

Temporal Emergence of Giant Vesicles Accompanied by Hydrolysis of Ammonium Amphiphiles with a Schiff-base Segment

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(Received July 28, 2004; CL-040888)

An aqueous dispersion of the ammonium amphiphile with a Schiff-base segment exhibited the formation and decomposition of giant vesicles with diameters of several micrometers 2–4 h after the preparation. The time course trace of the morphological changes of the self-aggregates by means of light microscopy, electron microscopy and ^1H NMR revealed that the temporal emergence of the giant vesicles was dependent on the progress of hydrolysis of the Schiff-base segment.

Morphological changes of giant vesicles (GVs) have drawn much attention from the aspect of cooperative dynamics of biomolecular membranes composed of amphiphilic molecules.¹ Such morphological changes are induced by the application of electric field, osmotic pressure, addition of electrolytes etc., and the real time observation by light microscopy enables us to reveal their mechanism of membrane dynamics.¹ Recently it was found that chemical formation of membrane molecules inside the vesicular membrane caused transformation of GV.²

Here we prepared amphiphile **1** in which the Schiff-base segment was incorporated into the hydrophobic chain close to the trimethylammonium polar head (Figure 1a). Although amphiphile **1** formed only a small vesicle, it was found that the dispersion of amphiphile **1** exhibited time-dependent morphological changes of the self-aggregates (small vesicle–giant vesicle–oil droplet), associated with the progress of the hydrolysis of the imine part of amphiphile **1** (Figure 1b). This is the first demonstration that the morphological changes of the self-aggregate in the dispersion of amphiphile **1** was induced by the change

of the relative amount of substrate **1** and the hydrolyzed products **2**, **3** as shown in Figure 1a.

N-[4-[2-(trimethylammonio)ethoxy]benzylidene]-4-octylaniline bromide **1** was synthesized by the dehydrocondensation of *N*-octylaniline **2** with 2-(4-formylphenoxy)ethyltrimethylammonium bromide **3** as described in the previous papers.^{2f,2g,3} Amphiphile **1** was dissolved in distilled water and sonicated for 30 s, affording a 10 mM aqueous dispersion. To discern the nature of the self-aggregates in the early stage of hydrolysis, the dispersion was examined with the aid of negative-stain transmission electron microscopy (TEM, JEM-1230, JEOL, Japan). The TEM sample was prepared by air-drying the suspension of **1** on the carbon-deposited Collodion membrane sustained over copper grids. Figure 1b (left) shows the TEM image of spherical aggregates with diameters in the range of 50–300 nm. Dynamic light scattering (DLS) measurement (Microtrac UPA150, NIKKISO, Japan) indicated that self-aggregates with the size of 89 nm (standard deviation = 76 nm) at $t = 10$ min were formed, supporting the above result. Accordingly, these aggregates can be assigned to small vesicles (SVs).³

Figure 1b also shows the photographs of the 10 mM dispersion of **1** observed under a differential interference contrast (DIC) microscope (BX51, Olympus, Japan) at $25 \pm 1^\circ\text{C}$ (Figure 1b, middle and right). The photographs showed that GV emerged and elongated up to ca. 5 μm in the second stage ca. 200 min after the mixing. After ca. 400 min, the emergence of oil droplets on the membrane of GV in the specimen was recognized, and eventually all the GV were converted to brilliant spots which were identified as oil droplets (Figure 1b right).⁴

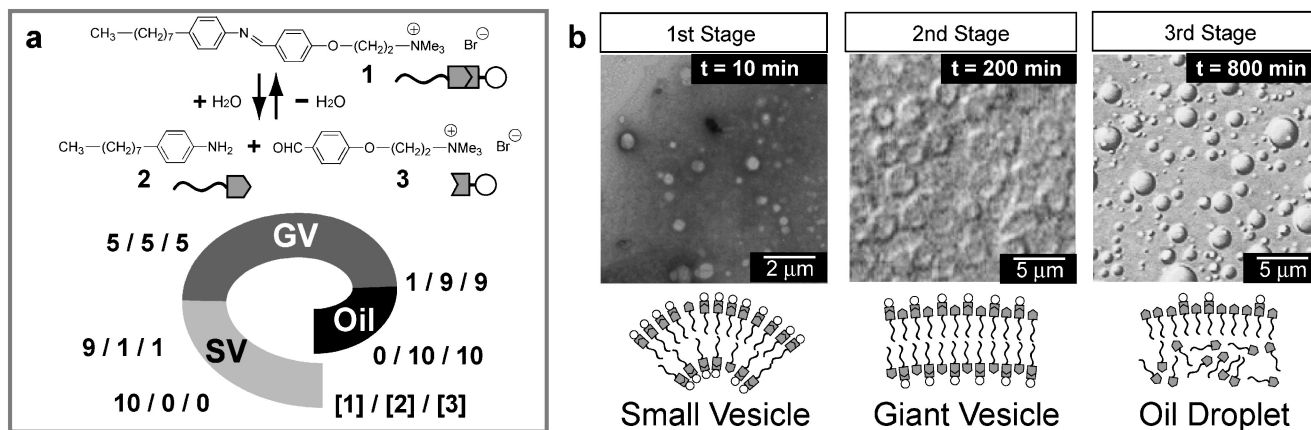


Figure 1. (a) Schematic illustration of amphiphile **1**, hydrophobe **2**, and hydrophile **3**. Black-filled blocks represent the imine part which separates into a hydrophobic amine part and formyl part. The white-filled part is a hydrophilic unit. Illustrated scheme of morphologies (SV; small vesicle, GV; giant vesicle, Oil; oil droplet) composed of **1**, **2**, and **3** with the ratio of [1] / [2] / [3] determined by ^1H NMR analysis is also shown. (b) TEM image of the first stage of morphologies in the 10 mM dispersion of **1** (1st stage). Phase contrast micrographs of giant vesicles (2nd stage) and oil droplets (3rd stage) in the dispersion containing **1**.

This morphological changes correspond to the third stage. The time dependence of the size population of self-aggregates in the dispersion of **1** was monitored by DLS. The average diameters of the self-aggregates were 610 ± 410 nm at the second stage and 640 ± 250 nm at the third stage, respectively.

In order to confirm that the morphological change of the self-aggregates was induced by the chemical transformation of amphiphile **1** to **2** and **3**, $^1\text{H NMR}$ of the dispersion was recorded on the same specimen as that of the light microscopic observation. The conversion of amphiphile **1** was monitored by a singlet signal at $\delta = 8.4$ ppm and $\delta = 9.8$ ppm which were assigned as the imine proton of amphiphile **1** and the formyl proton of hydrophile **3**, respectively.^{2f,2g} The conversion of **1** to **3** was determined to be 13, 46, and 88% ($\pm 3\%$) at the first, second and third stage, respectively. The pH value of the 10 mM aqueous dispersion of **1** remained in the range of 4.8–5.4 over all through stages.

On the basis of the above results, we may argue that the formation of GVs in the dispersion of **1** was induced by the generation of hydrophobe **2** and hydrophile **3** produced by the hydrolysis of amphiphile **1**. The hydrolysis of **1** is expected to proceed because the reactive part (the imine part) is located in the vicinity of the surface of GV and the hydrophile **3** can easily diffuse into water region from the intramembrane phase.^{2h,6} Since a mixture of **2** and **3** ($[\mathbf{2}]/[\mathbf{3}] = 10/10$ (mM)) forms only micrometer-sized oil droplets and no structured aggregates, we prepared a mixture of **1**, **2**, and **3** ($[\mathbf{1}]/[\mathbf{2}]/[\mathbf{3}] = 5/5/5, 5/5/0$ (mM)) and confirmed the generation of giant vesicles in the dispersion. The morphology of the dispersion of the mixture corresponds to that in the second stage.

The reversibility of the morphological change coupled with the hydrolysis of amphiphile **1** with the imine segment was confirmed by the following experiment. When the dispersion of **2** and **3** ($[\mathbf{2}]/[\mathbf{3}] = 100/100$ (mM)) was prepared at $25 \pm 1^\circ\text{C}$, GVs were generated from the surface of oil droplets in 30 min. The time course of $^1\text{H NMR}$ of the 200 mM dispersion of **2** and **3** showed that the conversion ratio of **1** was 49% ($\pm 2\%$) in the dispersion 24 h after the preparation.⁵ The formation of GVs from oil droplets was also recognized when the 10 mM oil emulsion, which was obtained by the completely hydrolyzed dispersion of **1** (24 h after preparation), was concentrated by 10 times.

The mechanism of the transient generation of GVs in this vesicular system is interpreted as follows. The generation of hydrophile **3** in bulk water through the hydrolysis of **1** flocculates vesicles of **1** due to the decrease of “thickness” (Debye length) of the electronic double layer on the vesicular surface.⁷ Simultaneously, hydrophobe **2** assists a fusion of small vesicles to GV (Figure 2) by forming an inverted-micellar structure within the membranes at the contacting surfaces of small vesicles (Figure 2, inset), since the polar head of **2** is smaller than that of **1**.^{7–9} As the ratio of **2** to **1** increases accompanied by the progress of hydrolysis, the decomposition of GVs proceeds and eventually the oil droplets were formed.

Although the precise structure and mechanism of self-aggregates at each stage is still veiled, it should be noted that this vesicular system shows temporal generation of GV composed by two simple molecules which do not form GV by itself. Moreover, we demonstrated for the first time that GV is formed accompanied by the hydrolysis of the Schiff base segment which

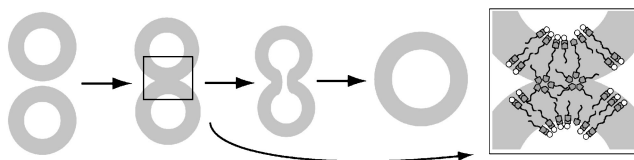


Figure 2. Schematic illustration of fusion of small vesicles through the formation of inverted-micellar structure (right) between the contacting membranes. Transformation from small vesicles to GVs is thus thought to occur.

is incorporated in amphiphile **1** and it is regenerated through the dehydrocondensation between hydrolyzed products **2** and **3**. It may be stated that the Schiff base segment which is buried in the self-aggregates operates as a clock to regulate the stages of the morphological changes.

This work was supported by Grant-in-Aid for Center of Excellence (No. 11CE2006) from the Ministry of Education, Culture, Sports, Science and Technology. T. T. is supported by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists (No. 08289).

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- 10 We thank Mr. Yasuhiko Saito (JEOL, Japan) for his skillful measurement in terms of transmission electron microscopy.