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Sulfatide incorporation effect on mechanical properties of vesicles

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ABSTRACT

The effect of the sulfatide incorporation on the nanomechanical properties of pure dipalmitoylphosphatidylcholine (DPPC) vesicles was studied using atomic force microscope surface. The forces, measured between an AFM tip and the vesicle, presented that the breakthrough of the tip into the vesicles occurred two times. Each breakthrough represented each penetration of the tip into each bilayer. Force data prior to the first breakthrough were fitted well with the Hertzian model to estimate Young's modulus and bending modulus of the vesicles. It was found that the incorporation led to decrease by around 90% in Young's modulus and bending modulus of the vesicles. The decrease appears to be attributed to the disruption of DPPC headgroup packing, which is caused by the larger hydration shell around the sulfatide headgroup.

1. Introduction

Sulfatides (galactosylceramidesulfates) are negatively charged glycosphingolipids, being minor constituents of most eukaryotic membranes, but are more abundant in the nervous system and especially important for brain myelin membranes [1]. In many cells sulfatides have been found to function as receptors for neurotransmitters, opiates, endorphins and a heat shock protein, Hsp70 [2–8]. In the myelin membranes sulfatides take part in contact formation between bilayers through interaction with galactoylceramide, a neutral glycosphingolipid [9–11]. This interaction is important for the stability of the myelin in the central nervous system and the myelin membranes are also highly enriched in both galactosylceramide and cholesterol [9,11]. It is becoming increasingly evident that glycosphingolipids participate in cell–cell interactions, possibly through defined membrane regions called glycosignalling domains [10]. Therefore, the understanding the physical behavior of sulfatides seems to play an important role in biological membranes.

Previous model membrane studies have shown that sulfatides are protected against antibody recognition in a sphingomyelin/cholesterol environment compared to a phosphatidylcholine/cholesterol environment [12], suggesting significant differences in the interaction with sphingolipid versus phospholipid rich membranes. Studies on the membrane properties of sulfatides have often been conducted on biological mixtures of sulfatides with a variety of acyl chains of different lengths and degree of hydroxylation [13,14]. The membrane properties of sulfatides have also been shown to be dependent on divalent cations [15,16]. However, little study has been performed about the effect of sulfatides on the physical properties of the membranes.

Mechanical properties of the membranes can be related to many aspects of the behavior of lipid vesicles, such as their formation, stability, size, shape, fusion, and budding processes [17]. Atomic force microscope (AFM) has proven to be an advantageous tool for measurements of forces of interaction between the AFM probe and the surface, giving information about the physical properties of sample surface [18–20]. On approach of the AFM probe tip to the sample, repulsive forces can be measured, such as electrostatics, salvation, hydration, and compression-related steric forces. The retraction force curves often show a hysteresis referred to as an adhesion pull-off event, which can be used to estimate the adhesion forces. Much experimental force data are now available in the literature, and theoretical models have been developed for the analysis of forces acting between two solid surfaces and to a lesser extent the properties of thin films [21–25]. In this work, we aim to investigate the effect of sulfatide on the mechanical properties of vesicle.

2. Experimental

Dipalmitoylphosphatidylcholine (DPPC) and sulfatides were purchased from Avanti Polar Lipids (Alabaster, AL), and used without further purification. The lipids were dissolved in chloroform and methanol (70:30) with a desired composition of sulfatides in

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the mixture of the lipids. The chloroform and methanol were subsequently removed using a dry stream of nitrogen. The lipid film was dried overnight at low pressure to remove any trace of impurities of the organic solvent. The films were immersed in a buffer solution containing 10 mM Hepes, 50 mM KCl, and 1 mM NaN_3 at pH 7. The lipid suspension was kept for at least 1 h above 10° above the gel–fluid transition temperature. During this period the suspension was vortexed several times. Large unilamellar vesicles were made by extruding the suspension through two stacked 100 nm pore size polycarbonate filters. For the composition, 0, 5, 10, 15, and 20 mol% were selected because it was found that the sulfatides were stably mixed with DPPC in their bilayer configuration up to 20 mol% [26]. Dynamic light scattering was used to measure the diameter of the vesicles (ELS-8000, Otsuka, Tokyo, Japan). The diameter for the all of vesicles was approximately 150 ± 20 nm.

AFM measurements were made at 25 °C using an optical lever microscope equipped with a liquid cell (Nanoscope v5.12, Veeco, Santa Barbara, CA). The contact mode topographic images were taken in the constant-deflection mode using the commercial cantilevers with typical radius of curvature of 20 nm and spring constants of 0.2 N/m. Mica was placed on the AFM scanner. The AFM cantilever was mounted onto a liquid cell and carefully lowered onto the O-ring, sealing it between the cell and the mica. After the mica was brought close to the AFM cantilever tip, the vesicle solution was injected into the fluid cell. The vesicle solution was allowed to incubate on mica at room temperature for 1 h. Excess vesicles were removed by flushing the fluid cell with the buffer solution. Force curves were obtained on and around the vesicles. However, only the force curves with two jump-in points and highest onset point will be accepted for further analysis. The force curves with two jump-on points correspond to measurement obtained directly on the vesicle and will represent the interaction between the tip and the vesicles.

Approaching force distance data was fitted to the Hertzian contact model assuming a spherical shape for the tip [27,28]. The indentation from the difference between the cantilever distance z–z_0 and cantilever deflection d–d_0 is described in the equation.

$$\begin{aligned} &|z - z_0| - (d - d_0) \\ &\quad = \delta = A(d - d_0)^{2/3} \\ &\quad = 0.825 \left[\frac{k^2 (R_{\text{tip}} + R_{\text{ves}})(1 - \nu_{\text{ves}}^2)^2}{E_{\text{ves}}^2 R_{\text{tip}} R_{\text{ves}}} \right]^{1/3} (d - d_0)^{2/3}, \end{aligned} \quad (1)$$

where δ is the indentation, E_{ves} is the Young's modulus of the vesicle, R_{tip} and R_{ves} are the radius of the tip and vesicle, respectively, ν_{ves} is the Poisson's ratio of the vesicle, and k is the cantilever spring constant. The vesicle radius is taken to be equal to $z_0/2$. Experimental data can be fitted to the equation with two fit parameters A and z_0. In general, the fitted z_0 value was found to be consistent with the visually examined contact point. Thus, z_0 is determined by the onset point of the repulsive force. E_{ves} is then computed from A (obtained by least square fitting). Only data with indentation less than 10 nm were used to ensure elastic behavior [27]. Bending modulus k_c is deducted from Young's modulus based on the equation:

$$k_c = \frac{E_{\text{ves}} h^3}{12(1 - \nu_{\text{ves}}^2)}, \quad (2)$$

where h is the bilayer thickness.

3. Results and discussion

In nanometer scale morphology, no significant difference from the incorporation of sulfatide into the vesicles was found. No difference was predicted, because extrusion above the transition

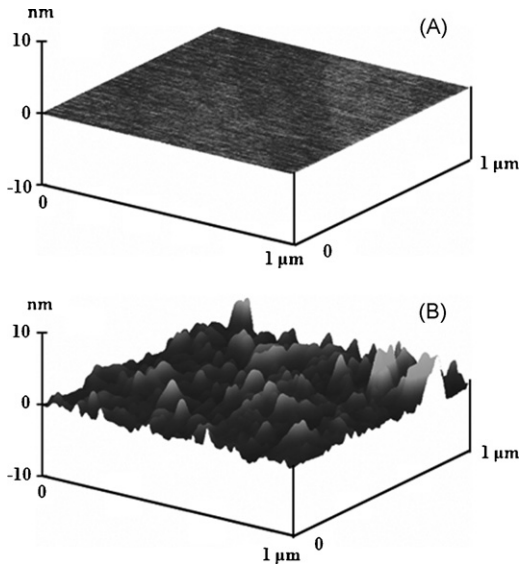

Fig. 1. AFM images (A) before the lipid vesicle adsorption and (B) after the lipid vesicle adsorption.

temperature of the lipids was performed in order to remove the effect of the change in the diameter on the mechanical properties. In this research, study was conducted only on the effect of the sulfatide incorporation on the properties. The AFM images were shown in Fig. 1. The image before the lipid vesicle adsorption is presented in Fig. 1(A). In Fig. 1(A), maximum step height is observed less than 1 nm, and cross-section width of most morphological bumps is found almost flat. Fig. 1(B) shows the image after the lipid vesicle adsorption. Step height reaches up to almost 10 nm and cross-section width of most bumps are found around 190 ± 60 nm.

The approaching force curve provides a wealth of stepwise mechanical deformation events on the lipid vesicles. Fig. 2(A) shows a deflection versus z position plot on vesicle made with 100% DPPC. Fig. 2(B) is a force versus distance curve based on the data from Fig. 2(A). Fig. 2(A) and (B) can be divided into four regions as labeled. In region (1), the non-contact region, the tip is far away from vesicle and the force between the tip and the vesicle is zero. Region (2) illustrates the elastic deformation of the vesicle under tip compression and therefore can be used to calculate the Young's modulus. Region (3) corresponds to further tip compression after the tip penetrates the vesicle's top bilayer. Region (4) reflects the cantilever deflection when it is in contact with the hard mica substrate after penetrating through the vesicle's bottom bilayer. Theoretically, the slope of region (4) should be 1.0 because the deflection of the cantilever is identical to the z-direction movement of sample on hard surface [29]. Based on the fitted data, a slope of 0.995 was obtained. The two jump-in points during the rise of the steric repulsion correspond to the tip penetrating the upper and lower portion of each vesicle, respectively [30]. Onset of repulsive regime on the bilayer found at 6.3 nm suggests that there is no bilayer adsorbed on the tip and force measurements were conducted on mica. This result is also supported from comparison of force curves made with a clear tip in pure water and with the tip that has been in the vesicle solution for several hours.

The slope of a force curve describes the elastic properties of a sample [27,29]. The slope values of the first repulsive region (2) and second repulsive region (3) before and after the first breakthrough point for different molar ratio sulfatide/DPPC systems are listed separately in Table 1. The experimental results shown are average values, and the range of the results is less than 3%. The values lay in the range of 0.63–0.67 and 3.2–3.4 N/m for the sulfatide/DPPC vesicles. By comparison, the slope values for the pure DPPC vesicles of