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What are the true values of the bending modulus of simple lipid bilayers?

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ABSTRACT

Values of the bending modulus K_C are reviewed, and possible causes for the considerable differences are discussed. One possible cause is the use of glucose and sucrose in the classical micromechanical manipulation and shape analysis methods. New data, using the more recent low angle X-ray method, are presented that do not support an effect of glucose or sucrose on K_C. Another possible cause is using an incomplete theory to interpret the data. Adding a tilt term to the theory clearly does not affect the value obtained from the shape analysis method. It is shown that a tilt term, using a value of the modulus K_θ indicated by simulations, theory, and estimated from order parameters obtained from NMR and from the wide angle X-ray method, should also not affect the value obtained using the micromechanical manipulation method, although it does require a small correction when determining the value of the area compressibility modulus K_A. It is still being studied whether including a tilt term will significantly affect the values of K_C obtained using low angle X-ray data. It remains unclear what causes the differences in the experimental values of K_C for simple lipid bilayers.

1. Introduction

The bending modulus K_C is a most important membrane mechanical property. Accordingly, it has been measured many times for many different lipid bilayers. Although uncertainties are typically reported at the 10% level, values obtained in different labs and with different measuring techniques typically differ by as much as a factor of two for the same lipid at the same temperature (Nagle, 2013; Marsh, 2006). As biological processes often involve transition states with curved membranes, that part of the activation energy would differ by a factor of two. For thermally activated processes, the predicted rate constant, depending as it does on the exponential of the activation energy, could easily be kinetically incompetent for the larger K_C value, while being quite feasible for the smaller value (Nagle, 2013). It is therefore of some biophysical importance to obtain more accurate values of K_C, as well as to alleviate the embarrassment that membrane researchers lack accepted values for a quantity that is recognized to be central.

The two most common methods for measuring K_C are micromechanical manipulation (MM) of giant unilamellar vesicles (GUV) (Rawicz et al., 2000; Henriksen and Ipsen, 2004; Vitkova et al., 2006; Shchelokovskyy et al., 2011; Evans and Rawicz, 1990), often called the pipette aspiration method, and fluctuating shape analysis (SA) of GUV (Meleard et al., 1998; Henriksen and Ipsen, 2002; Meleard et al., 1997; Pecreaux et al., 2004; Gracia et al., 2010) and many earlier references (Bouvrais, 2012; Vitkova and Petrov, 2013). A few results have been obtained by pulling cylindrical tethers from GUV (Heinrich and Waugh, 1996; Sorre et al., 2009; Tian et al., 2009) and a variety of other techniques are reviewed by (Dimova, 2014). Here we will focus on some MM and SA results as well as results from an X-ray method. Analysis of low angle diffuse X-ray scattering from oriented stacks of membranes has also been employed more recently (Lyatskaya et al., 2001; Liu and Nagle, 2004; Salditt et al., 2003; Li et al., 2006; Pan et al., 2008, 2009), and the values so obtained agree well with those reported in a classic MM paper (Rawicz et al., 2000). However, ignoring for the moment differences between different labs using the same method, the values obtained using SA are generally larger than those obtained using MM or X-ray methods (Nagle, 2013; Marsh, 2006). One possible reason may be related to different length scales of the measurements and the theory involved, as we review in Section IV. A more mundane possibility regards an experimental aspect of the two GUV methods to which we first turn in the next section.

2. What is the effect of sugar concentration c_s on the bending modulus?

The MM method typically uses a sugar solution to conserve the volume of the GUV (Vitkova et al., 2006; Evans and Rawicz,

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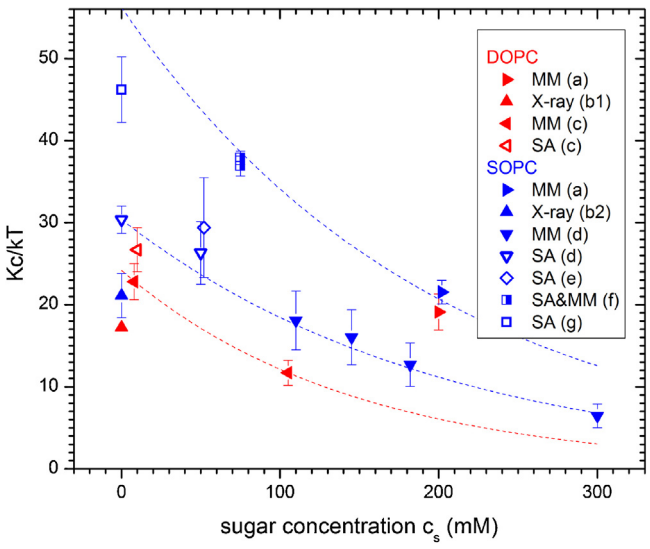

Fig. 1. Bending modulus K_C in thermal units kT versus sugar concentration c_s from literature values adjusted to $T = 30\,^{\circ}C$ using $-0.1/^{\circ}C$ (Pan et al., 2008) for lipid bilayers composed of DOPC (downward pointing triangles) and SOPC (upward pointing triangles). The lines are exponential fits as proposed for SOPC (Vitkova et al., 2006). The legend identifies the method of measurement and the reference, a = (Rawicz et al., 2000), $b1$ = (many results from this lab), $b2$ = (Kucerka et al., 2005), c = (Shchelokovskyy et al., 2011), d = (Vitkova et al., 2006), e = (Pecreaux et al., 2004), f = (Henriksen and Ipsen, 2004), g = (Genova et al., 2013).

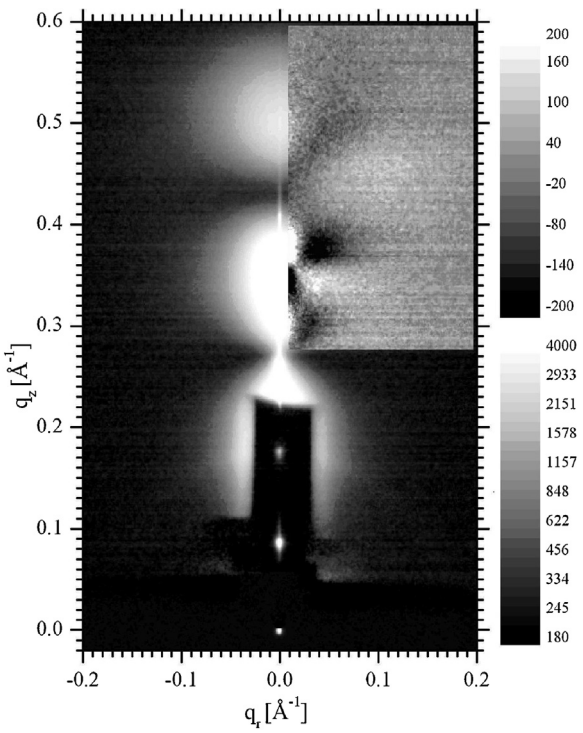

Fig. 2. X-ray scattering intensity from a stack of ~2000 oriented DOPC bilayers with glucose. The main panel shows the log of the intensity with the intensity scale shown at the lower right. The overlay in the upper right (positive q_r and $q_z > 0.28$ Å^{-1}) shows the residuals of the fit to the intensity in that region which is symmetrically equivalent to the intensity shown for negative q_r; the linear scale on the upper right shows that the residuals, though generally smaller than 2%, are non-random. A vertical molybdenum strip attenuates the $h = 1$ and 2 orders on the meridian and a thicker horizontal strip attenuates the beam near the bottom.

1990). Often a sucrose solution inside the GUV and a glucose solution outside the GUV is used to enhance visual contrast. A classic study used $c_s = 200$ mM sugar with the same molarity on both sides to ensure flaccid GUV with zero imposed surface tension or pressure; Fig. 1 shows the reported value (red right pointing triangle) of K_C/kT for DOPC, that has two double bonds in the two oleoyl (DO) hydrocarbon chains, and the value (blue right pointing triangle) for SOPC, that has a saturated stearoyl (S) chain that makes the SOPC bilayer a bit thicker and stiffer than DOPC (Rawicz et al., 2000). Also shown in Fig. 1 are the results from the first study that reported an effect of sugar concentration on SOPC (Vitkova et al., 2006). This study utilized the SA method for small sucrose concentrations (two open blue down triangles in Fig. 1) and the MM method (four solid down blue triangles in Fig. 1) for larger sucrose concentrations (fluorescent dye was used for contrast instead of glucose outside). These results, when interpolated at 200 mM, are about a factor of two smaller than the earlier results (Rawicz et al., 2000), perhaps attributable to differences in the way the two labs interpreted MM data. More importantly, an exponential decay with sugar concentration was indicated, as shown by the lower dashed blue line in Fig. 1. The same group, using the SA method exclusively, also reported a decreasing K_C with increasing sucrose concentration, although the decrease was only about half as large as in Fig. 1 (Genova et al., 2006). Subsequently, decreasing K_C was reported for other sugars (Genova et al., 2007), although, contrarily, maltose was reported not to decrease K_C (Genova et al., 2010). Fig. 1 also shows the most recent SOPC value (open square) with no sugar obtained after further development of the SA method (Genova et al., 2013).

An MM study of DOPC found that K_C at small $c_s = 8$ mM sucrose/8 mM glucose was twice as large as with 100 mM sucrose/110 mM glucose (Shchelokovskyy et al., 2011). As shown by the red dashed line in Fig. 1, that is also consistent with a strong exponential decay with sugar concentration; extrapolation to 200 mM gives a value three times smaller than the earlier DOPC result of (Rawicz et al., 2000). Interestingly, the same exponential dependence connects the earlier MM value for SOPC of (Rawicz et al., 2000) with the values of K_C reported by (Henriksen and Ipsen, 2004) using the MM method and by (Henriksen and Ipsen, 2002) using the SA method (half closed squares in Fig. 1). Finally, Fig. 1 shows that our X-ray results at zero sugar (upright triangles) agree well with the earlier MM results (right pointing triangles).

Not surprisingly, considering the current state of K_C results, there is the feeling that more experiments should be done (Genova et al., 2013; Dimova, 2014). However, instead of only exhorting others to do more experiments, we have been addressing this issue using the X-ray method.

3. New X-ray results for possible sugar effect

Briefly, four samples (i–iv) were made by mixing DOPC and sugar (first solubilized in heated trifluoroethanol or methanol) in excess 1:1 chloroform/(trifluoroethanol or methanol) organic solvent with mole ratios of (i) 0.12 glucose/DOPC, (ii) 0.12 sucrose/DOPC, (iii) and (iv) 0.22 glucose/DOPC. The mixtures were deposited on Si wafers using the rock and roll technique to achieve superior alignment in the stack of about 2000 bilayers (Tristram-Nagle, 2007). The dry sample was then hydrated in a humidity chamber in situ on the X-ray beamline. Hydration was conveniently even more rapid with sugar than for pure DOPC. Compared to the repeat spacing of fully hydrated DOPC, $D = 63.1$ Å, the repeat spacing increased to $D \approx 68$ Å for the lower concentrations in samples (i) and (ii), and for the higher concentrations in samples (iii) and (iv) D increased to 74 Å. Fig. 2 shows a grayscale image of the X-ray scattering. K_C/kT was obtained using our analysis procedure (Liu, 2003).

New X-ray results are shown in Fig. 3 along with some of the literature results already shown in Fig. 1. In order to facilitate comparison between various results, K_C has been normalized to 1 for