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Stroboscopic illumination study of the dynamics of fluctuating vesicles♣

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The dynamics of thermally induced shape fluctuations of stearoyl oleoyl phosphatidyl choline (SOPC) and dioleoyl phosphatidyl choline (DOPC) quasispherical vesicles was studied. Membrane fluctuations of giant unilamellar vesicles, prepared from these lipids were visualized and recorded in real time via phase contrast microscopy, for a continuous long time interval. The experimental equipment was improved by implementation of stroboscopic illumination based on a xenon flash lamp. In this way, the blur effect due to the finite integration time of the camera was removed and an instant picture of the fluctuating vesicle shape was achieved. The results permitted the estimation of the friction between the monolayers comprising the bilayer of the membrane, and the bending elasticity modulus of blocked exchange of molecules between the monolayers.

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1. Introduction

Cells are the basic building units of most living creatures, so they are an obvious object of interest when the properties and functions of living organisms are investigated. All biological cells, despite of their different functions, have a common feature – they have a plasma membrane and consist of other different intracellular membranes. So, the study of different properties of membranes is the object of investigations in many disciplines: biochemistry, biophysics, colloid chemistry, etc. which implies different approaches of analysis. The simplest model of the biological membrane is the so called model lipid membrane of a giant unilamellar vesicle (GUV), made artificially under laboratory conditions from amphiphilic molecules (lipids). These objects are very useful, especially for studying physical properties such as elasticity, shape transformations, permeability, fluctuation dynamics, etc.

A model for the dynamics of fluctuations of giant vesicles was first proposed by Schneider et al. [1]. They performed experiments by measuring the time autocorrelation function of the difference between two perpendicular diameters of the vesicle, then analyzed these, taking into account only the contribution of the second mode. Milner and Safran [2] introduced some corrections, and built a precise model of the dynamics of fluctuation of a giant vesicle. Considering a thin membrane, they predicted the relaxation of a fluctuating membrane to be solely influenced by the viscosity of the surrounding medium, the bilayer bending elasticity and the vesicle area to volume ratio. Firstly, the mono-exponential character of the shape fluctuation dynamics was confirmed using short time video image sequences of GUVs' thermally induced shape fluctuations [3]. A theoretical revision of the dynamics of the shape fluctuations of quasi-spherical giant vesicles was made [4, 5], and a possible influence of the friction between the monolayers of the bilayer of the vesicle membrane was predicted. Using the interlayer friction coefficient b_s measurements [6], the second dissipative mechanism was predicted to be considerable or negligible for the dynamics, depending on the fluctuation wavelength [4, 5, 7].

If we denote the angular-time autocorrelation function of the vesicle as $\xi(\gamma,\tau)$, the (*n, m*) Legendre function as $P_n{}^m$, the corresponding Legendre polynomial amplitudes as $\beta_n(\tau)$ and the *n, m* ($n \geq 2$ and $-n \leq m \leq n$) spherical harmonics amplitude as U_n^m then according to [3]:

$$\xi(\gamma,\tau) = \sum_{n\geq 2} \beta_n(\tau).P_n^0(\cos\gamma) \qquad (1)$$

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$$\left\langle U_n^m(t).U_n^m(t+\tau)\right\rangle = \frac{4\pi}{2n+1}\times\beta_n(\tau) \qquad (2)$$

The latest theoretical works [4, 5] predict a bi-exponential relation for the normalized amplitudes in the decomposition in Legendre polynomials:

$$\frac{\beta_n(\tau)}{\beta_n(0)} = (1-\chi_n).\exp[-\tilde{\Omega}_n.\tau] + \chi_n.\exp[-\tilde{\omega}_n.\tau] \qquad (3)$$

The coefficient χ_n^m depends on $\tilde{\Omega}_n^m$ and $\tilde{\omega}_n^m$.

As seen from equation (3), the fluctuation dynamics of a giant vesicle is described by two relaxation processes or two exponential decays. They account for the energy dissipation in the liquid phase on the both sides of the membrane and the influence of the friction between the two monolayers comprising the lipid bilayer. The experimental determination of the parameters of the two exponents permits the estimation of the bending elasticity modulus of the membrane for blocked exchange of the molecules (flip-flop) between the monolayers, comprising the lipid bilayer k_c^{bl} and the interlayer friction coefficient b_s [5].

2. Experimental equipment

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

The experimental equipment was improved using stroboscopic illumination from home-assembled commercial parts (a xenon flash lamp L6604, an external main discharge capacitor E7289-01 and a C6096 power supply, all from 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 were 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 to 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 that in the case of continuous illumination. The video signal from the camera was also fed to a frame grabber board (DT3155, Data translation, USA) mounted in a computer for proper digitization (768 x 576 8-bit pixels). The obtained digital data were further recorded on the hard disk drive of the PC. Every single image was acquired and recorded until the total number of images reached a given value (about 10,000). Despite the fact that the CCD has "square pixels", the images had to be corrected (via digital interpolation and resampling) for the difference of the scale factors in the 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 of the contour determination, mean squared amplitudes calculation and fitting procedure to determine the bending elastic modulus, k_c, and the dimensionless membrane tension, $\bar{\sigma}$, can be found in [7].

3. 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 and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine DOPC (C18:1/C18:1), from Avanti Polar Lipids Inc., USA, without any further purification. The giant vesicles studied in these experiments were prepared using the electroformation method [8]. The lipid was dissolved in chloroform 1 mg/ml. The experimental cell comprised two glasses (model 215 735 P0 covered with a 100 (+/-20) nm conductive layer of ITO, sheet resistance 100 Ohm/square), separated from each other by teflon spacers. The conductive wires (electrodes - one for each conductive glass) for the voltage applied from the generator were attached to the glasses of the experimental cell using conductive silver paste, and were isolated from the water medium with silicone. A number of small drops of the lipid solution were laid on the surface of the glasses of the experimental cell, in order to obtain as many lipid depots as possible for vesicle formation. After the entire evaporation of the solvent, the experimental cell was filled with doubly distilled water. Following the most suitable arrangement for our experiment regime, a low frequency (10 Hz) sinusoidal alternative voltage was applied (a step-like increase from 0.1 V PP (peak to peak) to 1.5 V PP) to the conductive glasses for about 5 hours, which led to the formation of giant vesicles, appropriate for our experiment. We choose giant (diameter of the order of 20-40 μm) fluctuating vesicles, without any visible defects.

4. Results and discussion

Using the realized stroboscopic illumination, the dynamics of the thermally induced shape fluctuations of a quasispherical vesicle was studied. Every image (40ms between two subsequent images) of the studied vesicle was recorded for a period of about 7 minutes, in which approximately 10 000 images were acquired.

An important criterion for selecting the vesicles is their stationarity during the experiment. In order to examine the time stationarity of the vesicles, the time dependence of the squares of the amplitudes of the second mode in the autocorrelation function decomposition in Legendre polynomials was plotted for every vesicle. In Fig. 1, both possible cases for this dependence are shown. The experimental data were fitted with a linear function of the type $y=a+bn$ ($n=0\div N_{max}$) and the values for the constants with their dispersions *a±Δa* and *b±Δb* were obtained.

The dispersion σ_y of a given variable *y* is expressed as

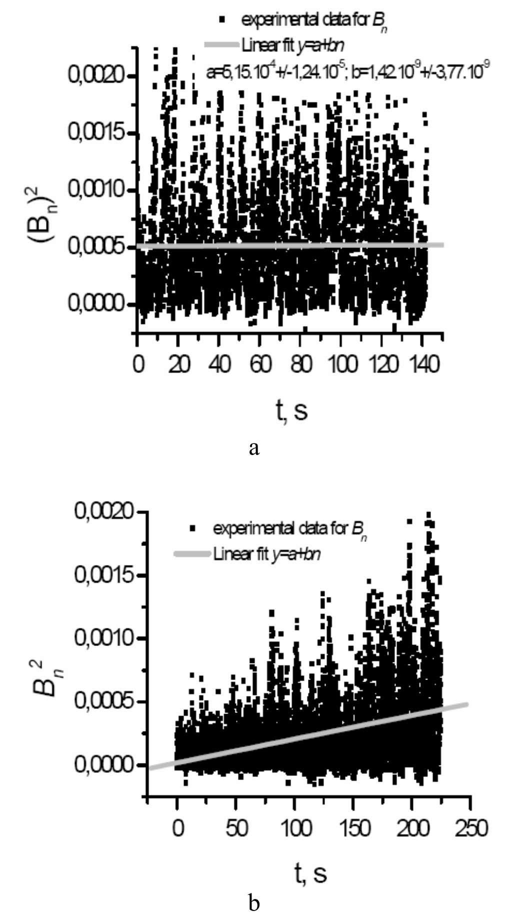
$$\sigma_y^2 = \frac{\sum (y_i - \bar{y})^2}{N} \tag{4}$$

Where N is the number of measurements and the dispersion $\sigma_{\bar{y}}$ of its mean value $\bar{y}$ is

$$\sigma_{\bar{y}}^2 = \frac{\sum (y_i - \bar{y})^2}{N(N-1)} \tag{5}$$

Or

$$\sigma_y = \sigma_{\bar{y}} \sqrt{N} \tag{6}$$



a

b

Fig. 1. Time dependence of the squares of the amplitudes of the second mode in the autocorrelation function decomposition in Legendre polynomials, with gray being a linear fit: a) stationary liposome, b) not stationary liposome.

We introduce the following criterion for the time stationarity of the vesicle:

$$\frac{|b|\,N_{\max}}{2} << \Delta a \sqrt{N_{\max}} \tag{7}$$

Where N_{max} is the maximal time value at which B_n (the amplitude of the second mode in the autocorrelation function decomposition in Legendre polynomials) is acquired.

Using the above criterion, we consider as time stationary and take into account in the further data processing only vesicles for which the value of $|b| N_{\max} / 2$ is at least one order of magnitude less than the value of $\Delta a \sqrt{N_{\max}}$.

The acquired experimental data show that for the liposomes with high enough precision of the amplitudes of the spherical harmonics, it is possible to distinguish two exponential decays. In Fig. 2, the time dependences of the normalized amplitudes of the second mode of the spherical harmonics are depicted on a semi logarithmic scale.

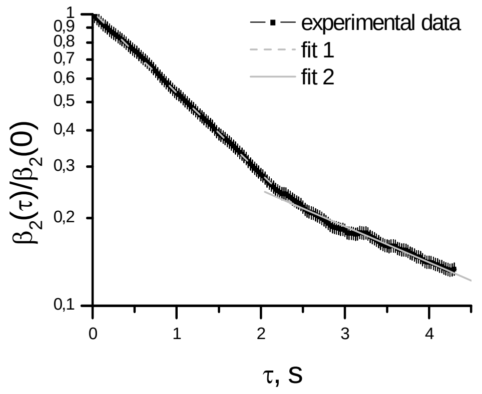


Fig. 2. Time dependence of the normalized amplitudes of the second mode of the spherical harmonics for a given vesicle, on a semi-logarithmic scale.

The experimental data were fitted with an equation of the type: $y=A\exp(-t_1\tau)+(1-A)\exp(-t_2\tau)$ and the coefficients:

$$A = \chi_n^m; \quad t_1 = \tilde{\omega}_n^m; \quad t_2 = \tilde{\Omega}_n^m$$

were obtained. Using a computer program, accounting for the exact relation between these coefficients and the constants k_c^{bl} and b_s, the bending elasticity modulus of the membrane for blocked flip-flop k_c^{bl} and an interlayer friction coefficient b_s are evaluated.

In Table 1, our estimations for the bending elasticity modulus of lipid membrane for blocked exchange of molecules between the two monolayers, comprising the lipid bilayer and the intermolecular friction coefficient, are given for both of the studied lipids.

Table 1. Bending elastic modulus for blocked flip-flop and intermolecular friction coefficient for both studied

lipids.

Lipid type	k^{bl}_c	b_s
SOPC	$[k^{fr}_c – 2\ k^{fr}_c]$ $(k_c^{fr}=0{,}95\text{x}10^{-19}\ J)$	$< 6.\ 10^7$ Ns/m^3
DOPC	$1{,}4\ k^{fr}_c$ $(k_c^{fr}=0{,}86\text{x}10^{-19}\ J)$	$2{,}6.\ 10^9$ Ns/m^3

Let us examine the two limiting cases for the ratio of the bending elastic modulus for free flip-flop to that for blocked flip-flop. In the theory of Helfrich [8], where the membrane was modeled via two thin elastic lamellae, the studied ratio is $k^{bl}_c = 4\ k^{fr}_c$. In the other limiting case, where the membrane is described in terms of the Petrov-Derzhanski-Mitov model and $d=\delta$ и $k_H=0$, then $k^{bl}_c = k^{fr}_c=0$ [9]. In other words, the bending elasticity modulus for blocked flip-flop must be in the range from that for free flip-flop to 4 times this modulus $k_c^{bl} \in [k_c^{fr} \div 4k_c^{fr}]$. Thus, the estimations obtained in the frames of our experiment are in a good agreement with the theoretical predictions, based on different models.

The estimation of the friction coefficient between the monolayers, comprising the lipid bilayer, is in a good agreement with the value obtained by Merkel et al., using a different experimental method - b_s~10^8 Ns/m^3 [6].

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