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Solution effects on the thermotropic phase transition of unilamellar liposomes

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We have investigated the effect of two monosaccharides, glucose and fructose, and two disaccharides, sucrose and trehalose, on the thermotropic phase transition of unilamellar extruded vesicles of DPPC. All the sugars investigated raise the main transition temperature (T_m) of some fraction of the lipid, but there are differences between the effect of glucose and the other three sugars. At low concentrations of glucose, T_m is lowered. At high concentrations of glucose there are two transitions, one with a low T_m and one with a high T_m. The data suggest that at low concentrations, all of the glucose present may bind to the bilayer and increase headgroup spacing by physical intercalation or increased hydration. The appearance of a T_m above that of pure hydrated DPPC suggests the possibility of the dehydration of some other population of phospholipid molecules. The other three sugars increase T_m, but at high concentrations of trehalose, sucrose, and fructose a second peak occurs at a low T_m. The other sugars appear to dehydrate the bilayer at low concentrations, but may show some binding or increased hydration of some portion of the lipid at very high concentrations. The sugar effects on unilamellar vesicles are strikingly different from the effects of these sugars on multilamellar vesicles.

Introduction

A number of recent studies, including one from this laboratory, have shown that chaotropic solutes (water structure 'breakers') and kosmotropic solutes (water structure 'makers') can affect the thermotropic phase transition temperature of pure phosphatidylethanolamine and phosphatidylcholine [1–9]. The suggestion has been made that these solutes exert their effects by being preferentially bound to or excluded from the phospholipid bilayer [3], by changes in the entropic contributions to bilayer structure due to changes in water structure [2], by osmotic dehydration of the headgroup [9] or by Hofmeister effects on the structure of water [6]. These explanations are not mutually exclusive.

Studies from this laboratory also have demonstrated that sugars have striking cryoprotective effects on fusion and leakage of contents from unilamellar vesicles [10,11]. At present, the mechanism of the cryoprotection is unknown, although there is some evidence that sugars may interact directly with the phospholipid during freezing [11,12]. Trehalose, sucrose, and to a lesser extent glucose and fructose have recently been shown to inhibit leakage of trapped solute from liposomes during a rapid shift through the thermotropic phase transition [13] and thus may have effects on bilayers at ambient temperatures as well as during freeze-thaw treatments.

Other studies have demonstrated that some sugars have the ability to stabilize proteins in solution [14–17] and during freeze-thaw treatments [18,19] and are also preferentially excluded from protein surfaces (have a negative preferential interaction parameter or preferentially 'hydrate' the protein) [14,15]. The suggestion has been made that the increase in protein thermal stability is related to the water structuring effect of the sugars rather than being due to some colligative property of the solute [16]. Preferential interaction studies [14,15] show a strong correlation between exclusion of sugars, their ability to prevent thermal denaturation of proteins, and the increase in surface tension of sugar solutions.

As part of a study of the stabilizing effects of sugars on liposomes, we have undertaken a systematic study of the effects of four sugars (trehalose, sucrose, fructose, and glucose) on the thermotropic phase transitions of

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phosphatidylcholine bilayers. We chose to study the effect of carbohydrates on unilamellar vesicles for several reasons. Some evidence in the literature suggested that in the preparation of MLV, sucrose was not distributed evenly between the bilayers, but tended to be concentrated in some regions of the MLV [20]. This led to the possibility that the increase in T_m seen was due to osmotic dehydration of some of the headgroups. However, it seems likely that carbohydrates do equilibrate throughout MLV following repeated cycles through T_m, which allows leakage across bilayers.

Secondly, unilamellar extruded vesicles more closely approximate both isolated biological membranes and whole cells than do MLV, and we are ultimately interested in the effect of solutes on living systems. Last, model studies of the effects of solutes on bilayer fusion and leakage have been carried out in this laboratory on unilamellar vesicles.

The present paper concerns the phenomenology of the sugar effects. The results suggest that, in accordance with other studies, sugars have a general effect on the thermotropic transitions of phospholipids that is consistent with dehydration of the bilayer and increased ordering of the acyl chains. The effect of a particular sugar is generally correlated with its water-structuring capacity, but there are individual differences between the sugars which suggest possible differences in binding, especially at higher concentrations. There are also significant differences between sugar effects on multilamellar vesicles, which have been used in previous studies, and the unilamellar vesicles used in the present study. The possible binding or exclusion of the sugars at the bilayer surface is presently being investigated.

Materials and Methods

All sugars were purchased from Pfanstiehl, Inc. (Waukegan, IL), except for a second sample of glucose which was purchased from Mallinckrodt, Inc., St. Louis, MO. The sugars were held under high vacuum at least 16 h and then stored in clean glass bottles. The purity of the sugars was checked by mass spectroscopy, and no contaminants were found. Results obtained using glucose obtained from two different sources were the same.

Dipalmitoylphosphatidylcholine (DPPC) was purchased in chloroform from Avanti Polar Lipids and stored at −20°C. Deionized distilled water was used for all preparations. The water and all solutions made with it were pH 5.5.

Liposomes were made by drying aliquots of DPPC in chloroform under dry nitrogen and then placing them in a high vacuum for at least 4 h. The lipid was rehydrated with water above the main phase transition temperature and vortexed several times to produce multilamellar vesicles (MLV). Unilamellar vesicles were produced by extruding the MLV at 60°C under dry nitrogen pressure through stacked polycarbonate filters with a 0.1 nm pore size [21].

Sugar solutions at a given weight% were made by weighing dry sugar and adding the appropriate volume of water and liposomes. Sugar solutions of a given molarity were made by diluting a solution of higher molarity with water and liposomes.

Differential scanning calorimetry was carried out using a high sensitivity calorimeter (Hart Series 7707, Hart Scientific, Provo, UT). Samples were scanned at a rate of 20°C/h, and data were collected every 20 s. The calorimeter has three sample cells and one reference cell, so each separate experiment included liposomes in water to compare with liposomes (from the same extrusion) in varying sugars at varying concentrations. About 3–4 mg lipid samples were used for each separate experiment and the lipid amounts were identical within each separate experiment. The volume in each ampoule was held constant at 0.3 ml. In this way we were able to detect reliably differences in T_m due to the presence of the sugar. Each experiment included at least four heating scans and cooling scans.

Osmolalities of sugar solutions were determined using a Wescor vapor pressure osmometer (Wescor, Inc., Logan UT, 84231) calibrated with standards of known osmolality.

Results and Discussion

Effects of sugars on thermotropic transitions

Multilamellar vesicles. A scan of MLV of DPPC in water is shown in Fig. 1. The size of the cooperative unit and the calorimetric enthalpy (ΔH_{cal}) of the transi-

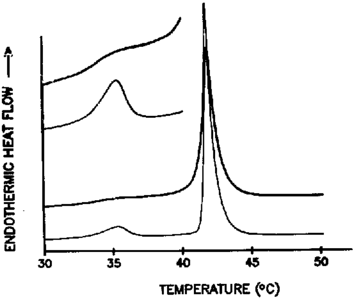

Fig. 1. Comparison of the thermotropic transitions of hydrated DPPC MLV (lower trace) and DPPC extruded vesicles (upper trace). Extruded vesicles have a slightly broadened transition, but the same T_m as MLV. The typical pretransition seen in MLV of saturated phosphatidylcholines is absent. The EV show an upward shift in heat capacity in the region of the pretransition (inset, enlarged 4×).

tion of the MLV in this study is in reasonable agreement with that found by Mabrey and Sturtevant [22].

Since other investigators have used MLV preparations for studying phospholipid–solute interactions, we have done calorimetric scans on DPPC MLV in the presence of several concentrations of trehalose and glucose for comparison with literature data. Multilamellar vesicles made in the presence of carbohydrate showed no difference in the transition between the first and subsequent heating scans; when carbohydrate was added externally, the MLV showed no further change in the transition following the first or second heating scan. Consistent with other reports [5,8,9,23], the sugars increased the temperature of both the pre- and main transitions. The concentration dependence of the upward shift in T_m in the presence of trehalose is not as pronounced for MLV as for EV (see below), even at the highest concentrations. There is no effect of 0.25 M trehalose on either the pre- or main transition.

Although the main transition of DPPC in the presence of 1M trehalose and glucose is slightly broadened (see also Ref. 23), it remains narrow and symmetrical and without any shoulders or separated peaks (scans not shown).

Effects of sugars on extruded vesicles

Establishment of equilibrium conditions. A small increase in heat capacity of the EV sample occurs immediately before the main transition, an event that may be analogous to the pretransition seen in MLV of phosphatidylcholines that have saturated acyl chains. Alternatively, this small change may reflect the change from the faceted (polygonal) shape of unilamellar vesicles of saturated phosphatidylcholines below their gel to liquid crystalline phase transition to the smooth spherical shape that they have above T_m [24,25] (Fig. 1).

The main transition of the EV preparation is narrow and symmetrical, with a T_m not significantly different from that of the MLV (Table I). Although there was no difference between the peak transition temperature of the EV and the MLV, the unilamellar EV had a broadened transition and a slight but significantly lower calorimetric enthalpy compared to the MLV (Table I). Extruded unilamellar vesicles showed a significant decrease in cooperativity (Table I) which can be appreciated by comparing the two scans in Fig. 1.

In each experiment carbohydrate was initially added to the outside of the vesicles. During the first heating scan, the liposomes underwent a transition and leaked sugar across the bilayer for about 25 min, allowing complete equilibration of the sugar concentration across the bilayer of unilamellar vesicles [13,26]. In each case where sugar was added outside the vesicles, there was an increase in T_m over the control on the first heating scan possibly due to osmotic dehydration consequent on the unequal concentration of carbohydrate across the bilayer. The extruded vesicles displayed a stable T_m during the following three cooling scans or three heating scans in the presence of trehalose, sucrose, and fructose. In a few initial experiments, vesicles were extruded in the presence of trehalose at various concentrations; in this case, all heating or all cooling scans of a sample showed identical transitions.

TABLE I

Transition properties of multilamellar and extruded vesicles of dipalmitoylphosphatidylcholine

The peak transition temperature is not significantly different for these two types of liposomes. However, the calorimetric enthalpy is slightly but significantly decreased for the unilamellar extruded vesicles (95% confidence limit) and the average size of the cooperative unit is decreased by almost half. The average size of the extruded vesicles is 105 nm as determined by photon correlation spectroscopy, thus the two halves of the bilayer are not equivalent in lipid content or packing.

	Multilamellar	Extruded
T_m (°C)	41.76 ± 0.01 (5)	41.68 ± 0.03 (17)
T_p (°C)	34.85 ± 0.27 (5)	–
ΔH_{cal} (kJ mol^{-1})	38.95 ± 0.92 (5)	34.94 ± 1.05 (4)
Size of cooperative unit	225	120

The exception to these general statements was found in the presence of glucose, which will be discussed more fully below.

Effects of trehalose, sucrose and fructose on extruded vesicles. An example of three samples of EV run simultaneously (Fig. 2) demonstrates that differences in T_m were easy to detect. At low concentrations of trehalose,

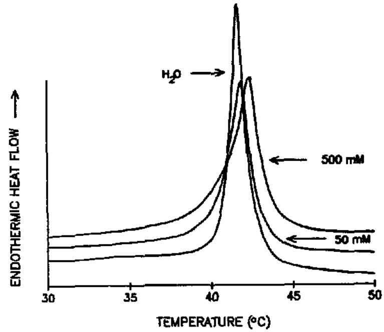

Fig. 2. Three samples of DPPC EV run simultaneously. The two scans in the presence of 50 mM and 500 mM trehalose show clear upward shifts in T_m. The transition is further broadened in the presence of the solute and shows an asymmetry on the low-temperature side in 500 mM trehalose.

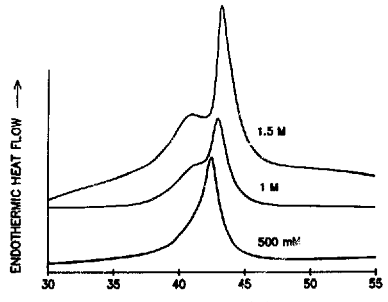

Fig. 3. With increasing concentrations of trehalose, the transition first shows an asymmetry on the low-temperature side which then becomes a shoulder and finally a distinct peak at the highest concentrations. The shoulder and peak are at a temperature below the hydrated DPPC transition temperature, suggesting binding of some of the sugar to some portion of the lipid. The same behavior was seen for EV in sucrose and fructose.

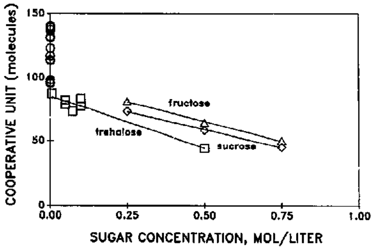

Fig. 4. The size of the cooperative unit of DPPC extruded vesicles was determined as in Ref. 22 in the presence of trehalose (□), sucrose (◇) and fructose (△). The size of the cooperative unit in water (○) averaged 120 molecules. Each symbol represents an individual sample.

sucrose, and fructose, the thermotropic phase transition of EV was symmetrical (Fig. 3), while at higher concentrations (e.g., 0.5 M sucrose, trehalose or 0.75 M fructose, Fig. 3) an asymmetry appeared on the low-temperature side of the scan. This asymmetry increased to a shoulder at still higher concentrations (e.g., 1 M sucrose, trehalose, 1.5 M fructose, Fig. 3) and a developed into a distinct peak at the highest concentration (e.g., 1.5 M sucrose or trehalose, Fig. 3). This second peak had a T_m below that of pure hydrated DPPC.

Since each concentration of each sugar was run only once or twice, we cannot make any statements about the effect of the sugars on the calorimetric enthalpy except to say that in comparison to the EV in water scanned at the same time, there was a variable slight decrease in the presence of the sugars. However, using the data from sugars at several different concentrations, it can be seen from Fig. 4 that sucrose, fructose, and trehalose caused a decrease in the cooperativity of the transition (see also Figs. 2, 3).

A comparison of the effect of trehalose, sucrose, and fructose on the increase in T_m in the presence of the solute shows that the disaccharides are similar to each other on a molar concentration basis, but have a greater effect than fructose at the same molar concentration (Fig. 5a). However, when sucrose and fructose, which

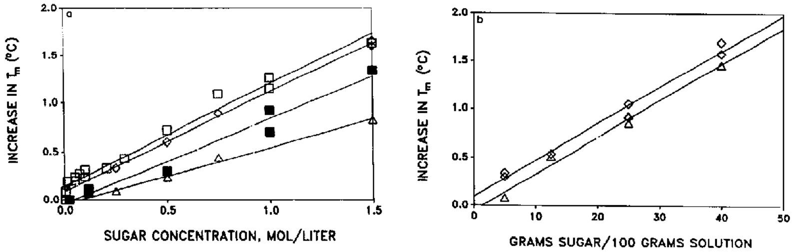

Fig. 5. (a) The increase in T_m of extruded vesicles of DPPC is monotonic in the presence of trehalose (□), sucrose (◇) and fructose (△). At the highest (weight%) concentrations of sugar, 1.5 M trehalose and sucrose, a second peak with a low T_m separates from the main high T_m peak. See Fig. 3 and text. DPPC MLV also show a monotonic increase in T_m in the presence of trehalose (■), but the effect is not as great as for EV. An analysis of co-variance showed that the data for EV in trehalose and sucrose are not significantly different from each other but each is significantly different from fructose. (b) The effect of the sugars is related to their water-structuring capacity rather than their molarity. Fructose is equally effective as sucrose on a weight% basis but only half as effective on a molar basis (see Fig. 5a). Each point on these graphs represent the average value of three heating scans made on a single sample of liposomes in sugar solution. The variation between the three heating scans lies within the symbols. An analysis of co-variance shows that the data for sucrose and fructose is not significantly different on a weight% basis.

have similar water structuring capacity on a weight% basis [16,27,28] are compared at the same weight% concentration, their effect on the T_m of DPPC is not significantly different (Fig. 5b).

The special case of glucose. Glucose, which has twice as much water-structuring capacity as fructose [27,28] might be expected to increase T_m of DPPC more than does fructose when the two sugars are compared on a weight% or molar basis. In fact, the effect of glucose on DPPC shows striking differences when compared to the other three sugars studied. At the lowest concentrations tested, 0.1 M and 0.25 M, DPPC extruded vesicles had a single, stable transition temperature below that of vesicles in pure water. On the second heating scan, when the 0.25 M glucose is osmotically equilibrated across the bilayer, a very small peak occurs at a higher T_m, but this higher peak disappears upon subsequent heating scans (Fig. 6a). This small peak at a higher T_m was not seen in 0.1 M glucose. At 0.5 M, there were two separate peaks on the second heating scan and upon repeated scanning the low-temperature transition grew at the expense of the high-temperature transition until the latter almost disappeared (Fig. 6b). At 0.75 M and 1.5 M, the low-temperature transition appears as a shoulder on the second heating scan. This shoulder develops into a separate low-temperature peak that grows at the expense of the high-temperature peak with repeated scanning. When the relative heights of the low- and high-temperature peaks are compared, a higher glucose concentration is seen to produce a more pronounced high-temperature transition (Fig. 6c).

A plot of the change in T_m against glucose concentration shows that glucose has dual effects on the phase transition properties of DPPC (Figs. 6, 7). At all concentrations of glucose, the T_m of a portion of the lipid is lowered, an effect that increases with repeated scanning of the sample. At the lowest concentrations of glucose, the lipid has a stable transition at a temperature below that of DPPC in pure water. As the concentration of glucose is increased, there is an increase in the portion of the DPPC that has an increased T_m; that portion, however, decreases in amount with repeated scanning of the sample as the portion of the lipid with a low T_m increases. The value of the increased or decreased T_m is also dependent on the glucose concentration. These data show that glucose does not have twice the effect of fructose on increasing the T_m of DPPC, but has the additional effect of lowering T_m of some portion of the lipid.

MLV and EV behave differently in sugar solutions. The effect of sucrose and trehalose on the T_m of multilamellar vesicles of phosphatidylcholines has been noted previously. DPPC and DMPC MLV showed an increase in the temperature of both the pre- and main transitions in sucrose solutions [9,18,23], and an increase of approximately 1°C in the sub-, pre-, and main transitions

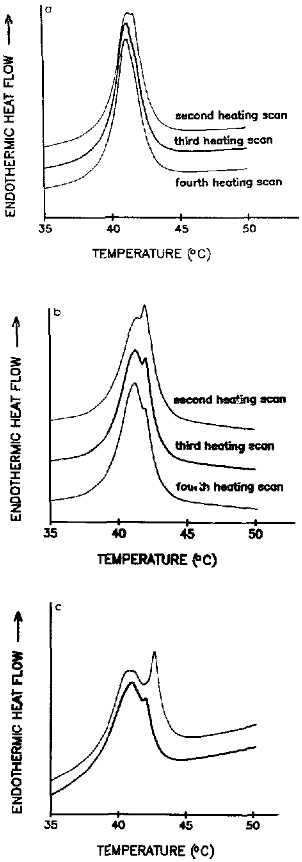

Fig. 6. Glucose interacts with DPPC EV differently than the other sugars tested. (a) On the second heating scan there is only a tiny peak at an elevated T_m in the presence of 0.25 mM glucose and the stable transition has a T_m below that in pure water. (b) Repeated scanning decreases the amount of lipid participating in the high temperature transition. At 0.5 mM glucose, the high T_m peak is more prominent than at 0.25 M glucose, but decreases in size with repeated scanning. (c) The high transition increases in temperature and calorimetric enthalpy with increases in glucose concentration. Shown are the fourth heating scans of vesicles in 0.5 M glucose (bottom) and 1 M glucose (top).

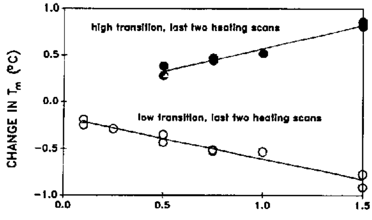

Fig. 7. Glucose lowers T_m of DPPC EV at concentration of 0.25 mol/liter or less. At higher glucose concentrations, two peaks are seen, one with a higher and one with a lower T_m than DPPC in water. The increase or decrease of T_m depends on the glucose concentration. Each symbol represents a transition from a single sample. The T_m on the last two heating scans falls within the symbol. The lines are from linear regressions of the data, r values 0.98.

in the presence of 1 M trehalose [5]. Our present data show that 1 M trehalose increases the pretransition temperature of DPPC MLV by 3°C and the main transition by almost 1°C. The effect of sugars on MLV reported by us and others [5,8,9,23] is significantly different from the effect of sugars on unilamellar vesicles.

Unilamellar vesicles exhibited a larger increase in T_m per increment concentration of trehalose (Fig. 5a) than did MLV. The apparent phase separation of different populations of DPPC which occurs in extruded unilamellar vesicles in the presence of sugars is absent in multilamellar preparations, even at the highest sugar concentrations. The source of this phase separation in EV at higher sugar concentrations is not clear: the sugar may be interacting differently with the inner and outer monolayers, and/or higher concentrations may foster binding between sugar and phospholipid.

Further, at no concentration does glucose lower the temperature of either the pre- or main transition of MLV. Therefore, the effect of glucose on the EV cannot be ascribed to the effect of some impurity. The fact that the transition of EV in glucose requires several scans for equilibration is not due to differences in glucose concentrations across the bilayer, since the other sugars equilibrated following one transition.

Mechanism of effects

Osmotic dehydration. It has been suggested in a study of carbohydrate effects on dimyristoylphosphatidylcholine MLV that the increase in the temperature of both the pre- and main transitions of multilamellar vesicles is due to osmotic dehydration of the headgroups as a consequence of a calculated decrease in water activity as sugar concentration is increased (see Fig. 6 in Ref. 9). In that study [9], all carbohydrates were equivalent in their effect when compared on the basis of a calculated water activity. The present study suggests that this is not the case, at least for unilamellar vesicles, for the following reasons. The effect of sucrose, trehalose, and fructose is much greater on EV than on MLV although the same solutions were used on both kinds of liposomes. Additionally, glucose at 0.1 M had no effect on the pre- or main transition of DPPC MLV while causing a decrease in T_m of extruded vesicles. Furthermore, we are able to detect a slight but repeatable increase in T_m at low concentrations of trehalose (5–15 mM) where the water activity must be very high, on the order of 99% of the activity of pure water.

We have determined the osmolality of weight% solutions of fructose, and sucrose using a vapor pressure osmometer. We found that although these two sugars have an equal effect on the T_m of DPPC at equivalent weight% (Fig. 5b), fructose exhibited a significantly higher osmotic pressure than sucrose (Fig. 8). These data show that the effect of sucrose and fructose on T_m of EV is not a colligative (osmotic) property of the solution.

Entropic contributions due to changes in water structure. It has been argued [2] that the stabilization of the lamellar phase of phosphatidylethanolamines by the chaotropic agents guanidine-HCl and urea is due to a reduction of the entropic contribution of water to the interaction of the hydrophobic surface of the bilayer with the aqueous phase. The reverse of these arguments could also be used for carbohydrates which are kosmotropes: an increase of water structure would increase the entropic contribution of the interaction of the bilayer surface with the aqueous phase, leading to the exclusion of water and increased dehydration of PE.

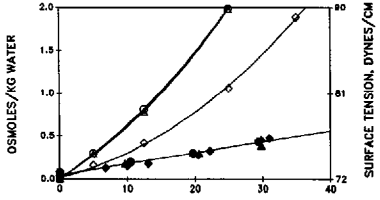

Fig. 8. The monosaccharides glucose (○) and fructose (△) have a higher osmolality than sucrose (◇) when compared on a weight% basis. However, all three sugars raise the surface tension of water (solid symbols) equally when compared on a weight% basis (data from Ref. 37). All lines are fitted by linear regression and have r values of 0.98 or better.

This effect of sugars has in fact been observed [4,6]. The lack of a demonstrated 'binding' of guanidine-HCL, urea, and SCN^- using ^{14}C-NMR [2], however, does not rule out a preferential binding in the meaning that Timasheff and his colleagues [29] use to describe solute–protein interactions. Such preferential binding can be no more than excess of the solute in the hydration sphere of proteins. At present there is no direct evidence to support either binding of exclusion of sugars at the bilayer/aqueous interface, so the question of entropic contributions to water structure must remain open.

Preferential interaction. The results of our studies presented above demonstrate that sugars have some effects (increased T_m, decreased enthalpy, decreased cooperativity) on the thermotropic phase transition of DPPC which suggest some dehydration and increased packing of the headgroups. These results are consistent with deuterium NMR studies which show an increase in order parameter of the acyl chains of palmitoyl-oleoylphosphatidylcholine in the presence of trehalose [30].

The increase of T_m of phosphatidylcholines in the presence of sugars might occur because of preferential exclusion of the solute and preferential hydration at the bilayer surface. This in turn could lead to increased headgroup packing to reduce the interaction of hydrophobic regions of the interface with the excess water, a situation analogous to the increased stabilization of proteins in sugar solutions [14,15]. Chaotropes and kosmotropes have long been known to affect protein denaturation or stability in aqueous systems.

The interaction of solutes with proteins can be described by the preferential interaction parameter which has been determined for a number of proteins and solutes by Timasheff and his colleagues in a large series of studies on water–protein–solute interactions [14,15, 29,31–36]. Briefly, the preferential interaction parameter is a measure of the exclusion of a solute from the surface or binding of a solute to the surface of a protein. Timasheff and his colleagues have found empirically that solutes that stabilize proteins in solution are preferentially excluded from the protein surface in aqueous solution, although the mechanism of exclusion is not clear. Sugars are preferentially excluded [14,15], and also increase the thermal denaturation temperature of proteins [16].

It has been suggested that the effect of sugars on protein stabilization by solute exclusion is due to the surface free energy perturbation of sugars which is reflected in the positive surface tension increment of water as a function of sugar concentration [14,15]. When the surface tension increment in the presence of sucrose, glucose, and fructose [37] is plotted against the weight% of sugar in solution, all are equal in their effect (Fig. 8). Thus, the effect of these three sugars on the higher temperature transition of DPPC can be correlated with their effect on surface tension of water, but not with osmolality of the solution (Fig. 9).

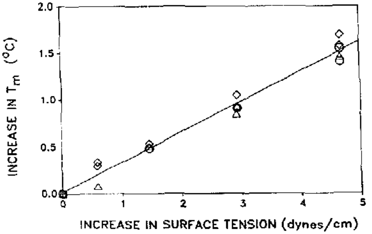

Fig. 9. The increase in T_m of the high-temperature transition of DPPC EV in glucose (○), fructose (△) and sucrose (◇) are directly correlated with the effect of these same sugars on the increase of the surface tension of water. The increase in surface tension for each weight% of sugar was calculated from the line in Fig. 8. The fitted line had an r value of 0.99.

Although it has been suggested that preferential interactions as demonstrated for proteins by Timasheff and his colleagues may occur between phospholipids and solutes [2–4], the only evidence presented so far is the effect of chaotropic and kosmotropic solutes on the transition temperatures of phosphatidylcholines and phosphatidylethanolamines (Refs. 2–9, this paper). These results are consistent with preferential interactions, but the only attempt to investigate the mode of interaction is inconclusive [2].

If the assumption is made that solutes that preferentially bind to the bilayer lower T_m by intercalation or increased hydration, and solutes that are preferentially excluded raise T_m by the mechanism described above, then the data presented here are consistent with the idea that trehalose, sucrose and fructose are largely excluded from the bilayer, but begin to show some binding at the highest concentrations studied. By this line of reasoning, glucose, on the other hand, would show binding to the bilayer at low concentrations and would be excluded only at higher concentrations. This result with glucose is consistent with a study of binding which compared trapped volumes as determined with calcein fluorescence with trapped volumes calculated directly from structural data [38]. At 0.1 M glucose, approximately 7 mol glucose per mol lipid were bound in excess of the glucose trapped in the vesicle interior. At the concentration of DPPC used in the present study, all of the glucose should be either trapped or bound when glucose is 0.1 M.

Interactions with frozen and dry bilayers

The effect of sugars on both the direction and the magnitude of the shift of the thermotropic transition of hydrated bilayers is strikingly different from the effect of sugar on the transition of very dry bilayers. For example, trehalose can lower the transition temperature of at least two different dry phospholipids by at least 80°C [39,40]. By contrast, trehalose increases T_m of hydrated bilalyers by about 1.5°C at the highest concentration attainable, 1.5 M.

Retention of trapped solute by dry liposomes depends with some specificity on the type of sugar used during lyophilization, while the increase in T_m seen in the present study depends only on the weight% of the sugar in solution. Other solutes, such as proline and dimethylsulfoxide also increase T_m of DPPC (unpublished data) and are cryoprotective for liposomes [11,41,42] but provide no stabilization of liposomes during freeze-drying [42]. We conclude that the basic mechanisms of interaction of sugars and other solutes are different in aqueous and dry systems.

Many questions remain unanswered by this study. The relatively small changes in T_m of phosphatidylcholine in unfrozen solution shown in this and other reports cannot be the source of the stabilization of liposomes by sugars during freeze-thaw experiments. However, they may cause changes in liposome behavior at ambient temperatures [13]. One can ask whether the apparent binding or exclusion shown by the calorimetric data are important in the cryoprotective effect of these solutes. Some experiments [11,12] suggest that there are direct interactions between sucrose or trehalose and the phosphate of the headgroup during freezing which are important in the cryoprotective effects of these sugars. Our calorimetric data suggest that for sucrose, trehalose, and fructose in unfrozen solution, preferential exclusion from the bilayer may be the predominant mode of interaction. However, at high concentrations of the sugars, the data also suggest that binding may be initiated. During the freezing process, the sugar concentration in the unfrozen fraction around liposomes increases to high levels. Perhaps it is binding at high concentration that will prove to be the important cryoprotective event.

Acknowledgments

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