Synthesis of a Bis-Urea Dimer and Its Effects on the Physical Properties of an Amphiphilic Tris-Urea Supramolecular Hydrogel

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Abstract: The successful development of stiff supramolecular gels is an important goal toward their practical application. One approach to stiffen supramolecular gels is to introduce covalent cross-links. The bis-urea dimer 2, having a structure similar to that of the low-molecular-weight gelator 1, was synthesized. Supramolecular hydrogels were formed from mixtures of 1 and 2 in appropriate ratios, with 2 acting as a covalent cross-linker to connect the fibrous aggregates formed by the self-assembly of 1. The introduction of these covalent cross-links greatly influenced the dynamic viscoelasticity of the supramolecular hydrogels. In the supramolecular hydrogel of 1 mixed with 5% 2, the storage modulus was 1.35 times higher than that of the supramolecular hydrogel of 1 alone, and the crossover strain was extended from 5% to over 20%. The supramolecular hydrogel of 1 and 2 was free-standing and supported 13 times its own weight.

Hydrogels have been attractive research subjects as soft materials because of their wide applicability in tissue engineering and drug delivery systems, among other uses. Fragility is a typical characteristic of hydrogels, which are mainly composed of water, and a common issue to be overcome before their application as practical materials. Several innovative strategies to improve the strength of polymer gels have been developed, including slide-ring gels with freely moveable cross-links, nanocomposite gels with organic/inorganic networks, double-network gels that combine rigid strong networks with flexible networks, and tetra gels with homogeneous tetrahedral structures.

Supramolecular gels, constructed by the self-assembly of small molecules known as low-molecular-weight gelators (LMWGs), have been proposed as smart materials. Their designability and stimuli responsiveness have found fascinating application in various fields, including the life sciences. The strengths of supramolecular gels are generally lower than those of polymer gels because their formation is based on weak noncovalent interactions. The fragility of supramolecular gels often prevents their practical application. Various attempts have been made to increase the strength of supramolecular gels. Post-polymerization techniques have produced stiffer supramolecular gels but impaired their original flexibility. Stiff supramolecular gels have been sometimes obtained from the structural modification of LMWGs or supplemental addition of appropriate chemicals. A general strategy to increase the strength of supramolecular gels would be quite useful. As a pioneering strategy, the viscoelastic properties of a supramolecular gel were controlled over the gelation kinetics.

In polymer gels, a common tactic used to increase their strength involves increasing the number of covalent cross-linking points. Although the introduction of covalent cross-links in a supramolecular gel should enhance its strength, no covalent cross-links have been reported in self-assembled supramolecular gels. Here, we describe a strategy for introducing covalent cross-links into a supramolecular gel based on a key material: a dimer in which two molecules of a LMWG are covalently connected through an appropriate linker. When the dimer and LMWGs co-assemble, the dimer dispersed throughout the fibers would serve as a covalent cross-link for the supramolecular gel (Figure 1). We recently developed C₃-symmetric tris-urea 1 as a LMWG, and investigated the viscoelastic properties of its supramolecular hydrogel. In this paper, we report synthesis of a bis-urea dimer 2, which structurally resembles a covalently linked dimer of 1, and examine the properties of the supramolecular hydrogels resulting from mixtures of 1 and 2.

We previously reported C₃-symmetric tris-urea compounds as suitable LMWGs that, upon structural modification, afforded various functional supramolecular gels. In these studies, it was found that co-assembly occurred for mixtures of structurally similar tris-urea compounds without self-sorting. Encouraged by these results, we envisioned the use of a dimer having a covalently linked LMWG structure as a covalent cross-linker for a supramolecular gel. Accordingly, bis-urea dimer 2 was designed based on amphiphilic tris-urea LMWG 1.

The bis-urea motifs of 1 are linked via a hydrophilic octaethylene glycol unit. In a preliminary experiment, we investigated the effect of adding a dimer connected through a tetraethylene glycol linker, but the resulting supramolecular hydrogel did not show desirable improvements in its properties over those of 1. We concluded that the length of the linker was insuffi-
cient to connect fibers through covalent cross-linking points. Therefore, the longer-chain-containing octaethylene glycol was selected as the linker. The synthesis of bis-urea dimer 2 was performed by the synthetic strategy depicted in Scheme 1. The core benzene units were covalently linked first, and subsequently, four ureide moieties were introduced. Reaction of octaethylene glycol[16] and 1,3,5-tris(bromomethyl)benzene[17] in the presence of NaH gave dimer framework 3 in 34% yield. Reaction of 3 and separately prepared urea derivative 4 afforded tetrakis-urea 5 in 24% yield. The desired bis-urea dimer 2 was

**Scheme 1.** Synthesis of bis-urea dimer 2.
obtained in 75% yield by ester hydrolysis under alkaline conditions, followed by acidification. The structure of 2 was confirmed by 1H and 13C NMR spectroscopy and electrospray ionization mass spectrometry (ESI-MS).

Amphiphilic C$_3$-symmetric tris-urea 1 acts as a low-molecular-weight hydrogelator, giving a viscous solution after dissolution in NaOH solution (>10 mM) while heating.\cite{13} The concentration of the NaOH solution affected the viscosity, which decreased with increasing NaOH concentration. (Figure S1)\cite{14} Addition of hydrochloric acid to this solution instantly afforded a supramolecular hydrogel, which could be quantitatively analyzed in terms of its viscoelastic properties using a rheometer, and was found to be sufficiently stiff.\cite{19} For example, the supramolecular hydrogel prepared from 1 (10 mM) and NaOH solution (10 mM) exhibits a high storage modulus (G') of 32.4 kPa at 1.0 Hz rad$^{-1}$. The concentration of 1 influences the stiffness of the resulting supramolecular hydrogel. The G' of the supramolecular hydrogel prepared from 1 (5 mM) and NaOH solution (10 mM) was 19.3 kPa at 1.0 Hz rad$^{-1}$ (Figure S2). Surprisingly, the concentration of the NaOH solution also strongly influences the stiffness of the supramolecular hydrogel, even if the final pH of each sample is equalized. The G' value of the supramolecular hydrogel prepared from 1 (5 mM) and NaOH solution (30 mM) is reduced to 4.5 kPa at 1.0 Hz rad$^{-1}$ (Figure S3).

In the gelation experiments of bis-urea dimer 2, mixtures of 2 and neutral or acidic water give insoluble suspensions, similarly to compound 1, as does a mixture of 2 (5 mM) and NaOH solution (10 mM) (Figure S4). A viscous solution is obtained from a mixture of 2 (5 mM) and NaOH solution (30 mM), and a supramolecular hydrogel does not form even after adding hydrochloric acid to the solution. Fibrous aggregates are observed in the SEM image of this viscous solution (Figure S5), but their aspect ratio is low in comparison with the fibrous aggregates found in the mixtures of 1 and NaOH solution. To date, we have been unable to establish conditions to form a supramolecular hydrogel from 2 alone.

We then focused on the preparation of supramolecular hydrogels from mixtures of 1 and 2, which could be obtained at appropriate ratios. We investigated the changes in the physical properties of supramolecular hydrogels of 1 that accompanied the addition of 2. As mentioned above, the rheological properties of supramolecular hydrogels of 1 are influenced by the concentrations of both the gelator (1) and base (NaOH). Therefore, the following procedure was established for the formation of the comparable supramolecular hydrogels of 1 and 2: 1) A viscous solution was prepared by heating a mixture of 2 (5 mM) and NaOH solution (30 mM). 2) A second viscous solution was prepared by heating a mixture of 1 (5 mM) and NaOH solution (<10 mM). The concentration of NaOH solution was changed according to the mixing ratio of 1 and 2. 3) The two viscous solutions were mixed in an optional ratio to prepare a mixture of 1 + 2 (5 mM) and NaOH solution (10 mM) (pH 6.0). 4) Hydrochloric acid was added to the solution, and a supramolecular hydrogel was formed spontaneously (pH 2.0). These supramolecular hydrogels formed from mixtures of 1 and 2, are expressed as [1 + 2 gel (x%)] according to the ratio of 2.

For example, [1 + 2 gel (5%)] refers to a supramolecular hydrogel prepared from 1 and 2 in a 95:5 ratio (Figure 2). The SEM image of a xerogel prepared from [1 + 2 gel (5%)] reveals fibrous aggregates with a high aspect ratio (Figure 2). It is difficult to identify the difference between the fibrous aggregates of 1 and 2 and those of 1 itself (Figure S6). The gel-sol phase transition temperature ($T_{gel}$) was determined by the inverse flow method.\cite{18} Both [1 + 2 gel (5%)] and [1 + 2 gel (0%)] demonstrate the same high $T_{gel}$ of 146 °C, above the boiling point of the medium, water. Therefore, from this result, it is still difficult to identify the effect of the bis-urea dimer 2 on the physical properties of the supramolecular hydrogel.

Rheological studies helped to understand the mechanical and viscoelastic properties of these supramolecular hydrogels. We prepared supramolecular hydrogels of 1 containing five different ratios of 2, i.e., with 1:2 ratios of 100:0 [1 + 2 gel (0%)], 97:3 [1 + 2 gel (3%)], 95:5 [1 + 2 gel (5%)], 91:9 [1 + 2 gel (9%)], and 87:13 [1 + 2 gel (13%)], and compared their rheological properties. All supramolecular hydrogels were prepared from the same concentrations of gelator (1 + 2 = 5 mM), NaOH, and HCl. All rheological measurements were performed at least three times, and each sample showed sufficient reproducibility. Initially, we measured the dynamic frequency sweep of the supramolecular hydrogels at a strain of 0.1% (Figures 3a and S7). The G' values of these supramolecular hydrogels are more than ten times higher than the loss modulus (G''), and both types of moduli are nearly independent of the frequency from 0.01 to 15 Hz rad$^{-1}$. As expected, the addition of 2 improves the stiffness of the resulting supramolecular hydrogel. The original G' value of 19.3 kPa is slightly improved to 20.5 kPa with the addition of 3% 2 [1 + 2 gel (3%)], and increases to 26.1 kPa with 5% 2 [1 + 2 gel (5%)]. The G' value tends to decrease beyond a content of 5% 2, affording values of 22.5 kPa and 14.9 kPa with the addition of 9% 2 [1 + 2 gel (9%)] and 13% 2 [1 + 2 gel (13%)], respectively. The G' value of the [1 + 2 gel (13%)] is lower than that of the original supramolecular hydrogel [1 + 2 gel (0%)].

Dynamic strain sweeps of supramolecular hydrogels demonstrate their typical elastic response (Figures 3b and S8).
linear region can be prolonged in comparison to the supra-molecular hydrogel of \[ \text{1 [1+2 gel (0%)]} \] by adding bis-urea dimer 2. The linear region is formally a region in which the value of \( \tan \theta \) (\( G' / G'' \)) is constant; however, supramolecular hydrogels in this research show a weak change in the value of \( \tan \theta \), even in the region that seems linear. Therefore, in this study, we evaluated the linear region from the strain crossover of \( G' \) and \( G'' \) (\( G' = G'' \) crossover). The \( G' \) and \( G'' \) values of \[ \text{1+2 gel (0%)} \] cross at 5% strain, whereas the crossover of \[ \text{1+2 gel (3%)} \] is extended to 15% strain. The largest crossover strain is found in \[ \text{1+2 gel (5%)} \], which exceeds 20% strain. In comparison with \[ \text{1+2 gel (5%)} \], the crossovers of \[ \text{1+2 gel (9%)} \] and \[ \text{1+2 gel (13%)} \] decrease slightly to 15% and 12% strain, respectively. In all cases, the addition of 2 extends the strain values of the crossover beyond that of the supramolecular hydrogel of 1 alone \[ \text{1+2 gel (0%)} \], which suggests that 2 does provide covalent cross-links for the supramolecular hydrogel of 1. The results from both the dynamic frequency sweep and dynamic strain sweep studies indicate that a 95:5 ratio of 1 and 2 \[ \text{1+2 gel (5%)} \] is adequate to improve the rheological properties. A similar tendency was observed for the supramolecular hydrogel prepared from 30 mm aqueous NaOH (Figures S9 and S10); however, the physical properties of the supramolecular hydrogel were more pronounced when prepared from 10 mm aqueous NaOH.

To confirm the actual strength of the supramolecular hydrogels of 1 and 2, we tested their ability to support added weight (Figure 4). A cylindrical \[ \text{1+2 gel (5%)} \] with a weight of 2.05 g and a height of 9.0 mm was prepared, and coins (4.5 g) successively stacked on the free-standing, stable hydrogel. The \[ \text{1+2 gel (5%)} \] was able to withstand the weight of six coins, which is 13 times its own weight. When supporting six coins, the height of the \[ \text{1+2 gel (5%)} \] sample was compressed to 6.3 mm, and when a seventh coin was placed on the stack, balance was lost and the stacked coins collapsed. On the other hand, an analogous cylindrical \[ \text{1+2 gel (0%)} \] sample was only able to withstand the weight of five coins (Figure S11).

In conclusion, we designed and synthesized the bis-urea dimer 2, which structurally resembles LMWG 1. Supramolecular hydrogels were obtained from mixtures of 1 and 2 in appropriate proportions. Rheology measurements revealed an improvement in the physical strength based on the addition of 2. Measurement of the dynamic frequency sweep showed that addition of 5% of 2 to 1 improved the value of \( G' \) by 1.35 times. Measurement of the dynamic strain sweep indicated that addition of 5% of 2 to 1 extended the crossover strain from 5% to over 20%. The supramolecular hydrogel of 1 and 2 was free-standing and could support 13 times its own weight. Optimization of the linker structure of the dimer to improve the hydrogels’ physical strength and application of this concept to other gelators are currently underway in our laboratory.

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**Conflict of interest**

The authors declare no conflict of interest.
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*The supramolecular hydrogel was prepared in aluminum cup (14 mm diameter) and used for measurement with a flat-plate geometry (8 mm as it was.*


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