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Editor's Choice

Mechanical Reinforcement of Supramolecular Hydrogel through Incorporation of Multiple Noncovalent Interactions

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Supramolecular hydrogel, comprising a three-dimensional network of self-assembled nanofibers, has recently emerged as a unique material for a variety of potential applications such as tissue engineering. One serious drawback of supramolecular hydrogels that sometimes limits their practical usage is their mechanical weakness compared with conventional polymer gels. Here we describe a stiff supramolecular hydrogel made of a zwitterionic amino acid-tethered amphiphilic molecule that self-assembles into nanofibers through multiple and orthogonal noncovalent interactions.

Supramolecular chemistry[1] has clarified the essence of a variety of molecular interactions in complicated biological systems and simplified artificial models over recent decades.[2,3] By regulating these intra- and intermolecular interactions, many complicated and beautiful molecular architectures can be created through noncovalent self-assembly of small molecules, in both natural[4] and artificial[5] supramolecular systems. It is believed that well-developed 3D structures in biological systems are responsible for sophisticated functions, and thus artificial molecular assembly is one of the central themes in modern chemistry. Interest in supramolecular hydrogels[6,7] comprising small molecules is now rapidly growing because of their potential as novel biomaterials that are applicable to intelligent drug release systems[8] and tissue engineering.[9] In these supramolecular gels, long-range interactions among small molecules and solvents may be involved to control the self-assembly, so that unique bulk materials can be produced. However, the mechanical fragility of supramolecular gels relative to conventional polymer gels can be a serious drawback for practical applications.[10] Although polymerization after self-assembly of small molecule gelators has been often carried out to enhance mechanical strength, in many cases it spoiled the flexible and rapid gel–sol response of the gels.[11] We describe herein that a chemical strategy for reinforcement of the mechanical stiffness of supramolecular hydrogels while retaining dynamic stimuli-responsive properties, by incorporating multiple and orthogonal molecular interactions into a small molecule.

In order to enforce the noncovalent interactions, we newly introduced electrostatic interactions by replacing the saccharide head group with a zwitterionic amino acid head based on the glycolipid hydrogelators **2** or **3** previously developed by our group[7f,7i,10e] (Figures 1a and 1b). In an amino acid hydrogelator **1**, intermolecular ion pairing between ammonium and carboxylate of the amino acid (L-lysine (Lys)) moieties may form at the surface of self-assembled fibers, on the basis of the bimolecular layer structure shown in Figure 1c. In addition, there are π–π stacking between fumaric double bonds, intermolecular hydrogen-bonding networks between fumaric amide units, and van der Waals packing around hydrophobic tails. The cooperative action of these four noncovalent interactions allows us to anticipate enhanced stabilization of the self-assembled gel fibers (Figure 1c).

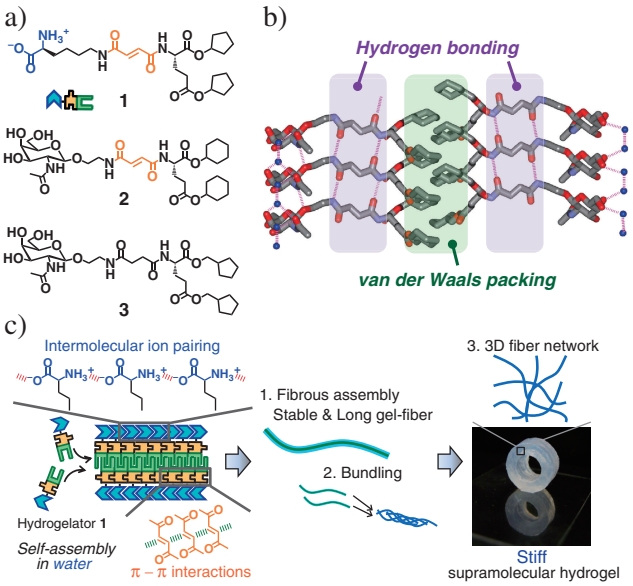
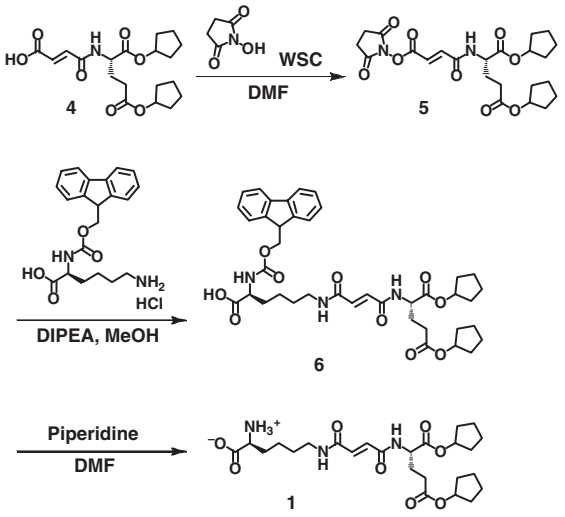


Figure 1. Design of zwitterionic supramolecular hydrogel. (a) Chemical structures of amino acid hydrogelator **1**, glycolipid hydrogelators **2** and **3**. (b) X-ray crystal structure of a glycolipid hydrogelator similar to **3** reported previously by us.[7f] (c) Schematic representation of the hierarchical self-assembly of **1** to form stiff supramolecular hydrogel using multiple and orthogonal molecular interactions.

The synthetic scheme of **1** is illustrated in Scheme 1. The dialkyl L-glutamate was connected to fumaric acid through an amide bond in the presence of a coupling reagent (WSC: water-soluble carbodiimide), and the resultant monocarboxylic acid **4** was converted to the activated *N*-hydroxysuccinimide ester **5**. The activated ester was condensed with a Fmoc–(αN)-L-lysine derivative, followed by deprotection of the Fmoc group by treatment with piperidine to afford **1**. As shown in the photograph in Figure 1c, we found **1** gave transparent hydrogels (critical gel concentration is 0.15 wt %).

To examine the mechanical strength of hydrogels **1**, **2**, and **3**, we conducted a rheological measurement. As shown in Figure 2a, the storage modulus (G') of hydrogel **1** was ca. six times higher than the loss modulus (G'') and both were almost independent of frequency, which clearly verifies the viscoelas-



Scheme 1. Synthesis of zwitterionic hydrogelator **1**.

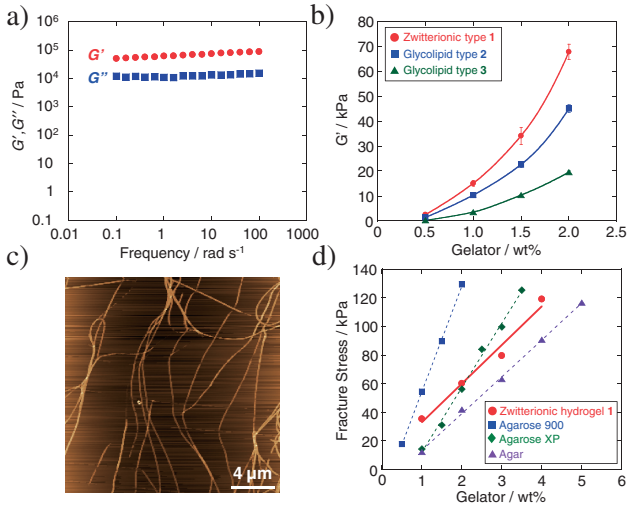


Figure 2. Mechanical strength of supramolecular hydrogels. (a) Storage (G') and loss (G'') moduli of zwitterionic hydrogel ([**1**] = 2.0 wt %/ion-exchanged water). (b) Gelator concentration dependence of storage modulus (G') of zwitterionic **1**, glycolipid **2**, and glycolipid **3** at room temperature. (c) Atomic force microscopy image of hydrogel **1** ([**1**] = 1.0 wt %/ion-exchanged water). (d) Gelator concentration dependence of fracture stress values of zwitterionic supramolecular hydrogel **1**, Agarose 900 (standard type, for electrophoresis), Agarose XP (low melting type, for electrophoresis), and agar (for bacterial cell culture) (ion-exchanged water, cylindrical hydrogels 9.8 mm in diameter and 6.0 mm thick) at room temperature.

ticity of hydrogel **1**. In addition, the G' value of hydrogel **1** showed larger values than the glycolipid hydrogels **2** and **3** as shown in Figure 2b. Also, the G' value of **2** was twice larger than that of **3** which lacked the fumaric double bond. These results suggest that both ion-pairing and π–π stacking interactions are important for the mechanical reinforcement of supramolecular hydrogels by enhancement stabilization of the self-assembled gel fibers. Indeed, well-developed fibers of **1** longer than 20 μm were observed by atomic force microscopy

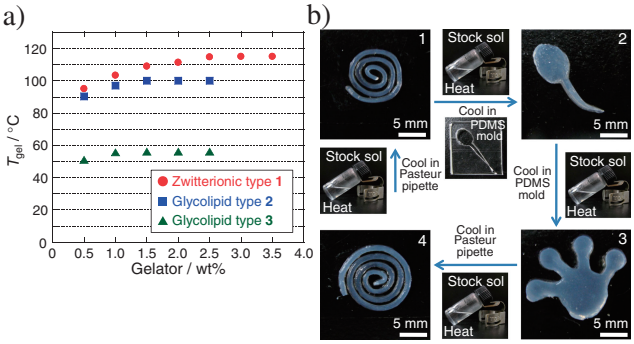


Figure 3. Thermal-responsive gel–sol transition of stiff supramolecular hydrogel **1**. (a) Gel-to-sol transition temperature (T_{gel}) depending on the gelator concentration for zwitterionic amino acid hydrogel **1**, and glycolipid gels **2** and **3**; at heating rate 1 °C min^{-1} in a sealed tube. (b) Photographs showing repeated fabrication of hydrogel **1** in various shapes using Pasteur pipettes or PDMS molds based on its reversible thermal-responsive gel–sol transition (1.0 wt %, ion-exchanged water).

(Figure 2c). The presence of π–π stacking interaction was supported by absorption and circular dichroism (CD) spectroscopies of **1** (Figure S1 in Supporting Information[12]). The absorption band due to the double bond of hydrogelator **1** revealed a hypochromic blue shift (2 nm in wavelength and 75% in absorbance) relative to that of monomeric **1** in MeOH. In addition, hydrogel **1** exhibited strong positive CD signals at 221 nm, whereas monomeric **1** showed negligible CD signals, similar to that of **1** in the sol state. This indicates that the fumaric amide moieties of **1** are stacked in a chiral fashion within the self-assembled nanofibers.

We also compared the fracture stress of the stiffest supramolecular hydrogel **1**, with conventional polymer gels that are widely used as matrices for DNA gel electrophoresis or bacterial cell culture. Figure 2d shows that the fracture stress of **1** increased linearly with increase of gelator content, similar to three polymer gels (agar for bacterial culture medium, agarose gel with low melting point (Agarose XP for electrophoresis of low MW nucleic acid), and the standard agarose gel (Agarose 900)). It is clear that the mechanical strength of hydrogel **1** was greater than that of agar, comparable with Agarose XP, and slightly less than Agarose 900 in the range from 1 to 4 wt %.

One of the advantages of supramolecular hydrogels is that they exhibit a macroscopic gel–sol phase transition in response to various stimuli. This phase transition is generally attributed to a stimuli-induced morphology change in the self-assembled fibers via modulation of noncovalent interactions. Indeed, by heating hydrogel **1** (0.5 wt %), we observed the gel-to-sol transition (T_{gel}) at 95 °C. Surprisingly, T_{gel} was greater than 100 °C (the boiling temperature of water) for a 1 wt % gel and reached 115 °C for more than 2.5 wt % gel, which are higher than for **2** (90–100 °C) or **3** (56 °C) (Figure 3a). This trend is consistent with the order of mechanical strength, again confirming the effect of the ion-pairing and π–π stacking interactions in enhancing the stability of the supramolecular hydrogel. Thanks to the sufficient stiffness and the reversible thermal gel–sol transition, a variety of shapes can be prepared from a single batch of hydrogel **1** using various molds, by repeating the gel-to-

sol transition as shown in Figure 3b. It is also noteworthy that the flexibility of the gel allows it to be shaped by rolling, and unique shapes with clear-cut edges are made possible by its mechanical toughness.

In conclusion, we demonstrated that rational accumulation of orthogonal noncovalent interactions such as ion pairing, π–π stacking, van der Waals, and hydrogen bonding, in supramolecular fibers can produce macroscopically stiff hydrogels comparable with conventional polymer-based hydrogels. We believe that this kind of stiff supramolecular hydrogel should be potentially useful for developing novel biomaterials. Further research on this topic is currently in progress.

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

