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Author(s)	Hamada, Tsutomu; Ishii, Ken-ichi; Sugimoto, Ryoko; Nagasaki, Takeshi; Takagi, Masahiro
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Description	

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Photochemical control on morphologies of a cell-sized synthetic vesicle

Tsutomu Hamada, Ken-ichi Ishii, Ryoko Sugimoto, Takeshi Nagasaki, Masahiro Takagi*
School of Materials Science, Japan Advance Institute of Science & Technology
1-1 Asahidai, Nomi City, Ishikawa 923-1292
Japan

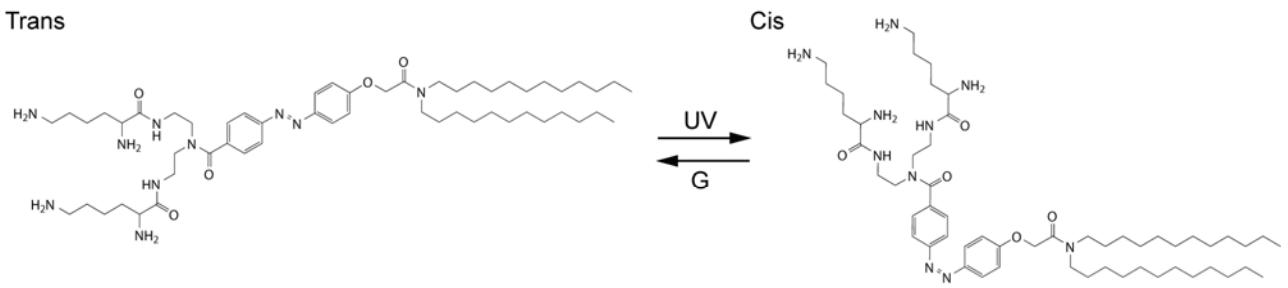
Abstract:
By using a synthetic photosensitive amphiphile containing azobenzene (KAON12), we developed a method for the photo-manipulation of lipid membrane morphology, in which the shape of a vesicle can be switched by light. Cell-sized liposomes are prepared from KAON12 and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). We conducted real-time observations of vesicular transformation in the photo-sensitive liposome by phase-contrast microscopy, and found that membrane budding transitions could be controlled by light. These transformations can be interpreted in terms of the change in the effective membrane surface area due to photoisomerization of the constituent molecules. We discuss the mechanism by considering the elastic free energy of the membranes.

1. INTRODUCTION

Living cells and cellular organelles, such as the nucleus, the mitochondrion, the golgi apparatus and endoplasmic reticulum, are commonly enclosed by a membrane with a lipid bilayer structure [1]. Cells actively change their structures in response to changes in the environment, such as chemical signals and/or physical conditions. Regulation of the lipid membrane structure is critical for many cellular processes, e.g., the budding-fission-fusion sequence of vesicular transport from the endoplasmic reticulum to the plasma membrane and the uptake of macromolecular aggregates via coated pits or phagocytosis [2,3]. The mechanical properties of the bilayer itself and/or embedded and associated proteins should play an important role in controlling these shapes [4].

Recently, cell-sized liposomes (>10 μm) have been actively studied as cell models due to their similarities to natural cell structures with regard to size and membrane composition [5]. Since they are large enough to allow direct microscopic observation of the membrane behaviors of individual vesicles, morphological dynamics in response to various internal and external stimuli have been investigated (e.g., temperature [6], chemical reaction inside or outside [7], polymerization of encapsulated cytoskeleton [8], addition of lanthanoid [9], osmotic stress [10,11] and interaction with solid-liquid interface [12]). The development of methods to control the vesicular morphology could help us to better understand the physico-chemical properties of membrane structures and to manipulate them as a micro-reactor [13,14]. Along these lines, we have developed a photo-manipulation method to control lipid vesicular shapes[15,16]. Light is an efficient experimental tool since energy can be supplied without contact and without changing the chemical composition of the medium. In these experiments, a photosensitive amphiphilic molecule could switch the shape of an assembled vesicle. Photoisomerization induced a change in membrane fluctuation behavior or a morphological transition between ellipsoid and bud shapes. The measurement of Π-A curve implied that membrane area expansion is one of the possible factors in the shape changes. The photo-induced change in membrane area was directly measured using micropipette aspiration technique. We will discuss the mechanism of these photo-induced transformations by comparing them to the changes in morphology in an osmotic shock experiment.

It should be noted that, in biological systems, changes in the conformation of photosensitive molecules embedded in membranes, such as rhodopsin, play important physiological roles [17]. Many studies have been conducted to prepare artificial photosensitive vesicles, which are also expected to be applied to drug delivery, and have reported changes in the permeability of ions and/or water-soluble compounds across the membrane upon photoisomerization [18,19]. In these studies, submicron vesicles have frequently been used, which implies that the direct observation of morphological change in individual vesicles is impossible.



Structural Formula 1: A photosensitive amphiphilic molecule (KAON12).

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2. EXPERIMENTAL SECTION

Materials

We designed and synthesized a photosensitive amphiphilic molecule containing azobenzene (KAON12); the conformation (trans or cis) of this molecule can be switched by light (Structural Formula 1) [20]. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was purchased from Avanti Polar Lipids. Deionized water obtained from a Millipore Milli Q purification system was used to prepare reagents.

Preparation of photosensitive cell-sized liposomes

Liposomes were prepared using the natural swelling method from a dry lipid film; a lipid mixture (KAON12 and DOPC) dissolved in 1:2 (v/v) methanol/chloroform in a glass test tube was dried under vacuum for 2 h to form thin lipid films. The films were then hydrated overnight with deionized water at 37 ℃. The final concentration was 0.5 mM lipids (KAON12 35~45%). Using a conventional microscopic method with fluorescent dyes to monitor lipid segregations within bilayer membranes, we confirmed that no segregation was detected on the KAON12/DOPC membrane surface.

Microscopic observation under photo-irradiation

The liposome solution (5 μl) was placed on a glass slip, covered with another smaller slip at a spacing of ca. 0.2 mm. We observed changes in the membrane morphology with a phase-contrast microscope (Olympus BX50, Japan) at room temperature and irradiated membranes through standard filter sets, (WU, Olympus; ex 330-385 nm, dichroic mirror 400 nm, em 420 nm and WIG, Olympus; ex 520-550 nm, dichroic mirror 565 nm, em 580 nm), with an extra-high-pressure mercury lamp (100 W) for photoisomerization. Irradiation time was within a second (no effect of sample heating). The ratios of trans-/cis-azo under UV and green illuminations are approximately 1/4 and 4/1, respectively [15]. The images were recorded on HDD at 30 frames/s.

Treatment with osmotic stress:

Liposome and 1 mM glucose solution were poured into a test tube and gently mixed by soft tapping. The difference in the molar concentration of glucose across the bilayer membrane was 0.5 mM.

3. RESULTS

Reversible control of membrane budding transitions

Figure 2 shows the results of the photo-irradiation on a prolate vesicle, which is spontaneously formed through natural swelling of the lipid film. In the past experiments on the swelling of lipid film, it is known that such kind of asymmetrical vesicles are generated rather frequently in addition to the symmetric spherical vesicles. In other words, deformed asymmetric vesicles are obtained without any specific experimental procedure. After UV irradiation, the prolate vesicle exhibits budding. Interestingly, the budded vesicle transforms back to the original ellipsoidal shape upon treatment with green light. This reversible change in morphology is observed more than ten times.

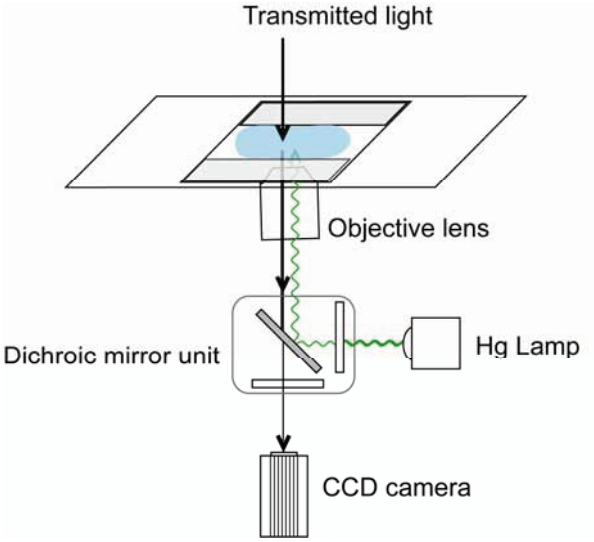

Figure 1. Experimental setup.

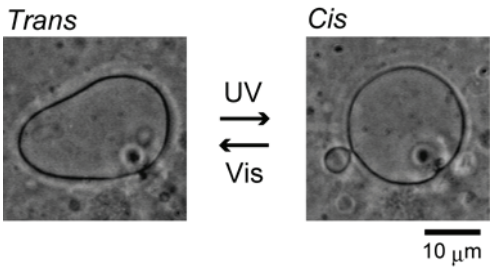

Figure 2. Photo-induced reversible prolate-bud transition in a cell-sized vesicle [15].

We found that photoisomerization induces several membrane transitions, such as stomatocyte, oblate, prolate and exo-bud, as shown in Figure 3. These photo-induced transformation can be attributed to a change in membrane surface area. Our experiments on π-A curves of a langmuir monolayer shows that the surface area per molecule in the cis-azo form is greater than that in the trans-azo form at the same pressure [15]. In addition, we measured the change in membrane area upon photoirradiation by a micromanipulation technique, and revealed that the average

ratio of the area expansion was ~3 % [16]. Notably, the volume change is negligible compared to the photo-induced change in surface area on the time-scale of our observation, due to the low permeability of water through a phospholipid bilayer membrane.

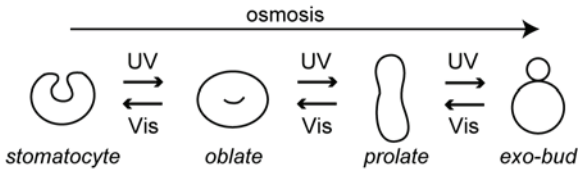

Figure 3. Photo-induced morphological transitions and transformation pathway in response to osmotic stress [16].

To better understand these photo-induced morphological transitions in terms of a change in membrane area, we investigated the behavior of the membrane morphology under osmotic stress [16]. When the liposomes are subjected to high osmolarity, the water efflux across the membranes reduces the inner aqueous volume, indicating that the liposomes acquire excess surface area. The excess area is a parameter that describes the membrane morphology using Helfrich bending energy,[21] defined by the ratio of area to volume, which is also called the reduced volume [22]. Within this context, the change in the interior volume with a constant area by osmosis is comparable to the change in area with a constant volume by photoisomerization.

The vesicular shapes observed in the osmotic evolution are consistent with the photo-induced morphological transitions (Figure 3). After osmotic pressure was applied, a thin flexible filament, most probably attached to the mother liposome, was observed in the vesicular space. The daughter filament gradually became thick, i.e., tubular structure with a micron-sized internal core. The contact point between the inner vesicle and the outer membrane started to open, and the shape of the liposome changed into the stomatocyte. After the invaginated area was completely opened, the liposome acquired an oblate shape. Finally, the oblate liposome elongated to be prolate, followed by an exo-bud. Thus, this change in morphology from an endo-bud to an exo-bud through stomatocyte, oblate and prolate occurs with an increase in excess area. These shapes are expected to have a minimum membrane bending energy for each excess area. Thus, the energetically stable shape shifted with a change in the membrane excess area due to osmotic stress.

Model of morphological transitions

The shape of liposomes can be deduced by an elastic bending energy [23]. Here we will adopt the bilayer coupling model to explain our experimental results. In this model, axisymmetric shapes of minimum bending energy was numerically calculated as a function of the reduced volume (i.e., area-to-volume ratio), v, and reduced area difference, Δa, defined as

$$v(x) \equiv \frac{V}{4\pi R_0^3/3},$$

$$\Delta a \equiv \frac{\Delta A}{\Delta A_{sphere}},$$

where R_0 is the equivalent sphere radius defined by $A = 4\pi R_0^2$, ΔA and ΔA_{sphere} are the area difference between each leaflet and that for spherical vesicles. We express the area expansivity of monolayers resulted from photo-isomerization using these variables. The Π-A measurements and microscopic observations confirmed that the effective cross-section of the photosensitive molecule changes between cis and trans isomers [15,16]. Let $A_{in/out}$ be the area of inner/outer leaflets, and let ΔS denote the area difference of the photosensitive lipid on each form. The changes in the membrane area upon photo-isomerization ratio x are

$$\frac{dA_{in/out}(x)}{dx} = N_{in/out}\alpha_{in/out}\Delta S \equiv \beta_{in/out}$$

where $N_{in/out}$ and $\alpha_{in/out}$ are the total lipid number and the ratio of the photosensitive lipid on each leaflet of bilayer membranes.

If one chooses $A = A_{in}$, one obtains

$$R_0(x) = \left[\frac{A_{in}(x)}{4\pi}\right]^{1/2} = \left[\frac{\beta_{in}x + A_{in}(0)}{4\pi}\right]^{1/2}.$$

Thus, the reduced volume as a function of x is

$$v(x) \equiv 3\sqrt{4\pi}V\left[\beta_{in}x + A_{in}(0)\right]^{-3/2}.$$

The reduced area difference is

$$\Delta a(x) = \frac{\Delta A(x)}{16\pi d(x)R_0(x)}$$

$$= \frac{(\Delta\beta x + \Delta A(0))(\beta_{in}x + A_{in}(0))^{1/2}}{8\sqrt{\pi}d(0)A_{in}(0)},$$

where we used the condition of incompressibility $d(x)A_{in}(x)=d(0)A_{in}(0)$.

Time scale for transformation

As described in the previous section, the driving force for the morphological transition is a bending energy: the stable morphology of vesicles is switched by photoisomerization. Here, we will confirm it from a simple estimation. We substitute the characteristic values of the experiment into the motion equation for a moving object in an aqueous solution:

$$\frac{\Delta U}{\Delta x} = \gamma V.$$

In the microscopic observations, liposomes took several seconds to transform their shapes, and the velocity V is around 10 μm/s. The coefficient of friction γ is proportional to the product of a viscous coefficient η (~ 10^{-3} $Nm^{-2}s$) and the length scale of the moving object nearly equal to Δx as $\gamma \sim \eta\Delta x$. If one takes Δx to be 5 μm, one obtains the energy $\Delta U \sim 60\ k_BT$, which corresponds to the order of the bending energies.

Enhanced water permeability of cis-azo membranes Under UV irradiation, the conformation of the cis-azo vesicles gradually shifts to a sphere over several tens of minutes, which is 10^3 longer than the time of the photoinduced transformation. The membrane composed of the cis isomer may have higher ion permeability than that of the trans isomer because of its bulky structure, as reported elsewhere [19]. A cis-azo vesicle can easily transfer water molecules across the membrane, and soon reaches an equilibrium morphology (i.e., a spherical shape with the lowest bending energy).

4. Conclusions

We conducted real-time observations of vesicular transformation in a liposome with an azo-lipid under photoirradiation. The results clearly demonstrate that the liposome exhibited various reversible transitions, such as exo- and endo-budding, due to photoisomerization of the constituent molecule. Since the course of the photo-induced shape transitions agreed well with the morphological changes due to the increase in excess area caused by osmotic pressure, the mechanism of these photo-induced transformations is interpreted in terms of the change in membrane surface area. Photons are potent stimuli that can be used for the timing-specific control of biological macromolecules. The present findings may provide insight into the biophysics of membrane mechanics and a basis for their wide practical applications with light.

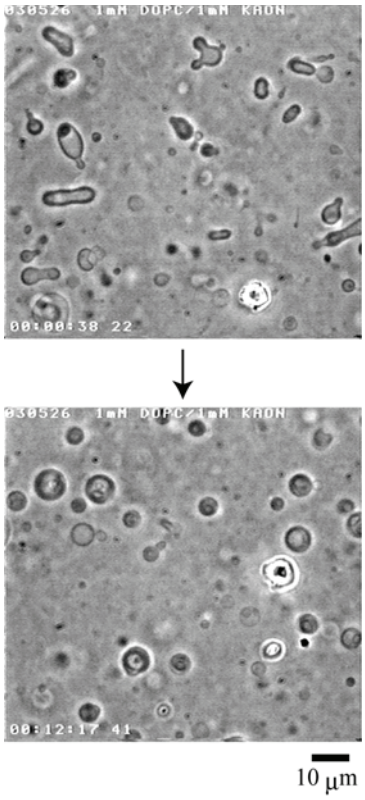

Figure 4. Transformation of vesicles with cis-azo form.

Acknowledgements

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