Encapsulation of Ferritin within a Hollow Cylinder of Glycolipid Nanotubes

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The iron storage protein, ferritin, was encapsulated into a one-dimensional hollow cylinder of a glycolipid nanotube (LNT), which was obtained by the self-assembly of synthetic glycolipid molecules in water under ambient conditions. This report gives the first example of protein encapsulation into a synthetic LNT.

The arrangement of functional biomolecules is a useful technique to fabricate nanometer-scale molecular devices. For example, the alignment of ferritin on a nanometer scale has attracted much attention from the viewpoint of applications to material sciences and biotechnologies.[1–3] Ferritin is an iron storage protein that has a spherical protein shell composed of twenty-four protein subunits.[4] The inner and outer diameters of ferritin are about 7 and 12 nm, respectively, and the inner cavity accommodates the ferrihydrite core in vivo.

Mann et al. have so far synthesized or biomineralize other inorganic materials such as semiconductors or metals and metal complexes in the inner cavity of ferritin instead of the ferrihydrite core.[5–7] Thus, one-dimensional (1-D) arrangement of ferritin is expected to provide a key component of single-electron devices, such as nanodots or quantum dots with 12-nm intervals. To realize such a 1-D alignment of ferritin, utilization of a template material with a high axial ratio is desired. Some kinds of polymer,[8] DNAs,[9] protein microtubules,[10] a virus with a tubular structure,[11] and lipid nanotubes (LNTs)[12–16] should be available for the template.

Among them, synthetic LNTs are useful, especially for macroscale biomolecules, since LNTs possess a well-defined hollow cylinder 10–100 nm wide. In addition, both the inner and outer surfaces of LNTs are hydrophilic. These characteristics are favorable for the 1-D encapsulation of hydrophilic biomacromolecules such as proteins. Furthermore, the organic template can self-assemble at ambient conditions and can be removed easily after alignment. Here we report the encapsulation of ferritins into the hollow cylinder of a synthetic LNT.

We prepared the LNT by the self-assembly of *N*-(11-*cis*-octadecenoyl)-β-D-glucopyranosylamine, **1** (Figure 1a). To obtain the LNT, we dispersed 1 mg of **1** in 20-mL water at 100 °C for 2 h. Then the aqueous dispersion was allowed to cool to room temperature with the rate of 2.5 °C/min at the first 30 min. After 1 day, we obtained an aqueous dispersion containing the LNTs. The average inner and outer diameters of the LNT were 61 [standard deviation (S.D.): 19 nm] and 200 nm (S.D.: 23 nm), respectively.[15] The length of the LNT ranges from ca. 500 nm to over 100 μm. A TEM image of the LNTs is shown in Figure 1b. Next, we freeze-dried the LNTs for 3 d and removed the water inside the hollow cylinder. The freeze-dried LNTs were added to the aqueous dispersion of ferritin (CALBIOCHEM, cadmium-free, from Equine Spleen). The concentration of the ferritin was adjusted to 5 mg/mL (pH = 6.9, [NaCl] = 15 mM). The ferritin was encapsulated into the hollow cylinder of the LNT by capillary force. Then we removed the ferritin remaining outside the LNT away by filtration and thorough washing. The LNT containing ferritins was measured by a transmission electron microscope (TEM, JEM-2010, 200 kV) equipped with an attached energy-dispersive X-ray spectroscope (EDX) (EDAX, DX-4). The ferrihydrite core is favorable for TEM measurements because Fe atoms in the ferrihydrite core efficiently shield the electron beam and give a high-contrast TEM image.

Figure 2a shows the LNT encapsulating ferritins in the hollow cylinder. The many black spots in the hollow cylinder are the ferric cores of ferritin. We confirmed the encapsulation of ferritins by EDX spectrum (inset), observing a Fe Kα peak derived from the ferric cores. The Cu Kα peak is the result of a TEM grid holding the LNTs.

Figure 2b shows a magnified image of the hollow cylinder and encapsulated ferritin molecules. Figure 2c shows a trace im-

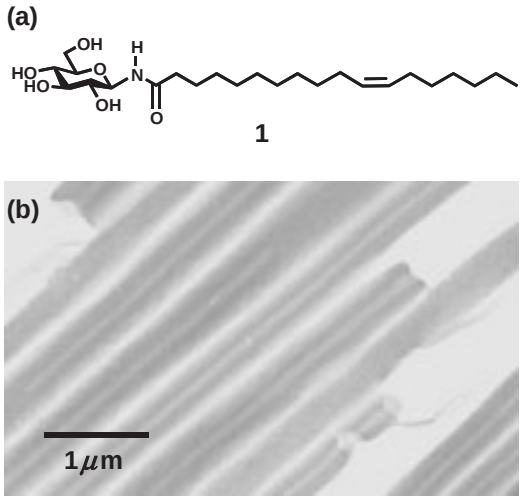

Figure 1. (a) Molecular structure of **1** as a building block of the LNT. (b) TEM image of the LNTs.

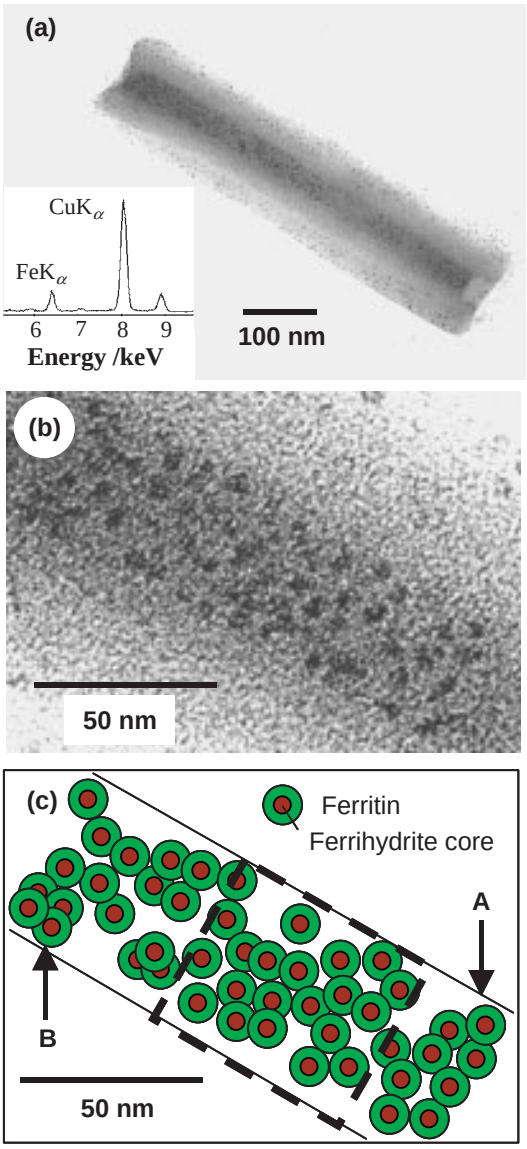

Figure 2. (a) TEM image of the LNT encapsulating ferritins in the hollow cylinder. (Inset) EDX spectrum of the same observation area. (b) Magnified TEM image of Figure 2a. (c) Trace image of Figure 2b. Encapsulated ferritin molecules are indicated by green circles with brown ferrihydrite cores. Explanations of the dashed square and arrows A and B are in the text.

age of Figure 2b. It should be noted that the black spots represent only ferric cores (7 nm) and no protein shells around the ferric core were observed by TEM. The inner diameter of the hollow of the LNT shown in Figure 2 was ca. 50 nm, and the outer diameter of the ferritin was 12 nm. Thus, at most, four ferritins can align in the normal direction to the inner surface of the LNT under the present conditions. An example of the corresponding part is indicated in Figure 2c (arrow A). We can also see the clustered black spots in the hollow of the LNT (arrow B in the Figure 2c). At the area where black spots clustered, we can consider that the ferritins were overlapped in the direction of the electron beam.

We then estimated the filling ratio of ferritin in volume. In the cylindrical volume indicated in the dashed square in Figure 2c (radius: 25 nm, length: 50 nm), approximately twenty ferritins can be counted. From the volume ratio between the cylindrical space and ferritins, the filling ratio was approximately 20% under our experimental conditions. To realize a 1-D array of ferritins in a single line, an LNT with a smaller inner diameter would be more appropriate. We are now trying to fabricate LNTs with a more appropriate hollow size with surface charges.[16]

In conclusion, we succeeded in encapsulation of ferritin into a 1-D hollow cylinder of synthetic LNTs. The present study demonstrates that synthetic LNTs can act as an organic template for functional large biomolecules over 10 nm.

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