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# Incorporation and Template Polymerization of Styrene in Single-walled Bilayer Membrane Nanotubes

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A facile method is demonstrated for fabricating nanofibrillar polymers using single-walled lipid bilayer nanotubes as templates. The versatility of this method is emphasized by easy incorporation of monomer into nanotubes, sufficient stability of template structures during polymerization, and easy removal of template lipids.

Organic nanotubes are now staged to revolutionize nanoarchitectonics because of their potential as alternatives to inorganic nanotubes[1] but with greater advantages, especially the high tunability in chemical functionality. Organic nanotubes can be roughly classified into biomolecules,[2] polymers,[3] and self-assemblies.[4] And among these, self-assemblies formed from lipid bilayer membranes offer great versatility as anisotropic organic media for various chemical reactions, in addition to their morphological properties. Lipid bilayer membranes have been used for the successful nanofabrication of various nanostructural polymers, e.g., polymerized nanohelical lipid bilayers,[5] helical nanoconductors by template polymerization,[6] nanocapsules from vesicles,[7] nanodisks from disk-like bilayers,[8] and so on.[9,10] However, there is no example of the preparation of nanotubular polymers by using lipid bilayer nanotubes as a template, although many types of nanotubular lipid bilayers have been developed by using polymerizable lipids.[4] Here we introduce the first example of nanofibrillar tubules by template polymerization with single-walled lipid bilayer nanotubes.

As the nanotube-forming lipid, we selected $N^2$,$N^3$-didodecyl-$N^1$-(3-pyridiniopropanoyl)-L-glutamine (***g*PP**) (Chart 1) because it has been known that amphiphlic derivatives of $N^2$,$N^3$-didodecyl-L-glutamine can form nanohelical and nanotubular aggregates.[1a,5] ***g*PP** was synthesized by previously reported procedure[5,11] and we established the preparation conditions for nanotube production in this study. The typical nanotube procedure is represented as follows: (1) 10 mg of ***g*PP** was dissolved in water (2.5 mL) at 60 °C by ultrasonication for 5 min and the obtained clear solution was kept at room temperature for 30 min. (2) 0.1 mL of a methanol solution with or without given amounts of monomer was added to the solution, and followed by further ultrasonication in an ice bath for 5 min. (3) Finally, the obtained solution was diluted with water to 0.5 mM ***g*PP** and aged at 10 °C for an hour.

Figure 1a shows a typical TEM image of the ***g*PP** aggregates without monomer prepared by the above-mentioned procedure. It is clearly seen that the obtained aggregates are 50–400 nm in length and 18.8 ± 1.9 nm in outer diameter. The tubular (cylindrical) structure can be assigned on the basis of the facts that a ring-shape morphology (arrow A) corresponding to a tube terminal end is seen and the corresponding tube cavity is filled by a staining reagent, indicating that the inside wall surface of the cavity is hydrophilic. In addition, the thickness of the white part (arrow B) is 6.0 ± 0.7 nm, which almost matches the bilength of ***g*PP**. These results strongly support that ***g*PP** can form nanotubules with a single-walled bilayer membrane structure.

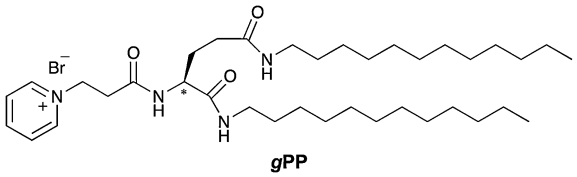


**Chart 1.**

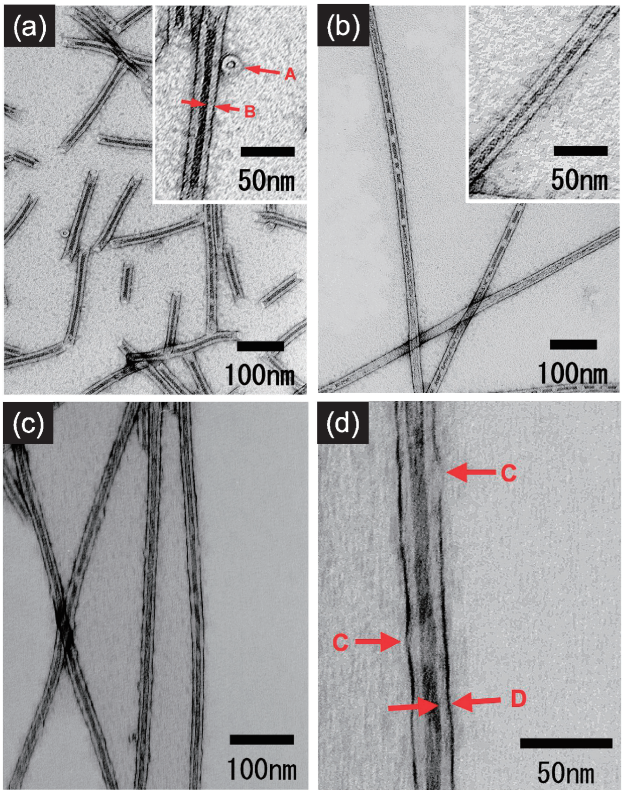


**Figure 1.** TEM images of ***g*PP** aggregates: (a) without a monomer; (b) with styrene (100 wt % for ***g*PP**); (c), (d) with styrene–divinylbenzene (65 wt % and 35 wt % for ***g*PP**, repsectively) after photoirradiated for 4 h. These samples were stained by 1.0 wt % uranyl acetate.

The loading of styrene into the ***g*PP** solution gave us unexpectedly interesting results. As shown in Figure 1b, one-dimensional growth of the aggregates was clearly found: e.g., increased from approximately 200 nm to 1 μm by addition of an

  

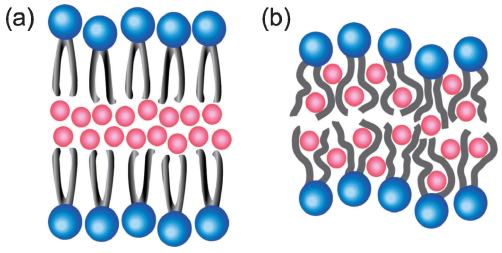


**Figure 2.** Schematic illustration of bilayer structures with monomers that are incorporated (a) at a hydrophobic boundary between the layers and (b) among the lipids.

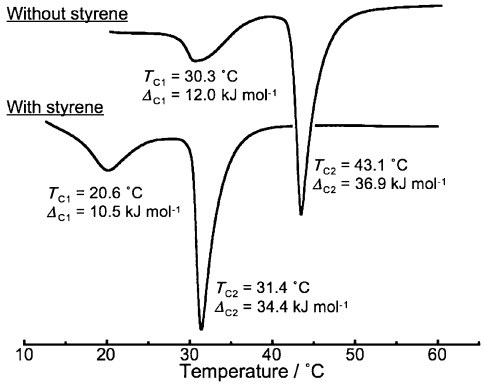


**Figure 3.** DSC thermograms of aqueous solutions of ***g*PP** (20 mM) with and without styrene (100 wt % for ***g*PP**).

equal amount of styrene for ***g*PP**. On the other hand, the tubular structure was perfectly maintained with the slight increases in the outer diameter (19.2 ± 1.3 nm). Similar one-dimensional growth of aggregates was observed with other hydrophobic monomers such as methyl acrylate and a styrene–divinylbenzene (65:35 w/w) mixture. These results indicate that hydophobic monomers can be well incorporated into the ***g*PP** aggregates and do not perturb the tubular structure but rather develop them.

The incorporation site of monomer should be discussed while two kinds of possible mechanisms will be proposed (Figure 2). To answer this question, the phase transition behaviors were investigated by DSC. As shown in Figure 3, the ***g*PP** solution showed a two-step phase transition with the peak-top temperatures at 30 ($T_{C1}$) and 43 °C ($T_{C2}$), which were assigned to a crystalline state I, crystalline state II, and a liquid crystalline state at temperatures below $T_{C1}$, between $T_{C1}$ and $T_{C2}$, and above $T_{C2}$, respectively. The monomer loading brought about a lowering of $T_{C1}$ and $T_{C2}$ by 10 and 12 °C, respectively but almost no significant change (within 10%) was observed in the phase-transition enthalpy ($\Delta_{C2}$). The values of $\Delta_{C2}$ were 36.9 and 34.4 kJ mol$^{-1}$ without and with styrene, which corresponded to those in an alkyl chain disordering based on the crystalline-to-liquid crystalline phase transition.[12] These results indicate that the incorporated styrene contacts at around the terminal moiety of the long-chain alkyl groups of ***g*PP** as the minimum contact area (Figure 2a) but not with the lateral face as the largest contact area (Figure 2b). Similar conclusions have been reported for monomer loading of vesicular and disk-like bilayer membrane systems,[8,9] although no direct evidence has been shown.

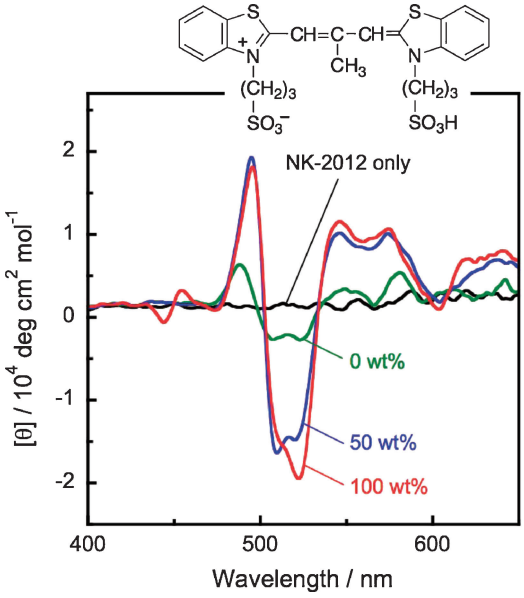


**Figure 4.** CD spectra of aqueous mixed systems composed of ***g*PP** and NK-2012 at 10 °C. [***g*PP**] = 0.5 mM, [NK-2012] = 0.025 mM, [styrene] = 0–100 wt % for ***g*PP**.

One-dimensional development of the ***g*PP** aggregates due to the incorporated styrene can be confirmed by using chirality induction. This is a useful method especially for chiral assemblies such as DNA, polypeptides, polysaccharides, and chiral lipid bilayers.[13] For this purpose, a cyanine dye, NK-2012 was selected. When an aqueous solution of NK-2012 was mixed with the ***g*PP** solution at 10 °C, an induction of chirality was observed in the absorption band of NK-2012 as shown in Figure 4. Since NK-2012 is achiral and the CD signals almost disappeared at temperature above $T_{C2}$ such as 60 °C, the induced CD can be explained by chiral interaction of NK-2012 on the ***g*PP** chiral aggregates.[14] Figure 4 also shows that the CD intensity increases five times with addition of styrene. These results suggest that one-dimensional growth of nanotubes by incorporation of styrene is accompanied by enhancement of chiral ordering among ***g*PP**.

Photoinduced polymerization of the incorporated monomer (styrene–divinylbenzene, 65:35 w/w) was carried out with Irgacure 369 ($\lambda_{max}$ = 321 nm) as a photoinitiator at 10 °C under an ultrahigh-pressure mercury lamp with a UV cut filter and monitored by the reduction in absorption at 247 nm related to the vinyl aromatic groups. More than 98% of the monomer was consumed (polymerized) after photoirradiation for 2 h (Figure S1).[15] The most significant result is that the original tubular morphology was perfectly maintained even after polymerization (Figures 1c and 1d): the inside cavity was maintained and the bilayer thickness (arrow D) was 7.2 ± 1.4 nm, indicating the inclusion of polymer while slightly more bulky moieties are partially created (arrow C). NMR spectroscopy showed that more than 98% of monomers disappeared to convert into polystyrene. We conclude that the nanotubes in Figures 1c and 1d are based on a hybrid structure of a lipid bilayer membrane with polymer.

The obtained polymer was gathered and purified by removal of ***g*PP** as follows: 20 mL of ethanol was added to 5 mL of the

  

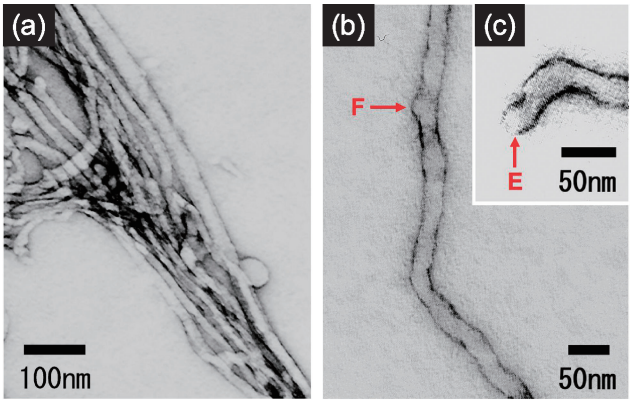


**Figure 5.** TEM images of poly(styrene–divinylbenzene) nanotubes after removal of ***g*PP**.

photoirradiated sample. The resultant precipitates were collected by centrifugation and the supernatant was analyzed by reversed-phase HPLC. The washing with ethanol and centrifugation were repeated until no more ***g*PP** was detected in the supernatant. In NMR spectroscopy, the final substance did not show any signal due to vinyl protons from monomers and pyridinium protons from ***g*PP** after washing, but included only the polystyrene-based proton signals ($\delta_{o\text{-Phe}} = 6.6$ and $\delta_{m\text{-},p\text{-Phe}} = 7.1$). TEM observation of the obtained polymer was carried out after by redispersion in ethanol and casting. Figure 5 shows that the polymer comprises nanosized fibrils with diameters of approximately 20–30 nm. The analogy with the original nanotubes is seen in the diameter, but the polymer looks rather soft and is not stained in the inside cavity, which can be explained by the fact that the poly(styrene–divinylbenzene) nanotubes are too hydrophobic in the cavity to incorporate the ionic staining reagent. The other analogy is the tube terminal end (arrow E) and partial formation of a slightly bulky moiety (arrow F) resembling those indicated with the arrows C in Figure 1d.

In conclusion, we have established a method to create nanofibrillar polymers by using single-walled lipid bilayer nanotubes as templates. This success was due to the use of ***g*PP** as the matrix bilayer tool, which provides easy morphological control, sufficient stability of the nanotubular structure, and facile removal after polymerization. To the best of our knowledge, it is the first successful example of fabrication of polymer nanotube by using lipid bilayer nanotubes as a template. A wide variety of nanostructural polymers would be created changing the preparation conditions such as temperature. The detailed temperature dependency of this template polymerization will be reported later.

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14 ***g*PP** showed a CD spectrum with $[\theta]_{280} = -2 \times 10^{-4}$ and $[\theta]_{200} = -1 \times 10^{-5}$ deg cm$^2$ dmol$^{-1}$ at 10 °C but almost no CD signal was observed at 60 °C. This is due to a thermotropic crystalline-to-liquid crystalline phase transition.

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

  