doi:10.1246/cl.2012.789
Published on the web July 28, 2012

Adhesion and Fusion of Two Kinds of Phospholipid Hybrid Vesicles Controlled by Surface Charges of Vesicular Membranes

Kentaro Suzuki,[1] Ryo Aboshi,[2] Kensuke Kurihara,[2] and Tadashi Sugawara*[1]
[1]*Research Center of Life Science as Complex Systems, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902*
[2]*Department of Basic Science, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902*

(Received April 9, 2012; CL-120312; E-mail: suga@pentacle.c.u-tokyo.ac.jp)

Vesicular adhesion and fusion of two kinds of hybrid vesicles composed of zwitterionic and anionic phospholipids were induced by a pH-change that caused a difference in vesicular surface charges. This facile vesicular fusion method can be applied to a substrate-transfer from a conveyer vesicle to a target vesicle.

Recently, giant vesicles (GV), which is a hollow supramolecular self-assembly of amphiphiles, have played an important role as a nanoreactor in which chemical reactions, including enzymatic reactions, occur efficiently.[1] In general, a GV consists of a semipermeable bilayer membrane which does not pass ions or large molecules. Therefore, development of a new transporting method of a substrate into GVs draws much attention not only from the aspect of construction of a successive model protocell[2] but also biomedical engineering or applications, e.g., drug delivery,[3] gene delivery,[4] and DNA computing.[5] However, spontaneous vesicular transport is difficult because of the large energetic barrier arising from the electrostatic repulsion between GVs with homopolar surface charge and the dehydration energy of a substrate required for passing itself through a hydrophobic membrane. In a biological system, smaller substrates like ions or polar molecules can be transported across membranes selectively through an ion channel. In the case of larger substrates, such as sugars, oligonucleotides, and proteins, they are transported by endocytosis. By mimicking these mechanisms from a chemical viewpoint, various transporting methods have been reported.[6–8] As for vesicular fusion,[9–11] a vesicular fusion is usually accompanied by adhesion with vesicles carrying a complimentary recognition site or an opposite surface charge.

In this paper, we explored a pH-change-triggered vesicular adhesion and fusion caused by two kinds of hybrid vesicles in a certain pH range. In order to transport substrates through this adhesion and fusion event, we are concerned with the acid dissociation equilibrium of the phosphate diester in phospholipids (Figure 1). Since the acid dissociation constant of the phosphate group in water is approximately 3, the phosphate group in basic or neutral water exists as the phosphate anion, and it is protonated in acidic water.[12] Namely, phosphocholine (PC) in 2-oleoyl-1-palmitoyl-*sn*-glycero-3-phosphocholine (POPC) has no effective charge in a neutral solution, but it becomes cationic at pH 3 by protonation, whereas about half of the ester group in 2-oleoyl-1-palmitoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (POPG) (sodium salt) remains anionic (Figure 1). Hence, while PC-rich and PG-rich vesicles do not adhere with each other in a neutral dispersion, they do interact and adhere at ca. pH 3.

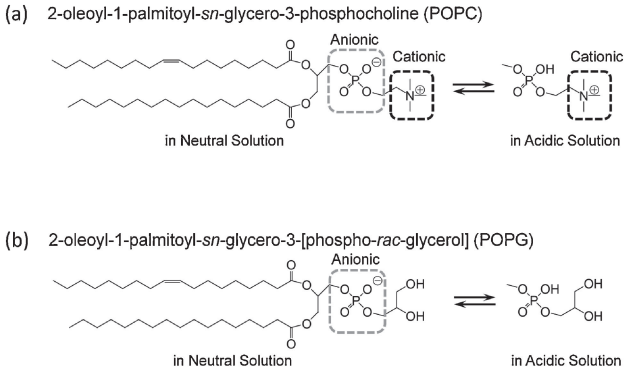

Figure 1. Change of the electric charge of phospholipids (a) POPC is zwitterionic in a neutral dispersion but it becomes cationic in an acidic dispersion. (b) POPG is anionic in neutral but nonionic in acidic dispersions.

The PC-rich GV [target GV] comprising POPC:POPG:cholesterol = 80:10:10 (mol %) was prepared by swelling the film with a 50 mM aqueous NaCl solution and the resulting dispersion was incubated at 23 °C until the adhesion experiment. On the other hand, PG-rich vesicles comprising POPG and cholesterol (POPG:cholesterol = 90:10) were prepared by film swelling as well, being stained by 0.1 mol % of a phospholipid tagged with a lipophilic fluorescent probe (2-(4,4-difluoro-5,7-dimethyl-4-bora-3*a*,4*a*-diaza-*s*-indacene-3-dodecaonyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine, β-BODIPY FL C12-HPC (Invitrogen)). The dispersion of PG-rich GVs was extruded twice by a syringe with a 0.2-μm pore membrane filter. By this treatment, the size distribution of the PG-rich GVs was shifted to a large vesicle (LV)-size [conveyer LV], which was unable to be detected under an optical microscope. The pH of the dispersions of target GVs and conveyer LVs stained with the β-BODIPY FL C12-HPC were adjusted to pH 3 and they were mixed in a frame-sealed incubation chamber ($17 \times 8 \times 0.25\,mm^3$) from the outlets placed at diagonal corners, respectively, by capillary force. Gentle mixing of both dispersions formed a boundary between two layers (Figure 2a). In the dispersion of target GV at pH 3, no aggregates were observed by phase contrast microscopy (the left-down layer in Figure 2b), or by fluorescent microscopy. The dispersion containing the conveyer LVs emitted green fluorescence as a whole (the right-up layer in Figure 2b); no GVs or aggregates were observed under phase contrast microscopy because the size of conveyer vesicles remained as the LV-size. These results mean that the aggregation between individual phospholipid vesicles did not occur in the separate layers. However, we noticed fluorescent target GVs

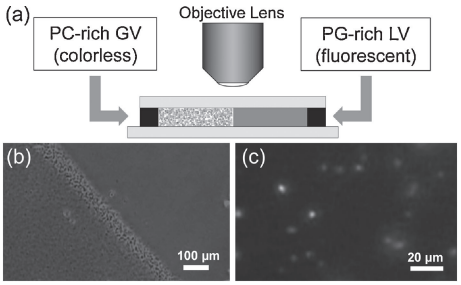

Figure 2. Temporal change of micrograms of a mixed dispersion of target GV and conveyer LVs. (a) Microchamber for mixing dispersions of target GV and conveyer LVs, the latter of which was stained by a fluorescent probe. (b) Fluorescence microgram of the boundary between target GVs and conveyer LVs 2 h after mixing. Target GVs became fluorescent due to the adhesion with fluorescent conveyer LVs. (c) A magnified image of target GV adhered by fluorescent conveyer LVs.

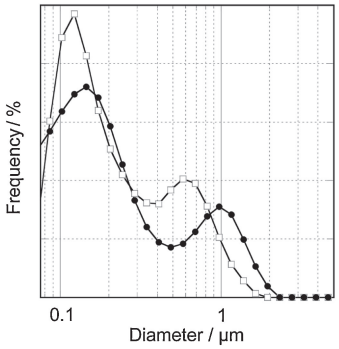

Figure 3. pH dependence of size distribution of target GVs and conveyer LVs in as dispersion Size-distribution obtained by mixing the individual dispersons at pH 7 (solid line connecting open squares) and at pH 3 (thick solid line connecting solid circles).

appeared at the boundary between two layers 2 h after the mixing. The expanded micrographic image of the target GV adhered by florescent conveyer LVs was shown in Figure 2c. The fluorescent target GV must be formed at least by the vesicular adhesion with fluorescent PG-rich LVs and it could be converted to a fused GV.

In addition, the size distribution of adhered vesicles was measured by dynamic light scattering experiments. The target GVs and the conveyer LVs were extruded by 1.2 and 0.2 μm-membrane filters, respectively, to be distinguished based on the vesicular size. Figure 3 shows the distribution of the target GVs and the conveyer LVs: The horizontal axis of Figure 3 corresponds to the logarithm of the vesicular size and the vertical axis corresponds to the intensity of scattered light which is proportional to a product of the vesicular volume and the frequency. A solid line connecting open circles in Figure 3 shows the vesicular size-distribution of both vesicles when mixed at pH 7. The two maxima correspond to the conveyer LV (ca. 0.12 μm) and to the target GV (ca. 0.6 μm), respectively. The plot drawn by thick lines connecting solid circles shows the size-distribution at pH 3 after 18 h. While the maximum in the smaller region remained almost the same, the maximum in the

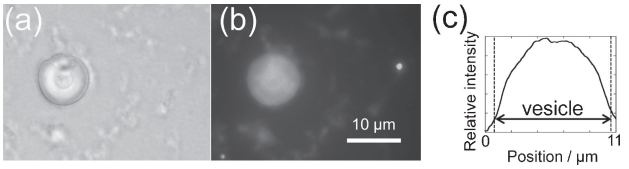

Figure 4. (a) Differential microscope image and (b) fluorescent microscope image of a fused target GV with fluorescent conveyer LVs after fluorescein transport by fusion. (c) Distribution of fluorescent intensity of the fused GV.

larger range was shifted to ca. 1 μm, which means that the adhesion between the target GVs and conveyer LVs, at least occurred.

In order to examine whether the adhesion is followed by the vesicular fusion, we prepared the conveyer LVs the inner pool of which was stained by fluorescein (a hydrophilic fluorescent probe) as follows. The thin PG film was swollen with a 500 mM aqueous solution of fluorescein and the exterior aqueous solution was diluted with water 20 times. This fluorescent PG-vesicular dispersion was filtered through a filter with a mesh of 0.2 μm so that vesicles cannot be observed under an optical microscope. It was then diluted with water 100-fold. Because the fluorescent background from the added outer aqueous solution was diluted about 8000-fold, the fluorescence microscopic observation was not influenced by the outer fluorescent dye. This dispersion of conveyer vesicles was mixed with a dispersion of target GVs (PC-rich GVs), and then 2 mM hydrochloric acid was added to adjust pH 3. When the mixed dispersion was incubated at 23 °C for 18 h, the inner pool of the GVs became fluorescent (Figures 4a and 4b). On the other hand, the incubated mixed dispersion of pH 7 did not show any change even after several days. The transport of fluorescein into these GVs at pH 3 was confirmed by the analysis of the fluorescence intensity along the diameter of the GV (Figure 4c). The maximum of the fluorescence intensity was detected at the middle along the diameter (see Supporting Information, Figure S1[15]). The result unequivocally indicates that two kinds of phospholipid hybrid vesicles not only adhered but also fused with each other in a specific pH region (ca. pH 3) where the surface charges of one kind of vesicle change to cationic, and the others remain anionic.

Adhesion and fusion events of a large number (50000 GVs) of target (PC-rich) GVs and conveyer (PG-rich) GVs were investigated on the basis of flow cytometric analysis.[13] Vesicular membranes of the target GV was stained by lipophilic fluorescence probe (2 mol % of BODIPY-red,[14] ethyl 10-[4-(4,4-difluoro-1,7-dimethyl-4-bora-3*a*,4*a*-diaza-*s*-indacene)-3,5-dimethylphenyl]decanate) and that of the conveyer vesicle was doped with a lipophilic quencher (20 mol % of 2,4-dinitro-1-octylbenzene), and the quench of the fluorescence intensity of the target GV was measured in terms of the decrease of the fluorescence intensity of each vesicle caused by the fusion with conveyer LVs. After these two kinds of vesicular dispersions were mixed at pH 7 and 3, respectively, and left standing still for 18 h, the forward scattered light (FS) intensity, corresponding to a size of GV, and the fluorescence (FL) intensity, corresponding to amount of the fluorescence probe per GV, of the vesicular mixture were measured (Figure 5). The size distribution of GVs after incubation (23 °C) at pH 3 increased slightly compared with that incubated at pH 7 (Figure 5a). On the other hand, the

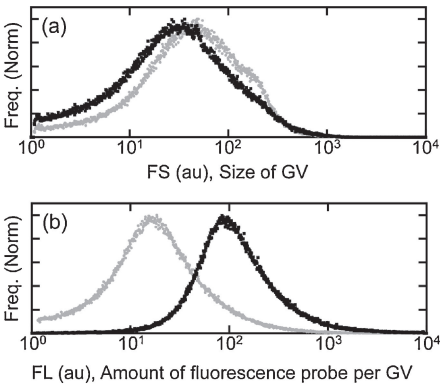

Figure 5. Histograms of the fused GVs after incubation at pH 7 (black dots) and pH 3 (gray dots). (a) Size distribution of GVs (FS), (b) amount of fluorescence probe per GV (FL). Note that the magnitude of frequencies in the two plots was normalized by the maximum value.

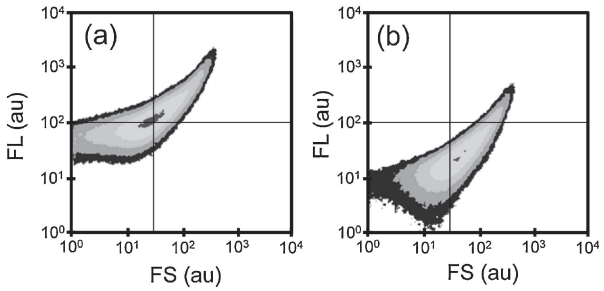

Figure 6. Density plots (2D contour map) of a flow cytometric analysis of dispersions of a mixture of target GVs with a lipophilic fluorescent dye and conveyer LVs with a lipophilic quencher incubated at pH 7 (a) and pH 3 (b). Cross bars in the both diagrams indicate the highest position in plot (a).

FL intensity of target GVs decreased by one tenth after being incubated at pH 3 compared with that at pH 7 (Figure 5b). A control experiment using conveyer LVs without the quencher caused almost no difference (Supporting Information, Figure S2[15]).

When the two-dimensional density plots of FS and FL intensities after incubation at pH 3 and 7 were examined precisely (Figure 6), the distribution along the FL axis after being incubated at pH 3 apparently broadened compared with that at pH 7. The broadening was not observed in the control experiment without the quencher (Supporting Information, Figure S3[15]). The above results mean that the quenching efficiency depends on the lamellarity of GVs. This is because conveyer LVs coat the surface of the target GV and the quenching occurs only in the outermost vesicular membrane. In the case of multilamellar GVs, fluorescent probes buried in the inside membranes are difficult to quench. Thus GVs with thin outer membranes are favorable to obtain high performance by our vesicular transport method since the efficiency depends on the lamellarity.

In conclusion, we found that the molecular transport of substrates from the conveyer vesicles to the target GV could be triggered by the pH-change through the vesicular adhesion and fusion processes. If transport of larger molecules, such as DNA, or enzymes, is feasible, this method will be widely applicable to biochemical or medical purposes.

This work was partially supported by a Grant-in-Aid for Scientific Research (B) (No. 23350060) from Japan Society for the Promotion of Science (JSPS).

References and Notes

1 D. M. Vriezema, M. C. Aragonès, J. A. A. W. Elemans, J. J. L. M. Cornelissen, A. E. Rowan, R. J. M. Nolte, *Chem. Rev.* **2005**, *105*, 1445.
2 a) V. Noireaux, A. Libchaber, *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 17669. b) K. Kurihara, M. Tamura, K.-i. Shohda, T. Toyota, K. Suzuki, T. Sugawara, *Nat. Chem.* **2011**, *3*, 775.
3 T. M. Allen, P. R. Cullis, *Science* **2004**, *303*, 1818.
4 a) E. W. F. W. Alton, P. G. Middleton, N. J. Caplen, S. N. Smith, D. M. Steel, F. M. Munkonge, P. K. Jeffery, D. M. Geddes, S. L. Hart, R. Williamson, K. I. Fasold, A. D. Miller, P. Dickinson, B. J. Stevenson, G. McLachlan, J. R. Dorin, D. J. Porteous, *Nat. Genet.* **1993**, *5*, 135. b) Y. Liu, L. C. Mounkes, H. D. Liggitt, C. S. Brown, I. Solodin, T. D. Heath, R. J. Debs, *Nat. Biotechnol.* **1997**, *15*, 167.
5 a) B. Bloom, C. Bancroft, in *DNA Based Computers V* in *DIMACS Series in Discrete Mathematics and Theoretical Computer Science*, ed. by E. Winfree, D. K. Gifford, The American Mathematical Society Press, New York, **2000**, Vol. 54, pp. 39–48. b) M. Takinoue, D. Kiga, K.-i. Shohda, A. Suyama, *Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys.* **2008**, *78*, 041921.
6 G. Das, P. Talukdar, S. Matile, *Science* **2002**, *298*, 1600.
7 a) F. M. Menger, D. S. Davis, R. A. Persichetti, J.-J. Lee, *J. Am. Chem. Soc.* **1990**, *112*, 2451. b) Y. Kobuke, K. Ueda, M. Sokabe, *J. Am. Chem. Soc.* **1992**, *114*, 7618. c) M. J. Pregel, L. Jullien, J.-M. Lehn, *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1637. d) F. Caschera, T. Sunami, T. Matsuura, H. Suzuki, M. M. Hanczyc, T. Yomo, *Langmuir* **2011**, *27*, 13082.
8 M. Mehiri, W.-H. Chen, V. Janout, S. L. Regen, *J. Am. Chem. Soc.* **2009**, *131*, 1338.
9 J. M. White, *Science* **1992**, *258*, 917.
10 *Membrane Fusion*, ed. by J. Wilschut, D. Hoekstra, Marcel Dekker, New York **1991**.
11 a) V. Marchi-Artzner, T. Gulik-Krzywicki, M.-A. Guedeau-Boudeville, C. Gosse, J. M. Sanderson, J.-C. Dedieu, J.-M. Lehn, *ChemPhysChem* **2001**, *2*, 367. b) S. Zellmer, G. Cevc, P. Risse, *Biochim. Biophys. Acta, Biomembr.* **1994**, *1196*, 101. c) N. Maru, K.-i. Shohda, T. Sugawara, *Chem. Lett.* **2008**, *37*, 340. d) D. Papahadjopoulos, W. J. Vail, C. Newton, S. Nir, K. Jacobson, G. Poste, R. Lazo, *Biochim. Biophys. Acta, Biomembr.* **1977**, *465*, 579. e) D. P. Pantazatos, R. C. MacDonald, *J. Membr. Biol.* **1999**, *170*, 27.
12 Y. Noda, *Bunseki Kagaku* **1987**, *36*, 403.
13 a) K. Sato, K. Obinata, T. Sugawara, I. Urabe, T. Yomo, *J. Biosci. Bioeng.* **2006**, *102*, 171. b) T. Toyota, K. Takakura, Y. Kageyama, K. Kurihara, N. Maru, K. Ohnuma, K. Kaneko, T. Sugawara, *Langmuir* **2008**, *24*, 3037. c) K. Kurihara, K. Takakura, K. Suzuki, T. Toyota, T. Sugawara, *Soft Matter* **2010**, *6*, 1888.
14 K. Yamada, T. Toyota, K. Takakura, M. Ishimaru, T. Sugawara, *New J. Chem.* **2001**, *25*, 667.
15 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

