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Sugars in the Aqueous Phase Change the Mechanical Properties of Lipid Mono- and Bilayers

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We applied two independent methods to measure the bending elasticity of SOPC lipid membranes in the presence of different sucrose concentrations in the aqueous phase. The micropipette technique was used to study the membrane bending rigidity in the concentration range of (0.11–0.30) mol/l of sucrose, while for sucrose concentrations 0 mol/l and 0.05 mol/l the thermal fluctuation analysis of quasi-spherical vesicles was applied. Both methods revealed a strong reduction of the bending elastic modulus, when sucrose is present in the water. Using micromanipulation of emulsion droplets, we investigated the sucrose influence on the stretching elasticity of SOPC lipid monolayers at the oil-water interface. Our results showed an almost two-fold reduction of the stretching elasticity modulus of the lipid monolayer in the presence of 0.18 mol/l of sucrose in the aqueous phase. The experimental results, reported here, reveal a strong influence of sugar molecules on the elasticity of lipid mono- and bilayers.

Keywords: emulsion droplets; giant vesicles; micropipettes; sugars; thermal fluctuations

1. INTRODUCTION

Various sugar-based molecules play an important structural and functional role in biological membranes [1]. Oligo- and polysaccharides (fructans) appear to produce a strong drought-protective effect in cellular membranes of plants [2,3]. It was found that fructans were able to stabilize the liquid-crystalline lamellar phase, which is consistent with a drought-protecting role in plants [4]. Small carbohydrates, such as

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trehalose and sucrose, have been reported to be efficient cryoprotectors, which is ascribed to the replacement of water at the membrane structure by molecules of these sugars [5,6]. The biological significance of carbohydrate molecules as well as their wide application in biophysical research determines the interest towards the sugar-membrane interactions. In the recent years they have become the object of intensive research in the literature. Vereyken *et al*. [4] found that fructans penetrate between the headgroups of different kinds of phospholipids even under conditions of very tight lipid packing. Dobereiner *et al*. [7] obtained a strong influence of glucose on the spontaneous curvature of liposomes. Demel *et al*. [2] measured a strong influence of fructans, as well as of small carbohydrates (mono- and disaccharides) on the tension of lipid monolayers on the air/water interface.

In the present study, we investigated the influence of some sugars (sucrose and fructose) on the elastic properties of model lipid membranes. It is well known that in the micromanipulation measurements of the bending elasticity of lipid bilayers there is a necessity to use an aqueous solution of an additive (usually sucrose or glucose) in order to assure the conservation of the vesicle volume [8,9]. That was one of the inspirations toward a profound investigation of the influence of these compounds on the elastic characteristics of lipid mono- and bilayers. In the present work we used two independent techniques for the determination of the elastic properties of lipid mono- and bilayers: the analysis of the thermally induced shape fluctuations of quasi-spherical vesicles [10–12] and the micropipette aspiration method [8,13]. The influence of sugars with different concentrations in the aqueous phase on the bending elasticity of lipid bilayers and on the stretching elasticity of lipid monolayers (the interface of emulsion droplets in our case) was investigated. A determination of the bending elasticity of lipid bilayers was carried out also in the presence of fructose in order to reveal if there was any specificity of the sugar effect on the membrane mechanical properties.

2. MATERIALS AND METHODS

All the experiments were performed with mono- or bilayers composed of l-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (C18:0/C18:1) (SOPC, Avanti Polar Lipids Inc., USA). The carbohydrates used in our experiments were sucrose and fructose (SigmaUltra® purity, 99.5% (GC), Sigma Aldrich Chemie, Germany). All chemicals were used without any further purification.

For the bilayer experiments giant unilamellar vesicles (Fig. 1b) were prepared by the gentle hydration method [14].

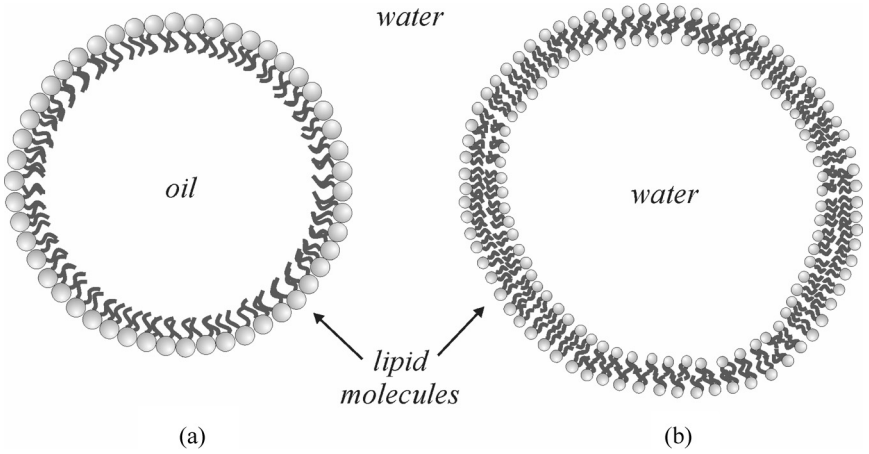

FIGURE 1 Schematic presentation of the structure of: (a) an emulsion droplet; (b) a giant lipid vesicle (equatorial cross-sections).

The emulsion droplets (Fig. 1a) were prepared following a standard procedure for preparation of emulsions [15]. First, SOPC was dissolved in *n*-dodecane (Sigma Aldrich Chemie, Germany) with concentration of 10^{-3} mol/l. Then, 2 ml of the lipid solution in the oil were added to 30 ml of the aqueous phase (deionised water or water solution of sucrose with concentration 0.18 mol/l, the concentration usually used in the micromanipulation measurements of the membrane elasticity). The mixture was then homogenized by a homogenizer (Ultraturrax, USA) at 15 500 min^{-1} for about 3 minutes. Only droplets without any visible defects and radiuses of the order of 10 μm were studied.

2.1. Thermally Induced Shape Fluctuation Method for the Measurement of the Bending Elasticity Modulus of the Lipid Bilayers of Quasi-Spherical Vesicles

Following the first detailed theoretical model of the thermally induced shape fluctuations of quasi-spherical lipid vesicles [16], a theoretical background for experimental procedures leading to precise measurements of the bending elastic modulus [11,12] was developed. The fundamental expression is [16]:

$$\left\langle \left| U_n^m(t) \right|^2 \right\rangle = \frac{k_B T}{k_c} \frac{1}{(n-1)(n+2)[\overline{\sigma} + n(n+1)]}, \qquad (1)$$

where $\left\langle \left| U_n^m(t) \right|^2 \right\rangle$ is the mean squared amplitude of $Y_n^m(\theta, \varphi)$ spherical harmonics, k_B is the Boltzmann's constant, T is the absolute temperature, k_c is the bending elasticity modulus of the membrane, n is the mode number and $\overline{\sigma} = \sigma R^2/k_c$ (or $\overline{\sigma} = \sigma R^2/k_c + 2c_0R + c_0^2R^2/2$, if the spontaneous curvature c_0 is different from zero) is the dimensionless membrane tension. In the Milner and Safran's model [16], for each of the fluctuation modes the time autocorrelation function is monoexponential:

$$\left\langle U_n^m(t) U_n^{m^*}(t + \Delta t) \right\rangle = \left\langle \left| U_n^m(t) \right|^2 \right\rangle \exp\left(-\frac{\Delta t}{\tau_n^m} \right) \tag{2}$$

with a correlation time τ_n^m for the amplitude $U_n^m(t)$ given by the expression:

$$\tau_n^m = \frac{\eta R^3}{k_c} \frac{2n+1}{(n-1)(n+2)[\overline{\sigma} + n(n+1)]} \left(2 - \frac{1}{n(n+1)} \right), \tag{3}$$

where η is the viscosity of the surrounding medium and R is the vesicle radius.

In fact, what is experimentally measured in the study of a fluctuating quasi-spherical giant vesicle (Fig. 2) is the quantity B_n [12], defined as:

$$B_n = \frac{2n+1}{4\pi} \left\langle \left| U_n^m(t) \right|^2 \right\rangle. \tag{4}$$

In most experiments, observation of the giant vesicle is performed by video microscopy. Unfortunately, the cameras used (CCDs or vacuum tubes) possess an intrinsic "defect" – the image presented to

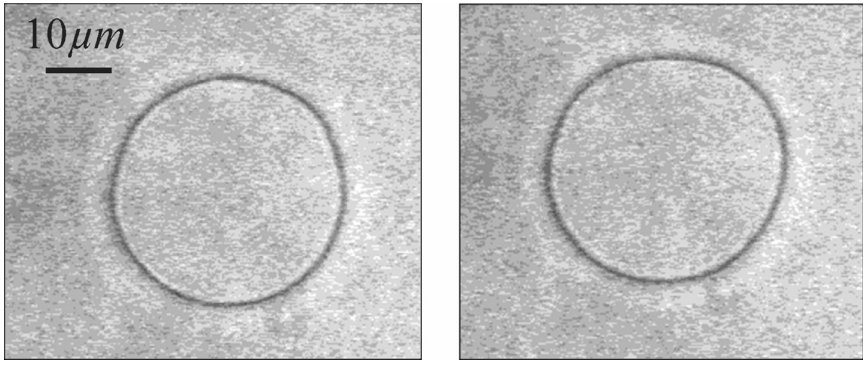

FIGURE 2 Two subsequent images of a fluctuating vesicle in phase-contrast microscopy (time interval of 1 s between them).

the observer (on the video monitor or in numerical form after digitalization by a frame grabber) reflects the integral energy accumulated on a given point (pixel) during the exposure time ($t_s = 40\,\text{ms}$ for the European TV standard). Thus, fast movements are smeared out and instead of the theoretical model amplitudes B_n one obtains $B'_n = f_n^{corr} B_n$, where the correction factor f_n^{corr} cannot be neglected and is calculated in [11] to be:

$$f_n^{corr} = 2\left(\frac{\tau_n^m}{t_s}\right)^2 \left[\exp\left(-\frac{t_s}{\tau_n^m}\right) - \left(1 - \frac{t_s}{\tau_n^m}\right)\right] \tag{5}$$

Two factors turn out to be of crucial importance for the precise determination of the bending modulus by the method of shape analysis of fluctuating quasi spherical giant vesicles. One of them is the dimensionless membrane tension $\overline{\sigma}$, which has its strongest influence on the low order modes, and the second is the effect of the video camera integration time, which has its highest influence on the fastest modes – the higher order ones.

In the present study this method was applied for the determination of the bending elasticity of SOPC bilayers at low concentrations (less than 0.1 mol/l) of the carbohydrate in the aqueous phase.

2.2. Micromanipulation Technique for the Determination of the Mechanical Properties of the Lipid Bilayers of Giant Vesicles and the Lipid Monolayers of Emulsion Droplets

The micromanipulation method [8,13] consists in sucking a vesicle with large enough size (radius $R_v \approx 10\,\mu\text{m}$) into a micropipette with small enough radius ($R_p \approx 4\,\mu\text{m}$) and determining its projected length ΔL (the length of the membrane, sucked into the micropipette) as a function of the sucking pressure Δp (the difference between the hydrostatic pressure inside the experimental cell and inside the pipette) (Fig. 3). The relation between these quantities is:

$$\frac{1}{2}\left[\left(\frac{R_p}{R_v}\right)^2 - \left(\frac{R_p}{R_v}\right)^3\right]\frac{\Delta L}{R_p} = \frac{k_B T}{8\pi k_c}\ln(\sigma) + \frac{\sigma}{k_s} + \text{Const}, \tag{6}$$

where k_B, T, and k_c are defined after Eq. (1), k_s is the stretching elasticity modulus of the lipid bilayer, ΔL is the projected length for the corresponding pressure Δp, and the membrane tension σ is expressed by Δp via the Laplace law:

$$\sigma = \frac{\Delta p R_v R_p}{2(R_v - R_p)} \tag{7}$$

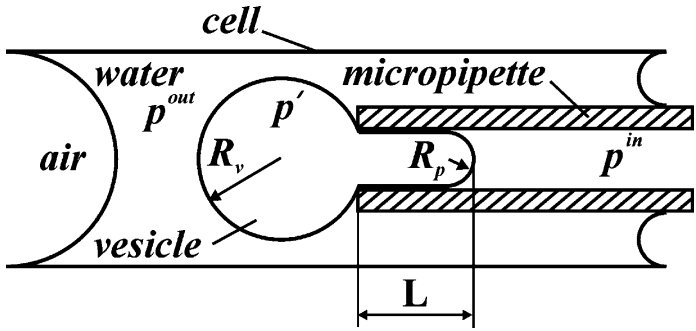

FIGURE 3 Scheme of the experimental set-up for micromanipulation measurements. A vesicle with a radius R_v is sucked into a micropipette with a radius $R_p < R_v$. The hydrostatic pressures inside and outside the micropipette and inside the vesicle are p_{in}, p_{out}, and p', respectively. The length of the membrane sucked into the micropipette is L.

The method permits the measurement of the bending elasticity k_c when σ is low enough and the stretching elasticity k_s when σ is high enough. It was used for the measurement of the bending elasticity k_c of lipid bilayers at higher concentrations (>0.1 mol/l) of the carbohydrate in the aqueous phase.

We applied this method also for the measurement of the elastic properties of a monolayer, covering an emulsion droplet of the kind "oil in water" (Fig. 4). In this case in relations Eqs. (6) and (7) R_d (the radius of the droplet) is substituted for R_v. Because of the amphiphilic nature of the SOPC molecules, they are able to adsorb on interfaces between hydrophilic and hydrophobic media (water/oil, for

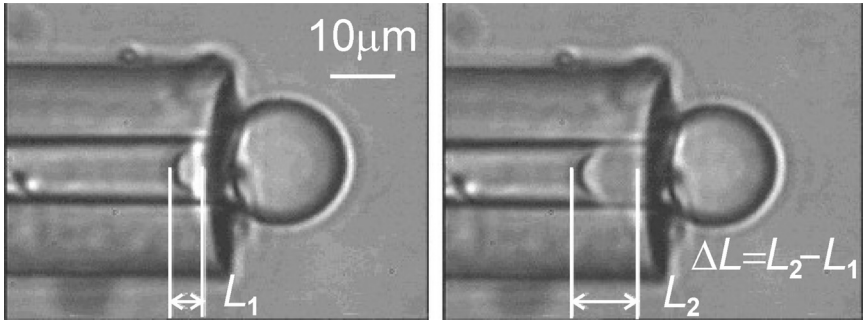

FIGURE 4 Two subsequent images of an emulsion droplet, aspirated in a micropipette, as observed by transmission optical microscopy.

example) and to form a lipid monolayer, characterized by its stretching elasticity (Fig. 1a). The experiments with emulsion droplets for the cases when the aqueous phase is pure water and when it contains a carbohydrate additive permit to answer the question whether sugar molecules have any measurable influence on the elastic properties of the lipid monolayers or not. Because of the necessity of additive in the aqueous phase, the micromanipulation of giant vesicles does not permit the determination of their mechanical properties in pure water. The observation of the emulsion droplets is possible via transmission microscopy due to the different refraction indices of the oil and the water (Fig. 4).

The emulsion droplets, produced for our experiments, had a relatively high interfacial tension (σ is of the order of 0.1 N/m). For such high values of the tension, only the stretching elasticity modulus k_s of the monolayer can be measured.

Substituting σ from Eq. (7) into Eq. (6), we obtain:

$$\frac{1}{k_s} = \frac{1}{2(R_p)^2}\left[1 - \frac{R_p}{R_d}\right]\left[\left(\frac{R_p}{R_d}\right)^2 - \left(\frac{R_p}{R_d}\right)^3\right]\frac{\partial(\Delta L)}{\partial(\Delta p)} \tag{8}$$

Thus, the experimental determination of the dependence $\partial(\Delta L)/\partial(\Delta p)$ enables the calculation of the stretching elasticity of the monolayer. The tension of the droplet can be estimated, too, using the value of the sucking pressure, at which the aspired part of the droplet has the form of a hemisphere (the projected length is equal to the radius of the pipette) according to the formula:

$$\sigma = \frac{\Delta p}{2\left(\frac{1}{R_p} - \frac{1}{R_d}\right)} - k_s \frac{R_p^2}{4R_d^2} \tag{9}$$

3. RESULTS AND DISCUSSION

The stretching elasticity modulae of SOPC monolayers on the emulsion droplets of the kind "dodecane in water" without sucrose and with 0.18 mol/l of sucrose in the aqueous phase were measured.

For each droplet four sucking pressures were chosen, an initial one Δp^1, two intermediate Δp^2 and Δp^3, and a final sucking pressure Δp^4, $\Delta p^1 < \Delta p^2 < \Delta p^3 < \Delta p^4$, and they were applied in the following sequence: Δp^1, Δp^2, Δp^3, Δp^4, Δp^3, Δp^2, Δp^1, etc. For each pressure of the sequence the projected length ΔL was measured. The obtained results are shown in Figure 5. When increasing the pressure Δp (i.e., in the parts Δp^1, Δp^2, Δp^3, Δp^4 of the sequence) the data for ΔL can be fitted with a straight line with some slope. When decreasing Δp (i.e., in

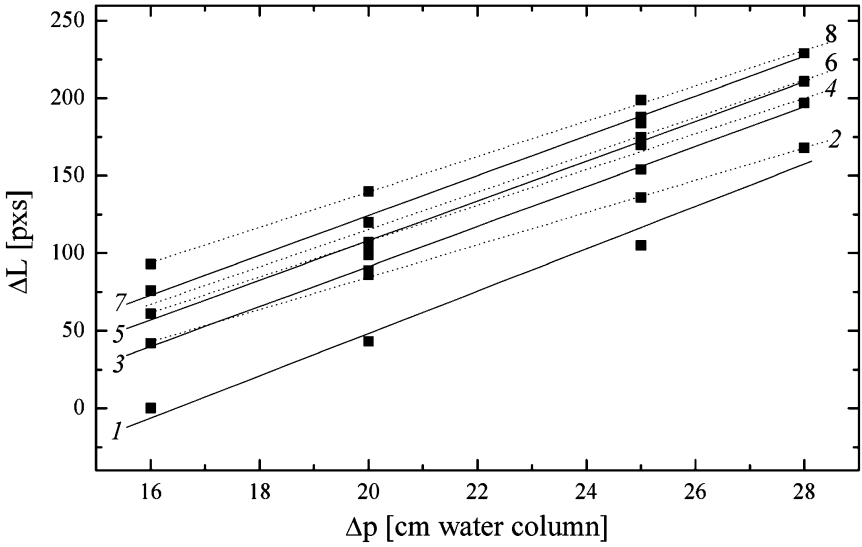

FIGURE 5 Experimental data for the projected length ΔL of an emulsion droplet with radius 16 μm sucked into a micropipette with radius 4.25 μm at zero sucrose concentration in the aqueous phase: ■ – experimental data for the projected length ΔL as a function of the sucking pressure Δp; — – the linear fit of the experimental data for the increasing parts of the pressure sequence (for details see the text). The lines are numbered left with odd numbers; · · · · – the same fit for the decreasing parts of the pressure sequence, numbered right with even numbers.

the parts Δp^4, Δp^3, Δp^2 and Δp^1 of the sequence) the data for ΔL can be fitted with a straight line with slightly lower slope. An exception are the points at the first increase and the first decrease of the pressure Δp. If they are approximated with straight lines, their slopes will be considerably higher. In the calculation of k_s we did not take into account the data from the first increase and the first decrease of Δp. We assumed the derivative $\partial(\Delta L)/\partial(\Delta p)$ from Eq. (8) to be equal to the slopes of the straight lines at the increases and the decreases of Δp. The difference between the two slopes is included in the experimental error of this derivative. By means of Eq. (8) the stretching elasticity k_s together with its experimental error was calculated. The difference in the slopes of the straight lines fitting the experimental data for ΔL, is a result of the increase of the droplet area with the time. An explanation of this phenomenon could be the additional adsorption of lipid molecules from the bulk of the droplet to its surface due to the considerable increase of the monolayer tension, when the droplet is

being sucked in the micropipette, which results in a decrease of the chemical potential of the lipid molecules building its monolayer.

The values of the stretching elasticity modulae k_s of the monolayers of emulsion droplets without sucrose and in the presence of 0.18 mol/l concentration of sucrose in the aqueous phase are presented in the Table 1. A k_s reduction of about 40% in the presence of sucrose with the mentioned concentration in the water environment is observed. This decrease is significantly greater than the error of the measurement. Our results revealed the strong influence of sucrose dissolved in the water environment on the stretching elasticity modulus of SOPC monolayers.

The bending elasticity of SOPC bilayers has been previously measured in the literature. By means of the thermal fluctuation method Méléard *et al*. [17] obtained $(1.27 \pm 0.07) \times 10^{-19}$ J for the bending elasticity modulus k_c of SOPC membranes in pure water. Evans and Rawicz [13] obtained a slightly lower value $(0.90 \pm 0.06) \times 10^{-19}$ J for the bending elasticity of the same bilayer using micromanipulation of giant lipid vesicles. The presence of sugars (sucrose inside the vesicle and glucose in the surrounding medium) in the micromanipulation experiment could be a possible explanation for the difference between these two values.

The evolution of the bilayer bending elasticity with the sucrose concentration was investigated also using the micropipette aspiration method. It has been shown [9], that a minimal concentration of additives in the aqueous phase exists, assuring that for additive concentrations higher than it the error of k_c and k_s measurements due to vesicle volume changes is less than 10%, typical precision for experiments of this kind. For SOPC bilayers this minimal concentration was estimated to be 0.1 mol/l. That is why we were able to measure the bending elasticity evolution by this method only for higher than 0.1 mol/l sucrose concentrations in the aqueous phase.

The experimental results for the bending elasticity modulus of SOPC membranes, containing different concentrations of sucrose in

TABLE 1 Experimental Results for the Stretching Elastic Modulus of SOPC Monolayers in Pure Water and in Presence of 0.18 mol/l Sucrose in the Aqueous Phase, obtained via Micropipette Aspiration Method

Sucrose concentration in the aqueous phase [mol/l]	k_s of SOPC monolayer
0	(64.3 ± 2.9) mN/m
0.18	(33.4 ± 2.7) mN/m

the aqueous phase as measured by the micromanipulation method are depicted in Figure 6.

Measurements to obtain the bending elasticity modulus of SOPC membranes at concentrations of sucrose in the water 0 mol/l and 0.05 mol/l were performed using thermal fluctuation analysis. These results are also presented in Figure 6. The results in the whole range of concentrations, achieved by both methods are fitted very well with an exponential curve, which gives opportunities these data to be used in other experiments of this type.

In order to verify if this sucrose effect on the bending elasticity of the SOPC membrane is a specific one, we performed an experiment via micromanipulation technique with fructose. A concentration of 0.18 mol/l of fructose in the aqueous phase was used. At these conditions we obtained for the bending elasticity the value $(0.51 \pm 0.05) \times 10^{-19}$ J. Using the same method and the same

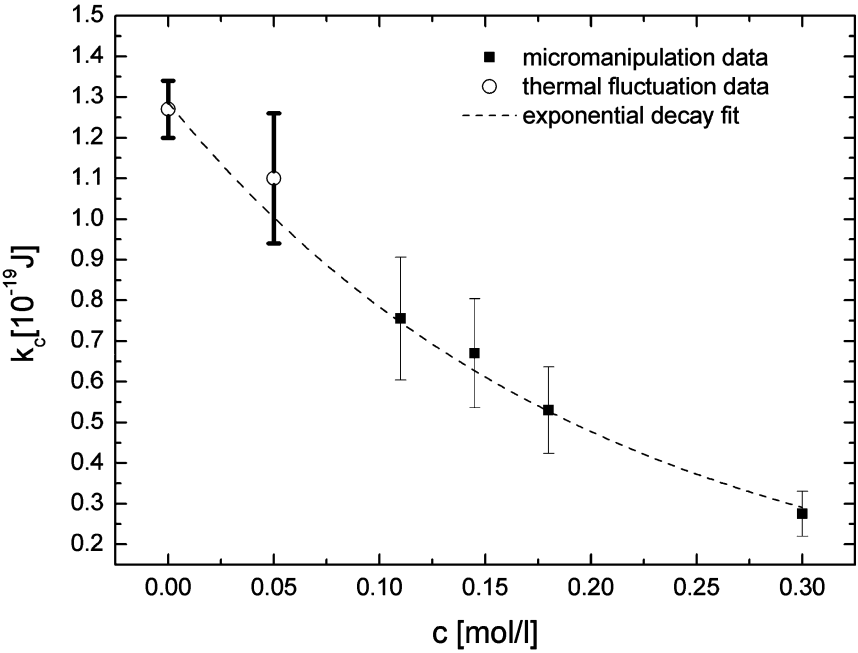

FIGURE 6 Bending elasticity of SOPC membrane as a function of the sucrose concentration in the aqueous phase. ■ – results obtained via micromanipulation of giant vesicles; ○ – experimental points obtained via analysis of the thermally induced shape fluctuations of quasi-spherical lipid vesicles; --- – exponential fit of the type $k_c(c) = k_c(0)\exp(-c/c_0)$ with $k_c(0) = (1.29 \pm 0.07) \times 10^{-19}$ J and $c_0 = 0.20 \pm 0.02$ mol/l.

concentration of sucrose, the result for this quantity was $(0.52 \pm 0.05) \times 10^{-19}$ J. Consequently in the frames of the experimental error there is no difference in the action of the sucrose and fructose on the membrane bending elasticity.

Our experimental results for the sugar influence on the membrane elastic properties are in good agreement with the results previously obtained by other authors for the influence of sugars on the membrane mechanical properties [2,7]. They show that the interactions of the carbohydrate molecules with the lipid layers cannot be neglected. Evidently, a part of these molecules builds up into the membrane, forming a kind of an additive in it. The concentration of the additive in the membrane is proportional to the concentration of the carbohydrate molecules in the aqueous phase. The decrease of the bending elasticity at low sugar concentrations in the water phase, when the concentration of the additive molecules in the membrane is also low is in agreement with the theoretical predictions for the influence of inclusions with low concentrations in the membrane on the value of its bending elasticity [18].

4. CONCLUSIONS

The dependence of the bilayer elasticity (bending and stretching) on the concentration of various sugars in the aqueous phase was investigated via two independent methods (giant vesicle micromanipulation and thermal fluctuation analysis). The sucrose effect on the lipid monolayer stretching elasticity was studied (up to our knowledge for the first time) via the emulsion droplet micromanipulation. All the results, obtained by us, reveal the strong effect of the sugar molecules on the lipid mono- and bilayer elasticity, despite the existing opinion for their neutrality, and raise points towards the theoretical elucidation of this significant influence. Finding the origin of the sugar influence on the membrane elasticity could lead to a better understanding of their biological role and significance. Experimental studies, permitting a strong reduction of the experimental error of the elasticity measurements, achieved by using stroboscopic illumination of a fluctuating vesicle are now in progress.

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