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THE BEHAVIOR OF WATER MOLECULES IN SEMI-CRYSTALLINE BILAYER STRUCTURE OF PHOSPHATIDYLETHANOLAMINE DSC study

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Abstract

The crystalline phase of dimyristoylphosphatidylethanolamine (DMPE)-water system was obtained by annealing the gel phase at around –5 °C for periods up to 30 days. It was investigated by differential scanning calorimetry and negative-stain electron microscopy, particularly focusing on the behavior of water molecules. The crystalline phase showed a two-dimensional ribbon-like structure composed of regularly-stacked lamellae with an interlamellar spacing narrower than that of the gel phase. The conversion of the gel to crystalline phases on annealing was accompanied by a change in the bonding model of water molecules from a loosely-bound interlamellar water to a more loosely-bound water outside the lamellae. Ice-melting curves were deconvoluted using a computer program and different structures of water were estimated from enthalpy changes of each deconvoluted component. In accordance with a micrograph, only the loosely-bound water of one molecule of H_2O per lipid was shown to be located between lamellae of the crystalline phase.

Keywords: crystalline phase, differential scanning calorimetry, dimyristoylphosphatidylethanolamine-water system, electron microscopy, interlamellar water molecules

Introduction

Phospholipids, main constituents of biomembranes, form the fundamental bilayer structure. There are a great variety of phospholipids, in which both phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are widely distributed as major phospholipid components of biomembranes. PE is characterized by a small geometrical size of the head group and intermolecular hydrogen-bonding formed between adjacent head groups [1]. These properties of PE cause a closer packing and less hydration of the head groups at bilayer surface, compared with the case of PC [2]. Based upon these differences in interactions of lipid–lipid and lipid–water, the gel phase of dimyristoyl (DM) PE transforms to the liquid crystal phase at 25 °C

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higher temperature than that of DMPC. Furthermore, our recent calorimetric and X-ray diffraction studies [3] have revealed that a fully hydrated DMPE gel phase interposes 6 interlamellar water molecules per lipid between adjacent bilayers, in contrast to 10 interlamellar water molecules for a fully hydrated DMPC gel phase [4, 5]. Figure 1 shows a relative enthalpy (ΔH) *vs.* temperature curve (t) of DMPE–water system previously reported by us [6]. The most remarkable feature of the DMPE–water system shown in Fig.1 is that in the presence of excess water, there are two types of crystalline phases of different stabilities. A stable one transforms directly into the liquid crystal phase without passing through the gel phase and a less stable one transforms into the liquid crystal phase via the gel phase [7, 8]. The behavior of the less stable semi-crystalline phase in DMPE–water system is similar to that of bilayer crystalline phase (so-called subgel phase) in DMPC–water system. Furthermore, our previous paper [6] has revealed that the stable crystalline phase of DMPE lacks freezable interlamellar water molecules, indicating that a conversion of the gel phase into a closely-packed crystalline state involves a change in water structure from the interlamellar to bulk free waters.

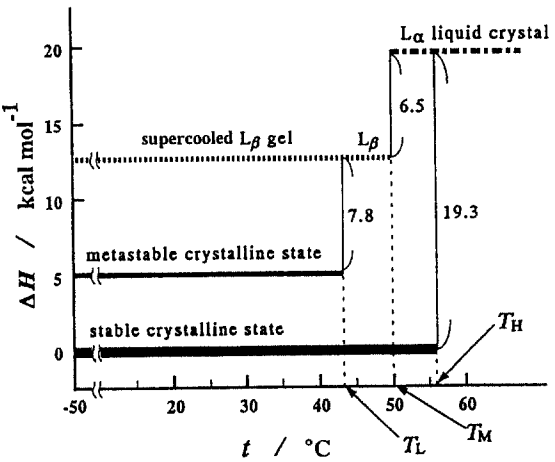

Fig. 1 Schematic diagram of relative enthalpy (ΔH) *vs.* temperature (t) curves in DMPE–water system. T_L, T_M and T_H represent transition temperatures of metastable crystalline-to-gel phases, gel-to-liquid crystal phases and stable crystalline-to-liquid crystal phases, respectively. Enthalpy changes associated with these transitions are shown in this figure (1 cal corresponds to 4.184 J)

In the present study, the interaction of DMPE and water molecules in the metastable crystalline phase shown in Fig. 1 was investigated from thermotropic behavior of the lipid phase transition and ice-melting of frozen water.

Materials and methods

1,2-dimyristoyl-3-*sn*-phosphatidylethanolamine (DMPE) was purchased from Sigma and used without further purification. Thin-layer chromatography of this

lipid showed a single spot. The DMPE, which was transferred to the high-pressure crucible cell of a Mettler differential scanning calorimeter, was dehydrated under high vacuum at a room temperature. The crucible cell containing the dehydrated DMPE was sealed off in a dry box filled with dry N_2 gas and then weighed by a microbalance (Mettler M3). A sample of DMPE–water mixture at a water content of 29.9% (=15[H_2O]/[lipid]), which provides a fully hydrated gel phase [3], was prepared by using a microsyringe to add a desired amount of water to the dehydrated compound (~50 mg). The sample, which was preheated at temperatures above the gel-to-liquid crystal transition, was cooled to –5 °C and annealed there for different periods up to 30 days. After annealing for a desired period, the sample was cooled to –60 °C for the differential scanning calorimetry (DSC) measurements. DSC measurements were carried out with a Mettler TA-4000 instrument at a heating rate of 1.0 °C min^{-1}.

Negative-stain electron microscopic experiments with sodium phospho-tungstate (*pH* approx.7) were performed with a JEOL JEM-2000EX electron microscopy operated at 200 kV at around 20 °C.

Results and discussion

Figure 2 shows typical thermotropic behaviors of DMPE and water at temperatures ranging from –40 to 80 °C observed for different periods of annealing at –5°C.

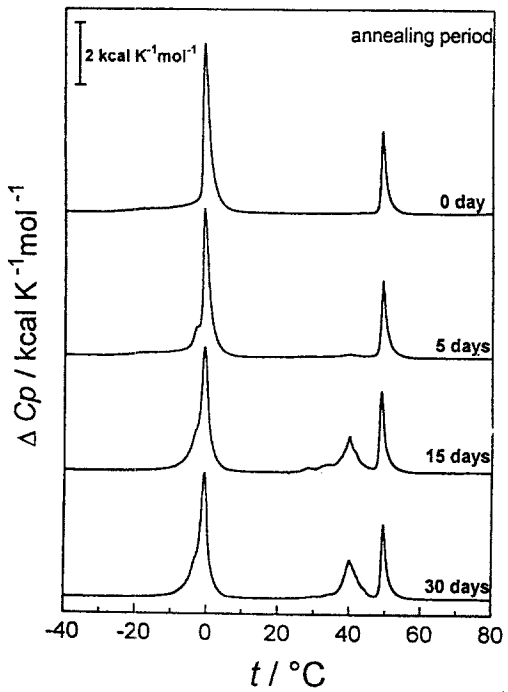

Fig. 2 Variation of thermotropic behavior of DMPE–water system with increase in period of annealing at –5 °C (1 cal corresponds to 4.184 J)

Focusing on the lipid phase transition, annealed samples (annealing periods of 5, 15, 30 days) show a low-temperature transition (T_L) at around 43 °C, successively followed by the gel-to-liquid crystal phase transition at 50 °C. The T_L transition peak grows with increasing annealing period up to 30 days, although the peak is slightly changing over the period from 15 to 30 days. Based on the ΔH *vs.* temperature curve shown in Fig. 1, the thermotropic behavior shown in Fig. 2 is explained as follows: the gel phase of the unannealed sample (annealing period of 0 day) exists in a metastable state at temperatures below the T_L transition and coverts gradually into a more stable state by annealing for adequate periods at temperature of –5 °C. The annealing temperature is quite different from that for a conversion of the gel phase to the most stable crystalline phase (Fig. 1), in which a two-step annealing of different temperatures was adopted for a nucleation (–50 °C) and nuclear growth (47 °C)[6].

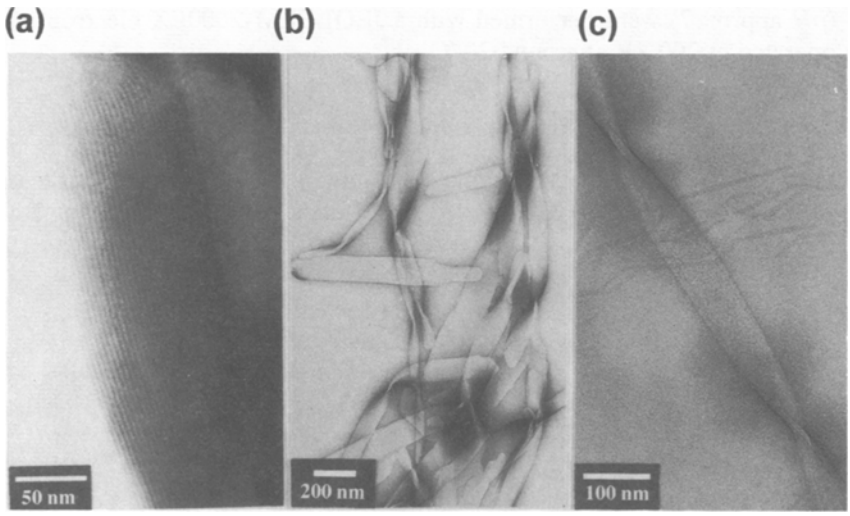

Fig. 3 Negative stain-electron micrographs of gel (a) and ribbon-like crystalline phases (b) of DMPE–water system. Enlarged micrograph (c) shows a part of ribbon-like structure

Electron micrographs of the more stable, new phase of the annealed sample are shown in Fig. 3, in comparison with that of the metastable gel phase of the unannealed sample. The profiles of these two phases fairly differ. The gel phase (Fig. 3a) shows a multilamellar structure composed of the lipid bilayers (white regions) and interlamellar water regions (black regions). In this contrast, the more stable phase (Fig. 3b) is most likely a two-dimensional, ribbon-like crystalline structure composed of 3–4 lamellae, in which the water layer sandwiched between the bilayers is detectable. However, an enlarged micrograph (Fig. 3c) of the more stable phase shows a narrowing of the water layer, compared with that of the gel phase.

In connection with a narrowing of the interlamellar water phase, the behavior of ice-melting peaks given in Fig. 2 is shown to change with increasing annealing pe-

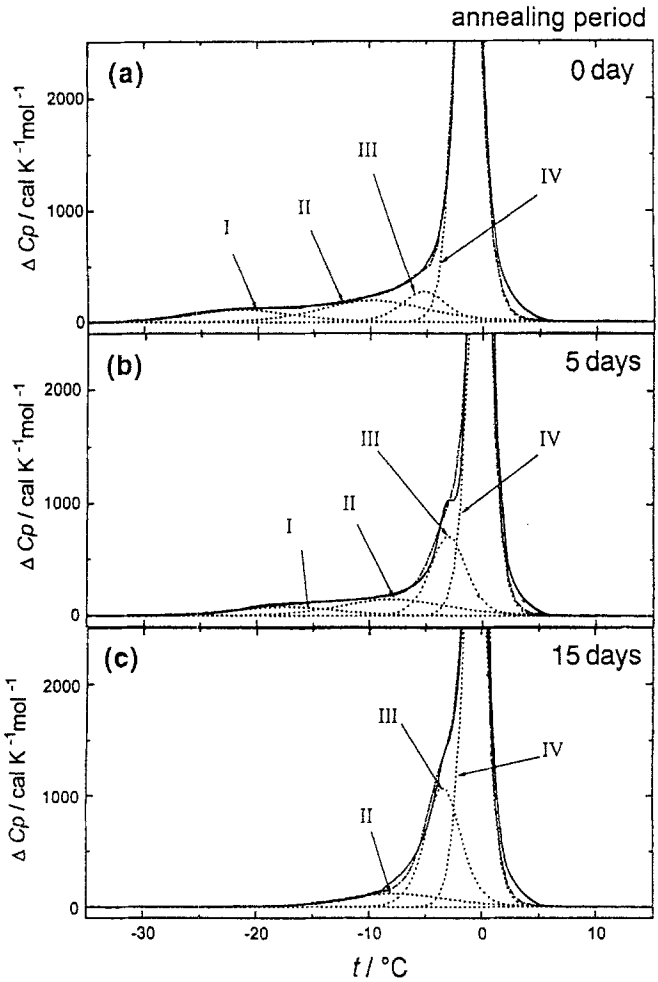

Fig. 4 Deconvolution analyses of ice-melting curves observed during the periods of annealing at –5°C. Four deconvoluted curves I, II, III and IV as well as a theoretical curve are shown by dotted lines (1 cal corresponds to 4.184 J)

riod, although a limiting ice-melting peak is obtained at the annealing period of 15 days. Thus, the conversion of the gel into more stable crystalline phases during annealing involves a change in the bonding model of water molecules. In order to make clear this phenomenon, each of ice-melting peaks shown in Fig. 2 was deconvoluted according to a computer program attached to a Microcal calorimeter [6]. Results of the deconvolution analyses are shown, in an enlarged scale, in Fig. 4. The present deconvolution analyses are based upon such a way as minimizing the total number of deconvoluted curves. For the unannealed gel phase (Fig. 4a), the ice-melting peak starting from around –35°C is deconvoluted into four components characterized by curves I, II, III and IV. Our previous paper [3] has revealed that

the deconvoluted ice-melting curves I, II and III originates from freezable water molecules, which are loosely bound to the lipid head groups and presumably exist between lamellae. Furthermore, according to our previous paper, the number of the freezable interlamellar water molecules in a fully hydrated gel phase of the present sample (29.9%) is revealed to be 3.7 H_2O per lipid. On the other hand, the deconvoluted ice-melting curve IV originates from bulk water. With an increase in annealing period, as shown in Fig. 4b and c, a marked growth of the deconvoluted curve III is observed, simultaneously with diminutions not only of the deconvoluted curves I and II but also of the deconvoluted curve IV. This behavior is more clearly recognized by Fig. 5 where enthalpy changes, ΔH_1, ΔH_2, ΔH_3 and ΔH_4, of the deconvoluted ice-melting curves I, II, III and IV per 1 mol of lipid are, respectively, plotted against annealing period. For the fully hydrated gel phase of unannealed sample shown in Fig. 5, a sum of the enthalpy changes of ΔH_1(5.6 kJ), ΔH_2(9.9 kJ) and ΔH_3(5.6 kJ), that is, 21.1 kJ per 1 mol of lipid, is shown to be occupied by 3.7 moles of freezable interlamellar water molecules mentioned above. Accordingly, from the average molar ice-melting enthalpy (5.7 kJ mol^{-1}) of these water molecules, the numbers of water molecules participating in the deconvoluted curves I, II and III are estimated to be approximately $1H_2O$, $1.7H_2O$ and $1H_2O$ per lipid, respectively. On the other hand, the more stable crystalline phase of the annealing period of 30 days shows about 3.7 times larger ΔH_3, compared with that of the gel phase, indicating that during the periods of annealing, the number of water molecules characterized by the deconvoluted curve III is increased by about $2.7H_2O$ per lipid. In this contrast, ΔH_1, ΔH_2 and ΔH_4 are decreased during the periods of annealing. From these decreased enthalpy values, the number of each of water mole-

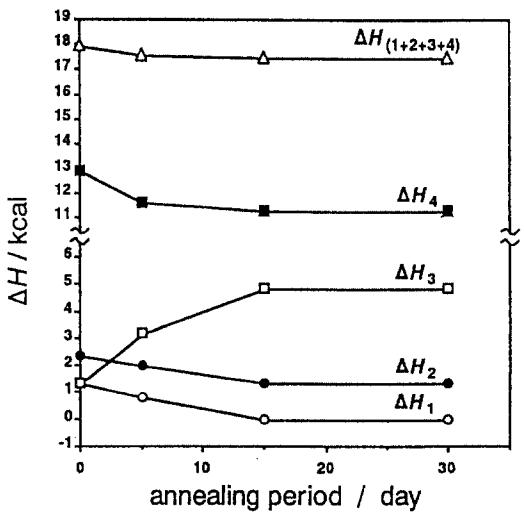

Fig. 5 Variation with increasing annealing period of enthalpy changes, ΔH_1, ΔH_2, ΔH_3 and ΔH_4, per 1 mol of DMPE for deconvoluted ice-melting curves I, II, III and IV respectively. $\Delta H_{(1+2+3+4)}$ represents a sum of these enthalpy changes (1 cal corresponds to 4.184 J)

cules characterized by the deconvoluted curves I, II and IV is estimated to be decreased by $1H_2O$, $0.7H_2O$ and $1H_2O$ per lipid, respectively. Thus, the total decreased number, $2.7H_2O$/lipid, of these water molecules is quite comparable to the increased number of the water molecules characterized by the deconvoluted curve III. Based upon the average standard deviation for the heat capacity functions of deconvoluted curves, the average standard deviation for the above-described number of water molecules is estimated to be 0.1. As noted above, the electron micrograph of crystalline phase (Fig. 3c) shows a narrowing of the interlamellar space, that is, a decrease in the amount of interlamellar water, compared with the gel phase. Most likely, this situation is attained by a release of the interlamellar water. From this viewpoint, the water characterized by the curve III is not considered the interlamellar water, because the number of thise water molecules is increased by annealing. Presumably, this water, characteristic of the crystalline phase, exists in a more loosely-bound water outside lamellae. In this connection, the ribbon-like structure of the crystalline phase shown in Fig. 3b suggests that there are a great number of bilayer surfaces exposed to bulky water phase. On this basis, the more loosely-bound water (characterized by the curve III) is presumed to interact with these bilayer surfaces outside lamellae. Therefore, only the loosely-bound water ($1H_2O$/lipid) characterized by the curve II is left as the interlamellar water of the crystalline phase, resulting in the narrow interlamellar space.

References

1 J. M. Boggs, Biochim. Biophys. Acta, 906 (1987) 353.
2 M. Kodama and T. Miyata, Colloids Surfaces, 109 (1996) 283.
3 M. Kodama, H. Aoki, H. Takahashi and I. Hatta, Biochim. Biophys. Acta, (1997) (accepted).
4 H. Aoki and M. Kodama, Thermochim. Acta, (1997) (accepted)
5 J. F. Nagle and M. C. Wiener, Biochim. Biophys. Acta, 942 (1988) 1.
6 M. Kodama, H. Inoue and Y. Tsuchida, Thermochim. Acta, 266 (1995) 373.
7 D. A. Wilkinson and J. F. Nagle, Biochemistry, 23 (1984) 1538.
8 S. Mulukutla and G. G. Shipley, Biochemistry, 23 (1984) 2514.