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Mechanical properties of protoamine-induced vesicles

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ABSTRACT
The change in the mechanical properties was an indicator to understand the effect of the agents on the biological membranes. The vesicles were prepared with dipalmitoylphosphatidylcholine (DPPC) and protoamine at their desired ratio and exposed to the approaching probe of the atomic force microscope (AFM). The movement of the probe was analyzed with the Hertzian model to estimate Young's modulus and Bending modulus. The properties became lower with the higher ratio of protoamine to DPPC, but were saturated at 0.5 of the ratio. These results appear to be the basis for the antimicrobial activity associated with protoamine, i.e. cellular penetration, on the physical properties of cell membranes.
Keywords – Protoamine, Vesicles, Young's modulus, Bending modulus

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I. INTRODUCTION

Protamine is currently used as an antimicrobial active against a variety of bacteria [1]. Approximately 70 % of protamine is composed of arginine, a strongly basic amino acid with a highly positive surface charge. The strong positive charge of protamine is thought to be important for bacterial access [2]. In fact, protamine can permeate bacterial membranes through electrostatic interactions with negatively charged bacterial coatings in both Gram-negative and Gram-positive bacteria [3,4]. However, the detailed behavior of bacterial cells and the mechanism of cellular penetration remain unclear.

Since the phospholipids distributed in the biological membranes are associated to the antimicrobial action through their physical behavior, the mechanical properties are critical to the biological phenomena [5-8]. Atomic force micro-scope (AFM) can provide the properties with the motion of an AFM probe [9-11]. AFM force data combined with the theories such as Johnson–Kendall–Roberts theory, Poisson-Boltzmann theory, and so on [12-16]. In this study, it was aimed to investigate the mechanical properties of protoamine-induced vesicles because little is known for protoamine metabolic-mechanism to cell membranes.

II. MATERIALS AND METHODS

Dipalmitoylphosphatidylcholine (DPPC) and protoamine were from Sigma Aldrich (St. Louis, MO). The vesicles were prepared in a desired ratio of DPPC and protoamine according to the procedures described in the previous research [17]. The vesicle diameter was between 130 and 170 nm from light scattering. An AFM probe was located in a liquid cell and approached to the mica. The vesicle solution was added to cover the mica surface completely. After 2 hours for the adsorption, the deflection data were collected between the probe and the vesicle [18]. The following theory was considered for the data with twice clear penetrations only.

The selection of data was for the analysis with the Hertzian model, which describes the elasticity of the sphere with the equation below [19,20].

$$\delta = 0.825\left[\frac{(1-\nu_{\text{ves}}^2)^2(R_{\text{tip}}+R_{\text{ves}})}{E_{\text{ves}}^2 R_{\text{tip}} R_{\text{ves}}}\right]^{\frac{1}{3}} F^{\frac{2}{3}} \quad (1)$$

$$\delta = \left|z - z_0\right| - \left(d - d_0\right),\ F = k(d - d_0)$$

where ν_{ves} is the Poisson's ratio of the vesicle, R_{tip} and R_{ves} are respectively the radius [m]

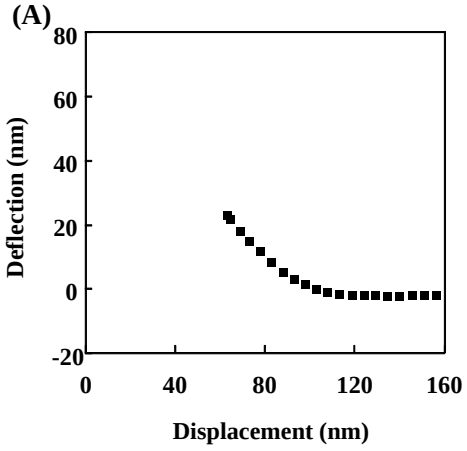
of the probe and vesicle, E_{ves} is the Young's modulus [Pa] of the vesicle, and k is the spring constant [N/m] of the probe. The indentation was acquired from the difference be-tween the probe displacement $|z - z_0|$ [m] and the probe deflection $(d - d_0)$ [m]. z_0 was the distance from the boundary between the regions, and d_0 was the deflection of the boundary. Therefore, the fitting of the experimental data was used through the least-square method to calculate E_{ves} using equation (1), which was applied to the equation below to estimate the bending modulus k_c [J]

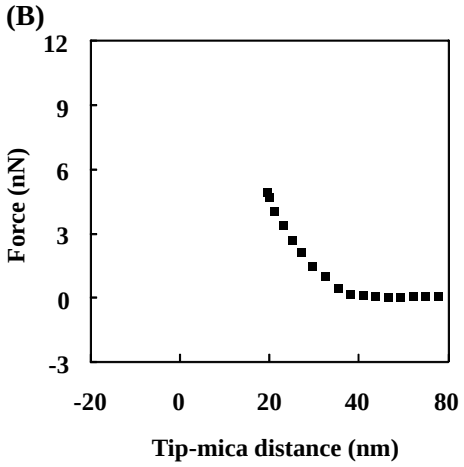
$$k_c = \frac{E_{ves} h^3}{12(1-\nu_{ves}^2)} \quad (2)$$

where h is the thickness [m] of the vesicle bilayer.

III. RESULTS AND DISCUSSION

The deflection data corresponded to the physical state of the vesicle. Fig. 1 (A) showed the deflection with respect to the displacement of the AFM probe. The force was acquired from the multiplication of the deflection by the probe spring-constant (Fig. 1 (B). The force curve was used to estimate Young's modulus as shown in Fig. 1 (C).





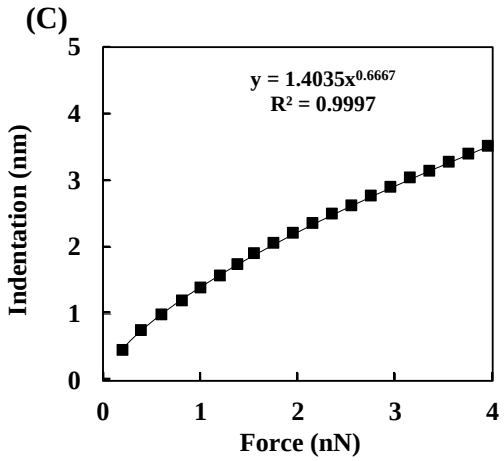


Figure 1. (A) Deflection with respect to displacement (z position) for vesicle at 0% protoamine; (B) force with respect to distance based on the data in (A); (C) indentation with respect to load force based on data of (A,B).

Table 1. Elasticity, Young's moduli (E_{ves}), and Bending moduli (k_c) for the ratio of protoamine to lipid.

Protoamine to Lipid	Elasticity	E_{ves} x 10^6 (Pa)	k_c x 10^{-19} (J)
0	0.7±0.01	81±2	11.3±0.3
0.1	0.68±0.01	79±2	11.0±0.3
0.3	0.66±0.01	77±2	10.6±0.3
0.5	0.64±0.01	75±2	10.3±0.3
0.7	0.64±0.01	75±2	10.3±0.3
1.0	0.64±0.01	75±2	10.3±0.3

The elasticity was explained with the slope of the force to the distance [15,19]. The slope corresponded to the resistance of each layer to the probe load. The slopes in Fig. 1 (B) are listed in Table 1 for the ratio of protoamine to DPPC, and averages with less than 3%. The exponential value (*b*) was 0.6667 from the fitting $\delta = AF^b$ to the data. In this formula, *A* is the proportional constant from several parameters shown in equation (1). Therefore, the justification of the elasticity was performed using the values of *b*. Using equation (2), the bending modulus was also estimated.

The increase in the ratio of protoamine to lipid lead to the decrease in Young′s modulus until the ratio was 0.5. No further decrease was observed above 0.5. The vesicle without protoamine showed 81 x 10^6 Pa as Young′s modulus, which was consistent with the previous research [20]. Therefore, both moduli were gradually decreased and saturated at 0.5 of the ratio, as shown in Table 1. The interference of protoamine to the lipid-headgroup arrangement may occur.

IV. CONCLUSION

The mechanical properties of DPPC vesicles were investigated for the protoamine ratio. The probe response was interpreted with the Hertzian model to evaluate the properties of DPPC vesicle neighboring with protoamine. The mechanical moduli were saturated at the protoamine ratio of 0.5. This result may be caused by the degree of the lipids associated with the protoamine at the ratio. This study may be basis for biological mechanisms related to cellular processes related to the antimicrobial activity of protoamine, i.e. cellular penetration.

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