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Quantitative study on the effects of sugars on membrane phase transitions – preliminary investigations

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Introduction

Severe dehydration is lethal for most biological species. However, there are a number of organisms or organelles which have evolved mechanisms to avoid damage during dehydration. One of these mechanisms is the accumulation of small solutes (such as sugars), which has been shown to preserve membranes by inhibiting deleterious phase changes at low hydration (eg [1]).

The effects of solutes on lipid phase transitions have been studied previously using a range of techniques capable of determining transition temperatures (eg DSC, FTIR). Although the effects are now well known, there is disagreement in the literature about the mechanism by which the solutes affect the lipid phase behavior. Specifically, there is debate about whether the effects are caused by direct interactions between the solutes and the lipids (eg [2-3]), or by non-specific effects related to the volumetric, osmotic and solution properties of the solutes (eg [4-7]).

Despite the interest in the problem, a systematic study of the effects of sugars on membrane phase transitions as a function of sugar:lipid ratio has yet to be done. Demé et al. have conducted a study of the effects of sugars on swelling at full hydration [8], but there has been no study of membrane phase behavior as a function of solute content and hydration. In this paper we report preliminary measurements in such a study. We use calorimetry to map out the phase diagram as functions of sugar:lipid ratio, hydration, and temperature; and SAXS to unambiguously determine the phase and the associated structural parameters.

Theory

The phase equilibrium of lipid/water mixtures is extremely complex, with contributions from van der Waals, entropic, and hydration forces. At low to intermediate hydrations the force balance is dominated by the strongly repulsive hydration force which can be written as (eg [9]):

$$P = P_o \exp\left(-\frac{d_w}{\lambda}\right) \qquad (1)$$

where P is pressure (force per unit area), P_o is the extrapolated pressure at zero separation, d_w is the separation between opposing bilayers, and λ is the decay length of the force.

This pressure is balanced in the lateral direction by a compressive stress in the plane of the membrane, which may be written as:

$$\pi = -Pd_w \tag{2}$$

This lateral stress is responsible for the elevation of the membrane transition temperature as a function of dehydration, via the two dimensional equivalent of the Clausius-Clapeyron equation:

$$\Delta T = \frac{T_o \Delta a}{2L}\pi \tag{3}$$

where T_o and L are respectively the transition temperature and latent heat at full hydration, Δa is the change in lipid area between the fluid and gel states, and ΔT is the change in the transition temperature.

The presence of solutes can be incorporated by allowing for osmotic and volumetric effects, leading to a relationship between interbilayer separation d_w and either osmotic pressure or hydration (see eg [5, 6]).

Methods

The synthetic phospholipid 1,2-Dilauroyl-sn-Glyero-3-Phosphocholine (DLPC) was obtained from Avanti Polar-Lipids and used without further purification. The dry lipid was hydrated by the addition of water to a known mass fraction. Samples with sucrose were prepared by hydrating the dry lipid with a specific mass of a sucrose/water solution at a known concentration, so the exact number ratio of sugar to lipid molecules could be calculated. Samples prepared at fixed osmotic pressures were hydrated to 50% by mass, then left to equilibrate over saturated salts at known humilities.

A Perkin-Elmer Differential Scanning Calorimeter (DSC) with an intercooler was used to determine phase transitions temperatures and enthalpies. Scanning rates of 10°/minute were used over the relevant temperature range (-40°C to 50°C). Scans were then repeated several times to ensure reproducibility. The onset temperature of the gel to fluid phase transition was determined from the scans and these were then used to determine the temperature range over which SAXS data were collected.

Small angle X-ray scattering (SAXS) was carried out with a Bruker NANOStar SAXS with an Anton Paar TCU-50 temperature controlled stage. The diffraction pattern at each temperatures was captured by a 2-D detector, radially averaged, and graphed as intensity vs q (magnitude of the scattering vector). Gaussian fits were made to the first order peaks to obtain the exact D-spacing ($2\pi/q$).

Results & Discussion

Figures 1a and 1b show the structural parameters for DLPC that are needed for the modeling of the phase transition as described in the theory. Figure 1a shows the measured repeat spacing along with the calculated water spacing and bilayer thickness as functions of hydration and Figure 1b shows the Lipid area as a function of hydration.

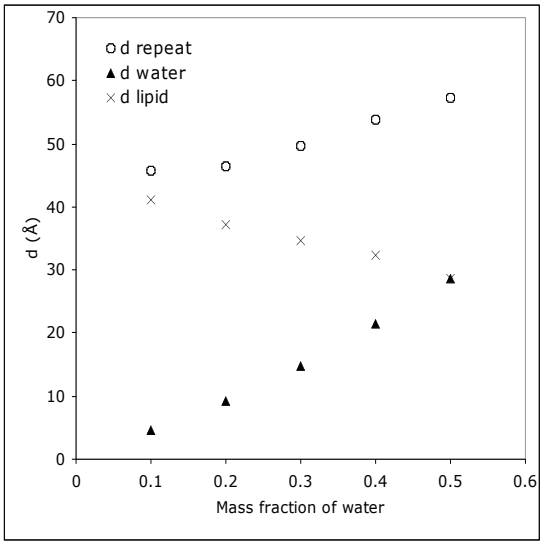

Fig. 1a.

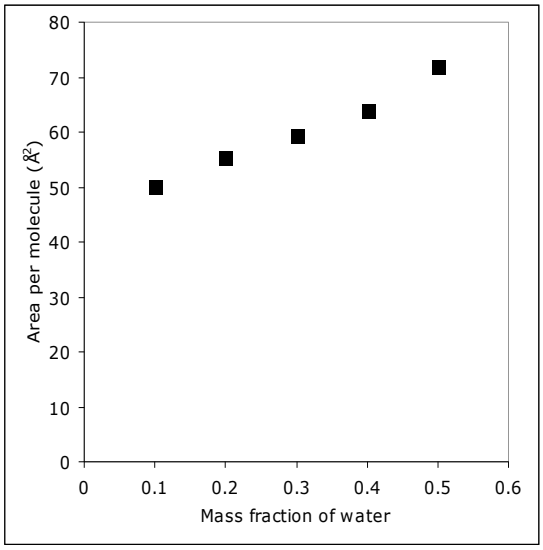

Fig. 1b

Figures 1a and 1b: Structural parameters for DLPC as a function of water content, as determined using SAXS.

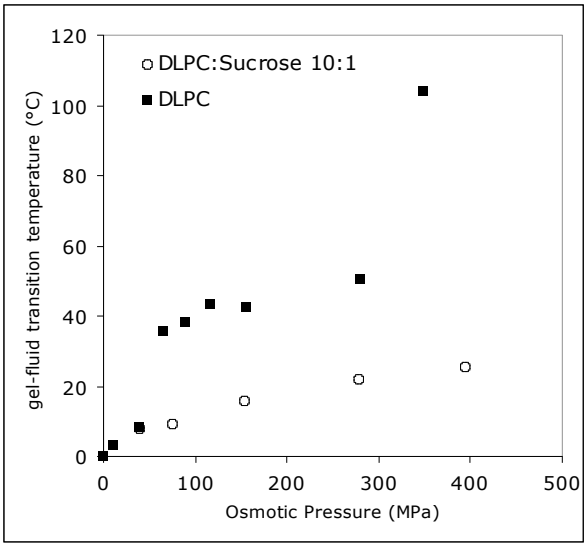

Figure 2: Transition temperature as a function of osmotic pressure for pure DLPC and for DLPC: Sucrose 10:1.

Preliminary experiments showing the effects of solutes on the transition temperature are shown in figure 2. Between approximately 100 and 300 MPa the presence of the sucrose reduces the membrane phase transition temperature by at least 15°C. Clearly even this small amount of sugar has a significant effect on the phase transition properties. Previous work has concentrated on the typical solute/lipid ratios found in desiccation and/or freezing tolerant organisms (typically lipid:sugar 1:1 or 1:2 (eg [11])).

Conclusions

These preliminary experiments show that even a sugar:lipid ratio of 1:10 has a significant effect on phase transition temperatures. This amount of sugar is much smaller than the typical amounts accumulated in dehydration tolerant and freezing tolerant organisms, and demonstrates the potency of this mechanism of protection. Further experiments at a range of sugar:lipid ratios are currently underway.

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