Optical Transformation and Fission of Single Giant Vesicles in Water by Radiation Pressure

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Unilamellar giant vesicles made of didodecyldimethylammonium bromide (DDAB) are very unique, since the vesicles show fusion of two vesicles and birthing of a daughter from a relatively large parent vesicle.[1,2] Fusion and birthing of DDAB vesicles need membrane−membrane interactions and subsequent structural transformation of the bilayer membrane. Nonetheless, its mechanisms including roles of membrane tension and osmotic pressure changes are still ambiguous. Furthermore, the role of a packing or stiffness factor of vesicles in membrane−membrane interactions is worth elucidating.[2,3] If one can control fusion or fission or both of single vesicles in a noncontact manner, a study on both molecular mechanisms of membrane−membrane interactions and physical−mechanical properties of vesicles will be greatly advanced. For noncontact manipulation of particles in solution, the use of the radiation pressure generated by light scattering by particles is the most probable candidate.[4] We thus explored transformation and fission of single DDAB vesicles in water by an infrared laser beam. In this paper, we report a role of the radiation pressure in manipulating and processing single DDAB vesicles in water.

DDAB (Aldrich, used as supplied) vesicles were prepared according to the literature.[2] Typically, 2.5 mg of DDAB was hydrated with an aqueous Tris/HCl buffer solution (pH 7.1, 4.5 mL) at 60 °C for 1 h. The vesicles were stable for several days at ambient temperature. The size of the vesicle ranged from several to several tens of micrometers. Fusion and birthing of the vesicles have been also confirmed in the presence of sodium acetate (0.2 mol/dm^3), as reported by Menger and Gabrielson.[1,2]

It has been reported that H_2O absorbs an incident 1064-nm laser beam, so that this might lead to temperature elevation of a sample solution.[5] To avoid thermal effects, we used D_2O as a transparent medium at 1064 nm. The sample D_2O solution (28 μL) was poured into a 6 mm i.d. Teflon O-ring cemented on a glass plate and covered with a thin glass plate. For laser manipulation, a 1064-nm laser beam (CW Nd:YAG laser, Spectron, SL902T) was introduced to an optical microscope (Nikon, Optiphot 2) and focused ($\sim$1 μm spot) onto the solution through an oil-immersion objective ($\times$100, N.A. = 1.30).[6] Actual laser power irradiated to the sample solution was estimated to be several hundreds of milliwatts.[7] Manipulation behavior was monitored by a CCD camera attached to the microscope. The microscope used was not a phase contrast mode, so that the images observed were not very clear. The images reported here were thus processed by an image processor (Argus-20, Hamamatsu Photonics). All experiments were performed at ambient temperature.

A typical example of the experiments is shown in Figure 1 (a−e). The focused laser beam was irradiated to the periphery of a vesicle (Figure 1a, right-handed side). Even when the microscope stage was scanned slowly along a lateral direction, the vesicle remained at the same position without any shape change, demonstrating laser manipulation of the vesicle in the $X-Y$ plane. With increasing the scan rate of the microscope stage (to the left-handed side), the vesicle transformed from a spherical to ellipsoidal shape (b and c). Since the vesicle experiences viscous resistance in water, the top front of the vesicle (right-handed side of the photographs) becomes a parabolic shape. At the scan rate (ν) of $\sim$6 μm/s, the vesicle was deformed considerably (d) and, finally, separated into two (e). In Figure 1e, a small vesicle can be seen at the laser spot (shown by right-handed side arrow), while the other vesicle transformed again to a nearly spherical shape within several seconds (without laser irradiation). These photographs demonstrate controlled transformation/fission of a single vesicle by the laser beam. So far, shape transformation/deformation of single giant vesicles has been reported on the basis of laser tweezers[8−10] or micropipet suction.[3] However, this is the first demonstration of laser-controlled fission of a giant vesicle. It is worth noting that the present results are not due to a thermal effect, because of the use of D_2O as the medium. Even if an absorbing species is contaminated, generated heat will be negligibly low since the absorbing volume is mostly D_2O and the vesicle wall is very thin (40 Å).[11] Thus, the driving force for transformation/fission of the vesicle will be the radiation pressure generated by light scattering.

The radiation pressure is exerted to a micrometer-sized particle, when light (wavelength = λ) is refracted by the particle (diameter = d ($>\lambda$)).[4] In the present case, however, both the inner and outer phases of the vesicle are D_2O and the vesicle wall is very thin, so that effective light refraction by the vesicle itself (Mie scattering) is not expected. On the other hand, the radiation pressure is also exerted on Rayleigh particles (i.e., $d < \lambda$) through the change in the polarizability of the particle.[12] Actually, such an example has been currently reported: 1064-nm laser-induced phase transition of polymer solutions[5] and single droplet formation from swelled micelles in water.[12] In the present case, we propose that Rayleigh scattering of the laser beam by the DDAB molecules composing the vesicle membrane wall plays an essential role. Namely, when ν is slow enough and the intermolecular forces between the DDAB molecules overcome viscous resistance experienced by the vesicle in water, the vesicle would be manipulated in the $X-Y$ plane, even by irradiation of the laser beam to the periphery of the vesicle (Figure 1a and b). With increasing ν, the nonirradiated part of the vesicle receives viscous resistance, while the irradiated volume remains at the same position owing to the radiation pressure exerted to the DDAB molecules in the vicinity of the laser focus, leading to deformation of the vesicle. This is what is observed in Figure 1d. Thus, transformation and fission of the vesicle is essentially ascribed to the radiation pressure and viscous resistance experienced by DDAB and the vesicle, respectively. If Stokes' law is applicable, the force between the DDAB molecules producing the bilayer membrane might be evaluated. Knowing the viscosity of water (η = 0.89 cP at 25 °C),[13] the radius of the vesicle ($r = \sim 3$ μm), and $\nu = \sim 6$ μm/s, we obtain the force ($F = 6\pi\eta r\nu$) to be in the

(1) Menger, F. M.; Balachander, N. *J. Am. Chem. Soc.* **1992**, *114*, 5862.
(2) Menger, F. M.; Gabrielson, K. *J. Am. Chem. Soc.* **1994**, *116*, 1567.
(3) Evans, E.; Needham, D. *J. Phys. Chem.* **1987**, *91*, 4219.
(4) *Microchemistry−Spectroscopy and Chemistry in Small Domains*; Masuhara, H., De Schryver, F. C., Kitamura, N., Tamai, N., Eds.; North-Holland: Amsterdam, 1994.
(5) Ishikawa, M.; Misawa, H.; Kitamura, N.; Fujisawa, R.; Masuhara, H. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 59.
(6) Kitamura, N.; Hayashi, M.; Nakatani, K.; Kim, H.-B. *Anal. Sci.* **1996**, *12*, 49.
(7) Misawa, H.; Koshioka, M.; Sasaki, K.; Kitamura, N.; Masuhara, H. *J. Appl. Phys.* **1991**, *70*, 3829.
(8) Moroz, J. D.; Nelson, P.; Bar-Ziv, R.; Moses, E. *Phys. Rev. Lett.* **1997**, *78*, 386.
(9) Bar-Ziv, R.; Frisch, T.; Moses, E. *Phys. Rev. Lett.* **1995**, 75, 3481.
(10) Angelova, M. I.; Pouligny, B.; Martinot-Lagarde, G.; Grehan, G.; Gouesbet, G. *Prog. Colloid Polym. Sci.* **1994**, *97*, 293.
(11) Bar-Ziv. R.; Moses, E. *Phys. Rev. Lett.* **1994**, *73*, 1392.
(12) Hotta, J.; Sasaki, K.; Masuhara, H. *J. Am. Chem. Soc.* **1996**, *118*, 11968.
(13) *Organic Solvent, Technique of Chemistry*; Riddick, J. A., Bunger, W. B., Eds.; Wiley-Interscience: New York, 1975; Vol. II.

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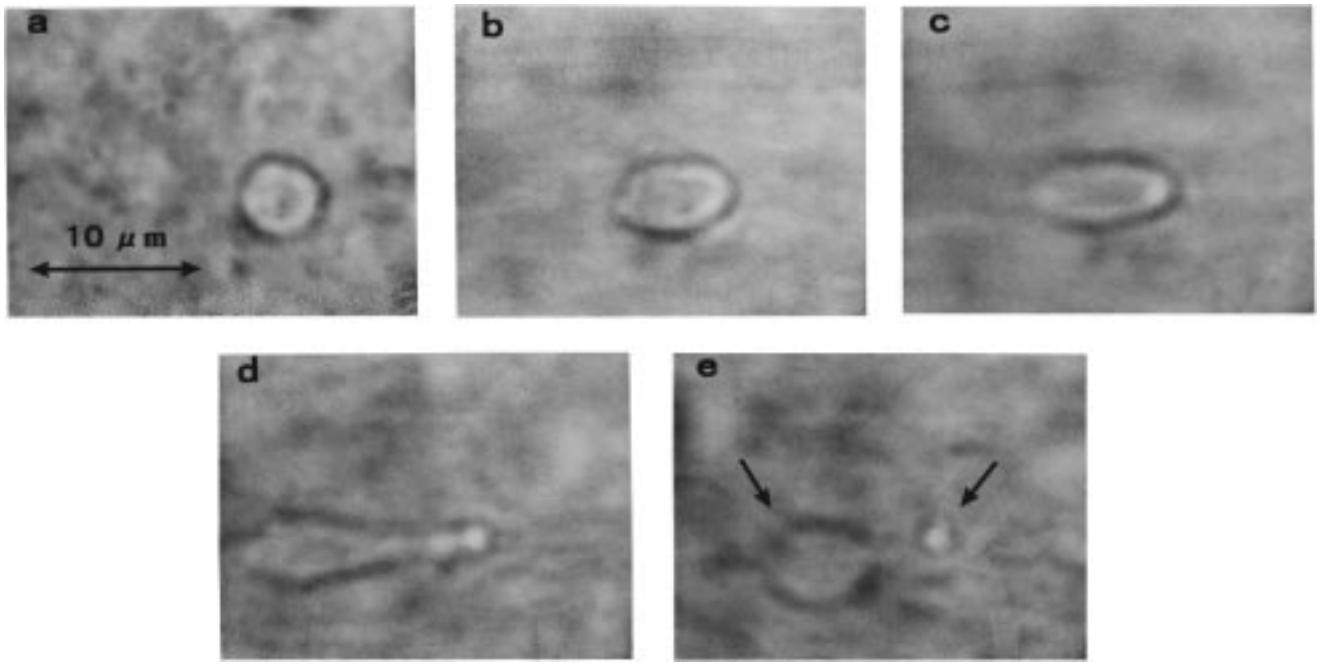

Figure 1. Optical transformation (a−c) and fission (d and e) of a single DDAB giant vesicle in D_2O. In b−e, the microscope stage was scanned to the left-handed side of the photograph with the vesicle being trapped by the 1064-nm laser beam. Scan rate = 6 μm/s (d and e). Laser power at 1064 nm was several hundreds of milliwatts.

order of 10^{-12} N ($\sim 0.3 \times 10^{-12}$ N). Although this is a rough evaluation, the observed value agreed quite well with those reported for phosphatidylcholine giant vesicles: $(0.4-1) \times 10^{-12}$ N.[8,10] Since we have not explored the laser-induced transformation/fission of vesicles other than DDAB, we cannot discuss its generality to other vesicles. In the present approach, the radiation pressure is used as a pin for fixing DDAB molecules at a laser focus and transformation/fission of the vesicle is induced by viscous resistance in water. The use of viscous resistance or flow has a high potential and has been employed to control conformations of DNA.[14] We thus expect a wide applicability of the present approach to control transformation/fission of various vesicles and living cells. Further sophisticated experiments using double-beam laser scanning manipulation−absorption/fluorescence spectroscopy[15] will engage advances in the relevant research fields, which is underway in this laboratory.

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(14) Perkins, T. T.; Smith, D. E.; Larson, R. G.; Chu, S. *Science* **1995**, *268*, 83.
(15) Yao, H.; Ikeda, H.; Inoue, Y.; Kitamura, N. *Anal. Chem.* **1997**, *68*, 44304.