Morphological Change of Giant Vesicles Triggered by Dehydrocondensation Reaction

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(Received December 5, 2001; CL-011226)

A giant vesicle composed of an amphiphile with a reactive site exhibited a morphological change when the vesicular amphiphile reacts with an added amphiphilic reaction-partner within its membrane.

A giant vesicle, which is composed of amphiphiles, is characterized by a spherical lamellar structure with diameters larger than 1 μm. Since the structure and the dynamic behavior of giant vesicles are similar to those of biological cell membranes, the vesicles have drawn much attention as a plausible model of an artificial cell.[1] Real time observations of their morphological changes induced by the variation in osmotic pressure, or temperature, or by additives have been reported.[2]

Herein we report the dynamic behavior of a giant vesicle consisting of amphiphiles with a reactive functional group at the end of the hydrophobic chain. Such a giant vesicle was found to show the morphological change, when an amphiphile bearing a functional group that can react with the vesicular amphiphile was added. This morphological change of the vesicle was triggered by a coupling reaction between two kinds of reactive amphiphiles to produce a monolayer-forming bolaamphiphile[3] which bears polar heads at both ends (Figure 1).

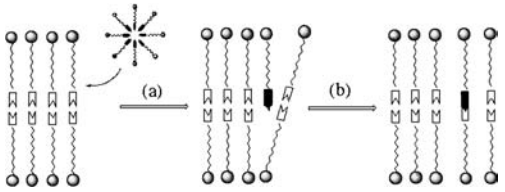

Figure 1. Formation of bolaamphiphile through coupling reaction between a reactive vesicular amphiphile and a reactive micellar amphiphile in a hydrophobic region of the vesicular membrane. (a) Incorporation of micellar amphiphile. (b) Coupling reaction between two kinds of reactive amphiphiles within the membrane to form a bolaamphiphile.

Our candidate for an amphiphile bearing a reactive group at the ω-position of a hydrophobic chain was aldehyde **1a**, which formed aggregates in 1 mM aqueous solution. Although the diameteres of the aggregates composed of **1a** were in the range of 0.2–1.1 μm,[4] the examination under an optical microscope showed that **1a** did not exist as vesicles but as oil-droplets. Therefore, we synthesized a double-chain amphiphile **1b** as the second component. Although **1b** itself formed mostly myelin-like aggregates, the mixture of **1a** and **1b** (5 : 1) turned out to form giant vesicles which were detectable by optical microscopy.[5] Although the mean diameter of giant vesicles is ca. 4 μm, one can find giant vesicles with diameters larger than 10 μm. In contrast, aniline-type amphiphile **2**, which was prepared as a reaction partner for **1a**, formed micelles with a diameter of 5.9 nm (SD = 1.3 nm, SD: standard deviation).

In addition, we prepared non-amphiphilic compounds bearing a reactive group, **3** and **4**, an amphiphile without a reactive group **5** and bolaamphiphilic diphenylazomethines **6a** and **6b**, as referential compounds (Figure 2).

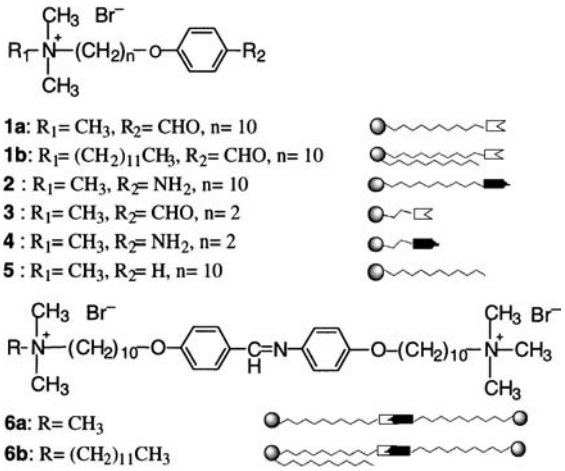

Figure 2. Compounds used in this work. Preparation of amphiphiles **1a**, **2** and **6a** was reported by Kunitake.[6]

After an aqueous solution of **2** (10 mM) was added to an equal volume of a dispersion of giant vesicles composed of **1a** and **1b** (2 mM), the UV spectrum of the mixture was monitored every 1 h for 24 h at room temperature (Figure 3). The absorption at 340 nm, which we assigned to diphenylazomethines **6a** and **6b**, increased as the condensation proceeded. The assignment of the absorption was confirmed by comparing the UV spectrum of an authentic mixture of **6a** and **6b** (5 mM : 1 mM).[7]

The conversion of the dehydrocondensation between **1a** (**1b**) and **2** was determined as a function of time from the molar absorptivity at 340 nm ($\varepsilon_{340} = 5500$) of the bolaamphiphilic mixture of **6a** and **6b** (5 : 1) (diamonds in Figure 3). Formation of the azomethines proceeded smoothly for the first 10 h, and then the equilibrium was reached at 34% of the conversion after 20 h. The conversion of the amphiphilic aldehydes to the azomethines increased up to 81% when a more concentrated solution of **2** (36 mM) was added to the giant vesicle dispersion (36 mM) of **1a** (**1b**).[8] In contrast, when non-amphiphilic aldehyde **3** and aniline **4** (1 mM : 5 mM) were mixed, a conversion of the corresponding azomethine was only 2.5% (triangles in Figure 3). On the basis of these findings, it may be concluded that the dehydrocondensation occurred even in aqueous solution when the bilayer membrane

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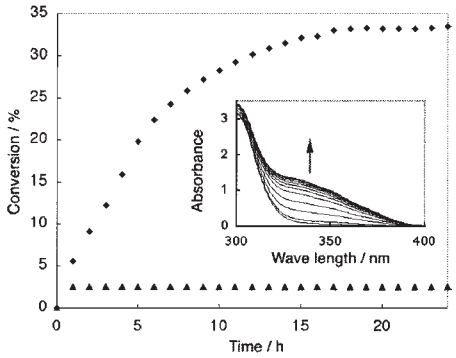

Figure 3. Dehydrocondensation between aldehyde derivatives **1a** (**1b**) or **3** and aniline derivative **2** or **4** at room temperature. Conversion to the azomethine derivatives was plotted as a function of time. Starting materials: **1a** (**1b**) and **2** (◆), **3** and **4** (▲). The inset shows the UV spectral change in the reaction mixture of **1a** (**1b**) and **2** at 2 h intervals.

provides an efficient hydrophobic reaction-environment as in the case of aldehyde-type amphiphiles **1a** (**1b**).

Two factors can account for our experimental results. One factor is that a high local concentration of the substrates in the membrane facilitates the collision between the two reactive groups. The other is that the hydrophobic environment shifts the equilibrium toward the product side by preventing the hydrolysis of the product, since the imine moiety of the product is located near the middle of the hydrophobic membrane.

The morphological change of giant multilamellar vesicles (GMVs) was monitored by means of differential interference contrast optical microscopy[9] at 23 °C after adding the reactive micellar amphiphile. The GMV consisting of amphiphilic aldehydes **1a** and **1b** (5 mM) showed two cytomimetic processes. The one is a ''birthing process'',[2d] which means the inner vesicle is released from the outer vesicle. The other is a''separation process'',[2e] in which the utmost outer layer peels away from the outer vesicle. These processes were found to occur as a reproducible phenomenon with the induction time of 7–20 min when a solution of aniline-type amphiphile **2** (10 mM) was added to an equal volume of a dispersion of the GMV (Figure 4).

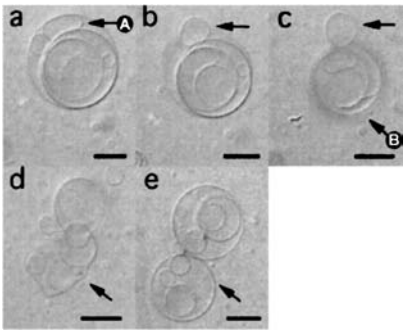

Figure 4. Morphological change of GMV at 0, 7, 7.5, 8.5, 9 min, (a)–(e), after the addition of a solution of **2**, respectively. ''Birthing'' occurred at site A and ''separation'' occurred at site B. The bar corresponds to 10 μm.

Furthermore, such morphological changes took place with almost no induction time in the case that bolaamphiphile **6a**, which is the product of the coupling reaction between **1a** and **2**, was added as an additive. In contrast, giant vesicles composed of **1a** and **1b** (5 : 1 mixture, 5 mM) did not show any distinct morphological change when an equal volume of a solution of non-reactive amphiphile **5** (10 mM) was added. These results strongly suggest that the formation of bolaamphiphiles **6a** (**6b**) within the bilayer membrane induces a cytomimetic process because the vesicular membrane is locally disturbed on the basis of the difference in the molecular shapes and the adhesion forces[6,10] between bilayer- and monolayer-forming amphiphiles.[11]

The novelty of this work lies in the fact that the dehydrocondensation between two amphiphiles occuring in the hydrophobic region of the vesicular membrane produces a new amphiphile and even induces the ''birthing'' and ''separation'' of giant vesicles. Namely, the reactive vesicle produces a substance to induce the morphological change within a membrane of itself. The difference in the ratio of components between the original vesicle and the exposed one may be examined in terms of a specific fluorescent probe which can monitor the chemical transformation. The investigation along this line is in progress in these laboratories.

This work was supported by grants-in-aid from the Center of Excellence (Study of Life Science as Complex Systems) of the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References and Notes

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3 J.-H. Fuhrhop and J. König, ''Membranes and Molecular Assemblies: The Synkinetic Approach,'' The Royal Society of Chemistry, Cambidge (1994).

4 The size of aggregates was measured by a dynamic light scattering method using a NIKKISO Microtrac UPA150 at room temperature. The solution of **1a** or **2** was sonicated for 5 min before analysis.

5 The solvent of an ethanolic solution of amphiphiles **1a** and **1b** was removed under reduced pressure to form a myelin-like film. Addition of deionized water to the resulting film at 23 °C afforded an aqueous dispersion of giant vesicles. All the giant vesicles described in this paper were prepared under the same condition.

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7 We also confirmed the formation of **6a** (**6b**) by ^{1}H NMR (in DMSO-d_6), monitoring the singlet signal ($\delta = 8.4$ ppm) which is characteristic of the imine proton of the azomethine derivative.

8 Since myelin-like aggregates and insoluble solids are formed under such a high concentration of amphiphiles, it is not appropriate to monitor the morphological change of giant vesicles.

9 An Olympus Power BX51 (obj. lens × 60) microscope was used.

10 Y. Liang, L. Wu, Y. Tian, Z. Zhang, and H. Chen, *J. Colloid Interface Sci.*, **178**, 703 (1996).

11 One of the plausible driving forces to promote ''birthing'' of inner vesicles is as follows. Provided that the bolaamphiphile which is formed in the vesicular membrane partly dissolves into the aqueous solution, the concentration of amphiphiles in a water pool inside of the vesicle becomes much higher than that of the aqueous solution. Therefore water penetrates into the inner water pool to induce ''birthing'' of inner vesicles through the disturbed membrane wall.