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Author(s)	Hamada, Tsutomu; Yoshikawa, Kenichi
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Description	

Peeling kinetics of giant multilamellar vesicles on a solid-liquid interface

Tsutomu Hamada, Kenichi Yoshikawa*

Department of Physics, Graduate School of Science, Kyoto University & CREST,
Kyoto 606-8502 Japan

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Abstract

We observed the dynamics of peeling of giant multilamellar vesicles interacting with a solid-liquid interface by using phase-contrast microscopy. When a multilamellar vesicle touches a surface, the outer layer peels away and the inner layer is exposed. There are two distinct pathways for this shape transformation that depend on the size of the vesicle. We theoretically discuss the mechanism and kinetics of peeling by considering the surface energy, line energy and bending energy.

* Corresponding author. Fax: +81-75-753-3779

E-mail: yoshikaw@scphys.kyoto-u.ac.jp

1. Introduction

Phospholipids tend to assemble to form micelles and vesicles in aqueous solutions due to their amphiphilic properties. Since phospholipids exhibit essentially the same bilayer structure as living cell membranes, they have been actively studied as a model of cell membranes and as a microreactor for biochemical reactions [1]. Vesicles, closed phospholipid membranes, range in size from 50 nm to 10 μm or greater, corresponding to the size of a typical cell. Giant vesicles (> μm) have low curvature and are stable enough for thermal fluctuation. They are large enough to allow real-time manipulation and real-time observation of transformation processes by optical microscopy [2,3]. There have been many theoretical [4,5] and experimental studies (caused by talin protein [6,7], amphiphilic polymers [8], temperature [9], laser tweezers [10] and osmotic pressure [11]) on the variation in vesicle morphology. However, most of these studies have focused on the morphology at equilibrium. Almost all phenomena concerning lipid membranes in living organism are time-dependent processes, and kinetical studies about simple vesicular system are invaluable. Very recently, a few reports have appeared on the time-dependent changes in vesicle morphology under nonequilibrium (theoretical study of vesicle motion induced by an adhesion gradient on a substrate [12], Brownian dynamics simulation of self-assembly kinetics [13], observations of transient pores in stretched vesicles [14, 15, 16] and dynamic behavior caused by interactions with surfactants [17]).

In this study, we conducted a microscopic observation of time-dependent changes in the morphology of giant multilamellar vesicles, which consist of multiple layers (phospholipid bilayers), on a solid-liquid interface (a glass surface). When vesicles interact with a glass surface, a pore is spontaneously generated on the outer layer and the inner layer transforms to become exposed. The dynamic changes in the morphology of multilamellar vesicles, including hole-opening and delamination, are important not only with regard to softmater physics, but also for biological studies, such as endocytosis, exocytosis and drug delivery.

2. **Experiment**

Giant vesicles were made from dioleoyl-phosphatidylcholine (DOPC) (ALEXIS) through a natural swelling method from dry lipid films [18, 19] by the following procedure: A 10 μL sample of 10mM DOPC in 1:2(v/v) methanol/chloroform in a glass test tube was dried to form a thin lipid film. The tube was placed in a desiccator for > 3 h to remove the organic solvent. The film was then swollen with 100 μL of 10 mM HEPES, 10 mM $CaCl_2$ solution for 1 h at 37 °C. After incubation, 10 μL of the solution was placed on a glass slip, covered with another smaller slip with 30-50 μm spacing, and sealed. These glass slips had been cleaned in advance with ethanol and dried. We observed the resulting giant vesicle with a phase-contrast microscope (Nikon TE-300) equipped with a Ti:Sa tunable laser (Model 390OS, Spectra Physics, 800 nm) pumped by a Millenia XS (Spectra Physics, 532 nm) and recorded on S-VHS videotape at 30 frames/s. We transported a single giant multilamellar vesicle in the solution to a glass surface using laser tweezers (0.3-0.5 W), and situated the vesicles on the surface by their spontaneous adhesion.

3. Results and discussion

Figure 1 shows typical phase-contrast images of the peeling of multilamellar vesicles interacting with a solid-liquid interface. A pore spontaneously opens in the outer layer, and the inner layer is exposed within a few min after the laser is withdrawn. The whole peeling process takes a few s. We found that there are two distinct processes in this transformation. The inner layer deviates greatly from a spherical shape to form a dual-spherical structure in Fig. 1 (a) (buckled peeling), while the inner layer remains spherical in Fig. 1 (b) (simple peeling). Furthermore, there is a trend in the peeling pathway. As shown in Figs. 1 (a) and (b), a large vesicle exhibits buckled peeling, while a small vesicle undergoes simple peeling. After careful observation, we consider that the peeling is generated layer by layer from the multilayer membrane, although at present we cannot give definite conclusion on this matter.

Next, we will analyze time-dependent changes in the peeling process. We define r_1, r_2, θ and ϕ as in Fig. 2 (a), where

$$r_1 \sin\theta = r_2 \sin\phi \,. \tag{1}$$

With reference to a report on the water permeability through phospholipids bilayer membrane [20], the volume change during the buckled peeling is estimated to be $\Delta V/V < 10^{-8}$. We measured the surface area of the inner layer during transformation and found that it remained essentially constant. Thus, Eq. (2) is used to describe the system:

$$4\pi r_0^{\,2} = 2\pi r_1^2(\cos\theta + 1) + 2\pi r_2^2(\cos\phi + 1), \tag{2}$$

where r_0 is the initial radius of the vesicle. By analyzing morphological changes using Eqs. (1) and (2), we deduced the time-dependent changes in r_1 and θ. Figure 2 (b) shows the change in the phase plane of r_1 and θ as evaluated from the experiments, and reveals that there is a clear difference in pathways. When the initial radius of the vesicle is large, the inner layer is depressed to undergo buckled peeling. When the initial radius is small, the value of the inner layer remains constant during peeling. There is a region of coexistence between buckled and simple peeling in the case of mid-sized vesicles.

We now discuss the mechanism of this difference in the transformation process in terms of the effect of surface energy from interaction with the glass surface, line energy of a pore in the outer layer and bending energy of a depression in the inner layer. Once vesicles are in contact with a glass surface, lipid molecules tend to spread onto the glass surface, driven by the enhanced stability of lipid on the glass surface. With a good approximation, closed membranes exhibit zero surface tension at equilibrium in aqueous solutions [21]. As reported, a pore opens in the bilayer when surface tension is generated (vesicles adhered to a substrate [14], sucked in a glass micropipette [22], shocked by an electric field [23] or trapped by laser tweezers [10]). Since the surface area of the outer layer shown in Fig. 2 (a) can be written as $2\pi r_1^2(\cos\theta + 1)$, the surface energy is written as

$$E_{\text{surface}} = \varepsilon \cdot 2\pi r_1^2(\cos\theta + 1)\,, \tag{3}$$

where ε is the energy difference per unit area for an outer layer membrane in the multilamellar vesicle to transfer onto the glass surface. Sandre et al. estimated the surface tension ($\varepsilon \sim 10^{-5}$ N/m; in the present paper we use the symbol "~" to indicate the order of parameters.) at which a pore can open in a unilamellar vesicle that has adhered to a substrate [14].

Next we consider the line energy; i.e. the free-energy cost per unit length of exposed edge. Edges are disfavored because of the high cost of either exposing the hydrophobic lipid chains to water or creating a highly curved rolled edge to hide them. The energy is written as

$$E_{\text{line}} = \gamma \cdot 2\pi r_1 \sin\theta\,, \tag{4}$$

where γ is the line tension required to form an exposed edge per unit length. Zhelev and Needham reported that the line tension of a single bilayer γ is $\sim 10^{-11}$ N [23].

The depressed region of the inner layer has a very high bending energy, and this local area is represented schematically in Fig. 2 (a). Under the assumption that the spontaneous curvature is zero, the energy can be written as

$$\begin{aligned} E_{\text{bending}} &= k_{\text{c}} \cdot \int \mathrm{d}S \left[\frac{1}{2}\left(\frac{1}{r_3} - \frac{1}{r_1 \sin\theta} \right) \right]^2 \\ &= \frac{\pi}{2} k_{\text{c}} r_1 r_3 (\pi - \theta - \phi) \sin\theta \cdot \left(\frac{1}{r_3^2} + \frac{1}{r_1^2 \sin^2\theta} - \frac{2}{r_1 r_3 \sin\theta} \right), \end{aligned} \tag{5}$$

where k_{c} is a bending elasticity ($\sim 10^{-19}$ J) [24]. ϕ can be described as a function of r_1 and θ from Eqs. (1) and (2), and we assume that $r_3 \approx 10$ nm, corresponding to the minimum radius restricted by their molecular structure. Thus, the bending energy can be written as a function of r_1 and θ. Since the bending energy of other parts of the inner and outer layers (~10^2 $k_{\mathrm{B}}T$) is much less than that of the depressed region (~10^5 $k_{\mathrm{B}}T$), we can ignore these effects. The total energy can be written as the sum of three energies:

$$U_{\text{total}} = E_{\text{line}} + E_{\text{surface}} + E_{\text{bending}} \,. \tag{6}$$

Figure 3 shows the total energy as a function of r_1 and θ with $\varepsilon = 2\times10^{-5}$ J/m, $\gamma = 5\times10^{-12}$ N, and $k_{\mathrm{c}} = 2\times10^{-19}$ J. The values of r_1 and θ change toward minimum energy. Before vesicles start to interact with the glass surface, the surface energy is zero, as shown in Fig. 3 (a). Since the initial state is stable, the shape of vesicles is unchanged. Interaction with a glass surface produces a surface energy, and the total energy changes from Fig. 3 (a) to Fig. 3 (b). The initial state becomes unstable and a pore can open in the outer layer. A comparison of Figs. 3 (b) and (c) shows how the total energy depends on the vesicle size. The surface energy scales to r_1^2, and the bending energy is proportional to r_1 (the second term ~ $1/r_1$ is negrigible in μm-sized vesicles) according to Eqs. (3) and (5). Thus, smaller vesicles show a greater effect of bending energy and tend to undergo simple peeling, while larger vesicles show buckled peeling. This model also explains the region of coexistence between buckled and simple peeling in Fig. 2 (b). The bending energy is proportional to the number of layers in the vesicle. Vesicles with a larger number of inner layers exhibit simple peeling, while those with fewer inner layers undergo buckled peeling. It is currently difficult to determine the exact number of inner layers experimentally, and the bending energy of inner layers cannot be determined exactly. Thus, the existence of an intermediate region can be attributed to the dispersion of multi-layers in the vesicle. A remaining problem is how to explain the mechanism by which vesicles interacting with a glass surface overcome the energy barrier to undergo peeling, on the order of 10^3 $k_{\mathrm{B}}T$ calculated from our model. The free energy barrier can be decreased by choosing appropriate values for γ, k_{c} and ε. However, it may be premature to seek the optimal values for reducing the free energy barrier, due to the large uncertainty in these parameters. In relation to the optical effect on the membrane, a recent study has reported that the energy due to the optical pressure is on the order of 10^3 k_BT under similar experimental conditions to the present study [25]. It may be valuable that such optical energy is nearly equal to the activation energy mentioned above.

Figure 4 examplifies the time development of θ during simple peeling. The typical kinetics of peeling can be characterized by a nearly constant rate of growth after nucleation. We consider the lamellar density of the vesicle $m(\theta, t)$ as an order parameter for a phenomenological discussion. The density $m(\theta, t)$ is not a conserved quantity in the present description. The time development of $m(\theta, t)$ is given as

$$\frac{\partial m(\theta)}{\partial t} = -L\frac{\delta F\{m(\theta)\}}{\delta m(\theta)} \,, \tag{7}$$

where $F\{m\}$ is the Ginzburg-Landau type free energy functional:

$$F\{m(\theta)\} = \int \mathrm{d}\theta \left(f\{m(\theta)\} + \frac{C}{2}(\nabla m)^2 \right), \tag{8}$$

where the first term in the integral is the free energy density on θ, and the second term describes the effect of a spatial gradient on the order parameter, which corresponds to the line energy. The following equation can be written from Eqs. (7) and (8):

$$\frac{\partial m(\theta)}{\partial t} = -L\left(\frac{\partial f(m)}{\partial m} - C\left(\frac{\cot\theta}{r_0^2}\frac{\partial m}{\partial \theta} + \frac{1}{r_0^2}\frac{\partial^2 m}{\partial \theta^2} \right) \right). \tag{9}$$

When a peeling wavefront exists at θ', Eq. (9) is transformed by the change of variables as $m(\theta,t) = m(\theta - v(\theta')t) = m(\psi)$, where $v(\theta)$ is the velocity of peeling at θ.

$$-\left(v(\theta') + \frac{LC\cot\theta'}{r_0^2} \right)\frac{\partial m}{\partial \psi} = -L\frac{\partial f(m)}{\partial m} + \frac{LC}{r_0^2}\frac{\partial^2 m}{\partial \psi^2}. \tag{10}$$

For simplicity, let us consider the force-order polynomial as $f(m)$ to describe the bistable system: $\partial f(m)/\partial m$ can be represented as

$$\frac{\partial f(m)}{\partial m} = a(m - m_1)(m - m_2)(m - m_0), \tag{11}$$

where m_1 and m_2 correspond to the order parameters of the metastable state, and coincide with the state before and after peeling. m_0 is the order parameter of the unstable state during peeling. Under the boundary condition $m(\infty) = m_1$, $m(-\infty) = m_2$, Eq. (10) has the solution of a traveling wave with an almost constant speed except at $\theta \sim 0$ and π.

$$v(\theta) \cong \frac{L}{r_0}\sqrt{\frac{aC}{2}}\left(m_1 + m_2 - 2m_0\right) - \frac{CL}{r_0^2}\cot\theta. \tag{12}$$

Although the range of θ is finite, this discussion is useful after a large enough nucleus is formed. Thus, the phenomenological meanfield theory semiquantitatively explains the observation of a constant speed of peeling, as shown in Fig. 4. In addition, Eq.(12) implies that the peeling speed in a smaller vesicle is higher than that of larger vesicles, in good correspondence with the experimental observation.

4. Concluding remarks

We have observed the peeling of multilamellar vesicles induced by interaction with a solid-liquid interface. The outer layer peels, and the shape of the inner layer changes so that it is exposed. Buckled peeling or simple peeling is observed depending on the vesicle size. A phenomenological meanfield theory can explain the difference in the kinetic pathway, as well as a nearly constant rate of the peeling kinetics. It is well known that neutral lipids tend to form multilamellar vesicles in aqueous solutions. The present findings are expected to provide insight into the physics of multilamellar vesicles and the basis for their wide practical applications.

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Figure captions

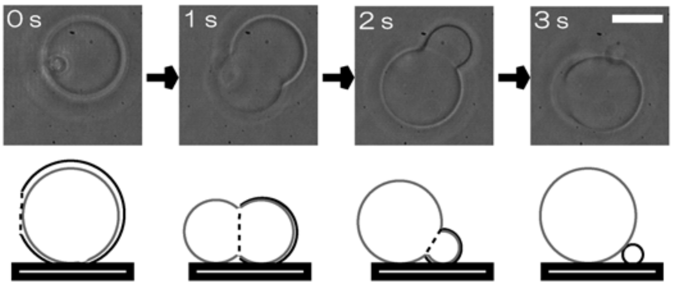
Fig. 1. Phase-contrast images of the peeling of vesicles interacting with a glass surface. In (a) (buckled peeling), the inner layer shows large deformation from aspherical shape. Conversely, in (b) (simple peeling), the inner layer retains its spherical shape. The scale bar is 10 μm.

Fig. 2. a) Schematic representation of a vesicle with two layers, together with variables for describing its geometry. b) Pathway for the change in $r_1$and θ during the peeling of vesicles interacting with a glass surface. Vesicles with a larger initial radius exhibit buckled peeling, while smaller vesicles undergo simple peeling. There is a region of coexistence between buckled and simple peeling in the case of mid-sized vesicles.

Fig. 3. a) Total energy of a vesicle with an initial radius of 10 μm. b) Total energy of a vesicle with an initial radius of 10 μm interacting with a glass surface. c) Total energy of a vesicle with an initial radius of 1 μm interacting with a glass surface. The energies are deduced from the model Eq. (6).

Fig. 4. An example of time development of θ during a simple peeling. The velocity $\mathrm{d}\theta/\mathrm{d}t$ is almost constant after the nucleation. Each vesicle showed different induction period.

(a) buckled peeling



(b) simple peeling

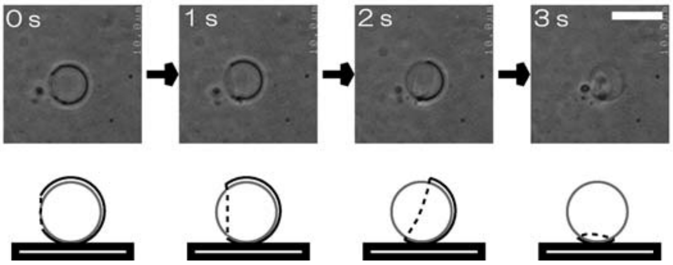


Figure 1.

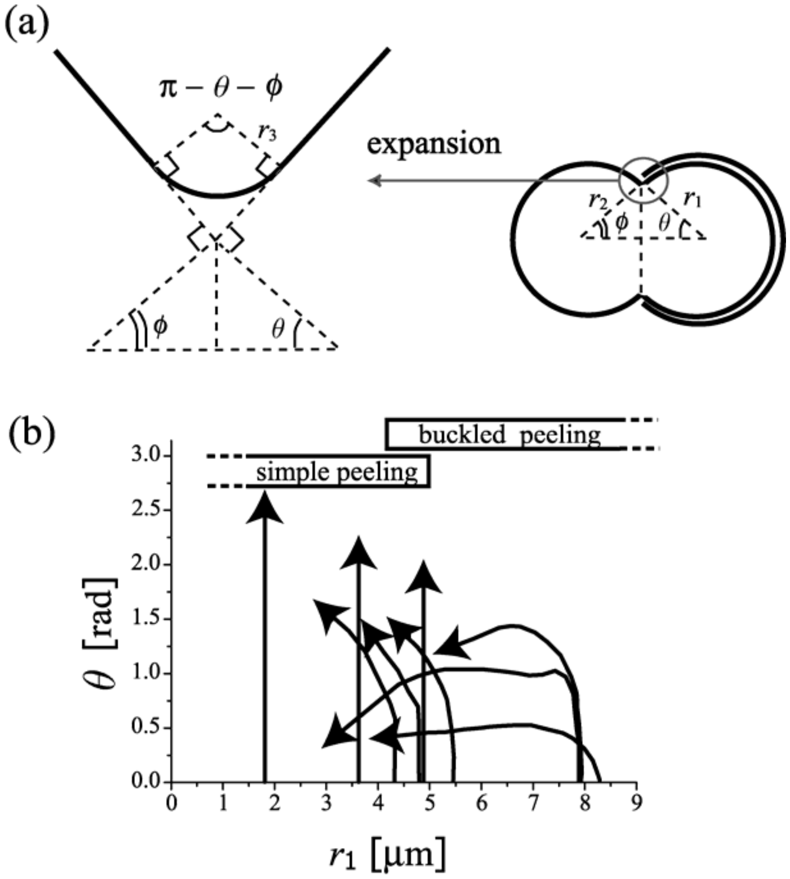

(a)
π − θ − ϕ
r_3
expansion
r_2
r_1
ϕ
θ
(b)
buckled peeling
simple peeling
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7
8
9
θ [rad]
r_1 [µm]

Figure 2.

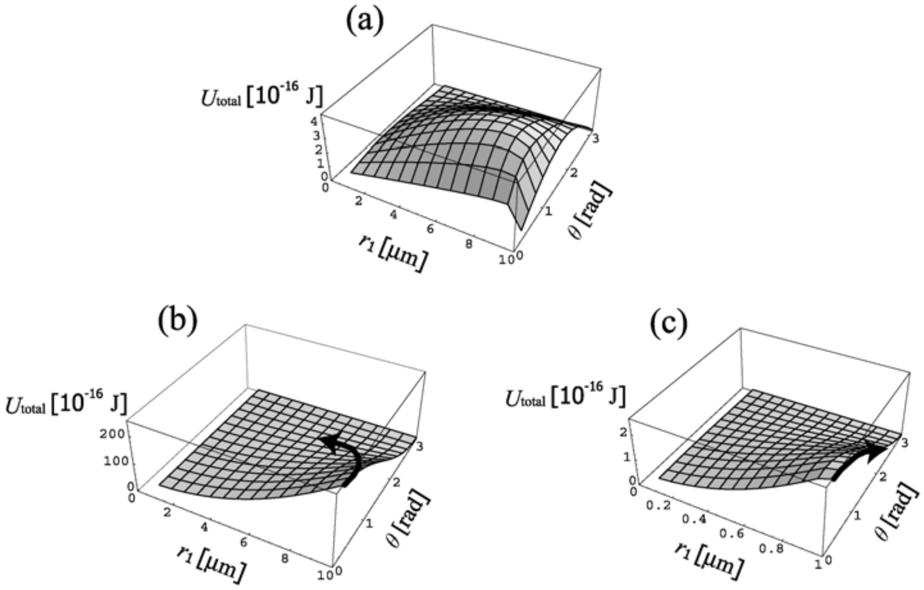

(a)
U_{total} [10^{-16} J]
4
3
2
1
0
0
2
4
6
8
10
r_1 [μm]
0
1
2
3
θ [rad]
(b)
U_{total} [10^{-16} J]
200
100
0
0
2
4
6
8
10
r_1 [μm]
0
1
2
3
θ [rad]
(c)
U_{total} [10^{-16} J]
2
1
0
0
0.2
0.4
0.6
0.8
1
r_1 [μm]
0
1
2
3
θ [rad]

Figure 3.

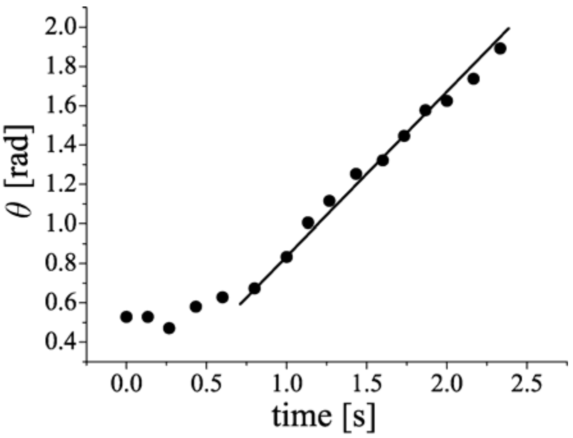


Figure 4.