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SUB-TRANSITION PHENOMENON OF THE "L-DIPALMITOYL PHOSPHATIDYLCHOLINE - WATER" SYSTEM: EFFECT OF WATER CONTENT AND ITS AGGREGATION STATE

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ABSTRACT

Thermoanalytical investigations on the two component system of L-dipalmitoyl phosphatidylcholine and water revealed that the sub-transition in the present system is remarkably affected by the water content as well as the condition of annealing treatment and also that the stable low-temparature phase obtained after the appropriate annealing shows the existence of the more loosely-bound water than that in the super-cooled L_{β}' phase.

INTRODUCTION

In recent years, Sturtevant et al (ref.1) have revealed that in the presence of water the L-DPPC, one of the main constituents of cell membranes, exhibits the new type of third phase transition, which has been now, generally, called as the "sub-transition", at the temperature below the so-called pretransition. Since then, the kinetic nature and the conformational change associated with the sub-transition phenomenon of the present system have been studied,mainly, in the region of high water content by several workers (refs.2-6).

In order to clarify the effect of the change of water content on the sub-transition phenomenon, the thermal analyses of about thirty specimens in the L-DPPC-water system, ranging from a completely dehydrated compound to about 87 g% water content, were carried out focusing our particular attention on the behaviours of the ice-melting.

EXPERIMENTAL

1,2-dipalmitoyl-sn-glycero-3-phosphotidylcholine (DPPC) was obtained from Sigma Chemical Co., and purified according to the procedure reported previously (ref.7) The purified L-DPPC was completely dehydrated under high vacuum at a temperature above its main transition temperature in the same manner as reported previously (ref.7). The completely dehydrated L-DPPC thus obtained were transferred into the high-pressure crucible cell of a Mettler differential scanning calorimetry (DSC), which was then sealed off in a dry box filled with dry N_2 gas.

Each thirty samples of L-DPPC-water mixture with different water contents

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were prepared by a controlled successive addition of a minute amount of water to the completely dehydrated compound by using a microsyringe.

All the samples were preheated at the required temperature above the main transition for about 2 h in order to assure homogeneous mixing, then being cooled down to about -20 °C and allowed to stand for at least 12 h.

In the first stage, the samples thus obtained, which correspond to the non-annealed samples, were supplied to the DSC measurements. After the DSC measurements (of the non-annealed samples) the thermal history for the samples was again followed by the same procedure as mentioned above, and then the sample temperature was raised up to 4 °C in order to finish the complete melting of the ice. The samples were then annealed by the thermal cycling between -4 and + 4 °C at the heating rate of 0.1 °C min^{-1} for about 4 days. After this annealing treatment, the samples were then again cooled down to -20 °C, maintained at this temperature for about 12 h, after which the DSC measurements for the annealed samples were carried out.

Thirty samples, which covered the water content from zero to about 87 g%, were prepared.

The differential scanning calorimetry (DSC) was performed with a Mettler DSC TA 2000 by using the high-pressure crucible in the temperature range from -20 °C to temperatures above the main transition at the heating rate of 0.2 °C min^{-1}.

RESULTS AND DISCUSSION

1. Condition of annealing treatment.

For the first time, we want to discuss the condition of annealing treatment, by which the transformation from the metastable super-cooled gel L_{β}' phase into the stable low temperature phase takes place. According to Fig.1, we want to illustrate the occurence of exothermic phenomena observed in the DSC curves of the sample at the water content of 14.85 g%. In this figure, DSC curve A shows the exothermic peaks ranging around +4 $\sim$-4 °C, successively followed by the

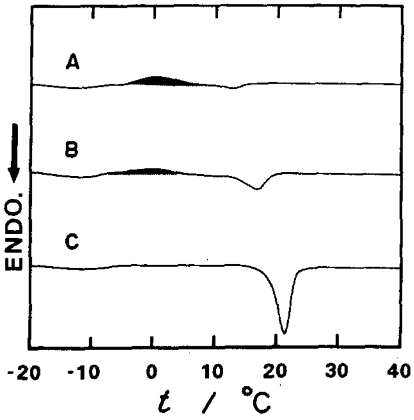

Fig. 1. Conditioning of the annealing treatment on the sub-transition phenomenon of the L-DPPC-water system at the water content of 14.85 g%. All samples A, B and C were stored initially at -20 °C for 12 h and samples B and C were then annealed by the repeated thermal cycling between +4 and -4 °C for 12 h and 4 days, respectively.

broad endothermic peak composed of, at least, two components (high and low temperature components). On the other hands, the annealed sample for 12 h shown in curve B exhibits a diminution of the exothermic effect and at the same time a growth of the high-temperature component of the broad endothermic peak shifting to the higher temperature, as compared with curve A for the non-annealed sample. Curve C for the annealed sample for 4 days shows a further development of the endothermic peak at 16.5 °C, which has been generally accepted as the sub-transition phenomenon, without any exothermic peak. Any change in the sub-transition peak was no more detected by the annealing time longer than 4 days.

As the results, the annealing condition necessary for the complete transformation into the stable low-temperature phase was confirmed to be the repeated thermal cycling between +4 and -4 °C for 4 days.

2. Dependence of the behavior of sub-transition on a variation of the water content.

In order to understand how the sub-transition peak changes with a variation of the water content of the sample, a series of typical DSC curves as a pair of the annealed and non-annealed samples is shown in Fig.2.

As shown in curves a ∿ m, all the non-annealed samples at the water content above ca 7 g% exhibits the broad, small endothermic peak starting around 4 °C and the broadness of these peak decreases with increasing the water content up to ca 19 g% (see curves a ∿ i), beyond which the same shape of the peak is preserved (see curves j ∿ m). As is mentioned above (see Fig.1), these broad peaks observed for the non-annealed samples continue to grow up to the sub-transition peak with an increased annealing time around the temperature region of +4 ∿ -4 °C.

When the curves a' and b' for the annealed samples are compared with a and b for the non-annealed samples at the same water content, the growth into the sub-transition peak by the annealing treatment is not observed up to ca 11 g% water content, beyond which the sub-transition peaks continue to grow and, at the same time, shift to higher temperature side with an increase in the water content up to ca 19 g% water content (see curves c' ∿ i'). Above this water content, both the peak area and the temperature of the sub-transition are nearly constant (see curves j' ∿ m'). All the obtained peak positions of samples a' ∿ m' give the sub-transition (Ts) curves shown in Fig.3.

3. Role of water on the sub-transition

In comparison with the molecular state in the L_{β}' gel phase, the carbonyl and polar head groups of L-DPPC molecules in the stable low temperature phase

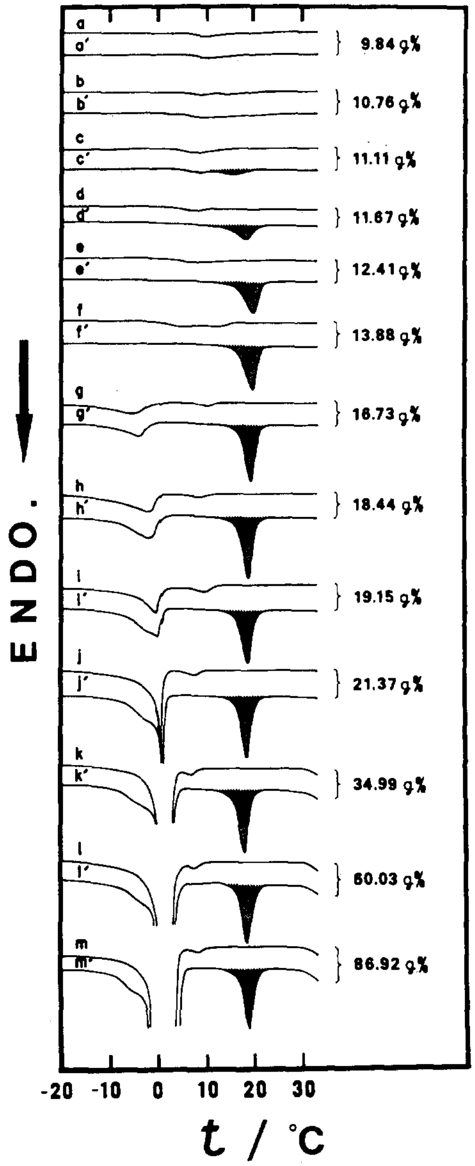

Fig. 2. Typical DSC curves around the sub-transition temperature with increasing amount of water (g%) indicated at the right hand side of each curve. Annealed (dash mark) and non-annealed samples with the same water content are compared in pairs. The endothermic peaks with hatched areas for the annealed samples correspond to the sub-transition.

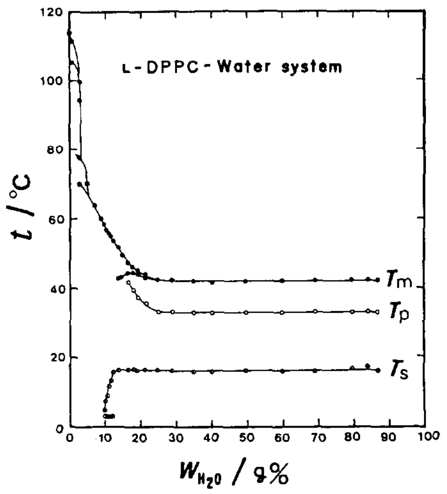

Fig. 3. Phase diagram of the L-DPPC-water system. T_m, T_p and T_s curves correspond to the main, pre-, and sub-transitions, respectively.

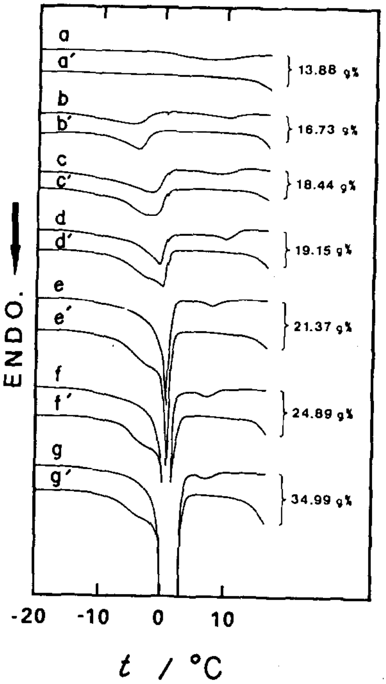

Fig. 4. Variation of the ice-melting DSC curves with an increase of the water content (g%) indicated at the right hand side of each curve. Annealed and non-annealed samples with the same water content are compared in pairs.

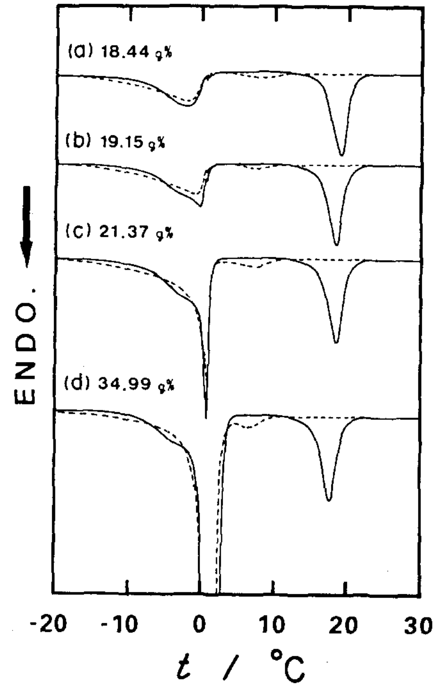

Fig. 5. Comparison of the ice-melting DSC curves between the annealed (—) and non-annealed (---) samples by overlaping with each other.

has been demonstrated to be in the more dehydrated state by few workers (refs. 3 - 5).

In connection with this fact, we should like to add some further informations obtained from the ice-melting DSC curves which were measured down to -20 °C.

Fig.4 shows a series of typical ice-melting curves as a pair of the annealed and non-annealed samples. As shown in curves a and a', any endothermic peak due to the ice-melting is not observed, regardless of the annealing procedure, up to ca 15 g% water content, beyond which both the annealed and non-annealed samples show the broad ice-melting peaks represented by curves b, b', c and c'. Accordingly all the water added below ca 15 g% water content is assumed to exist as a tightly-bound water (ref.8), while above this water content there exists a loosely-bound water (ref.8) showing the broad ice-melting peak which would be composed of plural components. At the water content of 19.15 g%, both the annealed and non-annealed samples exhibits a new type of ice-melting peak around 0 °C corresponding to the bulk free water, being superposed with the broad endothermic peak, as shown in curves d and d'. This finding of the existence of bulk water indicates that the phase separations in both samples take place at the water content around 18 ∿ 19 g% ; i.e., the low-temperature stable phase as well as the L_β' phase having the maximum amounts of the tightly-, and loosely-bound waters as the interlamellar water coexists with the free water.

Now, we should like to proceed to a discussion of the shape of ice-melting curve. When the ice-melting peaks are compared between the annealed and non-annealed samples, only distinct difference in the shape of the broad ice-melting peaks in the same temperature region is pointed out, as shown in Fig.5. That is, all the broad ice-melting peaks observed in the annealed samples are accompanied with a shoulder ranging -3 ∿ -6 °C, indicative of a growth of the high temperature region of the broad ice-melting peak by the annealing treatment This finding suggests that a part of the water existing as the loosely-bound water in the non-annealed sample (L_β' phase) is converted into a more loosely-bound water by the annealing treatment. This result may be closely related with the more dehydrated molecular state of the low-temperature stable phase at the temperature below the sub-transition, compared with the L_β' gel phase above this transition. In other words the shoulder of the ice-melting peak for the annealed sample seems to be corresponding to the water newly produced by the dehydration process from the carbonyl and polar head groups of the DPPC molecules.

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REFERENCES

1 S.C.Chen, J.M.Sturtevant and B.J.Gaffney, Proc. Nalt. Acad. Sci., USA, 77, 5060 (1980)
2 J.F.Nagle and D.W.Wilkinson, Biochemistry, 21, 3817 (1982)
3 H.H.Fuldner, Biochemistry, 20, 5707 (1981)
4 D.G.Cameron and H.H.Mantsch, Biophys. J., 38, 175 (1982)
5 M.J.Ruocco and G.G.Shipley, Biochim. Biophys. Acta, 684, 59 (1982)
6 M.Kodama, H.Hashigami and S.Seki, Biochim. Biophys. Acta (in press)
7 M.Kodama, M.Kuwabara and S.Seki, Biochim. Biophys, Acta, 689, 569 (1982)
8 M.Kodama, Y.Ogawa, M.Kuwabara and S.Seki, "Ions and Molecules in Solution", Studies in Physical and Theoretical Chemistry, Vol. 27 (1982) p.449, Elsevier Sci. Pub. Co. (Amsterdam)