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Enzyme Promotes the Hydrogelation from a Hydrophobic Small Molecule

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In this paper, we report on the use of an enzymatic process to assist the formation of supramolecular hydrogels from a hydrophobic compound. Supramolecular hydrogels,[1] composed of three-dimensional (3D) networks formed by the self-assembly of small molecules in aqueous solutions, have attracted intense research interest in recent years. They have been used as a platform for biosensing,[2] a 3D matrix for cell cultures,[3] an encapsulation material for drug release,[4] etc. Molecules that can form supramolecular hydrogels (so-called molecular hydrogelators) are usually amphiphilic, such as derivatives of amino acids,[5] peptides,[6] and carbohydrates.[7] Hydrogels formed by hydrophobic compounds have never been reported. Since hydrogels formed by hydrophobic compounds might be more stable than those formed by amphiphilic compounds, the formation of hydrogels from hydrophobic compounds is desired. In this study, we researched the use of a phosphatase to convert a hydrophilic precursor to a hydrophobic compound in a homogeneous mode, thus resulting in the formation of supramolecular hydrogels with good stability.

Enzymes favor the self-assembly of small molecules, and it has been proven that enzymatic conversions promote the formation of more ordered structures in supramolecular hydrogels.[8] We envisioned that the enzymatic process, in addition to helping form ordered structures, may also be the sole mechanism by which supramolecular hydrogels are formed in some special cases. To prove this hypothesis, we designed and synthesized **1** as a substrate of phosphatase. Hydrophobic **2** was generated by treatment with the enzyme. We thought that gels would be the result of this process because the enzyme produced **2** in a homogeneous mode, which promoted the formation of the 3D fiber networks that served as the matrix of the hydrogels.

The synthesis of **1** was easy and straightforward.[9] Treating L-tyrosine-OMe with Fmoc-OSu in the presence of $NaHCO_3$ resulted in the formation of **2** in high yield (93.4%). First, the phosphorylation of **2** was conducted with triethyl phosphite and I_2; treatment with TMSBr followed, affording title compound **1** in 85.5% yield. After obtaining both compounds, we tested the gelation ability of **2** and found that it would not form gels upon pH adjustment, changes in temperature, or sonication because of its poor solubility in aqueous solutions [<0.01 mg/mL in PBS buffer (pH 8.0); Figure 1A]. Next, we tried the enzymatic conversion. Treating a clear solution of **1** in PBS buffer with high concentrations of phosphatase (>16 units/mL) resulted in the formation of suspensions within 1 min (Figure 1C). Interestingly, opaque hydrogels were formed when lower concentrations of enzyme were used: final concentrations of 16 and 4 units/mL gave gel **I** (Figure 1D) and gel **II** (Figure 1E), respectively. Both gel **I** and gel **II** formed within 10 min, and HPLC results indicated that 64.3 and 62.8% of **1** had been converted at the gelling points of gels **I** and **II**, respectively.[9] The gels did not swell and were stable for at least 1 month at pH values from 0 to 9 (e.g., gel **II** in Figure 1F,G) below 25 °C. This observation clearly demonstrated that enzymatic conversion provides a sole mechanism for promoting the formation of self-assembled systems.

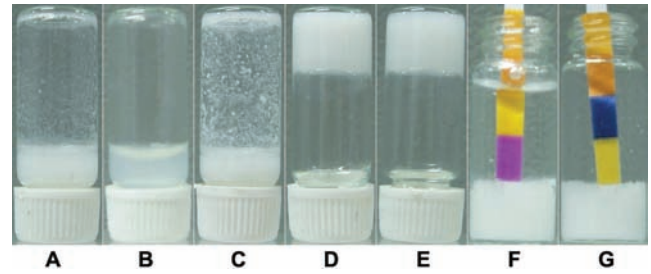

Figure 1. Optical images of 0.5 mL of (A) 1.0 wt % **2** in PBS buffer (pH 8.0) after sonication; (B) 1.0 wt % **1** in PBS buffer (pH 8.0); (C) suspension formed by the solution in (B) with enzyme (20 units/mL final concentration); (D) gel **I** formed by the solution in (B) with 16 units/mL of enzyme; (E) gel **II** formed by the solution in (B) with 4 units/mL of enzyme; and gel **II** in aqueous solution at (F) pH 0.0 and (G) 9.0.

Scanning electron microscopy (SEM) was used to characterize the gels. As shown in Figure 2A, large films with widths of hundreds of micrometers and thicknesses of ~1.8 μm provided the matrix of gel **I**. For gel **II** (Figure 2B), fibers with widths of 0.3−2 μm (arrows) and thin films with widths of tens to hundreds of micrometers were entangled with each other to form the 3D networks. Interestingly, nanoparticles with diameters of 100−800 nm and small pores or dimples adhered to the surfaces of the large films in gel **I** (Figure S-2A in the Supporting Information),[9] a phenomenon that was not observed for gel **II** (Figure S-2B).[9] Dark-field transmission electron microscopy (TEM) images indicated that the film structures in gels **I** and **II** consisted of fibers with widths of 0.45−2 μm (Figure 2C) and 200−600 nm (Figure 2D), respectively. The results obtained by SEM and TEM suggest that higher concentrations of enzyme lead to larger aggregates.

To understand the molecular arrangement of the fluorenyl groups in the solution and hydrogels, we used fluorescence spectroscopy to characterize the solution and hydrogels. As shown in Figure 3A, **1** in solution showed an emission peak centered at 310 nm, and this peak shifted to 323 and 361 nm in both gel **I** and gel **II** after enzymatic conversion, suggesting that the fluorenyl groups in the two gels overlapped in both antiparallel and parallel manners.[10] The higher intensities of the peaks at 323 nm relative to the peaks at 361 nm indicate that the fluorenyl groups favor the antiparallel overlap. The peaks at ~440 nm were from excimer emission of fluorenyl groups.[10] The higher intensities of the peaks in gel **I** relative to those in gel **II** implies that the fluorenyl groups are stacked more efficiently in gel **I**, which is consistent with the larger aggregates in gel **I** observed by SEM and TEM and the more opaque appearance of gel **I** in Figure 1D.

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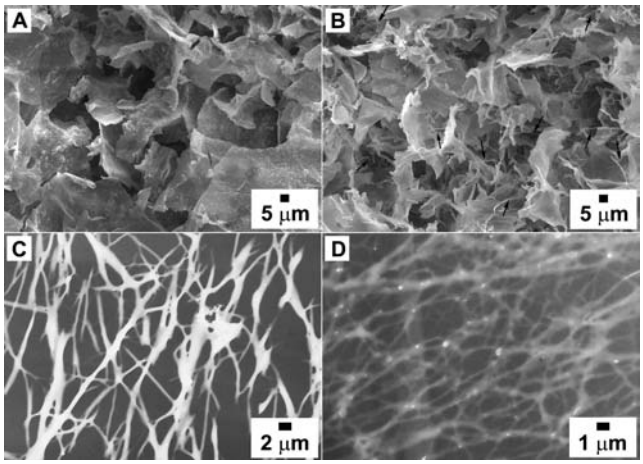

Figure 2. SEM images of balanced (A) gel **I** and (B) gel **II** and TEM images of (C) gel **I** and (D) gel **II** after 1 day.

We also used fluorescence microscopy to monitor the process of enzymatic hydrogelation (measured every 10 min after gelation). As shown in Figure 3B, the intensities of the peaks at 323 and 361 nm in gel **II** continued to decrease while the intensities of the peaks at ~440 nm increased. This result indicates that the enzyme continued to convert **1** into **2** in the initial stages, thus leading to more efficient stacking of fluorenyl moieties and more rigid hydrogels. The conversions stopped after 24 h, as proven by the HPLC results (Table S-3); the same **1/2** ratio existed in gel **II** after 24 and 48 h (i.e., there was ~3.9% **1** in the gel).[9]

We propose the following possible explanation of why the enzyme can help a "nonhydrogelator" form a hydrogel and how the nanofibers formed by hydrophobic **2** could be stable in aqueous solutions. As shown in Figure 4, on the basis of the spectroscopic analysis, most of the fluorenyl groups are stacked in an antiparallel mode, providing one of the major driving forces for nanofiber formation. We assume that the nanofibers were mainly formed by **2** and doped with hydrophilic **1**, making the nanofibers stable in aqueous solutions. **1** in the nanofibers can avoid being hydrolyzed by phosphatase, as demonstrated by the HPLC results (i.e., 3.9% of **1** was still intact in gel **II** after 24 and 48 h).[9] Nevertheless, only suspensions could be obtained from mixtures of different ratios of **1** and **2** upon sonication or by a heating−cooling cycle,[9] further demonstrating that the enzyme generates hydrophobic **2** in a homogeneous mode, thus assisting the formation of 3D fiber networks in supramolecular hydrogels.

In summary, we have demonstrated that an enzyme offers a sole mechanism for generating hydrophobic compounds in homogeneous modes, thus assisting the formation of 3D fiber networks and supramolecular hydrogels. This process provides a facial strategy for generating supramolecular hydrogels from hydrophobic molecules and offers more candidates for the generation of supramolecular hydrogels. What is more, there is potential for this approach to be developed into a convenient way to control the morphology

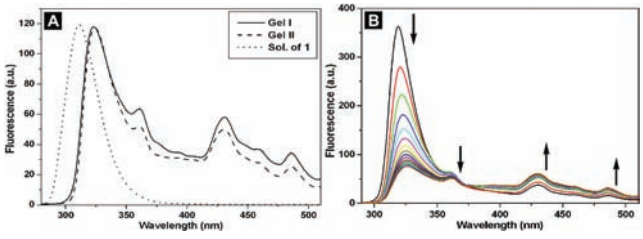

Figure 3. Emission spectra ($\lambda_{excitation}$ = 265 nm) of A) solution of **1**, gel **I**, and gel **II** and B) gel **II** at different time points (every 10 min).

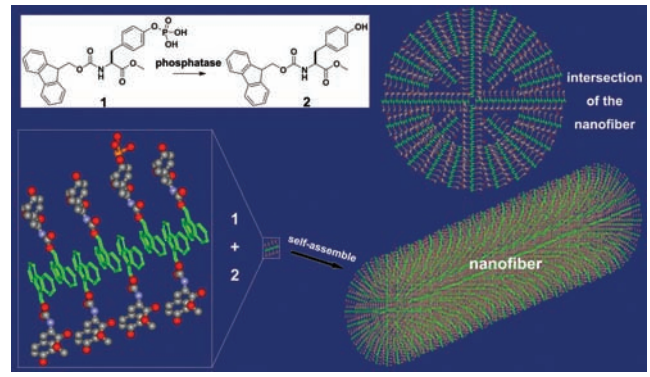

Figure 4. Schematic illustration of enzymatic conversion and proposed molecular arrangements of **1** and **2** in nanofibers (the antiparallel stacking of fluorenyl groups is highlighted in green).

of aggregates. The stable hydrogels formed by the enzymes could be developed into biomaterials that would be useful, for example, in drug delivery, enzyme immobilizations, and tissue engineering.

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Supporting Information Available: Synthesis and characterization of **1** and **2**, HPLC results, optical images of suspensions with different ratios of **1** and **2**, and SEM images of the two gels and the nanoparticles in gel **I**. This material is available free of charge via the Internet at http://pubs.acs.org.

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