adjacent molecules are linked into ribbons by water molecules. In solid phosphatidylethanolamines the ethanolamine groups link adjacent phosphate ribbons into a very rigid head group network which does not contain or require water molecules [19].

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