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A Novel Preparative Method of Silica Nanotubes by Utilizing Self-assembly and Disassembly of Peptide Amphiphiles

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The addition of 2,2,2-trifluoroethanol (TFE) induces both the transition from β-sheet to α-helix structure of peptides and disassembly of wormlike micelles of peptide amphiphiles. The hierarchical structural changes were utilized for the preparation of silica nanotubes from silica-micelle complexes with avoiding structural deterioration that is normally caused by thermal treatment. This method is advantageous for the preparation of silica nanotubes because of reusability of peptide templates, possible replication of the surface of β-sheet structure, and very mild conditions.

Silica nanotubes have received broad attention due to the potential applications not only in catalysis but also in medical and biological fields, such as DNA sensing[1] and drug carriers,[2] by modifying the outer surface and by encapsulating functional molecules in the inner pore. For these applications, control of the microstructure and biomodification of the surfaces of nanotubes is important. Silica nanotubes are normally prepared from soluble silica species by templating using collagen fibers,[3] organic gel filaments,[4,5] β-sheet peptides,[6,7] etc., as rod-like templates. However, because calcination around 500–600 °C is required to remove such organic templates, all the organic components burn off and the microstructures of silica tubes are varied. Therefore, milder methods for removing templates are required in order to make good use of the organic components and silica microstructures.

Here we report a new soft preparative method of silica nanotubes by utilizing self-assembly and disassembly of peptide amphiphiles (PAs). PAs are surfactants that have peptide chains in the hydrophilic regions, and some of the PAs are known to self-assemble into wormlike micelles.[8–12] Wormlike micelles of PAs with homogeneous and tunable diameter have peptides on the surface that can form secondary structures (α-helix, β-sheet, etc.) and exhibit bioactivity. Consequently, the micelles are well-suited as templates for preparing silica nanotubes. More importantly, self-assembled templates can be softly removed by disassembly.

Recently, Yuwano et al. reported the preparation of silica nanotubes with PA templates having lysines or histidines.[13] In the study, they found that no catalyst is required in the system because the amine moieties in lysines or histidines work as a catalyst for hydrolysis and condensation of silicon alkoxides. Though their study has exhibited advantages of PA micelles as templates, calcination was inevitably conducted in the process to remove wormlike PA micelles.

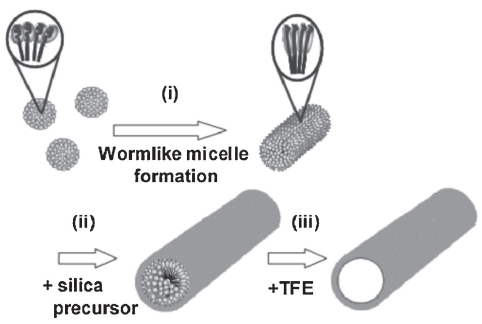
Generally, wormlike PA micelles are structurally stable compared to wormlike micelles of conventional surfactants because the β-sheet structure, based on intermolecular hydrogen bonding, stabilizes micelle structures. Thus, it is difficult to dissociate the wormlike micelles by increasing temperature of micelle solutions or adding alkyl alcohols to remove templates.

We have recently found that 2,2,2-trifluoroethanol (TFE) disassemble the wormlike micelles of peptide amphiphile C16-W3K by changing the peptide secondary structure from β-sheet to α-helix.[14] C16-W3K, consisting of a C16 alkyl tail and a peptide W3K [WAAAAKAAAAKAAAAKA] (W: tryptophan, A: alanine, and K: lysine) that has a high α-helical propensity,[15] has been reported to form wormlike micelles in an aqueous buffer solution with formation of a β-sheet structure, as reported by us.[16] Because C16-W3K has three lysines, that can act catalytically, and forms wormlike micelles at physiological pH (pH 7.4), it is expected to be an ideal template for preparation of silica nanotubes. In addition, the slow speed of the micelle formation, which is characteristic of C16-W3K, facilitates the control of the wormlike micelles. If the templates can be removed by disassembly of wormlike micelles by TFE, preparation of silica nanotubes without calcination should be achieved.

Here we propose a new method for the preparation of silica nanotubes by utilizing self-assembly and disassembly of C16-W3K as shown in Scheme 1.

Step (i) is the formation process of wormlike C16-W3K micelles, and step (ii) depicts the preparation of wormlike micelles coated with silica by hydrolysis and condensation of silica precursors. Step (iii) shows the process for removing the wormlike C16-W3K micelle template.

As the first step of the process, the templates of the wormlike micelles were prepared. The peptide amphiphile C16-



Scheme 1. Schematic of the formation of silica nanotubes.

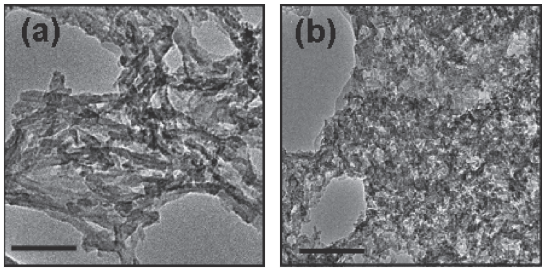


Figure 1. TEM images (without staining) of (a) silica-coated C16-W3K incubated for 10 min and the TMOS/PA molar ratio is one and (b) incubated for 10 min and the TMOS/PA molar ratio is three. Scale bar, 100 nm.

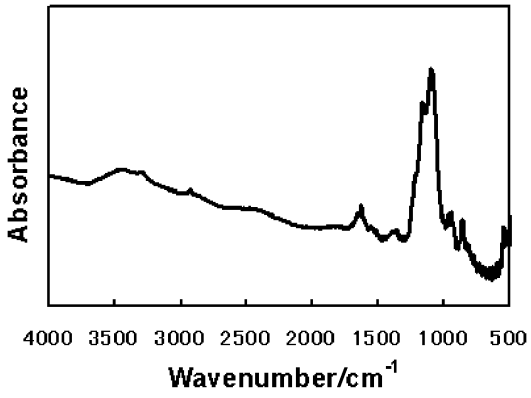


Figure 2. IR spectrum of the precipitates after the step (iii).

W3K was synthesized by Scrum Inc. by covalently bonding of the peptide W3K to the alkyl tail of C16 and verified by MALDI-TOF mass spectrometry. The template samples were prepared by adding C16-W3K to a buffer solution (pH 7.4) at a peptide concentration of about 125 μM and subsequently by incubating the solution at 50 °C for 1, 2, 5, 10, and 20 min to get short wormlike micelles. Networking of long wormlike micelles causes the gelation of the PA solution, which makes it difficult to mix PAs and silica precursors homogeneously. Short micelles are useful for preparation of detached silica nanotubes which are appropriate for the characterization. Because wormlike micelles of C16-W3K elongate with incubation time, the length of the wormlike micelle can be controlled by changing the incubation time.

Tetramethoxysilane (TMOS) was added to the solutions immediately after the incubation at 50 °C, and the mixtures were stirred at room temperature for two days. TMOS was chosen as a silica precursor because of its high hydrolysis rate. Neither acid nor base catalyst was added, and the molar ratio of TMOS to C16-W3K was 1, 2, 3, 5, and 20.

The transmission electron microscopy (TEM) image (without staining) of only the sample, prepared with C16-W3K incubated for 10 min and at the TMOS/PA molar ratio of one, shows mainly fibrous structures with a diameter of ca. 40 nm (Figure 1a). The fibrous materials have no branch and have uniform diameter. A small amount of membrane-like materials, presumed to be extra C16-W3K, were observed among the fibrils. The fibrous materials were presumed to be wormlike micelles coated with silica judging from its diameter. The images of the samples prepared with C16-W3K incubated for 1, 2, and 5 min show inhomogeneous structures including aggregated structures and very short fibrils (data not shown). The sample with C16-W3K incubated for 20 min was too viscous to mix the PA with TMOS homogeneously. Consequently, incubation time of 10 min is appropriate to prepare the templates of wormlike C16-W3K micelles. In the TEM images of the samples that have higher TMOS/PA molar ratios than one under the same incubation time (10 min), not only fibrous silica but also aggregated silica was observed. Figure 1b shows the TEM image of the sample (TMOS/PA = 3). Because it is difficult to isolate the wormlike micelles coated with silica from the mixture, the TMOS/PA molar ratio of one was found to be suitable for the process.

Even though C16-W3K was incubated only for 10 min before adding TMOS, the length of the fibrils was longer than 200 nm. This suggests that the wormlike PA micelles were elongated during the condensation of hydrolyzed species of TMOS due to the neutralization of the negatively charged hydrolysed TMOS and positively charged lysines in the W3K at the ends of the micelles. Though the incubation at 50 °C was applied here to make wormlike micelles, it has been found that C16-W3K also forms wormlike micelles at room temperature, though requiring longer than 10 days.[16] Thus, if the appropriate incubation time at room temperature can be found, the entire process should be performed at room temperature, which may facilitate establishment of a preparation system for silica nanotubes.

As the final step to prepare silica nanotubes, TFE was added to the solution to remove the templates. The concentration of TFE employed in solutions was 25%, because the value is most effective to dissociate wormlike C16-W3K micelles, as reported previously.[14] The solution was stirred for one day at room temperature. When the solution was left for a while after centrifugation, glittering precipitates were obtained in the solution. The IR spectrum (KBr disc) of the precipitates is shown in Figure 2. The IR spectrum shows two characteristic absorption bands at 1105 and 1165 cm^{-1}, assignable to the Si–O–Si stretching vibration. The broad bands at 3400 and 950 cm^{-1} are assigned to SiOH. Though very small sharp bands at 1630 and 2930 cm^{-1} are assigned to C=O and CH_2 in C16-W3K, respectively, the bands should be due to a small amount of C16-W3K that remained on the washed nanotubes. Because the silica-coated wormlike micelles are dispersed and cannot be isolated from the solution before adding TFE, it is inappropriate to directly compare the IR spectra of the samples before and after adding TFE. The washed sample virtually does not contain C16-W3K inside and outside of the nanotubes because the IR bands due to C16-W3K are so small.

The TEM image of the precipitates (Figure 3) shows the fibrils with an outer diameter of about 30–40 nm, judging from the TEM image. In the image at low magnification of the sample (Figure 3a), the membrane-like materials of C16-W3K observed in Figure 1a vanished, and the fibrous materials can be observed more clearly. In the fibrils, the linear areas of a light color with a diameter of ca. 10 nm were observed (Figure 3b). Because the TEM image was taken without staining, it was difficult to determine whether the PA templates were removed or not by the comparison between Figure 1a and Figure 3. However, as the IR result indicates that the precipitates consist of mainly silica, the

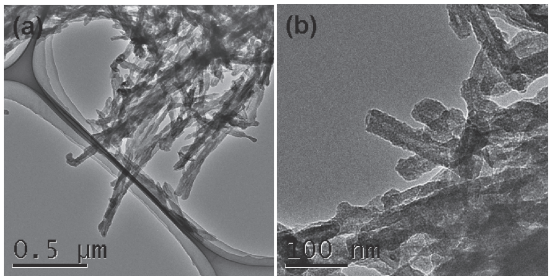


Figure 3. TEM images (without staining) of the precipitates (a) at low magnification and (b) at high magnification after the step (iii).

fibrils can be regarded as silica nanotubes. Because the inner pore diameter of ca. 10 nm, observed in the TEM image, roughly corresponds to the diameter of wormlike C16-W3K micelles measured by contrast variation SANS (small angle neutron scattering),[17] it is reasonable to conclude that silica layers are constructed on the outer surface of PA micelles.

In the process, hydrolyzed TMOS was condensed exclusively on the micellar surface due to the catalytic amino acids of W3K. Because the peptide forms relatively stretched structure (β-sheet) in the shell of the wormlike micelles, it is presumed that the outermost lysine mainly serves as a catalyst. Even though the wormlike micelles were covered with silica before adding TFE, we surmise that TFE can reach the interior from the ends or through the defects in silica layers and dissociate the wormlike PA micelles. The remarkable effect of TFE on the structural changes is explained by the presence of the hydrophobic TFE clusters.[18–20]

These IR and TEM results described above exhibit that the wormlike PA micelles inside the nanotubes can be removed by adding TFE, and silica nanotubes are prepared successfully without calcination. The TEM image exhibits relatively thicker silica walls if we consider the TMOS/PA ratio. A detailed explanation is not possible, but we may propose the following scheme. The PA solution is composed of monomeric PA, PA spherical micelles, and wormlike micelles. The initial hydrolysis and condensation of TMOS may occur randomly. Further condensation of dissolved silica species should preferentially occur on initially deposited silica which is thought to be mainly located on wormlike micelles because the catalytic lysine sites are denser on wormlike micelles. The control of wall thickness of silica nanotubes prepared with PA templates by changing the molar ratio of PA and TEOS, and volume fraction of ethanol in the solvent has been reported,[21,22] though there are differences between their study and ours in various points including PA types, silica precursor, solvent, template removal method, etc.

The results of the same procedure with the spherical micelles of C16-W3K in the sample without incubation showed no fibrous or spherical silica formation, which may be caused by the destabilization of the spherical micelles due to the interaction between the peptides and TMOS. We presume that stabilization of the micelles by β-sheet is important for the PA templates that are formed by the balance of various interactions.

The new method described here has several advantages including the use of a physiological pH of the solution, no need of catalysts, and an absence of a high-temperature process. Further, with this method through mild conditions, relatively expensive peptide amphiphiles can be reused and encapsulated drugs in the self-assembled templates can remain in the silica nanotubes after removing the templates. It is known that silicatein filaments and their subunits govern the enzymatic and structurally controlled synthesis of silica in vivo at ambient temperature and at near-neutral pH in a marine sponge,[23,24] which is similar to our system that the wormlike PA micelles serve as catalytic templates for the formation of silica layers at room temperature and at pH 7.4 in vitro. We believe that the new preparative method reported here is also fascinating as a model for understanding the mechanism of biomineralization.

In conclusion, structural changes of peptide amphiphiles with TFE are utilizable for the preparation of silica nanotubes while avoiding structural deterioration by thermal treatment. Because of the peptide secondary structure in the outer surface of the template and the mild condition of the preparation processes, the resultant silica nanotubes may exhibit structural uniqueness and open up new possibilities for these silica nanotubes in bioapplications.

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