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SUCCESSIVE PHASE-TRANSITION PHENOMENA AND PHASE DIAGRAM OF THE PHOSPHATIDYLCHOLINE-WATER SYSTEM AS REVEALED BY DIFFERENTIAL SCANNING CALORIMETRY

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A completely dehydrated dipalmitoylphosphatidylcholine (DPPC) was prepared with dehydration under high vacuum and at a temperature above its main transition temperature. Thermal analyses on about forty different samples of the DPPC-water system indicated that the main transition temperature decreased stepwise with an increase in the water content to the limiting temperature at 42.6°C, reflecting the thermal behaviors of a total of five endothermic peaks. The pretransition appeared at a water content above 17 g%, and the predominant role of 'newly incorporated water' between the bilayers of DPPC molecules at the pretransition was made evident.

Recently, by many investigators [1–8], it has been pointed out that phosphatidylcholine-water systems exhibit two successive phase changes which are refered to as the pretransition and the main transition, respectively. As to the mechanism of the pretransition, some workers have explained it from the viewpoint of roles of the interlamellar water incorporated between the bilayers of phosphatidylcholine molecules [3,5–7]. In particular, Janiak et al. [5] pointed out that the requirement for the appearance of the pretransition is the structurally bound water amounting to 20 g% water content which is surrounding the polar headgroups of phosphatidylcholine molecules in the L'_β phase [4]. This phase has been generally accepted as taking the bilayer lamellar structure of phosphatidylcholines at a temperature below the pretransition.

In our previous paper [9], a detailed thermoanalytical study on the surfactant*-water system elucidated that there appeared two successive kinds of transition, i.e., coagel-gel transition at lower temperature, and gel-liquid crystal transition at higher temperature, respectively. The former was found to be attributed to the incorporation of the additional water as the interlamellar constituent from the coexisting free (excess) water, and at the same time, to rearrangement of polar headgroups. The latter is generally explained as being the result of the partial melting of the hydrocarbon chains. Regarding that the present phosphatidylcholine-water system behaves similarly with the surfactant-water system studied by us, the pretransition seems to be corresponding to the coagel-gel transition and the so-called main transition to the gel-liquid crystal transition. Accordingly, our previous study on the surfactant-water system suggests that the excess free water coexisting with the

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* We have studied the system of water and dioctadecyldimethylammonium chloride (DOAC), which has two long hydrocarbon chains, as one of the appropriate model systems to DPPC.

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coagel phase plays an essential role for the appearance of the coagel-gel transition, not the 'bound water' * incorporated in the coagel phase.

In the present paper, we shall report our recent results of the thermal analyses on about 40 different specimens in the DPPC-water system, their compositions ranging from a completely dehydrated compound to about 90 g% water content. Based on the phase diagram obtained in this study, the nature of the pre- and main transitions will be discussed with particular attention to the thermal behaviors of the water contained in the present system.

Synthetic DPPC was obtained from Sigma Chemical Co., and was purified by recrystallization from chloroform/acetone solution. The conditions of complete dehydration were assessed by the use of a Cahn electrobalance Model 2000 and it was revealed that the complete dehydration was achieved only when the DPPC was dehydrated under high vacuum (10^{-4} Pa) and at a temperature 'above' its transition temperature due to the hydrocarbon chain-melting. Forty samples of the DPPC-water mixture, which covered the water content from zero to about 90 g%, were prepared by adding a controlled amount of water to the completely dehydrated compound with the use of a microsyringe. The differential scanning calorimetry (DSC) measurements were carried out with Mettler DSC TA 2000 in the temperature range from −20 to 140°C after the samples were preheated to about 100°C in order to assure homogeneous mixing.

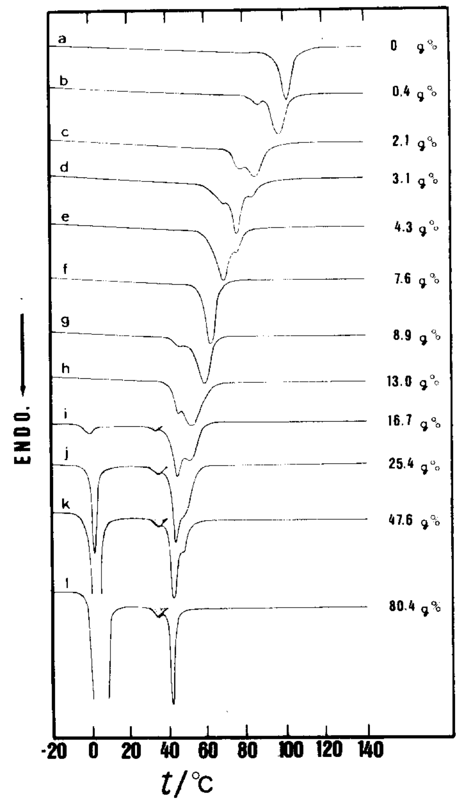

Fig. 1. Typical DSC curves at the scanning rate of 1 K·min^{-1} for 12 samples with increasing amounts of water (g%) indicated at the right hand side of each curve. The endothermic peak with dotted area corresponds with the pretransition.

Fig. 1 shows the representative DSC curves among the forty samples of the DPPC-water mixture in the heating process. Completely dehydrated DPPC exhibits only a pronounced, primary endothermic peak at 97.8°C (see curve a) due to the hydrocarbon chain-melting. The minute amount of water, which corresponds to the residual water retained in the compound by the dehydration under high vacuum 'below' the transition temperature, causes a new, second endothermic peak at about 84.6°C (see curve b). With further increase in water content up to about 90 g%, new, third, fourth and fifth endothermic peaks appear successively at lower temperatures in this order (see curves c–l); the disappearance of the endothermic peak at a higher temperature is replaced by the appearance and growth of the new peak at a lower temperature. When the water content in the mixture reaches about 50 g%, only the fifth peak is allowed to persist in the temperature region above 40°C (see curve l). The temperature of this transition (= at 42.6°C) remains nearly constant up to about 90 g%. Fig. 2 shows the phase diagram of the DPPC-water system obtained from all the DSC heating curves. The enlarged phase diagram in this figure indicates clearly the appearance of the successive phase transitions at the water content less than 10 g%. Similarly to our results, the X-ray observations of Tardieu et al. [4] on egg phos-

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* Janiak et al. (Ref. 5, p. 4580) do not distinguish the difference between the 'bound water' and the 'intermediate water' found in our previous work [9].

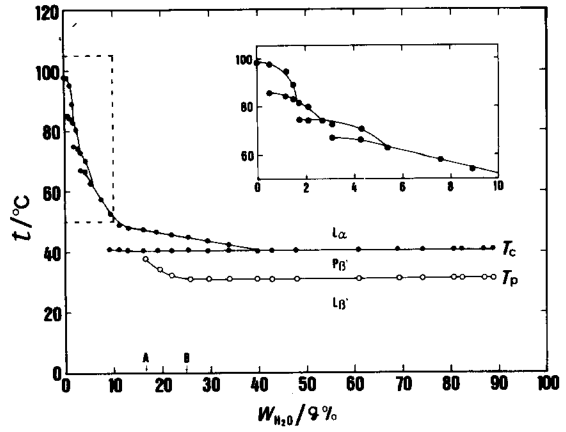

Fig. 2. Phase diagram of the DPPC-water system. ●, T_c curve; ○, T_p curve. At the water content (17%) indicated by mark A the phase separation to L_β' and the excess water phase takes place. At mark B (25 g%) the pretransition temperature reaches the limiting temperature of 32.3°C.

phatidylcholine-water system had revealed also the existence of the composite phases (H_α, Q_α and L_α) at the high-temperature high-lipid region, but their transition temperatures are now consistent with our results obtained on the synthetic DPPC employed in the present study. T_c curves in Fig. 2 express the variation of the main transition temperature with the water content. As is obvious from the T_c curve, the main transition temperature decreases stepwise to the limiting temperature of 42.6°C, reflecting the composite thermal behaviors of a total of five endothermic peaks; the limiting temperature can be attained not by a smooth direct shift of the primary endothermic peak itself (97.8°C) to that temperature (42.6°C) as depicted in many texts, but by the new appearance of the final fifth endothermic peak located just at the limiting temperature. This new finding is quite different from the results obtained by Chapman et al. [1].

When the water content reaches about 17 g%, there appears a small endothermic peak corresponding to the so-called pretransition at a temperature of 5°C lower than the main transition temperature (see curve i in Fig. 1). The transition temperature decreases gradually with an increase in water content to about 25 g%, beyond which the limiting temperature of this transition is observed at 32.3°C (see T_p curve in Fig. 2). It is interesting to note here that the endothermic peak for the pretransition shows itself for the first time at the same water content where the endothermic peak at about 0°C, due to the ice-melting, starts to appear (see curve i in Fig. 1). This fact indicates that additional water beyond 17 g% bears the phase separation; the so-called L_β' phase plus the phase of the excess free water. In the system of the water content below 17 g%, all the water may be allowed to exist as a kind of bound water which is incorporated between the bilayers in L_β' phase and does not crystallize on cooling down to −20°C. Recently, Inoko and Mitsui [6] have provided the interesting information that the increase in the repeat distance of lamellar phase in a DPPC-water system at the pretransition is due to the increase in the thickness of the water layer, indicating that the incorporation of additional water between the bilayers takes place at the pretransition.

Based on these facts, it may be said that in the present system, the excess free water coexisting with the L_β' phase (above approx. 17 g% water content) is incorporated between the bilayers at the pretransition and therefore the existence of this free water is a requirement for the appearance of the pretransition, which seems to account well for the present experimental results. That is, the simultaneous appearance of the endothermic peaks of the pretransition and of the ice-melting occurs at about 17 g% water content for the first time. P_β' phase [4], which has been accepted as the intermediate phase between the pretransition and the main transition, may be associated with the co-operational interaction between the polar head groups of DPPC and 'newly incorporated water' at the pretransition. This result seems to be inconsistent with the conclusions of Janiak et al. [5]. As mentioned above (see T_c curve in Fig. 2), the limiting temperature of the pretransition is obtained at about 25 g% content, showing that the interlamellar water content in P_β' phase may reach maximum value at this content, beyond which P_β' phase coexists with the phase of excess free water. This point is concordant with the observation by Janiak et al. [5].

References

1 Chapman, D., Williams, R.M. and Ladbrooke, B.D. (1967) Chem. Phys. Lipids 1, 445–474

2 Hing, H.J. and Sturtevant, J.M. (1972) J. Biol. Chem. 247, 6071–6075

3 Salsbury, N.J., Darke, A. and Chapman, D. (1972) Chem. Phys. Lipids 8, 142–151

4 Tardieu, A., Luzzati, V. and Reman, F.C. (1973) Mol. Biol. 75, 711–733

5 Janiak, M.J., Small, D.M. and Shipley, G.G. (1976) Biochemistry 15, 4575–4580

6 Inoko, Y. and Mitsui, T. (1978) J. Phys. Soc. Jap. 44, 1918–1924

7 Doniach, S. (1979) J. Chem. Phys. 70, 4587–4596

8 Inoko, Y., Mitsui, T., Ohki, K., Sekiya, T. and Nozawa, Y. (1980) Phys. Stat. Sol. (a) 61, 115–121

9 Kodama, M., Kuwabara, M. and Seki, S. (1981) Thermochim. Acta 50, 81–91