Shape Transformation of Adsorbed Vesicles on Oxide Surfaces: Effect of Substrate Material and Photo-Irradiation

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Shape transformation of phospholipid vesicles on oxide surfaces was investigated by a fluorescence microscope. The transformation of spherical vesicles to a planar lipid bilayer membrane spontaneously proceeded on mica and glass, while the intact vesicular layer formed on TiO₂. Interaction energy between the substrate and the bilayer, which was evaluated using the rigorously calculated Hamaker constant, was ~10 times larger on TiO₂ than on mica and SiO₂. The results seem inconsistent with the conventionally proposed adhesion induced tension model, in which stronger adsorption leads to easier planar membrane formation from vesicles, thus indicate that the shape transformation from vesicles to a planar membrane is dominated by the kinetic processes and the dynamics of the vesicles, rather than the adsorption state of individual vesicle. Area-selective SPB formation of adsorbed vesicles was induced by the irradiation of strong excitation light, which was assisted by the photo-induced expansion of SPB containing dye-labeled lipid molecules.

Key words: lipid bilayer membrane, liposome, mica, silicon oxide, titanium oxide, fluorescence microscope

1. INTRODUCTION

Lipid bilayer is a self-assembled structure of amphiphilic lipid molecules formed in aqueous solution. The lipid bilayer is a fundamental structure of a cell membrane, and the bilayers deposited on solid substrates are attractive interfacial systems between biological molecules and solid devices [1]. One of unique characteristics of the lipid bilayer is its flexibility in shape. The bilayer takes the form of a planar sheet, a sphere or a tube depending on the surrounding condition [1,2].

Spherical lipid bilayers in aqueous solutions are called lipid vesicles or liposomes. When a lipid vesicle reaches to a solid/liquid interface, the vesicle shape distorts due to the interaction between the bilayer and the solid surface, such as van der Waals, electrostatic and hydration forces. It is well known that a planar bilayer membrane forms from the vesicles on a hydrophilic substrate [1,3-7]. The vesicles adsorb on the substrate (Fig. 1a), fuse with each other and/or rupture (Fig. 1b), and finally transform to a planar bilayer membrane (Fig. 1c). The planar bilayer membrane at the solid/aqueous solution interfaces is called “supported planar bilayer (SPB)”. The SPB formation proceeds spontaneously, thus whether it occurs or not is determined by the surrounding condition. In case the rupturing step (Fig. 1b) is not triggered, the vesicles stably adsorb on the substrate and an intact vesicular layer forms (Fig. 1d) [3-5]. The shape of the bilayer on the substrate is affected by vesicle size, lipid component, temperature, ions in the solution, and substrate properties.

The shape transformation of the adsorbed vesicle has been considered theoretically [8] as well as investigate experimentally[3,4]. The most basic theory is called "adhesion induced tension model", which express the shape of the adsorbed vesicle including the effect of vesicle size, bilayer bending modulus and bilayer-substrate interaction energy [8]. This model indicates that stronger attraction leads to larger deformation of an adsorbed vesicle, and results in easier transformation to planar membrane.

In this study, we investigated the substrate effects on the bilayer shape transformation on mica, glass and TiO₂ surfaces. The transformation from adsorbed vesicles to SPB easily proceeded on the mica and glass surfaces, while the intact vesicular layer forms on the TiO₂

Fig. 1 Schematics of reaction processes during the shape transformation of lipid vesicles on a solid surface. (a–c) Formation processes of a planar bilayer membrane from adsorbed vesicles. (d) Intact vesicular layer, which forms if the step (b) is not triggered.
surface. The interaction energy between the substrates and the bilayer, which was evaluated from van der Waals energy using rigorously calculated Hamaker constant and hydration forces, showed that the adsorption energy of the bilayer on TiO$_2$ is 10 times larger than that on SiO$_2$ and mica. We also found that the photo-induced activation of molecular motion assisted the fusion between the SPB and adsorbed vesicles on mica surface. This photo-induced SPB formation proceeded area-selectively.

2. MATERIALS AND METHODS

2.1 Materials

Dipalmitoleoylphosphatidylcholine (DPOPC) and lissamine rhodamine B labeled dioleoylphosphatidylethanolamine (Rh-DOPE) were purchased from Avanti Polar Lipid Inc. and used without further purification.

2.2 Preparation of vesicle suspension

Multilamellar vesicle suspension of DPOPC (0.1 mg ml$^{-1}$) was prepared by agitating a vacuum-dried lipid film in a buffer solution (150 mM KCl, 40 mM MgCl$_2$, 5 mM HEPES/KOH (pH 7.2)). The suspension was frozen-and-thawed five times, and was extruded through a 200 nm- or 50 nm-mesh polycarbonate filter to obtain extruded unilamellar vesicles (EUV). The diameter of the EUV distribute around the filter mesh size [9]. We diluted the 0.1 mg ml$^{-1}$ vesicle suspension by the buffer solution before the deposition onto substrates. The details of the suspension preparation were described elsewhere [5,7]. After the substrate was incubated in the vesicle suspension, excess vesicles in the solution were removed by exchanging the suspension by the vesicle-free buffer solution.

2.3 Substrate cleaning

The top layers of a mica substrate were peeled off using a sticky tape to expose a fresh surface just before the incubation in the vesicle suspension. The glass cover slips were sonicated in acetone, methanol and MilliQ water, boiled in a piranha solution (conc. H$_2$SO$_4$ + 30% H$_2$O$_2$ (volume ratio of 4:1)), and sonicated in 0.02 M KOH aq. Caution: The piranha solution violently reacts with organic materials and extreme care must be taken at all time when handling. Single-crystal wafers of rutile-TiO$_2$(100) (Furuuchi Chemical Corp.) were immersed in 10% HF aq. and annealed at 700°C at 1.0 L min$^{-1}$ O$_2$ flow. The details of glass and TiO$_2$ cleaning were described in refs. [5] and [10], respectively.

2.4 Apparatus

The sample surfaces after the incubation in the vesicle suspension were observed by an epifluorescence microscope equipped with a 40× and 60× water-immersion objective lenses and a digital CCD camera. Vesicle suspension of DPOPC containing 1% Rh-DOPE (Ex 557 nm/Em 571 nm) was used for the fluorescence microscope observation. The sample was irradiated by a high-pressure mercury lamp through a 530-550 nm band-pass filter. The intensity of the excitation light from the mercury lamp was attenuated to 5.7 mW mm$^{-2}$ through ND filters for the imaging. The sample was irradiated by the excitation light of 247 mW mm$^{-2}$ for the photo-induced activation by removing the ND filters.

3. RESULTS AND DISCUSSION

3.1 Bilayer shape depending on substrate materials

Figure 2 shows the fluorescence microscope images of three oxide substrates after the incubation in the DPOPC-EUV suspension. The mica surface incubated at 40°C for 15 min was fully covered by a membrane with uniform brightness (Fig. 2a-1). It reasonably indicates that a planar bilayer forms on the surface. The bright spots in Fig. 2a-1 are unfused vesicles adsorbed on the SPB. The fluidity of the SPB was checked by following the process of fluorescence recovery after photobleaching (Figs. 2a-2-5).

(a) Mica surface incubated in a 200 nm-EUV suspension at 40°C for 15 min. (a-1) Before photobleaching. (a-2) 10 s, (a-3) 80 s, (a-4) 199 s and (a-5) 619 s after the photobleaching. (b) Glass surface incubated in a 200 nm-EUV suspension at 40°C for 60 min. (b-1) Before photobleaching. (b-2) 8 s, (b-3) 30 s, (b-4) 60 s and (b-5) 91 s after photobleaching. (c) TiO$_2$ surface incubated in the 200 nm-EUV suspension at 40°C for 15 min. (c-1) Before photobleaching and (c-2) 190 s after the photobleaching. (d) TiO$_2$(50 nm-EUV) surface incubated in a 50 nm-EUV suspension at 40°C for 15 min. (d-1) Before photobleaching and (d-2) 325 s after the photobleaching. The brightness of each sample is adjusted for facilitate visualization.
photobleaching (FRAP). The DPOPC-SBP is in the fluid liquid crystal (Lα) phase because the gel-Lα transition temperature of DPOPC is -36°C. When a part of the SPB is illuminated by a strong excitation light, the dye-labeled lipids in the illuminated region is quickly photobleached. The fluorescence signal from the bleached region temporally recovers if the fluid and continuous SPB exists on the substrate, because of the lateral molecular diffusion; bleached (dark) lipids go out of the bleached region and the fresh (bright) lipids come in the region. Figure 2a (a2-a5) shows the FRAP process of the SPB on mica. Almost complete fluorescence recovery in the bleached region indicates that a fluid and continuous SPB forms and that little immobile fraction exist. Similarly uniform and continuous full-coverage SPB was formed on the glass surface (Fig. 2b-1). Complete fluorescence recovery was also observed in the sequential images of FRAP (Fig. 2b, b2-b5). The SPB formation on the mica and the glass surfaces was little affected by the incubation time and lipid concentration, in the range of 10-60 min and 0.013-0.05 mg/ml, respectively. The results in Fig. 2a and 2b are reasonable compared with previous studies which showed unilamellar vesicles larger than a critical radius spontaneously transformed to SPB on mica and SiO2 surfaces [5-7,11].

On the TiO2(100) single crystal surface, however, fluorescence images completely different from that on mica and glass were obtained after the incubation in the DPOPC-EUV suspension in the similar condition (Fig. 2c-1). The visual impression of Fig. 2c-1 is grainy and textured compared with Fig. 2a and 2b. After a part of the sample in Fig 2c-1 was photobleached (Fig. 2c-2). The results in Fig. 2c indicate the formation of intact vesicular layer (Fig. 1d) on the TiO2(100) surface, and the adsorbed vesicles are immobile on the surface. Similar intact vesicular layer formed when the substrate was incubated in a 50 nm-EUV suspension instead of the 200 nm-EUV suspension (Fig. 2d). The results in Fig. 2c and 2d correspond to previously reported studies of DOPC vesicles on sputter-deposited TiO2 surfaces [3,4]. We recently reported that the SPB formation proceeds only when a step-and-terrace TiO2(100) surface was incubated in a sonicated unilamellar vesicle (~30 nm diameter) suspension with sufficiently high lipid concentration (>0.025 mg/ml) for more than 35 min [5].

3.2 Discussion from the view point of solid-bilayer interaction energy

The shape change of the adsorbed vesicles on solid surface has been explained by adhesion induced tension (AIT) model [8]. The adsorption state of a vesicle is expressed by a potential parameter w by

\[ w = \frac{WR_h^2}{k_b} \]  

where \( W \), \( R_h \) and \( k_b \) represent the interaction energy between a bilayer membrane and a solid surface, radius of the spherical vesicle, and bending modulus of the bilayer, respectively. A vesicle with a spherical shape without structural distortion (point contact to the substrate) has the value of \( w = 2.0 \), and larger value corresponds to larger distortion from sphere to scone and further to pancake. It is expected from eq. (1) that the stronger attraction between the bilayer and the substrate, and the larger vesicle size lead to more structural distortion of vesicle, thus result in easier planar membrane formation. We discuss in this section whether the results in Fig. 2 can be expressed by the AIT model.

The interaction energy (\( W \)) between two materials in an aqueous solution is expressed as the summation of van der Waals energy (\( W_{vdw} \)), electrostatic energy originated from the double layered force (\( W_{DL} \)) and hydration energy (\( W_{hyd} \)) [12]:

\[ W = W_{vdw} + W_{DL} + W_{hyd} \]  

The van der Waals interaction energy between two parallel planar surfaces is expressed as

\[ W_{vdw} = \frac{A}{12aD^2} \]  

where \( A \) represents Hamaker constant. Now we assume that two semi-infinite media "1" and "2" sandwich a planar slab of medium "3" of thickness \( D \). The value of \( A \) at the media 1/3/2 system is obtained by

\[ A_{ij} = A_{ij}(2D)\epsilon_{ij}^2 + A_{ij} \]  

\[ A_{ij} = \frac{3kT}{4} \left( \varepsilon_i(0) - \varepsilon_j(0) \right) \left( \varepsilon_i(0) - \varepsilon_j(0) \right) \]  

\[ A_{ij} = \frac{3h}{4\pi} \int R_i(v)R_j(v)dv \]  

where

\[ R_i(v) = \frac{2e^{\nu^2/v_i^2}}{v_i^2v_j^2} + \frac{2e^{\nu^2/v_j^2}}{v_j^2} + C_i + C_j + 2 \]  

(\( i = 1, 2 \), 7).

and \( \kappa, k, T, \varepsilon_j(0), h, v_i \) represent the inverse of Debye length, Boltzmann constant, temperature, the static dielectric constant of medium j (j = 1, 2, 3), Planck constant, and the absorption frequency of medium j in the UV range, respectively. The oscillator parameter \( C_i \) is represented by \( \nu = A/\nu_i \), where \( f_i \) is the oscillator strength of the absorption band, and \( C_i \) can be experimentally obtained by a "Cauchy plot"[13-15]. The parameters for the calculation of Hamaker constants are summarized in Table 1. A simplification called "Tabor-Winterton (TW) approximation" is applied generally to solve eq. (6) and (7) [12,13,16]. The TW-approximation gives a reasonable value of \( A_{ij} \) if the three media have similar absorption frequency (\( \nu \)) values, for example \( \nu = 3-10^3 \) s\(^{-1} \) for mica, SiO2, water and lipid. This method, however, seriously overestimate \( A_{ij} \) if the medium 1 has a small \( \nu \) value, such as TiO2 (Table 1). Therefore the \( A_{ij} \) is rigorously calculated by the method of Prieve and Russel (PR-method) [15] in this study. The detail of the calculation was described in ref. [5] and Supporting Information. The calculated values of \( A_{ij} \) for various oxide materials are shown in Table 1. Physical values of fused quartz (SiO2) are used for the alternate of glass. Figure 3a shows the \( W_{vdw} \) dependence on \( D \) calculated from eq. (3-7) based on the PR-method.
The plots of ZnO and Al₂O₃ are displayed for reference, as well as the substrate materials used in Fig. 2, mica, SiO₂, and TiO₂.

The second term in eq. (2) (\(W_{DL}\)) is neglected in this study, because the phosphatidylcholine headgroup of DPoPC is electrically neutral in the buffer solution in this study (pH 7.2).

The hydration energy (\(W_{hyd}\) in eq. (2)) was evaluated as follows:

\[
W_{hyd} = \frac{1}{2} \left( W_c \left( \frac{\rho e^{-\Gamma}}{\alpha} \right) - \frac{(kT)^2}{20kD^2} \cdot 2.7T_{Fe} \right) \tag{8}
\]

where \(\Gamma\), \(\alpha_p\), \(k_a\), \(k_b\) represent surface density of protruding head groups, protruding energy, area expansion modulus and bending modulus. The first term in eq. (8) represents the hydration energy originated from the structural water layers on a solid substrate [12]; the second and third terms arise from the repulsive forces due to the thermal motion of the bilayer membrane, peristaltic and protrusion, respectively. We took the typical values of \(W_c=6.0\) mJ m⁻² and \(\alpha_p=0.3\) nm, \(\kappa_a=2.5 \times 10^{-11}\) Jm⁻¹, \(k_b=0.15\) Jm⁻² and \(k_b=10^{-19}\) J from ref. [12], and used the value of \(\Gamma=1.6\) nm⁻² from the molecular occupying area of lipid (0.64 nm²/lipid) [17].

Figure 3b shows the total interaction energy (\(\hat{W}\)) plotted against the distance between the bilayer and the solid substrate calculated from eq. (2-8). The bilayer membranes on oxide surfaces has a minimum potential at \(D=1-2\) nm, which corresponds to the experimentally evaluated water layer thickness between the bilayer and the substrate (1.3-1.7 nm) [18,19]. The minimum energies and the distances at the potential minimum are -7.85 µJ m⁻² at 2.02 nm for mica, -9.71 µJ m⁻² at 1.94 nm for SiO₂, -99.6 µJ m⁻² at 1.08 nm for TiO₂.

The energy calculation in Fig. 3 seems inconsistent with the AIT model (eq. (1)), because the vesicles easily transformed to planar membrane on mica and SiO₂ (Fig. 2a and 2b), which have weaker interaction with the bilayer, whereas the intact vesicular layer forms on TiO₂ (Fig. 2c and 2d), on which bilayer is more strongly attracted. We should note that the AT model treats only the energetic aspect of static vesicle adsorption, and that the transformation of adsorbed vesicles to SPB is an irreversible and dynamic process largely containing kinetic aspect and other factors. We suppose that key factors for the transformation of adsorbed vesicles to a planar membrane are the inter-vesicle interaction and the interaction between the SPB and adsorbed vesicles rather than the adsorption energy of individual vesicle.

3.3 Photo-induced shape transformation of adsorbed vesicles

The shape transformation of the adsorbed vesicles is a thermally induced spontaneous reaction, thus the final state, a SPB or a vesicular layer, is difficult to control. Ujihara et al. recently reported that the lateral distribution of lipid molecules and the domain coverage in a binary SPB can be area-selectively controlled by the

![Fig. 3 Interaction energies of oxide/water/lipid membrane system using rigorously calculated Hamaker constant. (a) Van der Waals energy, and (b) total interaction energy including the energy from the surface hydration force.](image)
local excitation of the dye-labeled lipid molecules [6]. The proposed mechanism of the photo-induced excitation of the dye containing SPB is that a part of the energy which excited the fluorescent dye molecules is released through the activation of molecular vibration, translation and rotation. It is just like the local temperature around the dye-labeled lipid molecule increases due to the fluorescent excitation light. We applied this photo-activation to induce the shape transformation of the adsorbed vesicles area-selectively.

Figure 4a shows a fluorescence microscope image of a mica surface where the SPB and vesicular layer of DOPePC coexist. The vesicular layers are sometimes observed in the region near the sample edge even when almost whole the surface is covered with SPB, likely because of step bunches or contamination. This kind of intact vesicular layer is thermally stable, and can not be transformed to a planar membrane even if it is incubated again. A part of the surface in Fig. 4a was irradiated by strong excitation light of 247 mW mm\(^{-2}\), at room temperature (Fig. 4b). We found that the vesicular layer in the photo-irradiated region transformed to a planar membrane connected to the originally existing SPB (Fig. 4b-d). Photo-bleaching occurred at the irradiated region both in the SPB and the vesicular layer (Fig. 4b), but FRAP completely proceeded in whole photo-irradiated region because the vesicular layer transformed to a continuous SPB. It should be noted that this transformation from the vesicles to the planar bilayer was limited only at the light-irradiated region, and the adsorbed vesicular layer remained out of the light-irradiated region (Fig. 4b-d). This photo-induced SPB formation is assisted by the already formed SPB. The excitation light with the same intensity as Fig. 4b irradiated to the middle of the vesicular layer (Fig. 4e) did not cause the SPB formation (Fig. 4f-g). We observed the photo-induced shape transformation in-situ and found that the transformation proceeded always at the front of the planar domains; the already formed planar bilayer domains spread to the vesicular region, but no domain was newly generated in the vesicular region (data not shown).

We suppose that the planar bilayer formation is caused by the photo-induced expansion due to the local temperature increase through the excitation of the fluorescence dye. The expanded planar bilayer domain stimulates the rupture and fusion of the nearby adsorbed vesicles, which causes the chain-reaction of planar bilayer spreading (Fig. 4h). This photo-induced transformation did not occur when the sample was kept irradiated by the excitation light for the usual observation at the intensity of the 5.7 mW mm\(^{-2}\). However, the photo-induced expansion does not completely explain the phenomenon, because the SPB formation does not proceed by a simple heating of the sample, which causes the thermal expansion of the bilayer. The local orientation of the dye-labeled lipid molecule may change by the excitation, and induce the fusion between the SPB and adsorbed vesicles.

This photo-induced SPB formation is a quite interesting phenomenon to intentionally induce the SPB formation of adsorbed vesicles, and to make SPB bilayers heterogeneously. Several previous reports showed that the addition of bilayer fusion inducers, such as poly-ethylene glycol and Ca\(^{2+}\), into the buffer solution promotes the SPB formation by vesicle fusion. [11,20]. This method, however, is a homogeneous reaction. The photo-induced reaction shown in Fig. 4 achieves a spatially heterogeneous SPB fabrication, and can be applied to area-selective bilayer formation and bilayer membrane patterning.

4. SUMMARY

We have investigated shape transformation of vesicles on oxide surfaces, and the effect of substrate materials and photo-irradiation. The SPB formation
from the vesicles easily proceeded on the substrates with weaker attractive energy, e.g. mica and glass, while the intact vesicular layer formed on TiO$_2$, which has stronger attractive interaction with the bilayer, due to the large van der Waals energy. The results are inconsistent with the AIT model, and thus indicate that the shape transformation from vesicles to a planar membrane is dominated by the kinetic processes and the dynamics of the vesicles, such as adsorption, desorption, diffusion and fusion of vesicles, rather than the adsorption state of individual vesicle. Area-selective SPB formation of adsorbed vesicles was induced by the irradiation of strong excitation light, which was assisted by the photo-induced expansion of SPB.

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