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Interactions between soluble sugars and POPC (1-palmitoyl-2-oleoylphosphatidylcholine) during dehydration: vitrification of sugars alters the phase behavior of the phospholipid

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Abstract

The phase behavior of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) was characterized as a function of hydration in the presence of combinations of sugars representative of sugars found in seed embryos having differing degrees of desiccation tolerance. The tendency of the sugar mixes to vitrify was also monitored as a function of hydration. Using differential scanning calorimetry, it was found that all sugars diminished the increase in the gel-to-fluid phase transition temperature (T_m) of POPC that occurred upon dehydration of the pure lipid. These results are analyzed in terms of the osmotic and volumetric properties of sugars. Also, it was found that in those samples for which the glass transition temperature (T_g) was greater than the T_m of POPC, T_m was lowered by approx. 20 C° from the value for the fully hydrated lipid. X-ray diffraction data confirmed that acyl chain freezing was deferred to a lower temperature during cooling of vitrified samples. The significance of these results is discussed in terms of the ability of many organisms to tolerate desiccation.

Key words: Phosphatidylcholine; Sugar; Vitrification; Glass; Dehydration; Phase transition

1. Introduction

Sugars appear to play a role in the ability of certain organisms to survive extreme dehydration. Trehalose, a non-reducing disaccharide of glucose, accumulates to high concentrations in many desiccation tolerant organisms, including yeast, nematodes, brine shrimp cysts, and the resurrection plant *Selaginella lepidophylla* [1]. Seeds and pollen of many higher plants contain large amounts of sucrose or mixtures of sucrose and oligosaccharides [2], the levels of which correlate with desiccation tolerance [3,4]. Trehalose has the ability to prevent bilayer fusion and solute leakage in dried liposome preparations [5] and to preserve the functional integrity of sarcoplasmic reticulum vesicles during lyophilization and rehydration [6]. Other sugars have also been shown to stabilize dry liposomes and vesicles [7,8].

It has been suggested that the ability of sugars to stabilize dry liposomes is due, at least in part, to the sugars' ability to prevent the increased gel-to-fluid phase transition temperature, T_m, associated with the dehydration of phospholipids ([9], and references therein). When the phospholipids DPPC, POPC, and PS were dried in the presence of sugars, T_m did not increase, as it would have if the phospholipids had been dried alone [8,10]. Rather, T_m remained at the same temperature as for the fully hydrated lipid, or, in some instances, T_m was lowered by as much as 20

Abbreviations: DSC, differential scanning calorimetry; DPPC, dipalmitoylphosphatidylcholine; PS, phosphatidylserine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; T_m, gel-to-fluid phase transition temperature; T_g, glass transition temperature; gFW, wet weight (g) of samples.

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degrees below this value [8,10]. The preservation of T_m at the value for the fully hydrated lipid has been observed for DPPC dried with trehalose [10–16] and has been reported for DPPC dried with sucrose [17]. The effects of few other sugars have been tested [13], and no determination has been made of the efficacy of mixes of sugars similar to those found in desiccation tolerant seed embryos. It has been suggested that trehalose prevents increases in T_m during drying by hydrogen bonding to the phospholipids [1,13].

A recent hypothesis postulates that another important facet of sugars' ability to stabilize membranes during dehydration is their ability to vitrify, or enter the glass phase [18–20]. In the highly viscous vitrified state, molecular motions are hindered [21]; this may confer stability to the dried membrane [18]. Data suggest that the differential ability of dried sugars to stabilize liposomes and vesicles may correlate to differences in their glass-forming tendencies, as reflected by the glass-transition temperature, T_g [18]. Further, there is evidence of glass formation in desiccation tolerant seed embryos [20,22] and evidence that the glasses detected in the whole embryos are formed by the soluble sugar components [19].

To explore the hypothesis that the changes in sugar composition observed in seed embryos are involved in changes in the desiccation tolerance of plant membranes in vivo, we evaluated the interactions of a phospholipid, POPC, with the some of the sugars postulated to confer desiccation tolerance to seed embryos [3]. To study both postulated roles for sugars, we tested the ability of the sugars to prevent increases in the T_m of POPC, and we monitored the glass-forming properties of the sugars as a function of hydration. Finally, we examined the phase behavior of POPC in the presence of vitrified sugars and compared it to the phase behavior of POPC in the presence of non-vitrified sugar solutions.

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2. Materials and methods

2.1. Materials

POPC (1-palmitoyl-2-oleoylphosphatidylcholine), a mixed-chain, monounsaturated phosphatidylcholine, was chosen as a representative membrane phospholipid for this study. POPC obtained from Sigma (St. Louis, MO) and Avanti Polar Lipids (Birmingham, AL) was found to be pure by thin-layer chromatography and was used without further purification. The sugars glucose, sucrose, raffinose, and sorbitol were purchased from Sigma (St. Louis, MO) and used with no further purification.

Combinations of sugars used in this study simulate those found in seed embryos before and after the loss of desiccation tolerance. Seed embryos that have lost desiccation tolerance are typified by a high ratio of monosaccharides to sucrose [3]. The NT (non-tolerant) sugar mix consisted of glucose and sucrose (75:25, w/w). Desiccation-tolerant seed embryos contain high levels of sucrose and oligosaccharides, and no monosaccharides [3]. The DT (desiccation-tolerant) sugar mix contained sucrose and raffinose (85:15, w/w). When combined with POPC, NT sugars were mixed at a sugar-to-lipid ratio of 0.7:1 (w/w), and DT sugars were mixed at a sugar-to-lipid ratio of 2:1. These ratios approximate the sugar-to-total lipid ratios found in desiccation intolerant seedlings and desiccation tolerant seed embryos. In addition to samples described above, samples were prepared using sucrose and sorbitol with POPC at sugar-to-lipid ratios of 2:1 (w/w) for sucrose and 1:1 (w/w) for sorbitol.

2.2. Sample preparation

Samples for calorimetry were prepared by first drying POPC (in chloroform) under a stream of N_2 at 40°C. Solutions of the desired sugars dissolved in methanol/water 1:1 (v/v) were then added to the dried lipid film. After vortex mixing, the samples were thoroughly redried under a stream of N_2 at 40°C to remove methanol, then distilled water was added to excess. Aliquots of the sample solutions were transferred to preweighed pans and equilibrated from 3 to 10 d at 35°C over saturated salt solutions that generate the following relative vapor pressures: KNO_3, 0.90; KCl, 0.84; NaCl, 0.75; NH_4NO_3, 0.60; $Mg(NO_3)_2$, 0.52; K_2CO_3, 0.43; $MgCl_2$, 0.33; and LiCl, 0.12. Values for water activities above the saturated salt solutions were calculated from published values for the relative humidities at the specified temperature [23,32]. Water content and T_m remained constant after 3 d of equilibration. Following the equilibration period, sample pans were sealed and reweighed, and DSC measurements were performed. Dry weights were obtained after calorimetry by puncturing the sample pans and drying the samples in vacuo over fresh phosphorus pentoxide at 70°C for at least 16 h.

2.3. Calorimetry

DSC was performed using a Perkin-Elmer (Norwalk, CT) DSC-7. In a typical protocol, samples were cooled at a nominal rate of 200 C°/min to −150°C, equili-

brated at that temperature for several minutes until no further heat flow occurred, then heated at a scan rate of 20 C°/min to 80°C. Samples were then cooled at a rate of 20 C°/min to −150°C. In some experiments, samples were cooled and heated at rates of 0.5 C°/min, 2 C°/min, 5 C°/min, and 10 C°/min. In these samples, the measured T_m of the lipid did not deviate by more than 3 degrees from the values obtained at 20 C°/min. Glass transitions were much more pronounced in samples scanned at 20 C°/min than in those scanned at slower rates; therefore, for the purposes of this study, 20 C°/min was used as a standard scanning rate. In the wettest samples, in which the melting transition of ice overlapped the T_m of POPC, second heating scans were performed after cooling to only −15°C. This protocol avoided the freezing of water and revealed the phase transition of POPC. Duplicate samples were prepared and scanned for the various combinations of sugar, lipid, and water activity. All data used for analysis were taken from the heating scans, although data from cooling scans were used to check for hysteresis of the transitions. T_m represents the temperature of the peak maximum for the gel-to-fluid phase transition, and T_g represents the glass transition temperature for the sample, determined from the midpoint of the temperature range over which the change in specific heat occurred.

2.4. X-ray diffraction

Samples prepared for analysis by X-ray diffraction were desorbed above saturated salt solutions as described above except that the samples were equilibrated for 18 d at 20°C before loading into quartz X-ray capillaries (Charles Supper, Natick, MA). X-ray diffraction experiments were performed on the F3 station at the Cornell High Energy Synchrotron Source (CHESS). Sagittally-focused monochromatic radiation ($\lambda = 0.156$ nm) was passed through a 2.0 by 0.5 mm slit and focused at the film plane. The 0.5 mm slit dimension was used in the direction of focus. Diffraction powder patterns were recorded on 3 or 4 sheets of Kodak DEF-5 film, placed at a carefully measured specimen-to-film distance (10.1 cm), using typical exposure times of 10–20 min. Sample temperature was monitored with a thermocouple placed outside the capillary sample holder but in a position immediately adjacent to the portion of the sample in the X-ray beam. Temperature was regulated with an FTS air-jet cooling system and the temperature at the sample was compensated for a small (≈ 1 C°) difference between the temperature of a thermocouple positioned as described and one embedded in a lipid sample. Reported temperatures are ±1 C°. Samples were equilibrated for a minimum of 5 min at each temperature before the diffraction patterns were recorded.

3. Results

As determined using DSC, the T_m of fully hydrated POPC was −3°C; when POPC was dehydrated in the absence of sugars, the T_m rose to 61°C. When POPC was dehydrated in the presence of sugars, T_m did not exceed 6°C (Fig. 1). T_g of the sugars was monitored in the same samples and was found to increase with decreasing sample hydration, as expected (Fig. 1) [18,24]. DSC scans obtained from all samples were reproducible. A small hysteresis of lipid and sugar transitions between cooling and heating scans in the samples existed such that the onset temperature of the transition from the cooling scan was equal to the midpoint temperature of the transition from the heating scan. For samples in which the lipid T_m and the sugar T_g were approximately equal, the transitions were very broad and had a low enthalpy (Fig. 2B). These broad, low enthalpy scans were reproducible; however, based solely on the DSC data, it was not possible to identify the peaks as transitions of the lipid or the sugar. At hydrations less than approx. 6 wt%, for the samples containing either sucrose or the DT sugars, T_g was greater than the T_m of hydrated POPC. In these samples, the gel-to-fluid phase transition of the lipid was apparently lowered to approx. −25°C (Fig. 2C). The same phenomenon was observed for POPC dried in the presence of sugars found in desiccation intolerant seed embryos (NT sugars), but only at less than 1 wt% water (Fig. 1).

To confirm that the observed transitions around −25°C were due to the gel-to-fluid phase transition of POPC, we performed X-ray diffraction experiments on

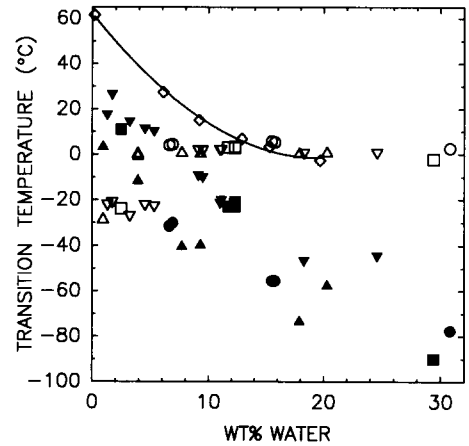

Fig. 1. Transition temperatures of POPC and sugars after dehydration. Open points represent the temperature (T_m) of the peak maximum for the gel-to-fluid phase transition of POPC measured in individual samples. Filled points represent the midpoint temperature (T_g) of the glass transition of the sugars in the same samples. Legend: ◇, pure POPC; ▽, ▼, DT sugars/POPC; △, ▲, NT sugars/POPC; □, ■, sucrose/POPC; ○, ●, sorbitol/POPC. Wt% water was calculated as (gH_2O/gFW)×100.

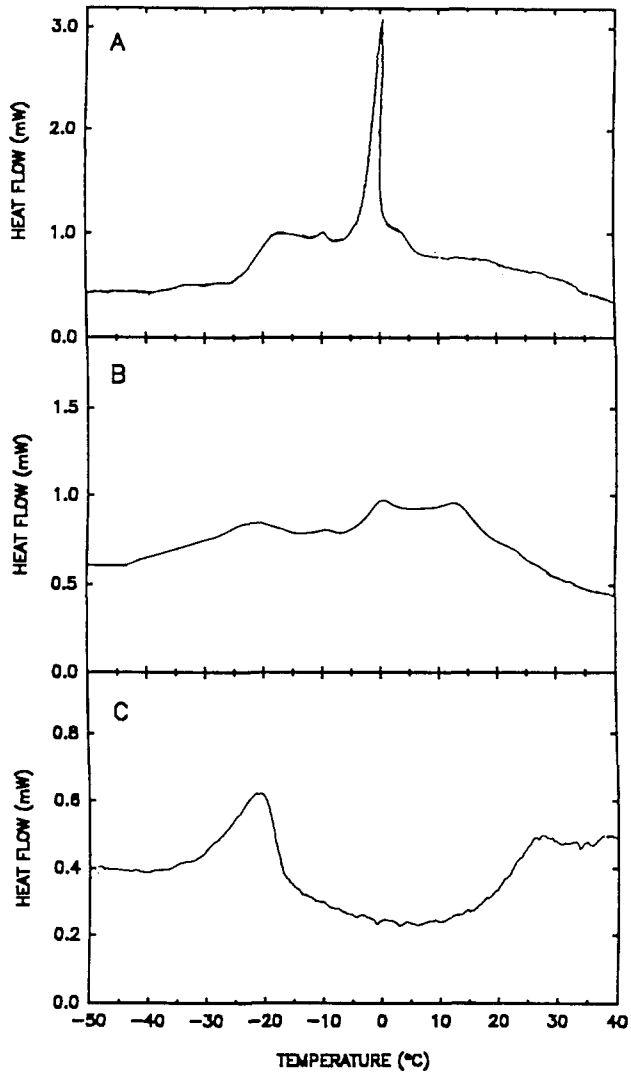

Fig. 2. DSC thermograms of sugar/POPC samples after dehydration. (A) DSC of POPC in the presence of sucrose after equilibration to a water activity of 0.60. The peak represents heat flow into the sample associated with the gel-to-fluid phase transition of POPC; T_m is taken as the peak maximum. T_g, the glass transition of the sugars, is taken as the midpoint of the deflection in the baseline centered around −23°C. (B) DSC of POPC in the presence of sucrose after equilibration to a water activity of 0.33. The warming scan reveals no peak that can be ascribed to a gel-to-fluid phase transition. (C) DSC of POPC dried in the presence of DT sugars to a water activity of 0.12. The peak centered at −21°C represents heat flow into the sample apparently associated with the gel-to-fluid phase transition. The deflection in the baseline centered around 22°C represents the heat flow into the sample associated with the melting of the vitrified sugars. Note that T_m of fully hydrated POPC is −3°C; T_m in the presence of the vitrified sugars has been lowered by almost 20 C°.

dehydrated sugar/POPC samples at a variety of temperatures above and below both the lipid T_m and the sugar T_g (Table 1). In all samples, at the starting temperature of 30°C, the small angle reflections consisted of a series of weak, diffuse, lamellar diffractions indexing in the sequence 1:1/2:1/3:1/4.... In several samples, we also detected additional reflections that do not index to lamellar, cubic, or hexagonal lattices. The origin of these reflections is not known; however, Quinn et al. [14] also detected additional unidentified reflections in DPPC/trehalose mixtures. During cooling of the samples to ≤ −10°C, the small angle reflections became diffuse and indistinct. This indicates poor long-range order perpendicular to the bilayer normal during the time course of the film exposure (10–20 min) and is considered to be a consequence of the method of sample preparation [14].

The sample containing DT sugars/POPC equilibrated to a water activity of 0.12 was in the L_α phase at 30°C as indicated by the small angle lamellar phase reflections and a diffuse wide-angle reflection at 0.41 nm arising from disordered acyl chain packing (Table 1). This wide-angle spacing is smaller than that normally observed for phospholipids, but it is similar to the value reported for POPC at 10 wt% water of 0.43 nm [25], indicating that dehydration and, possibly, the presence of sugars reduced the lateral spacing of the lipid, although the acyl chains were still fluid. At −10°C, the acyl chains remained in a disordered state, although with a slight reduction in the chain spacing to 0.40 nm. At −35°C, the diffraction pattern exhibited a sharp wide-angle reflection at 0.38 nm. The sharp nature of this reflection indicates an ordered state for

Table 1
Properties of sugar/POPC samples at various hydrations and temperatures

Sample (Water activity)	°C	Wide-angle spacing (nm)	Wide angle	Sugar T_g
DT/POPC	30	0.41	diffuse	
(0.12)	−10	0.40	diffuse	22°C
	−35	0.38	sharp	
DT/POPC	30	0.45	diffuse	
(0.75)	−10	0.41	sharp	−48°C
	−35	0.39	sharp	
NT/POPC	30	0.41	diffuse	
(0.12)	−10	0.40	sharp	crystals
	−23	0.39	sharp	
NT/POPC	30	0.44	diffuse	
(0.75)	−10	0.41	sharp	−72°C
	−35	0.41	sharp	
SUC/POPC	30	0.41	diffuse	
(0.12)	−10	0.39	sharp	crystals
	−23	0.39	sharp	
SUC/POPC	30	0.43	diffuse	
(0.75)	−10	0.41	sharp	−18°C
	−21	0.40	sharp	

Samples were equilibrated to water activities of either 0.12 or 0.75, then analyzed by X-ray diffraction and DSC. X-ray diffraction and T_g data were obtained on replicate samples. Evidence of crystallization was observed by X-ray diffraction. Note that in the DT sugars/POPC sample, for which T_g was greater than the T_m of fully hydrated POPC, freezing of the acyl chains was deferred to below −10°C. In these particular NT sugars/POPC and sucrose/POPC samples, crystallization of the sugars had occurred, and no lowering of the T_m of POPC below its hydrated value was observed.

the sample. Acyl chain spacing is smaller than previously reported for POPC in a gel phase (0.42 nm) [25]. This sample had no evidence of the presence of sugar crystals at any temperature.

As controls, we examined the NT sugars/POPC and sucrose/POPC mixtures as a function of hydration and temperature (Table 1). The diffuse wide-angle spacings observed in all samples at 30°C indicate that the lipids were in a disordered lamellar phase, although in the samples incubated at a water activity of 0.12, the wide-angle spacings had decreased as a result of dehydration. At higher hydrations (water activity = 0.75), during cooling to −10°C, POPC underwent a disorder-to-order transition of the acyl chains, as determined by the nature of the wide-angle signal. At these higher hydrations, T_g was less than T_m, regardless of the sugar compositions used.

To compare the effects of sugar composition in very dry sugar/POPC mixtures, we also examined NT sugars/POPC and sucrose/POPC, dehydrated to water contents less than 10 wt%, by X-ray diffraction. These samples had diffuse wide-angle reflections, indicative of the disordered lamellar phase, at 30°C (Table 1). At low hydrations, the samples of NT sugars/POPC and sucrose/POPC exhibited behavior different from that of DT sugars/POPC. In some samples, as determined using DSC, the T_g values were greater than −3°C (T_m of fully hydrated POPC), and the T_m of POPC was lowered to approx. −25°C. However, in other samples (Table 1), X-ray diffraction revealed extensive wide-angle reflections arising from crystalline sugars at all temperatures examined. In these samples, the T_m of POPC was similar to its hydrated value. This demonstrates that, although crystallization of sugars did not cause the T_m of POPC to increase above its hydrated value, it did preclude the lowering of T_m below the hydrated value that was observed in the presence of vitrified sugars. The failure to observe wide-angle reflections from sugar crystals in any of the samples containing DT sugars suggests that the combination of sugars associated with the desiccation tolerant state of seeds has less of a tendency to crystallize than do the sugars found in desiccation intolerant seed embryos or pure sucrose.

4. Discussion

The gel-to-fluid phase transition temperature of fully hydrated POPC was −3°C; in the presence of any of the combinations of sugars tested, T_m never rose above 6°C, regardless of the dehydrative stress imposed (Fig. 1). This differed from the behavior of pure POPC, for which T_m increased to 61°C after dehydration to 0.2 wt% water (Fig. 1). In order to explain the effect of the sugars, it is necessary to understand the effect of dehydration on the phase transition temperature for pure lipids.

At low hydrations, lipid/water mixtures are dominated by the strongly repulsive hydration force [26,27] that gives rise to a large compressive lateral stress in the membrane, as described by Wolfe [28]. This stress is responsible for the increase in T_m that occurs upon dehydration. The gel-to-fluid transition occurs at the temperature for which the entropy of the transition ($T\Delta S$) is greater than the enthalpy of the transition (the energy required for the lipid to expand in the plane of the bilayer). Increasing the compressive lateral stress increases the enthalpy of the transition; therefore, the entropy term must also increase (increase T) in order for the transition to occur. Thus, dehydration, and the induced membrane stress, causes the phase transition temperature to increase.

The situation described above is true for a pure lipid undergoing dehydration. The experiments presented in Fig. 1, however, show that the presence of sugars inhibits this dehydration-induced temperature increase at all water contents. In order to interpret the experimental results, we adopt the rationale of Wolfe [28], who modeled the dehydration characteristics and the induced lateral stress in the presence of ideal solutes. These ideas have since been extended to include the effects of the osmotically inactive volume [29,30], and are briefly summarized below.

For a multilamellar lipid-water-solute mixture, the chemical potential of water, Ψ, is related to the hydrostatic pressure, P, and the osmotic pressure, Π. At relatively low hydrations, the hydrostatic pressure is dominated by the hydration force, which is related to the interbilayer separation, y, by [29]:

$$\Psi = P - \Pi = -P_o \exp(-y/\lambda) - (\gamma n_s kT)/(V - b) \quad (1)$$

where λ is the characteristic decay length of the hydration force, γ is the activity coefficient of the solute (assumed to be 1, see below), n_s is the number of moles of solute, k is the Boltzmann constant, T is the temperature in Kelvin, V is the solution volume, and b is the osmotically inactive volume. For simplicity, this model assumes that surface tension and hydration force are independent of sugar concentration.

The volume of solution associated with each lipid is given by:

$$V = ay/2 = V_w + b = V_w + n_s v_s, \quad (2)$$

where a is the area per lipid molecule, y is the interbilayer separation, V_w is the water volume, and v_s is the solute partial volume. By inspecting this equation one can see that the effect of a non-zero n_s is quite substantial – it increases the separation between the bilayers at a particular water content and contributes an osmotic pressure (Eq. 1). The net effect of this is

that for a particular water content, the bilayer separation is larger (by a length $2n_s v_s/a$). This in turn reduces the repulsive force and, therefore, the lateral stress induced in the membrane. Thus, the effect of dehydration on the transition temperature is reduced. To illustrate the effect, consider the experimental system of the NT sugars/POPC (0.7:1, w/w). The unhydrated molecular volumes for glucose and sucrose are about 0.27 nm^3 and 0.52 nm^3, respectively. Taking a weighted average and multiplying by $n_s = 2.42$ (the molar ratio of sugars/POPC) gives a value of b (the volume of solute per lipid molecule) of about 0.8 nm^3 (assuming no water of hydration). This volume is equal to that of about 27 water molecules, which means that the interbilayer separation can never be reduced below about $y = 2b/a = 2.5$ nm [1].

The practical result of all this is that the hydration repulsion P is exceedingly small for all water contents and can be ignored in Eq. 1. The membrane stress (equal to the separation times the repulsion) is, therefore, also exceedingly small, and hence, the temperature of the transition is affected little by dehydration. For sugar/lipid ratios < 0.5 (w/w), the above theory has met with some success [29,30]. For the samples presented here, the sugar/lipid ratios are so high that the above theory cannot be quantitatively applied. The theory predicts virtually no change in the transition temperature for all water contents because the lateral stress is negligible. In practice, the transition temperatures are increased by a few degrees in the presence of these sugars at low water contents. Note that this increase ($\Delta T/T$) is only a few percent, so the error is quite acceptable. Such an error is expected from the underlying assumptions, which are that the hydration force parameters are unaffected by the presence of the sugars, and that the solution behaves ideally ($\gamma = 1$). At a water activity of 0.90, the sugar concentration is 2.8 M for the NT sugar/POPC samples, already in the non-ideal range.

Thus, the ability of sugars to reduce the phase transition temperature of phospholipids during dehydration is shown to largely be due to the osmotic and volumetric properties of the sugars. This is borne out by the fact that all of the sugar mixtures produced similar changes in the transition temperature. The values of b for these samples are within a factor of 2 of each other, so the effects on T_m are similar. It has been suggested that the ability of sugars to prevent the dehydration-induced increase in T_m is due to specific hydrogen bonding between the phospholipid headgroups and the sugar molecules [1,13]. If this were the most important effect of sugars, then at similar total solute volumes (i.e., concentration times molecular solute volume), some sugars should produce a substantially greater change in the T_m than others. Such specific sugar/lipid interactions may exist, but to first order, they probably contribute little to the effect of preventing increases in T_m.

The above analysis adequately describes the ability of the sugars to maintain the lipid T_m near that of the fully hydrated lipid. It does not, however, explain why, at very low hydrations for POPC in the presence of DT sugars, the T_m is reduced to below this value. This effect is observed only when the T_g of the sugar mixture is well above the lipid T_m (Fig. 2C). In cases where the two transitions are at similar temperatures (e.g., hydrations of approx. 8 wt% for sucrose/POPC), the DSC traces are too complex to unambiguously interpret (Fig. 2B).

Why the presence of a vitrified sugar solution lowers the T_m of POPC is uncertain to us. One might hypothesize that the increased viscosity around the lipid headgroups prevents or hinders the close approach of headgroups associated with the fluid-to-gel transition. If close approach were prevented, one might expect to see no decrease in wide-angle spacing in the X-ray diffraction pattern. However, as shown in Table 1, the wide-angle spacing did decrease during the disorder-to-order transition of the vitrified DT sugars/POPC sample. If close approach were merely hindered by the increased viscosity, one might expect to see a hysteresis of the transition, with T_m lowered during cooling and raised during warming. This was not observed using DSC. T_m occurred at approx. -25°C during both cooling and warming scans of the vitrified sample. It is conceivable that the lowering of T_m by vitrified sugars can be ascribed to changes in the surface tension between the sugar solution and lipid. A reduction in the solution/lipid surface tension would increase the optimal surface area per lipid molecule. This, in turn, would make the transition from gel to fluid phase energetically more favorable, thus lowering the transition temperature. The effect of vitrification on surface tension is unknown to us.

Crowe and colleagues [8,10] reported that POPC dried with trehalose had a T_m at -23°C, a value which agrees with our data for POPC dried with sugars that are vitrified above the T_m of the fully hydrated lipid. The water content of their sample was not stated; however, it was probably below 10 wt%, at which hydration the glass transition temperature of trehalose is approx. 17°C [18]. This is well above T_m of fully hydrated POPC, and could account for the depression of T_m to -23°C.

[1] The caveat here, of course, is that the sugar must remain evenly distributed. At very low hydration, it is possible that some of the sugar is excluded into a bulk crystalline phase. In such a situation, the value of b used here would be smaller, but the effect would remain strong.

The data found in the literature for the interactions of sugars and other phospholipids are more complicated. Most of the experiments have focused on trehalose and DPPC. Crowe and Crowe reported that trehalose lowered the T_m of dried DPPC to 24°C, which is below the T_m of 42°C for the fully hydrated lipid [10]. However, this lowering of T_m was only observed after heating the dried sample through the phase transition once; on the first heating scan of the DSC, the apparent T_m of DPPC was centered at 60.9°C. These data were interpreted to indicate that trehalose could only effectively incorporate itself into the lamellae and hydrogen bond to the phospholipid when the lipid was in the fluid phase. If trehalose was dried with the lipid in the gel phase, the spacing of the phospholipid headgroups was not optimal for hydrogen bonding with trehalose. We propose an additional interpretation. When a mixture of trehalose and DPPC is lyophilized, the sugar is freeze-concentrated and will have a T_g the same as that of a frozen trehalose solution, −29.5°C [24]. This is below the T_m of DPPC; therefore, no lowering of T_m will occur. During sublimation of the ice, T_g of the sugar increases to correspond to the T_g of trehalose at that low hydration; however, no translational diffusion of sugar molecules is possible until the sample has been heated through the T_g of the sugar-glass [24,31]. According to Crowe and Crowe [10], the water content of their dry sample was less than 1 wt% water. This would cause T_g to be around 72°C [18]. Thus, after the first heating scan, the sample is in the fluid phase, allowing molecular reorientations, and the T_g will be above the lipid T_m. Thereafter, the gel-to-fluid phase transition temperature is lowered to 24°C (see Fig. 1 in Ref. [10]). In contrast, samples of trehalose/DPPC that had been dried at 60°C had T_m at 24°C in the first and subsequent heating scans (Fig. 3, Ref. [10]). These samples were dried such that the T_g of trehalose would be that of the dry sugar, 72°C, before heating in the DSC. Thus, T_g was above T_m of the fully hydrated lipid, so T_m was lowered.

Several others have studied the interactions of dried trehalose and DPPC, but have not found that the T_m of DPPC was depressed below 42°C, the value for the fully hydrated lipid [11,12,14–16]. The hydrations reported for these samples were all 5–6 wt% water, corresponding to the dihydrate of DPPC. At these water contents, T_g of trehalose should be in the range of 40–50°C [18]. It is difficult to interpret these results unless one speculates that the water content of the samples was high enough so that T_g was slightly less than T_m of fully hydrated DPPC. In our samples, the effect of vitrified sugars was most clearly seen when T_g was several degrees or more above T_m, as in the DT sugars/POPC sample dried to a water activity of 0.12 (Fig. 2C). Perhaps the results with DPPC and trehalose have been unclear because T_g and T_m are converging at low hydrations.

The data presented here indicate that sugars from both desiccation tolerant and intolerant seeds, as well as pure sucrose and sorbitol, had the ability to prevent the increase in T_m normally caused by the dehydration of a phospholipid. This supports the recent proposition [29] that such effects may be a property of all sugars, and not solely due to specific interactions between certain sugars and phospholipids. We infer from this that changes in sugar composition associated with changes in desiccation tolerance are probably not needed to prevent increases in T_m. Rather, changes in sugar composition may be necessary to facilitate vitrification, both by raising T_g and preventing crystallization. We have shown that vitrified sugars interact in a different manner with membrane lipids than do non-vitrified sugars. Not all consequences of this effect are known to us, but it is conceivable that similar effects might occur between vitrified sugars and other cellular components. We believe that these effects could be important to the ability of some organisms to survive the loss of cellular water.

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