Successive Fusion of Vesicles Aggregated by DNA Duplex Formation in the Presence of Triton X-100

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Two kinds of large vesicles (average diameter: ca. 0.2 μm) bearing complementary DNA strands aggregated to form densely packed clusters. Addition of Triton X-100 (1.2 mM) as a fusogenic reagent to an aqueous suspension of the vesicle-clusters induced successive fusion of vesicles, leading to a giant multilamellar vesicle-like sphere with a diameter of ca. 3 μm.

Fusion of vesicles has drawn much attention because it is an indispensable process in a chemical model of a cell fusion or a drug delivery system.[1] Although a vesicle-fusion can be caused by addition of surfactants, e.g. sodium cholate or lysophospholipids, which perturb lipid membranes, a crucial step for the fusion event is that bimolecular membrane of neighboring vesicles are enforced to be located nearby through adhesion of vesicles.[2] In fact, firmly linked vesicles fuse spontaneously with each other without surfactants.[3] However, in the case of spontaneous or surfactant-assisted fusion of large vesicles (LVs, diameter: 0.1–1 μm), the transformation from LVs to giant vesicles (GVs, diameter: >1 μm) has rarely been observed. In the present study, we report a novel type of the vesicle-fusion accompanied by both the selective aggregation of vesicles tagged with complementary DNAs and the addition of Triton X-100, which is usually regarded as a surfactant to lyse vesicles (Figure 1a). This manipulation provides an efficient transformation method from LVs to giant multilamellar vesicle-like (GMV-like) spheres (Figure 1b) and it could be applied to a targeted vesicular reagent-delivery system.

Two kinds of DNA–cholesterol conjugates (Figure 1c), which can be incorporated into vesicle membranes, were synthesized according to a phosphoramidite method on a solid support; the base sequences of these DNA-tags are $^{5'}$ATGCGTCCATCACGA$^{3'}$ (**S1**) and $^{5'}$TCGTGATGGACGCAT$^{3'}$ (**S2**, complementary to **S1**), respectively.[4a] A phosphate buffer (1.0 mL, 100 mM, pH 7) was poured into lipid films (0.92 mg) composed of 1-palmitoyl-2-oleoyl-*sn*-3-phosphatidylcholine (POPC) and each of DNA–cholesterol conjugates (molar ratio was 1000:3), and the resulted suspensions of DNA-tagged vesicles were extruded through a polycarbonate membrane (pore size is 0.2 μm) five times. Mixing of these two suspensions of **S1**- and **S2**-tagged vesicles afforded closely packed clusters of vesicles after 3 h.[4b] The formation of vesicle-clusters was confirmed by transmission electron microscopy as shown in Figure 1d. Then, Triton X-100 was added to each of suspensions

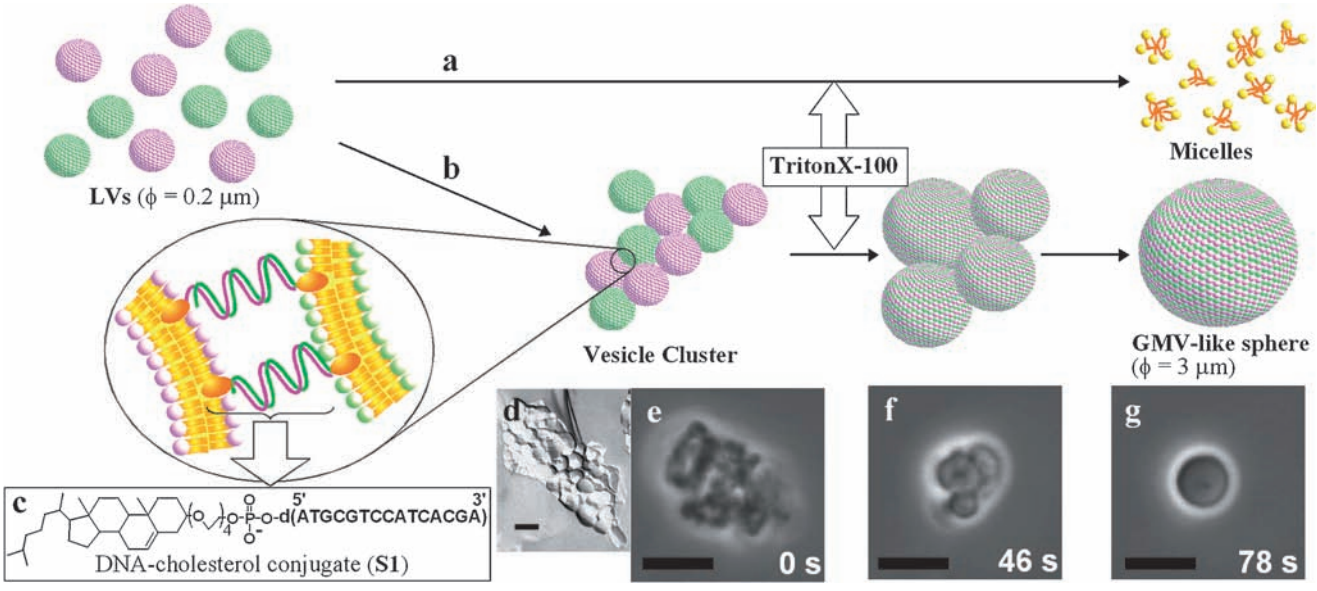

Figure 1. Schematic view of the transformation of DNA-tagged LVs in the presence of Triton X-100. (a) Micellization occurs in the absence of aggregation of LVs. (b) Successive fusion processes occurs to give a GMV-like sphere if LVs are associated via DNA duplexes. (c) A molecular structure of a DNA–cholesterol conjugate. (d) A freeze-fracture TEM image of a cluster of DNA-tagged LVs. The bar represents 200 nm. (e–g) Phase-contrast microscopic images of fusion dynamics of aggregated DNA-tagged vesicles in the presence of Triton X-100 (2.3 mM). Note that the time of (e) is set to 0 s. The bars represent 5 μm.

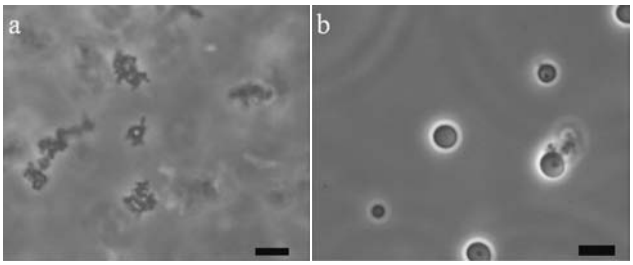

Figure 2. Phase-contrast micrographs of DNA-tagged vesicle-clusters in the presence of (a) 0 mM and (b) 1.2 mM Triton X-100. The bars represent (a) 10 μm and (b) 5 μm.

of the vesicle-clusters, and the mixtures were left standing over 3 h; the final concentration of POPC was 0.50 mM, and those of Triton X-100 were 0, 0.19, 0.56, 0.93, 1.2, 1.5, and 2.8 mM, respectively.

The transformation of clusters of DNA-tagged LVs in the presence of Triton X-100 was traced under a phase-contrast microscope (IX-71, Olympus Ltd., Japan). In the specimens containing Triton X-100 less than 0.19 mM, the clusters of vesicles remained intact (Figure 2a), whereas in the specimen with Triton X-100 more than 1.5 mM, all aggregates were lysed and a transparent solution was obtained. In contrast to these cases, spherical structures were generated in the specimens containing 0.56–1.2 mM of Triton X-100. In the 1.2 mM suspension, in particular, almost all vesicle-clusters turned into the spheres as shown in Figure 2b. Fluorescent microscopic analyses using membrane-stained or inner-pool-stained DNA-tagged LVs strongly suggest that these spheres are giant multilamellar vesicles (GMVs).[5] The average size of the generated GMV-like spheres was estimated from the micrographs to be 3.0 ± 0.7 μm ($N = 45$) and they persisted for at least 100 h. Thus, the transformation from aggregated DNA-tagged LVs to stable GMV-like spheres was clearly demonstrated.[6]

Real-time observation was conducted by means of a T-shaped mixing chamber[7] in order to elucidate the fusion process of aggregated LVs. The suspension of aggregated **S1**- and **S2**-tagged vesicles (Figure 1e) was applied to an open end of the mixing chamber and a 2.3 mM phosphate-buffered (100 mM, pH 7) solution of Triton X-100 was to the other open end. These two liquid phases merged at the center of the chamber, where a concentration gradient of Triton X-100 was generated. Fusion of aggregated vesicles was directly observed around the central region of the chamber under a phase-contrast microscope.

In the initial stage of the transformation, vesicles of about 1 μm emerged.[5] Because they were formed not only at the periphery of the cluster but also inside of it, the fusion of the original DNA-tagged vesicles proceeded simultaneously all over the cluster. The next event was a shrinkage of the whole cluster accompanied by further fusions of the vesicles, and the diameter of the resulting giant vesicles became as large as 2.2–3.1 μm (Figure 1f).[5] All the vesicles belonging to the cluster are eventually unified into a GMV-like sphere with a size of 3.8 μm (Figure 1g).[8] Thus, the transformation from the aggregated LVs to a GMV-like sphere in the presence of Triton X-100 comprised successive fusion processes of DNA-tagged vesicles.

On the other hand, the addition of Triton X-100 of 0.93 mM to nonaggregated DNA-tagged LVs caused not vesicle-fusion but micellization of LVs; the average diameter of the initial **S1**-tagged LVs monitored by the dynamic light scattering measurement (Microtrac UPA150, NIKKISO Ltd., Japan) was 231 nm, but it gradually decreased to 11 nm after 6 h.

In general, LVs consisting of phospholipids are transformed to micelles in the presence of Triton X-100 (Figure 1a). However, this pathway is turned out to be altered by self-aggregation of LVs via the duplication between complementary DNA-tags anchored in each vesicle (Figure 1b). We interpret the mechanism of the fusion as follows. In the course of partial micellization of vesicles by Triton X-100, defects in the vesicle membrane are developed and hydrophobic regions of the vesicle bilayer are exposed. If a neighboring membrane that is bridged by DNA duplexes exists, the exposed hydrophobic regions of the membranes will contact and reorganize themselves, leading to the fusion of the vesicles. A merit of our system is selectivity of aggregation of vesicles derived from DNA tags. Hence, the DNA-tagged vesicle could be utilized as a targeted reagent-carrier applicable to functional vesicular systems.[9]

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4 a) Syntheses of the conjugates are shown in Supporting Information which is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/. b) Aggregation of DNA-tagged vesicles occurred selectively according to their base sequences. For details of the aggregation of DNA-tagged vesicles, see: N. Maru, K. Shohda, T. Sugawara, *Nucleic Acids Symp. Ser.* **2004**, *48*, 95.
5 Fluorescent microscopic analyses are described in Supporting Information.
6 The best result was obtained by Triton X-100, although some other surfactants exhibited similar effects in the vesicle fusion. Polyoxyethylene (20) sorbitan monolaurate (Tween 20) and 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) transformed aggregated LVs to GMV-like spheres as well, but no fusogenic effects were detected in the case of lauryltrimethylammonium bromide and sodium cholate.
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