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Cholesterol influence on the bending elasticity of lipid membranes

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HIGHLIGHTS

- The influence of cholesterol on the elasticity of SOPC membrane was studied.
- Thermally induced shape fluctuation analysis was used.
- The experiments were performed in pure water environment.
- The presence of cholesterol stiffens the SOPC bilayer.

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ABSTRACT

The influence of cholesterol on the bending elasticity of lipid membranes from 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC) was studied. We used the analysis of thermally induced shape fluctuations of giant nearly-spherical lipid vesicles for the determination of the bending elastic modulus.

At relatively low concentrations (<10 mol %) of cholesterol in the SOPC membrane the bending elasticity modulus first increases with about 30% upon cholesterol increase and after that decreases to a value slightly lower than that of the pure bilayer. The increase of the cholesterol from 10 to 50 mol % leads to a threefold increase of the bending modulus compared to the value of the pure SOPC membrane.

1. Introduction

The lipid vesicles are closed structures formed by a double lipid layer [1]. They can be considered as the simplest model of a biological cell. Such objects are prepared from natural or synthetic lipids in laboratory conditions using different formation techniques [2,3]. The vesicle's membrane can be complicated by adding proteins, carbohydrates, cholesterol etc. to the lipid matrix in order to obtain model system resembling the real biomembranes.

The cholesterol is a vital component of the living cell membranes and its concentration could vary up to 50 mol% of the total lipid content [4].

The cholesterol has a significant ordering effect on the lipid hydrophobic chains. Its presence in the cell membrane leads to an increase of the lipid bilayer thickness and decrease of the area per molecule [5–8].

The effect of cholesterol on the mechanical properties of the lipid membranes is lipid-specific. For example the bending modulus of dioleoylphosphatidylcholine (DOPC) membranes does not change significantly after addition of cholesterol, while the sphingomyelin membranes become more flexible [5]. The presence of cholesterol strongly increases the bending modulus when both chains are fully saturated. When the lipid molecule possesses two unsaturated chains the bending modulus is practically not changed when cholesterol is added [6–8].

The data in the literature for the influence of cholesterol on the bending elasticity of SOPC membrane is quite scarce. The direct methods used for such systems (micropipette technique [18], thermal fluctuation analysis [19] tether formation [6,17]) present values for the bending elasticity modulus of the membrane for 1:1 SOPC/Cholesterol only and in presence of sugars (sucrose inside the vesicle and glucose outside of it, i.e. in nonsymmetrical conditions). Both sucrose and glucose have been shown to have a significant impact on the bending elasticity [12,13], which has to be separated from that of cholesterol. The aim of the present work is to study the influence of cholesterol on the elastic properties of SOPC membrane in a wide interval of concentrations in pure water environment via a direct and non invasive method as thermally induced shape fluctuation method. Furthermore, the applied method has been recently refined [21] to ensure improved reliability of the data.

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2. Theory

The description of the mechanical properties of lipid membranes proposed by Canham [14] and Helfrich [15] presents the elastic energy F_c per unit area of lipid membrane by the expression:

$$F_c = \frac{1}{2}k_c(c_1 + c_2 - c_0)^2 + \bar{k}_c c_1 c_2, \quad (1)$$

where c_1 and c_2 are the membrane principal curvatures in the point where F_c is determined, c_0 is the membrane spontaneous curvature, k_c is the bending elasticity modulus and $\bar{k}_c$ is the saddle splay bending elasticity modulus.

After the first detailed theoretical model of thermally induced shape vesicles fluctuations which has been proposed by Milner and Safran [16], experimental method, based on the analysis of the shape fluctuations of nearly-spherical vesicles were developed permitting the precise measurements of the bending elastic modulus [17,18].

Briefly, the membrane is considered as a closed surface $S(t)$, defined at a time t in the laboratory frame of reference XYZ, whose center is appropriately placed inside the vesicle. Let (θ,φ) be the polar angles of a direction, piercing the membrane at the moment t in a point with radius vector $R(\theta,\varphi,t)$, whose value can be presented as follows [16]:

$$R(\theta, \varphi, t) = R_0\left[1 + u(\theta, \varphi, t)\right], \quad (2)$$

where R_0 is the radius of a not fluctuating sphere with volume V, equal to that of the vesicle.

The function $u(\theta,\varphi,t)$ can be decomposed in a series with respect to the spherical harmonics $Y_i^j(\theta, \varphi)$:

$$u(\theta, \varphi, t) = \sum_{i=0}^{i_{max}} \sum_{j=-i}^{i} u_i^j(t)\, Y_i^j(\theta, \varphi) \quad (3)$$

The cutoff $i_{max} \sim R_0/\lambda$ is determined by λ appearing as some typical intermolecular distance.

The time mean squares of the amplitudes $\left\langle \left| u_i^j(t) \right|^2 \right\rangle$ satisfy the relation [16]:

$$\left\langle \left| u_i^j(t) \right|^2 \right\rangle = \frac{k_B T}{k_c} \frac{1}{(i-1)(1+2)(\bar{\sigma} + i(i+1))}, \quad (4)$$

where k_BT is the Boltzmann factor, $\bar{\sigma} = \frac{\sigma(R_0)^2}{k_c}$ is the normalized membrane tension, and σ is the membrane tension.

Thus from the fit of the experimentally determined mean square amplitudes with Eq. (4) one could calculate the elasticity k_c and the membrane tension, σ.

3. Materials and methods

The vesicles were prepared from l-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC) (C18:0/C18:1)-(Avanti Polar Lipids Inc. (USA)), containing appropriate quantities cholesterol (5-Cholesten-3β-ol, Sigma-Aldrich). The lipid was dissolved in chloroform with concentration 1 mg/ml. The cholesterol was dissolved in methanol with concentration 2 mg/ml. The final lipid–cholesterol solution was prepared by mixing SOPC and cholesterol solutions in the desired proportion for each cholesterol content. The electroformation cell, used for the preparation of the giant vesicles, consisted of two glass slides, coated with the transparent conductor indium tin oxide (thickness, 100 ± 20 nm, with resistivity of ~100 Ω/square) acting as electrodes and a polydimethylsiloxane (PDMS) spacer, preliminary treated to ensure no emission of impurities in the cell. A number of small drops of the final lipid/cholesterol solution were pipetted on the surface of the glasses of the formation cell in order to obtain as much as possible lipid depots for vesicle formation. The prepared in this way glasses were put under vacuum for at least 30 min. After the entire evaporation of the solvent the experimental cell was filled with double distilled water. 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 chose giant (diameter of the order of 20–40 μm) fluctuating vesicles without any visible defects-see Fig. 1.

All experiments were performed in double distilled water environment at 27 °C.

The samples of the fluctuating giant vesicles were observed under microscope (Axiovert 100, Zeiss, Germany, oil immersion objective Ph3 100× magnification) working in phase contrast regime. In all the experimental data, provided in this work, stroboscopic illumination was used to remove the artifact due to the finite integration time of the video camera. Stroboscopically illuminated sample presents an instant picture of the object to the observer [19]. The experimental equipment is based on xenon flash lamp L6604 (Hamamatsu, Japan) with damping vibration system, short light pulses (less than 3–4 μs long) and high input energy (2 J) [20].

An algorithm for digitalization and processing of image sequences of fluctuating vesicles with a detailed procedure for obtaining the mechanical constants of the vesicular membrane, applying strict objective criteria for qualification of the vesicle as a whole as well as for acceptance or rejection of a given contour of the sequence of recorded images [21] was used for all the experimental data presented in the work. The white noise contribution to the amplitudes of thermal shape fluctuations [21] was evaluated and taken into account in the reported values for the bending elasticity modulus.

4. Results and discussion

We performed systematic experiments with lipid membranes containing seven different cholesterol concentrations between 3% and 50%. Between 2 and 12 vesicles in pure water were observed and processed for each of the studied cholesterol concentrations.

Note that we measure experimentally the equatorial cross section radius in 128 equidistant directions from the center of the vesicle for every recorded image of the fluctuating quasi-spherical giant vesicles. Since the amplitudes of the thermal fluctuations are small enough, the time averaged angular autocorrelation function is decomposed into Legendre polynomials with amplitudes B_n, related to the mean squared amplitudes of spherical harmonics [17]:

$$\left\langle B_n(t) \right\rangle = \frac{(2n+1)}{4\pi} \left\langle \left| u_i^j(t) \right|^2 \right\rangle \quad (5)$$

Table 1 presents the determined values of B_n (up to $n = 19$ [21]) of three different vesicles with similar membrane tensions and different cholesterol concentration. As to be expected the amplitudes decrease with the increase of n. In most of the cases only the first 5 modes are most significant, however the account for all contributions up to the 19th mode increases significantly the accuracy of the method [18–22].

The obtained experimental values for the bending elasticity modulus, k_c as a function of the concentrations of cholesterol in the SOPC membrane are presented in Fig. 2.

Bivas and Meleard [22] have proposed a model description of the elastic properties of lipid membranes that predicts a reduction of the bending elastic modulus of a membrane with the increase of the concentration of a various additives to the lipid. The experimentally observed decrease of k_c as the cholesterol increases from

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