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Chemical Stimuli-Responsive Supramolecular Hydrogel from Amphiphilic Tris-Urea

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Hydrogels constructed from self-assemblies of low-molecular-weight hydrogelators (LMWHGs) are an attractive architecture in the field of supramolecular chemistry.^[1] Supramolecular hydrogels have great potential as smart materials.^[2] Stimuli-responsive gelation can be achieved as an advantageous function of non-covalent assemblies. A variety of supramolecular hydrogels that respond to stimuli, such as light,^[3] pH,^[4] chemical,^[5] ion,^[6] enzyme,^[7] and combinations of these^[8] have been reported. Despite these outstanding results, the rational design of LMWHGs is still challenging, and many of them are found by serendipity or from wide surveys. One practical strategy to make a LMWHG is the hydrophilic modification of a low-molecular-weight organogelator (LMWOG).^[9] Supramolecular hydrogels that respond to two different stimuli would be created by the introduction of a stimulus-responsive hydrophilic functional group onto another stimulus-responsive LMWOG. Herein, we report the synthesis of a LMWHG that is responsive to two chemical stimuli.

Previously, we have reported that a C_3 -symmetric trisurea LMWOG showed an anion-responsive gel–sol phase transition.^[10] Amphiphilic LMWHG **1** was designed by the introduction of hydrophilic glucoside groups onto the periphery of the hydrophobic LMWOG. The regulated aggregation of **1** in one dimension would be caused by the hydrophobic interactions of the central part of **1** and the intermolecular hydrogen bonding of the ureide groups. Saccharide is an appropriate functional group, because it can act not only as a hydrophilic group, but also as a probe for sugar-binding proteins (lectin). The ureide groups of **1** would function as anion-recognition sites. As a result, a self-assembled supra-

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molecular hydrogel of 1 would respond to both lectin and anions.

The LMWHG **1** was synthesized by condensation of two amines (**2** and **3**) and deprotection (Scheme 1). Penta-*O*-acetyl- β -D-glucopyranose was converted into amine **2** by a five-step synthesis. Amine **3** was prepared in two steps from 1,3,5-tris(bromomethyl)benzene.^[10b] The structure of **1** was



Scheme 1. Synthesis of LMWHG **1**. a) triphosgene (1.0 equiv), Et₃N (2.5 equiv), RT, 1 h, then **3** (0.33 equiv), Δ , 23 h, 55% yield; b) NaOEt (12 equiv), RT, 24 h, (repeat twice), 88% yield.

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determined using ¹H and ¹³C NMR spectroscopy and electrospray ionization (ESI) mass spectrometry (see the Supporting Information).

A pale-yellow transparent hydrogel was formed by mixing **1** with water, and leaving the mixture at ambient temperature for a few hours (gelation procedure I; Figure 1a). The



Figure 1. a) Photo of a hydrogel of 1 (2.0 wt %) prepared at ambient temperature over 3 h (inset) and a SEM image of the xerogel. b) Photo of a hydrogel of 1 (1.5 wt %), prepared by thermal treatment (inset), and a SEM image of the xerogel.

critical gelation concentration (CGC) of **1** was estimated to be 2.0 wt%. The thermal stability $(T_{gel})^{[11]}$ of the hydrogel was increased by increasing the concentration of **1** as a typical characteristic of the supramolecular gel $(T_{gel}; 38 \,^{\circ}\text{C}$ for 2.0 wt%, 64 $^{\circ}\text{C}$ for 4.0 wt%) (for details, see the Supporting Information). Narrow fibers (average diameter = 80 nm) were observed in the scanning electron microscope (SEM) images of the xerogel (Figure 1 a). The transparent hydrogel of **1** gradually lost its transparency under ambient conditions, and changed into an opaque gel over a period of several months. Xerogels prepared from the opaque gels showed thicker fibers (up to 800 nm) than those from early transparent gels (see the Supporting Information, Figure S1).

Thermal dissolution and cooling of an aqueous mixture of **1** also afforded a pale-yellow transparent hydrogel, and the CGC of **1** was improved to 1.5 wt% (gelation procedure II; Figure 1b). Slightly higher T_{gel} values were observed than those for gelation procedure I (T_{gel} ; 40 °C for 2.0 wt%, 66 °C for 4.0 wt%; for details, see the Supporting information). The gel obtained using this thermal procedure was stable, and kept its form and transparency for more than a year under ambient temperatures. SEM images of the xerogel showed intertwining nanofibers with diameters between 100 and 250 nm (Figure 1b).

The dense glucosides on the surface of the fibrous aggregates of **1** should work as recognition sites for lectin, with an interaction appearing as a macroscopic alteration of the hydrogel. Well-established concanavalin A (ConA) was selected as a lectin for the mixed gelation experiments with **1**.^[12] Opaque gels were formed by mixing **1** (2.0 mg, 0.85 µmol) and ConA (<0.35 mg, <13.6 nmol as a monomer) in water (100 µL), and leaving the mixture at ambient temperature for a few hours. However, mixtures of **1** (2.0 mg, 0.85 µmol) and more than 0.35 mg of ConA (>13.6 nmol) in water

(100 µL) afforded cloudy sols. The addition of appropriate amounts of ConA enabled a reduction in the CGC of 1. A mixture of 1 (1.0 mg, 0.43 µmol) and ConA (0.10~0.20 mg, $3.9 \sim 7.8$ nmol) in H₂O (100 µL) afforded a gel. The amounts of ConA also influenced the $T_{\rm gel}$ values of the hydrogel (for details, see the Supporting Information). The value of $T_{\rm gel}$ was increased by adding an appropriate amount of ConA. The value of T_{gel} of an opaque gel, prepared by mixing 1 (2.0 mg, 0.85 µmol) and ConA (0.20 mg, 7.8 nmol) in water (100 µL), was estimated at 85 °C, which is about 50 °C higher than the value of T_{gel} of 1 alone. Further addition of ConA led to a decrease in the value of $T_{\rm gel}$, and finally afforded the sol discussed above. A suitable amount of ConA would moderately cross-link the fibrous aggregates of 1 to reinforce the thermal stability of the gel, whereas an excess of ConA could lead to an excessive cohesion of the fibers to obstruct any gelation (Figure 2). SEM images of the sol containing 1 and ConA showed only unidentified larger objects, and little production of nanofibers was observed (see the Supporting information, Figure S2).



Figure 2. Schematic representation of the gel-sol phase transition triggered by ConA and saccharide.

The addition of a saccharide to an aqueous mixture of 1 and ConA, which gave a cloudy sol in the absence of saccharide, would afford a gel, depending on the association ratio of the saccharide with ConA (Figure 2). The results of gelation experiments of 1 (2.0 mg, 0.85 µmol), ConA (0.5 mg, 19.6 nmol), and various types and amounts of saccharides in water (100 µL) are summarized in Table 1. An opaque gel was obtained in the presence of 1 equivalent of α-methyl-D-mannoside (Me-α-Man) for ConA. The strong binding of Me- α -Man to ConA $(K_a = 1.1 \times 10^4 \text{ m}^{-1})^{[12]}$ would partially prevent the interaction between 1 and ConA, and thus enable the formation of a gel. The T_{gel} value of the opaque gel was estimated to be 80°C. The addition of an excess of Me- α -Man (10 equivalents or more for ConA) to an aqueous mixture of 1 and ConA afforded a semitransparent gel. The transparency of the gel would be restored from the negation of the interaction between 1 and ConA. This negation was reflected in the T_{gel} value of the semitransparent gel (T_{gel}=55°C). Furthermore, reconstructed intertwining nanofibers were observed in the SEM images of the xerogel (see the Supporting information, Figure S2). Addition

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Table 1. Gelation of 1 in the presence of ConA and saccharide.^[a]

Saccharide	ConA (equiv)					
	1.0	3.0	4.0	10	260	
Me-a-Man	OG	OG	OG	G	G	
Me-a-Glc	S	OG	OG	OG	G	
Man	S	S	OG	OG	G	
Isomal	S	S	OG	OG	G	
Glc	S	S	S	OG	G	
Gal	S	S	S	S	S	
L-Glc	S	S	S	S	S	
Mal	S	S	OG	OG	G	
Me-β-Glc	S	S	S	S	OG	

[a] A mixture of 1 (2.0 mg), ConA (0.5 mg), and saccharide in H₂O (100 μ L) was kept at ambient temperature for 3 hours. G=semitransparent gel, OG=opaque gel, S=cloudy sol, Me- α -Man= α -methyl-D-mannoside, Me- α -Glc= α -methyl-D-glucoside, Man=D-mannose, Isomal= isomaltose, Glc=D-glucose, Gal=D-galactose, L-Glc=L-glucose, Mal=D-maltose, Me- β -Glc= β -methyl-D-glucoside.

of a large excess of α -methyl-D-glucoside (Me- α -Glc, 3 equiv for ConA) was required to form a gel, since the binding constant of Me- α -Glc to ConA ($K_a = 3.0 \times 10^3 \text{ M}^{-1}$)^[12] is smaller than that of Me- α -Man to ConA. The formation of a gel was achieved by adding 4 equivalents of D-mannose (Man) or isomaltose (Isomal) to ConA. The addition of 10 equivalents of D-glucose (Glc) was required to form an opaque gel. These amounts were adequate, compared with their binding constants to ConA (Man: $K_a = 2.2 \times 10^3 \,\mathrm{M}^{-1}$, Isomal: $K_a = 1.7 \times 10^3 \,\mathrm{m}^{-1}$, Glc: $K_a = 8.0 \times 10^2 \,\mathrm{m}^{-1}$).^[12] The transparency of these gels containing Me-a-Glc, Man, Isomal, or Glc improved by increasing the number of equivalents, with semitransparent gels eventually being obtained. In contrast with the above results, the addition of saccharides with no- or lesser binding ability for ConA, such as Dgalactose (Gal) or L-glucose (L-Glc), did not form a gel, even when a large excess was added (up to 260 equivalents for ConA). The unknown binding constant of a saccharide with ConA could be approximated using this gelation procedure. Opaque gel formation was achieved by adding 4 equivalents of D-maltose (Mal). This result resembles those from gelation experiments in the presence of Man and Isomal. The binding constant of Mal with ConA was inferred to be around $1.7 \sim 2.2 \times 10^3 \,\text{M}^{-1}$. An opaque gel was formed in the presence of 260 equivalents of β-methyl-D-glucoside (Me-β-Glc). This result indicates that the binding constant of Me- β -Glc to ConA is much smaller than that of Glc to ConA. However, the value should be a finite number. The successive addition of saccharide to a solvated mixture of 1 and ConA in water gave the same results as shown in Table 1, although the gelation required a longer period of time (Figure 3).

The hydrogel of **1** exhibited a gel-to-sol phase transition that was responsive to anions. Subsequent addition of anions, such as chloride ions from NaCl, to a hydrogel of **1** gradually led to a phase transition at the point of contact between the gel and the salt. A variety of anions showed such a phase transition, and the amounts required were similar (Table 2). The hydration of anions may be the cause of the



Figure 3. Photographs of rheological alteration of a mixture of 1 and ConA by addition of saccharides (260 equiv for ConA).

Table 2. Anion responsive gel-to-sol phase transition.^[a]

Salt	1 (equiv)					
	0.25	0.50	1.0	2.0		
NaF	_	G	OG	V		
NaCl	-	G	OG	V		
NaBr	-	G	G	V		
NaI	-	G	G	G		
NaOAc	-	G	G	V		
Na_2SO_4	-	OG	V	V		
$NaClO_4$	-	G	G	G		
KCl	-	G	OG	V		
NH ₄ Cl	-	G	OG	V		
MgCl ₂	OG	V	V	V		
$MgSO_4$	SG	V	V	V		
$CaCl_2$	SG	V	V	V		

[a] Stock solution of salt was placed on the top of the hydrogel of 1 (2 wt%). G=gel, OG=opaque gel, SG=soft gel, V=viscous suspension.

low selectivity of the phase transition. The addition of two equivalents of monovalent anions was sufficient to attain a gel-to-sol phase transition. This is reasonable in that a smaller amount of bivalent anions than monovalent anions led to this phase transition. In exception, iodide and perchlorate ions had a low capability for the phase transition because of their low affinity for the ureide moiety of **1**. These phase transitions took place by adding smaller amounts of alka-line-earth metal salts than those of alkali metal salts. For instance, addition of 1.0 equivalent of NaCl maintained the gel, whereas addition of 0.5 equivalents of MgCl₂ caused a viscous suspension. The interaction between the alkaline-earth metal ions and the glucoside moiety of **1** may also affect the phase transition.

An assessment of different types of mineral water, according to their hardness, was realized by mixing samples with $\mathbf{1}$ (Figure 4). One particularly prevalent anion in mineral water is sulfate ions, along with minor components such as chloride, fluoride, and nitrate ions. The principal cationic elements in mineral water are sodium, potassium, magnesium, and calcium. The amounts of magnesium and calcium ions were found to increase with increasing hardness, whereas the quantities of sodium and potassium ions in each mineral water remained approximately the same. Therefore, the alkaline-earth metal salt-sensitive and anion-nonspecific-responsive character of $\mathbf{1}$ is suited for the detection of the





Figure 4. Photographs of mixtures of **1** and mineral waters. a) Crystal Geyser (soft water), b) Paradiso (moderately hard water), c) Wattwiller (hard water), and d) Contrex (hard water).

hardness of mineral waters. Crystal Geyser, with a total hardness of 38 mg L^{-1} (soft water), gave a transparent gel on mixing with 2.0 wt% of **1**. A mixture of **1** and Paradiso, which is classified as a moderately hard water (total hardness=290 mg L⁻¹), formed an opaque gel. Cloudy sols were obtained from mixtures of **1** with hard-water samples, such as Wattwiller (total hardness=627 mg L⁻¹) or Contrex (total hardness=1551 mg L⁻¹). The hardness of water was distinguished from the three different states observed, i.e., a transparent gel, an opaque gel, and a cloudy sol.

In conclusion, we have synthesized a LMWHG **1** based on the design of the amphiphile. The hydrogel showed that the gel-to-sol phase transition responded to two different types of stimuli: lectin and anion. These results demonstrate the potential of supramolecular gels as innovative chemical sensors.

Experimental Section

Gelation Procedure I: Mixing at Ambient Temperature

A mixture of **1** and H₂O (200 μ L) (ϕ =6.2 mm) was left at ambient temperature in a test tube. The viscosity of the mixture increased over time, and gel formation was complete after 2~3 hours. Gel formation was evaluated by the "inverted tube" test. A mixture remaining at the top of an inverted test tube was defined as a gel.

Gelation Procedure II: Thermal Treatment

A mixture of **1** and H₂O (200 μ L) (ϕ =6.2 mm) was heated in a test tube at 90 °C. The obtained solution was gradually cooled to ambient temperature. Gel formation was evaluated by the inverted-tube test. A mixture remaining at the top of an inverted test tube was defined as a gel.

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