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Bending elasticity of lipid membranes in presence of beta 2 glycoprotein I in the surrounding solution

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Abstract. Thermally induced shape fluctuations of giant quasi-spherical lipid vesicles are used to study the bending elasticity modulus k_c of a phospholipid (PHLP) membranes in presence of beta 2 glycoprotein I (β2-GPI) in the aqueous solution which surrounds the vesicle's membrane. The bending elastic modulus k_c of PHLP – protein membrane was obtained for different mass concentrations of β2-GPI for pure neutral SOPC membranes and for mixed SOPC: Cardiolipin negatively charged membranes. The experimental results for the bending elastic modulus k_c of the PHLP membranes does not show dependence on the concentration of β2-GPI in the range from 5.5 to 55 μg/ml, when β2-GPI is present in the aqueous solution surrounding the vesicle's membrane. Obtained results are in good agreement with predictions, based on different experiments, explaining the mechanism of binding of β2-GPI to neutral membranes.

1. Introduction

The lipid bilayer is a thin membrane made of two layers of lipid molecules. These membranes are flat sheets that form a continuous barrier around cells. The cell membrane of almost all living organisms and many viruses are made of lipid bilayers, as are the membranes surrounding the cell nucleus and other sub-cellular structures. Biological membranes typically include several types of lipids and integral membrane proteins. The mechanical properties of model lipid membranes containing biologically relevant proteins are of great interest, because they give valuable information on the way to more profound understanding of properties and functioning of the living cell.

β2-GPI is a soluble 50-54 kDa plasma glycoprotein, primarily synthesized in the liver. It acts as a natural anticoagulant [1, 2]. The physiological function of this protein is not fully understood, but it is known that β2-GPI is involved in variety of physiologically relevant processes such as blood coagulation, clearence of phosphatidylserine (PS) and cardiolipin (CL) containing liposomes from the circulation, membrane budding process [3] and diseases, such as the modulation of platelet-depending thrombosis, atherosclerosis and autoimmune diseases, such as antiphospholipid syndrome [4].

β2-GPI is present in human blood plasma at a concentration of 150-300 μg/ml [5]. The isoelectric point of β2-GPI is at pH between 5-7, so the protein is negatively charged at physiological pH 7.4 [6,

7]. This protein can interact with the PHLP membranes by electrostatic interactions and hydrophobic loops [6].

β2-GPI is composed of four domains, which are structurally similar, the fifth domain contains an extra C-terminal loop, which is necessary for PHLP binding [6]. Both ionic and hydrophobic interactions of the fifth domain are important for binding of β2-GPI to PHLP membranes. In contrast to other apolipoproteins, lipid-free β2-GPI does not self-associate in aqueous solution. The positively charged multilysine rich region on fifth domain interacts with negatively charged lipid head-groups and hydrophobic flexible loop putatively inserts into the PHLP layer.

The crystal structure of β2-GPI shows an overall fish-hook-like appearance with dimensions of 130 Å in vertical direction and 85 Å in horizontal direction [5], as indicated on the figure 1.

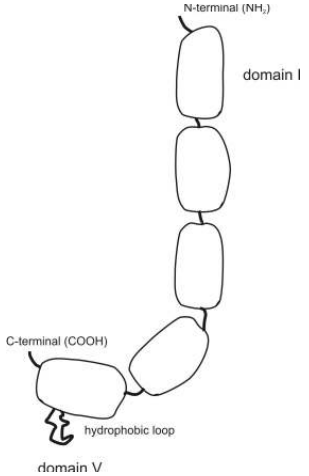

Figure 1. Crystal structure of β2-GPI.

The structural and biochemical data indicate a relatively simple membrane binding mechanism. Positive charges on domain V interact with the anionic PHLP head-groups and flexible loop putatively inserts into the PHLP layer; at the interface region between the acyl chains and phosphate head-groups of the PHLPs, thus anchoring itself to the membrane [5].

To better understand how β2-GPI affects the mechanical properties of model lipid membranes we can apply the experimental technique of thermally induced shape fluctuations of giant PHLP quasi-spherical vesicles.

2. Theory

The first theoretical models for the mechanical properties of lipid membranes proposed by Helfrich [8] and Evans [9] describe the elastic energy per unit area of lipid membrane, F_c by the expression:

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

where: c_1 and c_2 are the membrane principal curvatures, c_0 is the spontaneous curvature, and k_c and $\bar{k}_c$ 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 has been proposed by Milner and Safran [10], the experimental procedures, based on the analysis of thermally induced shape fluctuations of quasi-spherical vesicles were developed for the precise measurements of the bending elastic modulus [11, 12]. The fundamental expression of thermally induced shape fluctuations as in [10] is:

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

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

In fact what is measured in the experiment of fluctuating quasi-spherical giant vesicle is the equatorial cross section radius. It is shown in [11] 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 like:

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

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 4π comes from the different normalizations of Legendre polynomials and spherical harmonics.

3. Materials and Methods

β2-GPI was isolated from pooled human plasma and was concentrated using Microcon centrifugal filter device with YM-30 Ultracel membrane [13]. Aliquots of β2-GPI in PBS (1.2 mg/ml) were stored at –20°C. The final concentration of β2-GPI in the experiments was 5.5 μg/ml, 27.5 μg/ml and 55 μg/ml, which is approximately half of the free β2-GPI concentration in human plasma. Before the start of electroformation procedure, the stock suspension of 1.2 mg/ml of β2-GPI was diluted in sucrose solution to a final concentration.

For vesicle preparation we used the lipids: 1-stearoyl-2oleoyl-sn-glycero-3-choline (SOPC) and 1',3'-bis[1,2-dioleoyl-sn-glycero-3-]-sn-glycerol (cardiolipin, CL) purchased from Avanti Polar Lipids. All lipids were dissolved in chloroform in concentration of 1 mg/ml. Two different lipid mixtures were prepared: first mixture of 9:1 volume ratio of SOPC:CL (~5.5 mol% of CL) and second mixture of 4:1 volume ratio of SOPC:CL (~11.6 mol% of CL).

For electroformation we used two different cells. In figure 2 is shown the cell, made out of getinaks (micarta). Glued glasses were coated with transparent indium tin oxide conductor (ITO), which acts as an electrode. The distance between the two ITO glasses was 4 mm. Over the electrodes an ac voltage was applied, to start the electroformation process.

In figure 3 is shown a smaller electroformation cell. The tube's cap was pierced to insert four Pt electrodes. The electrodes were 3-4 mm apart. We used 4 electrodes to increase the quantity of PHLP in the whole volume and consequently to increase the number of electroformed GUVs.

Lipid mixture of SOPC and CL was put in small drops over ITO glass electrodes, and similarly, on every Pt electrode. The electroformation cell or tube cap with Pt electrodes was put in vacuum for 15-20 minutes to evaporate the organic solvent (chloroform). After the entire evaporation of the organic solvent, the cell was assembled and filled with desired aqueous solution (double distilled water or mixture of sucrose solution and phosphate buffer saline).

The cell was connected to the function generator (HP 33120A) with output termination of 50 Ω.

The preparation procedure for GUVs formation is summarized in table 1. All the experiments were done at room temperature.

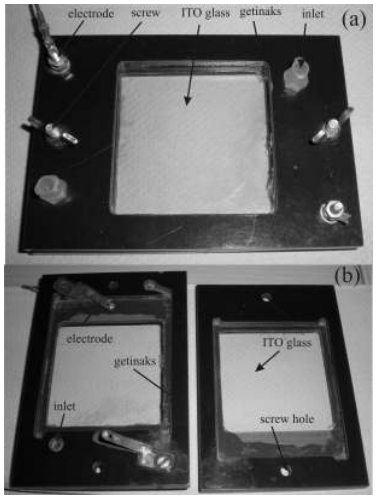

Figure 2. Electroformation cells with ITO electrodes.

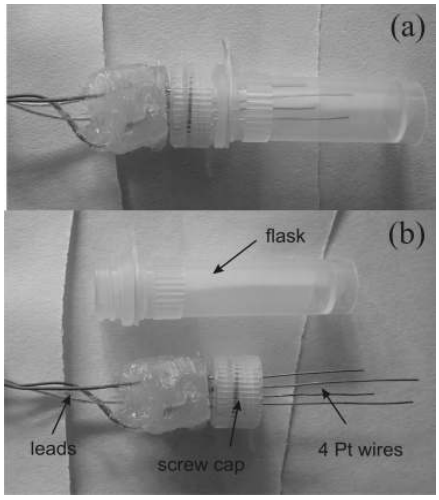

Figure 3. Electroformation cell with 4 Pt electrodes.

A: Assembled electroformation cell; B: Disassembled electroformation cell.

Table 1. Electroformation procedure for forming SOPC:CL GUVs.

ac voltage, peak-peak [mV]	Frequency [Hz]	Time duration [min]
100	10	5
200	10	5
300	10	15
600	10	60
1500	10	120

4. Results and Discussion

In the process of studying the properties of lipid vesicles an important element is their formation procedure. In order to increase the quality and quantity of lipid vesicles and to reduce the impurities in the solution, we constructed seven different modifications of electroformation cells:

A. Four platinum wires for electrodes in glass flask;
B. Four platinum wires for electrodes in plastic vial;
C. Two ITO glasses for electrodes with a rubber O-ring as a spacer;
D. Two ITO glasses for electrodes with a FFKM O-ring (Trelleborg sealing solutions) as a spacer;
E. Two ITO glasses for electrodes in getinaks frame
F. Two ITO glasses for electrodes in plexiglass frame
G. Two stripes of ITO glass for electrodes in glass flask.

In order to compare the seven different modifications of the electroformation cells we study the time dependence of their resistance. Every cell was thoroughly washed with solvents (chloroform and methanol) and distilled water. After the entire evaporation under vacuum the cells were filled with pure double distilled water and connected to a digital Lock-in amplifier (Stanford Research Systems, model SR830). A 280 Ω resistor was connected in series to the electroformation cell. At given output voltage (40 mV_{RMS} @ 1 kHz sine) from the Lock-in amplifier, a voltage drop on the resistor was measured for 5 hours (approximately twice the electroformation time). The obtained results are normalized and plotted in figure 4.

The electroformation cell conductivity was measured consequently, as the voltage drop across on the 280 Ω resistor. As can be seen the conductivity of double distilled water

increased with time. The increase of conductivity could come due to impurities, which are emitted into the pure water. The impurities could originate from materials used for cell assembly or from electrodes.

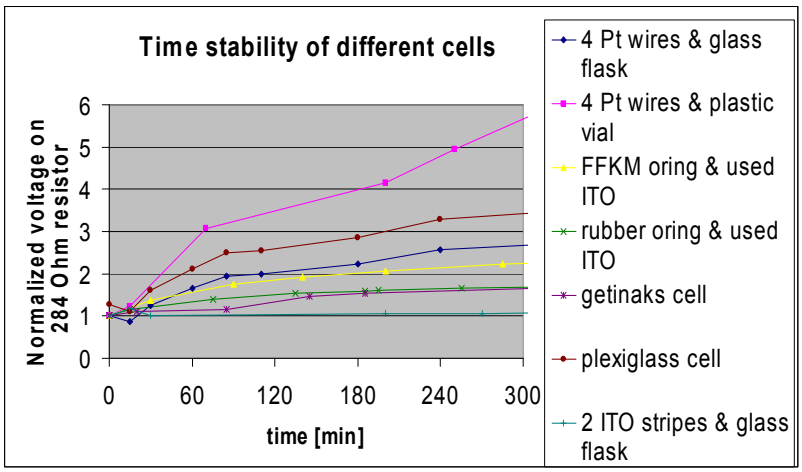

Figure 4. Normalized time dependence of the resistance of different cell modifications.

As it can be seen from figure 4 the most stable with time is the cell G - two stripes of ITO glass for electrodes in a glass vial, followed by the cell E- two ITO glasses for electrodes in getinaks frame and C- two ITO glasses for electrodes with a rubber O-ring as a spacer. It is also seen that the cells E and C show very similar dependencies.

The obtained experimental data give important information for the time dependence of the cell resistance of the most frequently used electroformation cells and their modifications, used by a lot of scientific groups, dealing with lipid vesicles and give motivation for usage of one cell in spite of another for every different experiment and system.

Using the method of thermally induced shape fluctuation of giant quasi spherical PHLP vesicles, we obtained the values for the bending elastic modulus k_c for different systems of interest: at three different concentration of β2-GPI present in the aqueous solution 5.5 μg/ml; 27.5 μg/ml and 55 μg/ml for pure neutral SOPC membrane and mixed SOPC: CL in 9:1 volume ratio of negatively charged membrane. The obtained results for the mean values of k_c for different studied systems are summarized in table 2.

For every system the mean value of the bending elastic modulus and its standard deviation is calculated from the Gaussian distributions (with their mean values and standard deviations). For calculating the mean values of k_c, the number of vesicles for every system was at least 7 and up to 30.

From the obtained results we can conclude that the bending elastic modulus of PHLP membranes, surrounded by glycoprotein β2-GPI does not depend on the concentration of β2-GPI in the range 5.5 – 55 μg/ml (the protein was added directly into 280 mmol/l sucrose solution used for formation of giant PHLP vesicles).

The obtained values for the bending elastic modulus k_c of the studied systems are higher than the approximate value for elastic modulus of pure SOPC membranes in of 280 mmol/l sucrose solution, which is $k_c \approx (0.75 \pm 0.05) x 10^{-19} J$ [14].

In many studies the interaction of β2-GPI with liposome membranes containing negatively charged PHLPs (CL, PS, PA, PI) was shown. The interaction with neutral PHLPs (PC, PE, SM) was marginal [16, 17, 7]. But from PHLP monolayer surface pressure study [15], it was shown, that β2-GPI also interacted with neutral (DPPC) lipid monolayer, not only with negatively charged (DPPS) lipid monolayer. The interaction was indicated by an increase in initial surface pressure of lipid monolayer.

Our results indicate that there was some interaction between β2-GPI and neutral PHLP membranes. The interaction could be observed through an increase of bending elastic modulus k_c, obtained from thermally induced shape fluctuation method. k_c does not depend much on β2-GPI concentration.

Table 2. The mean bending elastic modulus k_c of giant vesicles for different studied systems.

Phospolipid content	β2-GPI	Aqueous solution	Bending elastic modulus x 10^{-19} J	Number of studied vesicles
SOPC	5,5 μg/ml	280mM sucrose	$\langle k_c \rangle = 1{,}152 \pm 0{,}05$	7
SOPC	27,5 μg/ml	280mM sucrose	$\langle k_c \rangle = 0{,}98 \pm 0{,}051$	8
SOPC	55,5 μg/ml	280mM sucrose	$\langle k_c \rangle = 1{,}082 \pm 0{,}042$	30
SOPC:CL = 9:1	5,5 μg/ml	280mM sucrose	$\langle k_c \rangle = 1{,}030 \pm 0{,}05$	11
SOPC	0; (control)	280mM sucrose	$\langle k_c \rangle = 1{,}002 \pm 0{,}053$	20

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