ELSEVIER

Biochimica et Biophysica Acta 1328 (1997) 197–206

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Effect of water on lamellar structure of DPPC/sugar systems

H. Nagase [a,*], H. Ueda [a], M. Nakagaki [b]

[a] *Department of Physical Chemistry, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142, Japan*
[b] *Tokyo Institute of Colloid Science, 502 Higashi-Nakano 4-4-3, Nakano-ku, Tokyo 164, Japan*

Received 27 March 1997; accepted 3 April 1997

Abstract

The ability of two monosaccharides, four disaccharides and one trisaccharide, to lower the transition temperature of L-α-dipalmitoyl phosphatidylcholine (DPPC) was investigated using differential scanning calorimetry (DSC) and the ability of these sugars to change the lateral packing of the acyl chains of DPPC was investigated using wide-angle X-ray diffraction. The sugars affected the gel–liquid crystal transition temperature (T_c) of DPPC when the water content of the DPPC/sugar systems was less than 20 wt.%. Specifically, T_c of the DPPC without sugar increased to approximately 106°C, the T_c of the DPPC/monosaccharide system remained almost constant at 43°C and of the DPPC/disaccharide or trisaccharide systems decreased to approximately 24°C. In the dehydrated state, di- and trisaccharides caused looser packing of the DPPC hydrocarbon chains than the monosaccharides did, and the sugars affected the packing mode in different ways. The addition of water caused this difference in the sugars' effects on the packing mode to disappear and further addition of water caused the effect of sugar to almost disappear. Thus, the addition of water to a DPPC/sugar system weakens the interaction between the sugar and lipid and strengthens the DPPC chain packing. © 1997 Elsevier Science B.V.

Keywords: Phase transition temperature; Phospholipid; Sugar; Differential scanning calorimetry; X-ray diffraction; Lamella

1. Introduction

Reports have shown that several kinds of disaccharides prevent fusion among vesicles and the leakage of materials entrapped in vesicles during freeze-drying [1–3]. In particular, trehalose, which is a non-reducing disaccharide of glucose and is found in anhydrobiotic organisms [4], has proved to be effective in preserving the structural and functional integrity of biological membranes against freeze-drying [5]. These effects have been postulated to arise because the sugar replaces the water of hydration as the membranes are dehydrated. That the gel–liquid crystal transition temperature (T_c) of DPPC/sugar system is similar to that of hydrated DPPC [4] supports this 'water replacement' hypothesis [4]. The effect of trehalose on the transition temperature (T_c) of DPPC has been analyzed in freeze-dried [3,6–8], air-dried [9] and heat-dried [10] systems; however, the degree depression of T_c differed between drying methods. This difference in T_c may be due to differences in sample preparation or thermal history. Crowe and Crowe showed that a difference in thermal history caused a difference in T_c [6].

The lowering of T_c of a DPPC/sugar system was considered to be due to the formation of hydrogen

Abbreviations: DPPC, L-α-dipalmitoyl phosphatidylcholine; DSC, differential scanning calorimetry; T_c, gel–liquid crystal transition temperature; T_g, glass transition temperature

* Corresponding author. Fax: (+81) (3) 3786-0036; E-mail: nagase@hoshi.ac.jp

PII S0005-2736(97)00088-6

bonds between the OH groups of the sugar and the polar head groups of DPPC [11].

Although monosaccharides prevent fusion of membrane vesicles, they have been shown to be much less effective than disaccharides on the prevention of leakage [1,3]. Sun et al. have shown that in a dry liposome with disaccharide, fusion and solute leakage of liposome clearly occurred above, but only to a very small degree below the glass transition temperature (T_g) of the disaccharide [12]. Monosaccharides depress the T_c of DPPC to nearly equal that of fully hydrated DPPC, but disaccharides depress the T_c of DPPC to below the T_c (42°C) of hydrated DPPC by approximately 18°C [3,6,11]. Koster et al. have shown that in order to lower the T_c of the lipid in a lipid/sugar mixture, the glass transition temperature of the sugar must be higher than the T_c of the lipid [13]. However, Crowe et al. have proposed that both glass formation of the sugar and direct interaction of the sugar and lipid are required to lower the T_c of the lipid in a lipid/sugar mixture [14].

Sugars are known to stabilize phospholipid bilayers in dry state and this stabilization is related to the prevention of an increase in T_c of the phospholipid [15]. However, at which step of the dehydration the effect of the sugar appears and how the transition temperature changes with a decrease in water content remains to be clarified.

NMR and X-ray diffraction studies have revealed the existence of the Lκ-phase and the Lλ-phase of a freeze-dried DPPC/trehalose mixture prepared using organic solvents [16]. The Lκ-phase was characteristic of an Lβ-phase (for nomenclature see [17]) and the Lλ-phase was characteristic of disordered acyl chains and the choline head group which showed hindered molecular motions compared to DPPC alone [16]. The authors noted that the Lκ-phase was a normal gel phase, namely Lβ-phase, but that the Lλ-phase differed to a normal liquid crystal phase. Quinn et al., in a real-time X-ray diffraction study, showed that the transition temperature of a freeze-dried DPPC/trehalose mixture prepared from a hydrated state was a typical gel (Lβ)–liquid crystal (Lα) transition temperature [18]. The packing mode of a DPPC/sugar system prepared by freeze-drying has been investigated [16,18]. Although phase diagrams and the stoichiometry of a DPPC/trehalose system prepared by heating under vacuum have been investigated [10], the packing mode of molecules in this DPPC/trehalose system has yet to be examined.

In the present study, DPPC was used as a membrane model. The lipid was mixed with various sugars at a molar ratio of 2.6 mole sugar per mole of DPPC and was dried by heating under vacuum. Using differential scanning calorimetry (DSC) and wide-angle X-ray diffraction, the thermal behavior of various DPPC/sugar/water systems and the packing mode of DPPC at different phases was investigated.

2. Materials and methods

L-α-dipalmitoyl phosphatidylcholine (DPPC), glucose (GLU), rhamnose (RHA), trehalose (TRE), maltose (MAL), melibiose (MEL) and raffinose (RAF) were purchased from Sigma Chemical Company. Sucrose (SUC) was purchased from Wako Pure Chemical Industries Ltd. The materials were used without further purification. Water was purified using the Milli-Q Labo (Millipore Ltd.).

DPPC (150 mg) in chloroform was dried at 20°C by rotary evaporation and then dried at 60°C for 12 h under vacuum. The dried thin filmy DPPC was hydrated with either water or with one of the sugar solutions (6 ml) at a molar ratio 2.6 (mol sugar/mol DPPC). The dispersions were equilibrated at 60°C for 3 h with shaking. Water was removed by evaporation at 35°C and the samples were heated to 90°C to obtain a powdery material. The powdery material was cooled to room temperature in air. These samples are hereafter referred to as the 'powdery samples'. The samples were stored in powder form at −18°C before use. The powdery samples were then transferred to either an aluminum pan with volume of 25 μl for DSC or a capillary for X-ray diffraction. Some of these powdery samples were further dried for 3 h at 110°C under vacuum and then immediately sealed using a sealer or by flaming. These samples are hereafter referred to as the 'dried samples'. Water was added using Micropet pipettes (Beton, Dickinson and Company) to some of the unsealed dried samples at 24°C. After sealing, the samples were swollen for 3 h at 60°C. These samples are hereafter referred to as the 'swollen samples'. The water content of the powdery samples was determined by the Karl Fischer method using a moisture meter CA-06 (Mitsubishi

Kasei Corp.). The water content of the dried samples and the swollen samples were gravimetrically estimated from the weight of the powdery sample.

DSC measurement was performed using a DSC 8240D under the TAS 200 thermal analysis system (Rigaku Corp.). The powdery samples were loaded at 24°C. The powdery sample was sealed at 24°C. The dried sample was sealed immediately under nitrogen gas after heating under vacuum. The samples were cooled to 0°C using a CryoCool CC100II under nitrogen gas and then immediately heated from 0 to 110°C at a heating rate of 2°C/min. The transition temperature was estimated using the peak top temperature. The second scan was carried out immediately after the first scan. Phase diagrams were constructed using data obtained from the second DSC scan because the addition of water to the dried samples was used to hydrate the sugar, as previously reported [10]. Alumina was used as the reference criterion.

Wide-angle X-ray diffraction patterns were recorded at 24°C using a RINT 1400 X-ray diffractometer (Rigaku Corp.). X-ray measurement was carried out at 1°/min from 2.5° to 40° of the 2θ range. Measurement was performed at 60 kV and 200 mA. X-ray capillaries were 2 mm in diameter. Peak separation was carried out after excluding the background signal. Peak fitting with two peaks was performed using four parameters; intensity, half width, *d*-value and Gauss ratio and peak fitting software supplied with the Rigaku analysis system.

3. Results

3.1. Phase diagram of DPPC / sugar / water system

3.1.1. Phase diagram of DPPC / glucose / water system

DSC thermograms of DPPC/GLU/water systems that had a molar ratio of sugar to DPPC of 2.6 and that contained different amounts of water are shown in Fig. 1. When the water content was 18.5 wt.%, a single endothermic peak was observed at 45.5°C. Above 18.5 wt.%, water, a small peak appeared on the lower temperature side and the two peaks successively decreased as the water content increased, below 18.5 wt.% water, one peak with a weak shoulder peak on the lower temperature side was observed.

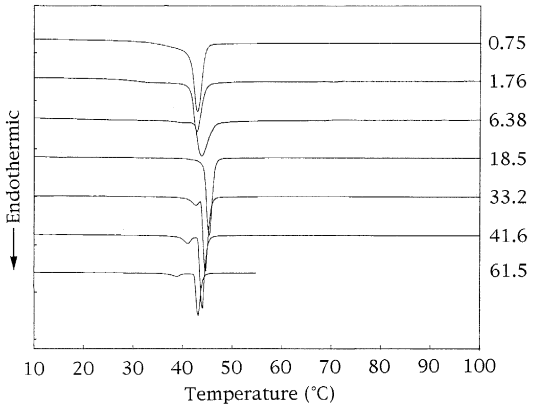

Fig. 1. DSC thermograms of DPPC/glucose/water system which had a GLU/DPPC ratio of 2.6 and different water contents ranging between 0.75 and 61.5 wt.% as indicated on the right side of each curve.

Following a small decrease, the peak temperature increases slightly.

The phase diagrams of the DPPC/GLU/water systems were obtained from the peak top temperature of DSC curves, as shown in Fig. 2. In this figure, the gel–liquid crystal transition and pretransition temperatures, denoted by T_c and T_p, respectively, of the DPPC/water system were also plotted (shown as closed circles). The thermal behavior of the DPPC/water system is in close agreement with that reported in the literature [19,20]. As shown in Fig. 2, when the water content was greater than 20 wt.% water, T_c and T_p of the DPPC/GLU/water system

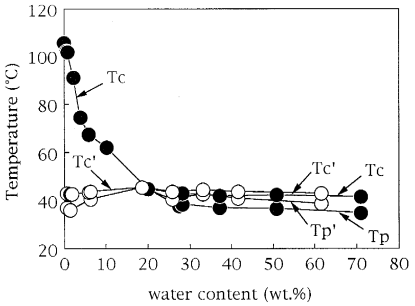

Fig. 2. Phase diagrams of DPPC/water system with and without glucose. ○, DPPC/GLU/water system; ●, DPPC/water system. T_c and T_p are gel–liquid crystal transition temperature and pretransition temperature of DPPC without sugar, respectively. T_c' and T_p' are gel–liquid crystal transition temperature and pretransition temperature of DPPC/monosaccharide/water systems, respectively.

gradually decreased as the water content increased. In addition, T_c and T_p of the DPPC/GLU/water system were higher than those of the DPPC/water system. At 41 wt.% water T_c and T_p of the DPPC/GLU/water system were 1.7 and 3.9°C higher than those of the DPPC/water system.

An increase in the T_c and T_p of DPPC caused by sucrose in a hydrated unsonicated DPPC/sucrose mixture [21] and by trehalose in DPPC multilamellar vesicles with trehalose [22] has been previously observed. Furthermore, increases in the T_c of DPPC have been shown to depend on the amount of sugar present in the system [23].

The T_c of both DPPC with and without GLU were equal when the water content was approximately 20 wt.% water. The T_c of the DPPC without GLU increased by 61.0°C from 44.7°C at 20.1 wt.% to 105.7°C at 0.1 wt.%, while T_c of the DPPC with GLU decreased by only 2.4 to 43.0°C at 0.8 wt.% after passing through a maximum value of 45.4°C at 18.5 wt.%.

3.1.2. Phase diagram of DPPC / maltose / water system

DSC thermograms of the DPPC/MAL/water systems that had a molar ratio of sugar to DPPC of 2.6 and that contained various amounts of water are shown in Fig. 3. When the water content was 18.4 wt.%, two endothermic peaks were observed; one was at 50.2°C and the other at 43.6°C. The lower peak was very weak. Above 18.4 wt.% water, the higher peak gradually decreased and the lower peak slightly decreased as water content increased. Below 10.1 wt.% water, two endothermic peaks appeared. The lower transition temperature decreased to 28.1°C and the higher transition temperature increased to 70.2°C as water content decreased.

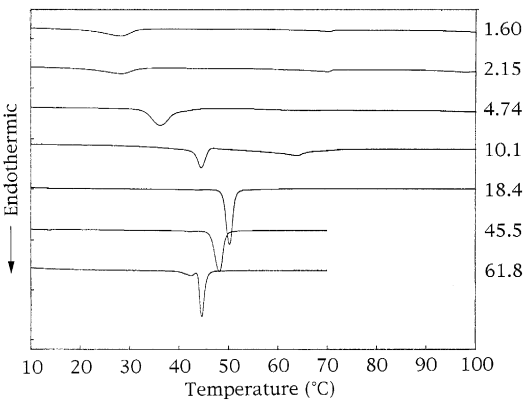

Fig. 3. DSC thermograms of DPPC/maltose/water system with 2.6 molar ratio (MAL/DPPC) and different water content for DPPC between 3.47 and 61.8 wt.% indicated at the right side of each curve.

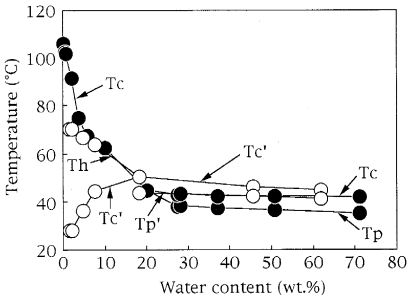

Fig. 4. Phase diagrams of DPPC/water systems with and without maltose. ○, DPPC/MAL/water system; ●, DPPC/water system. T_c and T_p are gel–liquid crystal transition temperature and pretransition temperature of DPPC/water system without sugar, respectively. T_c', T_p' and T_h are gel–liquid crystal transition temperature, pretransition temperature and higher transition temperature of DPPC/MAL/water system, respectively.

The phase diagrams of the DPPC/water system and DPPC/MAL/water systems are shown in Fig. 4. Above 20 wt.% water, T_c and T_p of the DPPC/MAL/water system gradually decreased as water content increased and T_c and T_p of the DPPC/MAL/water system were higher than those of the DPPC/water system. At 46 wt.% water T_c and T_p of the DPPC/MAL/water system were 3.8 and 5.3°C higher than those of the DPPC/water system. The T_p of the DPPC/MAL/system increased slightly and its peak area gradually decreased as water content decreased. The peak almost disappeared at approximately 18 wt.% water. When the water content was 20 wt.%, T_c of the DPPC/MAL/water system was equal to that of the DPPC/water system. Below 20 wt.% water, T_c of the DPPC/MAL/water system decreased to 28.1°C and higher transition temperature (T_h) of the DPPC/MAL/water system increased to 70.2°C.

3.1.3. Phase diagram of DPPC / sugar / water system

Fig. 5 shows the phase diagrams of the DPPC/sugar/water systems obtained from the peak

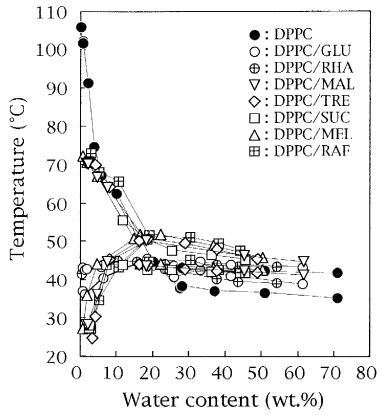

Fig. 5. Phase diagrams of DPPC/water systems with and without sugar.

top temperature of the second scan DSC curves. The thermal behavior of DPPC/RHA/water system was similar to that of the DPPC/GLU/water system. The T_c and T_p of DPPC/RHA/water system were observed above 20 wt.% water and T_c and T_p increased and then T_p approached T_c and finally disappeared at approximately 18 wt.% water as water content decreased. After passing through a maximum value of 43.0°C at 17.8 wt.%, T_c slightly decreased to 41.6°C at 0.3 wt.%.

The thermal behavior of DPPC/di- or trisaccharide/water systems was similar to that of the DPPC/MAL/water system. The T_p of the DPPC/di- or trisaccharide/systems increased slightly and its peak area gradually decreased as water content decreased. The peak almost disappeared at approximately 18 wt.% water. T_c of the DPPC/di- or trisaccharide/systems increased slightly as water content decreased. Below approximately 18 wt.% water, two transition temperatures were observed. The higher transition temperature increased to approximately 73°C and lower transition temperature (T_c) decreased to approximately 24°C as water content decreased.

3.1.4. Transition temperature of powdery and dried DPPC / sugar / water system

The gel–liquid crystal transition temperatures of the powdery and dried samples are shown in Table 1. For powdery samples, T_c of DPPC decreased to 43.9 ± 1.4°C (T_c) regardless of the kind of sugar in the sample. Compared to T_c (67.3°C) of the DPPC/water system, the sugars in each of the powdery samples lowered T_c by 23.4 ± 1.4°C.

T_c of dried DPPC without sugar was 105.7°C at 0.1 wt.% water. T_c of dried samples containing monosaccharide was 42.3 ± 0.7°C while T_c of those containing di- and trisaccharides was 26.9 ± 2.2°C (T_c). The di- and trisaccharides decreased T_c of DPPC by approximately 15°C more than the monosaccharides. Thus, the difference in transition temperature between the DPPC/monosaccharide/water systems and DPPC/di- or trisaccharide/systems was readily apparent when these samples were dried. T_c of the DPPC/trehalose/water system was higher in the powdery samples, but conversely lower in the dried samples than that of the other sugars.

3.2. X-ray diffraction

3.2.1. Powdery samples

The wide-angle X-ray diffraction patterns of the powdery samples are shown in Fig. 6. At approxi-

Table 1
Gel–liquid crystal transition temperature (T_c) of dried and powdery samples

Sugar	Dried sample		Powdery sample	
	T_c (difference) (°C)	Water content (wt.%)	T_c (difference) (°C)	Water content (wt.%)
−	105.7 (−)	0.1	67.3 (−)	4.8
Glucose	43.0 (−62.7)	0.8	43.8 (−23.5)	6.4
Rhamnose	41.6 (−64.1)	0.3	42.8 (−24.5)	4.4
Trehalose	24.7 (−81.0)	3.5	45.3 (−22.0)	8.2
Maltose	28.1 (−77.6)	1.6	44.5 (−22.8)	7.7
Sucrose	27.3 (−78.4)	2.3	43.9 (−23.4)	7.3
Melibiose	27.9 (−78.7)	0.8	43.9 (−23.4)	4.9
Raffinose	27.3 (−78.4)	3.0	43.3 (−24.0)	10.6

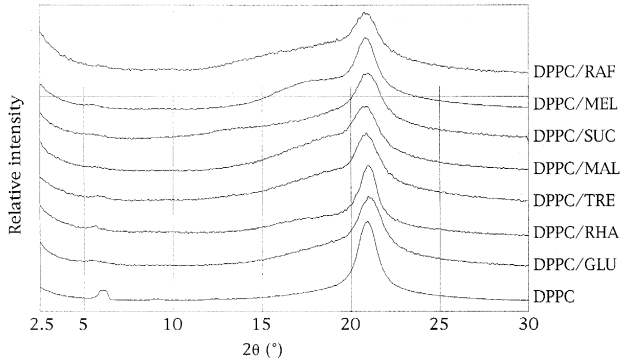

Fig. 6. Wide-angle X-ray diffraction patterns of powdery DPPC/water systems with and without sugar.

mately $2\theta = 21°$ ($d = 4.2$ Å) a peak, with a broad peak on the lower 2θ-angle side, was observed for each powdery DPPC/sugar/water system. The powdery DPPC/water-only system showed one peak at the same 2θ angle (21°). Thus, the broad peak of the powdery DPPC/sugar/water systems are representative of the effect of the sugars on the chain packing of DPPC. This effect was largely independent of the kind of saccharide in the system, which is in agreement with the DSC data in which T_c of the powdery DPPC/sugar/water systems are approximately 44°C regardless of the kind of sugar in the sample (see Section 3.1.4).

3.2.2. Swollen samples

The wide-angle X-ray diffraction patterns of the swollen DPPC/sugar/water systems that contained approximately 20 wt.% water are shown in Fig. 7. In

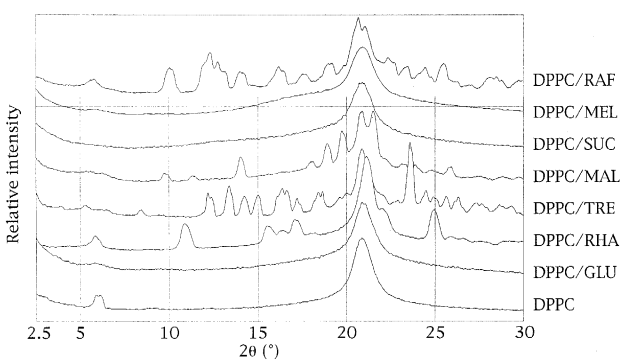

Fig. 7. Wide-angle X-ray diffraction patterns of swollen DPPC/water systems with and without sugar. Water content was approximately 20 wt.%.

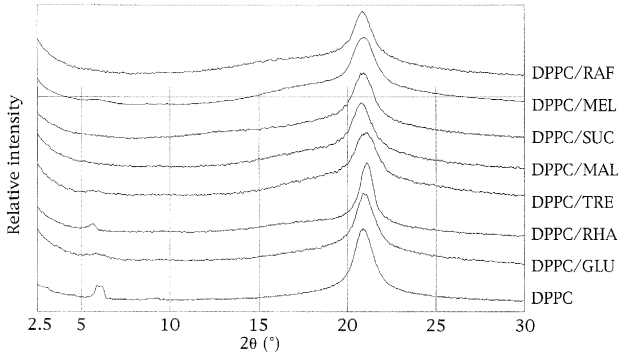

Fig. 8. Wide-angle X-ray diffraction patterns of swollen DPPC/water systems with and without sugar after heating up to the dehydration temperature. Water content was approximately 20 wt.%.

the swollen DPPC/RHA, TRE, MAL or RAF/water systems, many peaks appeared. These peaks were most likely due to the hydrate crystal of each sugar, since RHA, TRE, MAL and RAF form mono-, di-, mono- and penta-hydrates, respectively. Thus, excess sugar was present in these systems. Once the samples containing the sugar hydrates were heated above the dehydration temperature of the crystalline hydrates, many peaks disappeared except for a peak at $2\theta = 21°$ ($d = 4.2$ Å), as shown in Fig. 8.

3.2.3. Dried samples

Fig. 9 shows the wide-angle X-ray diffraction patterns of the dried DPPC/sugar/water systems. For the DPPC/monosaccharide/water systems, the peak pattern hardly changed compared with the peak

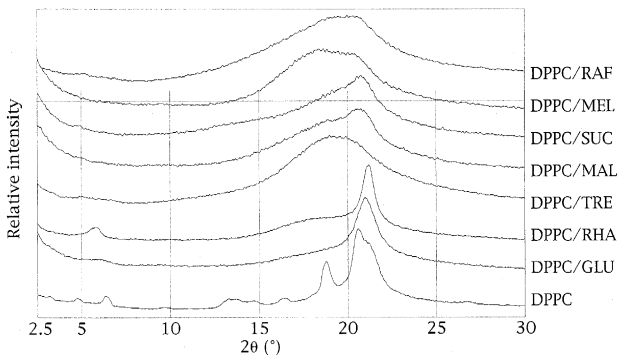

Fig. 9. Wide-angle X-ray diffraction patterns of dried DPPC/water systems with and without sugar. Water content of DPPC system with GLU, RHA, TRE, MAL, SUC, MEL and RAF is 0.7, 0.3, 3.4, 1.4, 2.0, 0.7 and 2.8 wt.%, respectively.

pattern of the same system in powdery form shown in Fig. 6. In contrast, for the dried DPPC/di- or trisaccharide/water systems, the peak intensity of the lower angle side increased and the peak intensity at $2\theta = 21°$ ($d = 4.2$ Å) decreased. When the water content of DPPC/di- or trisaccharide/water systems was greater than the water content shown in Fig. 9 of each system, the X-ray diffraction pattern of the DPPC/di- or trisaccharide/water systems was identical to that of the corresponding powdery samples (data not shown). The water content shown in Fig. 9 was less than that at 24°C estimated by the phase diagrams in Fig. 4, indicating that the dried DPPC/di- or trisaccharide/water systems were in a liquid crystal phase above T_c. Distinct differences were observed between the diffraction patterns of the different dried DPPC/sugar/water systems.

As seen in Fig. 9 the DPPC/water-only system showed many X-ray diffraction peaks. This result indicates that the dried DPPC/water system is in crystal form. It is known that DPPC/water system without sugar is in crystal form when the water content is as low as 1 or 2 wt.% [17]. Thus, the DPPC sample without sugar in the present study was in crystal form. Sugars prevent the DPPC from crystallization caused by dehydration.

3.2.4. Sugar induced expansion of lateral packing of DPPC hydrocarbon chains

The peak at approximately 21° ($= 2\theta$) seen in the X-ray diffraction patterns of the DPPC/sugar/water systems examined in the present study corresponds to a subcell which is a cell formed by the periodicity of the lateral packing of the methylene groups of hydrocarbon chains [24]. A single peak indicates that the subcell is hexagonal and two peaks indicate that the subcell is non-hexagonal [25,26]. Assuming that the peak of the DPPC/sugar/water system consists of two overlapping peaks at around 21° ($= 2\theta$), the peak should be able to be separated into two peaks. The relationships between the diffraction pattern and the subcell after peak fitting with two peaks are shown in Fig. 10.

The intensity fraction (IF) of the lower 2θ-angle peak was defined as follows in the present study; $IF = X/(X + Y)$, where X and Y are the intensity of each peak after peak fitting (see Fig. 10). Fig. 11 shows the intensity fraction of the dried, powdery and swollen (about 20 wt.% water) DPPC/sugar/water systems. The intensity fraction of the DPPC/di- or trisaccharide/water systems increased as the water content decreased. However, this increase was small for DPPC/monosaccharide/water systems. In partic-

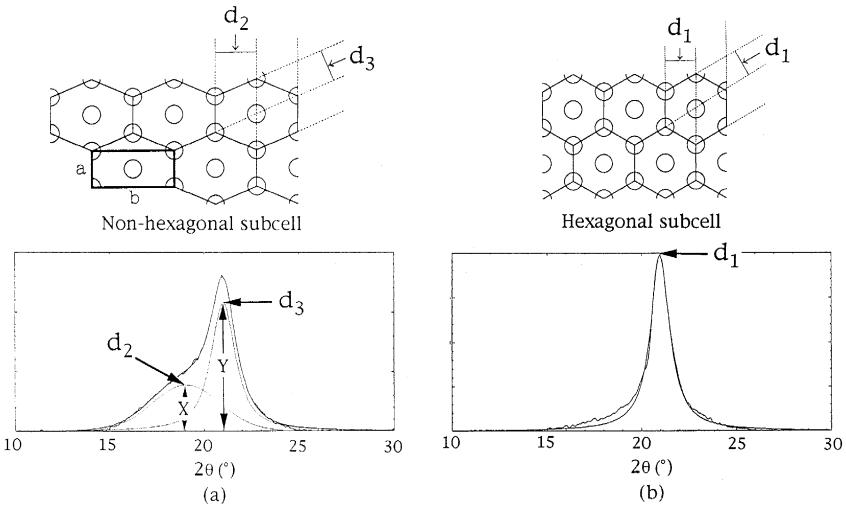

Fig. 10. Relationship between X-ray diffraction pattern and subcell formed by hydrocarbon chains. a: non-hexagonal subcell obtained from two peaks in powdery DPPC/MAL/water system. b: hexagonal subcell obtained from one peak in swollen DPPC/MAL/water system. Open circles indicate hydrocarbon chains viewed along chain axis. X and Y are intensity of the two peaks. a and b are lattice constants of rectangular subcell.

ular, in the dried DPPC/MEL/water system, the intensity of lower peak is stronger than that of the higher peak. Although the intensity fraction of the dried DPPC/sugar/water systems differed between each system, the intensity fraction decreased and the difference narrowed as water content increased.

The lattice constants, a and b, of the rectangular subcell in Fig. 10 were calculated using d-values, d_1 or d_2 and d_3, after peak fitting. Lattice constant b was obtained from the equations $b = 2d_1$ and $b = 2d_2$. Lattice constant a was obtained from the equations:

$$a = 2d_1/\sqrt{3} = b/\sqrt{3} \quad \text{(for hexagonal subcell)}$$

$$a = 2 \times d_2 \times d_3 \times \sqrt{1/(4 \times d_2 \times d_2 - d_3 \times d_3)}$$

(for non-hexagonal subcell).

For the non-hexagonal subcell, two pairs of lattice constants were calculated; one pair for when $d_2 > d_3$ and one pair for when $d_3 > d_2$. A simulation of the pattern of a subcell that consisted of lateral packing of CH_2 group showed that if $d_2 > d_3$, then the intensity of the lower angle peak would be weaker than that of the higher angle peak and if $d_3 > d_2$, then the reverse would occur. The result obtained from the inequality $d_2 > d_3$ was in accordance with the experimental data (see Fig. 10a). Fig. 12 shows the lattice constants, a and b, of DPPC/sugar/water systems that contained different amounts of water. In the powdery and swollen DPPC/water-only systems, the relationship between a and b is simply $a = b/\sqrt{3}$ because the subcell is hexagonal. As indicated by the closed Greek cross in Fig. 12, a and b were 4.90 and 8.49 Å, respectively. In all the dried, powdery and swollen DPPC/sugar/water systems, a and b were

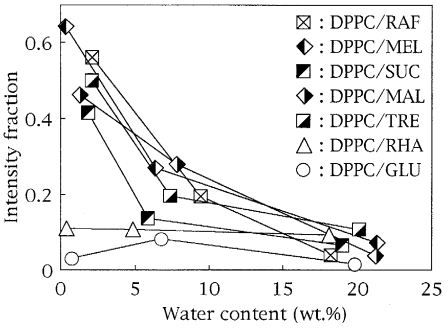

Fig. 11. Relationship between intensity fraction and water content of DPPC/sugar/water system.

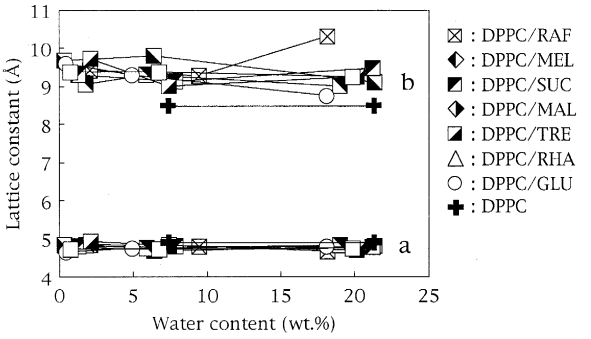

Fig. 12. Relationship between lattice constants (a, b) and water content of DPPC/sugar/water system.

4.8 ± 0.2 and 9.6 ± 0.6 Å, respectively. Thus, the addition of sugar to DPPC had almost no effect on lattice constant a, but b increased by approximately 1.1 Å.

4. Discussion

The decrease in the transition temperature (T_c) of DPPC upon addition of sugar appeared when the water content was less than approximately 20 wt.%, as seen in Figs. 2 and 4. The number of bound water is known to be 9–12 mol water/mol lipid (ca. 18–23 wt.% water) [27,28]. This water content is equal to the water content (ca. 18–20 wt.% water) at which T_c of the DPPC/water system is equal to T_c of the DPPC/sugar/water system. The rising of T_c in a DPPC/water system is thought to be caused by approaching of hydrocarbon chains when the system is dehydrated [28]. Therefore, sugars cause loose packing of the DPPC hydrocarbon chains. Sugars effectively prevent the approach between hydrocarbon chains due to the dehydration of bound water.

When bound water is removed, less than 20 wt.% water, GLU replaces the bound water and interacts with the phosphate groups of DPPC via hydrogen bonds, as IR spectra have shown [6]. This interaction leads to loose packing of the DPPC hydrocarbon chains. The T_c of our system with GLU was roughly constant (44.1 ± 1.3°C) when the water content less than 20 wt.%. In this sense, the effect of GLU on DPPC is similar to that of bound water on DPPC. This result supports the 'water replacement hypothesis' presented by Crowe and Crowe [4].

When the water content was less than 20 wt.%, two endothermic peaks were observed in the DPPC/di- or trisaccharide/water systems. Two endothermic peaks have also been reported for a heat-dried DPPC/TRE (=1/1) mixture [10], a freeze-dried DPPC/TRE (=1/2.2) mixture [29] and a DPPC/SUC (=1.02/0.98) mixture [30]. The T_c of the dried samples in the present study (43.0°C for DPPC/GLU and 28.1°C for DPPC/MAL) were nearly equal to that of vacuum-dried DPPC/sugar mixtures after freeze-drying (44.1°C for DPPC/GLU system [3] and 26.8°C for DPPC/MAL system [12]). Thus, sugars are apt to depress the T_c of DPPC under low hydration conditions (water content below 20 wt.%) and to raise the T_c of DPPC in hydrated state (water content above than 20 wt.%). The fall in T_c is to be caused by direct interaction between DPPC and sugar [11]; the rise in T_c may be explained by the competition of water for the hydrogen bonding sites on the phospholipid [10].

Koster et al. have proposed a novel explanation for the mechanism by which the lipid T_c in lipid/sugar systems is lowered [13]. They showed that in order to lower the T_c of the lipid in lipid/sugar systems, the sugar must be in a glassy state when the lipid is in a liquid crystal state. Crowe et al. have stated that glassy sugar is necessary, but not sufficient, to lower the lipid T_c [9]. In the present study, the monosaccharides do not satisfy this necessary condition, because the glass transition temperatures of GLU and RHA are 36 and 0°C, respectively [31] and the T_c of DPPC in the DPPC/GLU or RHA/water systems is approximately 42°C. On the other hand, the glass transition temperatures of RTE, MAL, SUC, and MEL are 77, 92, 67 and 91°C, respectively [31]. These sugars satisfy the necessary condition and as shown the T_c of DPPC in these DPPC/sugar/water systems did fall markedly.

The effect of the sugars on the packing of DPPC when the water content was low was characterized by the appearance of a broad peak at the lower 2θ-angle side. In previous the X-ray diffraction studies, freeze-dried DPPC/TRE mixtures prepared using organic solvents [16] and water [18] showed a single peak at 4.11 and 4.01 Å, respectively. The powdery sample of pure DPPC below T_c had a hexagonal subcell, while the other powdery DPPC samples with sugar had non-hexagonal subcells. Thus, the sugars affected the chain packing of the powdery DPPC samples. The appearance of a peak at lower 2θ-angle side indicates the expanded subcell (see Fig. 10).

The effect of sugars on subcell is most likely caused by the interaction between the sugar and hydrophilic groups of DPPC [6]. The effects in the powdery sample were almost independent of the kind of saccharide. When the water content approximately 20 wt.%, the broad peak observed at the lower angle side in the powdery DPPC/sugar/water systems almost disappeared and the effect of the sugars on the diffraction pattern of DPPC was hardly visible (see Fig. 8). This suggests that at a water content of 20 wt.%, sugars hardly interact with DPPC.

The addition of 2.6 times sugar to DPPC results in an excess of sugar, which exists outside the lamellar structure and forms a hydrate upon the addition of water, as discussed previously [10].

In the dried DPPC/monosaccharide/water systems, the diffraction pattern hardly changed compared with that of the corresponding powdery systems (see Figs. 8 and 9). On the other hand, in the dried DPPC/di- or trisaccharide/water systems the diffraction pattern was extremely broad compared with that of the corresponding powdery systems (see Figs. 8 and 9). The results of dried DPPC/sugar/water systems suggest that a DPPC with monosaccharide sample is still in gel phase, but DPPC with di- or trisaccharide sample reaches liquid crystal phase by dehydration. A normal liquid crystal phase, which can be seen at above T_c in a DPPC/water system, is characterized by a broad single peak at approximately 4.6 Å ($2\theta = 19.3°$) [25]. Thus, a liquid crystal phase above the T_c of a DPPC/di- or trisaccharide/water systems is not accordance with the normal liquid crystal phase. The various diffraction patterns in Fig. 9 imply that the packing modes in these liquid crystal phases are different.

The addition of water to the dried DPPC/sugar/water systems caused an increase in T_c and a decrease in intensity fraction (see Fig. 11). This indicates that the addition of water weakened the interaction between the sugar and phospholipid. Consequently, the chain packing was strengthened.

Lattice constants a (4.90 Å) and b (8.49 Å) in the powdery DPPC/water-only system were slightly longer than a (4.84 Å) and b (8.38 Å) of the

hexagonal subcell in the hydrated DPPC in Pβ′ phase [25]. In the powdery DPPC/sugar/water systems, lattice constant a (4.8 ± 0.2 Å) was shorter than lattice constant a (5.21 Å) of hydrated DPPC in Lα phase [25] but lattice constant b (9.6 ± 0.6 Å) was longer than lattice constant b (9.02 Å) of hydrated DPPC in Lα phase [25]. The area of the rectangular subcell of the powdery DPPC/water systems was 41.6 Å^2. The area of the subcell of the DPPC/GLU/water system was 44.4 Å^2 for the dried sample and 44.2 Å^2 for the powdery sample. The area of the dried DPPC/TRE/water system was the largest area in the all the dried samples. The area of the subcell of the DPPC/TRE/water system was 47.9 Å^2 for the dried sample and 43.3 Å^2 for the powdery sample. The difference between the areas of the powdery and dried DPPC/GLU/water systems was 0.2 Å^2 and for DPPC/TRE/water systems was 4.6 Å^2. Upon dehydration, the lateral packing of DPPC/GLU/water system hardly changed, while that of the DPPC/TRE/water system expanded by 4.6 Å^2. This expansion of the rectangular subcell of the DPPC/TRE system was larger than for any of the other DPPC/sugar/water/systems. The increase in area caused loose lateral packing of the DPPC hydrocarbon chains and may have resulted in the observed decrease in T_c of DPPC (see Table 1).

5. Conclusion

Upon dehydration, the interaction between DPPC and sugar increases, resulting in loose packing of the DPPC hydrocarbon chains. The addition of water weakens the interaction and strengthens the chain packing. Trehalose most strongly interacts with DPPC in DPPC/sugar systems in dehydrate state.

References

[1] L.M. Crowe, C. Womersley, J.H. Crowe, D. Reid, L. Appel, A. Rudolph, Biochim. Biophys. Acta 861 (1986) 131–140.
[2] T.D. Madden, M.B. Bally, M.J. Hope, P.R. Cullis, H.P. Schieren, A.S. Janoff, Biochim. Biophys. Acta 817 (1985) 67–74.
[3] K. Tanaka, T. Takeda, K. Fujii, K. Miyajima, Chem. Pharm. Bull. 40 (1992) 1–5.
[4] J.H. Crowe, L.M. Crowe, Science 223 (1984) 701–703.
[5] J.H. Crowe, L.M. Crowe, S.A. Jackson, Arch. Biochem. Biophys. 220 (1983) 477–484.
[6] L.M. Crowe, J.H. Crowe, Biochim. Biophys. Acta 946 (1988) 193–201.
[7] C.W.B. Lee, J.S. Waugh, R.G. Griffin, Biochemistry 25 (1986) 3737–3742.
[8] M. Sakurai, H. Kawai, Y. Inoue, A. Hino, S. Kobayashi, Bull. Chem. Soc. Jpn. 68 (1995) 3621–3627.
[9] J.H. Crowe, F.A. Hoekstra, K.H.N. Nguyen, L.M. Crowe, Biochim. Biophys. Acta 1280 (1996) 187–196.
[10] M. Nakagaki, H. Nagase, H. Ueda, J. Membr. Sci. 73 (1992) 173–180.
[11] L.M. Crowe, J.H. Crowe, D. Chapman, Arch. Biochem. Biophys. 236 (1985) 289–296.
[12] W.Q. Sun, A.C. Leopold, L.M. Crowe, J.H. Crowe, Biophys. J. 70 (1996) 1969–1976.
[13] K.L. Koster, M.S. Webb, G. Bryant, D.V. Lynch, Biochim. Biophys. Acta 1193 (1994) 143–150.
[14] L.M. Crowe, D.S. Reid, J.H. Crowe, Biophys. J. 71 (1996) 2087–2093.
[15] J.H. Crowe, L.M. Crowe, J.F. Carpenter, A.S. Rudolph, C.A. Wistrom, B.J. Spargo, T.J. Anchordoguy, Biochim. Biophys. Acta 947 (1988) 367–384.
[16] C.W.B. Lee, S.K.D. Gupta, J. Mattai, G.G. Shipley, O.H. Abdel-Mageed, A. Makriyannis, R.G. Griffin, Biochemistry 28 (1989) 5000–5009.
[17] A. Tardieu, V. Luzzati, F.C. Reman, J. Mol. Biol. 75 (1973) 711–733.
[18] P.J. Quinn, R.D. Koynova, L.J. Lis, G.B. Tenchov, Biochim. Biophys. Acta 942 (1988) 315–323.
[19] M. Kodama, M. Kuwabara, S. Seki, Biochim. Biophys. Acta 689 (1982) 567–570.
[20] M. Nakagaki, T. Handa, K. Ohashi, Yakugaku Zasshi 99 (1979) 1–7.
[21] G. Strass, P. Schurtenberger, H. Hauser, Biochim. Biophys. Acta 858 (1986) 169–180.
[22] A.S. Rudolph, B. Goins, Biochim. Biophys. Acta 1066 (1991) 90–94.
[23] B.Z. Chowdhry, G. Lipka, J.M. Sturtvant, Biophys. J. 46 (1984) 419–422.
[24] S. Abrahamsson, B. Dahlen, H. Lofgren, I. Pascher, Prog. Chem. Fats Lipids 16 (1978) 125–143.
[25] M.J. Ruocco, G. Shipley, Biochim. Biophys. Acta 691 (1982) 309–320.
[26] K. Harlos, Biochim. Biophys. Acta 511 (1978) 348–355.
[27] M. Kodama, H. Hashigami, S. Seki, J. Colloid Interface Sci. 117 (1986) 497–504.
[28] J.H. Crowe, L.M. Crowe, J.F. Carpenter, C.A. Wistrom, Biochem. J. 242 (1987) 1–10.
[29] T.D. Tsvetkov, L.I. Tsonev, N.M. Tsvetkova, R.D. Koynova, G.B. Tenchov, Cryobiology 26 (1989) 162–169.
[30] M. Hentschel, P. Miethe, H.W. Meyer, Biochim. Biophys. Acta 980 (1989) 169–174.
[31] Y. Roos, Carbohydr. Res. 238 (1993) 39–48.