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Sugars favour formation of hexagonal (H_{II}) phase at the expense of lamellar liquid-crystalline phase in hydrated phosphatidylethanolamines

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The disaccharides, sucrose and trehalose, markedly decreased (up to 17–13C°) the temperature of the lamellar to hexagonal ($L_\alpha \rightarrow H_{II}$) phase transition and simultaneously increase by 2–4 C° the temperature of the lamellar gel to lamellar liquid-crystal ($L_\beta \rightarrow L_\alpha$) phase transition in hydrated dihexadecylphosphatidylethanolamine and distearoylphosphatidylethanolamine. These two transitions merge and convert into a single L_β–H_{II} phase transition when dispersed in 2.4 M sucrose. These results are inconsistent with recent reports by Crowe et al. (1987) Biochem. J. 242, 1–10; (1988) Biochim. Biophys. Acta 947, 367–394) which suggest that trehalose stabilizes the L_α phase relative to the H_{II} phase and shifts upwards beyond detectability the L_α–H_{II} transition. The present results are considered as a manifestation of the Hofmeister effect in which the sugars act as kosmotropic reagents stabilizing the structure of bulk water. This tends to decrease the area of contact between the lipid and the aqueous phases and favours the H_{II} and L_β phases relative to L_α phase. This hypothesis is consistent with the effects of chaotropic reagents on the L_α–H_{II} phase transition (Yeagle and Sen (1986) Biochemistry 25, 7518–7522) and on the stability of the lamellar phase of dipalmitoylphosphatidylcholine (Oku and MacDonald (1983) J. Biol. Chem. 258, 8733–8738).

Much of the recent interest in sugar–membrane interactions is due to the ability of certain sugars to protect cell membranes from injuries resulting from freeze-drying and re-hydration. Sugars such as trehalose are believed to be natural cryoprotectants and their metabolism in some organisms has been correlated with the ability of these organisms to survive dehydration (anhydrobiosis) [1–5]. An explanation of the sugar protective action at low hydration levels is based on the 'water replacement' hypothesis which assumes that the carbohydrates can substitute for water bound to macromolecular and membrane surfaces [4,6]. Numerous studies on membranes and proteins have provided support for this hypothesis (see, for example, Refs. 5–9 for recent reviews and references).

In view of the possible involvement of the lamellar to hexagonal ($L_\alpha \rightarrow H_{II}$) phase transition in the mechanism of cryodamage [7] the effect of sugars on this transition is also of considerable interest. Recent studies have found that trehalose stabilizes the lamellar phase of hydrated phosphatidylethanolamine at the expense of H_{II} phase and is capable of maintaining these lipids in lamellar phase at temperatures well above the L_α–H_{II} phase transition [8–11]. In this report we present calorimetric and X-ray data that contradict these findings and show that sucrose and trehalose have precisely the opposite effect. According to our results, these sugars favour formation of H_{II} at the expense of L_α. They strongly decrease the temperature of the L_α–H_{II} phase transition until there is complete abolition of the intermediate L_α phase and the appearance of a direct gel–H_{II} transformation at high sugar concentrations.

The phosphatidylethanolamines (DSPE, DHPE) (both puriss. grade from Fluka) were hydrated in solutions of sucrose or trehalose in doubly distilled deionised water for 15 min at 85°C and shaken 5–10

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; DSPE, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; DHPE, 1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine; L_α, lamellar liquid-crystalline phase; L_β, lamellar gel phase; L_c, lamellar crystalline (sub-gel) phase; H_{II}, inverted hexagonal phase; DSC, differential scanning calorimetry.

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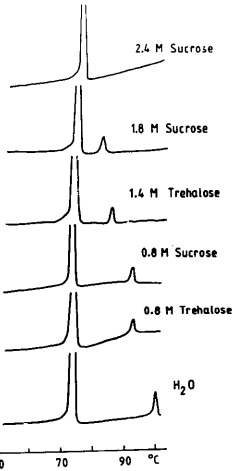

Fig. 1. DSC scans (excess specific heat versus temperature) of DSPE dispersions in aqueous solutions of sucrose or trehalose. The large endotherms at lower temperature correspond to $L_\beta \rightarrow L_\alpha$ transitions, the smaller endotherms at higher temperature reflect $L_\alpha \rightarrow H_{II}$ transitions. The single transition in 2.4 M sucrose corresponds to a direct $L_\beta \rightarrow H_{II}$ transformation.

times on a vortex mixer at this temperature. Lipid dispersions prepared by this procedure were found to have reproducible thermal behaviour as was indicated by successive DSC scans. The lipid concentrations were 0.03 wt% in the DSC and 25 wt% in the X-ray measurements.

The calorimetric scans were recorded with a high-sensitivity differential adiabatic scanning calorimeter (DASM-1M [12]) at a heating rate of 0.5 C°/min. Low- and wide-angle X-ray measurements were performed at the Daresbury synchrotron laboratory using conditions described elsewhere [13].

Dispersions of the phosphatidylethanolamines in excess water equilibrated at high temperature prior to the measurements are known to display a phase sequence of the type $L_\beta \rightarrow L_\alpha \rightarrow H_{II}$ during heating scans [14–16]. A large endothermic peak corresponding to the melting L_β–L_α transition is followed by a much smaller L_α–H_{II} endotherm. High-sensitivity DSC scans through these transitions in DSPE and DHPE are shown in Figs. 1 and 2, bottom. The peak temperatures of the L_α–L_β and L_α–H_{II} transitions were 74.4°C and 100.2°C, respectively, in DSPE dispersions, and 66°C and 85°C, respectively, in DHPE dispersions. These values agree closely with previously reported transition temperatures of these lipids in unbuffered water [15]. Calorimetric scans of lipid dispersions containing increasing amounts of sucrose and trehalose clearly show that these disaccharides shift to lower temperatures the L_α–H_{II} transition and at the same time increase slightly the temperature of the L_β–L_α transition in both DSPE and DHPE (Figs. 1 and 2). In 2.4 M sucrose these two transitions merge and convert into a single transition centred at 78.6°C in DSPE and at 68°C in DHPE (Figs. 1 and 2, top). This transition can be expected to reflect a direct $L_\beta \rightarrow H_{II}$ transformation. Similar effects of these sugars have also been observed with synthetic, stereochemically pure glycolipids which display $L_\beta \rightarrow L_\alpha \rightarrow H_{II}$ phase sequences in excess water (Koynova, R.D., unpublished DSC measurements).

The melting phase transition in DSPE dispersions in 0, 1.2 and 2.4 M sucrose was investigated by low- and wide-angle X-ray scattering. Structural parameters were determined and phases were assigned according to conventional procedures [17]. As sugars appeared to facilitate the formation of the sub-gel phase prior to the

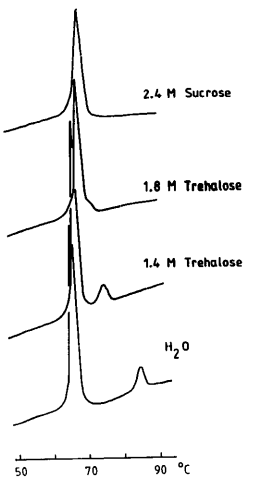

Fig. 2. DSC scans of DHPE in aqueous solutions of sucrose and trehalose (see the text and the legend to Fig. 1 for details).

measurements the lipid dispersions were pre-heated to 85°C. Both static measurements at constant temperatures below and above the transitions and time-resolved measurements during temperature scans at 10 C°/min showed a L_β–L_α transition in 0 and 1.2 M sucrose. The long spacing of the L_β phase was 6.3 nm at 5 C° below the transition, and the long spacing of the L_α phase was 5.4 nm at 5 C° above the transition. Increase of sucrose concentration up to 1.2 M seemed to decrease the long spacings by about 0.1 nm. This change, however, was comparable with the error margin of the measurements. In 2.4 M sucrose, the melting transition proceeded as a direct transformation of the L_β phase into a hexagonal phase. The latter phase was characterised by low-angle reflections at 6.4, 3.7 and 3.2 nm (at 5 C° above the transition). Its lattice spacing (distance between axes of water cylinders) was $a = 7.4$ nm ($a = 2d/\sqrt{3}$ where $d = 6.4$ nm).

The present measurements provide clear evidence that sucrose and trehalose strongly decrease the temperature of $L_\alpha \rightarrow H_{II}$ transition. These disaccharides decrease the temperature range of existence of the L_α phase and close the 'gap' between gel and H_{II} phases at about 2.4 M sucrose. This results contradicts the conclusions of Crowe et al. [8–11] that trehalose stabilizes the lamellar liquid-crystalline phase in phosphatidylethanolamines and shifts upwards to indetectability the $L_\alpha \rightarrow H_{II}$ transition. We are unable to account for this inconsistency. Our observations are consistent with the recently published results of Bryszewska and Epand [18] that disaccharides and sugar alcohols lower the bilayer to hexagonal phase transition temperature of dielaidoylphosphatidylethanolamine. The influence of sugars on the phase behaviour of DSPE and DHPE is very similar both qualitatively and quantitatively to the effect of NaCl described by Seddon et al. [15]. In both cases the L_α phase in completely eliminated and direct $L_\beta \rightarrow H_{II}$ transitions appear at nearly the same temperature in saturated solutions of either NaCl or sucrose. However, the intermediate L_α phase of synthetic glycolipids is fully abolished in significantly less than saturated sugar solutions (unpublished measurements).

While the protective action of sugars at low levels of hydration is most probably due to hydrogen bonding with the lipid head groups as envisaged by the 'water replacement' concept, their strong effect on the $L_\alpha \rightarrow H_{II}$ transition might also involve indirect (Hofmeister) interactions since it is exerted in excess water. A large number of experimental studies show that the properties of the interface between aqueous and non-aqueous phases can be markedly affected via indirect interactions with kosmotropic and chaotropic reagents [19]. These reagents stabilize or destabilize, respectively, the structure of bulk water and thus influence the properties of interfacial water (see Ref. 19 for a comprehensive review of the various manifestations of the Hofmeister effect and possible mechanisms of indirect interactions between Hofmeister solutes and interfaces).

There is now ample evidence that many cryoprotectants, sugars among them, are kosmotropic, water-structure making reagents [19]. By stabilizing the structure of bulk water these substances tend to reduce the area of the unfavourable interfaces between aqueous and lipid phases. This tendency must favour formation of H_{II} phase at the expense of the L_α phase since the lipid surface areas are known to be smaller in the former phase compared to the latter [16,20]. Although reliable theoretical estimates of the free energies involved in this process do not seem possible at the present stage, it is clear nevertheless from the experimental data that the degree of stabilization of water structure by sugars should be sufficiently great as to affect appreciably the energetic balance between the L_α and H_{II} phases and decrease significantly the temperature of the transition between them. The same 'kosmotropic' effect might be responsible also for the slight upward shift of the $L_\beta \rightarrow L_\alpha$ transition. This shift is much smaller than in the previous case since the enthalpy of this transition is correspondingly much greater than that of the $L_\alpha \rightarrow H_{II}$ transition. Similar upward shifts have been reported also for fully hydrated DPPC. Sucrose elevates the pre-transition and the main transition of DPPC [21,22] while 1 M trehalose increases the sub, pre- and main transitions of DPPC by about 1 C° [23]. It is important to note that the hypothesis proposed here employs the kosmotropic properties of the sugars only and does not necessarily require involvement of direct interactions between them and the lipid head groups. Such interactions, however, cannot be excluded or assessed as insignificant from the presently available data.

It could be expected from these considerations that chaotropic water-structure breaking reagents which belong to the opposite side of the Hofmeister series must also have an opposite effect on the L_α–H_{II} transition. That this is indeed the case has already been demonstrated by Yeagle and Sen [24] who have found that the L_α phase of soya phosphatidylethanolamine was stabilized relative to the H_{II} phase by addition of chaotropic reagents (guanidine hydrochloride, urea and NaSCN). Strong chaotropes such as NaSCN increase the L_α–H_{II} transition by more than 60 C° [24]. In this case, the destabilisation of water structure by the chaotropic reagents allows for greater interface areas of the lipid head groups and favours the L_α phase relative to the H_{II} phase. Concurrent with this are also the results of Oku and MacDonald [25] that strong chaotropes promote micellisation of the lamellar phase of phosphatidylcholines. Here again, the chaotropes favour larger head group areas and, consequently formation of micelles at the expense of lamellar phase.

A tentative general description of the Hofmeister effect on the lipid phase behaviour emerging from these

studies can be formulated as follows. Kosmotropic reagents (sugars and possibly other cryoprotectants) stabilize water structure and thus favour smaller lipid head group areas. This leads in particular to a preference for H_{II} phase relative to the L_α phase and to a decrease of the $L_\alpha \rightarrow H_{II}$ temperature (Figs. 1 and 2). Chaotropic reagents must have an opposite effect on the lipid phase behaviour as they destabilize water structure and allow in this way larger head group areas. This results in a preference for the L_α phase relative to the H_{II} phase [24] and for the micellar phase relative to the lamellar phase [25].

It could be expected also that some chaotropic reagents might be able to induce appearance of an intermediate L_α phase in lipids which display direct $L_c \rightarrow H_{II}$ or $L_\beta \rightarrow H_{II}$ transitions in pure water. Our preliminary experiments with galactolipids characterised by $L_c \rightarrow H_{II}$ transitions provide indications that high concentrations of the chaotropic SCN^- do induce appearance of an intermediate phase separating the lamellar crystalline and H_{II} phases.

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