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CALORIMETRIC INVESTIGATION OF CONVERSION TO THE MOST STABLE SUBGEL PHASE OF PHOSPHATIDYLETHANOLAMINE-WATER SYSTEM

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Abstract

The conversion of either the gel or the liquid crystal phase to the most stable subgel phase in dimyristoylphosphatidylethanolamine (DMPE)–water system at a water content of 25 mass% was studied by differential scanning calorimetry and isothermal calorimetry. The calorimetric experiments were performed for two samples depending on whether the thermal treatment of cooling to –60°C was adopted or not. In DSC of varying heating rate, exothermic peaks due to the partial conversion were observed at either temperatures just below the gel-to-liquid crystal phase transition at 50°C or temperatures where the liquid crystal phase is present as a metastable state. The enthalpies of conversion for both the gel and the liquid crystal phase were measured directly by the isothermal calorimetries at 47 and 53°C, respectively, where the exothermic peaks were observed by DSC and were compared with the enthalpy difference between the gel and subgel phases and that between the liquid crystal and subgel phases.

Keywords: DMPE–water system, DSC, isothermal calorimetry, subgel phase

Introduction

Phospholipids are major components for a bilayer structure of biomembranes. Diacylphosphatidylethanolamine, as well as diacyl-phosphatidylcholine, constitutes the majority of the total phospholipids in biomembranes. When these phospholipids are suspended in aqueous medium, they are present in multilamellar dispersions, and the lipid bilayer lamellae usually exist in either a gel or a liquid crystal phase depending on temperature. However, it is generally accepted that the gel phase is metastable. Thus, the gel phase converts into a more stable phase, generally called a subgel phase. However, the conversion is achieved only when the thermal annealing adopted for the gel phase is adequate. From this viewpoint, in our previous studies, the annealing conditions necessary for the conversion of the gel to subgel phases have been investigated for various phospholipid–water systems [1–10].

In Fig. 1, the relative enthalpy of resultant subgel phases of dimyristoylphosphatidylethanolamine (DMPE)–water system (25 mass% of water) obtained by adequate annealing at different temperatures [4, 5, 9, 10] are shown by comparison with

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the gel and liquid crystal phases. As shown in Fig. 1, the subgel phase of the DMPE–water system is present in two types, designated the *L*- and *H*-subgel phases [11–13]. Furthermore, a noticeable point in Fig. 1 is that the liquid crystal phase is also present as a metastable supercooled state although its occurrence is limited to temperatures between the T_M (gel-to-liquid crystal phase) and T_H (*H*-subgel-to-liquid crystal phase) transitions. This suggests the possibility for the metastable liquid crystal phase to convert to the *H*-subgel phase, similarly to the gel phase previously reported by us [4, 9, 10]. From this viewpoint, in the present study, focusing on the processes of nucleation and nuclear growth [13], the conversion of the liquid crystal to the *H*-subgel phase in a full hydration [9] for the DMPE–water system was investigated by differential scanning calorimetry (DSC) and isothermal calorimetry and compared with the conversion of the gel to the *H*-subgel phase.

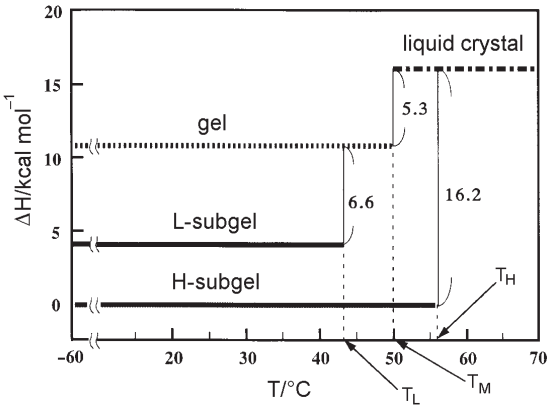

Fig. 1 Schematic diagram of the relative enthalpy (Δ*H*) *vs.* temperature (*T*) for the DMPE–water system at water content of 25 mass%, constructed from the transition enthalpies and temperatures associated with the lipid phase transitions. The designations T_L, T_M, and T_H show the phase transitions of the *L*-subgel-to-gel, gel-to-liquid crystal, and *H*-subgel-to-liquid crystal phases (1 cal= 4.184 J)

Materials and methods

1,2-Dimyristoyl-sn-glycero-3-phosphatidylethanolamine (DMPE) used in the present study was purchased from Sigma Co. (St. Louis, MO). The DMPE (approximately 30 mg) in a high pressure crucible cell (for a DSC apparatus) was dehydrated under a high vacuum for at least 3 days and then the crucible cell containing the dehydrated lipid was sealed off in a dry box filled with dry N_2 gas and weighed with a microbalance. By reference to the water distribution diagram previously reported by us [14], a fully hydrated sample at a water content of 25 mass% [(g water)/(g lipid+g water)] was prepared by adding the desired amount of water to the dehydrated lipid and annealed by repeating thermal cycling at temperatures above and below the T_M transition of the gel to the liquid crystal phase to ensure homogeneous mixing.

DSC was performed with a Mettler TA-4000 apparatus for the sample in the high pressure crucible cell (pressure resistant to 10 MPa) at different heating rates of 0.1, 0.2, 0.5, 1.0, and 1.5°C min^{-1}. The Mettler apparatus makes it possible to be used for isothermal calorimetry where the magnitude of baseline noise is a level of $2 \cdot 10^{-6}$ cal s^{-1} (Figs 3 and 4), so that it was used in an isothermal mode at desired temperatures to measure the enthalpies of conversion of either the gel or the liquid crystal phase to the respective *H*-subgel phases.

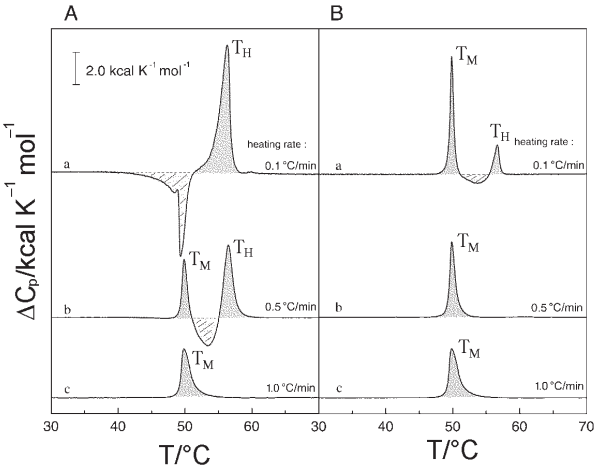

Fig. 2 Typical DSC curves at varying heating rate for the DMPE–water system at 25 mass%. A is results for the sample which was cooled to –60°C before the beginning of DSC, and B is results for sample which has not experienced such a low temperature cooling. The designations T_M and T_H show the endothermic transition peaks of the gel-to-liquid crystal and the *H*-subgel-to-liquid crystal phases, respectively. Heating rate (°C min^{-1}): a – 0.1, b – 0.5, c – 1.0

Results and discussion

In Figs 2A and B, results of DSC of varying heating rates are compared for two samples A and B of the DMPE–water system at a water content of 25 mass%. These samples differ in the history of thermal treatment. Thus, the sample A was cooled from a liquid crystal phase temperature of 70 to –60°C and was then kept at the temperature for at least 6 h before beginning the DSC. However, the sample B has never experienced such a low temperature. Thus, the sample B was cooled from 70 to 20°C, immediately after which the DSC was started.

When the sample A is heated at a rate as slow as 0.1°C min^{-1}, as shown in Fig. 2A(a), the exothermic peak is observed to extend over a wide temperature range of 43 to 50°C at a temperature just below the T_M transition and the peak is successively followed by the endothermic peak of the *H*-subgel-to-liquid crystal phase (T_H) transition. Nearly the same thermal behavior is observed at a heating rate of

0.2°C min^{-1} (no data in Fig. 2A). However, as shown in Fig. 2A(b), for a rate of 0.5°C min^{-1}, the exothermic peak is observed in a temperature region between the T_M and T_H transition peaks. For rates higher than 1°C min^{-1}, neither exotherm nor endotherm other than the T_M transition peak is observed, as shown in Fig. 2A(c). On the other hand, for the sample B, as shown in Fig. 2B(a), a faint exothermic peak is observed at temperatures between the T_M and T_H transition peaks only when a heating rate is as slow as 0.1°C min^{-1}. However, above this rate, only the T_M transition peak is observed, as shown in Fig. 2B(b) and (c).

In our previous papers [4, 9, 10], a two-step annealing for the processes of nucleation at around –60°C and nuclear growth at temperatures of 45 to 48°C just below the T_M transition was adopted to complete the conversion of the gel to the *H*-subgel phase. The annealing conditions were based upon the fact that the exothermic phenomenon due to the stabilization of the gel phase is observed only for the sample which has experienced the low temperature. The same behavior is confirmed in Fig. 2A(a) for the sample A of the present study. Another point noticeable for the sample A is that as shown in Fig. 2A(b), the exothermic phenomenon is observed at the liquid crystal temperature in the place of the gel temperature when the higher heating rate of 0.5°C min^{-1} was used. Furthermore, by reference to Fig. 1, it becomes evident that the exothermic event occurs at specific temperatures where the liquid crystal phase is present as a metastable state. In DSC, the period for a sample to stay at a desired temperature becomes longer as the heating rate is slower. This indicates

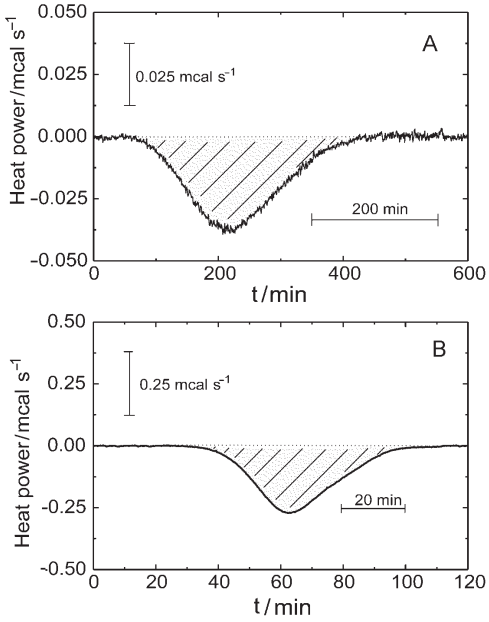

Fig. 3 Isothermal calorimetries at (A) 47°C of the gel phase temperature and (B) 53°C of the liquid crystal phase temperature for the samples which have experienced the cooling to –60°C. The differential heat power is shown as a function of time

that the stay for the heating rate of 0.5°C min^{-1} is long enough to facilitate nuclear growth at the liquid crystal temperature, but is not enough for the gel temperature. As a result, as shown in Fig. 2A(b), no exothermic event is observed at the gel temperature as long as the heating rate of 0.5°C min^{-1} is used in the place of that of 0.1°C min^{-1}. However, the stay for the heating rates higher than at least 1°C min^{-1} is too short even for the liquid crystal temperature, so that no exothermic event is observed over all the temperatures, as shown in Fig. 2A(c). These results suggest that nuclear growth for the sample A proceeds at the liquid crystal temperature more rapidly than at the gel temperature. On the other hand, the faint exothermic event for the sample B shown in Fig. 2B(a) suggests that nucleation occurs at the limited temperature ranges where the liquid crystal phase exists in the metastable state.

On the basis of the results of DSC, isothermal calorimetries were performed for the samples A and B at two different temperatures of 47 and 53°C where the exothermic phenomenon was observed. Thus, in the case of the sample A, it was heated from –60 to 47°C at a rate as high as 20°C min^{-1}, and after achieving complete return to baselines the differential heat power between the sample and reference cells was measured as a function of time. The same procedure was applied to the isothermal calorimetry of sample A at 53°C. Results of the calorimetries at 47 and 53°C for the sample A are shown in Fig. 3. In Fig. 3A at 47°C of the gel temperature, the exothermic effect is shown to appear in about 40 min after flat base-

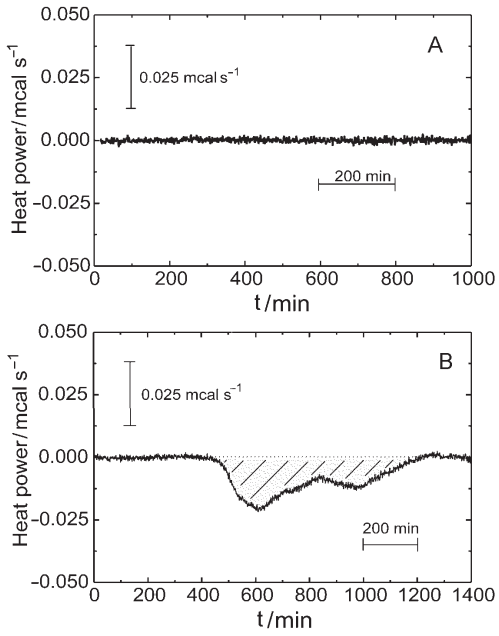

Fig. 4 Isothermal calorimetries at (A) 47°C of the gel phase temperature and (B) 53°C of the liquid crystal phase temperature for the samples which were cooled from 70°C to the respective desired temperatures. The differential heat power is shown as a function of time

lines were attained and then to continue over periods of about 400 min. On the other hand, in Fig. 3B at 53°C of the liquid crystal temperature, the exotherm begins in 20 and then completes in 80 min. Thus, both of the periods necessary for the beginning and the completion at the liquid crystal temperature are shorter, compared with the corresponding periods at the gel temperature. However, the amount of heat released per second is fairly greater for the liquid crystal temperature than for the gel temperature. These results are consistent with those obtained by DSC, i.e., the rate of nuclear growth at the liquid crystal temperature is faster than that at the gel temperature.

Figures 4A and B show results of the isothermal calorimetries performed at 47 and 53°C for the sample B which was cooled from 70°C to the respective desired temperatures. In Fig. 4A for the gel phase temperature of 47°C, no exothermic effects are observed up to at least 2 weeks studied. However, in Fig. 4B for the liquid crystal temperature of 53°C, exothermic processes of two-step modes continue up to periods of 15 h after flat baselines extending over periods of 7 h. Considering that the sample B has not experienced the low temperature, it is presumed that nucleation for this sample B proceeds slowly during the waiting as long as 7 h observed before the beginning of the exothermic processes. Furthermore, the two-step heat effect in Fig. 4B suggests that on the way of attainment of the final destination of *H*-subgel phase, there is a relatively stable intermediate state. Thus, the liquid crystal phase of the sample B converts to the most stable *H*-subgel phase by way of the intermediate state. The conversion process is distinct from that for sample A at the same liquid crystal temperature shown in Fig. 3B. Table 1 summarizes the enthalpies of conversion of either the gel or the liquid crystal phase to the respective *H*-subgel phases, which were calculated by integration of the exothermic peaks observed in the present isothermal calorimetries (Figs 3A, 3B and 4B).

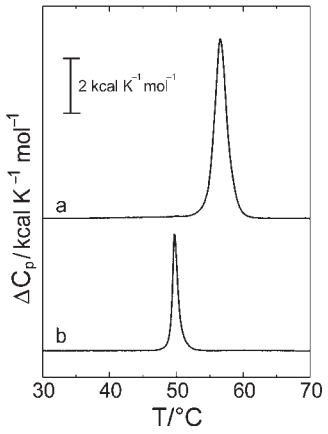

Fig. 5 Typical DSC curves at a heating rate of 0.5°C min^{-1}, observed after the isothermal calorimetries. Curve a is common for the samples which present the exothermic effects shown in Figs 3A, 3B, and 4B. Curve b is obtained from the sample, for which no heat effect is observed as shown in Fig. 4A

Table 1 Enthalpies of conversion (ΔH) of either the gel or the liquid crystal phase to the respective *H*-subgel phases, obtained by isothermal calorimetries at 47 and 53°C, respectively

T/°C	Enthalpies of conversion[a] ΔH/kcal (mol of lipid)$^{-1}$	
	Sample A[b]	Sample B[c]
47	10.0	0
53	16.1	16.9

[a]The mean fluctuation in the individual enthalpies is approximately ±0.2 kcal mol^{-1} lipid
[b]Sample A was kept at –60°C for at least 6 h and was then heated to the respective desired temperatures of 47 and 53°C for the isothermal calorimetry
[c]Sample B was cooled from 70°C of the liquid crystal temperature to the respective desired temperatures for the isothermal calorimetry

After the isothermal calorimetries, all the samples were directly cooled to 20°C and DSC was then started in a heating direction. Figure 5 shows two typical DSC curves a and b characterized by the T_H transition at 57°C and the T_M transition at 50°C, respectively. Curve a is common to the samples which all showed the exothermic effect in the isothermal calorimetries (Figs 3A, 3B and 4B), indicating a complete conversion to the *H*-subgel phase. Curve b is a result for the sample which did not show any heat effect in the isothermal calorimetry (Fig. 4A). The transition enthalpies obtained by the DSC, shown in Fig. 1, were compared with the conversion enthalpies by the isothermal calorimetry shown in Table 1 and the following results were summarized. The enthalpy difference between the gel and *H*-subgel phases estimated from the T_H and T_M transition enthalpies is 10.9 (=16.2–5.3) kcal per mole of lipid and is close to the enthalpy of conversion of the gel to the *H*-subgel phase (at 47°C), 10.0 kcal per mole of lipid, for the sample A shown in Table 1. Similarly, the enthalpy difference between the liquid crystal and *H*-subgel phases, 16.2 kcal mol^{-1} lipid, comparable to the T_H transition enthalpy, is nearly equal to the enthalpies of conversion of the liquid crystal to the *H*-subgel phase (at 53°C) which are 16.1 and 16.9 kcal mol^{-1} for the samples A and B, respectively, as shown in Table 1.

References

1 M. Kodama, H. Hashigami and S. Seki, Thermochim. Acta, 88 (1985) 217.
2 M. Kodama, Thermochim. Acta, 109 (1986) 81.
3 M. Kodama, H. Hashigami and S. Seki, J. Colloid Interface Sci., 117 (1987) 497.
4 M. Kodama, H. Inoue and Y. Tsuchida, Thermochim. Acta, 266 (1995) 373.
5 H. Aoki and M. Kodama, J. Thermal Anal., 49 (1997) 839.
6 M. Kodama, T. Miyata and T. Yokoyama, Biochim. Biophys. Acta, 1168 (1993) 243.
7 M. Kodama, H. Aoki and T. Miyata, Biophys. Chem., 79 (1999) 205.
8 M. Kodama and H. Aoki, Recent Res. Dev. Biophys. Chem., 1 (2000) 27.
9 M. Kodama, H. Kato and H. Aoki, Thermochim. Acta., 352–353 (2000) 213.
10 M. Kodama and H. Aoki, in 'Thermal Behavior of Dispersed Systems', N. Garti, Ed., Marcel Dekker Inc., New York 2000, p. 247.
11 H. Chang and R. M. Epand, Biochim. Biophys. Acta, 728 (1983) 319.

12 S. Mulukutla and G. G. Shipley, Biochemistry, 23 (1984) 2514.
13 D. A. Wilkinson and J. F. Nagle, Biochemistry, 23 (1984) 1538.
14 M. Kodama, H. Aoki, H. Takahashi and I. Hatta, Biochim. Biophys. Acta, 1329 (1997) 61.