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Creation of Hierarchical Polysaccharide Strand: Supramolecular Spinning of Nanofibers by Microfluidic Device

Munenori Numata,* Yusuke Takigami, and Momoko Takayama
Graduate School of Life and Environmental Science, Kyoto Prefectural University, Shimogamo, Sakyo-ku, Kyoto 606-8522

(Received September 29, 2010; CL-100836; E-mail: numata@kpu.ac.jp)

We have demonstrated that the creation of hierarchical polysaccharide architectures can be achieved by using a fluidic liquid–liquid interface as a dynamic template for the supramolecular spinning of polysaccharide nanofibers. The individual polysaccharide nanofibers were entangled to form extended network structures in the macroscopic strand, which is never produced through conventional renaturing of polysaccharide. The wrapping of a synthetic functional polymer by the polysaccharide also proceeded on the fluidic liquid interface, leading to the creation of polysaccharide strand having unique functionalities such as conductivity or fluorescence arising from the entrapped polymer.

Polysaccharides are an abundant organic material existing on the earth, and therefore novel strategies to utilize them as practical materials have been strongly desired. Herein, we demonstrate a novel strategy to create polysaccharide strands through the supramolecular spinning of polysaccharide nanofibers, where fluidic liquid–liquid interface acts as a dynamic template for extending the nanofiber up to a macroscopic strand.

β-1,3-Glucans adopt a well-regulated triple-helix superstructure in nature. The triple-helix structure is stable in water, whereas it dissociates into the single-stranded chain in DMSO (denature). Upon addition of water to the DMSO solution, single-stranded chains self-organize to form the original triple-helix (renature) (Figure 1a).[1] So far, we have demonstrated that β-1,3-glucans can wrap synthetic functional polymers during renaturing to form water-soluble one-dimensional inclusion complexes.[2] Based on this fundamental wrapping property of β-1,3-glucans in a homogeneous solution, we have recently exploited the observation that the wrapping can also proceed on the O/W emulsion surface to create micellar structure, on which β-1,3-glucan wraps hydrophobic polymer dissolved in the oil droplet and forms polymeric network structure. These results imply that the oil/water interface acts as a temporal template for the supramolecular wrapping of β-1,3-glucan and the transcription of the three-dimensional shape of the liquid–liquid interface into the polymeric network eventually occurs.[3] The wrapping by β-1,3-glucans on the emulsion surface stimulated us to create novel polymeric architectures by using liquid–liquid interface with diverse shape, dimension, and size. Accordingly, we have begun to explore the possibility that the wrapping on a fluidic liquid–liquid interface instead of the static emulsion surface enables β-1,3-glucan chains to form the junction of the triple-helix structure through spinning of the single-stranded nanofibers, which would lead to the creation of macroscopic strands consisting of polysaccharide nanofibers.[4]

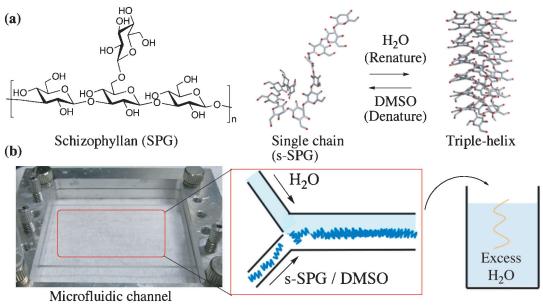

Figure 1. Chemical structure of β-1,3-glucan (SPG) and its renature and denature processes (a). Concept of the supramolecular spinning of polysaccharide nanofibers (b).

The spinning of β-1,3-glucan nanofibers was carried out with a microfluidic channel with a 100 µm wide, 40 µm deep, 120 mm long channel pattern and Y-shape junction (Figure 1b). Several concentrations of DMSO solutions containing schizophyllan (SPG: $M_w = 1150000$), a kind of β-1,3-glucan, were prepared and the solution and distilled water were oppositely charged into the microfluidic channel. The resultant solution containing 50 vol % of DMSO was further diluted with excess distilled water.[5] The renature of single-stranded SPG (s-SPG) to triple-helix SPG (t-SPG) occurred on the water–DMSO fluidic interface, being affected by the concentration of s-SPG as well as the flow rates of two layers.

As a preliminary condition, the flow rate was fixed at 10 µL min^{-1} for two layers, and the concentration effects of s-SPG on the self-assembling structures were thus investigated using 0.2, 10, 20, and 30 mg mL^{-1} s-SPG DMSO solutions.[6] To visualize the obtained structure by confocal laser scanning microscopy (CLSM) and by naked eye, rhodamine-labeled SPG was first employed. For 20 and 30 mg mL^{-1} of s-SPG solutions, after 25 min flow, the creation of strands extending to several centimeters in length was confirmed by naked eye under UV light irradiation (365 nm) as shown in Figures 2a and 2b. In the case of s-SPG concentration below 10 mg mL^{-1}, we could not confirm any strand structure by CLSM or by naked eye. Even in the case of 20 mg mL^{-1} s-SPG solution, the obtained polysaccharide strands shown in Figure 2a were unstable and tended to dissociate to small fragments within 5 h, giving clear solutions. When the s-SPG concentration was increased to 30 mg mL^{-1} however, the strand structure became

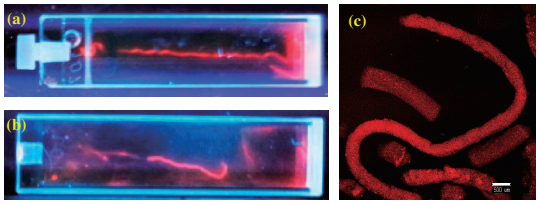

Figure 2. Photoimages of the polysaccharide strands obtained from (a) 20 and (b) 30 mg mL^{-1} s-SPG DMSO solutions; these images were taken after 3 min from pouring the strand into water. (c) CLSM images of the strand obtained by using 20 mg mL^{-1} of s-SPG DMSO solution (bar: 500 µm).

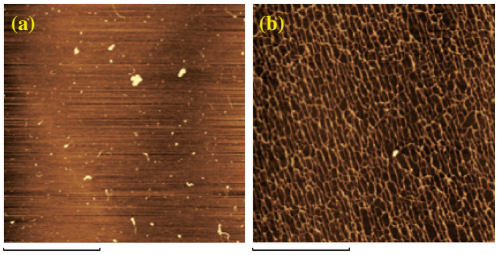

Figure 3. AFM images of SPG fibers obtained from 0.2 mg mL^{-1} of s-SPG solution (a) and the network structure obtained from 10 mg mL^{-1} of s-SPG solution (b), mica substrate, bar: 2 µm.

rather stable and maintained its shape for more than one month. As shown in Figure 2c, CLSM image revealed that the widths of the obtained strands are almost constant and the fluorescence arising from rhodamine homogeneously appears in the strand, suggesting that SPG chains self-organize to form a regular nanostructure in the strand.

To clarify the detail formation mechanism and how s-SPG chains interact with each other in the strand, AFM observation was carried out for the clear solution samples obtained from 0.2, 10, and 20 mg mL^{-1} s-SPG DMSO solutions.[7] As shown in Figure 3a, in the case of low concentration of s-SPG, i.e., 0.2 mg mL^{-1}, individual fibrous structures with 0.6–1.0 nm heights were observed, supporting the view that the usual renature from s-SPG to t-SPG would proceed on the DMSO–water fluidic interface. On the other hand, in the case of 10 and 20 mg mL^{-1} s-SPG solutions, network structures were formed as shown in Figures 3b and S1.[8] From the height profile of the network structure, it is revealed that t-SPG chains are entangled with each other to form the network structures (Figure S3[8]). As a reference experiment, when 20 mg mL^{-1} s-SPG solution was mixed with the same amount of distilled water without using a microfluidic channel, individual fibrous structures were observed. These results strongly support the view that the fluidic liquid–liquid interface is indispensable for the creation of the network structures; that is, the renature of s-SPG chains proceeds continuously on the water–DMSO interface, allowing s-SPG chains to self-organize to a macroscopic strand. Although we could not obtain AFM images for the strand obtained from 30 mg mL^{-1} s-SPG solution due to its stable strand structure, the network structures would be extended all over the strand, where each s-SPG chain is fastened to each other, endowing the strand with structural stability.

β-1,3-Glucans can accommodate various synthetic polymers in their helical structures to form one-dimensional inclusion complexes.[2] Based on the established wrapping ability of s-SPG, we thus tried to functionalize the SPG strand by using water-soluble poly(phenylenevinylene) (PPV) as a guest polymer, where nanofunctionalities arising from PPV can be magnified through the network structures. According to the experimental conditions described above, DMSO solution of s-SPG (20 mg mL^{-1}) and aqueous solution of PPV (10 mg mL^{-1}) was charged into the microfluidic channel, expecting that SPG would wrap PPV on the DMSO–water interface to form strands with fluorescence properties arising from PPV. The obtained solution gave an induced CD signal at the UV–vis absorption region of PPV, indicating that SPG actually wraps PPV chains on the liquid–liquid interface (Figure 4). As shown in the CLSM image, fluorescence from PPV is overlapped with the strand image. These results reasonably lead to the conclusion that PPV chains are insulated into the helical

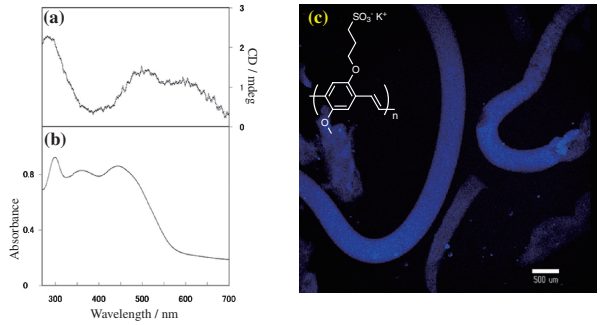

Figure 4. CD (a) and UV–vis (b) spectra of SPG/PPV complex formed on the liquid–liquid interface in the channel; 1.0 cm cell, r.t. CLSM image of the strand containing PPV (inset: chemical structure of PPV).

structure of SPG, making polymeric network structure, which homogeneously extends over the whole strand at the macroscopic region.

In conclusion, we have successfully demonstrated that supramolecular polysaccharide strands can be created on a fluidic liquid–liquid interface, making continuous spinning of the polysaccharide nanofibers under nonequilibrium control possible. The supramolecular wrapping of a synthetic functional polymer also occurs on the fluidic interface. The sophisticated combination of the wrapping by SPG and the spinning of SPG has potential to amplify self-assembling events at nanolevel as well as nanofunctionalities.

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7 For the sample obtained from 20 mg mL^{-1} solution, the clear solution obtained after decomposition of the strand was casted on mica surface.
8 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

