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Novel Anisotropic Supramolecular Hydrogel with High Stability over a Wide pH Range†

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The hydrolysis of the carboxylic ester bond, by a base or catalyzed by an enzyme under weak basic conditions, serves as the only path to obtain a novel anisotropic supramolecular hydrogel that is stable over a wide pH range. This result not only expands the molecular scope of supramolecular hydrogelators but also illustrates the design principles for creating pH-stable supramolecular soft materials.

Supramolecular hydrogels, resulting from the self-assembly of certain small organic molecules (i.e., hydrogelators) driven by noncovalent interactions, have emerged as a type of versatile soft material and have found applications in many areas.[1] Because of their inherent and excellent biocompatibility and biodegradability, supramolecular hydrogels have shown promise in becoming a useful alternative to polymeric hydrogels.[2] For example, supramolecular hydrogels are being explored to serve as scaffolds for regenerative medicine,[3] wound healing,[4] biomineralization,[5] vehicles for controlled drug release,[6] matrices for protein microarrays,[7] a low-cost platform for screening enzyme inhibitors or enzyme detection,[8] and components for enzyme mimetics.[9]

Forming a hydrogel is the first step in developing supramolecular hydrogels as useful soft materials. There are several different ways to generate supramolecular hydrogels. A commonly used method involves dissolving hydrogelators into an aqueous solution and changing the temperature, pH, or ionic strength to initiate molecular self-assembly in water, resulting in hydrogelation.[1] This kind of approach, which works well for most hydrogelators, has some inherent disadvantages for certain hydrogelators, for example, a hydrogelator with exceedingly low solubility (or unusually high hydrophobicity) or having the potential to form precipitates instead of a hydrogel as a result of the change in temperature, ionic strength, or pH. These molecules have the potential to self-assemble in water despite their poor aqueous solubility. One approach is to dissolve them in a polar organic solvent and then mix this solution with water to form nanostructures or hydrogels. For example, in work on the self-assembly or hydrogelation of NH_2-Phe-Phe-COOH,[10] Fmoc-Phe-Phe,[11] Fmoc-Phe(F5),[12] GSH-pyrene,[13] and NH_3^+-Phe-Phe-CO-NH_2,[14] an organic solvent (usually 1,1,1,3,3,3-hexafluoro-2-propanol or DMSO) is always necessary to assist the dissolution of these compounds. Despite its effectiveness, this approach inevitably brings small amount of organic solvent into the hydrogels, which changes, if not completely disturbs, the biocompatibility or rheological behavior of the resulting hydrogels.

To explore new methods for making hydrogels, we and others have been developing new ways to induce hydrogelation via chemical or enzymatic conversion. For example, the phosphorylation of tyrosine residues on hydrogelators offers precursors that are soluble at physiological pH. Then a phosphatase can hydrolyze the phosphoric monoester and convert the precursors to less soluble but amphiphilic hydrogelators, which self-assemble in water to form supramolecular nanofibers and result in hydrogels.[15] Besides the route of dephosphorylation, other chemical or enzymatic paths should also be suitable for the generation of supramolecular hydrogels, which have been less explored.[16]

In this work, we explored the paths for generating the hydrogel of a small molecule (**2**). Because of its high hydrophobicity, neither the change in pH nor the change in temperature creates the hydrogel of **2**. However, a simple chemical modification of **2** offered

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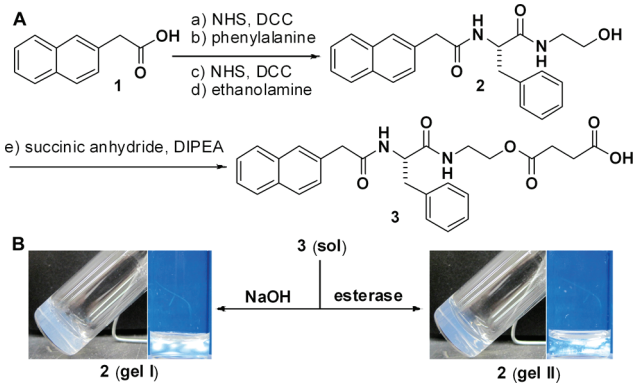

Figure 1. (A) Synthesis route to **2** and **3**. (B) Reactions to convert the precursor (**3**) to the hydrogelator (**2**) under basic conditions (pH 9) to afford gel I or via hydrolysis catalyzed by esterase (1 U/μL, 10 μL) at pH 7.5 to give gel II (optical images: left, normal; right, through a pair of crossed polarizers).

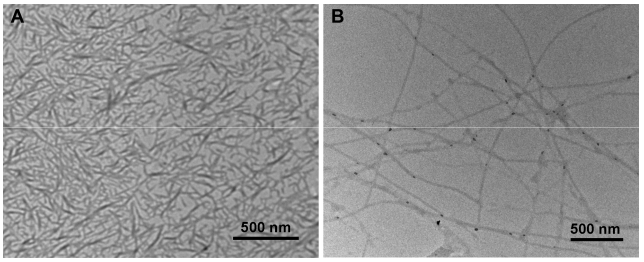

Figure 2. TEM images of the matrices in (A) gel I and (B) gel II (aged for 7 days).

molecule **3** (i.e., the precursor of **2**) with excellent solubility at physiological pH, and a chemical (i.e., a strong base) or a biological catalyst (i.e., an esterase) triggered the hydrolysis of the carboxylic ester bond of **3** to produce **2**, which self-assembled into a supramolecular hydrogel. Because **2** lacks a carboxylic acid group or an amine group, the hydrogel of **2** exhibits extraordinary stability over a wide pH range, which is crucial for some applications of biomaterials.[17] In addition, unlike most of the supramolecular hydrogels, the hydrogel of **2** is anisotropic (i.e., it exhibits birefringence), which is associated with the order of the nanofibers. Besides the identification of a novel hydrogelator, this work illustrates a powerful strategy for evaluating the ability of hydrophobic molecules to form supramolecular hydrogels, which may provide a new direction for designing supramolecular materials based on small-molecule therapeutics because a large number of them are quite hydrophobic.

Figure 1 shows the structures and the syntheses of **2** and **3**. Two cycles of activation of **1** by *N*-hydroxysuccinimide (NHS) and coupling with the amine group on phenylalanine and ethanolamine, respectively, give **2** in 91% yield (Scheme S1). Finally, the reaction between succinic anhydride and **2** results in **3**, which exhibits a drastically increased aqueous solubility (> 20 mg/mL) compared to that of **2** (< 20 μg/mL). After obtaining pure compounds **2** and **3**, we tested the ability of **2** to form a hydrogel and found that it failed to form a hydrogel via changing the temperature or adjusting the pH. Unlike **2**, compound **3** dissolves easily in a weak basic solution (pH 7.5) at the concentration of 0.8% (w/v). The addition of a small amount of base (NaOH, 1.0 M, 10 μL)

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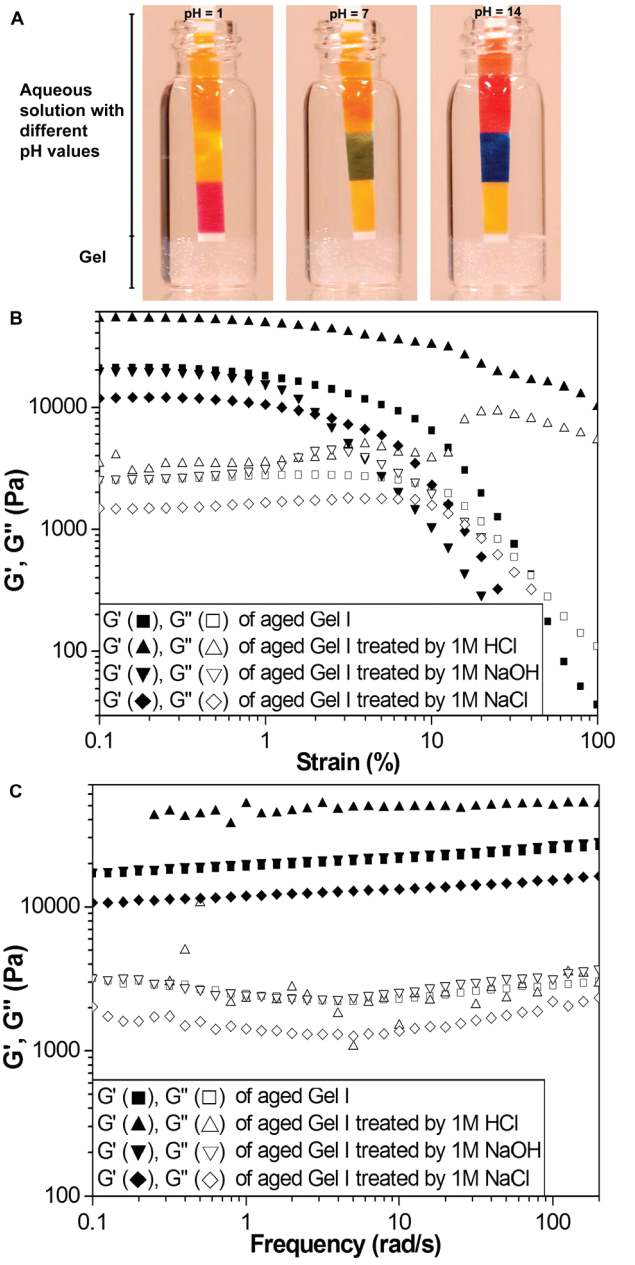

Figure 3. (A) Optical images of the gels of **2** immersed in solution with different pH values. (B) Dynamic strain sweeps (at 6.28 rad/s) of aged gel I (400 μL, 0.8% (w/v)), without treatment and immersion in 1.0 M HCl (20 mL), 1.0 M NaOH (20 mL), and 1.0 M NaCl (20 mL) for half an hour. (C) Dynamic frequency sweeps of corresponding gel I.[19]

to reach pH 9 or esterase (1 U/μL, 10 μL) in 400 μL of the solution of **3** causes the hydrolysis of **3**. The loss of succinic acid from **3** affords **2** in almost quantitative yield (Figure S1), which self-assembles in water to result in a clear hydrogel that shows strong birefringence (Figure 1B) without a noticeable pH change. The use of NaOH to hydrolyze **3** helps to determine the hydrolysis rate (Figure S2) and to estimate the critical gelation concentration (CGC) of **2** to be 0.16% (w/v) (Figure S3). We designate the hydrogels formed by the addition of NaOH and esterase as gels I and II, respectively.

We used a dynamic time sweep experiment to determine the gelling point upon the addition of base or enzyme. The gelling points of gels I and II were about 1 and 38 h, respectively (Figure S4), which match the observation in the TEM images of

the two hydrogels (Figure 2). According to the TEM results, gel I, which formed faster, consists of short fibers (< 500 nm) with more randomly interwoven networks (Figure 2A). Gel II, which formed more slowly, containing long fibers (> 2 to 3 μm) with less-dense networks (Figure 2B).

In addition, increasing the quantity of reagents for ester bond hydrolysis can speed up the hydrolysis and thus gelation. Upon the addition of a larger amount of NaOH, gel I forms within 3 min (video in Supporting Information). Besides controlling the gelation speed, different hydrolysis routes may be applied to different applications. For example, gelation catalyzed by esterase can proceed under physiological condition, which will be useful in encapsulating biomacromolecules. Moreover, we can tailor the properties of the hydrogels with different microstructures for different applications by adding different catalysts at various quantities.

When immersed in both highly acidic and highly basic solutions, the hydrogels of **2** showed excellent stability (Figure 3A). To understand the stability of the hydrogel of **2** further, we quantified its rheological properties after the gels were treated with HCl (1.0 M) or NaOH (1.0 M). The dynamic strain sweeps and dynamic frequency sweeps reveal that aged gel I retains its rheological properties after immersion in solutions (20 mL) with various pH and a salt solution (20 mL) for half an hour (Figure 3B,C). The gel in basic solution (pH 14) maintains the G' and G'' values that are comparable to those of native gel I, although the critical strain seemed to decrease from 40 to 4%. The acid-treated gel shows an increase in the storage modulus of up to at least 2.5 times the G' value of native gel I. Besides, upon treatment of the 1.0 M HCl solution, the volume of gel I changes little (Figure S5). However, gel I treated with a 1 M NaCl aqueous solution exhibits a slight decrease in viscoelasticity. The above results suggest that the acid may increase the hydrogen bonding among the hydrogelators and water.

Because the formation of gel II is slow enough to allow us to monitor the optical and spectroscopic changes over time, we synchronized the time-dependent CD spectra and the time-dependent birefringence of gel II to examine the gel-formation process (Figure 4). In the first 12 h, the CD data hardly showed peaks below 210 nm and we observed no birefringence. However, after that, the birefringence as evidenced by the negative peak at around 209.6 nm and the positive peak at 195.8 nm also appeared. Afterward, the birefringence increases and these two peaks become larger and sharper. According to Boden and co-workers' pioneering work,[18] the appearance of these two peaks correspond to the formation of the β-sheet. In addition, the increase of anisotropy likely contributes to the higher positive peak at 230 nm. Therefore, these results suggest that the birefringence is associated with the formation of the β-sheet (Figures 4 and S6).

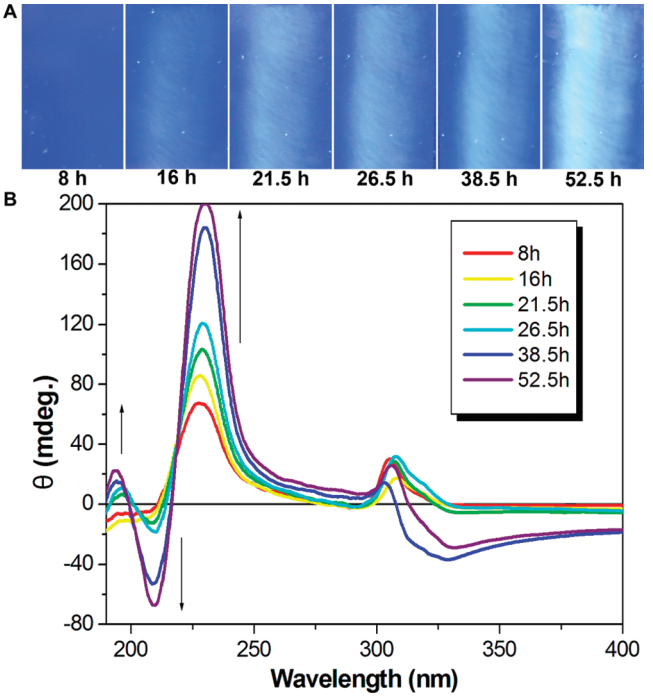

Figure 4. (A) Optical images of gel II (formed by the catalysis of esterase) under cross-polarized light at different time points and (B) the corresponding CD spectra.

In conclusion, we have demonstrated that the controlled hydrolysis of a carboxylic ester bond by a base or an enzyme can act as a simple trigger to initiate self-assembly and form supramolecular hydrogels, even if the molecule itself is unable to form hydrogels by directly changing the pH or temperature. The replacement of a carboxylate group with a hydroxyl group on the hydrogelator not only results in a supramolecular hydrogel that is stable over a wide pH range but also offers a soft supramolecular material that is insensitive to ionic strength. These properties may be particularly useful in designing a robust drug release system that can maintain a constant release rate against abrupt changes in the environment, which is a subject that is currently under investigation.

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Supporting Information Available: The experiment details, NMR, hydrolysis product analysis, hydrolysis rate, CGC test, dynamic time sweep, video of fast gelation, swelling test, and the normalized intensities of CD. This material is available free of charge via the Internet at http://pubs.acs.org.

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