Influence of Cholesterol on the Elastic Properties of Lipid Membranes

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Abstract. Thermally induced shape fluctuations of giant quasi-spherical lipid vesicles are used to study the influence of the cholesterol, incorporated in the lipid membranes, on the bending elasticity modulus $k_c$ of the lipid membrane. The influence of cholesterol is investigated throughout a considerably wide interval of concentrations. The values of the bending elastic modulus for 10, 20, 30 and 50 mol\% of cholesterol in the SOPC membrane are obtained as a mean weighted value of 6-11 vesicles for each system. The dependence of the bending elasticity modulus on the concentration of cholesterol in the lipid membrane is obtained. At low concentration of cholesterol in the SOPC membrane (10 mol \%) a decrease of the bending elasticity modulus is observed, compared to pure SOPC membrane. At high cholesterol content (50mol\% and above) a twofold increase of the bending modulus is obtained. The data for $k_c$ for mixed SOPC - cholesterol membrane is compared to the results obtained by different methods on different lipid matrices.

1. Introduction

The simplest model of the biological cell is the so-called lipid vesicle (figure 1). It is a closed structure formed by a double lipid layer (bilayer). Such objects are prepared from natural or synthetic lipids in laboratory conditions using different formation techniques [1, 2]. By means of adding proteins, carbohydrates, cholesterol etc. to the lipid matrix the studied object is subsequently complicated in order to obtain model system resembling the real membranes.
Cholesterol (figure 2) is a vital component of the living cell membranes varying up to 50 mol % of the total lipid content [3]. It is necessary for the synthesis of hormones of the adrenal cortex - cortisone, corticosterone, aldosterone, male hormones - testosterone, androsterone, and female sex hormone - estrone, estriol, estradiol, progesterone, vitamin D and bile acids. Cholesterol is considered as a key molecule in the formation of small raft domains, providing a favorable nanoenvironment for the biochemical function of membrane proteins. At the high concentrations it is found in the cell membranes (close to 50 mol %) cholesterol separates the phospholipids so that the fatty acid chains can't come together and decreases the temperature of lamellar liquid crystal – gel phase transition.

These are just some of the reasons for the constant scientific interest towards this molecule and its influence on different physicochemical properties of the lipid membranes.

There are many studies available in the literature, investigating the influence of cholesterol on the mechanical properties of lipid membranes using different experimental methods as micromanipulation [4], tether pulling [5], vesicle deformation induced by electric fields [6], nuclear magnetic resonance (NMR), X-ray diffraction [4, 8-10], etc. These studies demonstrate that cholesterol has significant ordering effect on the lipid hydrophobic chains and its presence in the cell membrane leads to an increase of the lipid bilayer thickness and decrease of the area per molecule. It is also shown in the literature that the effect of cholesterol is lipid-specific. For example, the bending modulus of dioleoylphosphatidylcholine (DOPC) membranes does not change significantly after addition of cholesterol, but the sphingomyelin membranes become more flexible [6]. The presence of cholesterol strongly increases bending modulus when both chains are fully saturated, but not at all when there are two unsaturated chains [7, 8].

NMR measurements of acyl-chain order parameter of lipids with perdeuterated chains show that cholesterol disorders the lipid chain below the main transition and orders the chain above it [11 Ipsen).

In this work we focus our attention on the influence of cholesterol on the elastic properties of SOPC lipid membranes in pure water environment. Using the non-invasive method - thermally
induced shape fluctuations of nearly spherical vesicles (liposomes) we measure the bending elastic modulus $k_c$ of lipid vesicle’s membrane in presence of different percentage of cholesterol in it.

2. Theory

The first theoretical models for the mechanical properties of lipid membranes proposed by Helfrich [12] and Evans [13] describe the elastic energy per unit area of lipid membrane, $F_c$, by the expression:

$$F_c = \frac{1}{2} k_c (c_1 + c_2 - c_0)^2 + \kappa c_1 c_2$$

(1)

where: $c_1$ and $c_2$ are the membrane principal curvatures, $c_0$ is the spontaneous curvature, and $k_c$ and $\kappa$ are bending and saddle bending elasticity 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 has been proposed by Milner and Safran [14], the experimental procedures, based on the analysis of thermally induces shape fluctuations of quasi-spherical vesicles were developed for the precise measurements of the bending elastic modulus [15, 16]. The fundamental expression used by the authors is [14]:

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

(2)

where $\left\langle |U_n^m(t)|^2 \right\rangle$ is the mean squared amplitude of the membrane fluctuations’ decomposition in spherical harmonics $Y_n^m(\theta, \phi)$, $k_B$ is the Boltzmann's constant, $T$ is the absolute temperature, $m$ and $n$ are the numbers, characterizing the given mode and $\sigma = \sigma R^2 / k_c$ is the dimensionless membrane tension (an adjustable parameter depending on the membrane tension and the difference of the lipid molecules between the inner and the outer layer of the lipid bilayer).

In fact what is measured in an experiment of fluctuating quasi-spherical giant vesicle is the equatorial cross section radius in 128 equidistant directions from the center of the vesicle for every recorded image. In spherical coordinates the radius of the vesicle in given direction can be written by the expression:

$$\rho(\phi, t) = R [1 + u(\pi / 2, \phi, t)]$$

(3)

where $R$ represents the radius of a sphere with equal volume and $u(\theta = \pi / 2, \phi, t)$ is a function describing the membrane fluctuations, $\theta = \pi / 2$ represents the equatorial cross section of the vesicle with the plane, passing through the vesicle centre, parallel to XY plane of the coordinate system. It is assumed that the amplitudes of the fluctuations are small compared to the vesicle radius, $|u(\theta, \phi, t)| << 1$.

The normalized angular autocorrelation function of the vesicle radius is given by the expression:

$$\xi(\gamma, t) = \frac{1}{R^2} \left[ \frac{1}{2\pi} \int_0^{2\pi} \rho(\phi + \gamma, t) \rho^*(\phi, t) d\phi - \rho^2(t) \right]$$

(4)
It is shown in [15] that the time averaged angular autocorrelation function can be decomposed into Legendre polynomials with amplitudes $B_n$, related to the mean squared amplitudes of spherical harmonics:

$$B_n = \frac{2n+1}{4\pi} \left\langle |j_n^m(t)|^2 \right\rangle$$  \hspace{1cm} (5)

Taking into account the relations (4), (5) and (2) we can calculate the bending elasticity modulus of the membrane from the decomposition of the angular autocorrelation function of the equatorial cross-section radius as following:

$$k_c = \frac{1}{B_n}  \frac{k_BT}{4\pi} \frac{(2n+1)}{(n-1)(n+2)(\sigma + n(n+1))}$$  \hspace{1cm} (6)

In all the experimental data provided in this work stroboscopic illumination was 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 [17-19].

3. Materials and Methods

All the experiments are performed with vesicles composed of SOPC (1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (C18:0/C18:1), Avanti Polar Lipids Inc., USA). The cholesterol (5-Cholest-3β-ol) used is product with high purity > 99% (GC): (Sigma - Aldrich). All the chemicals are used without any further purification.

The giant vesicles, studied in these experiments are prepared via modified electroformation method. The lipid is dissolved in chloroform 1 mg/ml. The cholesterol is dissolved in methanol 2 mg/ml. The final lipid–cholesterol solution for electroformation is prepared by mixing SOPC and cholesterol in the desired proportion for every cholesterol content. The electroformation cell, used for all experimental procedures is shown on figure 3. Glasses, coated with transparent conductor, indium tin oxide (ITO; thickness of 100 ± 20 nm, resistivity of 100 Ω/□) are acting as electrodes.

![Figure 3 (a). An image of the electroformation cell.](image1)

![Figure 3 (b). An image of the electroformation set-up (electroformation cell connected to the generator.](image2)

A number of small drops of the final 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. The prepared in this way glasses are put under vacuum for at least 30 minutes. After the entire evaporation of the solvent the experimental cell is filled with double distilled water. We establish the most suitable for our experiment regime of electroformation. A low frequency (10 Hz) sinusoidal alternative voltage was applied (1.5 V PP) to the conductive glasses overnight. This procedure leads to the formation of
vesicles, appropriate for our experiment. We choose giant (diameter of the order of 20-40 \( \mu \)m) fluctuating vesicles without any visible defects. The samples of the fluctuating giant vesicles were observed under phase contrast microscope (Axiovert 100, Zeiss, Germany, oil immersion objective Ph3 100x magnification). The experimental equipment was improved using stroboscopic illumination, based on xenon flash lamp L6604 Hamamatsu, Japan) with damping vibration system, short light pulses (less than 3-4 \( \mu \)s long full width at half maximum) and high input energy (2 J) [19].

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).

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 \( \sigma \), can be found in the article of Faucon et al. [15].

4. Results and Discussion
The analysis of thermally induced shape fluctuations of giant vesicles is used to study the influence of cholesterol on the elasticity of lipid membrane.

Table 1. Bending elasticity modulus of SOPC membrane, containing different concentration of cholesterol. The first column shows the molar percentage of cholesterol in the membrane, the second column shows the mean weighted value of the bending elasticity modulus and the third column shows the number of vesicles over which the mean is calculated.

| Cholesterol concentration (mol %) | Weighted mean value of the bending elasticity modulus \(( k_c \times 10^{-19} (J) \)) | Number of vesicles |
|----------------------------------|---------------------------------|-------------------|
| 0                                | 1.7±0.1                         | 10                |
| 10                               | 1.56±0.05                       | 11                |
| 20                                | 1.98±0.11                       | 11                |
| 30                                | 2.02±0.09                       | 10                |
| 50                                | 2.67±0.13                       | 6                 |

The obtained experimental data for the bending elasticity modulus, \( k_c \), for different concentration of cholesterol in the lipid membrane (0 - 50 mol %) are presented in table 1. The values for the bending elasticity modulus for every cholesterol concentration in the lipid membrane are calculated as a weighted average value of about 6-11 vesicles. The value for the bending elasticity modulus of pure SOPC membrane in double distilled water, measured by the same method is given for comparison. All the experiments are performed in double distilled water environment.

As it can be seen from the obtained data for low cholesterol concentration (10 mol %) the bending elastic modulus decreases in comparison with that of pure lipid membrane. The theoretical studies for the influence of different admixtures on the elastic properties of lipid membranes [20] predict that for all the admixtures, influencing the elastic modulus of lipid membranes at low concentrations in the membranes reduction of the bending elastic modulus should be observed. For the substances that gradually reduce the bending elastic properties with the increase of its concentration in the membrane this result is obvious and experimentally proven. For the case of admixtures causing an increase of the elastic modulus of lipid membranes at high concentrations in the membrane (such as cholesterol) this result is specific and proves the theoretical predictions. Up to our knowledge this is the first experimental prove of the theory for membranes, containing substances increasing the bending elasticity at high concentration in the membrane.
At higher cholesterol content (20 mol % and above) the value for the bending elastic modulus increases with the increase of cholesterol percentage in the lipid membrane. At the highest cholesterol content, studied by us (50 mol %) the value for the bending elasticity modulus is approximately two times higher than that of pure SOPC membrane. The obtained result is in very good agreement with the data, achieved by fitting the liquid crystalline theory with LAXS diffuse scattering data for SOPC membranes, containing cholesterol [21] as well as with the experimental data, obtained by different working groups for the influence of POPC (1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) [10] and DMPC (1,2-Dimyristoyl-sn-glycero-3-phosphorylcholine) [22] on the elastic properties of lipid membranes.

5. Conclusion
The thermally induced shape fluctuation study of the elastic properties of cholesterol containing membranes shows that cholesterol gradually increases the bending elastic modulus of SOPC lipid membrane at concentrations above 20 mol % in the membrane. This result is in good agreement with the existing in the literature data provided by different experimental techniques and for other types of lipid membranes. At low cholesterol content in the membrane (10 mol %) the value of the bending elastic modulus is reduced. This result is in good agreement with the theoretical predictions.

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