Chemistry and Physics of Lipids 196 (2016) 76–80

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Sugar does not affect the bending and tilt moduli of simple lipid bilayers

John F. Nagle*, Michael S. Jablin, Stephanie Tristram-Nagle

Department of Physics, Carnegie Mellon University, Pittsburgh, PA 15213 USA

ARTICLE INFO

Article history:
Received 16 December 2015
Received in revised form 25 January 2016
Accepted 29 January 2016
Available online 17 February 2016

Keywords:
Mechanical properties
Bending modulus
Tilt modulus
Lipid bilayers

ABSTRACT

The diffuse X-ray scattering method has been applied to samples composed of SOPC, DOPC, DMPC, and POPC with added sugar, either sucrose, glucose, fructose, maltose, or trehalose. Several sugar concentrations in the range 200–500 mM were investigated for each of the lipid/sugar samples. We observed no systematic change in the bending modulus K_C or in the tilt modulus K_θ with increasing sugar concentration. The average values of both these moduli were the same as those of the respective pure lipid controls within statistical uncertainty of 2%. These results are inconsistent with previous reports of sugar concentration dependent values of K_C.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

The bending modulus K_C is a fundamental mechanical property of membranes that has overarching biophysical relevance. It has been a concern (Nagle et al., 2015; Nagle, 2013) that, even for simple lipid bilayers, there are significant differences in the reported values for K_C. An hypothesis for the disparate values is that the true bending modulus may not only be a property of the intrinsic lipid bilayer but may also vary upon adding sugar to the aqueous environment. If true, this would affect the values of K_C determined by the classical methods of shape analysis (Meleard et al., 1998, 1997; Henriksen and Ipsen, 2002; Pecreaux et al., 2004; Gracia et al., 2010; Bouvrais, 2012; Vitkova and Petrov, 2013) and mechanical manipulation (Rawicz et al., 2000; Henriksen and Ipsen, 2004; Vitkova et al., 2006; Shchelokovskyy et al., 2011; Evans and Rawicz, 1990). Both methods have typically used sugar to improve optical contrast. The disparity in K_C values could then have arisen because different studies have used different sugar concentrations. In this report we test this hypothesis using the method of low angle diffuse X-ray scattering from oriented stacks of membranes to measure K_C (Lyatskaya et al., 2001; Liu and Nagle, 2004; Salditt et al., 2003; Li et al., 2006; Pan et al., 2008, 2009; Jablin et al., 2014). The most recent extension of this method also determines the tilt modulus K_θ (Jablin et al., 2014; Jablin, 2015), so we report the effect of sugar on this modulus that only our method has been able to determine experimentally. We conclude by briefly discussing why the shorter length scale probed by X-rays compared to the classical methods is unlikely to alter our conclusion that sugar has no effect on true bending moduli.

2. Experimental methods

Lipids studied were SOPC, DOPC, DMPC, and POPC obtained from Avanti Polar Lipids. Sugars studied were sucrose, glucose, fructose, maltose, and trehalose obtained from Sigma–Aldrich. Samples were made by first mixing lipid and sugar (first solubilized in heated trifluoroethanol or methanol) in excess 1:1 vol:vol chloroform/(trifluoroethanol or methanol) organic solvent. Mole ratios of sugar to lipid n_S are given in Table 1. The mixtures were then deposited on Si wafers using the rock and roll technique, creating stacks of about 2000 aligned bilayers (Tristram-Nagle, 2007). Dry samples were then hydrated in a humidity chamber *in situ* on the X-ray beamline. Hydration was conveniently even more rapid and, importantly, proceeded further with sugar than for pure lipid. Table 1 shows the repeat spacing D which contains a bilayer and its associated water. Previous studies obtained the mole ratio n_W of water/lipid for fully hydrated D spacings (Nagle and Tristram-Nagle, 2000). For samples with different D spacings, n_W was calculated, using the previously established result that the area per lipid remains the same within the investigated D spacing range (Nagle and Tristram-Nagle, 2000). Dividing the sugar/lipid ratio n_S by n_W gave the sugar/water ratio that is converted to aqueous sugar concentration C_S, listed in Table 1. This concentration is an average concentration of sugar in

* Corresponding author.
E-mail address: nagle@cmu.edu (J.F. Nagle).

http://dx.doi.org/10.1016/j.chemphyslip.2016.01.003
0009-3084/© 2016 Elsevier Ireland Ltd. All rights reserved.

Table 1
List of lipid/sugar samples and their exposures at different repeat spacings D, corresponding to sugar concentrations C_S. Fully hydrated D values are indicated by *, and w estimates the relative goodness of the tilt-independent fit to the different exposures.

Lipid	Sugar	n_S	D (Å)	C_S (mM)	w
SOPC	None	0	65.8*	0	0.33
	Sucrose	0.21	65.0	428	0.26
			70.9	343	0.34
			70.5	348	0.31
	Glucose	0.22	68.6	395	0.49
			74.2	328	0.28
	Fructose	0.22	65.8	440	0.27
			73.8	332	0.15
DOPC	None	0	63.5*	0	0.33
	Sucrose	0.21	65.6	368	0.26
			69.3	323	0.29
	Fructose	0.21	65.9	364	0.13
			67.0	349	0.13
			62.0	425	0.29
	Maltose	0.17	64.4	316	0.75
			67.5	281	0.54
			70.0	259	0.69
			72.4*	241	0.70
	Trehalose	0.17	64.3	316	0.51
			67.6	259	0.65
			66.5	282	0.62
DMPC	None	0	62.7*	0	0.11
	Sucrose	0.21	65.5	357	0.32
	Glucose	0.19	63.3	412	0.08
			66.8	362	0.16
	Fructose	0.19	66.3	368	0.18
			67.3	355	0.31
POPC	None	0	65.1*	0	0.42
	Maltose	0.17	64.4	326	0.25
			68.6	280	0.37
			67.5	291	0.31
			69.6	271	0.37
	Trehalose	0.17	63.4	339	0.40
			65.9	308	0.27
			66.7	299	0.30

the water that includes both the water in the interfacial headgroup region and in the ample water space between neighboring bilayers in the well hydrated bilayer stacks.

X-ray scattering data were taken at G1 station at the Cornell High Energy Synchrotron Source (CHESS), following published protocols (Liu and Nagle, 2004). A sample was placed in a hydration chamber maintained at 30 °C, and it was hydrated through the vapor phase. All bilayers were in the fluid phase (relative humidity >99%) for all reported results. The X-ray wavelength was either 1.177 Å or 1.108 Å. During an X-ray exposure, the incident angle was continuously varied by rotating the sample between −1.6° and 7°. The lamellar repeat D spacing was increased by increasing the current through a Peltier cooler in contact with the bottom of the silicon wafer holding the sample; this cooling of the sample compared to the vapor increased the effective relative humidity at the sample. Diffuse scattering data were fit using a new analysis method that obtains both the bending modulus K_C and the tilt modulus K_θ as the parameters that provide the best fit to the measured intensity (Jablin et al., 2014; Jablin, 2015). The data were also fit with $K_\theta$fixed to a very large value, thereby effectively removing tilt from the analysis; these tilt-independent results for K_C agreed well with the earlier analysis method that did not incorporate tilt in the elasticity model (Liu and Nagle, 2004). Some samples were better fit than others; the inverse of the root mean residual sum of squares was used to assign a relative weight with values w shown in Table 1.

3. Results

Table 1 lists samples analyzed for this study. Most combinations of lipid and sugar were measured with several different values of the repeat spacing D in order to obtain several sugar concentrations C_S for the same sample. Usually, the sample was allowed to gradually become more hydrated, although some decreases in D were deliberately induced by manipulating the Peltier current. The time sequence for the exposures of each lipid/sugar sample followed the order shown in Table 1. For the controls with no sugar, the values for the D spacing shown in Table 1 are the fully hydrated values, D^*, that have been well established in these and previous studies (Nagle and Tristram-Nagle, 2000). For some of the sugar samples, it was verified that the largest reported D spacing was near its fully hydrated value D^*, although time did not permit accurate determinations of D^* for all samples. Nevertheless, we estimate for the concentrations of sugar shown in Table 1 that the fully hydrated D^* for the DMPC samples was about 69 Å, for DOPC samples D^* was about 73 Å, for POPC samples D^* was about 72 Å,

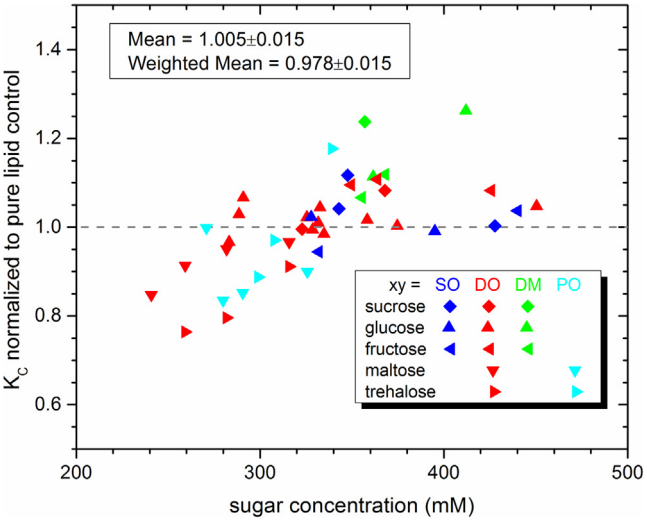

Fig. 1. Normalized K_C values for the combination of the sugars listed in the rows of the legend and for the xyPC lipids indicated in the columns of the legend. Normalization was to each pure lipid control. Values were obtained from tilt-independent fits. The DOPC/glucose results were previously published (Nagle et al., 2015).

Download English Version:

https://daneshyari.com/en/article/1251694

Download Persian Version:

https://daneshyari.com/article/1251694

Daneshyari.com