doi:10.1246/cl.2012.954

Self-assembling Property of 6,6′-Di-*O*-octyltrehalose under Aqueous Conditions

Manami Kanemaru, Kazuya Yamamoto, and Jun-ichi Kadokawa*
Graduate School of Science and Engineering, Kagoshima University, 1-21-40 Korimoto, Kagoshima 890-0065

(Received June 20, 2012; CL-120663; E-mail: kadokawa@eng.kagoshima-u.ac.jp)

In this study, we synthesized a new trehalose-based amphiphile, 6,6′-di-*O*-octyltrehalose, from trehalose by five reaction steps. The SEM and TEM images of the sample prepared by drying its aqueous dispersion showed the formation of morphologically controlled hollow rod nanoaggregates.

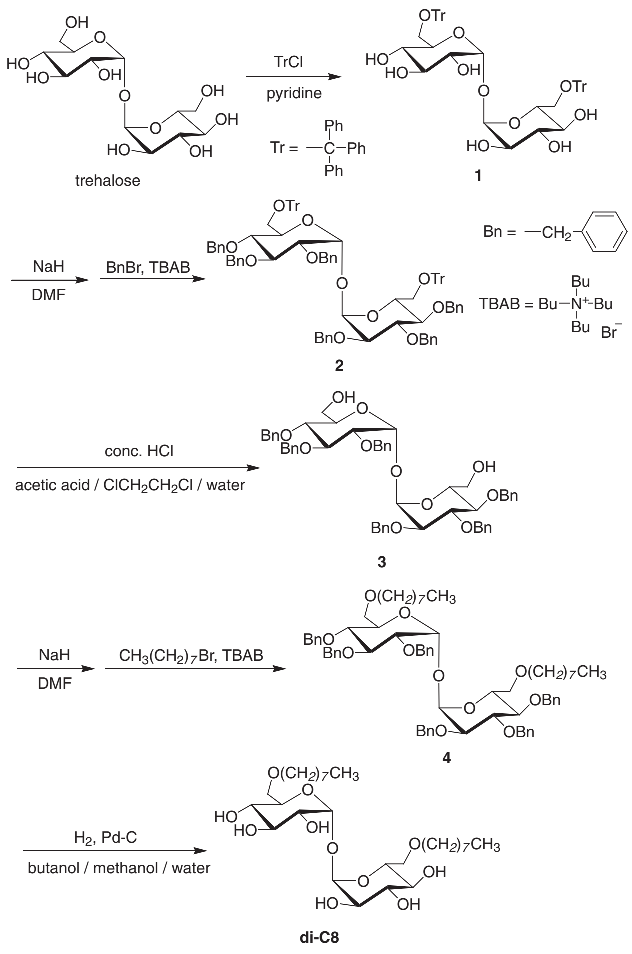
Amphiphilic molecules, of which soap is a typical example, possess antagonistic hydrophilic and hydrophobic moieties in the same molecule. In aqueous media, such molecules self-assemble into diverse aggregate morphologies, depending on the molecular shape and solution conditions.[1] Because natural amphiphiles containing carbohydrates as a hydrophilic part such as glycolipids exhibit important in vivo functions in living systems,[2] synthetic carbohydrate-based amphiphiles have been studied extensively to exhibit a large variety of self-assembling morphologies.[1b,3] For example, the self-assembling properties and applications, as surfactants, of fatty acid esters of disaccharides such as sucrose and trehalose have been studied.[4,5] Moreover, the sucrose fatty acid esters are commercially used as effective additives as emulsifiers in various food industries.[6] We have considered that carbohydrate ether amphiphiles, which have the structures composed of a hydrophilic carbohydrate residue with hydrophobic alkyl chains by ether linkages, exhibit different self-assembly from such carbohydrate ester amphiphiles, owing to the more simple structure and exclusion of hydrogen bonding by the carbonyl group. On the basis of this viewpoint, in the previous paper, we reported morphologically controlled self-assembly of a mixture of 6-*O*- and 6′-*O*-hexadecylsucroses under aqueous conditions.[7]

Trehalose is a nonreducing disaccharide, in which two glucose units are linked through an α,α-1,1′-glycosidic linkage.[8] Because trehalose has been produced industrially from starch by enzymatic catalyses and has become available at lower costs, it is regarded as a new renewable resource comparable to sucrose. Naturally occurring glycolipids containing a trehalose residue (trehalolipids) are found in nature as an example of which is trehalose 6,6′-dimycolate. This is the most reported trehalolipid and is a α-branched-chain mycolic acid in which the 6-position of each glucose unit is esterified.[9] Therefore, studies on synthesis and self-assembling properties of new amphiphiles consisting of a trehalose residue as the hydrophilic part considerably contribute to the development of practical emulsifiers and food additives. Although the preparation and properties, as surfactant, of synthetic trehalose ester amphiphiles were reported so far,[5] to the best of our knowledge, trehalose ether amphiphiles had hardly been investigated. In the previous paper, we reported a study on the self-assemblies in a series of new trehalose monoalkyl ether amphiphiles under aqueous conditions, which were 6-*O*-alkyltrehaloses having different lengths of alkyl chains, i.e., 8, 10, 12, 14, and 16, connecting to a trehalose residue at the 6-position by an ether linkage.[10] The analytical results indicated that the self-assembly of 6-*O*-dodecyltrehalose was completely different from that of the other derivatives. The former material primary formed spherical micelles in water, which further assembled according to the face-centered cubic organization by drying process from the aqueous dispersion. On the other hand, the other derivatives formed vesicle-type particles via the formation of lamellar-like planes, which were further merged with each other probably by fusion of the planes to construct larger aggregates.

In this letter, we report the synthesis and self-assembling behavior of a new trehalose ether amphiphile, i.e., 6,6′-di-*O*-octyltrehalose (**di-C8**), which is composed of two 6-*O*-octylglucose residues linked through an α,α-1,1′-glycosidic linkage, and accordingly has a symmetric molecular structure. Consequently, this material exhibited morphologically controlled self-organization under aqueous conditions to form rod-shaped aggregates. The morphology of the aggregates was completely different from that obtained from the previously reported 6-*O*-alkyltrehaloses under the same aqueous conditions.[10]

The synthesis of **di-C8** was carried out from trehalose in five steps according to Scheme 1, which were the successive ditritylation at 6- and 6′-positions, benzylation of other free hydroxy groups, detritylation, dietherification at 6- and 6′-posititons, and debenzylation. The first three reactions were already conducted to give the intermediate **3** for the synthesis of 6-*O*-alkyltrehaloses in our previous study.[10] The following dietherification using 1-bromooctane and debenzylation were carried out according to the usual manner to give **di-C8** in 19.1% overall yield from **3**.[11]

The structure of the product was confirmed by the ^{1}H NMR and MALDI-TOF MS measurements. All the signals in the ^{1}H NMR spectrum (CD_3OD, Figure S1a[11]) were assignable to the structure of **di-C8** as follows; δ 0.81 (t, 6H, CH_3, $J = 6.6$ Hz), 1.13–1.36 (m, 20H, $-(CH_2)_5-CH_3$), 1.44–1.49 (m, 4H, $-O-CH_2-CH_2-$), 3.21–3.25 (m, 4H, H-4,4′,6_a,$6'_a$), 3.33–3.46 (m, 6H, H-2,2′, $-O-CH_2-CH_2-$), 3.50–3.59 (m, 2H, H-6_b,$6'_b$), 3.67 (t, 2H, H-3,3′, $J = 9.4$ Hz), 3.80–3.83 (m, 2H, H-5,5′), 4.98 (d, 2H, H-1,1′, $J = 3.6$ Hz). The dietherified structure was confirmed by the detection of a set of glucose signals and the integrated ratio of the signal due to CH_3 protons of the octyl groups to the signal due to two anomeric protons of the glucose residues, which was 3:1. The MALDI-TOF MS showed a significant peak corresponding to the molecular mass of [**di-C8**]Na^+ (found; m/z 589.6440, calcd; m/z 589.3564). To further confirm the structure, the product was acetylated with acetic anhydride in the presence of pyridine. The ^{1}H NMR spectrum in $CDCl_3$ (Figure S1b[11]) of the acetylated derivative showed that the chemical shifts of the signals assignable to the H-6 and H-6′ protons were not different from those before acetylation (Figure S1a[11]), whereas the signals due to other positions (besides anomeric signals) were shifted to lower magnetic field. This data indicated that both 6- and 6′-positions



Scheme 1. Synthesis of 6,6′-di-*O*-octyltrehalose (**di-C8**).

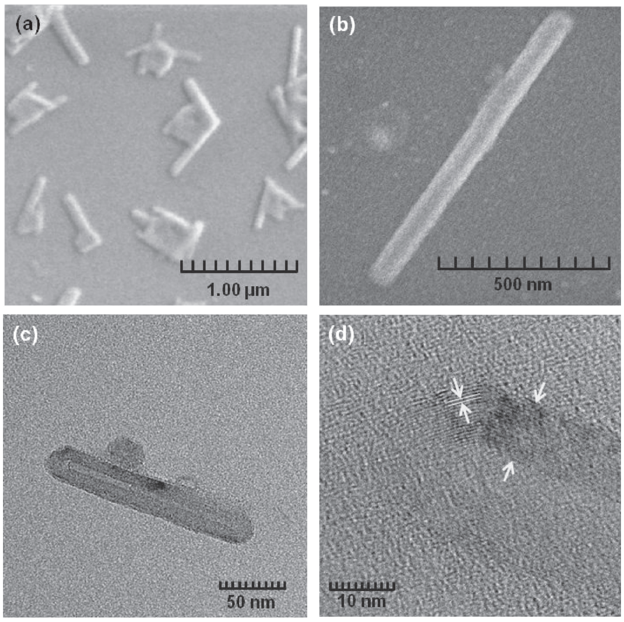


Figure 1. SEM (a and b) and TEM (c and d) images of the samples prepared from dispersions of **di-C8** in water (1.0×10^{-5} mol L^{-1}).

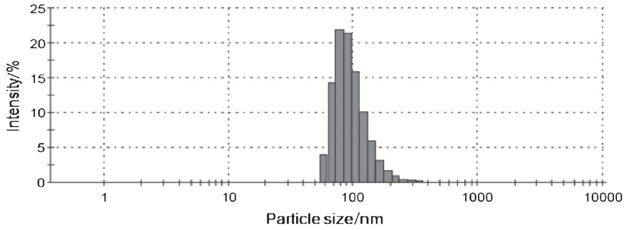


Figure 2. DLS profile of **di-C8** in water (1.0×10^{-5} mol L^{-1}).

were etherified in the aforementioned product, and accordingly, was not acetylated by acetic anhydride. All the above analytical and acetylation results can be taken to support the structure of **di-C8**. Then, the product was subjected to the following self-assembling investigations under aqueous conditions.

In the SEM images of a sample on aluminum plate (Figures 1a and 1b), which was prepared by drying a dispersion of **di-C8** in water (1.0×10^{-5} mol L^{-1}), the rod-shaped nanoaggregates with ca. 30–100 nm in width and ca. 300–800 nm in length were seen. To further confirm hierarchical structures of the nanoaggregates, the TEM measurement of the sample was conducted. The dispersion of **di-C8** in water (1.0×10^{-5} mol L^{-1}) was placed on a carbon film-coated grid. After negative-staining was carried out, the TEM sample was prepared by drying the preparative material. The TEM image of the **di-C8** sample (Figure 1c) exhibited a hollow rod with ca. 30 nm in width and ca. 150 nm in length. Furthermore, the magnified TEM image (Figure 1d) showed that the rod was constructed from lamellar-like planes probably by self-organization of the alternating hydrophilic trehalose and hydrophobic octyl layers. These SEM and TEM results suggested that the self-assembling process of **di-C8** under aqueous conditions was completely different from that of the previously reported 6-*O*-alkyltrehaloses under the same conditions.[10] Although the formation of the lamellar-like morphologies was also confirmed in such self-assembling studies of 6-*O*-octyl-, decyl-, tetradecyl-, and hexadecyltrehaloses, the planes constructed the vesicle-like particles, but the rod-like aggregates. Because the conditions in drying of the dispersion of **di-C8** for the preparation of the SEM and TEM samples may affect the precise morphology of the rod-shaped nanoaggregates such as width and length, the measurements using the samples prepared by the more regulated drying process are under the way. The SEM images of the other 6,6′-di-*O*-alkyltrehaloses, i.e., 6,6′-di-*O*-butyl- and 6,6′-di-*O*-hexyltrehaloses (Figure S2[11]) showed uncontrolled morphologies of the nanoaggregates. These results suggested that alkyl chain lengths in 6,6′-di-*O*-alkyltrehaloses strongly affected the self-assembling properties under aqueous conditions, and thus, the balance of the hydrophilic trehalose part and hydrophobic octyl part in **di-C8** is probably more favorable for the controlled self-assembly than that of others under aqueous conditions to form the rod-shaped aggregates.

The DLS result of the dispersion of **di-C8** in water (1.0×10^{-5} mol L^{-1}) (Figure 2) showed a monomodal profile with relatively narrow distribution. An average size of nanoaggregates was 99.4 nm with the polydispersity index value of

0.417, which probably corresponded to the average size in the widths and lengths of the rod-shaped nanoaggregates observed in the SEM images.

In conclusion, we reported herein the self-assembly of 6,6′-di-*O*-octyltrehalose under aqueous conditions. The derivative was synthesized from trehalose in five reaction steps. The structure of the product was confirmed by ^{1}H NMR and MALDI-TOF MS measurements as well as acetylation experiments. The SEM images of the sample prepared by drying aqueous dispersion of the product showed rod-shaped nanoaggregates ca. 30–100 nm in width and ca. 300–800 nm in length. The TEM images exhibited hollow rods, constructed from lamellar-like planes probably by self-organization of the alternating hydrophilic trehalose and hydrophobic octyl layers. Further study on revealing the detailed self-assembling process of the present material is now in progress.

References and Notes

1 a) G. S. Kwon, *Crit. Rev. Ther. Drug Carrier Syst.* **2003**, *20*, 357. b) T. Shimizu, M. Masuda, H. Minamikawa, *Chem. Rev.* **2005**, *105*, 1401. c) Z. Ge, S. Liu, *Macromol. Rapid Commun.* **2009**, *30*, 1523.

2 a) M. Corti, L. Cantù, P. Brocca, E. Del Favero, *Curr. Opin. Colloid Interface Sci.* **2007**, *12*, 148. b) D. Kitamoto, T. Morita, T. Fukuoka, M.-a. Konishi, T. Imura, *Curr. Opin. Colloid Interface Sci.* **2009**, *14*, 315.

3 a) T. Shimizu, *Macromol. Rapid Commun.* **2002**, *23*, 311. b) J.-H. Fuhrhop, T. Wang, *Chem. Rev.* **2004**, *104*, 2901.

4 a) V. Molinier, B. Fenet, J. Fitremann, A. Bouchu, Y. Queneau, *J. Colloid Interface Sci.* **2005**, *286*, 360. b) V. Molinier, P. J. J. Kouwer, J. Fitremann, A. Bouchu, G. Mackenzie, Y. Queneau, J. W. Goodby, *Chem.—Eur. J.* **2006**, *12*, 3547. c) V. Molinier, P. J. J. Kouwer, J. Fitremann, A. Bouchu, G. Mackenzie, Y. Queneau, J. W. Goodby, *Chem.—Eur. J.* **2007**, *13*, 1763. d) J. W. Goodby, V. Görtz, S. J. Cowling, G. Mackenzie, P. Martin, D. Plusquellec, T. Benvegnu, P. Boullanger, D. Lafont, Y. Queneau, S. Chambert, J. Fitremann, *Chem. Soc. Rev.* **2007**, *36*, 1971. e) Y. Queneau, S. Chambert, C. Besset, R. Cheaib, *Carbohydr. Res.* **2008**, *343*, 1999.

5 a) L. Choplin, V. Sadtler, P. Marchal, D. Sfayhi, M. Ghoul, J.-M. Engasser, *J. Colloid Interface Sci.* **2006**, *294*, 187. b) Y.-E. Sun, W.-S. Xia, G.-J. Tao, F. Qin, J. Chen, *Eur. Food Res. Technol.* **2009**, *229*, 403. c) L. Schiefelbein, M. Keller, F. Weissmann, M. Luber, F. Bracher, W. Frieß, *Eur. J. Pharm. Biopharm.* **2010**, *76*, 342.

6 I. Kralova, J. Sjöblom, *J. Dispersion Sci. Technol.* **2009**, *30*, 1363.

7 M. Kanemaru, S.-y. Kuwahara, K. Yamamoto, Y. Kaneko, J.-i. Kadokawa, *Carbohydr. Res.* **2010**, *345*, 2718.

8 S. Ohtake, Y. J. Wang, *J. Pharm. Sci.* **2011**, *100*, 2020.

9 A. Franzetti, I. Gandolfi, G. Bestetti, T. J. P. Smyth, I. M. Banat, *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 617.

10 M. Kanemaru, K. Yamamoto, J.-i. Kadokawa, *Carbohydr. Res.* **2012**, *357*, 32.

11 Supporting Information is also available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.