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Phase structures and transitions in fully hydrated diacyltrehalose

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Real time X-ray diffraction was used to examine the gel bilayer to disordered bilayer phase transition in fully hydrated dipalmitoyltrehalose. The L_β to L_α phase transition was shown to proceed via a second-order thermodynamic process involving incommensurate mesophase bilayer repeat structures and the formation of an intermediate rectangular acyl chain packing subcell. This phenomenon has only been previously shown to occur for dihexadecylphosphatidylcholine (DHPC) and dipalmitoylphosphatidylcholine (DPPC) dihydrates undergoing stepwise (i.e., noncontinuous) temperature changes. It can thus be inferred that the presence of trehalose–trehalose intra-bilayer interactions is a sufficient condition to modify the acyl chain structural rearrangements within the bilayer as a function of temperature.

Introduction

The non-reducing disaccharide of glucose, trehalose, has been implicated in the survival of organisms after complete dehydration [1–4]. In addition, trehalose has been shown to be effective as a cryo-protectant in membrane systems [2,5–8]. Recently, physical studies have attempted to probe the structural implications of these phenomena [9–12]. Tenchov and co-workers provided clues as to the inconsistencies in thermodynamic parameters previously reported for these systems [13,14].

It was shown [13,14] that the state of the lipid bilayer, and the method required to mix trehalose with these molecules, influenced the eventual bilayer phase state and transition properties. The technique which produced the least reproducible results involved in the formation of these mixtures was the mixing of trehalose and lipids in an organic solvent which was subsequently removed from the system. The hydration of lipids in 1 M solutions of trehalose in water with subsequent dehydration using lyophilization or desiccation produced bilayer structures and transitions which were reproducible. The phase state of the lipid dispersion before dehydration and trehalose content influenced the transition in the subsequent mixture. We have examined the structural details of these phenomena using real time X-ray diffraction [15]. These results were consistent with the observations from scanning calorimetry [13,14].

Still to be determined is the exact mechanism of the influence of trehalose on bilayer packing and membrane function. Part of this answer must consist of the influence of trehalose–trehalose interactions in the organization of the membrane. Trehalose, alone does not show transition properties in the thermal region important to lipids and membranes. We have thus chosen to examine a synthetic lipid consisting of dipalmitoyltrehalose. This lipid can be dispersed in water such that the ensuing bilayer phases take on the thermal packing characteristics of the acyl chains. In addition, a detailed study of the L_β to L_α transition in this system indicates an intermediate chain packing consisting of a rectangular unit subcell. Other observations of intermediate packing in the L_β to L_α phase transition were reported for DHPC [16] and DPPC dihydrates [15]. It was thus evident that trehalose–trehalose (and perhaps carbohydrate–carbohydrate) intra-bilayer headgroup interactions modify the response of membrane structure to thermally enhanced molecular motions.

Materials and Methods

Dipalmitoyltrehalose was prepared using procedures previously described [17]. Lipid dispersions were produced by suspending the lipid in water at a concentration of 80% wt. water/total wt. The samples were heated at 65 °C, which was above their expected transition temperature, for one hour and then stored at

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approx. 0 °C. The sample was equilibrated at room temperature before examination in the X-ray beam. The resulting sample appeared to be a homogeneous lipid/water mixture with no evidence of residual lipid powder.

X-ray diffraction patterns were obtained using the 0.150 nm X-radiation at station 7.25 of the Science and Engineering Research Council Daresbury (U.K.) Laboratory synchrotron radiation source [18]. A cylindrically bent single crystal of Ge [19] and a long flat mirror were used for monochromatization and horizontal focussing. A Keele University flat plate camera with a sample path of 1 mm was used. Scattered X-rays were recorded on a linear detector constructed at the Daresbury Laboratory. The dead time between data acqusition frames was 50 μs with a collection time of 2.0 s for each frame. X-ray scattering has been recorded as a function of tan 2θ where θ is the scattering angle and plotted as a function of reciprocal space ($S = 1/d = 2\sin\theta/\lambda$) using teflon ($1/S = 0.48$ nm) as a calibration standard [20]. All mesophase and subcell spacings were calculated using Bragg's Law.

Temperature scans were produced by water baths connected internally to the sample holder mount of the X-ray camera. The temperature of the sample was monitored internally using a thermocouple placed adjacent to the sample in the X-ray sample holder.

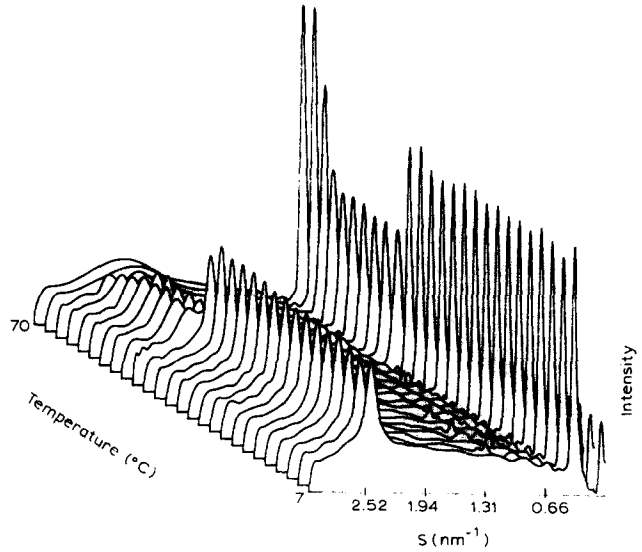

Fig. 1. X-ray diffraction patterns of dipalmitoyltrehalose dispersed in 80% wt. water/total wt. obtained during a linear (with time) temperature scan of approx. 8.3 C °/min beginning at approx. 7 °C. Every tenth frame of 2.0 s duration from a total data set of 255 frames is shown. Each diffraction pattern displayed after that obtained at 7 °C represents an increase in temperature of 2.52 °C over previous diffraction pattern.

Results and Discussion

Real time X-ray diffraction techniques have been used to study fully hydrated samples of dipalmitoyltrehalose (DPT). A gel state bilayer or L_β phase was observed as the initial structure at approx. 7 °C. The $L_\beta \rightarrow L_\alpha$ phase transition was then monitored (Figs. 1 and 2). An examination of the wide-angle X-ray scattering (WAXS) peaks indicated that the single sharp peak at 0.400 nm ($S = 2.50$ nm^{-1}) acyl chain packing characteristic of the L_β phase acyl chain packing transformed to the single broad peak at 0.464 nm ($S = 2.16$ nm^{-1}) characteristic of the L_α phase acyl chain packing via a phase containing acyl chains packed

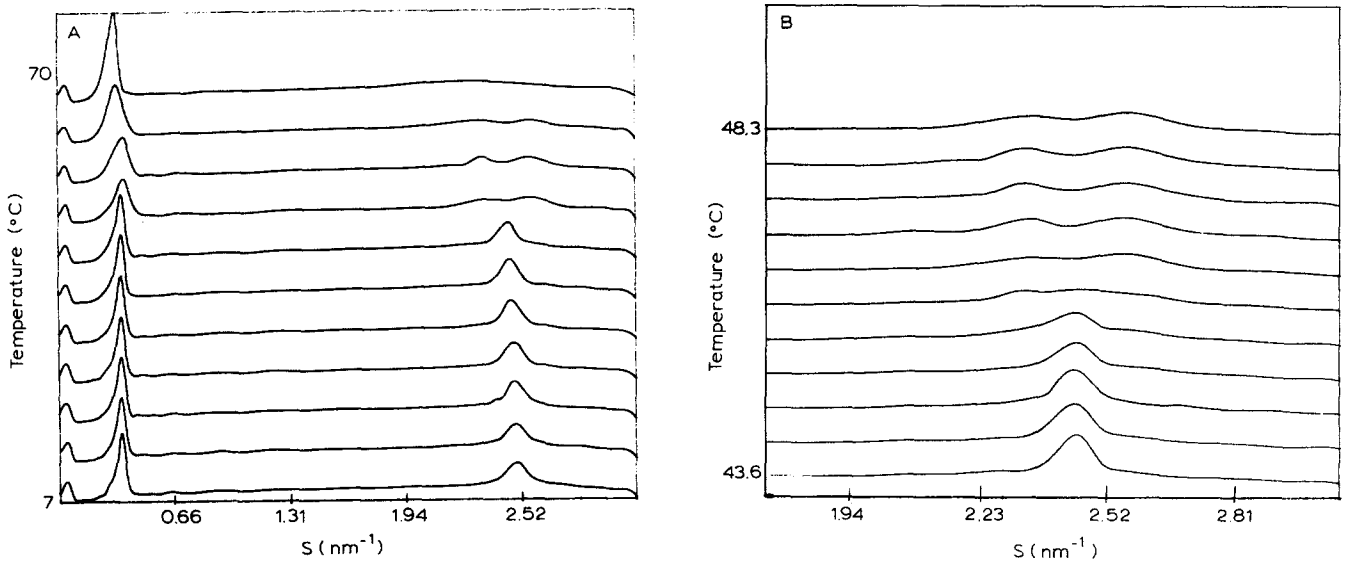

Fig. 2. (A) Selected X-ray diffraction patterns from the data set in Fig. 1 plotted as a function of inverse d-spacing. Every twenty-fifth frame of the data set is shown. Each diffraction pattern displayed after that obtained at 7 °C represents an increase in temperature of 6.3 °C over the previous diffraction pattern. (B) An expansion of the wide-angle diffraction patterns for every second frame (0.47 C ° temperature increase per diffraction pattern) from this data set between 43.6 °C and 48.3 °C.

in a rectangular subcell as indicated by the presence of two sharp WAXS peaks at 0.393 and 0.437 nm ($S = 2.54$ and 2.29 nm^{-1}, respectively). It is unlikely that the two WAXS peaks in this intermediate phase are due to coexisting acyl chain subcells since they do not correspond to either the L_β or L_α acyl chain WAXS peaks. It is also unreasonable to speculate that the acyl chain packing decreased in the L_β phase to the 0.39 nm WAXS peak. The presence of all three WAXS peaks from the acyl chain packing of the L_β and intermediate phase is a further indication that the intermediate phase is not just a region of coexistence of the L_β and L_α phases. The transition from the L_β to the intermediate phase from 45 to 47 °C (see Fig. 2B) involved a two-state or first-order thermodynamic process as deduced from the presence of coexisting L_β and intermediate acyl chain WAXS peaks in this temperature range. The intermediate phase and the L_α phase acyl chain packing structures were found to coexist upon further heating from 64 to 68 °C before the final evolution into a single L_α phase thus proceeding via another two-state or first-order thermodynamic process.

The thermodynamic classification of the $L_\beta \rightarrow L_\alpha$ acyl chain phase transition is more complicated. If the intermediate acyl chain packing phase is an equilibrium phase then the $L_\beta \rightarrow L_\alpha$ acyl chain phase transition simply involves two first order thermodynamic processes. However, it is not clear that the intermediate acyl chain packing phase is not just a kinetically derived intermediate rather than an actual phase. An intermediate state, between the gel and liquid-crystalline bilayer phases, consisting of acyl chains packed in a rectangular subcell has been previously observed for DPPC and DHPC bilayers at low hydration [15,16]. It has also been previously shown that DPPC dihydrate produced this intermediate acyl chain packing only if incubated for some time at the appropriate temperatures during stepwise changes in temperature but not during a continuous temperature scan [15]. However, all possible conditions (i.e., rate of temperature change, hydration, etc.) necessary for the formation of this type of intermediate phase were not rigorously studied [15]. Since we observed the presence of an intermediate acyl chain packing phase during the $L_\beta \rightarrow L_\alpha$ acyl chain phase transition while the sample was undergoing a temperature scan we can infer that a similar intermediate phase should be present during static temperature studies of the $L_\beta \rightarrow L_\alpha$ acyl chain phase transition. The substitution of the phosphatidylcholine head group by trehalose clearly enhanced the kinetic formation of this intermediate phase. However, it can be predicted based on the data for low hydration DPPC and DHPC bilayers [15,16], that the acyl chain phase intermediate would not be observed if the fully hydrated DPT sample was subjected to a rapid temperature jump (e.g., approx. 5–10 C°/s). Therefore, if it is assumed that the acyl chain intermediate state is simply an intermediate between the L_β and L_α phases then we must classify the $L_\beta \rightarrow L_\alpha$ transition as a second order thermodynamic process. In any phase transition or critical phenomenon, the presence of an intermediate or intermediates between the initial and final states is classified as a second-order transition process as distinguished from a first order process which proceeds via the coexistence of the initial and final states [21].

The appearance of the two wide-angle peaks characteristic of a rectangular acyl chain subcell was accompanied by a broadening of the small-angle diffraction peak characteristic of the mesophase lamellar repeat spacing. A visual examination of the temperature sequence of the first-order small-angle X-ray peaks (Fig. 2) indicates that this broadening may be due to the coexistence of the initial and final mesophase bilayer repeat structures. Specifically, this single broad small-angle diffraction peak may be a composite of diffraction peaks from both the L_β and L_α bilayer repeat structures. It is unlikely that the broadening is due to increased thermal motion of the molecules in the lipid bilayer since the small-angle diffraction peak of the final L_α phase was narrower then that observed in the intermediate region, although broader than that observed for the L_β phase. Unfortunately, the presence of only three small-angle diffraction peaks for each of the phase regions percludes the possibility of a determination of the electron density profile for each of the phases present. The bilayer repeat transition thus may involve incommensurate structures consisting of an L_β phase with a repeat spacing of ≈ 5.7 nm ($S = 0.18$ nm^{-1}) and an L_α phase with a repeat spacing of 6.1 nm ($S = 0.16$ nm^{-1}). The presence of only mesophase lamellar repeat spacings for the L_β and L_α phases re-enforces an assumption that the intermediate acyl chain packing is an intermediate rather than equilibrium phase.

Conclusions

We have observed a second order $L_\beta \rightarrow L_\alpha$ phase transition involving incommensurate mesophase bilayer repeat structures for dipalmitoyl trehalose in excess water which proceeds via an intermediate acyl chain subcellar packing involving the expansion and contraction of the two-dimensional axis of the initial hexagonal subcell. The appearance of the L_α bilayer repeat and acyl chain subcellar structures occurred simultaneously after the disappearance of the intermediate rectangular acyl chain subcell. The appearance of an intermediate rectangular phase was previously described for $L_\beta \rightarrow L_\alpha$ phase transitions in dehydrated DHPC and DPPC [15,16]. We can infer that the trehalose–trehalose intrabilayer interactions are as strong as those present between phosphatidylcholine bilayers at low hydration. The formation of a temperature induced intermediate

rectangular acyl chain subcell must be a manifestation of thermal disorder in the lipid within the bilayer. The strong interaction between headgroups allows for a re-orientation within the bilayer such that expansion of the headgroup spacings was not uniform but along a preferred direction which probably depended on the headgroup structure itself. Spectroscopic and detailed X-ray diffraction studies are needed to fully explore this phenomenon.

It is interesting to speculate on the importance of this phenomenon for membranes. The action of trehalose in counteracting the effect of dehydration on membrane may be one of retaining a membrane bilayer structure in which inter-connected membrane components do not simply become a lipid/protein mixture upon the withdrawal of water. In this manner, membrane protein functionality would be preserved even after the membrane underwent dehydration and subsequent re-hydration.

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