

Electron density analysis of the effects of sugars on the structure of lipid bilayers at low hydration – a preliminary study.

T. Lenné^a, B. Kent^b, K.L. Koster^c, C.J. Garvey^d and G. Bryant^b

^a *Functional Ecology Group, RSBS, ANU, Canberra, ACT 0200, Australia.*

^b *Applied Physics, RMIT University, Melbourne, Vic, 3000, Australia*

^c *Australian Nuclear Science and Technology Organization, Menai, Australia*

^d *Department of Biology, The University of South Dakota, Vermillion, SD, USA*

Small angle X-ray scattering is used to study the effects of sugars on membranes during dehydration. Previous work has shown that the bilayer and chain-chain repeat spacings of DPPC bilayers are relatively unaffected by the presence of sugars. In this work we present a preliminary analysis of the electron density profiles of DPPC in the presence of sugars at low hydration. The difficulties of determining the correct phasing are discussed.

1. Introduction

Sugars and other small solutes have been shown to have an important role in improving the tolerance of a range of species to desiccation and freezing [1]. In particular it has been shown that sugars can stabilize membranes in the fluid membrane phase during dehydration [2], and in the fully dehydrated state [3]. Equivalently, at a particular hydration, the presence of sugars lowers the transition temperature between the fluid and gel phases.

There are two competing models for explaining the effects of sugars on membrane phase transition temperatures. One, designated the water replacement hypothesis (WRH) [1, 3-4], states that sugars hydrogen bond to phospholipid headgroups, thus hindering the fluid-gel phase transition. One version of this model suggests that certain sugars (such as trehalose) achieve the measured effects by inserting between the phospholipid head groups [4].

An alternative model explains the observed effects of sugars in terms of the sugars' effect on the hydration repulsion [5] that develops between opposing membranes during dehydration. The hydration repulsion leads to a lateral compressive stress in the bilayer which squeezes adjacent lipids more closely together, resulting in a transition to the gel phase. When sugars are present, their osmotic and volumetric effects reduce the hydration repulsion, reduce the compressive stress in the membranes, and therefore tend to maintain the average lateral separation between lipids [6-8]. This model is called the hydration forces explanation (HFE).

We recently showed that neither mono- nor di- saccharides affect the average distance between lipid chains in the bilayer, supporting the predictions of the HFE [9]. In this paper we further investigate the effects of sugars on membrane structure by conducting electron density analysis of recent data. This preliminary analysis sheds additional light onto the effects of sugars on membrane structure.

2. Methods

The experimental methods have been described in detail elsewhere [9]. DPPC, 1,2-dipalmitoylphosphatidylcholine, (Avanti) and glucose (Sigma) were used without further purification. DPPC was suspended in an appropriate amount of glucose solution to achieve the desired glucose:DPPC molar ratio in the range from 0:1 to 1:1. Further milli-Q water was added to ensure the sample was in excess. Samples were mixed by freeze-thawing, vortex mixing and centrifugation, and equilibrated for 1 week at 23 °C over saturated NaCl, with a relative humidity (RH) of 75%. Equilibrated samples were placed in 1.5 mm quartz X-ray capillaries (Wolfgang Muller Glas Technik) and sealed using silicone (Pro Seal).



Small angle X-ray (SAXS) experiments were carried out on the ChemMatCARS 15ID-D beamline at the Advanced Photon Source, Argonne National Laboratory. Diffraction patterns were recorded on a Bruker 6000 CCD detector over the Q range 0.046 to 1.7 Å⁻¹. All exposures were 1 s duration, following 5 minutes equilibration at 20 °C.

3. Results

Figure 1 shows representative SAXS patterns for a sample of DPPC with 0.1 glucose molecules per lipid. The higher order reflections are magnified in the inset. The positions of each peak are simple multiples of the primary reflection, characteristic of a lamellar structure. Four reflections are sufficient to obtain detailed electron density profiles.

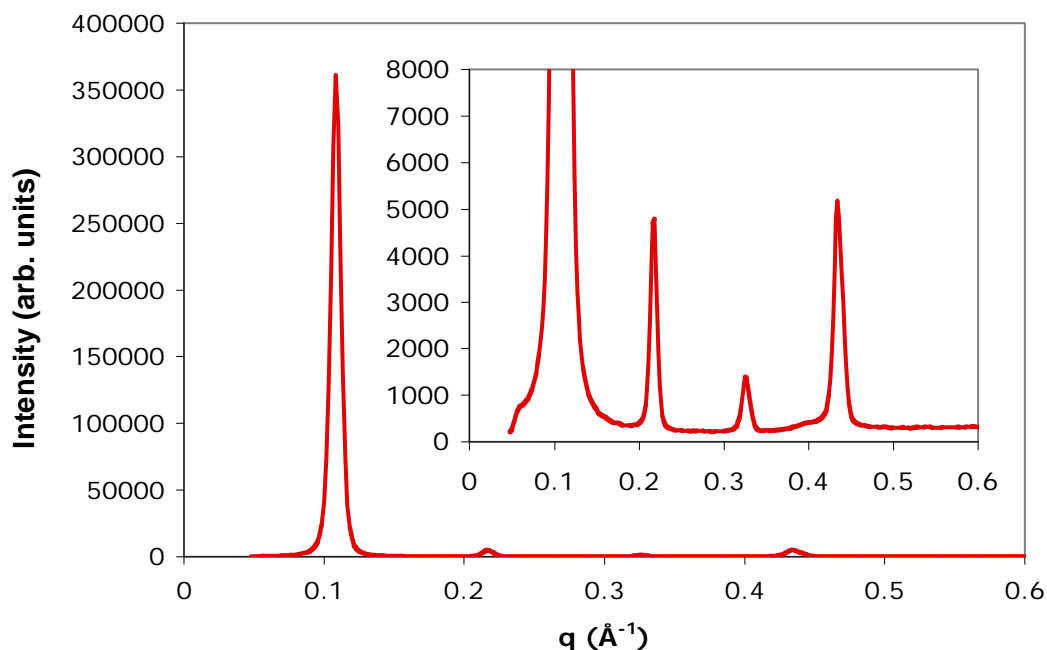


FIG. 1: SAXS reflections for DPPC with 0.1 glucose molecules per lipid. Sample was equilibrated to 75% RH. Measurements were carried out at 20°C, where the lipid is in the gel phase. Inset is on an expanded y scale.

The determination of the electron density profiles follows the method of Harper [10]. For the Lamellar phase the electron density profile (EDP) is given by:

$$\rho_{\text{lam}}(\mathbf{r}) = \rho_{\text{avg}} + \sum_{i=1}^{\infty} A_i \cos\left(\frac{2\pi i x}{d}\right)$$

where ρ is the electron density, d is the main lamellar repeat spacing and x is in the direction perpendicular to the membrane. ρ_{avg} may be set to zero for an arbitrary electron density scale. The Fourier coefficients A_i are related to the normalized integrated intensities of the diffraction rings at a particular scattering vector q by:

$$A_i = \sqrt{\frac{I_q \sin \theta}{m}}$$

where m is the multiplicity factor (ie the number of orientations of a crystal that yield a given reflection), and θ is the scattering angle of the reflection. For SAXS, $\sin \theta \approx \theta$.

The determination of the electron density profile relies on a determination of the phasing – the relative phases (positive or negative) of the diffracted orders. For four diffraction orders there are 16 possible phasings. However, only 8 of these are unique (the others being the negative equivalents). Figure 2 shows the 8 possible phasings for the data shown in figure 1.



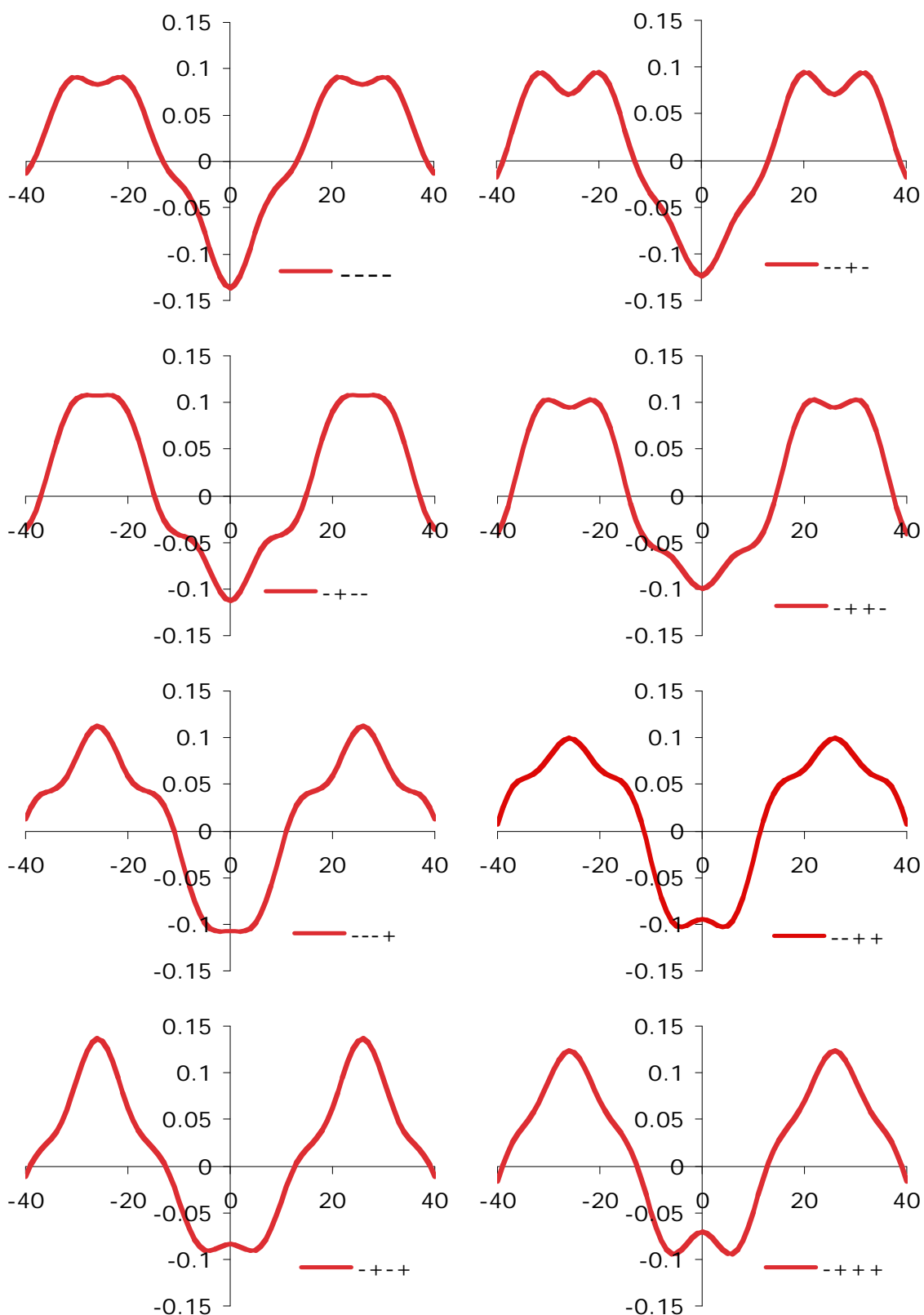


FIG. 2: Electron Density Reconstructions of the gel phase of DPPC at 20°C with 0.1 glucose per lipid, at 75% RH. The phasings of the 4 reflections are indicated in the legends.



In order to determine which is correct, we consider the membrane geometry. The EDP represents the ED as one passes from the water on one edge of the membrane, through the membrane (with the midpoint at the centre of the bilayer) through to the other side of the membrane. The EDs of each chemical group are known, so the EDP must satisfy the conditions: (i) it must have a minimum at the terminal methyl groups (in the middle of the membrane) - this eliminates --++-, -++- and -+++ and ---+; (ii) it must have maxima at the positions (on either side) of the phospholipid head groups - this condition eliminates ----, --+- and -+--. This leaves only -+-. Figure 3 shows the EDPs using this phasing for 5 glucose concentrations. Although the highest glucose ratio yields slightly higher phospholipid peaks and a slightly lower methyl trough, the differences are of the same order as the uncertainties of the reconstruction method (which are related to the uncertainties in determining the relative peak heights). This shows that the presence of the glucose has little effect on the EDP, and therefore little effect on the phospholipid headgroups.

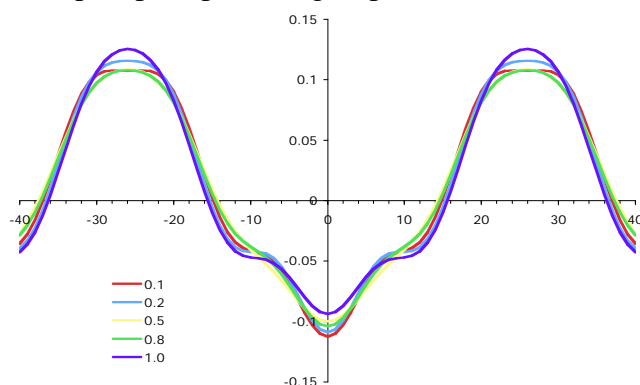


FIG. 3: Electron Density Maps of DPPC with the glucose:lipid molar ratios indicated.

4. Conclusions

This preliminary analysis suggests that the presence of glucose does not significantly affect the bilayer structure, consistent with the predictions of the HFE. Future studies will expand this analysis to a wider range of hydration and include both mono- and di-saccharides, and include a full analysis of the uncertainties in the reconstruction.

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