JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 9, No. 2, February 2007, p. 427 - 430

Monosaccharides (fructose, glucose) and disaccharides (sucrose, trehalose) influence the elasticity of SOPC membranes

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The elasticity of SOPC lipid membranes in presence of monosaccharides (fructose, glucose) and disaccharides (sucrose, trehalose) was studied by thermally induced shape fluctuations of giant quasi-spherical vesicles. The vesicles were prepared in aqueous solutions with different sugar concentrations (0-400mM), using an electro-formation technique. Stroboscopic illumination was applied in order to remove the video camera integration time smearing. The experimental results show that all the sugars studied influence the bending elasticity k_c of SOPC membranes. The strength of the effect depends on the type of the sugar and its concentration.

(Received November 1, 2006; accepted December 21, 2006)

Keywords: Elasticity, Sugars, Lipid membranes

1. Introduction

Despite their differing functions, all biological membranes have a common structure: a very thin film of lipid and protein molecules, which are able to move about in the plane of the membrane.

Under laboratory conditions, a wide range of artificial membranes can be made from amphiphilic molecules (lipids), which assemble in water to build bilayer membranes and vesicles. In addition to their applications in the medical, food, and cosmetics industries, these lipid bilayers and vesicles are simple models of biological membranes and cells, especially for studying physical properties such as shape transformations, elasticity and transport. In this work, we studied some biologically significant monosaccharides: glucose and fructose and disaccharides: sucrose and trehalose and their influence on the elasticity of the lipid membrane. It has been experimentally proved [1] that disaccharides have a stabilizing effect on biological membranes.

Glucose, a monosaccharide (or simple sugar), is one of the most important carbohydrates in biology. The cell uses it as a source of energy and a metabolic intermediate. Glucose is one of the main products of photosynthesis and starts cellular respiration in both prokaryotes and eukaryotes. Glucose contains six carbon atoms and an aldehyde group (Fig. 1 a) and is therefore referred to as an aldohexose. The glucose molecule can exist in an open-chain (acyclic) and ring (cyclic) form, the latter being the result of an intramolecular reaction between the aldehyde C atom and the C-5 hydroxyl group to form an intramolecular hemiacetal. In water solution, both forms are in equilibrium, and at pH 7 the cyclic one is the predominant.

Fructose (or levulose) is a simple sugar (monosaccharide) found in many foods and one of the three most important blood sugars along with glucose and galactose. Honey, tree fruits, berries, melons and some root vegetables, such as beets, sweet potatoes, parsnips and onions, contain fructose, usually in combination with sucrose and glucose. Fructose is a levorotatory monosaccharide with the same empirical formula as glucose ($C_6H_{12}O_6$) but with a different structure (Fig. 1b). Pure fructose has a sweet taste similar to cane sugar, but with a "fruity" aroma. Although fructose is a hexose (6 carbon sugar), it generally exists as a 5-member hemiketal ring (a furanose). This structure is responsible for the longer metabolic pathway and higher reactivity compared to glucose.

Sucrose (common name: table sugar, also called saccharose) is a disaccharide (glucose + fructose) with the molecular formula $C_{12}H_{22}O_{11}$ (Fig. 1 c). It is best known for its role in human nutrition.

Trehalose, also known as mycose, is a 1-alpha (disaccharide) sugar found extensively but not abundantly in nature. It is thought to be implicated in anhydrobiosis - the ability of plants and animals to withstand prolonged periods of desiccation. The sugar is thought to form a gel phase as cells dehydrate, which prevents disruption of internal cell organelles by effectively splinting them in position. Trehalose is a non-reducing sugar formed from two glucose units joined by a 1-1 alpha bond giving it the name of α-D-glucopyranogluco-pyranosyl-1,1-α-D-glucopyrano-side (Fig. 1d). The bonding makes trehalose very resistant to acid hydrolysis, and therefore stable in solution at high temperatures, even under acidic conditions.

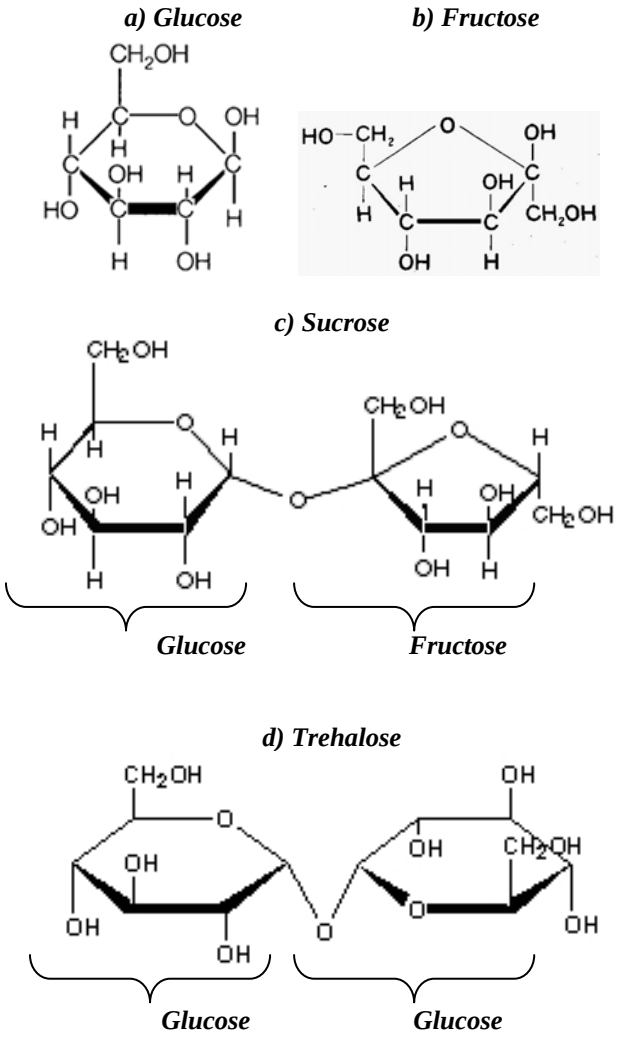

Fig. 1. Structure of the studied saccharides.

The first theoretical models for the mechanical properties of lipid membranes proposed by Helfrich [2] and Evans [3] describe the elastic energy per unit area of the lipid membrane, F_c by the expression:

$$F_c = \frac{1}{2} k_c \left(c_1 + c_2 - c_0\right)^2 + \overline{k}_c c_1 c_2 \tag{1}$$

where: c_1 and c_2 are the membrane principal curvatures, c_0 is the spontaneous curvature, and k_c and $\overline{k}_c$ are the bending and saddle bending elastic moduli of the lipid bilayer, respectively. The spontaneous curvature of a symmetric membrane vanishes, $c_o = 0$.

After the first detailed theoretical model of thermally induced shape fluctuations was proposed by Milner and Safran [4], experimental procedures, based on the analysis of thermally induced shape fluctuations of quasispherical vesicles, were developed for precise measurements of the bending elastic modulus [5, 6]. The fundamental expression used by the authors was [4]:

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

where $\left\langle \left|U_n^m(t)\right|^2 \right\rangle$ is the mean squared amplitude of the spherical harmonic $Y_n^m(\theta,\varphi)$, k_B is Boltzmann's constant, T is the absolute temperature, n is the mode number and $\overline{\sigma} = \sigma R^2 / k_c$ (or $\overline{\sigma} = \sigma R^2 / k_c + 2c_0 R + c_0^2 R^2 / 2$, if $c_0 \neq 0$) is the dimensionless membrane tension.

In fact what is measured in an experiment of a fluctuating quasi-spherical giant vesicle is the equatorial cross section radius. It is shown in [5] that its time averaged angular autocorrelation function is a sum of Legendre polynomials with amplitudes B_n, related to the mean squared amplitudes of the spherical harmonics:

$$B_n = \frac{2n+1}{4\pi} \left\langle \left|U_n^m(t)\right|^2 \right\rangle \tag{3}$$

where the factor $2n+1$ is due to the $2n+1$ different m-modes for a given n, and 4π comes from the different normalizations of the Legendre polynomials and spherical harmonics.

In all the experimental data provided in this work, stroboscopic illumination was used to remove the artifact due to the video camera integration time and present an instant picture of the object to the observer [7, 8].

2. Experimental equipment

The samples of the fluctuating giant vesicles were observed using a phase contrast microscope (Axiovert 100, Zeiss, Germany, objective LD Ph2 63x NA 0.75) using home-assembled stroboscopic illumination (xenon flash lamp L6604, external main discharge capacitor E7289-01, power supply C6096, all by Hamamatsu, Japan) synchronized with the vertical sync pulses coming from the CCD video camera controller (C2400-60, Hamamatsu, Japan). According to the Hamamatsu data sheet, the light pulses are less than 3-4 μs long (full width at half maximum) at 2 J input energy.

The pulsed light of the stroboscopic illumination is irritating for the eyes, so the samples were observed on an attached TV monitor. Due to the "sample and hold" effect of the CCD matrix, the picture on the monitor appears like continuous illumination. The video signal from the camera was also fed to a frame grabber board (DT3155, Datatranslation, USA) mounted in a computer for a proper digitization (768 x 576 8-bit pixels). The obtained digital data were further recorded on the hard disk drive of the PC. Every second, an image was acquired and recorded till the total number of images reached a preliminary given value (about 400 or so). Although the CCD had "square pixels" the images had to be corrected (via digital interpolation and resampling) for the sdifference of the scale factors in x and y directions, due to the mismatch of the CCD's pixel shift clock (in the CCD camera

controller) and the pixel acquisition clock (in the frame grabber).

The value of the scale factor was determined by the ratio of the above mentioned clocks, taken from the respective data sheets and verified by x and y calibration using an object micrometer rule oriented in the respective directions. Further details on the contour determination, mean squared amplitudes calculation and fitting procedure for determining the bending elastic modulus, k_c and the dimensionless membrane tension, $\bar{\sigma}$, can be found in the article of Faucon et al. [5].

3. Materials and methods

All the experiments were performed with bilayers composed of l-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC, Avanti Polar Lipids Inc., USA). In our experiments, we used glucose, fructose, sucrose and trehalose (SigmaUltra® purity, 99.5% (GC), Germany). All chemicals were used without any further purification. The giant vesicles studied in these experiments were prepared using an electroformation method [9]. The lipid was dissolved in chloroform 1mg/ml. With this lipid solution, a number of small drops were laid on the surface of the glasses of the experimental cell (covered by a conductive ITO layer, working as an electrode) in order to obtain as much as possible of the lipid deposits for vesicle formation. After the entire evaporation of the solvent, the experimental cell was filled with sugar solution with concentrations 0 - 400 mM in double distilled water. A low frequency (10Hz) sinusoidal alternative voltage (from 100mV PP (peak to peak) to 1.5V PP) was applied to the conductive glasses for about 5 hours, which led to the formation of giant vesicles, appropriate for our experiment. We chose giant (diameter of the order of 20-40 μm) fluctuating vesicles without any visible defects.

4. Results and discussion

The results for the bending elasticity modulus, k_c, measured via the analysis of thermally induced shape fluctuations of giant vesicles for different concentration of glucose, fructose and trehalose in the aqueous phase (0 - 400 mM) are presented in Fig. 2. The data for sucrose, as published in [10], are depicted for comparison. The values for the bending elasticity modulus for every sugar concentration were calculated as weighted average values for about 10-30 vesicles. The common trend (except for 400 mM glucose) is that sugars reduce the bending modulus with increasing sugar concentration. We believe that the higher than expected value of the bending modulus at a 400 mM glucose concentration is due to the relatively small number of vesicles studied at this particular concentration, all of which probably contained two bilayers. The experimental value of the bending modulus, divided by 2, is also depicted in Fig. 2 for comparison.

It was implicitly considered in the literature that sugars (glucose and fructose) do not influence the bending and stretching elastic modulus of lipid bilayers and were widely used to increase the contrast in almost all micropipette experiments [11-15] and thermal fluctuation experiments [16, 17].

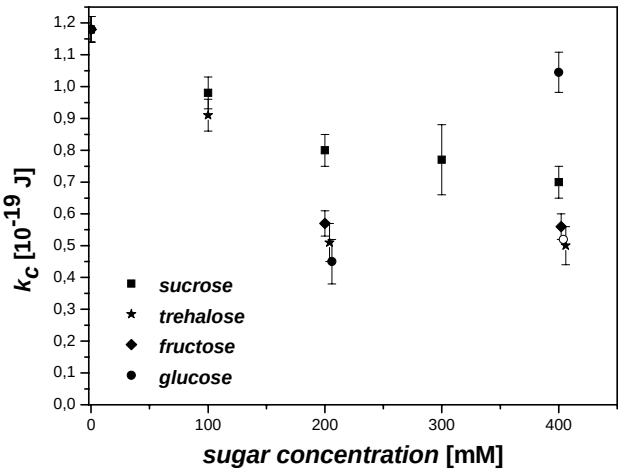

Fig. 2. Bending elastic coefficient k_c of a SOPC membrane as a function of the sugar concentration in the aqueous phase, for different types of sugar.

Our studies reveal that using sugars for image contrast leads to an underestimation of the bending elasticity modulus, dependant on the concentration used.

What are the reasons for the influence of sugars on the bending elasticity modulus?

Data from freezing in lipid lamellar phases [18] show that sucrose and trehalose increase the hydration of the lamellar phase, disrupt the water structure and thus reducing the repulsive hydration interaction between membranes.

Results of a molecular simulation of a phospholipid bilayer [19] reveal that the interaction of disaccharide molecules with the bilayer occurs at the surface of the bilayer, and is governed by the formation of multiple hydrogen bonds to fit onto the surface topology of the bilayer, often interacting with up to three different lipids simultaneously. At high disaccharide concentrations, the results of simulations indicate that disaccharides can serve as an effective replacement for water under anhydrous conditions.

Evidently, sugars perturb the lipid/water structure in the hydrophilic region of the membrane, and this leads to a consequent change in the membrane bending elasticity.

Acknowledgements

This study was supported by Grant No.F-1506 from the Ministry of Education and Science of Bulgaria.

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