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THE OPENING-UP OF LIPID BILAYER VESICLES BY GUEST MOLECULES: THE ADSORPTION ISOTHERM AND NUMERICAL CALCULATIONS

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Spherical vesicles in aqueous solution, made of bilayer lipid membranes, undergo shape transformations to open cup-like vesicles, due to the addition of guest molecules such as the protein talin. A semi-quantitative analysis of the opening up of the vesicles is made, based on the adsorption isotherm of guest molecules to the rims of the opening vesicles. The cuplike vesicles are represented as a partial sphere cut by a plane. The line tension of the rims is reduced greatly by adsorbed talin, and is determined from the statistical mechanics of the adsorption equilibrium. The total free energy of a cuplike vesicle is lower than that of the original spherical one. A more precise numerical evaluation of the shape of the open vesicles is also made. In this, we use the line tension and the equivalent spontaneous curvature of the membrane as theoretical parameters. The analysis reproduces the observed shapes of the cuplike vesicles well.

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1. Introduction

Because of its softness, fuzzy nature, flexibility, and lack of a strict theoretical background, the physics of soft condensed matter has its own characteristics compared to solid state physics. However, the softness, fuzzy nature, and flexibility are required or realized in many fields of science and technology, and especially in living materials. With the above features in these fields, soft materials physics has been studied extensively. Contemporary topics in soft materials physics are polymers, liquid crystals, amphiphiles, complex fluids, and biomaterials such as proteins, DNA, etc.

In this paper, we present a theoretical study of the opening up of lipid vesicles. So far, such vesicles have been studied as topologically closed items, and their opening up has not been observed as a stable form. Thus, closed vesicles made of a lipid bilayer membrane have been studied extensively due to their importance in biology, in industries such as food, paint, cosmetics, pharmacology, and so on. Closed lipid vesicles are stable, with rather long lifetimes, and are used for practical purposes. Seifert and Lipowsky presented a review of experimental and theoretical works on such vesicles [1]. The stability and long lifetime of the closed vesicle is especially important for a biological cell, in maintaining the separation between its interior and exterior. In reality, making holes in the surface of a lipid spherical vesicle is rather difficult. In the electroporation method, application of a strong electric field can make a temporary hole. However, the pore disappears immediately when the electric field is reduced below some threshold value [2]. Osmotic shock [3], laser tweezers [4] and adhesion [5] have also been developed to open transient holes in membranes.

Recent studies have shown, however, that some organic chemical agents such as the protein talin [6] or detergents [7] are capable of inducing a stable hole or holes in lipid membranes.

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The spherical vesicles transform into cuplike ones, as tube-like shapes or bilayer sheets. Although the precise mechanism has not yet clarified, the oligopeptide melittin (a bee toxin) induces hemolysis, so that its addition can make a hole in a red shell membrane [8]. The shapes of the observed holes have been interpreted on the basis of the mechanical balance between the bending rigidity of the membrane and the line tension - the energy cost per unit length of the edges of the holes. Assuming a value for the line tension energy on a small circular hole in a spherical vesicle, one can show that a quasi-stable hole opens in the membrane under an excess inner pressure [9]. However, this model can only explain transient holes, because the leakage of water through the holes may reduce the pressure difference across the membranes.

In this paper, we will briefly review the experimental results on the opening up of lipid vesicles by adsorbed guest molecules. Next, we will present an analysis of the stability of the cuplike vesicles, caused by guest molecules adsorbed on the rims of vesicles, based on statistical mechanics. In the first part of the theory, for simplicity, a partial sphere cut by a plane will be taken as a model for the opened vesicles, to illustrate the adsorption isotherm using the statistical mechanical model. The stability of the opened vesicles will be analysed by minimizing the total free energy. The origin of the line tension by the adsorption isotherm will be clarified. Next, a more precise numerical analysis of the shapes of the open vesicles will be performed, by assuming the line tension and the bending rigidity of the membrane. The shape of an open vesicle with a single hole will be calculated, using two parameters - the spontaneous curvature and the line tension of the rims of the open vesicles. The shapes obtained by numerical calculation will be compared with the observed shapes of cuplike vesicles.

2. Review of experimental results

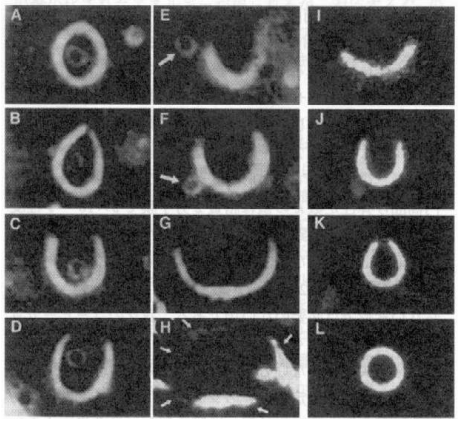

Fig. 1. Observed opening up of vesicles, showing the shape changes induced by changes in concentration. A to H are for increasing concentrations, and H to L for decreasing concentrations [6].

As already reported [6], when a small amount of the protein talin was added to a spherical lipid vesicle system, opening up of the vesicles was observed above a threshold concentration of talin, of the order of micro moles, as shown in Fig. 1. As well as the vesicles shown in Fig. 1, which were taken by dark field microscopy, vesicles with two and three holes were also observed. These coexisted with spherical vesicles when the concentration was not high [6]. By switching from a dark field microscope to a fluorescence microscope, talin could be shown (due to the fluorescent nature active talin) to be adsorbed on the rims of the cuplike or sheet like vesicles,. As shown in Fig. 1, the shape of the cuplike vesicles changed reversibly when the concentration of talin was changed. Just above a threshold concentration, a small hole opened up. Its size increased progressively from B to H. Upon decreasing the concentration from H to L, the hole shrank reversibly, until the original spherical vesicle was re-formed. This result shows that the shape of the cuplike vesicles was

determined by the concentration of talin. Similar characteristics of the opening up of vesicles have also been observed for other lipid and surfactant systems [7].

3. The adsorption isotherm of talin to cuplike vesicles

For cuplike vesicle formation in the lipid and talin system, Suezaki and co-workers have clarified the origin of the shape change of the vesicle by the adsorption isotherm of talin between the periphery of the cup-like vesicle and the aqueous solvent [10]. Here we will reformulate the theory starting from the simplified free energy description, so that it may be comprehensible to readers other than physicists. Before going into the total free energy description, we briefly review the bending elastic energy of the vesicle membrane. Helfrich first described this as follows [11]:

$$E_{bend} = \frac{k_c}{2}\iint \left(c_x + c_y - c_0\right)^2 dxdy + k_g \iint c_x c_y dxdy \tag{1}$$

where k_c and k_g are the cylindrical bending modulus and the Gaussian bending modulus, respectively. The factors c_x, c_y, and c_0 are the two principal curvatures and the spontaneous curvature, respectively. The integrals are taken on the surface of the vesicle. We will neglect the term involving the Gaussian bending modulus in the following calculation.

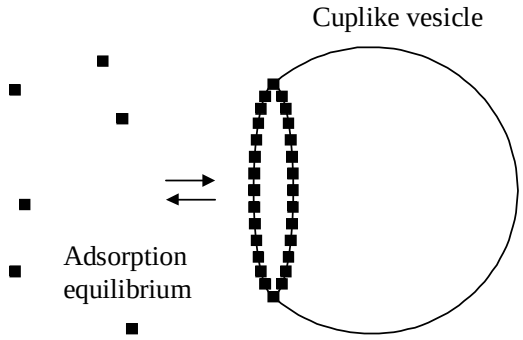

Fig. 2. Schematic picture of the adsorption isotherm of talin to the rim of a cuplike vesicle.

In this paragraph, we will briefly discuss the reason why we can neglect the Gaussian bending energy term. Previously, many workers tried to develop theoretical explanations of the complicated phase behaviours of colloidal systems such as microemulsions, lamellar layers etc. The factors k_c and k_g were treated as independent theoretical parameters [12]. It is important to notice, however, that the Gaussian bending modulus is the elastic modulus for the shear deformation of the membrane surface. The experimental condition for the lipid membrane is that it is in the liquid phase [6, 7], and the lipid bilayer membrane is not resistant to shear stress. The theory of elastic membranes [13] cannot answer this problem. For a reliable description of liquid membranes, Petrov and co-workers described the membrane in the liquid phase via a surface elasticity model, using dumbbell molecules [14]. One of the present authors revisited the model of Petrov *et al.*, and examined the physical nature of the Gaussian bending modulus [15]. According to this analysis, the value of the Gaussian bending modulus, k_g cannot be of the same order of magnitude as that of major bending modulus, k_c. Instead, the value of k_g should be at most two orders smaller than k_c. This is due to the shear free condition of the lipid membranes. Thus, in the first order approximation of the theory, we will neglect the Gaussian bending modulus in further discussions. However, in a more detailed investigation of the vesicle topologies, it plays a considerable role in the formation of opened vesicles, as will be discussed later.

When we analyze the adsorption isotherm of a cuplike vesicle, we will regard it as a partial sphere cut by a plane. Fig. 2 shows this schematically, together with the adsorption scheme. The bending energies E_{sphere} and E_{cup} are represented simply as

$$E_{sphere} = 2\pi k_c \left(2 - c_0 R_0\right)^2 \tag{2}$$

$$E_{cup} = 2\pi k_c \left(2x - c_0 R\right)^2 \tag{3}$$

where $x=R_0/R$ is the relative curvature of the cuplike vesicle [10]. The factors, R_0 and R are the radii of curvature of the original sphere and the cuplike vesicle, respectively.

Before proceeding further, we evaluate the mechanical condition for creating the open vesicle. By postulating the line tension, γ, the line tension energy E_{rim} is written as

$$E_{rim} = 4\pi\gamma R_0 \sqrt{1 - x^2} \tag{4}$$

To have a positive solution for the relative curvature x, the following equation should hold:

$$\frac{\partial\left(E_{cup} + E_{rim}\right)}{\partial x} = 4\pi\left[2k_c\left(2x - c_0 R_0\right) - \frac{\gamma R_0 x}{\sqrt{1-x^2}}\right] = 0 \tag{5}$$

In order to possess a positive solution of x, the condition is stated simply as

$$2x > c_0 R_0 \tag{6}$$

This means that the frustrated bending moment should work so as to expand the original spherical vesicle to form the open one.

Now, we denote X, N, n_0 and n as the numbers of total talin, and adsorbed talin, and the numbers of original spherical vesicles and cuplike vesicles, respectively. Then, the free energy F of the total system can be written as:

$$\begin{aligned} F = {} & (X - N)kT \log c_{X-N} + NkT \log c_r + -\varepsilon_0 N + n\left(E_{cup} - E_{sphere}\right) \\ & - kT\left[n \log n + \left(n_0 - n\right)\log\left(n_0 - n\right)\right] \end{aligned} \tag{7}$$

where

$$c_{X-N} = \frac{X - N}{V} \tag{8}$$

$$c_r = \frac{1}{v_t} \tag{9}$$

are the concentrations of talin in the aqueous solution and of talin at the rim of the cuplike vesicle. The factors V and v_t are the total volume of the system and that of the molecular volume of talin, respectively. The factor ε_0 in Eq. (7) is the affinity free energy of talin from water to the rim of the cuplike vesicle. Here, we neglect the adsorbed number of talin on the surface of the cuplike vesicle. It can be shown to be negligible because of the large value of ε_0 estimated from the observed threshold concentration of talin.

The numbers N and n are determined so as to minimize the free energy F. Now, we perform the minimization of the free energy. Firstly, we show the minimization procedure by N as follows;

$$\frac{\partial F}{\partial N} = \mu_{ad} - \mu_{bulk} = 0 \tag{10}$$

where

$$\mu_{ad} = kT \log\left(\frac{c_r}{e}\right) - \varepsilon_0 + 8\pi k_c n\left(2x - c_0 R_0\right)\frac{\partial x}{\partial N} \tag{11}$$

$$\mu_{bulk} = kT \log c_{X-N} \tag{12}$$

are the chemical potentials of adsorbed talin and that dissolved in the aqueous solvent, respectively.

Next, the number n of cuplike vesicles should also be determined, so as to minimize the free energy F. This is written as

$$\frac{\partial F}{\partial n} = E_{cup} - E_{sphere} + 8\pi n k_c \left(2x - c_0 R_0\right)\frac{\partial x}{\partial n} + kT \log\left(\frac{n}{n_0 - n}\right) = 0 \tag{13}$$

The above equation can be interpreted in another way: the relative curvature, x, is determined as a function of the concentration, $C_X=X/V$. Namely, it describes the mechanical balance of the cuplike vesicle.

Lastly, we mention the minimized free energy value, F_{min}. Using Eqs. (7), (10) and (13), we obtain the following result:

$$\frac{F_{\min} - F_{N=0}}{XkT} = 1 - \frac{C_{th}}{C_X} - \log\left(\frac{C_X}{C_{th}}\right) < 0 \tag{14}$$

where

$$C_{th} = \frac{1}{e v_t}\exp\left(-\frac{\varepsilon_0}{kT}\right) \tag{15}$$

is the threshold concentration for formation of the cuplike vesicles. From the observed value of the threshold concentration, ε_0 is estimated to be approximately $20kT$. In Eq. (14), the term of the order of n has been neglected, because it is small compared to the terms in Eq. (14). Eq. (14) shows that the formation of cuplike vesicles lowers the free energy, and the open vesicles are more thermodynamically stable than the system of spherical vesicles. The large value of ε_0 means that the adsorbed number of talin to the bulk membrane surface can be neglected to a first approximation, although we have not shown this explicitly here.

Before closing this section, we will obtain the line tension as a function of the concentration explicitly, as follows. From Eq. (5),

$$\gamma = 2k_c\left(2x - c_0 R_0\right)\frac{\sqrt{1-x^2}}{R_0 x}. \tag{16}$$

By defining σ as the number of talin molecules per unit length of the rim, we obtain $N = nl\sigma$, where $l = 4\pi R_0\sqrt{1-x^2}$ is the length of the rim per open vesicle. From Eqs. (10)-(12), (15) and (16), the line tension turns out to be

$$\gamma = \sigma kT \log\left(\frac{c_{th}}{c_X - c_{th}}\right). \tag{17}$$

Therefore, the line tension is a decreasing function of the concentration of talin.

4. Numerical analysis of the shapes of the opening up vesicles

In the previous section, we could successfully understand the cuplike vesicle formation based on the adsorption isotherm. However, the vesicle shape was assumed qualitatively to be a partial sphere. In reality, the shape is non-spherical, as shown in Fig. 1. In this section and the following one, we will present a numerical calculation of the shapes of the open vesicles.

The equilibrium shape of the closed vesicles has been thoroughly studied from the theoretical viewpoint, following the pioneering work in this field of Helfrich [11]. A variety of vesicle shapes and shape transformation pathways under various conditions have been explained using the Helfrich free energy. This is given by Eq. (1), and/or by the more realistic ADE model [16]. The standard procedure for calculating the equilibrium shape is the variational method. By applying this to the energy functional of the bending elastic energy, one can derive the Euler-Lagrange equation, from which the equilibrium shape of the vesicle is obtained. Recently, Tu and Ou-Yang [17] have applied this method to membranes with free edges. Using the Helfrich free energy, they derived the Euler-Lagrange equation and the boundary conditions holding at the membrane edges. Umeda *et al.* [18] modified these equations to include the ADE model, and obtained open shapes in good agreement with the observed ones.

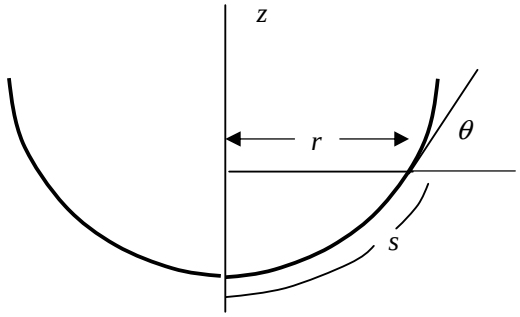

Fig. 3. Schematic picture of the axisymmetric cuplike vesicle s is the distance from the bottom along the meridian line θ is the tangent angle, as shown in the figure r is the distance from the axis of rotation.

Here we use the simpler model, i.e. Helfrich's free energy (with spontaneous curvature, (Eq. 1)), and describe the shape equation and the boundary conditions for the open vesicles. The analysis is restricted to axisymmetric deformation, to simplify the expressions.

By assuming the line tension energy γ at the rim of the cup, we may write the energy of a cuplike vesicle in the following form:

$$W = E_{bend} + \gamma \int dl = 2\pi \int \left[\frac{k_c}{2} (2H - c_0)^2 + k_g K \right] r ds + 2\pi \gamma r_0 , \tag{18}$$

where

$$H = \frac{1}{2}\left(c_x + c_y\right) = \frac{1}{2}\left(\frac{\sin\phi}{\mathrm{r}} + \frac{d\phi}{ds} \right) \qquad \text{and} \qquad K = c_x c_y = \frac{\sin\phi}{r} \frac{d\phi}{ds} \tag{19}$$

are the mean curvature and the Gaussian curvature of the surface, respectively. The factors r, s, and ϕ are the geometrical parameters shown in Fig. 3. The factor r_0 in Eq. (18) is the radius of the hole. The equilibrium shape of the vesicle is obtained by minimizing W for a given membrane area $A = 4\pi R_0^2$. The shape equation and the boundary conditions for open vesicles are derived from the expression

$$\delta(W + \lambda A) = 0 , \tag{20}$$

where λ is the Lagrange multiplier. Equation (20) leads to the following Euler-Lagrange equation in the surface:

$$2k_c\Delta H + k_c(2H - c_0)(2H^2 - 2K + c_0 H) - 2\lambda H = 0, \tag{21}$$

where $\Delta = (1/r)d(r(d/ds))/ds$ is the Laplace-Beltrami operator on the surface [17]. This equation corresponds to the force balance per unit membrane area in the direction normal to the surface, and it is the same shape equation as that for closed vesicles [18]. The boundary conditions for Eq. (21) on the rim are

$$k_c\left(2H - c_0\right) + k_g \frac{\sin\phi}{r} = 0, \tag{22}$$

$$2k_c \frac{dH}{ds} + \gamma \frac{\sin\phi}{r} = 0, \tag{23}$$

$$\frac{k_c}{2}(2H - c_0)^2 + k_g K + \lambda + \gamma \frac{\cos\phi}{r} = 0. \tag{24}$$

Physically, Eqs. (22) and (23) correspond to the moment balance and the balance of the shear force per unit length of the boundary, respectively. Equation (24) is associated with the membrane tension. For more precise descriptions of these equations, see references [17] and [19]. Note that the parameters γ and k_g are involved only in the boundary conditions. The equilibrium shape of the open vesicles is obtained by solving Eq. (21) with the boundary conditions Eqs. (22)-(24), and the constraint $A = 2\pi\int rds = 4\pi R_0^2$.

5. Results of numerical calculations

In Fig. 4, we show the result of a numerical calculation using Eq. (21), with the boundary conditions (22)-(24). The parameters used in the calculations were $c_0 R_0$ = 1 and k_g = 0. Figure 4a shows the radius of the hole as a function of the line tension, and Fig. 4b depicts the opening up shapes of the representative points A, B, and F in Fig. 4a. Open vesicles are obtained when γ is smaller than k_c / R_0.

The total energy *W* of Eq. (18) is shown in Fig. 5, as a function of the line tension γ. In this figure, the solid line shows the energy profiles of the open vesicles shown in Fig. 4, and the dotted line is for a spherical vesicle.

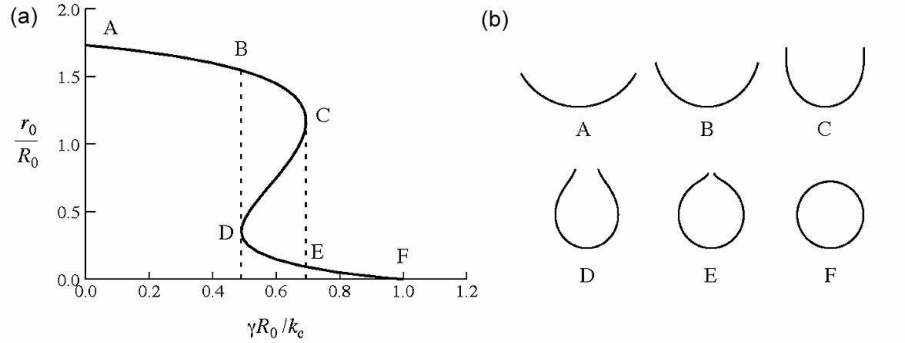

Fig. 4. The shapes of open vesicles, as obtained by numerical calculations (a) Normalized size of the hole r_0 / R_0 versus normalized line tension $\gamma R_0 / k_c$. (b) Vesicle shapes at the points indicated by the letters in (a).

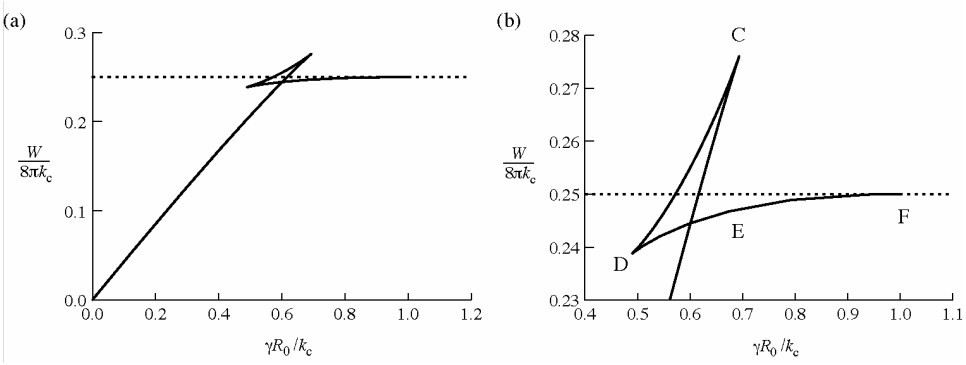

Fig. 5. Calculated total energy W. Dotted line: the energy of a sphere with the same area (a) Normalized energy of the open vesicle shown in Fig. 4. (b) Magnification of (a). Solid line: the energy of the open vesicle.

From Fig. 4 and the energy profiles shown in Fig. 5, we see that the opened shapes bifurcate from a sphere at point F ($\bar{\gamma} = \gamma R_0 / k_c$ = 1.0). As $\bar{\gamma}$ decreases, the hole becomes larger and the total energy W is reduced from that of a spherical vesicle. This result coincides with that from Sec. 3, i.e/ that open vesicles are more thermodynamically stable than spherical ones, when the talin concentration is higher than a threshold value. This is because the line tension γ decreases as the concentration of talin increases (see Eq. (17)). However, the $\bar{\gamma}$-r_0 curve in Fig. 4 folds at D and C, and there are three solutions for 0.49 < $\bar{\gamma}$ < 0.69. Fig. 5 indicates that open vesicles with shapes between C and D are unstable. Therefore as $\bar{\gamma}$ decreases, a first-order transition occurs and the cup-like vesicles discontinuously expand their holes to assume the shapes shown in B-C of Fig. 4b. Then they assume a dish-like shape as shown in A.

Similar results were obtained for calculations with $c_0 R_0 \neq 1$. The bifurcation to the open shapes occurred at $\bar{\gamma} = 2 - c_0 R_0$ from a sphere with a hole continuously or discontinuously expanded as $\bar{\gamma}$ decreased. However, we could not find open shapes for $c_0 R_0 > 2$. The inequality $c_0 R_0 < 2$ may be a necessary condition for the formation of open vesicles. This suggestion is also supported by the analysis described in Sec. 3. When the hole is infinitesimal small, i.e. when x ≈ 1, condition (6) reduces to $c_0 R_0 < 2$. This means that a frustrated bending moment is needed for the opening up of vesicles, as stated in Sec. 3.

The calculated shapes shown in Fig. 4b are very close to those shown in Fig. 1. However, vesicles similar to those in C and D were also observed in experiments, and the change of the shape seems to be continuous, in contrast to the expected discontinuous change in Fig. 5. Because realistic colloidal systems are often realized as metastable states, some of the observed vesicles in Fig. 1 might correspond to the calculated vesicles. Furthermore, we also calculated the shape of the open vesicles using the ADE model [19] instead of employing the spontaneous curvature model of bending elastic energy. Although the results showed discontinuous shape changes as well, the pattern of shape transformations predicted by the ADE model seemed to be closer to the observed pattern than to the results of the spontaneous curvature model. For a more precise stability criterion, the study of the open vesicles is just starting, and we need more experimental and theoretical studies in future work.

For larger values of the line tension γ, the hole becomes very small, as shown in E of Fig. 4 since the line tension tends to minimize the hole perimeter. In Fig. 6, we show the enlarged picture of a nearly spherical vesicle with a small opening. The surface around the hole protrudes, where the large negative curvature $d\theta / ds$ at the opening compensates for the positive curvature $\sin\theta / r$ in Eq. (17). This shape is very different from the ideal one assumed in Sec. 3, where a sphere is cut by a plane. Although very small holes are not detectable, the observed membrane shape seems not to be spherical, but to protrude around the hole. Therefore, some of the consequences obtained in Sec. 3

may require modification, at least for vesicles having very small holes. A full analysis using non-spherical shapes will be a topic for future work.

Fig. 6. The shape of a small opening in a nearly spherical surface. The parameters are $c_0 R_0 = 1$, $k_g = 0$ and $\gamma R_0 / k_c = 0.9$.

Because we neglected the effect of k_g in the calculation used to obtain Fig. 6, the orifice of the opening opens as shown in the figure. The effect of k_g is also evaluated in our calculation [18]. The orifice is more smoothed out when k_g is negative, and more curled up when it is positive. Although the resolution of the optical observations made so far is not good enough to distinguish these differences, precise observation and comparison with the theoretical calculation will reveal the true nature of the Gaussian bending modulus [15].

6. Concluding remarks

In the first part of this paper, we reported a physico-chemical mechanism for the opening up of lipid vesicles, including a rim adsorption of guest molecules and shape changes, according to statistical mechanics. It was shown that the concentration of guest molecules on the rims of the opened up vesicles determined their shapes. In other words, the line tension is controlled by the adsorption isotherm of the guest molecules. For simplicity in this analysis, the opened vesicles were assumed to be partial spheres. By this simplified model, we could clearly understand the interplay between the adsorption isotherm and the torque balance of the opened vesicles.

In the last part of this paper, the precise shapes of the opened vesicles were analyzed by numerical calculations based on the spontaneous curvature derived originally by Helfrich [11]. Depending on the line tension and the spontaneous curvature of the membrane, cuplike vesicles were predicted. These shapes coincided with observed pictures [6], [7]. Theoretical analysis, however, showed that the shape changes were expected to be discontinuous, from cuplike vesicles to shallow dish-like shaped ones.

Although the observed pictures [6], [7] seem to show a continuous change, more precise observations, and a comparison of the theories of the spontaneous curvature model and the ADE model should be made. The bending energy of thin elastic materials can be described well by the traditional spontaneous curvature model [13]. Helfrich nicely described the bending energy of lipid membranes by extending the elastic energy of a liquid crystal to the monolayer of surfactant membranes, and introduced the concept of spontaneous curvature [11]. Because the elastic energy of liquid crystals is that of the elastic materials in origin, Helfrich's bending energy is based on the elastic theory of materials.

The ADE model of the area difference of monolayers of lipid bilayers describes the spontaneous curvature in a different way. This model seems to be more analytically gentle than the spontaneous curvature model, as far as we see from the numerical calculations shown in this paper and those in another report using the ADE model [18].

The calculation was made only for axisymmetric shapes. However, non-axisymmetric vesicles have also been observed. The analysis of such non-axisymmetric vesicles will be a future problem.

Acknowledgments

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