Self-assembling Lipid Microtubules Based on Cyclobolaphile That Mimics Archaeal Membrane Lipid

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This paper describes the first preparation of lipid microtubules that are composed of macrocyclic lipids. The lipid contains diacetylenic alkyl chains in a hydrophobic segment and phosphatidylcholine as a hydrophilic headgroup. The structural features of the assembly include: (i) monolayer wall, (ii) micron scale length, (iii) nano scale diameter, and (iv) elasticity.

In this paper, we describe the first representative example of self-assembling lipid microtubules that have been constructed from macrocyclic lipids. Results presented herein challenge the hypothesis that macrocyclic framework is theoretically ill-suited to the formation of tubules,[1] which has been an exclusive property of single-chain and double-tailed amphiphiles, i.e., acyclic amphiphiles.[2]

Our design of the lipid has been inspired by macrocyclic membrane lipids found in archaea that has considerable resistance to extreme environment.[3,4] The design principle that we have used for the construction of cyclobolaphile[5] **1** incorporates structural elements that are found in an archaeal membrane lipid[3] and 1,2-bis(tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine ($DC_{8,9}PC$)[2b]. In essence, **1** includes macrocyclic structure as well as (i) diacetylenic units and (ii) chirality that are requisite for the formation of tubules composed of $DC_{8,9}PC$.[2b] Based on this design principle, we have synthesized cyclobolaphile **1** (Figure 1).[4a]

To probe the ability of **1** to self-assemble, we adopted a modified "non-shaken mechanical dispersion method."[6] In brief, **1** (5.1 mg, 4.8 μmol) was dissolved in a mixture of chloroform/methanol (2/1, v/v, 18 mL) and a portion (1.8 mL) of the solution was placed in a petri dish (glass). The solvent was then evaporated under a stream of nitrogen and the resulting thin film was dried in vacuo (2 h). After adding slowly house-deionized Milli-Q water (2.0 mL) to the film, the aqueous solution was incubated for 1 month at ambient temperature.

First, the micrometer level morphology of the aggregates in the aqueous solution was probed by differential interference contrast (DIC) microscopy. The DIC image showed rod-like assemblies with an apparent diameter of about 1 μm and a length of several ten micrometers (Figure 2a).

Next, we examined the nanometer level structure by transmission electron microscopy (TEM, JEM-1010, JEOL) and scanning electron microscopy (SEM, JSM-5510, JEOL). The

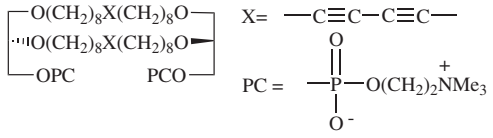

Figure 1. Molecular structure of cyclobolaphile **1**.

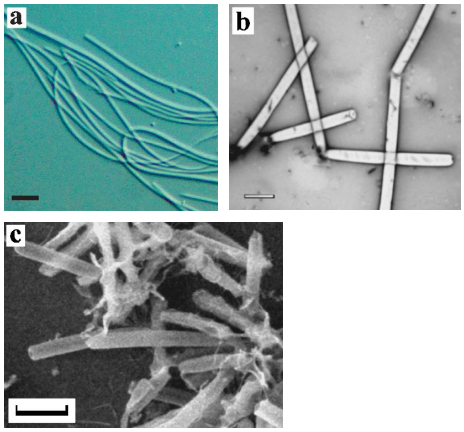

Figure 2. Micrographs of lipid assemblies: (a) Differential interference contrast micrograph; bar represents 10 μm. (b) Transmission electron micrograph (3% uranyl acetate); bar represents 1 μm. (c) Scanning electron micrograph; bar represents 1 μm.

TEM image showed rod-like assemblies having an average diameter of ca. 400 nm and a length of several micrometers (Figure 2b); the SEM image revealed Au-coated cylinders with an average diameter of ca. 300 nm (Figure 2c).[7] The SEM image also shows that the lumen of the cylinders demonstrates that the cylinders are open-ended.[8] Thus, the SEM image provides support for the hollow tubular structure. We, however, cannot conclude that the assemblies are tubules from these results. The reason for our consideration is based on the SEM and TEM specimen preparation, i.e., (i) both the preparations subject the specimen to high vacuum, and (ii) especially, the SEM protocol involves metal coating process. Thus, both the preparation protocols yield, in a sense, an artifact that may not exactly reflect the 3-D structure of the assemblies in aqueous solution.

To gain further insight into whether or not the rod-like assemblies are hollow, we have examined the aggregates by in situ tapping-mode atomic force microscopy (AFM).[9,10] Examination of the rod-like assembly by AFM, using a set point amplitude of 2.3 V, revealed that a height and diameter of the assembly were 93 and 678 nm, respectively (Figure 3a).[11] At this point in time, we have hypothesized that enhanced pressure on the surface of the assembly should significantly reduce its height, if the assemblies have a hollow tubular structure. With this in mind, we examined this assembly by AFM measurement, using a set point amplitude of 1.4 V. As shown in Figure 3b, this examination exhibited that the assembly was flattened with a height of 9.6 nm. Space-filling model of the minimum energy configuration of cyclobolaphile **1** predicts a lipid length of 4.8 nm, when the alkyl chains are fully extended. On the basis of these numbers, we reason that the flattened area of the assembly is double-layer. Sub-

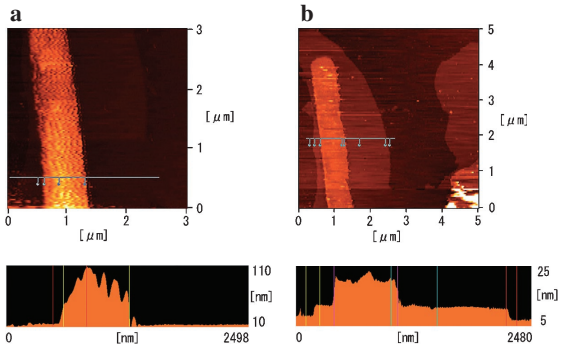

Figure 3. AFM micrographs of assemblies based on cyclobolaphile **1**. The height along the trace drawn through the micrograph is also indicated. Set point amplitude was fixed at (a) 2.3 V and (b) 1.4 V.

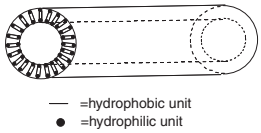

Figure 4. Schematic diagram of a possible cyclobolaphile.

sequent AFM measurement that employed a set point amplitude of 2.3 V again, revealed the restoration of height (ca. 90 nm, data not shown). Taken together, we consider that the assembly is an elastic unilamellar tubule (Figure 4).[12]

In principle, this communication presents, to the best of our knowledge, the first demonstration of the self-assembling lipid microtubules based on macrocyclic lipid. Efforts currently in progress aim at examining thermostability and polymerization of the tubule.

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5 a) Amphiphilic molecules containing a polar head group at the end of a hydrophobic segment have been termed "bolaamphiphiles" or "bolaphiles." While amphiphiles having a macrocyclic ring as a hydrophobic segment have been termed "macrocyclic bolaamphiphiles," we prefer to adopt the abbreviated and more readily pronounceable term, "cyclobolaphile." b) For "bolaamphiphiles" see: J.-H. Fuhrhop and J. Mathiewu, *Angew. Chem., Int. Ed. Engl.*, **23**, 100 (1984). c) For "bolaphile" see: N. Jayasuriya, S. Bosak, and S. L. Regen, *J. Am. Chem. Soc.*, **112**, 5844 (1990). d) For "macrocyclic bolaamphiphiles" see: F. M. Menger and X. Y. Chen, *Tetrahedron Lett.*, **37**, 323 (1996).

6 "Liposomes: A Practical Approach," ed. by R. R. C. New, IRL, Oxford (1990), p 39.

7 The apparent diameter of the rod-like assemblies that are found in the TEM image is not consistent with that in the SEM image. One plausible reason for this difference is that the freeze-drying process of the SEM specimen preparation may shrink the diameter.

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9 Fleshly cleaved mica was used as the substrate for AFM observation. Micrographs were recorded with a SPI4000-SPA400 scanning probe microscope (Seiko Instruments Inc.) at 20 °C. A 100-μm-long Si_3N_4 cantilever having a spring constant of 0.09 N/m was used.

10 The AFM images suggest that the rod-like assembly lies on a lipid sheet with a thickness of ca. 3.9 nm. We presume that successful AFM measurement of the rod-like assembly is partly due to the immobilization of the assembly on this sheet during scanning. The formation mechanism of the sheet remains to be clarified.

11 The apparent diameter of the assembly was much larger than those found in the TEM and SEM images. We presume that the observed increase in a diameter of the tubule stems from (i) swelling due to hydration, (ii) deformation of tubular structure by absorption onto the monolayer sheet, or (iii) deformation by pressure produced by the tip during scanning.

12 The ripple section was observed, when a set point amplitude of 2.3 V was used (Figure 3a). Currently we believe that this phenomenon is due to elasticity of the tubule.

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