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A Novel System of Self-Reproducing Giant Vesicles

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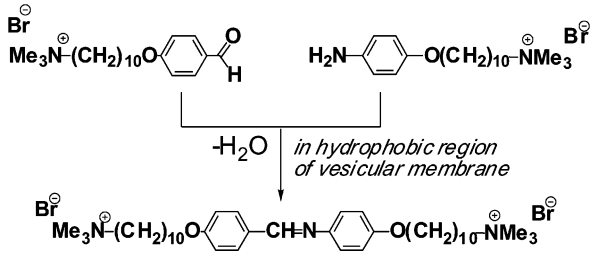
Abstract: Novel self-reproducing giant vesicles, consisting of a vesicular amphiphile with an imine group in its hydrophobic chain, were constructed. This vesicular amphiphile, the product of a dehydrocondensation reaction between amphiphilic aldehyde and a lipophilic aniline derivative, could be prepared within the giant vesicles. When a protected form of the aldehyde precursor was added to a suspension of giant vesicles containing the lipophilic aniline precursor and a catalyst, dehydrocondensation between the two precursors took place inside the vesicles and produced the same amphiphile as the one which constitutes the original vesicle. The newly formed amphiphiles self-assembled in the inner water pool to form small vesicles, which were eventually extruded through the outer layer of the original vesicle to the bulk water. Accordingly, this kinetic system can be designated as a self-reproducing system of giant vesicles.

Introduction

Giant vesicles (GVs) are composed of amphiphiles and have spherical lamellar structures with diameters larger than 1 μm. Since the structure and the dynamic behavior of GVs are similar to those of biological cell membranes, such vesicles have drawn much attention as plausible models for artificial cells.[1] GVs undergo characteristic morphological changes induced by variations in osmotic pressure or temperature or by the presence of additives.[2] It is particularly interesting that morphological changes can also be induced by chemical transformation of the amphiphiles within the vesicular membrane.[3]

Recently, we discovered that a vesicular membrane serves as an efficient reaction environment for an imine-coupling between a benzaldehyde-type amphiphile and an aniline-type amphiphile to produce a bolaamphiphile,[4] which forms a monolayer membrane due to the presence of polar heads at both ends of the hydrophobic chain (Scheme 1).[5] Although this kinetic system undergoes morphological changes that result in an increase in the number of vesicles, it cannot be regarded as a self-reproducing system because the product of the coupling reaction is not a component of the original vesicle.

Scheme 1. Formation of Bolaamphiphile Occurring in Vesicular Membrane



Herein we report a novel self-reproducing system of giant vesicles as represented in Figure 1. An amphiphile, **V**, bearing an imine group in its hydrophobic chain, was found to form GVs when 5 mol % of cholesterol was added as a stabilizer. Since amphiphile **V** is a coupling product of amphiphilic aldehyde **A** and 4-octylaniline (**B**), if amphiphilic aldehyde **A** is added to the suspension of GVs containing lipophilic aniline **B**, amphiphile **V** can be prepared by the dehydrocondensation between above two precursors as in the case of the precedent example. In this sense, these two precursors could be regarded as nutrients for amphiphile **V**. To prevent the dehydrocondensation between **A** and **B** from occurring in the bulk water, we protected the formyl group of **A** with a 1,3-dioxolane group to produce "locked" precursor, **A′**. In addition, we incorporated **C**, the catalyst for the removal of the protective group into the vesicular membrane (Figure 1). In fact, ^{1}H NMR spectroscopy revealed that additional **V** molecules were produced under the above reaction condition.

This finding prompted us to use differential interference contrast optical microscopy to monitor such morphological changes. When the reaction partner **A′** was added to a suspension of giant vesicles containing **B**, a drastic morphological

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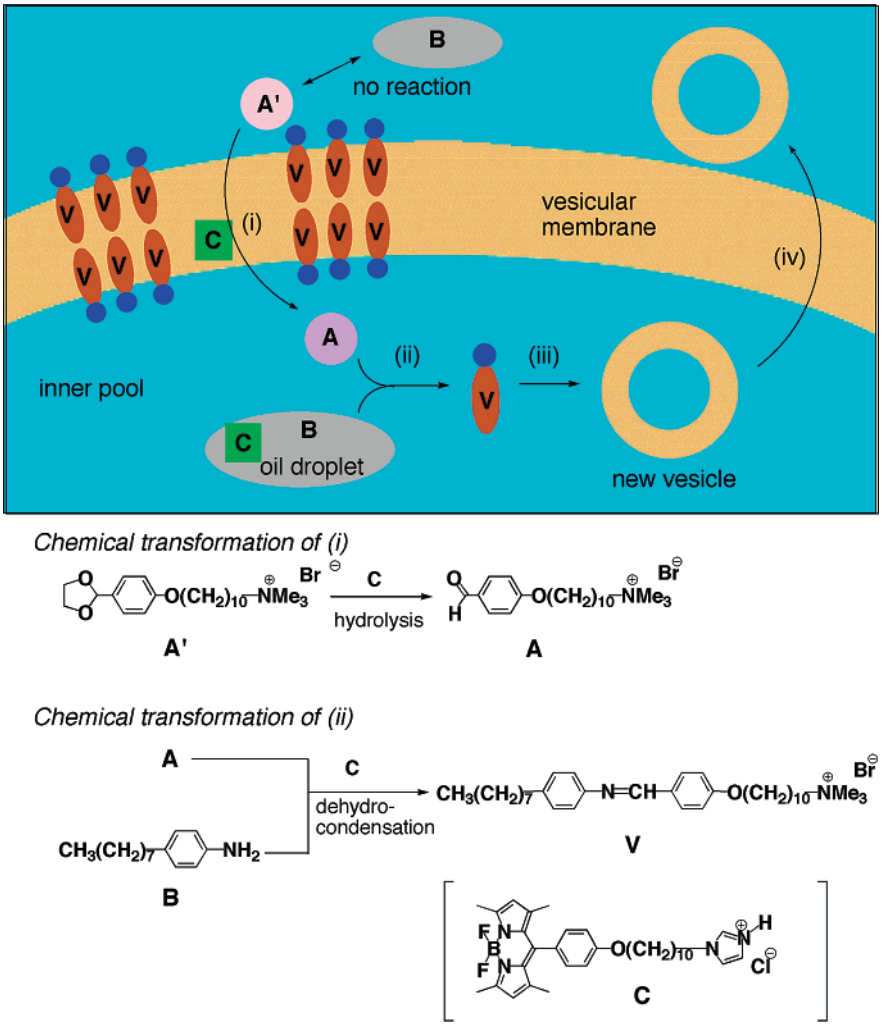

Figure 1. Schematic illustration of the self-reproducing giant vesicles: (i) locked precursor **A′** is incorporated into a vesicle composed of **V** and catalyst **C** and is unlocked to produce reactive precursor **A**; (ii) **A** reacts with lipophilic precursor **B** inside the vesicle to form vesicular molecule **V**; (iii) new vesicles are generated as **V** is produced; (iv) generated vesicles are extruded through the membrane to the bulk water.

change, such as *birthing*[2d] or *separation*,[2e] took place inside the original vesicle. Such a reproducing system can be regarded as a more progressed system compared to the previously reported ones[3a] because the reproducing process takes place within the vesicle, accompanying the transfer of chemical substances from the bulk water to the inner water pool of the vesicle through the vesicular membranes.

Results and Discussion

1. Synthesis and Properties of Amphiphiles. Amphiphilic precursor **A** was synthesized according to the procedure reported by Kunitake.[6] Precursor **A** and vesicular amphiphile **V** were synthesized according to the procedures outlined in Scheme 2. The 1,3-dioxolane derivative of 4-(10′-bromo-*n*-decyloxy)benzaldehyde was treated with trimethylamine to afford locked precursor **A′**. A dynamic light scattering measurement revealed that locked precursor **A′** self-assembled to form micelles (diameter, 4 nm; standard deviation = 1 nm). In contrast, lipophilic precursor **B** was dispersed in water as an emulsion of oil droplets. The acid-catalyzed dehydrocondensation between **A** and **B** in EtOH produced azomethine derivative **V**. The synthetic amphiphile **V** turned out to form giant vesicles when 5 mol % of cholesterol was mixed as an additive.

Catalyst **C**, in which a catalytic moiety (imidazolium hydrochloride) and a green fluorescent BODIPY[7] moiety are connected by a long alkyl chain, was prepared as shown in Scheme 3. The presence of a fluorescent probe in catalyst **C** is advantageous because the probe enables us to visualize the site where the catalytic reaction takes place. Condensation of the starting benzaldehyde derivative[6] with 2,4-dimethylpyrrole gave a dipyrromethane intermediate, which was treated with $BF_3 \cdot Et_2O$ to form a BODIPY derivative. A nucleophilic substitution reaction between imidazole and the fluorophore with a bromodecyloxy tail and subsequent acidification afforded catalyst **C**. The absorption maximum in the fluorescent spectrum of catalyst **C** was observed at 500 nm, and the emission maximum appeared at 519 nm. The fluorescence quantum yield for catalyst **C** was determined to be 0.58 upon excitation at 500 nm by using *N*,*N*′-bis(1-hexylheptyl)-3,4:9,10-perylenebis(carboximide) as a reference compound.[8]

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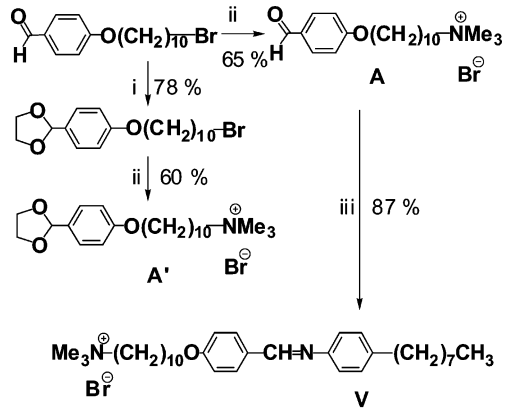
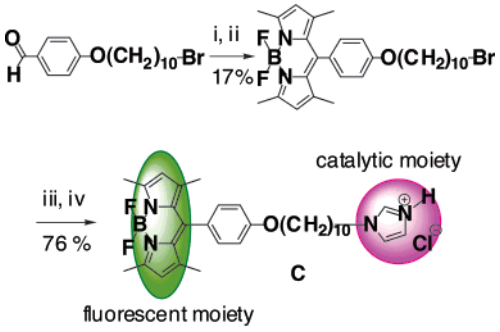
Scheme 2. Synthesis of Vesicular Molecule **V** and Its Precursors, **A**, **A′**, and **B**



(i) $HOCH_2CH_2OH$, cat. PTSA / C_6H_6, reflux, 15h
(ii) NMe_3 / H_2O, 80°C, 30h
(iii) 4-octylaniline **B**,cat. AcOH / EtOH, reflux, 12h

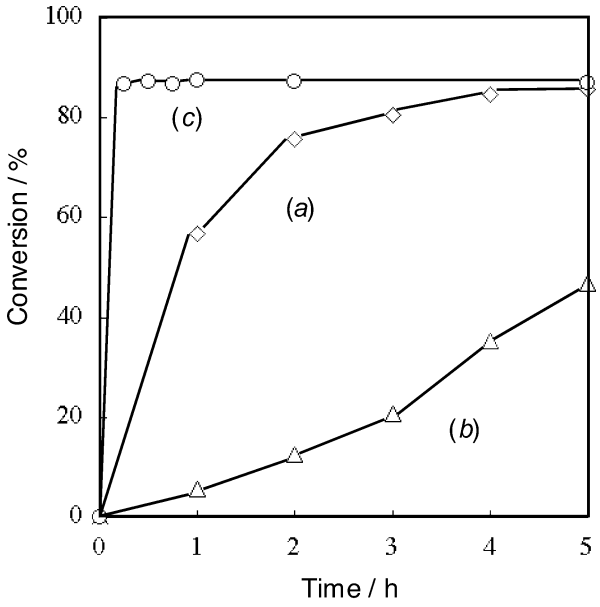
Scheme 3. Synthesis of Catalytic Amphiphile **C**



(i) 1. 2,4-Dimethylpyrrole (2eq), cat. TFA / CH_2Cl_2, r.t, 12h. 2. DDQ, r.t, 3h
(ii) $BF_3 \cdot OEt_2$, Et_3N / $CHCl_3$, r.t, 8h
(iii) 1. Imidazole (5eq) /EtOH, reflux, 12h. 2. NaOH aq
(iv) 1N-HCl

2. Formation of Vesicular Amphiphile in Water. The formation of vesicular amphiphile **V** by dehydrocondensation between **A** and **B** in water was monitored by ^{1}H NMR spectroscopy. The progress of the conversion under the various reaction conditions is plotted in Figure 2. When unlocked precursor **A** and lipophilic **B** were mixed, the amount of product **V** increased smoothly for the first 2 h, and the equilibrium was reached after 5 h (plot a). On the other hand, **V** formed much more slowly in the reaction between locked precursor **A′** and **B** (plot b). In contrast, the reaction was greatly accelerated by the addition of 5 mol % of catalyst **C** and equilibrium was reached after 15 min (plot c). These results show that catalyst **C** catalyzed not only the cleavage of the protective group but also the imine-coupling reaction.

3. Preparation of Nutrient-Including Giant Vesicles (NIGVs). It is interesting to study the dynamic behavior of the giant vesicle composed of **V** and a catalytic amount of **C** when precursors **A** and **B** are added. Since lipophilic **B** formed relatively large oil droplets, it dissolved only slightly into the vesicular membrane composed of **V** when added to the bulk water. To overcome this difficulty, we prepared a GV consisting of **V**, **C**, and **B**. In other words, we set up a situation in which one of the precursors of the vesicular molecule had been incorporated into the vesicle in advance. We prepared GVs composed of a mixture of **V**, **B**, **C**, and cholesterol (20:20:1:2) according to the film-swelling method, and after the suspension of GVs stood for 2 days, we found that most of them included oil droplets in their inner water pool (Figure 3). [9] It was revealed by fluorescence microscopy that the catalyst **C** was distributed not only in the original vesicular membrane but also in oil droplets included in the vesicle.



a: **A** + **B** → **V**

b: **A'** → **A**, followed by **A** + **B** → **V**

c: $\mathbf{A'} \xrightarrow{\mathbf{C}} \mathbf{A}$, followed by $\mathbf{A} + \mathbf{B} \xrightarrow{\mathbf{C}} \mathbf{V}$

Figure 2. Formation of **V** from various combination of starting materials: (a) **A** and **B** (◇); (b) **A′** and **B** (△); (c) **A′**, **B**, and 5 mol % of **C** (○).

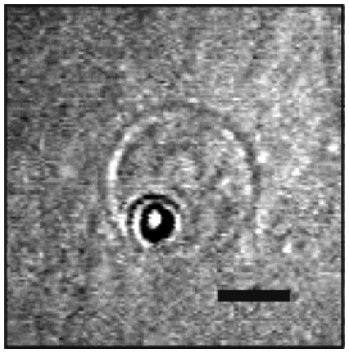


Figure 3. Differential interference contrast optical micrograph of a NIGV composed of **V**, **B**, **C**, and cholesterol (20:20:1:2). An oil droplet with a diameter of ca. 5 μm is included in the inner water pool of the vesicle, which has a diameter of 20 μm. The scale bar corresponds to 10 μm.

(9) Oil droplets were not present in the inner water pool of the giant vesicles immediately after they were produced by the film-swelling method. We assume that the oil droplets separated from the vesicular membranes over time because the membranes were supersaturated with **B**. We observed oil droplets not only in the inner water pool but also in the bulk water, owing to the separation of oil droplets into the outer water phase.

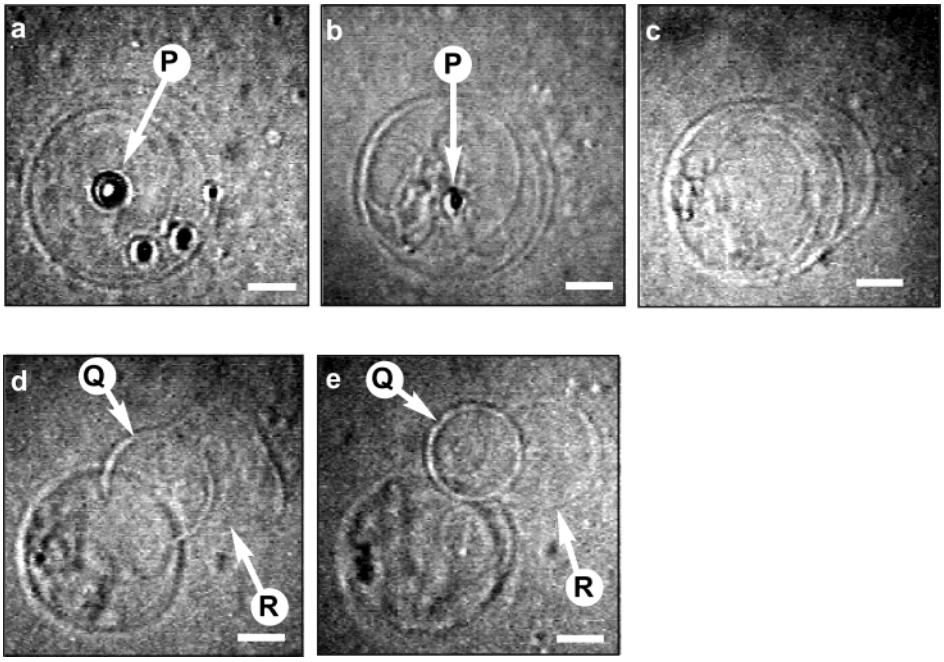


Figure 4. Differential interference contrast optical micrographs of morphological changes in a NIGV: (a)−(c) images obtained 10, 20, and 23 min, respectively, after addition of a micellar solution of **A′**; (d), (e) images showing the release of inner vesicles at 26 min after the addition of **A′**. The scale bars correspond to 10 μm. The vesicular membranes formed at site P, birthing occurred at site Q, and separation occurred at site R.

Table 1. Types of Aggregates Formed from Aqueous Mixtures of **V**, **B**, **C**, and Cholesterol

rujln	ratio of components (**V**:**B**:**C**:cholesterol)	shape of aggregate
1	40:0:1:2	GV
2	30:10:1:2	GV
3	20:20:1:2	GV including oil droplets (NIGV)
4	10:30:1:2	oil droplet
5	0:40:1:2	oil droplet

To examine the ratio of the compositions for preparing GVs that contained oil droplets**,** we systematically varied the ratio and monitored the types of aggregates that were produced (Table 1). **V**/**B** ratios larger than 1 (runs 1 and 2) produced GVs that contained no oil droplets, while ratios smaller than 1 produced only oil droplets (runs 4 and 5). However, whenever the ratio was close to 1 (run 3), most of GVs include oil droplets. On the basis of these findings, we concluded that the oil droplets that separated from the vesicular membrane were composed mainly of lipophilic aniline **B**. Since **B** is one of the components, or "nutrients," of vesicular amphiphile **V**, we designate these GVs as nutrient-including giant vesicles (NIGVs).

4. Morphological Change in NIGVs. The morphological changes in NIGVs (**V**:**B**:**C**:cholesterol = 20:20:1:2) were monitored by means of differential interference contrast optical microscopy at 23 °C after the addition of a micellar solution of **A′**. Although all the NIGVs underwent some kinds of morphological change, the mode of the morphological change depended on the volume ratio of the outer vesicle to the included oil droplets.

Among NIGVs, GVs containing several oil droplets exhibited dramatic dynamics (Figure 4). About 10 min after **A′** was added, the oil droplets inside NIGV started to decrease in size, and after 20 min, even the largest oil droplet was about to disappear. In compensation for the disappearance of the oil droplets, new vesicles were generated inside the original NIGV and the inner vesicles came out through the outer membrane (birthing[2d]) at the site Q. Accompanied by the birthing process, the peeling of the outermost layer of the original vesicle (separation[2e]) also took place at the site R. When the same experiment was carried out on the NIGVs including several oil droplets, they also exhibited the same dynamics, that is, the membrane formation from the included oil droplets and the subsequent morphological change. The result supports that the dynamics depicted in Figure 4 is a reproducible phenomenon.

To clarify that the composition of the extruded vesicles are the same as that of the original ones, we set up a simplified model reaction system shown in Figure 5a. In the current vesicular system, catalyst **C** was dissolved in the oil droplets in the inner water pool as well as in the membrane of the vesicle (Figure 1). In the model system, however, **C** was dissolved only in the oil droplets of lipophilic precursor **B**. The dynamics of the oil droplets after the addition of a solution of locked precursor **A′** was monitored by mean of both fluorescence microscopy and differential interference contrast optical microscopy.

When a solution of **A′** was added to the suspension of **B** containing fluorescent catalyst **C** (site L), vesicular membranes were generated from the hydrophobic surface of the oil droplets and they grew as the oil droplets decreased in size (Figure 5b−d) with high reproducibility. In a control experiment carried out on the right side of the same slide (site R), no morphological changes occurred when **A′** was added to a suspension of oil droplets of **B** without containing **C** (Figure 5e). The above results unequivocally demonstrated that the vesicles were generated only after locked **A′** had been transformed to reactive **A** in the presence of catalyst **C**,[10] giving rise to the vesicular amphiphile **V** through the dehydrocondensation.

The dynamics discovered in the above experiments rationalizes the vesicular reaction system schematically depicted in Figure 1. Namely, when amphiphilic precursor **A′** is added to

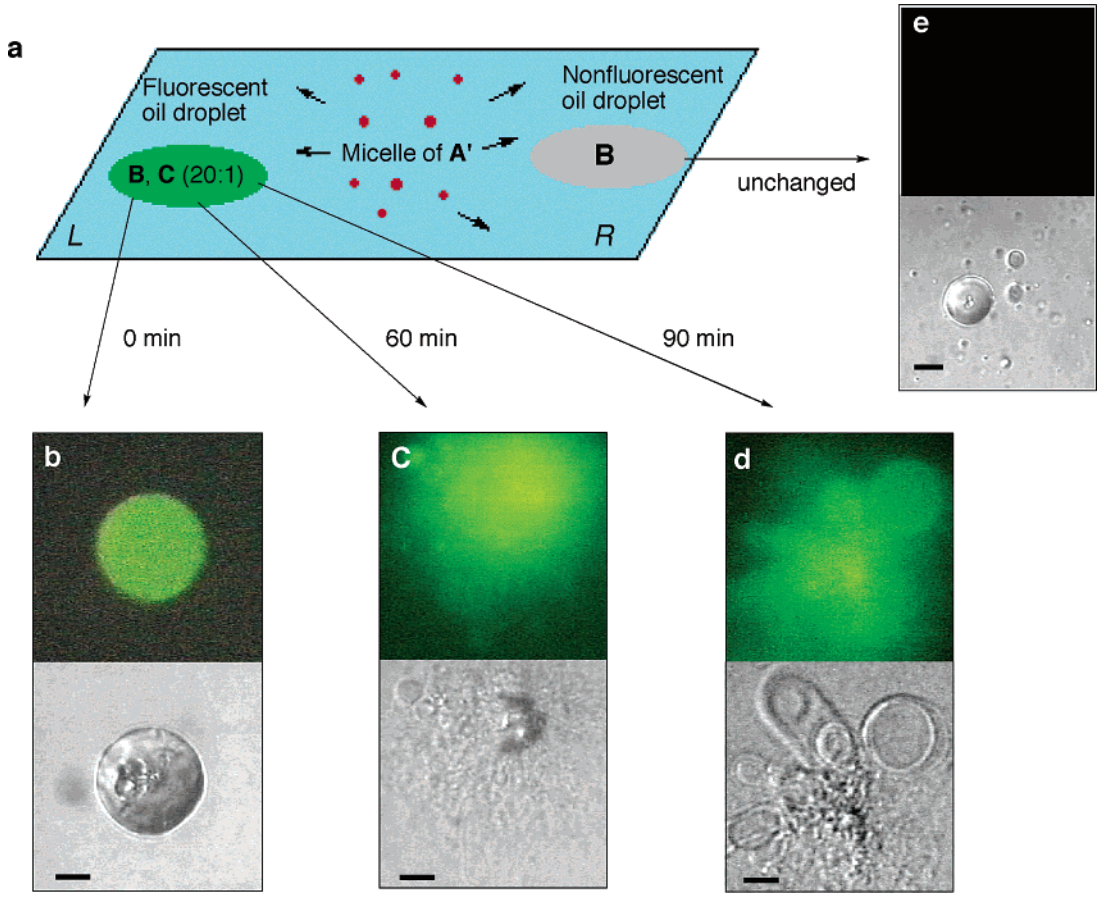


Figure 5. (a) Schematic illustration of the mixing chamber used to observe the two kinds of oil droplets: **B** plus **C** (green circle), **B** (gray circle). (b)−(d) Representative microscopic images of the morphological changes in the oil droplet composed of **B** and 5 mol % of **C** at site L at 0, 60, and 90 min, respectively, after the addition of a solution of **A′** (red dots). (e) Representative microscopic image of the oil droplet composed of only **B**. The scale bars correspond to 10 μm. The upper images were recorded by fluorescence microscopy and the lower by differential interference contrast optical microscopy.

the bulk water, it dissolves into the vesicular membrane containing catalyst **C** and is converted to deprotected precursor **A**. Then vesicular amphiphile **V** is produced in the inner water pool of NIGV through the dehydrocondensation between **A** and lipophilic precursor **B** which exists as the encapsuled oil droplet, as proved by the model experiment (Figure 5). It should be noted that the addition of the amphiphilic precursor **A′** triggers the increase of the number of GVs through birthing and separation phenomena. Although all the extruded vesicles are not necessarily the newly formed vesicles but may contain some preexisting ones in the original vesicles of a multi-lamella type, it can be said that the composition of the extruded vesicles are the same as the original ones. Accordingly, the present vesicular system can be regarded as a self-reproducing one because the GV of the first generation separated into GVs of the second generation composed of the same amphiphile as the first generation.

5. Significance of the Self-Reproducing System. The most characteristic feature of morphological changes in our system is that they were induced by a chemical transformation occurring in the inner water pool of the GV. In their pioneering work on giant multilamellar vesicles, Menger et al. have reported that the birthing of a GV composed of didodecyldimethylammonium bromide (DDAB) takes place immediately after the addition of octyl glucoside, which does not react with DDAB.[2d] In contrast, the morphological changes in our system occurred only after an induction period of ca. 20 min, as in case of our previously reported system.[4] The induction period corresponds to the time period necessary for the production of a sufficient amount of **V** that is enough to induce the morphological changes. Jaeger et al. have reported that vesicles are destroyed by the reaction with 2-chloroethyl phenyl sulfide[11] or with *O*-methyl *S*-benzyl phenylphosphonothiolate[3d,11] because the vesicular molecule is transformed into a nonamphiphilic molecule. In light of these experimental results, the morphological changes we observed in our reaction system may have resulted from the amphiphilic nature of **V**.

Our reaction system can be described as a novel self-reproducing system because the new vesicles are composed of the same component as the original vesicle. Although the self-reproduction of an oleic acid/oleate giant vesicle has been reported,[3a] one can argue that the alkaline hydrolysis of oleic anhydride really occurs within the vesicular membrane. The advantage of our system is that we visually confirm the generation of the new GVs within the original vesicle.

(10) To confirm that catalyst **C** firmly adhered to the oil droplets, we carried out the following experiment. We used a semipermeable membrane (SPECTRUM Por6RC) to separate a suspension of fluorescent oil droplets composed of **B** and 5 mol % of **C** from a suspension of nonfluorescent oil droplets of **B** alone. Even after standing for 5 days, the oil droplets without **C** did not fluoresce at all. This result shows that **C** has sufficient affinity for the hydrophobic emulsion.

(11) Jaeger, D. A.; Li, B. *Langmuir* **2000**, *16*, 5−10.

Wick et al. have reported a vesicle-producing system in which two kinds of precursors, a lisophospholipid and a fatty acid, and an enzyme catalyst are artificially incorporated into a giant vesicle by means of microinjection.[12] Although new GVs are generated by the formation of a double-chain phospholipid, they are not released in this case. In contrast, the characteristic of our system is that the inner vesicles were released, especially when amphiphilic precursor **A′** was added to the bulk water. The dynamics is extremely interesting from the viewpoint that they bear a topological resemblance to some cellular processes, such as the budding of yeast.[13] Moreover, it is advantageous to supply GVs continuously with amphiphilic precursor as nutrients for developing a robust and recursive self-reproducing system.

It is also to be noted that the current system is interesting from a viewpoint of the chemical complex system.[14] A microscopic-level chemical transformation, occurring within a vesicle, induces a macroscopic-level morphological change in the vesicle. This morphological change, in turn, affects the microscopic process because the change modifies the reaction environment itself. If these two dynamics, molecular transformations and morphological changes, are suitably balanced, the self-reproducing vesicular system renders an essence of the cellular dynamics which draws a recursive trajectory.

Summary

We constructed a novel self-reproducing vesicular system in which a giant vesicle produced daughter vesicles in its inner water pool by means of a coupling reaction between the two precursors of the amphiphile that constituted the original vesicle. The generation of the new vesicles in the inner water pool and their release to the bulk water were visually confirmed. Such dynamics are extremely interesting in that they bear a topological resemblance to some cellular processes.

Experimental Section

General. All commercially available reagents were purchased from Tokyo Kasei Co. or Aldrich Co. and were used without further purification. Reaction solvents were distilled. ^{1}H NMR spectra were recorded on a JEOL GSX-270 spectrometer. UV−vis spectra were recorded on a JASCO V-570 spectrometer. Fluorescence spectra were recorded on a Shimadzu RF-503A spectrometer. High-resolution fast atom bombardment mass spectra (HRMS-FAB) were recorded on a JEOL JMS-700 spectrometer with *m*-nitrobenzyl alcohol as matrix.

[10-[4′-(1″,3″-Dioxolan-2″-yl)phenoxy]decyl]trimethylammonium Bromide (A′). 4-(10-Bromo-*n*-decyloxy)benzaldehyde (683 mg, 2.0 mmol), which was prepared according to a literature procedure,[6] and ethylene glycol (140 mg, 2.3 mmol) were dissolved in benzene (7.5 mL), and *p*-toluenesulfonic acid monohydrate (38 mg, 0.2 mmol) was added. The mixture was refluxed for 15 h and then cooled. The resulting solution was diluted with benzene, washed with 10% aqueous sodium hydroxide and brine, dried over Na_2SO_4, filtered, and concentrated. The crude mixture was purified by recrystallization from THF/*n*-hexane to afford the 1,3-dioxolane derivative (601 mg, 78%) as colorless crystals. The obtained 1,3-dioxolane derivative (385 mg, 1.0 mmol) was added to a 30% aqueous solution of trimethylamine (10 mL), and the suspension was heated at 80 °C for 30 h. After the suspension was cooled, water and excess trimethylamine were removed under reduced pressure, and the resulting solid was washed with acetone to afford **A′** (224 mg, 65%).

^{1}H NMR (270 MHz, DMSO-d_6): 7.32 (2H, d, J = 8.6 Hz), 6.89 (2H, d, J = 8.6 Hz), 5.62 (1H, s), 4.05−3.87 (4H, m), 3.01 (9H, s), 1.58−1.74 (4H, m), 1.20−1.46 (12H, m). HRMS−FAB (*m*/*z*): $[M - Br]^+$ calcd for $C_{22}H_{38}NO_3$, 364.2852; found 364.2863.

[10-[4′-(4″-Octylphenylimino)phenoxy]decyl]trimethylammonium Bromide (V). Unlocked precursor **A** (400 mg, 1.0 mmol), which was prepared by the literature procedure,[6] and 4-octylaniline (**B**) were dissolved in absolute ethanol (2 mL), and a catalytic amount of acetic acid was added. The mixture was refluxed for 12 h and then cooled. After the evaporation of ethanol, the residue was washed with acetone to afford amphiphilic imine derivative **V** (512 mg, 87%).

^{1}H NMR (270 MHz, $CDCl_3$): 8.39 (1H, s), 7.82 (2H, d, J = 8.9 Hz), 7.18 (2H, d, J = 8.1 Hz), 7.12 (2H, d, J = 8.1 Hz), 6.96 (2H, d, J = 8.9 Hz), 4.02 (2H, t, J = 6.3 Hz), 3.55 (2H, m), 3.44 (9H, s), 2.61 (2H, t, J = 7.8 Hz), 1.88−1.20 (28H, m), 0.88 (3H, t, J = 6.6 Hz). HRMS-FAB (*m*/*z*): $[M - Br]^+$ calcd for $C_{34}H_{55}N_2O$, 507.4314; found 507.4329.

1-[10′-{4″-(1‴,3‴,5‴,7‴-Tetramethyl-4‴,4‴-difluoro-4‴-bora-3a‴,4a‴-diaza-*s*-indacen-8‴-yl)phenoxy}decyl]imidazole Hydrochloride (C). 4-(10-Bromo-*n*-decyloxy)benzaldehyde (342 mg, 1.0 mmol) and 2,4-dimethylpyrrole (190 mg, 2.0 mmol) were dissolved in dichloromethane (25 mL), and the solution was degassed by bubbling nitrogen (30 min). One drop of trifluoroacetic acid was added, and the solution was stirred for 12 h at room temperature under a nitrogen atmosphere. Then, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.25 g, 1.1 mmol) was added, and the mixture was stirred for 3 h. The reaction mixture was washed with saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by alumina column chromatography using $CHCl_3$ as an eluent to afford a dipyrromethane derivative as a colored oil. The product was dissolved in chloroform (10 mL), together with $BF_3{\cdot}Et_2O$ (0.72 mL, 3.8 mmol) and triethylamine (0.50 mL, 6.8 mmol), and the mixture was refluxed for 1 h. The reaction mixture was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The fluorescent oil was purified by silica gel column chromatography using hexane/ethyl acetate to afford a fluorescent alkyl bromide (73 mg, 13%) as a vermilion powder.

^{1}H NMR (270 MHz, $CDCl_3$): 7.14 (2H, d, J = 8.6 Hz), 6.98 (2H, d, J = 8.6 Hz), 5.97 (2H, s), 4.00 (2H, t, J = 6.6 Hz), 3.41 (2H, t, J = 6.7 Hz), 2.55 (6H, s), 1.90−1.73 (4H, m), 1.58−1.25 (18H, m). HRMS-FAB (*m*/*z*): $[M]^+$ calcd for $C_{29}H_{38}BBrF_2N_2O$, 558.2229; found 558.2232.

The alkyl bromide (50 mg, 0.09 mmol) was mixed with imidazole (68 mg, 1 mmol) in EtOH (0.5 mL), and the mixture was heated at 80 °C for 12 h. After the mixture was cooled, diethyl ether was added, and the organic layer was washed with 0.5 N aqueous NaOH, dried over Na_2SO_4, filtered, and concentrated. After the evaporation of diethyl ether, the fluorescent N-substituted imidazole (37 mg, 76%) was obtained as a vermilion powder.

^{1}H NMR (270 MHz, $CDCl_3$): 7.47 (1H, s), 7.14 (2H, d, J = 8.9 Hz), 7.05 (1H, s), 6.98 (2H, d, J = 8.9 Hz), 6.90 (1H, s), 5.97 (2H, s), 4.00 (2H, t, J = 6.6 Hz), 3.93 (2H, t, J = 7.0 Hz), 2.55 (6H, s), 1.88−1.56 (4H, m), 1.53−1.18 (18H, m). HRMS-FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{32}H_{42}BF_2N_4O$, 547.3420; found 547.3436.

Finally, the N-substituted imidazole (35 mg, 0.06 mmol) was dissolved in 1 N hydrochloric acid, and the solvent was removed under reduced pressure to afford fluorescent imidazolium hydrochloride **C** (37 mg, quantitative) as a vermilion powder.

^{1}H NMR (270 MHz, $CDCl_3$): 9.66 (1H, br, s), 7.38 (1H, s), 7.14−7.11 (3H, m), 6.98 (2H, d, J = 8.6 Hz), 5.97 (2H, s), 4.34 (2H, br, s), 4.00 (2H, t, J = 6.4 Hz), 2.54 (6H, s), 2.00−1.72 (4H, m), 1.55−1.18 (18H, m). UV−vis (CH_2Cl_2): λ_{max} (log ϵ) 500 (4.7), 336 nm (4.0). Fluorescence (CH_2Cl_2): λ_{em} 519 nm (λ_{ex} 475 nm). HRMS-FAB (*m*/*z*): $[M - Cl]^+$ calcd for $C_{32}H_{42}BF_2N_4O$, 547.3420; found 547.3409.

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Dynamic Light Scattering (DLS) Measurement. An aqueous solution of **A′** (10 mM) was used to perform DLS experiments on a NIKKISO Microtrac 150 at room temperature after the solution had been sonicated for 5 min.

^{1}H NMR Spectroscopic Monitoring of the Formation of Vesicular Molecule V. A 40 mM D_2O solution of amphiphilic precursor **A′** or **A** was prepared. Lipophilic precursor **B** containing 5 mol % of catalyst **C** in the case of b in Figure 2 was dissolved in ethanol, and then the solvent was removed under reduced pressure. To the residue was added D_2O, and the mixture was sonicated to prepare a 40 mM aqueous dispersion of oil droplets. Then, 5 mL of the solution of **A** or **A′** and an equal volume of the dispersion of oil droplets of **B** (with **C** in the case of b) were combined and then stirred at room temperature. Aliquots (50 mL) of the reaction mixture were withdrawn by micropipet every 15 min, and the reaction solvent was rapidly removed at room temperature under reduced pressure. The residual mixture was dissolved in 500 μL of DMSO-d_6 for measurement of the ^{1}H NMR spectra of the reaction mixture on a JEOL GSX-270 spectrometer. The conversion from **A′** and **B** to **V** or from **A** and **B** to **V** was monitored by means of the ratio of the signal area of the imine **V** to that of the starting material at the initial stage.

Preparation and Observation of Nutrient-Including Giant Vesicle (NIGV). A 20:20:1:2 mixture of **V**, **B**, **C**, and cholesterol (a membrane stabilizer) was dissolved in ethanol. Removal of the solvent under reduced pressure resulted in the formation of a myelin-like film. Addition of deionized water to the resulting film at 45 °C produced a suspension of giant vesicles. After the suspension was left standing for 48 h, NIGVs were observed with an Olympus BX51 (obj. lens × 40) differential interference contrast microscope equipped with an image-recording and -processing system.

Mixture of a Solution of NIGVs with a Solution of Locked Precursor A′ in a Mixing Chamber. An aqueous dispersion of NIGV (2.5 mM, 15 μL) was mixed with an aqueous solution of **A′** (10 mM, 15 μL) at 23 °C, in a mixing chamber made of a glass slide and a cover slide, which were firmly fixed together with spacers.[2g] A NIGV dispersion was injected from an open site of the mixing chamber. Then, a solution of **A′** was placed on the opposite open site of the chamber. These two solutions were gently mixed by the surface tension and thereby a concentration-gradient of **A′** was spontaneously generated in the chamber. The dynamic behavior of the NIGVs was monitored with an Olympus BX51 (obj. lens × 40) equipped with an image-recording and -processing system.

Optical Microscopic Observation of Catalyst-Dependent Generation of Vesicular Membranes. An aqueous dispersion of fluorescent oil droplets composed of **B** and 5 mol % of **C** (2.5 mM, 10 μL) was placed on one side of a rectangular glass plate (24 × 60 mm), and an aqueous dispersion of the nonfluorescent oil droplets composed of **B** (2.5 mM, 10 μL) only was placed on the other side. Then, an aqueous solution of **A′** (10 mM, 10 μL) was placed on the middle of the plate, and the plate was covered with a cover glass. The morphological changes in the oil droplets on the two sides after contact with the solution of **A′** were monitored with an Olympus BX51 (obj. lens × 40) microscope equipped with a halogen-lamp and a color CCD camera connected to an image-recording and -processing system. An Olympus WIB filter set (ex = 460−490 nm; em > 515 nm) was used for recording the fluorescent images.

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Supporting Information Available: Representative images of morphological changes in the NIGVs releasing no vesicles, as well as the relevant discussion in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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