

PHASE TRANSITION PHENOMENA INDUCED BY THE SUCCESSIVE APPEARANCES OF NEW TYPES OF AGGREGATION STATES OF WATER MOLECULES IN THE “L-DIPALMITOYLPHOSPHATIDYLCHOLINE–WATER” SYSTEM *

MICHIKO KODAMA

*Department of Chemistry, Faculty of Science, Kansai Gakuin University, Nishinomiya 662
(Japan)*

(Received 17 July 1986)

ABSTRACT

Thermal analysis of the aggregation state of water molecules in the “L-dipalmitoylphosphatidylcholine (DPPC)–water system revealed that in the low-temperature crystal phase below the sub-transition temperature, there are three kinds of interlamellar water, i.e., tightly, loosely, and more loosely bound water, which are incorporated between the bilayers of L-DPPC molecules, while the $L\beta'$ gel phase lacks the third bound water molecule. Furthermore, we found that both the sub- and pre-transitions below the main transition temperature appear, for the first time, at water contents of around 11 and 18 g%, respectively, just at which point the new types of water structure occur. These findings indicate that the condition necessary for the appearance of these two kinds of phase transition is the existence of the characterized aggregation state of water molecules, that is, more loosely bound water for the sub-transition and bulk free water coexisting with the $L\beta'$ gel phase for the pre-transition.

INTRODUCTION

Recently, in connection with the rapid progress of physicochemical investigations on biological membranes, the phase transition phenomena in systems composed of water and lipids, one of their main constituents, have attracted the attention of many investigators [1–5], and a large number of these have been concerned with the configurational changes of lipid molecules alone. However, it can be said that in cell membranes the presence of water enables, for the first time, the lipid molecules to form highly organized aggregates represented by the bilayer lamellar structure of gel and liquid crystal phases. Therefore, in studies of the phase transition phenomenon in

* Dedicated to Professor Syûzô Seki in honour of his contribution to Calorimetry and Thermal Analysis.

water–lipid systems it would be necessary to take into account the role of water molecules [6–8].

From this viewpoint, we investigated, in more detail, the successive appearance of new types of aggregation states of water molecules in the binary system water–L-dipalmitoylphosphatidylcholine (DPPC), a typical lipid, with varying water content [9–13]. Based on these results, the present paper reviews the phase transition phenomena, such as sub- and pre-transitions, focusing on the role of water molecules.

RESULTS AND DISCUSSION

Figure 1 shows the phase diagram obtained from the heating DSC curves of about 40 specimens in the L-DPPC–water system ranging from a completely dehydrated compound up to about 95 g% water content. As shown in Fig. 1, and as is well known for the present system, there are three kinds of successive phase transition due to the structural changes of the bilayer crystal $\rightarrow L\beta'$ gel, $L\beta'$ gel $\rightarrow P\beta'$ gel and $P\beta'$ gel $\rightarrow L\alpha$ liquid crystal phases on heating, which are generally called sub-, pre-, and main-transitions, respectively [14–18]. However, such a complete sub-transition temperature

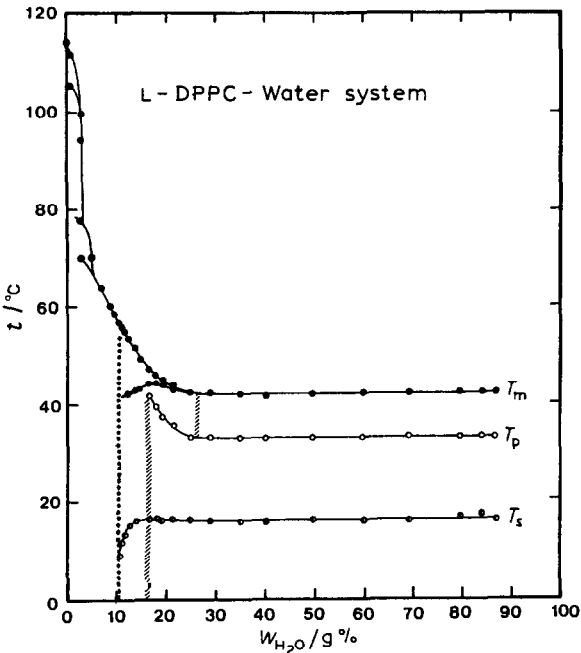


Fig. 1. Phase diagram of the L-DPPC–water system. Details are given in the enlarged phase diagram of Fig. 4.

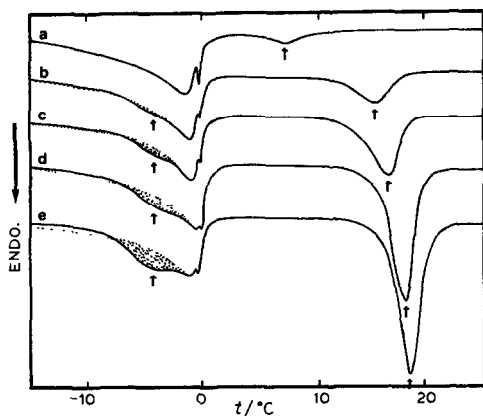


Fig. 2. Growth process of the ice-melting shoulder (·····) derived from the more loosely bound water associated with gradual transformation into the thermodynamically stable, bilayer crystal phase, with an increased time (a → e) of annealing. Annealing time (h): (a) 0; (b) 17; (c) 24; (d) 72; (e) 96.

curve covering almost all the water content region shown in Fig. 1 has not been reported yet. Therefore, in this respect, the phase diagram obtained by us may be said to be the first to be made up of a complete set of the three kinds of phase transition curves, in preference to those of other workers.

On the other hand, it is already known that on cooling the $L\alpha$ liquid crystal phase at a temperature above the main transition temperature is easily converted into the $P\beta'$ gel, followed by the $L\beta'$ gel phase. However, the final transformation into the bilayer crystal phase is not achieved, even on cooling to -100°C . This fact indicates that $L\beta'$ gel phase can exist as a metastable supercooled state down to this temperature. Therefore, it is necessary to give the appropriate annealing treatment to this supercooled phase in order to attain complete transformation into the thermodynamically stable bilayer crystal phase. Figure 2 shows the process of transformation due to the annealing discovered by us [12], in which with an increased annealing time, the small endothermic peak located around 8°C becomes larger together with a gradual shift of the transition temperature to the higher temperature side, finally reaching the limiting sub-transition temperature at 16.5°C . These findings indicate that annealing for up to 4 days gives complete transformation into the bilayer crystal phase.

In order to distinguish the thermodynamic stability of the phases in the present system, we have prepared a schematic diagram of Gibbs free energy (G) vs. temperature (T) on the basis of the DSC heating curves (see Fig. 3). In this figure, reflecting the thermal behavior of Fig. 2, a number of metastable phases appear showing the transition temperatures T_s' and T_s'' , which are finally transformed into the most stable phase corresponding to the bilayer crystal due to adequate annealing time.

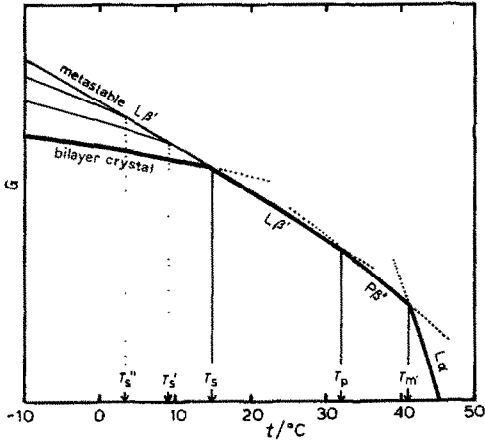


Fig. 3. Schematic diagram of the Gibbs energy (G) vs. temperature (T) curve in the L-DPPC-water system. T_s , T_p and T_m are the sub-, pre- and main-transition temperatures, respectively. T_s' and T_s'' represent the transition temperatures of the metastable phases situated between the supercooled $L\beta'$ gel and the stable bilayer crystal phases.

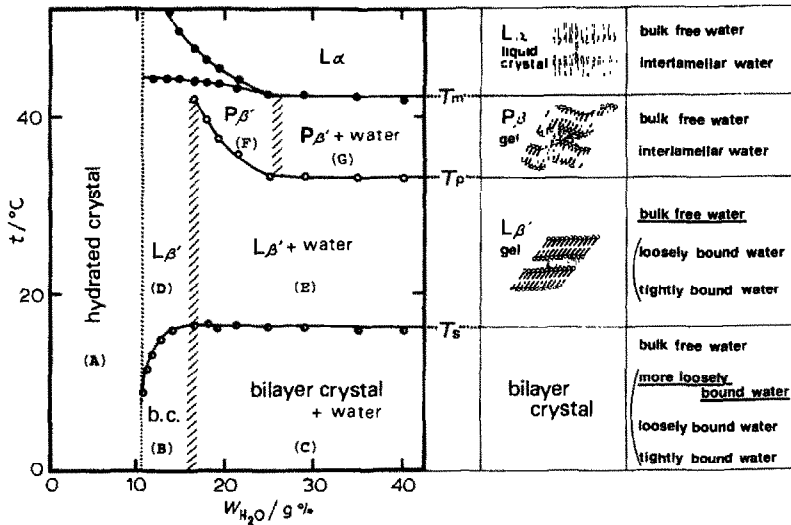


Fig. 4. Enlarged phase diagram of the L-DPPC-water system at temperatures below 50°C and a water content below 40 g%. A list of classifications of the water structure at each phase is also presented on the right-hand side of this phase diagram. The water content shown by the dotted and hatched lines indicates the initial water content, at which the new types of water structure appear for the first time. Furthermore, with the aid of these lines and the transition temperature curves, the phase diagram at temperatures below the main-transition can be divided into seven categories: A, B, C, D, E, F and G.

Now we refer to the dotted and hatched lines in the phase diagram of Fig. 1 which represent the initial water contents at which there are successive occurrences of the new types of aggregation state of water molecules. It is interesting to note here that both the sub- and pre-transitions start to appear, for the first time, at the water contents shown by the dotted and hatched lines, respectively, indicating the simultaneous occurrence of the new types of water structure and that of the phase transition at the same water contents. These new findings suggest the essential role of water molecules in the phase transitions in the present system. Before proceeding to a discussion at this point, we should like to provide the following results of the thermal analysis of the aggregation state of water molecules based on the enlarged phase diagram shown in Fig. 4.

Changes in aggregation state of water molecules with varied water content in the L-DPPC-water system

(a) In the region of water content up to 11 g% shown by the dotted line in Fig. 4, all the water added to the sample exists as non-freezable water, which is not crystallized, even on cooling to -100°C . This water can be assumed to exist as interlamellar water which is interposed between the bilayers of the L-DPPC molecules and is strongly bound to their polar head-groups. In this sense, we will hereafter call this non-freezable water "tightly bound water".

(b) The limiting uptake of tightly bound water is attained at a water content of approx. 11 g%, after which freezable waters, shown by a series of typical ice-melting DSC curves in Fig. 5, are furnished.

Here, we call attention to the facts that at a water content of approx. 11 g% the sub-transition from the bilayer crystal to the $L\beta'$ phases appears for the first time, and furthermore the $L\beta'$ gel phase is allowed to persist in the supercooled state down to -100°C unless the appropriate annealing is

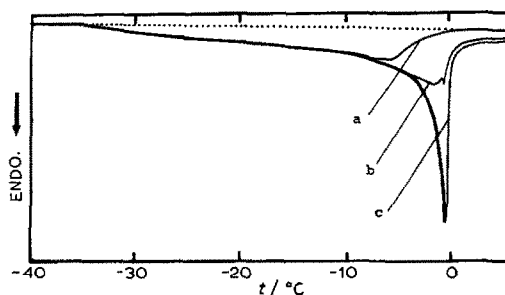


Fig. 5. Three typical kinds of DSC curve due to the melting of frozen waters which appear successively with increasing water content: (a) broad ice-melting peak alone ($11 < W_{\text{H}_2\text{O}}/\text{g}\% < 18$), (b) broad ice-melting peak followed by the small sharp one ($18 < W_{\text{H}_2\text{O}}/\text{g}\% < 21$), (c) broad ice-melting peak overlapped by the large sharp one ($W_{\text{H}_2\text{O}}/\text{g}\% > 21$).

adopted. In connection with these facts, the thermal behavior of the ice-melting peak in the bilayer crystal and supercooled $L\beta'$ phases was investigated in order to compare the difference in water structure between both phases and the following results were elucidated.

(c) Water added above 11 g% up to 18 g% water content in both phases causes growth of the broad ice-melting peak starting from approx. -35°C , represented by curve (a) of Fig. 5. The appearance of this ice-melting peak suggests the existence of further interlamellar water composed of a mixture of components in both phases. This water is assumed to be weakly bound to the polar groups of the L-DPPC molecules, i.e., so-called loosely bound water, in comparison with tightly bound water.

(d) The amount of secondary-bound water also reaches a limiting value at a water content of approx. 18 g%, above which the addition of more water furnishes the sharp ice-melting peak located around 0°C shown in curve (b) of Fig. 5. This finding indicates the occurrence of phase separation: two phase systems take place, i.e., bulk free water coexisting with the $L\beta'$ gel or the bilayer crystal phases. As is obvious from curve (c) of Fig. 5, above 21 g% water content it becomes impossible to separate these two kinds of ice-melting peaks into the broad and sharp components.

In conclusion, the present results on the thermal analysis of the water state suggest that in both phases the process of the occurrence of new types of aggregation state of water molecules proceeds in the same mode, i.e., tightly bound, loosely bound and finally bulk free waters on increasing water content.

When the broad ice-melting peak is compared, in more detail, between the $L\beta'$ gel and the bilayer crystal phases by reference to Fig. 6, we notice

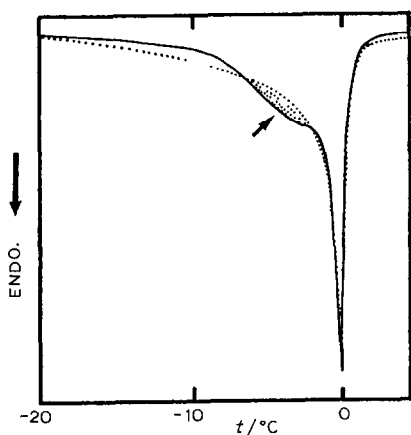


Fig. 6. Comparison of the ice-melting peak around -20 – 0°C between the stable bilayer crystal (—) and the supercooled $L\beta'$ gel phases (\cdots). The ice-melting shoulder (\rightarrow) indicates the existence of more loosely bound water in the bilayer crystal phase.

that this broad peak in the bilayer crystal phase exhibits a shoulder ranging from -6 to -2°C , indicating the existence of the additional interlamellar water in a freer aggregation state which is hereafter referred to as more loosely bound water, as compared with loosely bound water. In connection with this finding, we again want to pay particular attention to the ice-melting DSC curves shown in Fig. 2 where the ice-melting shoulder (dotted mark) continues to grow with increasing annealing time, indicating the growth of the more loosely bound water with the gradual transformation into the bilayer crystal phase. A comparison of the broad ice-melting peak between both phases suggests that in the bilayer crystal phase there are three kinds of interlamellar water, i.e., tightly, loosely, and more loosely bound waters, while the $L\beta'$ gel phase is lacking in the last, more loosely bound water.

Based on the results of the thermal analysis of the water state, at the second stage, we should like to proceed to a discussion of the nature of the phase diagram considering the role of water molecules.

Nature of the phase diagram considering the role of water molecules

(a) In the region of 0–11 g% water content, corresponding to region A of Fig. 4, it can be assumed that hydrated crystals exist with different amounts of tightly bound water which is interposed between the bilayers up to 5 H_2O per mole of L-DPPC. These phases transform directly into the liquid crystal $L\alpha$ phase.

(b) In the region of 11–18 g% water content and at temperatures below the sub-transition temperature (see region B of Fig. 4), there is a bilayer crystal phase which also incorporates the interlamellar water existing as the two kinds of loosely bound water, more loosely and loosely bound, in addition to the existing tightly bound water. Therefore, the structural difference between the hydrated crystal and bilayer crystal phases may be concerned, mainly, with a looseness in the lateral packing of the polar head-groups of L-DPPC molecules depending upon whether the additional interlamellar water is incorporated or not.

(c) At water contents above 18 g% (see region C), the bilayer crystal phase, which incorporates the maximum amount of interlamellar water composed of 5 H_2O tightly-bound and 4 H_2O both kinds of loosely-bound, coexists with the bulk free water.

Here, looking at the $L\beta'$ gel phase which appears above 18 g% water content and above the sub-transition temperature, it is worth noting that this gel phase lacks the more loosely bound water. Furthermore, as is mentioned above, this water tends to increase with the conversion of the supercooled $L\beta'$ gel phase to a more ordered molecular state, finally becoming the bilayer crystal phase, due to the annealing treatment. If we take into consideration that the polar groups of L-DPPC molecules in the

bilayer crystal phase are in a more dehydrated state than those in the $L\beta'$ gel phase [15–17], the more loosely bound water discovered by us exactly corresponds to the “newly produced water” caused by the dehydration process of the polar groups of the L-DPPC molecules. Therefore, it may be said that the annealing in the present system not only brings about conversions of the aggregation state of the L-DPPC molecules, but also of the water molecules.

Judging from these facts, the key role of the more loosely bound water in the appearance of the sub-transition is pointed out:

(d) At an elevated sub-transition temperature, this bound water has greater interaction with the polar groups of L-DPPC molecules, simultaneously accompanying the conversion into loosely bound water. As a result, the $L\beta'$ gel phase appears, which is in the loose packing state of the L-DPPC molecules.

On the other hand, the $L\beta'$ gel phase in region D reaches the final liquid crystal $L\alpha$ phase directly, while that in region E passes the additional gel phase, $P\beta'$, before attaining the final state. This difference can be assumed to depend upon whether the $L\beta'$ gel phase coexists with the bulk free water or not. That is, as is shown in Fig. 4, the pre-transition starts to appear at the same water content of approx. 18 g% (see ref. 10) where the $L\beta'$ gel phase having the limiting amount of interlamellar water (5 H_2O tightly bound + 4 H_2O loosely bound) coexists with the bulk free water for the first time. This finding indicates that the condition necessary for the appearance of the pre-transition is the existence of excess free water which leads to the possibility of further incorporation of water molecules between the bilayers. Similarly to the sub-transition, the key role of the bulk free water on the appearance of the pre-transition is as follows:

(e) At the elevated pre-transition temperature, this free water is newly incorporated between the bilayers and as a result, a periodically rippled lamellar structure characteristic of the $P\beta'$ gel phase appears, caused by the cooperational interaction of the “newly incorporated water” with the polar groups of L-DPPC molecules.

Consistent with our findings, Inoko and Mitsui [19] have revealed the sudden increase in the thickness of the water layer at the pre-transition temperature, which may be caused by the “newly incorporated water” discovered by us.

SUMMARY

(1) With an increase in water content, the new types of aggregation state of water molecules occur in the order of tightly bound, both kinds of loosely bound and finally bulk free waters; (2) The mode of the transformation process from the ordered crystal up to the final liquid crystal phases can be

classified into three categories, depending upon the water contents, at which new types of water state start to appear; (3) In connection with point (2), the existence of the corresponding types of water state enables, for the first time, the occurrence of the gel phases such as $L\beta'$ and $P\beta'$. In other words, this finding points out the necessity of an adequate aggregation state of water molecules for sub- and pre-transitions to appear, that is, the more loosely bound water incorporated between the bilayers for the former and bulk free water coexisting with the $L\beta'$ gel phase for the latter.

REFERENCES

- 1 D. Chapman, R.M. Williams and B.D. Ladbooke, *Chem. Phys. Lipids*, 1 (1967) 445.
- 2 V. Luzzati, in D. Chapman (Ed.), *Biological Membranes*, Academic Press, New York, 1968, p. 71.
- 3 A. Tardieu, V. Luzzatti and F.C. Reman, *J. Mol. Biol.*, 75 (1973) 711.
- 4 M.J. Janiak, D.M. Small and G.G. Shipley, *Biochemistry*, 15 (1976) 4575.
- 5 E. Jüngens, G. Höhne and E. Sackmann, *Ber. Bunsenges. Phys. Chem.*, 87 (1983) 95.
- 6 V.A. Parsebian, N. Fuller and R.P. Rand, *Proc. Natl. Acad. Sci.*, 76 (1979) 2750.
- 7 L.J. Lis, M. McAlister, N. Fuller, R.P. Rand and V.A. Parsegian, *Biophys. J.*, 37 (1982) 657.
- 8 L. Guldbbrand, B. Jonsson and H. Wennerstrom, *J. Colloid Interface Sci.*, 89 (1982) 532.
- 9 M. Kodama, M. Kuwabara and S. Seki, *Biochim. Biophys. Acta*, 689 (1982) 567.
- 10 M. Kodama, Y. Ogawa, M. Kuwabara and S. Seki, *Ions and Molecules in Solution, Studies in Physical and Theoretical Chemistry*, Vol. 27, Elsevier, Amsterdam, 1982, p. 449.
- 11 M. Kodama, H. Hashigami and S. Seki, *Biochim. Biophys. Acta*, 814 (1985) 300.
- 12 M. Kodama, H. Hashigami and S. Seki, *Thermochim. Acta*, 88 (1985) 217.
- 13 M. Kodama, H. Hashigami and S. Seki, *J. Colloid Interface Sci.*, (1986) in press.
- 14 S.C. Chen, J.M. Sturtevant and B.J. Gaffney, *Proc. Natl. Acad. Sci. U.S.A.*, 77 (1980) 5060.
- 15 H.H. Fuldner, *Biochemistry*, 20 (1981) 5707.
- 16 M.J. Ruocco and G.G. Shipley, *Biochim. Biophys. Acta*, 684 (1982) 59.
- 17 D.G. Cameron and H.H. Mantsch, *Biophys. J.*, 38 (1982) 175.
- 18 L.T.M. Saraga and G. Madelmont., *J. Colloid Interface Sci.*, 99 (1984) 420.
- 19 Y. Inoko and T. Mitsui, *J. Phys. Soc. Jpn.*, 44 (1978) 1918.