Effects of Vitrified and Nonvitrified Sugars on Phosphatidylcholine Fluid-to-Gel Phase Transitions

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ABSTRACT DSC was used to study the ability of glass-forming sugars to affect the gel-to-fluid phase transition temperature, T_m, of several phosphatidylcholines during dehydration. In the absence of sugars, T_m increased as the lipid dried. Sugars diminished this increase, an effect we explain using the osmotic and volumetric properties of sugars. Sugars vitrifying around fluid phase lipids lowered T_m below the transition temperature of the fully hydrated lipid, T_o. The extent to which T_m was lowered below T_o ranged from 12° to 57°, depending on the lipids' acyl chain composition. Sugars vitrifying around gel phase lipids raised T_m during the first heating scan in the calorimeter, then lowered it below T_o in subsequent scans of the sample. Ultrasound measurements of the mechanical properties of a typical sugar-glass indicate that it is sufficiently rigid to hinder the lipid gel-to-fluid transition. The effects of vitrification on T_m are explained using the two-dimensional Clausius-Clapeyron equation to model the mechanical stress in the lipid bilayer imposed by the glassy matrix. Dextran and polyvinylpyrrolidone (PVP) also vitrified but did not depress T_m during drying. Hydration data suggest that the large molecular volumes of these polymers caused their exclusion from the interbilayer space during drying.

INTRODUCTION

The stabilizing effect of sugars on dehydrated membranes and liposomes has been extensively documented in recent years (Caffrey et al., 1988; J. H. Crowe et al., 1984, 1992; Koster et al., 1994; Sun et al., 1996; Suzuki et al., 1996). A wide range of scientists are interested in this subject, including biologists studying the ability of several organisms to survive extended periods of desiccation and those interested in the use of liposomes in drug delivery systems and other technological applications. One aspect of stability in the dry state is the prevention of lipid fluid-to-gel phase transitions, and the associated leakage through the membrane (J.H. Crowe et al., 1989, 1992). Many studies have demonstrated that soluble sugars have the ability to prevent increases in the gel-to-fluid phase transition temperature T_m of phospholipids during dehydration (Crowe and Crowe, 1988; Koster et al., 1994). This effect can largely be explained by the theory of Bryant and Wolfe (Bryant and Wolfe, 1992; Wolfe and Bryant, 1999), which describes how nonspecific osmotic and volumetric effects of sugars prevent the close approach of adjacent bilayers and thus reduce the mechanical stresses that occur when bilayers are brought close together. This theory suggests that these effects would be seen with any small solutes of comparable size that partition into regions near membranes. Another theory (Crowe and Crowe, 1988; J. H. Crowe et al., 1988, 1996, 1998) stresses the importance of specific hydrogen bonding between some sugars and lipids. Where such bonding occurs to a sufficiently large extent, it may modify mechanical stresses in membranes via mechanisms discussed later.

In a previous paper, we described the effect of vitrified sugars on the phase behavior of a representative phosphatidylcholine (PC), 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) (Koster et al., 1994). We demonstrated that all sugars tested were able to reduce dehydration-induced increases in T_m and that sugars that vitrified around fluid phase POPC during dehydration had the additional ability to lower the T_m to ~22° below T_o, the gel-to-fluid transition temperature of the lipid in excess water. We have since found that this effect occurs for other molecular species of PC: vitrification of sugars around fluid-phase lipids results in a lowering of the lipid T_m below T_o (Koster and Anderson, 1995). This effect has also been confirmed by Zhang and Steponkus (1996), who have suggested a model to explain the observed behavior.

The goal of the current study was to further explore the mechanism by which vitrified sugars alter the phase behavior of phospholipids. We used DSC to extend our findings to several other molecular species of PC, using different sugars and glass-forming polymers. Furthermore, we used ultrasound to study the elastic properties of a relevant vitrified sugar to determine if the glass is sufficiently rigid to account for the observed changes in the lipid-phase transition temperatures.

THEORY

Increase in the gel-to-fluid transition temperature due to dehydration

The effect of dehydration on membrane phase transitions is fully described elsewhere (Bryant and Wolfe, 1992; Wolfe and Bryant, 1999). Only a brief summary will be given here.

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As a fully hydrated membrane in the fluid phase is cooled, it reaches a temperature (T_o) at which it undergoes a transition to the gel phase. This transition is accompanied by the liberation of heat (called the latent heat L of the transition) and a reduction in cross-sectional area per lipid. The change in energy (heat) is balanced by the change in entropy between the high-entropy fluid phase (where the chains are free to move) and the low-entropy gel phase (where the chains are frozen). The equilibrium phase transition occurs at a temperature T_o where

$$T_o \Delta S = L$$

where ΔS is the change in entropy between the two phases.

When cells, liposomes, or other membranous systems are dehydrated, bulk water is removed, and osmotic contraction occurs. When the hydration is below ~0.2 gH_2O/dry weight (g) of samples (gDW), membranes and other components are brought into close proximity. When the distance between the membranes is reduced to the order of 1 nm, the strong hydration repulsion opposes further removal of water (Rand and Parsegian, 1989). This generates a suction in the water phase between the membranes, which induces a lateral compressive stress π in the plane of the membrane (i.e., $\pi/2$ in the plane of each monolayer, in the case of bilayers) (Wolfe, 1987).

Because the area per lipid in the gel phase is smaller than in the fluid phase, a sufficiently large compressive stress can cause the membrane to undergo the transition to the gel phase at temperatures higher than T_o. This effect can be described (to first order) by the two-dimensional equivalent of the Clausius-Clapeyron equation (Bryant and Wolfe, 1992):

$$\Delta T = \frac{T_o \Delta a}{2L} \pi \quad (1)$$

where ΔT is the change in the transition temperature associated with a total lateral stress π, and $\Delta a = (a_f - a_g)$ is the difference in area per lipid between the fluid (f) and gel (g) phases.

Using Eq. 1, for a given lateral stress the increase in the membrane transition temperature can be calculated. For typical values of these parameters for phospholipids, this equation predicts that the transition temperature will rise by ~0.5° for each mN/m of applied lateral stress (Wolfe and Bryant, 1999).

Elastic properties of solids

When a force is applied to one face of an isotropic solid, the stress is given by (e.g., Jastrzebski, 1987)

$$\sigma = F/A$$

where F is the applied force, and A is the area over which the force is applied. The response of the solid to this stress is called the strain ϵ, which measures the change in length, Δl, relative to the original length l_o:

$$\varepsilon = \Delta l / l_o .$$

The ratio of the stress to the strain is a property of the material known as Young's modulus (Y):

$$Y = \sigma/\varepsilon$$

If the length of an object changes in response to an applied stress, then the other dimensions will also change (e.g., an object that is stretched will become thinner). The amount of that change is determined by Poisson's ratio μ, which is the ratio of the fractional change in diameter (d) to the fractional change in length:

$$\mu = \frac{\Delta d / d_o}{\Delta l / l_o}$$

Ultrasound

In a solid, sound waves can travel either as longitudinal waves or transverse (shear) waves, with velocities v_l and v_t, respectively. The velocities in an isotropic solid are related to the density ρ and the elastic properties Y and σ. The relevant equations are (e.g., Ensminger, 1988)

$$v_l = \left\{ \frac{Y(1-\mu)}{\rho(1+\mu)(1-2\mu)} \right\}^{1/2} \qquad v_t = \left\{ \frac{Y}{2\rho(1+\mu)} \right\}^{1/2}$$

Thus, if one can measure v_l, v_t, and ρ for a solid, Young's modulus and Poisson's ratio can be calculated. In practice, the velocities are measured by generating the appropriate wave in a solid, measuring the time taken for the wave to travel through a known distance of the sample, then calculating the velocity.

By knowing Young's modulus, we can determine if the sugar-glass is rigid enough to hinder the gel-to-fluid phase transition in the membrane, and therefore account for the observed change in T_m in the presence of a vitrified sugar.

MATERIALS AND METHODS

Calorimetry

DSC was used to measure the thermal properties of mixtures of PC, water, and either sugars or larger polymers. Samples were prepared using several molecular species of PC mixed with a variety of aqueous sugar solutions. The PC molecular species used in this study had the following acyl chain compositions: 1,2-dipalmitoyl-phosphatidylcholine (DPPC) = 16:0/16:0, 1,2-dimyristoyl-phosphatidylcholine (DMPC) = 14:0/14:0, 1-steroyl-2-oleoyl-phosphatidylcholine (SOPC) = 18:0/18:1, 1-oleoyl-2-palmitoyl-phosphatidylcholine (OPPC) = 18:1/16:0, POPC = 16:0/18:1, and 1,2-dioleoyl-phosphatidylcholine (DOPC) = 18:1/18:1. Lipids were obtained from Avanti Polar Lipids (Birmingham, AL); these were found to be pure by thin-layer chromatography and were used without further purification. The sugars used (Sigma Chemical Co., St. Louis, MO) included glucose, trehalose, and a mixture of sucrose and raffinose (85% sucrose and 15% raffinose by weight). The latter is modeled on sugars found in desiccation-

tolerant maize embryos (Koster and Leopold, 1988). The polymers dextran and polyvinylpyrrolidone (PVP) were obtained from Sigma and had average molecular weights of 40,000.

The sample preparation procedure has been described before (Koster et al., 1994, 1996). In brief, lipid in chloroform was dried under a stream of N_2, then resuspended in a solution containing the appropriate sugar or polymer dissolved in water:methanol (1:1, v/v). Samples containing lipid/sugar mixtures had a 2:1 sugar:lipid weight ratio, and samples containing lipid/polymer mixtures had a 3:1 polymer:POPC weight ratio. The resultant suspensions were vortex-mixed to disperse the lipid and solutes, then the samples were dried in vacuo at 60°C to remove the methanol. The dry lipid-solute mixtures were resuspended in purified water (Nanopure; Barnstead, Dubuque, IA) and vortex-mixed. Control samples containing only lipid or polymer were prepared using the same procedure.

The samples were then loaded into preweighed DSC volatile-sample pans as previously described (Koster et al., 1994, 1996). Samples in excess water were sealed immediately after loading into the pans. To obtain a range of sample hydrations, DSC pans were incubated at 28°C above saturated salt solutions that generate known relative vapor pressures (Rockland, 1960). Low water vapor pressures dehydrated the samples; the osmotic pressures (Π) within the samples at equilibrium were calculated using the following equation (Nobel, 1983), and assuming that the partial molar volume of water (V_w) does not change at low hydrations:

$$\Pi = -\frac{RT}{V_w}\ln\left(\frac{RH}{100}\right)$$

RH refers to the percentage relative humidity above the saturated salt solutions (Rockland, 1960). The salts used in these experiments and the osmotic pressures they generate at 28°C are KNO_3 = 11 MPa, NaCl = 40 MPa, NH_4NO_3 = 65 MPa, $Mg(NO_3)_2$ = 88 MPa, K_2CO_3 = 117 MPa, $MgCl_2$ = 156 MPa, LiCl = 283 MPa, and KOH = 351 MPa. After incubation for 1 week at the designated relative vapor pressures, the pans were sealed and reweighed before calorimetry. Dry weights were obtained after calorimetry by puncturing the lids and drying the samples for at least 16 h at 70°C over P_2O_5 in vacuo.

Calorimetry was carried out using a DSC-7 (Perkin-Elmer, Norwalk, CT) with liquid N_2 cooling. Sealed samples were loaded at 25°C, cooled at a nominal rate of −200°/min to −100°C, then scanned while heating at 20°/min to 120°C. Multiple scans of the same sample, if necessary, were carried out using the same protocol. Samples with nominal hydrations of 0.0 gH_2O/gDW were scanned after drying in vacuo at 70°C over P_2O_5. Data obtained from the heating scans were analyzed using the software provided by Perkin-Elmer for the model 1020 controller. T_m represents the temperature of the peak maximum for the lipid gel-to-fluid phase transition, T_o represents T_m of the lipid at full hydration, and T_g represents the midpoint temperature of the glass-melting endotherm.

In a previous paper (Koster et al., 1994), a number of different scan rates (between 0.5°/min and 20°/min) were used. During warming scans, the higher scan rates overestimated the transition temperatures by ~3° because of thermal disequilibrium in the calorimeter. However, as the glass transition is difficult to detect at slower scan rates, 20°/min was used here. Because the transition temperatures were all measured under the same conditions, comparison among different samples is not affected by the scan rate.

Ultrasound

The sample used for ultrasound measurements consisted of a mixture of sucrose and raffinose (85:15 by weight), at a hydration of 0.11 gH_2O/gDW. Macroscopic (1–2-cm-thick) samples were prepared by heating the concentrated sugar-water mixture until all of the sugar had dissolved, then cooling it in a freezer at −15°C. The resulting glass was stable up to ~0°C. Measurements were made at approximately −10°C. Attempts to make similar macroscopic samples of glass from pure sucrose were unsuccessful because of its tendency to crystallize.

Ultrasound measurements were made using a DIA sonograph medical ultrasound probe (Nuclear Enterprises Limited) at 2.5, 3.3, and 5 MHz. To check the methodology, measurements of other materials (e.g., ice, steel, aluminum) were made. The results of these measurements compared favorably with values in the literature.

Because ultrasound waves are absorbed very quickly in air, there must be good coupling between the transducer and the sample, which is normally achieved by using a coupling medium (such as oil) between the transducer and the sample. For these samples this was not possible, so coupling was achieved by placing a steel block in contact with the solution before vitrification. After the sample had cooled and vitrified, the metal was in good contact with the sample. The sound waves were generated perpendicular to the surface of the solid (using oil as the coupling medium), and 180° reflections were observed from both the steel and sample surfaces. By varying the width of the steel plate, a sample could be made so that clear reflections were observed from both the metal and the bottom of the glass. Using the thickness of the sample, the speed of sound could be calculated for the material, and Young's modulus could be estimated.

RESULTS

Figs. 1–5 show transition temperatures as a function of hydration for a range of different sugar/PC combinations. In each graph the main-chain melting transition temperature, T_m, of the lipid without sugar (*open squares*), the T_m of the lipid in the presence of sugar (*open diamonds*), and the midpoint glass transition temperature, T_g, of the sugar matrix (*filled circles*) are shown. Samples dried in vacuo at 70°C to achieve a nominal hydration of 0.0 gH_2O/gDW are represented by open inverted triangles (lipid T_m) and filled inverted triangles (T_g) (Figs. 1 and 2). The figures are shown in order of decreasing value of T_o, the lipid gel-to-fluid phase transition temperature in excess water (see Table 1). Each of the figures shows results for lipids mixed with (*a*) trehalose and (*b*) sucrose/raffinose. Figs. 1, 2, and 4 also show results for lipid mixed with glucose (*c*).

Glass-melting endotherms were detected in most of the sugar-containing samples. In samples containing excess water, T_g corresponded to the T_g of the freeze-concentrated sugar solution (Levine and Slade, 1988), between −25° and −45°C for the sugars used in these experiments. Ice-melting endotherms were also observed in these samples. In samples that had no ice-melting endotherm, values of T_g increased with decreasing sample hydration (Figs. 1–5, *filled symbols*). For trehalose, T_g in oven-dried samples (at a nominal hydration of 0.0 gH_2O/gDW) attained values between 82° and 88°C (Figs. 1 *a* and 2 *a*). For the sucrose-raffinose mix, T_g in oven-dried samples was 55–60°C (Figs. 1 *b* and 2 *b*). For glucose, T_g in oven-dried samples ranged between 34° and 36°C (Figs. 1 *c* and 2 *c*). These data are in agreement with previously published values for T_g of these dehydrated sugars (Levine and Slade, 1992; Koster et al., 1996). The trehalose values measured here are lower than the reported T_g of anhydrous trehalose, 114°C (L. M. Crowe et al., 1996), and probably reflect the absorption of water

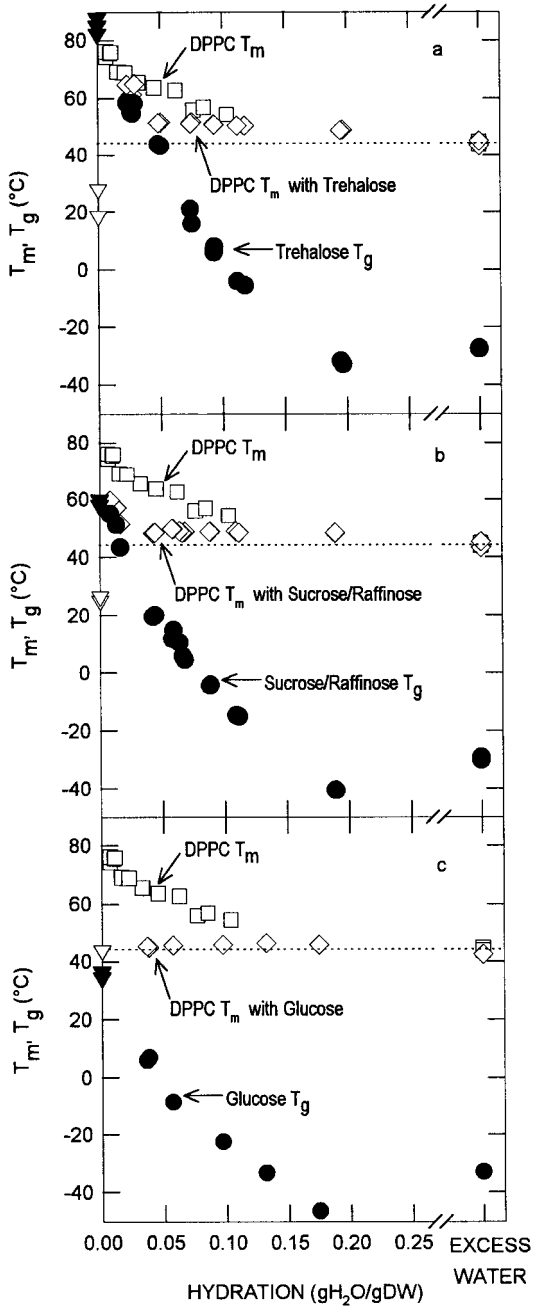

FIGURE 1 Transition temperatures as a function of hydration for DPPC and (*a*) trehalose, (*b*) sucrose/raffinose, and (*c*) glucose. For all figures: □, T_m of the lipid in the absence of sugar; ◇, T_m of the lipid in the presence of sugar; ●, midpoint T_g of the sugar-glass transition. The dotted line designates T_o, the lipid gel-to-fluid phase transition temperature at full hydration. Hydration values were calculated based on dry weights obtained after samples were dried at 70°C in vacuo with P_2O_5. Points at 0.0 gH_2O/gDW (▼, T_g; ▽, T_m) were taken from samples dried at 70°C in vacuo before calorimetry.

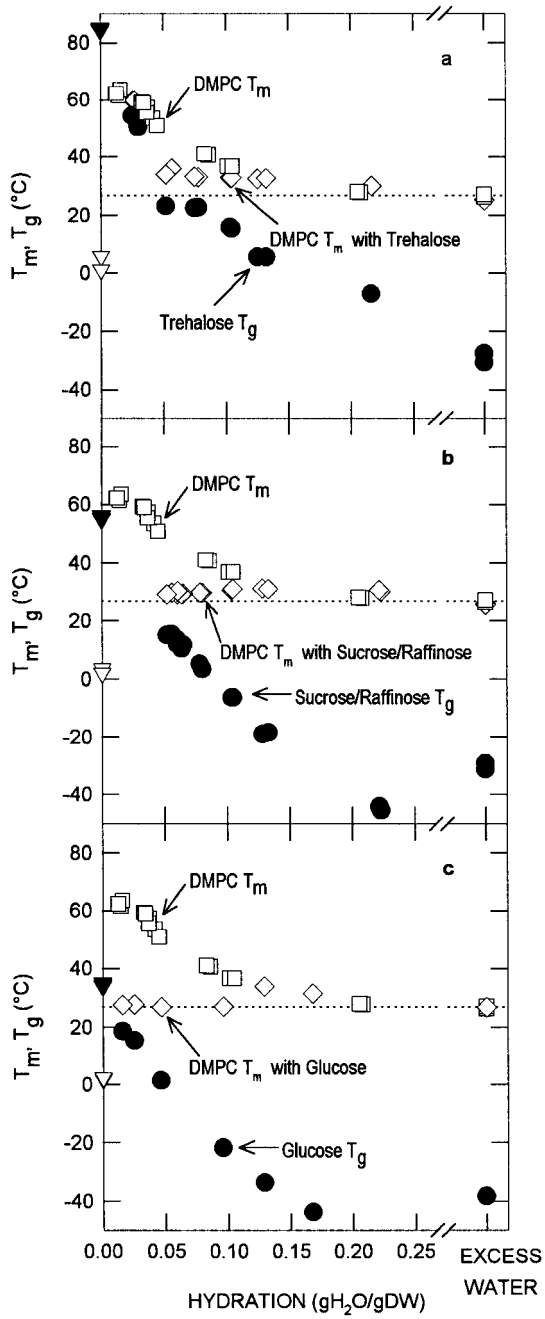

FIGURE 2 Transition temperatures as a function of hydration for DMPC and (*a*) trehalose, (*b*) sucrose/raffinose, and (*c*) glucose. For all figures: □, T_m of the lipid in the absence of sugar; ◇, T_m of the lipid in the presence of sugar; ●, midpoint T_g of the sugar-glass transition. The dotted line designates T_o, the lipid gel-to-fluid phase transition temperature at full hydration. Hydration values were calculated based on dry weights obtained after samples were dried at 70°C in vacuo with P_2O_5. Points at 0.0 gH_2O/gDW (▼, T_g; ▽, T_m) were taken from samples dried at 70°C in vacuo before calorimetry.

vapor by the samples after their removal from the vacuum oven.

For all PC molecular species tested, T_m rose as the pure lipid was progressively dried (Figs. 1–5, *open squares*), as has previously been reported for both saturated and unsaturated species of PC (Chapman et al., 1967; Lynch and Steponkus, 1989; Collins et al., 1990; Webb et al., 1993; Koster et al., 1994; Ulrich et al., 1994). T_m of the pure lipids

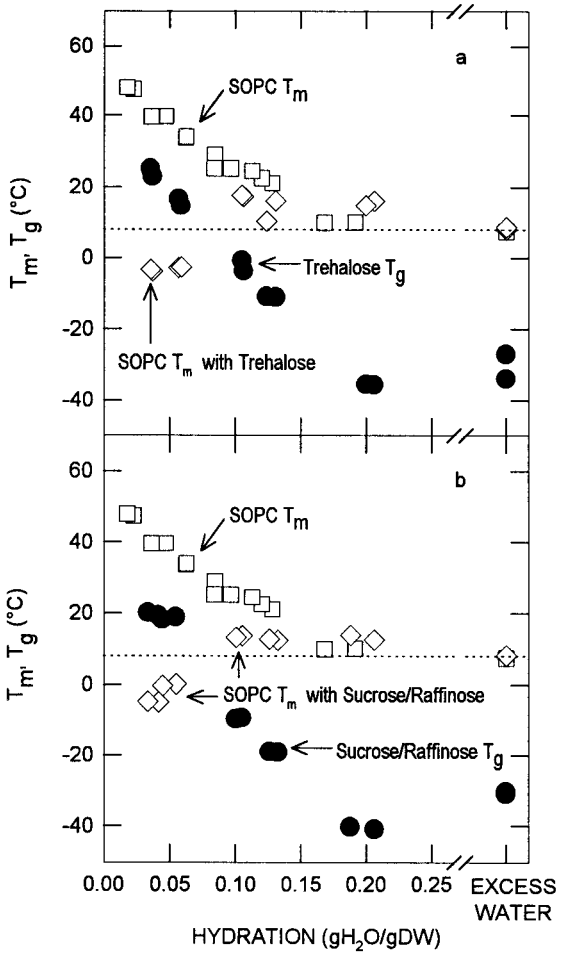

FIGURE 3 Transition temperatures as a function of hydration for SOPC and (*a*) trehalose and (*b*) sucrose/raffinose. For all figures: □, T_m of the lipid in the absence of sugar; ◇, T_m of the lipid in the presence of sugar; ●, midpoint T_g of the sugar-glass transition. The dotted line designates T_o, the lipid gel-to-fluid phase transition temperature at full hydration. Hydration values were calculated based on dry weights obtained after samples were dried at 70°C in vacuo with P_2O_5.

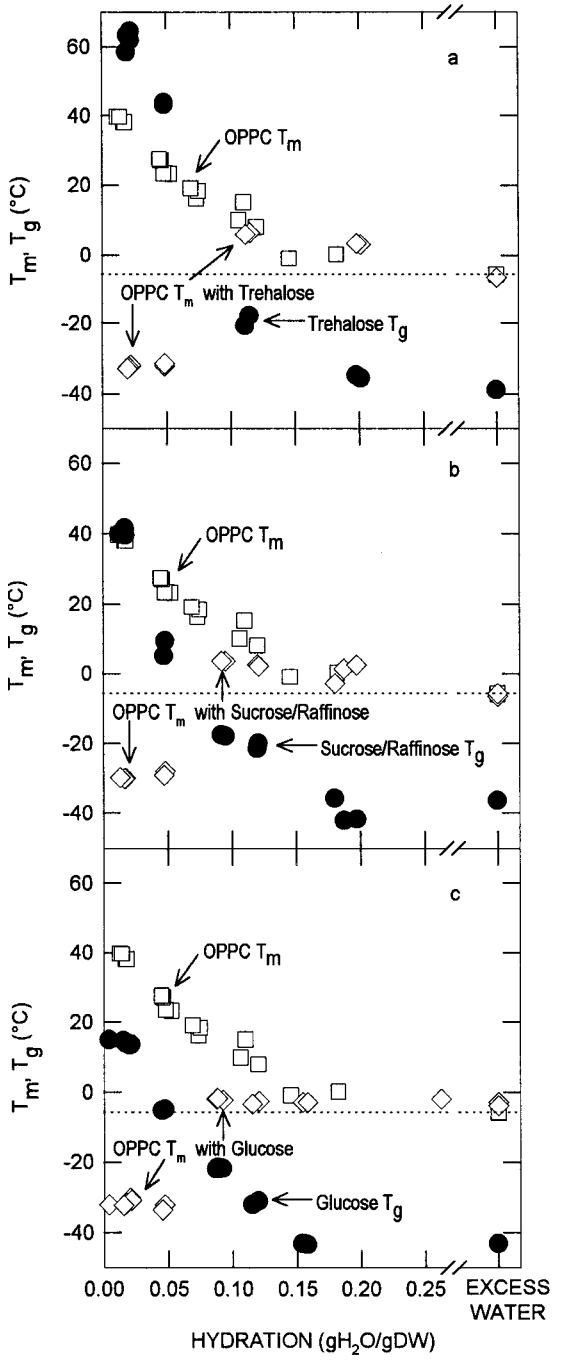

FIGURE 4 Transition temperatures as a function of hydration for OPPC and (*a*) trehalose, (*b*) sucrose/raffinose, and (*c*) glucose. For all figures: □, T_m of the lipid in the absence of sugar; ◇, T_m of the lipid in the presence of sugar; ●, midpoint T_g of the sugar-glass transition. The dotted line designates T_o, the lipid gel-to-fluid phase transition temperature at full hydration. Hydration values were calculated based on dry weights obtained after samples were dried at 70°C in vacuo with P_2O_5.

was not significantly affected by dehydration until sample water contents reached values less than 0.2 gH_2O/gDW (about seven to nine water molecules per lipid, or an interbilayer separation of ~0.8–1 nm); below this value, T_m increased with dehydration (Figs. 1–5, *open squares*).

PCs dried in the presence of sugars displayed more complex phase behavior that varied according to the state of the sugars and the thermal history of the sample. When the PCs were dried in the presence of sugars, T_m increased by 0–12° for samples in which $T_g < T_o$ (Figs. 1–5, *open diamonds*). The extent of this dehydration-induced elevation in T_m was largest for lipids dried with trehalose, followed by the sucrose/raffinose mixture, then glucose, which showed an increase of less than ~3° for all samples. The observed increases are in all cases considerably less than those observed for the pure lipid at the same sample water content (Figs. 1–5, compare *open diamonds* to *open squares*).

Three of the lipids (SOPC, OPPC, DOPC) were always in the fluid phase during drying at 28°C. When the samples were at sufficiently low hydrations that T_g of the sugars was above T_o, the measured transition temperature T_m of the

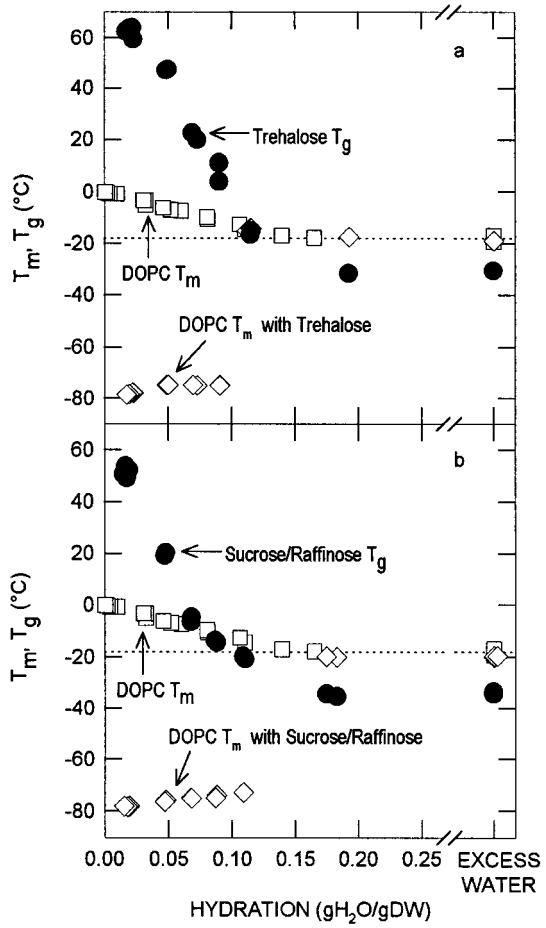

FIGURE 5 Transition temperatures as a function of hydration for DOPC and (*a*) trehalose and (*b*) sucrose/raffinose. For all figures: □, T_m of the lipid in the absence of sugar; ◇, T_m of the lipid in the presence of sugar; ●, midpoint T_g of the sugar-glass transition. The dotted line designates T_o, the lipid gel-to-fluid phase transition temperature at full hydration. Hydration values were calculated based on dry weights obtained after samples were dried at 70°C in vacuo with P_2O_5.

lipids was lowered dramatically below T_o, so that $\Delta T = (T_m - T_o)$ was in the range $-12°$ to $-55°$ (Figs. 3-5 and Table 1). In other words, when the sugars vitrified near or between fluid phase bilayers, the lipid transition from the fluid to the gel phase was deferred to a lower temperature. The extent of the vitrification-induced depression of T_m below T_o varied with the acyl chain composition of the PC (Table 1). The lowered T_m in these vitrified samples was reproducible and showed minimal hysteresis between cooling and heating scans, in agreement with previous results for POPC (Koster et al., 1994). Typically, the lipid phase transitions measured in the presence of vitrified sugars occurred over a broader temperature range (~20°) and had a decreased transition enthalpy when compared to the gel-to-fluid transitions measured in nonvitrified samples. As previously reported for POPC (Koster et al., 1994), samples in which T_g was approximately equal to T_o had complex thermograms that were impossible to unambiguously interpret. Data from these scans are not shown in the figures.

In the case of DPPC (Fig. 1), the samples were in the gel phase during dehydration, and the initial scan for samples in which $T_g > T_o$ was different from the second and subsequent scans. DMPC dried with trehalose at 28°C entered the gel phase when dried to hydrations less than ~0.1 gH_2O/gDW because dehydration caused T_m to increase to 30°C (Fig. 2 *a*). For these few samples, just as for DPPC, the initial scan for samples in which $T_g > T_o$ differed from subsequent scans. The graphs for DPPC and DMPC (Figs. 1 and 2) show data obtained from the first heating scan (the second and subsequent scans will be discussed later). At hydrations where the T_g of the sugars was lower than the T_o of the lipids, T_m increased to a small extent with dehydration, just as it did for the other lipids. However, when samples were dehydrated sufficiently that T_g was above T_o, the T_m of the lipids encased in the vitrified sugars was increased to temperatures just above T_g (Figs. 1, *a* and *b*, and 2 *a*). During subsequent cooling of the samples, the sugars vitrified around fluid phase lipids, and T_m was depressed below T_o, to ~25.6°C for DPPC and 5.7°C for DMPC. This is similar to the behavior reported by others for DPPC (Crowe and Crowe, 1988; J. H. Crowe et al., 1996).

To confirm that the behavior of the lipid in vitrified sugars depended on whether the lipid was in the fluid or gel phase during dehydration, DPPC was dehydrated with trehalose over $CaSO_4$ at 50°C, so the lipid was in the fluid phase during drying. This preparation attained a water content of 0.01 gH_2O/gDW. During the first and subsequent heating scans, T_g was found to be 74°C, which is well above T_o for DPPC, and T_m was 25°C, equal to the value found in the second scan for samples prepared at 28°C. Further confirmation comes from the samples of DPPC and DMPC that were oven-dried at 70°C and, hence, were in the fluid phase during drying (Figs. 1 and 2, *inverted triangles*). With the exception of DPPC dried with glucose, T_g was above T_o for all samples, and T_m was depressed below T_o on the initial scan.

As a further test, and given the proximity of the T_o of DMPC (26°C) to the drying temperature of 28°C, samples of DMPC with trehalose were dried at 20°C, a temperature at which this lipid is clearly in the gel phase, to several water contents less than 0.04 gH_2O/gDW. When these samples were scanned in the calorimeter, T_g was found to be above T_o for all samples, and T_m was elevated to ~60°C in the first heating scan. In all subsequent heating scans, after trehalose had vitrified around the fluid phase lipid during cooling, T_m was lowered to ~6.6°C, a value consistent with the samples that were dehydrated in the fluid phase.

Fig. 6 shows the effects of the polymers (Fig. 6 *a*) dextran and (Fig. 6 *b*) PVP on the T_m of POPC. As for the smaller sugars, the glass transition temperature T_g rose with decreasing hydration. Unlike the small sugars, however, the presence of the polymer did not prevent the increase in T_m

TABLE 1 Measured and calculated parameters for vitrified PC-sugar mixtures

Lipid	T_o (°C)	n	T_m (glass)	n	ΔT_m	Error ΔT_m	L (±3) (kJ/mol)	Δa (nm^2)	Error Δa	$-\pi$ (mN/m)	Error π
DPPC	44.2	10	25.6	13	−18.6	4.0	35	0.15	0.03	45	23
DMPC	26.0	6	5.7	11	−20.3	3.2	24	0.12	0.03	45	24
SOPC	8.1	5	−4.2	4	−12.3	1.2	25	0.11	0.05	32	22
POPC	−2.6	10	−24.4	28	−21.8	1.9	21	0.17	0.05	33	17
OPPC	−6.0	3	−30.7	12	−24.7	2.0	19	0.17	0.05	34	18
DOPC	−18.8	5	−76.0	20	−57.2	2.8	33	0.19	0.05	(130)	(53)

T_m(glass), gel-fluid transition temperature in the presence of sugar glass; T_o, gel-fluid transition temperature in excess water; n, number of samples; ΔT_m, T_m(glass) − T_o; L, latent heat of gel-fluid transition in excess water; Δa, difference in lipid cross-sectional area between the fluid and gel phases; π, lateral stress in the bilayer. The error in ΔT_m was calculated from the standard deviations of T_o and T_m(glass) for all samples. L is estimated from the range of values found in the LIPIDAT data base (Caffrey, 1993). The error was estimated at ±3 kJ/mol. Data for Δa are scarce, and the values in the table have been estimated from various measurements made by a number of authors (Lis et al., 1982; Rand and Parsegian, 1989; De Young and Dill, 1988; Nagle et al., 1996; Janiak et al., 1976, 1979; and references contained in these papers). Good estimates can be made for only two lipids, DPPC and DMPC. For the other lipids the estimates are less reliable and a nominal uncertainty in Δa of 0.05 nm^2 has been assigned for calculation purposes.

observed for the pure lipid. Instead, for all sample hydrations less than excess water, T_m of POPC in the presence of the polymer was actually greater than T_m for the pure lipid (Fig. 6). Vitrification of the polymers also had no effect on the phase behavior of the lipid, in agreement with previous work (J. H. Crowe et al., 1996). This will be discussed in detail below.

To help us understand the effect of the vitrified sugars on the lipid phase transitions, ultrasound was used to measure the elastic properties of a glass formed of sucrose and raffinose (85:15, w/w, 0.11 gH_2O/gDW). The longitudinal velocity in the sugar-glass was found to be 3500 ± 400 m/s, and the glass had a density of 1.6 ± 0.1 g/cm^3. Because of the high sound absorption of the glass, no accurate measurement of the transverse wave could be made. However, a good estimate of Young's modulus (the important parameter for our purposes) can still be made. For an isotropic material Poisson's ratio lies between the values of 0 and 0.5; $\mu = 0$ means that the thickness does not change when the length changes, and $\mu = 0.5$ implies that the change in width is half the change in length. Neither of these is physically plausible. Most materials lie in the range between $\mu = 0.15$ (e.g., concrete) and $\mu = 0.43$ (e.g., hard rubber); values of typical materials are 0.365 (ice), 0.23 (soda glass), and 0.4 (nylon) (e.g., Jastrzebski, 1987). With these values, the upper and lower bounds of Young's modulus can be established. This leads to a value for Young's modulus of $Y = 15$ GPa, with an error of about ±10 GPa, which is sufficiently accurate for our analysis (see below). This compares, for example, with a value of 9 GPa for ice (Hobbs, 1974).

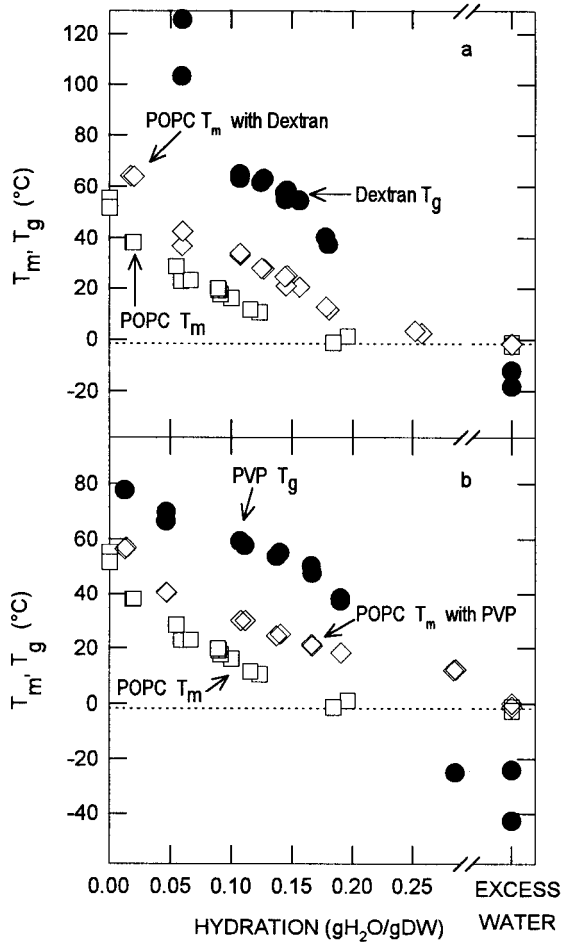

FIGURE 6 Transition temperatures of POPC and large polymers after dehydration. □, T_m of the lipid in the absence of the polymer; ◇, T_m of the lipid in the presence of polymer; ●, midpoint T_g of the polymer glass transition. The dotted line designates T_o, the lipid gel-to-fluid phase transition temperature at full hydration. Hydration values were calculated based on dry weights obtained after samples were dried at 70°C in vacuo with P_2O_5.

ANALYSIS AND DISCUSSION

There are three important effects demonstrated in the experimental results. We discuss each of them in turn, together with an analysis of the mechanisms.

Reduction in the dehydration-induced increase in T_m

The first effect is the reduction in the dehydration-induced increase of the lipid T_m in the presence of small solutes. Figs. 1–5 show that each of the three sugar mixtures tested achieved this. This can be explained by the application of a simple physical model (Bryant and Wolfe, 1992; Wolfe and Bryant, 1999). In brief, if there is no solute present, dehydrated membranes are brought into close proximity to each other, where the hydration repulsion induces a compressive stress in the membrane, which makes the gel phase energetically favorable at higher temperatures. The two-dimensional Clausius-Clapeyron equation (Eq. 1) can be used to estimate the increase in T_m as a function of dehydration.

If a sample containing small solutes is dehydrated, the osmotic and volumetric effects of the solutes mean that the distance between membranes is larger, and the inter- and intramembrane stresses smaller, than they would be if the solute were not present (for details see Bryant and Wolfe, 1992; Wolfe and Bryant, 1999). The lower lateral stress in the membrane implies that the dehydration-induced increase in T_m will be less than it would be in the absence of solutes, as modeled by Eq. 1.

According to this model the effectiveness of solutes in reducing the dehydration-induced increase in T_m is a combination of their osmotic properties (roughly related to the number of molecules) and their volumetric properties (how large the molecules are). The fact that glucose was the most effective at reducing T_m for these samples is consistent with this model. The samples were all made with a sugar:lipid weight ratio of 2:1. This translates to sugar:lipid molar ratios of ~4.4:1 (trehalose), ~4:1 (sucrose/raffinose mixture), and ~8:1 (glucose). The glucose samples had twice the number of sugar molecules per lipid, and so at a given hydration, the osmotic effects will be larger for glucose than for the other samples. This is evident in Figs. 1, 2, and 4, where the presence of glucose caused T_m to be roughly equal to T_o (within 3°) for all hydrations where vitrification did not occur. A simple application of the physical model described by Wolfe and Bryant (1991) predicts virtually no temperature increase under these conditions. The small discrepancy is due to the approximations inherent in the model. This is discussed in detail elsewhere (Koster et al., 1994).

For the samples containing trehalose or sucrose/raffinose, the lipid T_m increased as a function of dehydration, but the increase was considerably less than in the absence of solutes. The osmotic effects for trehalose and sucrose/raffinose are roughly the same, given that they have almost the same solute/lipid ratio. However, the sucrose/raffinose mixture reduced T_m more than trehalose did. The presence of raffinose is crucial here—it is considerably larger than those of trehalose and sucrose, and its volumetric properties become critical. Each unhydrated raffinose molecule has a volume equal to ~30 water molecules (compared to ~18 for trehalose and sucrose) (Carpita et al., 1979). The presence of raffinose between neighboring membranes limits how close the membranes can come and therefore how large the stress in the membranes becomes. The fact that in the mixture used here (sucrose:raffinose = 85:15, w/w) there is only about one raffinose molecule for every 2.5 lipids moderates this effect somewhat.

The reduction in T_m in the presence of solutes is only significant if the solutes remain in the region between the bilayers. If the solutes are large enough (e.g., polymers), then they will be excluded from these interbilayer regions (LeNeveu et al., 1976; Rand and Parsegian, 1989; Wolfe and Bryant, 1999), and the effects are quite different (see below). The fact that the effect of the sucrose:raffinose mixture is stronger than for trehalose indicates that the raffinose molecules are not strongly excluded from between the membranes, as large polymers would be (see below).

Note that this analysis does not exclude the possibility of specific hydrogen bonding between the lipids and sugars. If such hydrogen bonding did occur to a substantial degree, then it would have a number of effects, such as modifying the hydration force between bilayers (and hence membrane stress) and altering the values of L and Δa in the Clausius-Clapeyron equation. The extent of such hydrogen bonding and the degree to which it may vary among different sugars are, however, very difficult to quantify.

Depression of T_m below T_o as a result of vitrification

All of the vitrified sugars tested in these experiments had the ability to lower the gel-fluid transition temperature below T_o, the transition temperature for the fully hydrated lipid. Trehalose and the sucrose/raffinose mixture, because of their higher molecular weights, were able to vitrify at higher water contents and, thus, lowered the T_m of all of the lipids tested (Figs. 1–5). Glucose, with its lower molecular weight, never had a T_g greater than 36°C; therefore, its T_g was never above the T_o of DPPC, and vitrification of glucose had no effect on the phase behavior of this lipid (Fig. 1 *c*). Vitrified glucose, however, was able to lower the T_m of other lipids with which it was tested, namely DMPC (Fig. 2 *c*) and OPPC (Fig. 4 *c*). For DMPC, the T_g of glucose was only above T_o in the samples that had been dried at 70°C in vacuo with P_2O_5 to achieve the lowest possible water contents, so the observed depression of the T_m of DMPC only occurred in the samples at the nominal hydration of 0.0 gH_2O/gDW (Fig. 2 *c*). For OPPC, however, the T_g for glucose was above the T_o of the lipid for water contents of 0.05 gH_2O/gDW and below, so the depression of the lipid T_m was observed at several hydrations (Fig. 4 *c*).

The qualitative effects seen here are not specific to any particular sugar or lipid acyl chain composition. If $T_g < T_o$, then vitrification of the sugar has no effect on the lipid T_m. If $T_g > T_o$, and vitrification occurs when the lipids are in the

fluid phase, then the presence of the glass depresses the fluid-to-gel phase transition temperature by an amount that is chain length dependent. However, this depression is not significantly dependent on the sugar used. Where depression occurred, the variation among the three sugars used was $\pm 2°$.

Thus, for the sugars and lipids tested, the sugars depressed T_m below T_o when T_g was greater than T_o. This is consistent with the observations of Zhang and Steponkus (1996), who also found that depression of T_m below T_o only occurs if the lipid is in the fluid phase when the interlamellar layer vitrifies. If the membrane is in the gel phase when the glass is formed, T_m is elevated above T_o, as seen here for DPPC (Fig. 1, *a* and *b*) and DMPC (Fig. 2 *a*). Note that we use T_o as an unambiguous reference point. However, it may not be T_o that is the critical temperature, but the extrapolated T_m of the lipid-sugar mixture at that hydration. Because these two temperatures are within several degrees of each other in all cases, and the transitions are broad, we cannot distinguish between these two possibilities without further experimentation. Crowe and Crowe (1988) have observed similar effects—they found that if DPPC/trehalose mixtures were dehydrated in the gel phase, then T_m was raised to ~60°C on the initial scan but was depressed to 24°C on subsequent scans.

Zhang and Steponkus (1996) proposed the following mechanism to explain this effect, which has been more fully elaborated by Wolfe and Bryant (1999). A glass (being a solid) can support a substantial anisotropic stress. The presence of the glassy matrix impedes the conformational change associated with the lipid phase transition. Consider the case where the lipids are in the gel phase when vitrification occurs. As the temperature rises the lipids tend to expand in area, and the area change is large when going from the gel to the fluid phase. As the temperature is raised above T_o, the presence of the glass impedes the transition to the fluid phase—because the glass is a solid it resists the lipid expansion. The glass exerts a compressive stress on the lipids. Because of Newton's laws, the compressive stress in the lipids must be balanced by a tensile stress in the glass. As the temperature is raised further, a point is reached where the tendency of the lipid to expand is sufficient to overcome the presence of the glass, and the gel-fluid transition can occur. T_m is thus elevated above T_o (see Eq. 1). If, on the other hand, the lipids are in the fluid phase when vitrification occurs, cooling below T_o creates a tensile stress in the membranes and a compressive stress in the glass, and T_m will be depressed. To summarize, the effect of intermembrane vitrification is to tend to keep the membrane lipids in the phase they were in when the intermembrane solution vitrified.

We can use the Clausius-Clapeyron equation to analyze the data in a systematic way. From the data, $\Delta T/T_o$ can be estimated (see Table 1). The measurement of Young's modulus gave a value of $Y = 15 \pm 10$ GPa for a typical sugar-glass. The value is likely to be similar (within the errors) for sugar-glasses composed of different sugars. However, Young's modulus is a function of temperature, which we discuss later.

To compare quantitatively with theory, we need to accurately know the difference in cross-sectional area per lipid molecule between the gel and fluid phases. The most accurately known area change is that for DPPC, where $\Delta a \approx 0.15$ nm^2 (Nagle, 1993; Nagle et al., 1996). If we use $T_o = 44.2$°C (Table 1) and $L \approx 35$ kJ/mol (value averaged from values found in the LIPIDAT database; Caffrey, 1993), then from Eq. 1, $\pi/\Delta T \approx 2.4$ mN/K. If the glass were to support the stress of a membrane down to 18.6° below T_o (i.e., $\Delta T = -18.6°$), this would correspond to a lateral stress of $\pi \approx 45 \pm 23$ mN/m (see Table 1 for a discussion of the errors). If this lateral stress were supported over half the thickness of the interlamellar separation (on the order of ~0.5 nm), this would lead to a stress of 90 MPa on the intermembrane layer. For $Y = 15$ GPa this represents a strain in the glass of ~0.6%. To determine the maximum strain, we take the lower bound of the estimate of Young's modulus ($Y = 5$ GPa), which yields a strain in the glass of ~1.8%. This level of strain can easily be supported by a solid.

We can now extend this analysis to the other lipids. As can be seen from Table 1, the ΔT values for four of the lipids are in reasonable agreement (DPPC, DMPC, OPPC, POPC), between $-18.6°$ and $-24.7°$. SOPC ($\Delta T = -12.3°$) and DOPC ($\Delta T = -57.2°$) are significantly different (note: data for POPC are from Koster et al., 1994). How can these different numbers be reconciled? The model suggests that when the stress in the glass reaches some critical value (e.g., the 90 MPa calculated above for DPPC), then the transition will occur. If we assume that Young's modulus is the same for all glasses, and that the interlamellar separations are similar for all lipids when vitrification occurs, then the simple model suggests that (to first order) the transition will occur when the lateral stress in the membranes is the same. The calculated lateral stresses for the lipids studied are shown in Table 1. With the exception of DOPC, they lie in the range between 32 and 45 mN/m (Table 1). Thus, within the experimental uncertainties, these are in good agreement and provide good support for the simple model.

The calculated value of tensile stress for DOPC is much larger, ~130 mN/m. This large discrepancy is caused by a number of assumptions breaking down. First, we have assumed that Young's modulus is independent of temperature; however, Young's modulus normally increases with decreasing temperature. This variation can be large—for example, for aluminum, Y doubles as the temperature is reduced from 60°C to −15°C (Jastrzebski, 1987). For DOPC the transition occurs at a much lower temperature than for the other lipids, so one would expect Y to be significantly larger. If, for example, the value of Y were doubled in the calculation in Table 1, the calculated tensile stress would be

halved. Direct measurement of the temperature dependence of Young's modulus for relevant sugar-glasses is required before this analysis can be taken further.

Second, the analysis uses the two-dimensional Clausius-Clapeyron equation, which is an approximation that applies only for small changes in temperature. For the other lipids $\Delta T/T$ is on the order of 0.1 or less, so the equation may be reasonably used. For DOPC, however, $\Delta T/T = 0.22$, so it is unreasonable to expect the Clausius-Clapeyron equation to be quantitatively accurate. Finally, the analysis also assumes that the thickness of the intermembrane vitrified layer is the same in all cases, which is unlikely to be the case. Given the large uncertainties in some of the quantities used in the calculations, especially the area changes of the lipids, further refinements of this model are not warranted at this stage. More experiments are needed to refine the various parameters and provide a more stringent test of the model.

Despite these uncertainties, this analysis demonstrates that the depression of the transition temperature below the fully hydrated value T_o in the presence of a glassy matrix can be explained using the simple physical model proposed by Zhang and Steponkus (1996). The measurement of Young's modulus for a typical glass, together with the application of the Clausius-Clapeyron equation, demonstrates that the variation among different phosphatidylcholine acyl chain compositions and different vitrifying sugars can largely be accounted for by the model. One would expect that the elevation of T_m above T_o by vitrification near gel-phase lipids can also be explained by this model, though more experiments are needed to test this.

The fact that the depression in T_m is approximately constant whether the vitrifying sugar is trehalose, glucose, or a mixture of sucrose and raffinose (Figs. 1–5) demonstrates that the depression of T_m is, to first order, a nonspecific effect of the vitrifying sugar. The small differences among the sugars can be explained, for instance, by small differences in the elastic properties of the glasses. The only requirement is that the vitrification occurs between the lipid membranes. If the vitrifying solute is excluded, then vitrification will have no direct effect on the lipid T_m (see below).

Effect of polymers on T_m

Fig. 6 shows two important aspects of the effects of large polymers on the gel-to-fluid transition temperature. First, dehydration in the presence of large polymers increases T_m rather than decreasing it, and this effect is relatively small; second, vitrification of the polymers has no significant effect on lipid T_m.

In recent papers, Crowe and co-workers (J. H. Crowe et al., 1996, 1998; Oliver et al., 1998) also found that vitrification of large polymers has no significant effect on the T_m of a PC during drying, and they use this result to question the proposal by Koster et al. (1994) that vitrification of sugars such as trehalose can depress T_m below T_o. Crowe and co-workers argue that if vitrification is the cause of the depression of T_m, then the effect should also be apparent for polymers that have high glass transition temperatures. From the fact that this is not the case they infer that vitrification "clearly cannot account for depression of T_m" (Crowe et al., 1998). We examine this analysis here.

First, Koster et al. (1994) suggested that vitrification of the sugars studied was necessary to depress T_m below T_o, the transition temperature of the fully hydrated lipid. Crowe and co-workers misinterpreted that paper, claiming that it says that "the carbohydrate T_g must exceed the membrane T_m in order to prevent elevation of T_m" (Oliver et al., 1998). These authors appear to confuse two separate but related effects: 1) Small solutes diminish the elevation of T_m above T_o during drying. This effect was observed for all sugars tested by Koster et al. (1994) and occurs without vitrification. 2) When sugars vitrify near fluid-phase bilayers, T_m is lowered below T_o. The data reported by Crowe and coworkers (J. H. Crowe et al., 1996, 1998; Oliver et al., 1998) are consistent with these two effects. Each of these effects can be explained using simple physical models, but the mechanisms are different.

Second, Koster et al. (1994) made no claims about the effects of vitrifying polymers, only about the small sugars used in that study. The two cases are dissimilar, as we shall now discuss. For a solute to have any substantial direct effect on membrane phase properties, the solute must remain very near the membranes. When a membrane/small solute/water system is frozen or dehydrated, the water and solutes are not distributed uniformly. When frozen, for example, ice forms in bulk phases, and the intermembrane regions contain concentrated solutions, but no ice (Yoon et al., 1998). Similarly, when such a system is dehydrated, small solutes may remain between the membranes. However, if the solutes are large enough (e.g., large polymers), they will be excluded and sequestered in a bulk phase, as shown by the routine use of polymers to dehydrate membranes and liposomes (LeNeveu et al., 1976; Arnold and Gawrisch, 1993).

In the case of small solutes that remain in the interbilayer region during dehydration, vitrification will have a profound effect (as described above). Polymers excluded from interbilayer regions will have little direct effect on the bilayers, and vitrification of the polymer will have no direct effect on bilayer phase behavior. The presence of the excluded polymer in the sample will have indirect effects—for example, by changing the overall water chemical potential and further dehydrating the membranes, as seen in Fig. 6. The vitrification of a bulk polymer phase may also affect the equilibration of membrane-rich phases and so may have a small effect on measured transition temperatures.

The nonspecific osmotic and volumetric effects that cause the reduction in T_m in the presence of solutes (Wolfe

and Bryant, 1999) can only occur if the solutes remain between the membranes at close approach. Similarly, the proposal by Koster et al. (1994) that vitrification of sugars is the cause of the depression of T_m below T_o, and the model proposed by Zhang and Steponkus (1996) to explain this effect, refer only to cases in which vitrification occurs in the region between membranes. As Wolfe and Bryant (1999) have explicitly shown recently, large polymers do not fall into this category.

Because the polymers used in our experiments are very large (average MW = 40,000), they are excluded from the intermembrane regions at low hydrations. In excess water, the lipid transition temperatures measured were the same with or without the polymer present (Fig. 6). As the sample hydration was reduced, the membranes were brought into close proximity, excluding the polymers into macroscopic volumes. The polymers have an osmotic effect (they sequester some of the water), so at a particular sample hydration, the lipid hydration will be lower in the presence of the polymer than in a pure lipid sample. Thus the lipid with the polymer present was dehydrated and had a higher T_m than the pure lipid.

This can be clearly seen by looking at the data in another way—Figs. 7 and 8 show the data for POPC with dextran and PVP, respectively. Each of the graphs has osmotic pressure on the horizontal axis. In each case part *a* of the figure shows the hydration of POPC (gH_2O/g lipid) and the polymer (gH_2O/g polymer), part *b* shows the hydration of the mixture (gH_2O/gDW), and part *c* shows the lipid transition temperature with and without polymer. If the polymers are excluded from the interbilayer regions at low water content, then it should be possible to predict the water content of the mixed sample by knowing that of the pure samples. Using the data in Figs. 7 *a* and 8 *a*, and knowing that the samples consisted of a 3:1 weight ratio of polymer to lipid, the predicted sample hydration can be calculated. This is shown in Figs. 7 *b* and 8 *b*.

As can be seen, the predictions are in good agreement with the experimental hydrations. For the dextran/POPC mixtures (Fig. 7) the agreement is good over the whole hydration range, although there is some scatter. For PVP/POPC mixtures (Fig. 8), the agreement is excellent at high pressures (low hydration), but at lower pressures (higher hydrations) the simple calculation overestimates the hydration. This implies that at high hydrations the PVP molecules were not completely excluded. The average radii of gyration of both PVP and dextran with MW = 40,000 are calculated to be ~5–6 nm (Grulke et al., 1999). These data are consistent with the idea that the polymers are excluded from the interbilayer regions at low hydrations (where the interbilayer separation is 1 nm or less). In excess water the interbilayer separation for POPC is ~3 nm (estimated from data for DOPC and DPPC; Rand and Parsegian, 1989). Because the polymers are polydisperse, there will be a range of particle sizes, and many of them may be small enough to fit between the bilayers at high hydration. Detailed polydispersity data are not available for these samples, but it is likely that the PVP used was more polydisperse than the dextran used and had a larger number of smaller polymers that can fit between the bilayers at high hydration. Thus at

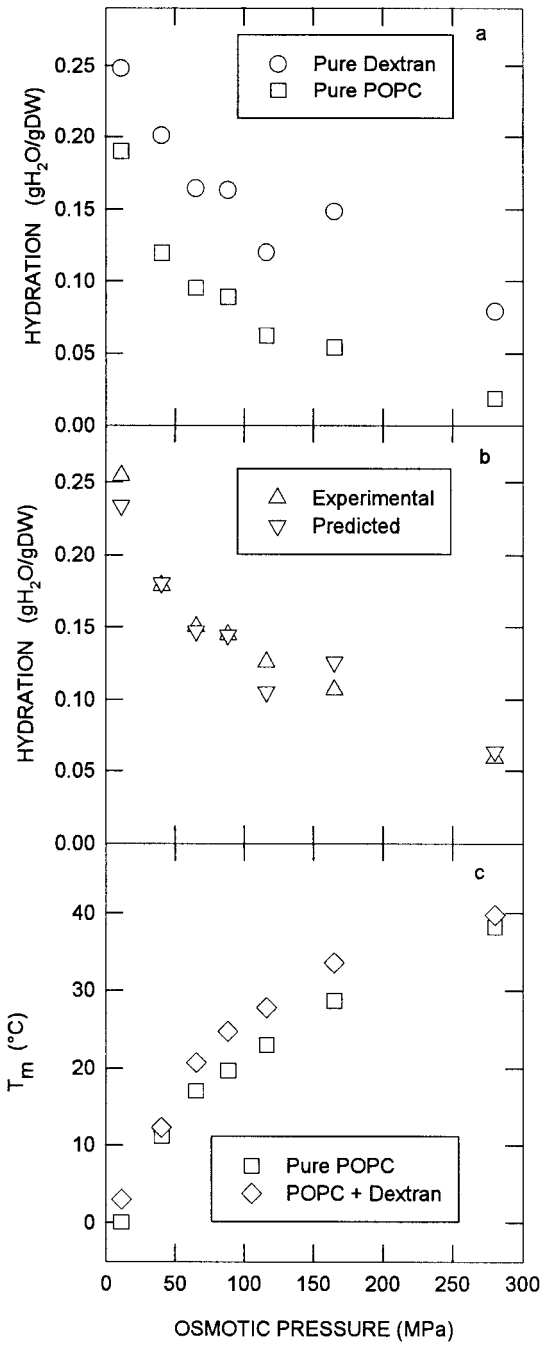

FIGURE 7 (*a*) Hydrations of dextran and POPC as functions of osmotic pressure. (*b*) Experimental and predicted hydrations of the dextran/POPC 3:1 mixture as functions of osmotic pressure. (*c*) T_m of POPC in the presence and absence of dextran, as functions of osmotic pressure. Each point represents a mean value (n = 2). Most of the coefficients of variation for these values were less than 1%, and none exceeded 10%.

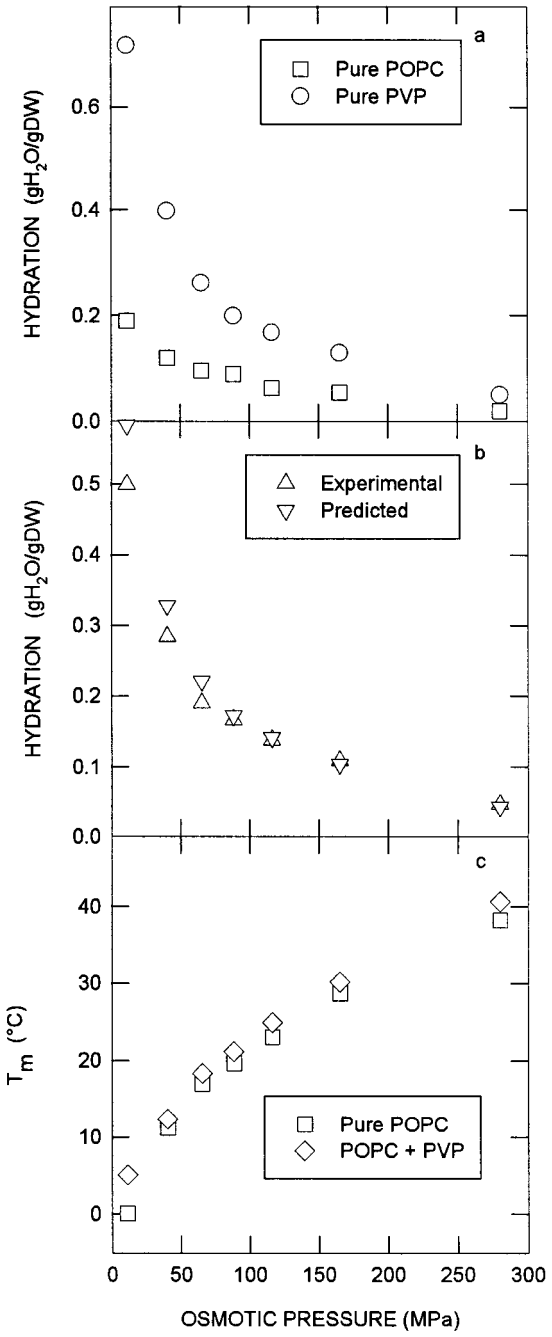

FIGURE 8 (*a*) Hydrations of PVP and POPC as functions of osmotic pressure. (*b*) Experimental and predicted hydrations of the PVP/POPC 3:1 mixture as functions of osmotic pressure. (*c*) T_m of POPC in the presence and absence of PVP, as functions of osmotic pressure. Each point represents a mean value ($n = 2$). Most of the coefficients of variation for these values were less than 1%, and none exceeded 10%.

high hydration, the water was probably shared between the polymers and the lipids, and the experimental hydration was lower than that predicted from the exclusion model. At the low hydrations that are particularly important in anhydrobiology and cryobiology, however, both polymers were clearly excluded from the interlamellar regions.

Fig. 6 shows that the phospholipid T_m was significantly increased by the presence of the polymer, as expected, given that nonpenetrating polymers are sometimes used to dehydrate lipids (LeNeveu et al., 1976; Rand and Parsegian, 1989; Arnold and Gawrisch, 1993). The effect can be better understood by plotting T_m versus osmotic pressure (Figs. 7 *c* and 8 *c*). For both polymers, the graphs show that the phase transition temperature difference between pure lipid and lipid/polymer was small in all cases, with the transition temperature in the presence of the polymer being only slightly higher (~5°). The presence of the polymer thus dehydrated the lipid more than would have been the case if the polymer were not there. This effect on T_m is the reverse of what is observed for small solutes because the polymers are largely excluded from the interbilayer region.

The different effects of sugars and polymers on lipid bilayers are illustrated schematically in Fig. 9. At high sample hydrations, small sugars and large polymers can fit between bilayers and have no significant effect on the lipid T_m (Fig. 9, *regions A* and *A′*). At intermediate hydrations, the effects of small sugars and polymers are very different. As sample hydration decreases, the small sugars remain between the bilayers, where they prevent the close approach of opposing bilayers that would, in the absence of solute, cause T_m to increase (Fig. 9 *a*, *region B*). Thus, as predicted by the model of Bryant and Wolfe (1992), T_m does not rise much above T_o. As samples containing large polymers are dehydrated, however, the polymers are excluded from the interlamellar space, where they sequester water and effectively further dehydrate the lipids. This results in a slight elevation of T_m (Fig. 9 *b*, *region B′*). If the solutes vitrify at a temperature above T_o, which might happen at low hydrations for sugars, the effects of small solutes and polymers are again different. If the small solutes vitrify between the bilayers while the lipids are in the fluid phase, the glass mechanically impedes the lipid transition to the gel phase during cooling, and T_m is depressed below T_o (Fig. 9 *a*, *region C*). In the case of polymers, vitrification has no additional effect on the lipid T_m because the glassy polymers have been excluded from the interlamellar space (Fig. 9 *b*, *region C′*).

CONCLUSIONS

In this paper we have demonstrated several things:

1. For lipids with the sugars trehalose, sucrose/raffinose, and glucose, the reduction in T_m relative to lipids without sugars is consistent with the sugars' osmotic and volumetric properties, as predicted by Bryant and Wolfe (1992).

2. When the T_g of the sugars is greater than the lipid transition temperature in excess water T_o, then T_m is depressed below T_o (if vitrification occurs when the acyl chains are fluid) or elevated above T_o (if vitrification occurs when the acyl chains are frozen).

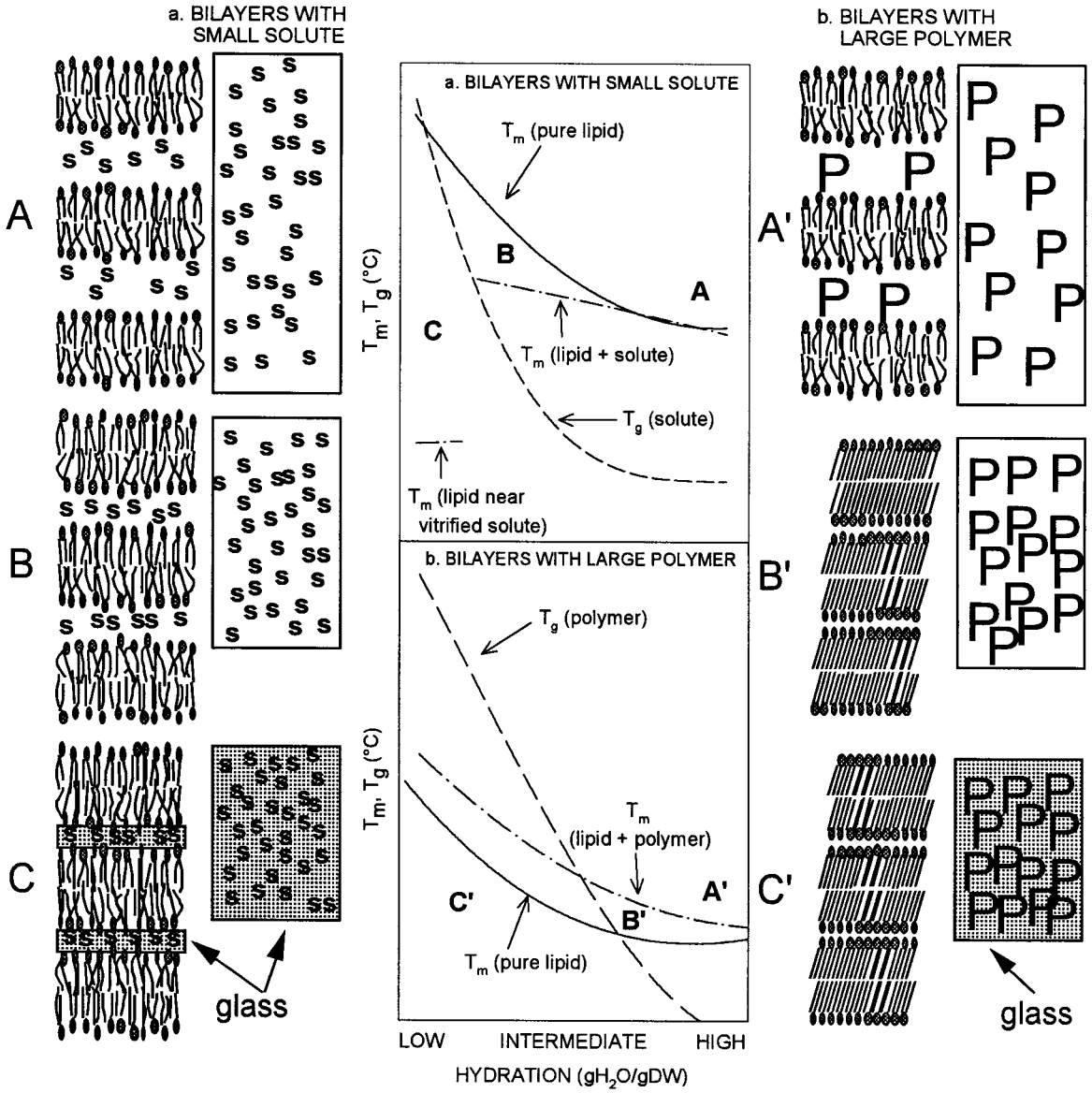

FIGURE 9 Solute partitioning and vitrification during dehydration of lipid/water/solute mixtures for (*a*) small solutes (e.g., sugars) and (*b*) large solutes (e.g., polymers). The cartoons to the left (*a*) and right (*b*) of the phase diagrams represent model membrane and solute behaviors at high (*A, A′*), intermediate (*B, B′*), and low (*C, C′*) sample hydrations and correspond to the regions of the phase diagrams having the same label. At high hydration the bilayers are separated by large distances, and solutes may enter the regions between the bilayers (*A, A′*). At intermediate hydrations the average lipid area decreases, and the bilayer thickness increases. If solutes are small enough to remain between the bilayers (*B*), the interbilayer separation will be larger than if the solutes are excluded (*B′*), and the lipids will remain in the fluid phase (*B*) rather than entering the gel phase (*B′*). At low hydrations the solutes may vitrify. If the solutes remain between the bilayers, then vitrification may occur there, hindering the transition to the gel phase (*c*). If the solutes are excluded, vitrification will not occur near the bilayers and will not affect the lipid phase behavior (*C′*).

3. This effect is independent of the type of sugar used, as predicted by the mechanism proposed by Zhang and Steponkus (1996). The differences among the lipids can be qualitatively explained using the Clausius-Clapeyron equation.

4. The effect of large solutes (e.g., polymers) that are excluded from the intermembrane regions is to osmotically dehydrate the lipids to a small degree, causing a slight increase in T_m.

5. Vitrification of large polymers, which does not occur in the interbilayer region, has little effect on the phase transition properties of the lipids.

6. All of the effects described here are consistent with simple thermodynamics as described by Wolfe and Bryant (1999) and are consistent with data reported by other researchers (J. H. Crowe et al., 1996, 1998; Zhang and Steponkus, 1996; Oliver et al., 1998).

Vitrification of sugars is increasingly recognized as an important component of stability in the dry state, both for anhydrobiotic organisms (Sun et al., 1998; Wolkers et al., 1998) and for storage of foods and pharmaceuticals (Slade and Levine, 1995; Potera, 1998). We have shown that if vitrification occurs in the intermembrane region, the glassy state mechanically hinders conformational changes of lipid bilayers and, thus, can alter the phase behavior of the membranes. This mechanical effect of the vitrified sugars is different from the well-known ability of nonglassy sugars to prevent dehydration-induced increases in T_m and should be considered a separate factor contributing to the ability of sugars to stabilize dry systems.

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