Formation of Micrometer-sized Supramolecular Assemblies with Unique Morphologies from Triple-chain Lipids with Two Sugar Head Groups

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Triple-chain lipids with two sugar head groups spontaneously formed the giant vesicles in aqueous media, and their morphologies were remarkably affected by the structure of the sugar head group as well as the incubation temperature.

One of the most interesting areas in supramolecular chemistry is the construction of bilayer assemblies with a variety of morphologies, including spheres, tubules, and helical structures.[1] In particular, studies on closed bilayer assembles with sizes of 1–100 μm, so-called "giant vesicles," have attracted much attention in recent years.[2] Giant vesicles are useful models and tools for studies on cell membrane structures and function, since their large size allows not only the direct observation of their dynamic features by optical microscopy, but also easy modification by micromanipulation. They have also been used as microreactors into which various reagents, substrates, proteins, and nucleic acids can be microinjected.[3] Although there have been numerous reports on giant vesicle morphologies and their transformation,[4] most of the work has been carried out by limited kinds of double-chain lipids, such as dioleylphosphatidylcholine (DOPC).[4e–4g] In particular, giant vesicles formed with triple-chain lipids are rarely mentioned, although such giant vesicles can be expected to show unique morphologies and profiles based on the closer packing of their hydrophobic chains in the vesicle membrane.[5] In this letter, we report for the first time the formation of giant vesicles from triple-chain lipids with two sugar head groups and their morphological changes as affected by the incubation temperature.

We chose compounds **1a** and **1b** with three octadecyl chains and two sugar head groups (Figure 1) as triple-chain lipids. Here, the two sugar head groups can give the triple-chain molecule enough hydrophilicity for the spontaneous formation of the giant vesicles in aqueous media. These two sugar head groups may also provide useful information about the effects of intramolecular hydrogen bonding between the sugar head groups on the morphology of the giant vesicles. Compounds **1a** and **1b** were prepared in nine steps from 1-*O*-octadecylglycerol as a starting material, via an intermediate compound with three octadecyl chains and two methoxycarbonyl groups.[6] The structures of compounds **1a** and **1b** were characterized by ^{1}H NMR, mass, and IR spectra. The gel-to-liquid crystalline phase-transition temperatures of the hydrated lipids **1a** and **1b** were estimated to be 56.1 and 56.2 °C by DSC measurements, respectively. A typical procedure for the preparation of the micrometer-sized assemblies from **1a** and **1b** is as follows: a methanol–chloroform (1:1) solution of lipid **1a** or **1b** (2.5×10^{-4} M in 1 mL) in a test tube was slowly evaporated in vacuo at ambient temperature. Thin lipid films were obtained after drying in vacuo overnight, and water (1 mL) including sucrose[7] (100 mM) and $MgCl_2$[8] (1 mM) was added.

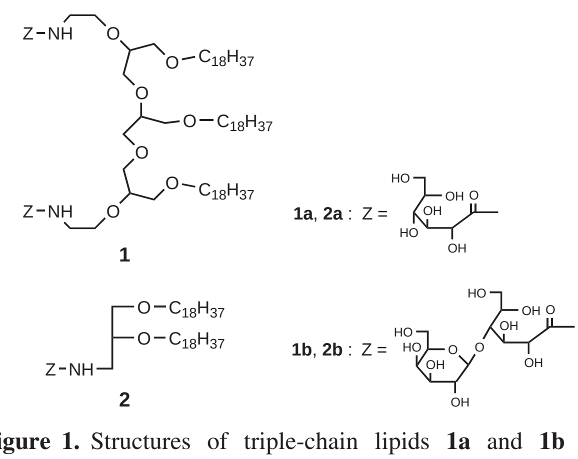

Figure 1. Structures of triple-chain lipids **1a** and **1b** and double-chain lipids **2a** and **2b**.

After an incubation for 2 h at 70 °C, which is approximately 14 °C higher than the phase-transition temperatures, the mixture was cooled down to ambient temperature. Figure 2 shows phase-contrast microscopic images of the assemblies formed with lipids **1a** and **1b**. Both **1a** and **1b** mainly formed spherical giant vesicles with an average diameter of 10 μm. The formation of the giant vesicles from **1a** and **1b** was also confirmed by the encapsulation of the water-soluble fluorescent probe, calcein, into the interior of the assemblies. Figure 3 shows fluorescence microscopic images of the assemblies formed with **1a** and **1b**. These assemblies were prepared in the presence of calcein, and then the fluorescence due to calcein in the bulk phase of the assemblies was quenched by the addition of $CoCl_2$ through Co^{2+}-calcein complexation.[9] The images clearly indicate that assemblies formed with **1a** and **1b** have an internal aqueous phase which can trap the calcein. On the other hand, the corresponding double-chain lipids with one sugar head groups **2a** and **2b** (Figure 1), which were synthesized for comparison, did not spontaneously form the giant vesicles under the same conditions. These results demonstrate that the molecular shape and amphiphilicity of **1a** and **1b** are suitable for the spontaneous formation of giant vesicles.

Interestingly, when the incubation temperature was decreased to 57 °C, which is slightly higher than the phase-transition temperatures of hydrated **1a** and **1b**, a remarkable difference in the morphology between **1a** and **1b** was observed: **1a** formed pearl-on-string assemblies[4f] together with the spherical giant vesicles, but **1b** formed tube-like assemblies as well as the spherical giant vesicles (Figure 4). Here, the average diameters of the spherical giant vesicles formed with **1b** (3 μm) were smaller than those of the spherical giant vesicles from **1a** (10 μm). This result suggests that the curvatures of the former vesicle membranes are higher than those of the latter.

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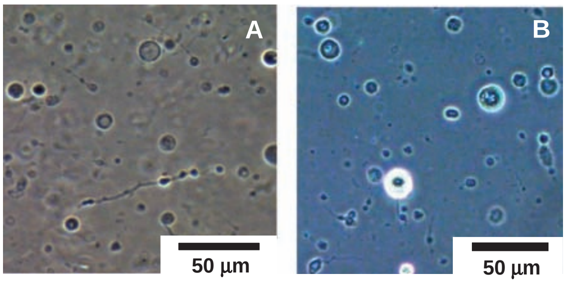

Figure 2. Phase-contrast microscopic images of assemblies formed with (A) **1a** and (B) **1b** incubated at 70 °C in water including sucrose (100 mM) and $MgCl_2$ (1 mM).

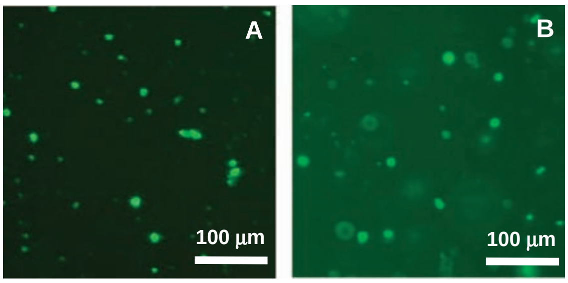

Figure 3. Fluorescence microscopic images of assemblies formed with (A) **1a** and (B) **1b** incubated at 70 °C in water including calceine (0.1 mM), sucrose (100 mM), and $MgCl_2$ (1 mM).

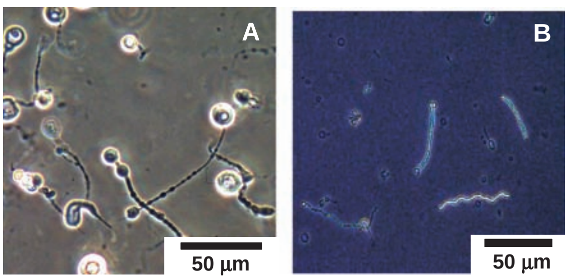

Figure 4. Phase-contrast microscopic images of assemblies formed with (A) **1a** and (B) **1b** incubated at 57 °C in water including sucrose (100 mM) and $MgCl_2$ (1 mM).

An increase in incubation temperature over the range of 57 to 70 °C decreased the ratio of the pearl-on-string assemblies to the spherical vesicles for **1a** and the ratio of the tube-like assemblies to the spherical vesicles for **1b**. At the same time, the average diameter of the spherical vesicles formed with **1b** increased with an increase in the incubation temperature, whereas that of the spherical vesicles from **1a** barely changed. Eventually, the incubations of **1a** and **1b** at 70 °C generated almost the same assemblies in shape and size, spherical giant vesicles with an average diameter of 10 µm. These findings may suggest that intra- or inter-molecular hydrogen bonding between the sugar head groups in the triple-chain lipid affects the morphology of the micrometer-sized assemblies. Incubations at 70 °C may cause the cleavage of the intra- or inter-molecular hydrogen bonding between the sugar head groups of both **1a** and **1b**, thus canceling the original difference in the effective cross-sectional area of the hydrophilic part in the vesicle membrane between those compounds. Detailed studies on the mode and temperature dependence of intra- or inter-molecular hydrogen bonding between the sugar head groups of **1a** and **1b** are now under investigation.

In conclusion, we have demonstrated that triple-chain lipids bearing two sugar head groups spontaneously formed giant vesicle in water including sucrose and $MgCl_2$. The morphology of such vesicles was found to be remarkably affected by the structure of the sugar head group in the constituent lipid as well as the incubation temperature. Work is currently in progress to gain further insight into the factors that control the morphology of these bilayer assemblies by changing the alkyl chain length and sugar head group structure.

References

1 a) J.-H. Fuhrhop, W. Helfrich, *Chem. Rev.* **1993**, *93*, 1565. b) M. Antonietti, S. Förster, *Adv. Mater.* **2003**, *15*, 1323. c) T. Shimizu, M. Masuda, H. Minamikawa, *Chem. Rev.* **2005**, *105*, 1401.
2 a) F. M. Menger, M. I. Angelova, *Acc. Chem. Res.* **1998**, *31*, 789. b) H. Hotani, F. Nomura, Y. Suzuki, *Curr. Opin. Colloid Interface Sci.* **1999**, *4*, 358. c) P. L. Luisi, P. Walde, T. Oberholzer, *Curr. Opin. Colloid Interface Sci.* **1999**, *4*, 33.
3 a) D. T. Chiu, C. F. Wilson, F. Ryttsen, A. Stromberg, C. Farre, A. Karlsson, S. Noldholm, A. Gaggar, B. P. Modi, A. Moscho, R. A. Garza-Lopez, O. Orwar, R. N. Zare, *Science* **1999**, *283*, 1892. b) P. Walde, in *Giant Vesicles*, ed. by P. L. Luisi, P. Walde, John Wiley & Sons, **2000**, Vol. 6, Chap. 22, p. 297. c) K. Tsumoto, S. M. Nomura, Y. Nakatani, K. Yoshikawa, *Langmuir* **2001**, *17*, 7225. d) A. Fischer, A. Franco, T. Oberholzer, *ChemBioChem* **2002**, *3*, 409.
4 a) J. Käs, E. Sackmann, *Biophys. J.* **1991**, *60*, 825. b) E. Farge, P. F. Devaux, *Biophys. J.* **1992**, *61*, 347. c) H. Hotani, F. Nomura, Y. Suzuki, *Curr. Opin. Colloid Interface Sci.* **1999**, *4*, 358. d) H.-G. Döbereiner, in *Giant Vesicles*, ed. by P. L. Luisi, P. Walde, John Wiley & Sons, **2000**, Vol. 6, Chap. 10, p. 149. e) O. Sandre, C. Ménager, J. Prost, V. Cabuil, J.-C. Bacri, A. Cebers, *Phys. Rev. E* **2000**, *62*, 3865. f) T. Tanaka, Y. Tamba, S. M. Masum, Y. Yamashita, M. Yamazaki, *Biochim. Biophys. Acta* **2002**, *1564*, 173. g) K. Akiyoshi, A. Itaya, S. M. Nomura, N. Ono, K. Yoshikawa, *FEBS Lett.* **2003**, *534*, 33.
5 a) Y. Sumida, T. Oki, A. Masuyama, H. Maekawa, M. Nishiura, T. Kida, Y. Nakatsuji, I. Ikeda, M. Nojima, *Langmuir* **1998**, *14*, 7450. b) Y. Sumida, A. Masuyama, M. Takasu, T. Kida, Y. Nakatsuji, I. Ikeda, M. Nojima, *Langmuir* **2000**, *16*, 8005. c) Y. Sumida, A. Masuyama, M. Takasu, T. Kida, Y. Nakatsuji, I. Ikeda, M. Nojima, *Langmuir* **2001**, *17*, 609.
6 Y. Sumida, A. Masuyama, H. Maekawa, M. Takasu, T. Kida, Y. Nakatsuji, I. Ikeda, M. Nojima, *Chem. Commun.* **1998**, 2385.
7 J. P. Reeves, R. M. Dowben, *J. Cell. Physiol.* **1969**, *73*, 49.
8 K. Akashi, H. Miyata, H. Itoh, K. Kinosita, Jr., *Biophys. J.* **1998**, *74*, 2973.
9 N. Oku, D. A. Kendall, R. C. MacDonald, *Biochim. Biophys. Acta* **1982**, *691*, 332.

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