Does maltose influence on the elasticity of SOPC membrane?

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Abstract. Thermally induced shape fluctuations of giant quasi-spherical lipid vesicles are used to study the influence of the disaccharide maltose, dissolved in the aqueous solution, on the curvature elasticity \( k_c \) of a lipid membrane. The influence of the carbohydrate solute is investigated throughout a considerably wide interval of concentrations. The values of the bending elastic modulus for 200 mM and 400 mM of maltose in the water solution are obtained. The data for \( k_c \) in presence of maltose is compared with previously obtained results for this constant for the most popular hydrocarbons: monosaccharides glucose and fructose and disaccharides sucrose and trehalose. It is shown that the presence of maltose, dissolved in the aqueous phase surrounding the membrane does not influence on the bending elasticity with the increase of its concentration in the aqueous solution. Up to our knowledge this is the first sugar that does not show decrease of the bending elastic modulus of the lipid membrane, when present in the water surrounding it in concentration up to 400mM.

1. Introduction

The biological cell is the functional basic unit of life. It is the functional unit of all known living organisms. It is the smallest unit of life and is often called the building block of life. That is why the biological cell appears to be the most natural object of interest when properties and functions of living organisms are investigated. The biological cell besides its plasma membrane consists of organelles, most of which are or contain membrane structures. This is the basic motivation for the profound study of variety of physical, chemical, mechanical, rheological, etc. properties of biomembranes. Usually it is realized by the means of modeling them with much more simple and easy for theoretical description model objects and subsequently complicating the system in order to obtain model systems resembling afterwards the real membranes. The simplest models of the biological cells are the lipid vesicles (figure 1). They are closed structures formed by a model lipid membrane (a lipid bilayer). They are prepared from natural or synthetic lipids in laboratory conditions using different formation techniques.

This work is a continuation of our previously made study of the influence of carbohydrates on the elasticity of model lipid bilayers. Carbohydrates are made of 3 elements, carbon, hydrogen and oxygen. Along with proteins and fats they comprise the major components of living matter and are used for maintenance of cellular functional activities and as reserve and structural material for cells. Carbohydrates are formed by green plants in the process of photosynthesis. Humans and animal life obtain most efficiently the carbohydrates they need from the plant world. Carbohydrates provide energy for all the processes in the living cell. All life activities - digestion, blood circulation, heart activity, thinking, walking, etc. are dependent upon carbohydrates.
Carbohydrates are also known as saccharides and are classified according to numbers of single carbohydrate molecules in each chemical structure as monosaccharides, disaccharides and polysaccharides. Mono- and disaccharides can be grouped together because of their common features. They are water soluble, and they have a sweet taste and a crystalline structure, share the suffix “ose” meaning sugar and are called sugars. Monosaccharides are the only sugars that can be absorbed and utilized by the body. Disaccharides and polysaccharides are ultimately broken down into monosaccharides in the digestive process before they can be utilized by the body. Disaccharides are also divided into reducing and non reducing. Typical representatives of non reducing disaccharides are sucrose and trehalose. Typical representatives of reducing disaccharides are maltose and lactose.

Using the thermally induced shape fluctuation method we studied profoundly the influence of the most popular monosaccharides fructose and glucose and most popular no reducing disaccharides sucrose and trehalose [1, 2] and we know that all of them influence on the bending elastic modulus of the lipid membrane. The bending elastic modulus $k_c$ decreases sufficiently with the increase of the sugar concentration in the aqueous for the membrane solution. In this work we focused on another disaccharide - maltose, which is a typical representative of reducing disaccharides (figure 2).

Maltose or malt sugar is the least common disaccharide in nature. It is present in germinating grain, in a small proportion in corn syrup, and forms on the partial hydrolysis of starch. Maltose, the disaccharide obtained by enzyme-catalyzed hydrolysis of starch, consists of two D-glucopyranoses joined by a 1,4'-beta-glycoside bond. Both maltose and cellobiose are reducing sugars because the anomeric carbons on the right-hand sugar are part of a hemiacetal. Maltose, however, is digested without difficulty and is fermented readily.

### 2. Theoretical models

The first theoretical models for the mechanical properties of lipid membranes proposed by Helfrich [3] and Evans [4] describe the elastic energy per unit area of lipid membrane, $F_\varepsilon$, by the expression:

$$ F_\varepsilon = \frac{1}{2} k_\varepsilon \left( c_1 + c_2 - c_0 \right)^2 + \bar{k}_\varepsilon c_1 c_2 $$

(1)

where: $c_1$ and $c_2$ are the membrane principal curvatures, $c_0$ is the spontaneous curvature, and $k_\varepsilon$ and $\bar{k}_\varepsilon$ are bending and saddle bending elastic moduli of lipid bilayer, respectively. The spontaneous curvature of a symmetric membrane in a symmetric environment vanishes, $c_0 = 0$.

After the first detailed theoretical model of thermally induced shape fluctuations proposed by Milner and Safran [5], the experimental procedures, based on the analysis of thermally induces shape...
fluctuations of quasi-spherical vesicles are developed for the precise measurements of the bending elastic modulus \([6, 7]\). The fundamental expression used by the authors is \([5]\):

\[
\left\langle U_n^m(t)^2 \right\rangle = \frac{k_BT}{k_B} \frac{1}{(n-1)(n+2)(\sigma + n(n+1))}
\]

where \(\left\langle U_n^m(t)^2 \right\rangle\) is the mean squared amplitude of the spherical harmonic \(Y_n^m(\theta, \phi)\), \(k_B\) is the Boltzmann’s constant, \(T\) is the absolute temperature, \(n\) is the mode number and \(\sigma = aR^2/k_c\) (or \(\overline{\sigma} = aR^2/k_c + 2c_0/2 + c_0R^2/2\), if \(c_0 \neq 0\)) is the dimensionless membrane tension.

In fact what is measured in an experiment of fluctuating quasi-spherical giant vesicle is the equatorial cross section radius. It is shown in \([6]\) that its time averaged angular autocorrelation function is a sum of Legendre polynomials with amplitudes \(B_n\), related to the mean squared amplitudes of spherical harmonics:

\[
B_n = \frac{2n+1}{4\pi} \left\langle U_n^m(t)^2 \right\rangle
\]

where the factor \(2n+1\) is due to the \(2n+1\) different \(m\)-modes for a given \(n\) all of them having the same mean squared amplitude and \(4\pi\) comes from the different normalizations of Legendre polynomials and spherical harmonics.

In all the experimental data provided in this work stroboscopic illumination is used to remove the artifact due to the video camera integration time. Stroboscopically illuminated sample presents an instant picture of the object to the observer \([8, 9]\).

### 3. Experimental equipment

The samples of the fluctuating giant vesicles were observed under phase contrast microscope (Axiovert 100, Zeiss, Germany, objective LD Ph2 63x NA 0.75).

The experimental equipment was improved using home-assembled stroboscopic illumination using commercial parts (xenon flash lamp L6604, external main discharge capacitor E7289-01, power supply C6096, all by Hamamatsu, Japan). The flash of the stroboscopic illumination was synchronized with the vertical 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 should be observed on an attached monitor. Due to the “sample and hold” effect of the CCD matrix the picture on the monitor is like in the case of a continuous illumination. The video signal from the camera was fed also to a frame grabber board (DT3155, Data translation, USA) mounted in a computer for a proper digitization (768 x 576 8-bit pixels). The obtained digital data was further recorded on the hard disk drive of the PC. Every second an image was acquired and recorded till the total number of images reaches a preliminary given value (about 400 or so). Nevertheless that the CCD has "square pixels" the images have to be corrected (via digital interpolation and resampling) for the difference 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 to determine the bending elastic modulus, \(k_c\), and the dimensionless membrane tension \(\overline{\sigma}\), can be found in the paper \([6]\).
4. Materials and Methods

All the experiments were performed with bilayers composed of l-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine ((C18:0/C18:1) SOPC, Avanti Polar Lipids Inc., USA). The hydrocarbons used were products with high purity > 99% (GC): D-(+)-Maltose monohydrate (Sigma - Aldrich). All the chemicals were used without any further purification.

The giant vesicles, studied in these experiments were prepared using a modification of the electroformation method. The lipid is dissolved in chloroform 1 mg/ml. A simple electroformation cell, used for all experimental procedures is shown on figure 3. The frame of the cell was made out of electro technical sheet getinaks (micarta). It is a laminated material obtained by hot pressing of paper impregnated with a thermosetting phenol-formaldehyde- or epoxy-based binder. Glasses, coated with transparent conductor, indium tin oxide (ITO; thickness of 100 ± 20 nm, resistivity of 100 Ω/□) acting as an electrode, were glued on the both sides to the cell’s frame. The electrodes were 3 to 4 mm apart (d=3 or d=4 mm). Over the electrodes an ac voltage is applied, while the electroformation process is started.

A number of small drops of the lipid solution are laid on the surface of the glasses of the experimental cell in order to obtain as much as possible lipid depots for vesicle formation. Meanwhile a solution with the desired concentration is prepared in fresh double distilled water. After the entire evaporation of the solvent the experimental cell is filled with this previously made sugar solution. We established the most suitable for our experiment regime for electroformation. A low frequency (10 Hz) sinusoidal alternative voltage is applied (a step-like increase from 0.1 V PP (peak to peak) to 1.5 V PP) to the conductive glasses for about 4 hours. This procedure leads to the formation of vesicles, appropriate for our experiment. We choose giant (diameter of the order of 20-40 µm) fluctuating vesicles without any visible defects.
5. Results and Discussion
The analysis of thermally induced shape fluctuations of giant vesicles is used to study the influence of one typical reducing disaccharide - maltose on the elasticity of lipid membrane. The obtained experimental data for the bending elasticity modulus, $k_c$ for different concentration of the carbohydrate in the aqueous phase ($0 - 400 \text{ mM}$) are presented in Table 1. The values for the bending elasticity modulus for every sugar concentration are calculated as a weighted average value of about 10-15 vesicles.

Comparing the obtained results we can conclude that in the frames of the error the values for the bending elastic modulus for the different concentrations of maltose in the solution are the same. So, we can claim that in such concentrations ($0 – 400 \text{ mM}$) in the surrounding medium of the membrane the maltose does not influence on its bending elasticity.

Table 1

| Maltose concentration in the aqueous solution | 0 mM (pure SOPC membrane) | 200 mM | 400 mM |
|---------------------------------------------|---------------------------|--------|--------|
| Bending elastic modulus of the lipid membrane $k_c$ | $(1.17 \pm 0.10) \times 10^{-19} J$ | $(1.26 \pm 0.04) \times 10^{-19} J$ | $(1.23 \pm 0.05) \times 10^{-19} J$ |

Previously obtained [1]

This result is getting even more interesting in comparison with our previous results, obtained using the same method for different sugars. On the figure 4 the data for 4 different sugars are given: monosaccharides fructose and glucose and non-reducing disaccharides sucrose and trehalose [2]. For easy comparison the data for maltose is depicted on the same figure with “◦”.

Figure 4. Bending elastic modulus $k_c$ of SOPC membrane as a function of the carbohydrate inclusion concentration in the aqueous phase for different types of carbohydrates: monosaccharides - glucose and fructose and disaccharides- sucrose, trehalose and maltose.
We can conclude from the received experimental data that the reducing disaccharide maltose has different behaviour in the proximity of the lipid molecules, comprising the bilayer structure than the other type of sugars: monosaccharides fructose and glucose and non-reducing disaccharides sucrose and trehalose.

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