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MELTING OF WATER ABSORBED BY PHOSPHOLIPID BILAYERS : Effect of an Unsaturated Hydrocarbon Chain

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SUMMARY

Characteristics of melting curves of water in the phosphatidylcholines - water system were investigated with special reference to the effect of a highly unsaturated acyl group, arachidonic acid,attached to the position of glycerol. The arachidonic acid lowers not only the temperature of transition from gel to liquid crystal, but also the melting point of absorbed water. The result is interpreted by the penetration of water to the hydrophobic cavity in the sample, as previously postulated to explain the adsorption isotherms of 1S-2A-PC.

INTRODUCTION

Natural phospholipid molecules usually contain unsaturated hydrocarbon chains at the β position of glycerol. The unsaturated groups of phospholipids lower the phase transition temperatures and give fluidity to phospholipid membranes.

We have been studying structures and function of triphosphoinositide(PIP_2) whose physiological role has attracted special interest recently.[1] It is known that one of the major unsaturated acyl groups in a PIP_2 molecule is an arachidonic acid which has four double bonds in the hydrocarbon chain.

We have supposed that the arachidonic acid not only lowers transition temperatures of lipid bilayers but also raise permeability of water molecules and small ions due to the bulky conformation of the unsaturated hydrocarbon chain. In this paper, we report the characteristics of melting of water absorbed by phospholipids and discuss the state of water in the phospholipid bilayers.[2]

First, we compare the melting curves of water absorbed by phosphatidylcholines(PC_s) having different acyl groups,then we

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show the DSC curves of PIP_2 - water system ,then we discuss briefly differences of the melting characteristics of water between PC and PIP_2.

MATERIALS AND METHODS

We used commercially available PC_S to investigate the effect of an unsaturated hydrocarbon chain, because we could not obtain PIP_2 with definite acyl chains. Dipalmitoyl phosphatidylcholine(DPPC) and 1-stearoyl-2-arachidonoyl phosphatidylcholine(1S-2A-PC) were purchased from Sigma Chem.Co., and Avanti Polar Lipid , INC., respectively, and used without further purification. Chloroform absorbed by the commercial samples were removed under a vacuum of 10^{-3} mmHg for more than 24 hours at a room temperature. PIP_2 was extracted from bovine brain by the method of Folch. Absorption of water by dry samples were carried out by putting the sample in a saturated vapor for presetted hours.

Differential scanning calorimetry(DSC) was carried out by using DSC of Seiko I. & E. Ltd. Usually, sample weights of phospholipids were 3-5mg, and the heating rate was 1 °C/min.

RESULTS AND DISCUSSION

Fig.1 shows melting curves of water in DPPC. The melting curves of water in DPPC have been reported by many workers, and the present result is nearly the same as the result reported earlier by D.Chapman[3].

At a preliminary stage of our DSC study on a 1S-2A-PC - water system, we obtained a result as shown in Fig.2. However, with a careful protection to light in the preparatory procedure of the sample, our successive study on the same system revealed that the correct endothermal peaks for the pure 1S-2A-PC - water system are given as in Fig.3.

The reason for this conclusion is as follows. X-ray diffraction study clarified that the endothermal transition at 10 °C for dry sample is a gel to liquid crystal transition of 1S-2A-PC,[4] and the transition temperatures decrease to -8 °C for the sample with a water content of 9 wt% and to -13 °C for the samples of 21, 30, 39 wt% of water as shown in Fig.3.

It was found that the endothermal transition at -13 °C disappears by an irradiation of ultraviolet light. Special caution was not paid to protect degradation of samples at the preliminary

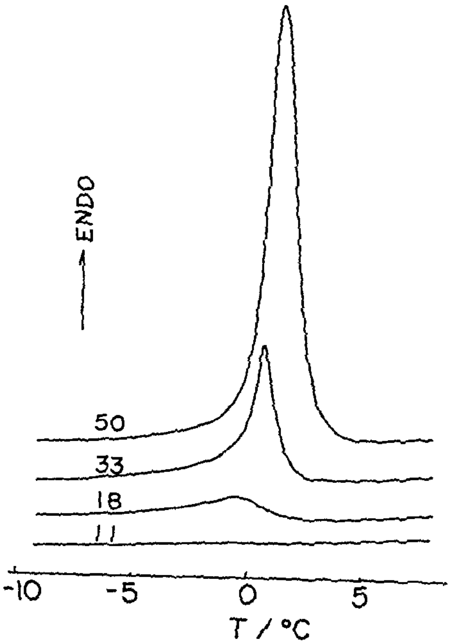

Fig.1. DSC curves for DPPC - water system. Numbers in the figure are weight per cent of water to DPPC.

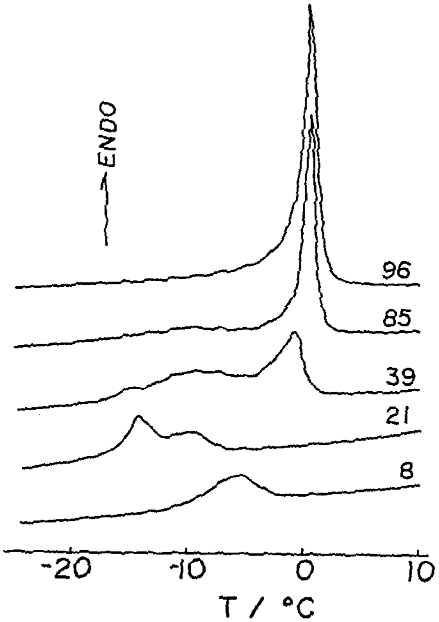

Fig.2. DSC curves for 1S-2A-PC - water system. Lot number of 1S-2A-PC; #180-204PC-53.

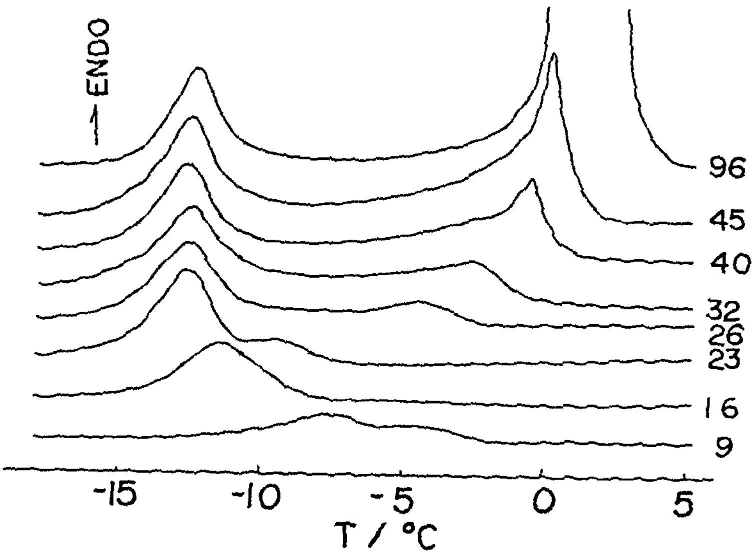

Fig.3. DSC curves for 1S-2A-PC - water system. Nondegraded sample, lot number; #180-204PC-64.

experiment, and degradation of the arachidonic group might occur during the water absorbing process to the sample. For further discussion, we use the result in Fig.3 which shows the melting behavior of nondegraded 1S-2A-PC - water system.

First, we discuss the phase transition enthalpies from gel to liquid crystal of 1S-2A-PC. Although some ambiguity is left in the procedure of the decomposition of the endothermal peak curves , we can obtain the transition enthalpies with enough accuracy for the following discussion. Fig.4 shows the transition enthalpies for the sample with varied content of water. The transition enthalpy increases from 2 kcal/mol of lipid to a value larger than 5 kcal/mol for a fully hydrated state. The transition enthalpies for 1S-2A-PC are smaller than those for DPPC as shown in Fig.4, resulting much smaller transition entropies for 1S-2A-PC than those for DPPC, though transition temperatures of 1S-2A-PC are lower than those of DPPC. The result can be explained if we assume that the gel state of 1S-2A-PC is more disordered than the gel state of DPPC.

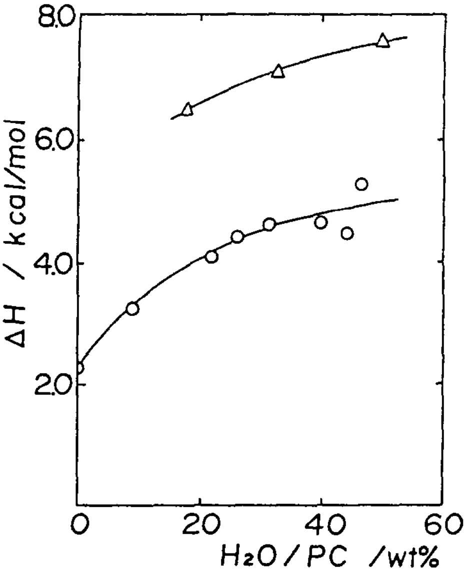

Fig.4. Enthalpies of transitions from gel to liquid crystal for 1S-2A-PC (o) and for DPPC (Δ) as functions of water content.

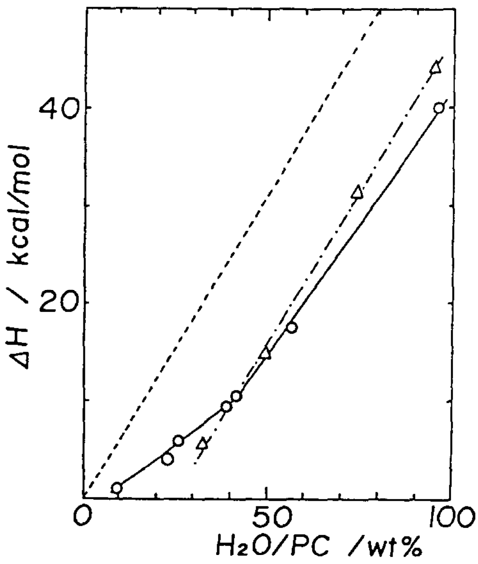

Fig.5. Melting enthalpies of water absorbed by 1S-2A-PC (o) and DPPC (Δ). Dotted line; melting enthalpy of pure water

Next, let us examine the melting of water in a 1S-2A-PC - water system. Freezable water is clearly observed in the DSC curve in Fig.3,even for a sample of water content of 9.5 wt%, while the endothermal peak is scarcely observed in the DSC curve for a DPPC - water system at such a low content of water as shown in Fig.1.

Fig.5 represents melting enthalpies of water per a mole of lipid as a function of water content. The dotted line in the figure indicates the case in which all the water in the sample is free. Experimental data for 1S-2A-PC fit into two straight lines with different inclinations. One line which fits into the data for higher content of water, and the other with a smaller inclination angle fit into the data for lower content of water. The break point is at a water content of about 45 wt%. The data for DPPC above a water content of 30 wt% fit well into a line parallel to the line for pure water. Contrasting with this, the line for 1S-2A-PC is not parallel to these lines, even at higher contents of water. The result shows that partially restricted water exists in the 1S-2A-PC - water system up to water content of 100 wt%.

Previously, we speculated that water is trapped in a hydrophobic cavity, in order to explain the adsorption isotherms of 1S-2A-PC.[5] The smaller melting enthalpies of water in the lower region of water content can be explained by the same reason, too.

Finally, we discuss briefly the melting of water in the PIP_2 - water system. Fig.6 shows one example of the DSC curves for varied water content for the PIP_2 - water system. Only one endothermal peak around 0°C are observed in these curves,and notable increases of the base lines which correspond to increases of heat capacities are observed, implying partial melting of absorbed water by PIP_2.

However, physico-chemical properties of PIP_2 are very sensitive to the preparatory procedure of the sample, and we observe another endothermal peak at -15°C for a different lot of preparation, and complete understanding of the melting of water in this system is left for the subject for a future study.[6]

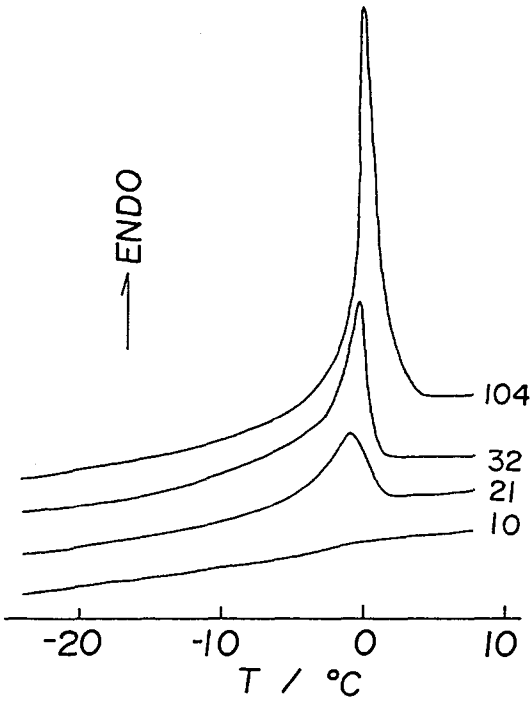

Fig.6. DSC curves for PIP_2 - water system.

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