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Temperature change of the lamellar structure of DPPC/disaccharide/water systems with low water content

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Abstract

Temperature change in L-α-dipalmitoyl phosphatidylcholine (DPPC)/disaccharide systems with low water content (less than 8 wt.%) was investigated using X-ray diffraction within a range of two transition temperatures. X-ray diffraction above the higher transition temperature showed a broad symmetric peak, indicating the Lα phase. Below the higher transition temperature, two overlapping diffraction peaks were observed. After peak separation, temperature change in these systems was analyzed using peak parameters of the two peaks. Peak parameters of the lower angle peak changed continuously up to and above the higher transition temperature, suggesting the systems to be in a liquid crystal phase below the higher transition temperature. Fourier-transform infrared (FT-IR) spectra of the DPPC/trehalose system with 5.5 wt.% water showed the wave number of asymmetric stretching of phosphate groups to change at the lower transition temperature and that of symmetric stretching of CH_2 groups, to change between the lower and higher transition temperatures. Thus, below the lower transition temperature, the system is shown to be in a gel phase. Conformational change in phosphate groups occurred at the lower transition temperature. Within the lower and higher transition temperatures, two phases were found to coexist and transition from the gel phase to Lα phase to occur continuously. Above the higher transition temperature, the system is in the Lα phase.

Keywords: Phase transition temperature; DPPC; Disaccharide; DSC; X-ray diffraction; Fourier-transform infrared (FT-IR)

1. Introduction

Several sugars are effective for stabilizing liposomes during freeze-drying or freeze-thawing [1–4]. Trehalose is very effective not only for stabilizing liposomes but preserving their morphology as well during freeze-drying [5,6]. Maltose also effectively stabilizes freeze-dried liposomes [1,2,7]. These effects are thought to result from reduction in gel–liquid crystal transition temperature (T_c) [2,8], which in turn depends on thermal treatment of a sample [8].

Sugar in a glassy state prevents leakage of trapped materials more effectively than in a non-glassy state [9]. In a DPPC/trehalose mixture [8], the disaccharide in a non-glassy state lowers the T_c of DPPC to 70°C, whereas in a glassy state, to 24°C. Thus, to lower T_c of a lipid to 24°C, when the sugar is in a

Abbreviations: DPPC, L-α-dipalmitoyl phosphatidylcholine; DSC, differential scanning calorimetry; T_c, gel–liquid crystal transition temperature; T_H, higher transition temperature; T_L, lower transition temperature; T_g, glass transition temperature of sugar

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glassy state, the lipid must be in a liquid crystal state [10].

Dried DPPC/trehalose mixture prepared by heat application under vacuum was recently shown to have two transition temperatures, 24°C and 70°C [11]. Two transition temperatures were also noted in the second scan. Although these features were not noted by Crowe et al. [8] or Tanaka et al. [2], two endothermic peaks have been observed in a freeze-dried DPPC/trehalose (1/2.2) mixture [12] and DPPC/sucrose (1.02/0.98) mixture [13].

X-ray diffraction analysis of DPPC/di-, or trisaccharide systems prepared by heating under vacuum has indicated such systems to be in a liquid crystal phase at approximately 24°C [14]. Two broad peaks appeared at approximately 4.2 Å and 4.6 Å in this phase, thus showing it to be at variance with the liquid crystal phase ($L\alpha$) in hydrated DPPC, that is above the gel–liquid crystal transition temperature [14]. The temperature range in which a normal liquid crystal phase may be observed has get to be determined. The liquid crystal phase in the present system is at variance with those reported by Lee et al. [15] or Quinn et al. [16], since the diffraction patterns of the latter represent the $L\alpha$ phase.

For clarification of the effects of sugar on membrane transition temperatures, the L-α-dipalmitoyl phosphatidylcholine (DPPC) lipid model was used to characterize the phase of the heat-dried DPPC/trehalose or maltose system below and above the transition temperatures using wide angle X-ray diffraction and Fourier-transform infrared (FT-IR) spectroscopy.

2. Experimental

2.1. Materials and sample preparation

DPPC (Nichiyu Liposome), trehalose (TRE, Sigma) and maltose (MAL, Sigma) from commercial sources were used without further purification. Water was purified using Milli-Q Labo (Millipore).

DPPC (150 mg) in chloroform was dried at 20°C by evaporation and again for 12 h at 60°C under vacuum. This was followed by hydration with 6 ml water or a solution with a sugar to DPPC molar ratio of 2.6. Dispersion was equilibrated at 60°C for 3 h with shaking. Water was removed by evaporation at 45°C and all samples were heated to 90°C for their conversion to powder, followed by cooling to room temperature in air. These powders constituted what is hereafter referred to as the 'powdery system'. The samples were transferred to an aluminum pan for DSC or a capillary for X-ray diffraction, heated at 90°C for 1 h and sealed immediately using a sealer or flame, respectively. These samples comprised the 'heated system'. Water content of the powdery system was determined by the Karl Fischer method using the moisture meter, CA-06 (Mitsubishi Kasei). Water content of the heated system was found based on that of the powdery system by gravimetry.

2.2. Differential scanning calorimetry (DSC)

DSC was performed using a DSC 8240D in the TAS 200 thermal analysis system (Rigaku), at a heating rate of 2°C/min from 0°C to 110°C. The transition temperature was determined based on the peak top temperature. Alumina served as the reference.

2.3. Wide-angle X-ray diffraction

Wide-angle X-ray diffraction was recorded between 14°C and 80°C using a RINT 1400 X-ray diffractometer (Rigaku). X-ray measurement was carried out at 1°/min from 2.5° to 40° of the diffraction angle at various temperatures produced using hot air, at 60 kV and 200 mA. Each X-ray capillary was 2 mm in diameter. Peak separation was done subsequent to background signal elimination and peak fitting with two peaks was conducted based on intensity, half width, *d*-value and Gauss ratio, using the software provided with the Rigaku analysis system. Relative intensity (I/I (at the lowest temperature)), half width and *d*-value served as the peak parameters for a diffraction peak.

2.4. FT-IR spectroscopy

The powdery DPPC/water and powdery DPPC/TRE/water systems were each placed between two potassium bromide plates. The former was sealed with a presser. The latter was dried for 1 h at 40°C and immediately sealed in the same manner.

FT-IR spectra were obtained using a Micro 20 (JASCO) equipped with DSC apparatus (Metller), at a heating rate of 4°C/min, from 25°C to 105°C.

3. Results

3.1. Powdery DPPC / disaccharide / water systems

3.1.1. DSC

DSC endothermic curves for the powdery DPPC system containing 7.3 wt.% water, powdery DPPC/MAL system 7.5 wt.% water and the powdery DPPC/TRE system 5.9 wt.% water are shown in Fig. 1. Second and third scannings were carried out immediately after the first. The gel–liquid crystal transition temperature (T_c) of DPPC was 65.9°C and was consistent with that of DPPC containing 7.6 wt.% water [17]. In the powdery DPPC/disaccharide/water systems, lower and higher transition temperatures, designated as T_L and T_H, were observed.

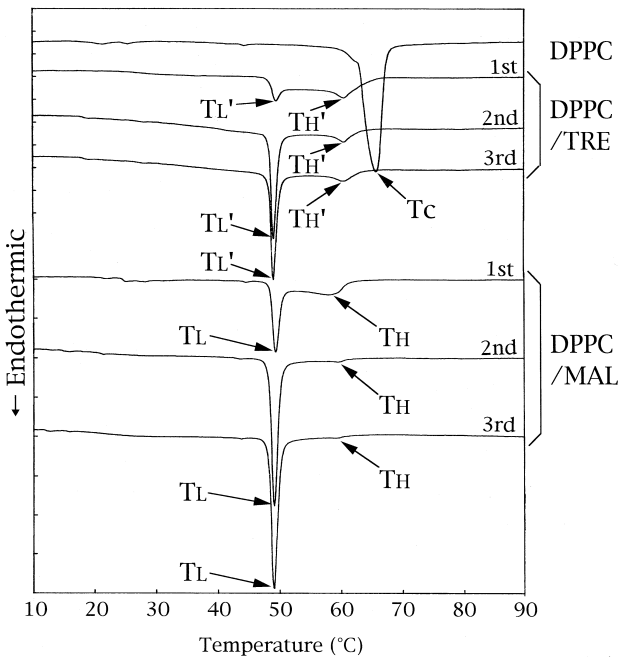

Fig. 1. DSC thermograms of the powdery DPPC system containing 7.3 wt.% water, powdery DPPC/maltose system 7.5 wt.% water and powdery DPPC/trehalose system 5.9 wt.% water. T_c is the gel–liquid crystal transition temperature of DPPC/water. T_L and T_H are the lower and higher transition temperatures, respectively, of the DPPC/maltose/water system. T'_L and T'_H are the lower and higher transition temperatures, respectively, of the DPPC/trehalose/water system.

In the powdery DPPC/MAL/water system, T_L was 49.2°C and T_H 57.8°C for the first scan from 0°C to 110°C. T_L and T_H were 49.0°C and 59.2°C for the second scan, respectively and 49.0°C and 59.4°C for the third, respectively. T'_L and T'_H were also observed for the powdery DPPC/TRE/water system as 49.2°C and 60.8°C for the first scan. Neither of these temperatures changed for the second or third scanning.

3.1.2. Wide-angle X-ray diffraction analysis

Fig. 2 shows diffraction patterns at various temperatures of the powdery DPPC system containing 7.4 wt.% water, powdery DPPC/MAL system 7.7 wt.% water and powdery DPPC/TRE system 6.0 wt.% water. The diffraction peak of the powdery DPPC system was symmetric at 20.9° (2θ) at 24°C, indicating the gel to be in the Lβ phase (for nomenclature see Ref. [18]). This peak shifted to the lower angle side with increase in temperature. At 70°C, (above T_c, 65.9°C), a broad symmetric peak appeared at 19.2° (2θ), demonstrating the powdery DPPC/water system to be in the Lα phase above T_c. The diffraction patterns of the powdery DPPC/MAL/water system at 24°C and powdery DPPC/TRE/water system at 23°C indicated asymmetric peaks with a broad peak in each case at the lower angle side at 21.0° (2θ). In the powdery DPPC/disaccharide/water systems, the higher angle peak shifted to the lower angle and whose intensity decreased with rise in temperature. That of the lower angle peak increased with temperature. Above DPPC/MAL T_H (57.8°C) and DPPC/TRE T'_H (60.8°C), a broad symmetric peak was noted at 19° (2θ). These peaks were essentially the same as that of the Lα phase [19].

In Fig. 3, a comparison is made of the peak parameters (relative intensity, half width and *d*-value) for the powdery DPPC/disaccharide/water systems and powdery DPPC/water system. Fig. 3b shows the peak parameters of the powdery DPPC peaks and higher angle peak of the powdery DPPC/disaccharide/water systems following peak separation. *d*-Values of the three systems gradually increased with temperature and steeply so at T_c in the powdery DPPC/water system and T_H and T'_H in the powdery DPPC/disaccharide/water systems. The half width of each system decreased slightly with rise in temperature and steeply increased at T_c for the powdery DPPC and at T_H and T'_H for the powdery DPPC/dis-

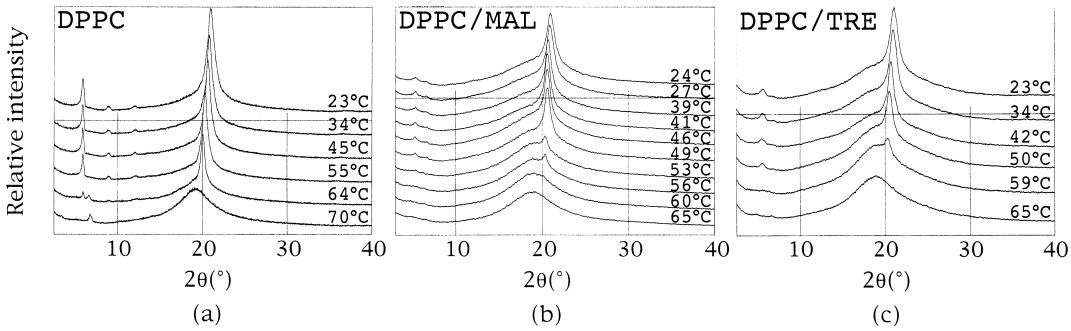

Fig. 2. X-ray diffraction patterns of (a) powdery DPPC system containing 7.4 wt.% water, (b) powdery DPPC/maltose system 7.7 wt.% water and (c) powdery DPPC/trehalose system 6.0 wt.% water at various temperatures. T_c is 65.9°C for DPPC system with 7.3 wt.% water. T_L and T_H are 49.2°C and 57.8°C, respectively, for the DPPC/maltose system containing 7.5 wt.% water. T'_L and T'_H are 49.2°C and 60.8°C, respectively, for the DPPC/trehalose system containing 5.9 wt.% water.

accharide/water systems. Relative intensity of the powdery DPPC/water system gradually decreased with temperature, while that of the powdery DPPC/disaccharide/water systems decreased and slightly increased at T_H and T'_H, respectively. Change in half width and *d*-value at T_H and T'_H in the powdery DPPC/disaccharide/water systems was consistent with that at T_c in powdery DPPC.

Fig. 3a shows parameters of the lower angle peak of the powdery DPPC/disaccharide/water systems after peak separation. *d*-Value and relative intensity increased and half width decreased with rise in temperature. Parameters of the lower angle peak changed gradually at T_H and T'_H and steep change could not be detected at T_H or T'_H, in contrast to the higher angle peak.

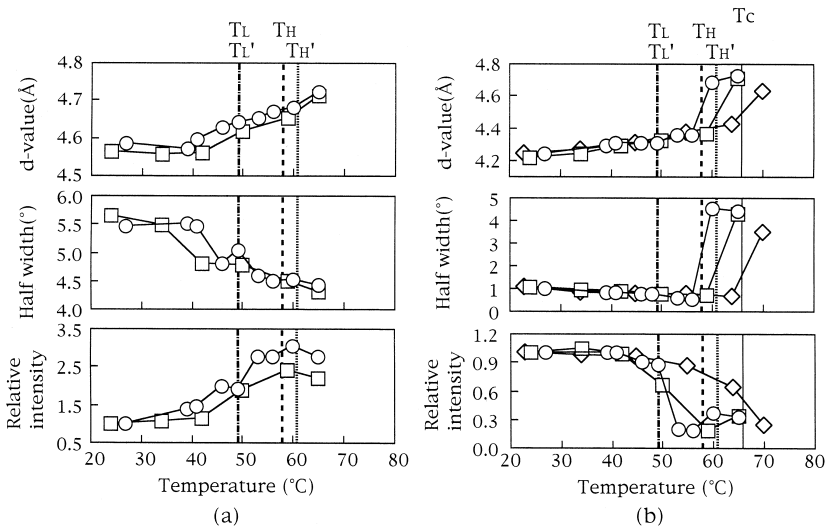

Fig. 3. Peak parameters of powdery DPPC/water system (◇), powdery DPPC/maltose/water system (○) and powdery DPPC/trehalose/water system (□). (a) parameters of the lower angle peak of DPPC/disaccharide/water systems, (b) parameters of the peak of the DPPC/water system and higher angle peak of the DPPC/disaccharide/water systems. Peak parameters are relative intensity [I/I (the lowest temperature)], half width and *d*-value. Broken bold lines indicate T_L (49.2°C) and T_H (57.8°C) of the DPPC/maltose/water system and dotted bold lines, T'_L (49.2°C) and T'_H (60.8°C) of the DPPC/trehalose/water system. Thin lines indicate T_c (65.9°C) of DPPC.

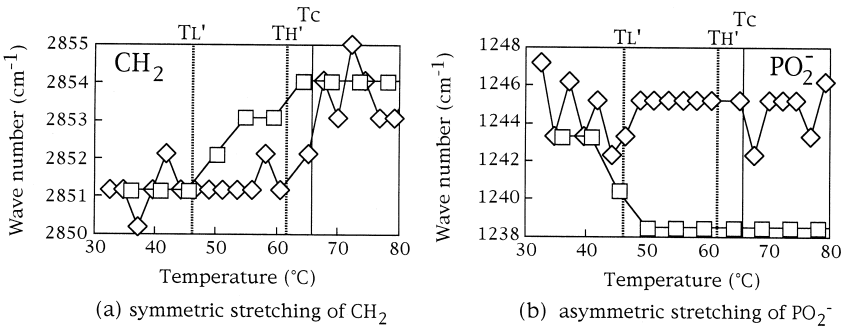

Fig. 4. Temperature-dependent change in wave numbers of peak tops for FT-IR spectra in the powdery DPPC/water system (◇) containing 7.4 wt.% water and powdery DPPC/trehalose system (□) 5.5 wt.% water. (a) and (b) show symmetric stretching of CH_2 and asymmetric stretching of PO_2^- of DPPC, respectively. T'_L (46.2°C) and T'_H (61.5°C) are lower and higher transition temperatures, respectively. T_c (65.8°C) is the gel–liquid transition temperature of the DPPC system.

3.1.3. FT-IR spectroscopy of powdery DPPC / water and powdery DPPC / trehalose / water systems

Fig. 4 shows the results of FT-IR spectroscopy for the powdery DPPC system containing 7.4 wt.% water and the powdery DPPC/TRE system 5.5 wt.% water. Wave numbers of peak tops are plotted in this figure. That of symmetric stretching of CH_2 groups in the DPPC/TRE/water system increased from T'_L to T'_H and in the powdery DPPC/water system, at T_c. The wave number of asymmetric stretching of phosphate groups in the DPPC/TRE/water system decreased at T'_L but showed no definite change in the DPPC/water system.

3.2. Heated DPPC / disaccharide / water systems

3.2.1. DSC

DSC endothermic curves of the heated DPPC system containing 0.2 wt.% water, heated DPPC/MAL system 2.0 wt.% water and heated DPPC/TRE/water system 3.2 wt.% water are shown in Fig. 5. T_c (104.2°C) of the heated DPPC system was 38.3°C higher than that (65.9°C) of the powdery DPPC system. The increase in T_c indicates the powdery DPPC/water system to be dehydrated [15]. T_L and T_H of the heated DPPC/MAL system were evident at 26.4°C and 69.4°C, respectively. These transition temperatures remained essentially constant in the second scan. Powdery system, dehydration caused increase in T_H and decrease in T_L. In the heated DPPC/TRE system, T'_L and T'_H were observed. In the first scanning from 0°C to 110°C, T'_L was 44.5°C and T'_H 64.8°C, and in the second immediately following, T'_L decreased by 18.6°C to 25.9°C (T''_L) and T'_H increased slightly by 1.2°C to 66.0°C. T''_L did not change and T'_H increased slightly in the third scanning. The small frames in Fig. 5 indicate magnified DSC curves. A shift in the base line was observed, possibly indicating the glass transition of the disaccharide. T_g was calculated based on the midpoints of this shift and for maltose and trehalose, were 90.0°C and 95.0°C, respectively.

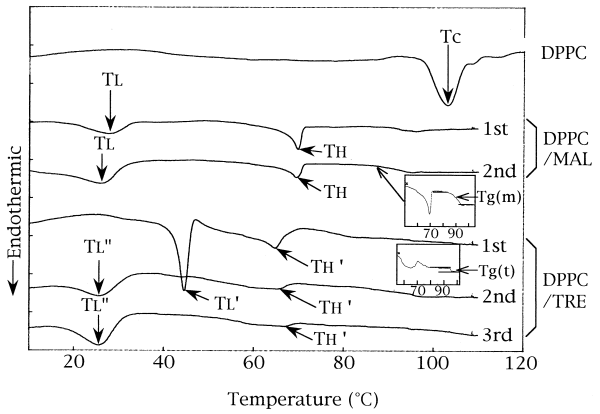

Fig. 5. DSC thermograms of the heated DPPC system containing 0.2 wt.% water, heated DPPC/maltose system 2.0 wt.% water and heated DPPC/trehalose system 3.2 wt.% water. T_c is 104.2°C for the heated DPPC/water system. T_L and T_H are the lower and higher transition temperatures, respectively, of the DPPC/maltose/water system. T'_L and T'_H are the lower and higher transition temperatures, respectively, of the DPPC/trehalose/water system. T'_L: first scanning, T''_L: second and third scannings. Small frames show 2nd and 4th scannings of the DPPC/maltose and DPPC/trehalose systems from 60°C to 100°C. T_g(m) and T_g(t) indicate glass transition temperatures of maltose and trehalose, respectively.

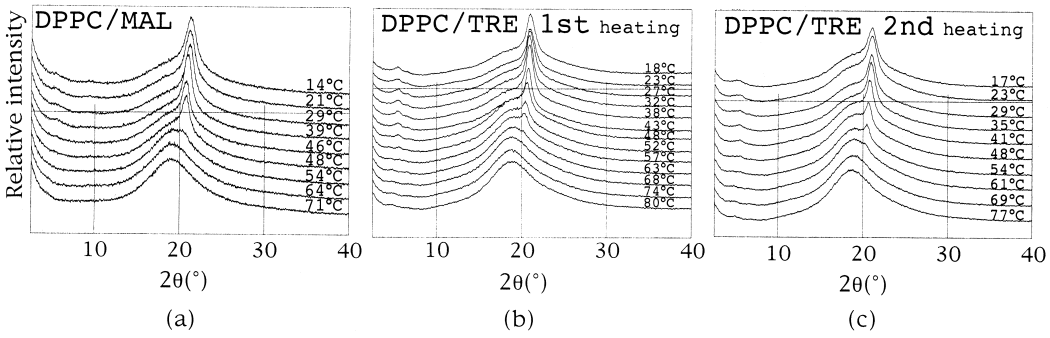

Fig. 6. X-ray diffraction patterns of the (a) heated DPPC/maltose system containing 2.1 wt.% water, (b) and (c) heated DPPC/trehalose/water system 3.2 wt.% water. (b) diffraction of 1st heating, (c) diffraction of 2nd heating. T_L and T_H of 1st scanning of DPPC/maltose system are 26.4°C and 69.4°C, respectively. T'_L and T'_H of 1st scanning of DPPC/trehalose system are 44.5°C and 64.8°C, respectively. T''_L and T''_H of 2nd scanning of DPPC/trehalose system are 25.9°C and 66.0°C, respectively.

3.2.2. Wide-angle X-ray diffraction analysis

Fig. 6a shows diffraction patterns at various temperatures of the heated DPPC/MAL system containing 2.1 wt.% water. Fig. 6b and c show diffraction patterns at various temperatures of the heated DPPC/TRE system containing 3.4 wt.% water. Diffraction was measured twice since T_L obtained in the second DSC scanning differed from that of the first scanning. At the lowest temperature, the diffraction pattern of the heated DPPC/disaccharide/water systems showed an asymmetric peak at 21.0° (2θ) and a broad peak on the lower angle side. The higher angle peak shifted to the lower angle side, its intensity decreased with temperature, as did also that of the lower angle peak. Above T_H (69.4°C), T'_H (64.8°C) and T''_H (66.0°C), a symmetric broad peak in each case appeared at 19.0° (2θ) and was similar to that of the Lα phase, as evident from Fig. 2a for the powdery DPPC system above T_c.

Fig. 7 presents a comparison of peak parameters for the heated DPPC/MAL/water system and powdery DPPC/MAL/water system. In Fig. 7b, peak

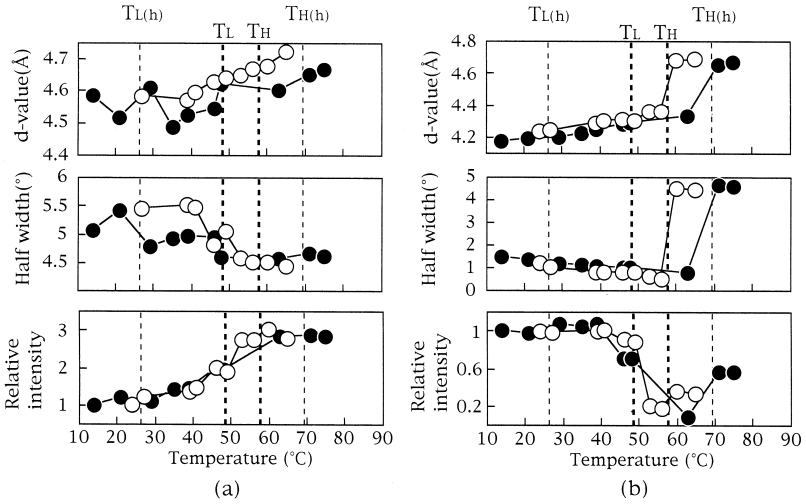

Fig. 7. Peak parameters of the powdery DPPC/maltose/water (○) and heated DPPC/maltose/water systems (●). (a) parameters of lower angle peak (b) parameters of the higher peak. T_L and T_L(h) are the lower transition temperatures of the powdery and heated systems, respectively. T_H and T_H(h) are the higher transition temperatures of the powdery and heated systems, respectively. Broken bold lines indicate T_L and T_H of the powdery DPPC/maltose/water system and broken thin lines, T_L(h) and T_H(h) of the heated DPPC/maltose/water system.

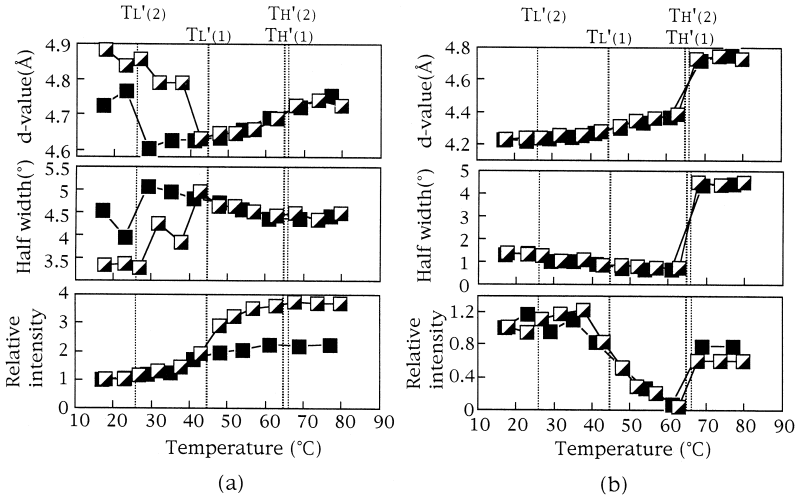

Fig. 8. Peak parameters of the heated DPPC/trehalose system containing 3.2 wt.% water. ◪: Peak parameters for 1st heating, ■: peak parameters for 2nd heating. (a) Peak parameters for the lower angle peak, (b) peak parameters for higher angle peak. Dotted bold lines indicate transition temperatures ($T'_L(1)$, $T'_H(1)$) for 1st heating and dotted thin lines, transition temperatures ($T'_L(2)$, $T'_H(2)$) for 2nd heating.

parameters of the higher angle peak are shown. *d*-Value of either system gradually increased with temperature and steeply so at higher transition temperature in the powdery system, T_H, and at higher transition temperature in the heated system, $T_H(h)$. The half width of each system decreased slightly with temperature and steeply increased at T_H and $T_H(h)$. The relative intensity of the two systems decreased with rise in temperature and increased at T_H and $T_H(h)$. Change in the half width and *d*-value at $T_H(h)$ in the heated system was in agreement with that at T_c in the powdery DPPC system.

Fig. 7a shows parameters of the lower angle peak. *d*-Value gradually increased with temperature in both systems. Half width was roughly constant above T_L for the powdery system and above $T_L(h)$ for the heated system. Relative intensity in the heated system changed more gradually with temperature, compared to the powdery system.

Fig. 8 shows parameters for the heated DPPC/TRE/water system. The systems prior to the first and second heat applications are referred to hereafter as systems 1 and 2, respectively. Fig. 8b shows parameters of the higher angle peak. *d*-Value and half width of systems 1 and 2 changed gradually with rise in temperature and increased steeply at $T'_H(1)$ and $T'_H(2)$. Relative intensity of either system decreased at approximately 38°C and increased at $T'_H(1)$ and $T'_H(2)$. Change in *d*-value and half width of systems 1 and 2 was essentially the same as noted for the gel to liquid crystal transition in DPPC without sugar. Change in relative intensity of systems 1 and 2 was basically that for DPPC with maltose (Fig. 3b and Fig. 7b) while differing with that for DPPC without sugar (Fig. 3b).

Fig. 8a shows parameters for the lower angle peak of systems 1 and 2. Half width and *d*-value changed markedly at $T'_L(1)$ and $T'_L(2)$, but not significantly at $T_H(1)$ and $T_H(2)$. Relative intensity of systems 1 and 2 started to increase at 38°C.

4. Discussion

Powdery DPPC without sugar changed near T_c (65.9°C) from a gel to liquid crystal phase (Fig. 1). One symmetric diffraction peak each appeared for the gel and liquid crystal phases, (Fig. 2a), indicating lateral packing of DPPC hydrocarbon chains without sugar in both phases to be hexagonal [20,21]. In the powdery DPPC/water system at T_c, a hexagonal subcell with *d*-value of 4.2 Å in the gel rapidly changed to an expanded hexagonal subcell with *d*-value of 4.6 Å in liquid crystal.

In the DPPC/disaccharide/water systems, symmetric broad peaks were evident above T_H as seen in

Figs. 2 and 6, indicating that above T_H, these systems are in the normal liquid crystal phase ($L\alpha$ phase). The $L\alpha$ phase has been observed above T_c in a freeze-dried DPPC/TRE mixture [16].

Asymmetric peaks below T_H (Figs. 2 and 6) are characteristic of the DPPC/disaccharide/water system. The heated vacuum dried DPPC/disaccharide system with water content less than 10 wt.% water showed the same asymmetric diffraction patterns at 24°C [14]. Temperature-dependent change in parameters of higher angle peaks of powdery DPPC/disaccharide/water system was the same as that for the powdery DPPC/water system below T_c (see Fig. 3b), indicating that below T_H, the powdery DPPC/disaccharide/water system is in a gel state. Parameters of the lower angle peak of the powdery DPPC/disaccharide/water systems changed continuously at T_H (see Fig. 3b), indicating the liquid crystal phase below T_H. The gel and liquid crystal phases would thus appear to coexist below T_H of the powdery DPPC/disaccharide/system. From T_L to T_H, relative intensity of higher angle peaks decreased and that of lower angle peaks increased at the same time (see Fig. 3). The transition from gel phase to liquid crystal phase thus apparently occurs continuously between T_L and T_H.

Dehydration with change of the powdery system (Fig. 1) to the heated system (Fig. 5) caused decrease in T_L and increase in T_H and thus also caused expansion of change in peak parameters, that occurred below T_H for the higher peak angle (Fig. 7b) and above T_L for the lower peak angle (Fig. 7a). Dehydration is thus responsible for the transition from the gel to liquid crystal phase of DPPC/disaccharide/water systems. Fig. 3a shows the parameters of the lower angle peak to change gradually near T_L. No marked change in hydrocarbon chains was observed at T_L. Definite change in the parameters of higher angle peak was detected at T_H, but none similar to this could be seen at T_L, as shown in Fig. 3b. The endothermic peak at T_L may thus not necessarily correspond to change in the melting of DPPC hydrocarbon chains. Wave number change of phosphate groups was noted at lower temperature than that of the CH_2 groups (see Fig. 4). Change in state at lower transition temperature is thus shown not to be due to chain melting but change in head group conformation. A similar difference between these two stretchings has also been reported for an isolated membrane [22]. Leslie et al. suggest the lipid head group undergo conformational change prior to acyl chain melting. In the present study, the broad endothermic peak of the first scan of the powdery DPPC/MAL and DPPC/TRE systems (see Fig. 1) indicated successive chain melting, this being consistent with the increase in relative intensity of the lower angle peak with rise in temperature from T_L to T_H (see Fig. 3a). A broad transition region from 47°C to 57°C has also been noted for a freeze-dried DPPC/trehalose mixture [16]. Tsvetkov et al. [12] and Tsvetkova et al. [23] measured DSC of a freeze-dried DPPC/trehalose mixture at various molar ratios and found two transition temperatures. With continuity of transition to the liquid crystal phase taken into consideration (see figure 4 in Ref. [12]), the higher transition temperature may have been the same as T_H in this study.

Fig. 5 shows that for the heated DPPC/TRE system containing 3.2 wt.% water, the lower transition temperature, T_L', in the first scanning differs from that (T_L'') in the second scanning conducted after heating to 110°C in the first scanning. T_L'' in the second scanning was 18.6°C lower than that of the first scanning. This was not observed in the heated DPPC/MAL/water system. *d*-Value and half width at the first heating also differed from those at the second heating below $T_L'(1)$ in Fig. 8a. The present X-ray diffraction analysis results were in agreement with those of DSC. Koster et al. [10] state that, for the lipid/saccharide system to be in a stable state, it is required for the saccharide to be in a glassy state when the lipid is in a liquid crystal state. The glassy transition temperatures of maltose (90.0°C) and trehalose (95.0°C) in each heated system were lower and higher, respectively, than sample preparation temperatures. The heated DPPC/MAL system thus satisfies this condition of Koster et al., but not the heated DPPC/TRE system. The glassy transition temperatures of maltose and trehalose in the heated systems in this study exceeded those in the literature [24]. That water content of excess saccharide in the systems is less than that of the sample owing to competition of water by the lipid and excess saccharide may be explanation for this [11].

In two previous studies, decrease in transition temperature in the second scanning was observed for

a freeze-dried DPPC/trehalose mixture [2,8]. Similar lowering of the transition temperature has also been observed for freeze-dried DPPC/maltodextrins liposomes [25]. In the first scanning [25], one transition temperature was observed above 65°C between 20°C and 110°C, whereas in the second scanning, below 30°C in this temperature range. These two systems [2,8] differed in the number of transitions and transition temperature, in contrast to the present systems with two transition temperatures. This possibly may be due to sample preparation. Their samples formed unilamellae while those here, multilamellae.

5. Conclusions

DPPC/trehalose and maltose/water systems containing low water content (less than 8 wt.%) have two transition temperatures, T_L and T_H. The systems are in a gel phase with a partially liquid crystal phase below T_L and in a liquid crystal phase above T_H. From T_L to T_H, the gel and liquid crystal phases coexist in the systems, and transition from one phase to the other occurs continuously between T_L and T_H. Above T_H, the systems are in the normal liquid crystal phase ($L\alpha$ phase). Dehydration by heat application causes the gap between lower and higher transition temperature to widen. As temperature increases, conformational change of phosphate groups occurs at the lower transition temperature, leading to disorder of the hydrocarbon chains between the two transition temperatures.

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