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Using β-Lactamase to Trigger Supramolecular Hydrogelation

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This communication reports the rational design and synthesis of a β-lactam conjugate, which transforms into a hydrogelator by catalysis of a β-lactamase and yields a supramolecular hydrogel. β-Lactam antibiotics (e.g., penicillins and cephalosporins), a major class of antimicrobial agents in clinical use for treating bacterial infections, rely on the strained β-lactam ring to react with penicillin-binding proteins (PBPs) to inhibit cell wall synthesis and growth of bacteria.[1] β-Lactamases hydrolyze the four-membered β-lactam ring and cause the most widespread antimicrobial drug resistance.[2] Thus, it is essential to detect the presence of β-lactamases and screen their inhibitors.[3] Although fluorescent (e.g., genotyping based on polymerase chain reaction (PCR)) or colorimetric assays (e.g., using nitrocefin as indicator) are able to perform such tasks,[4] a simple, rapid, and accurate assay is desirable because colorimetric assay fails in a colored medium and PCR remains costly and time-consuming. Therefore, we chose to use the event of hydrogelation to report the presence of β-lactamases because the formation of supramolecular hydrogels[5,6] offers several advantages as an assay for an enzyme: (i) It is easy to determine a macroscopic change such as hydrogelation (even in a colored medium) by the naked eye, thus eliminating the need of any instrument; (ii) an enzyme can catalyze either bond formation or bond cleavage to trigger hydrogelation,[7,8] which makes this strategy suitable for a wide range of enzymes; and (iii) the hydrogel enlists water as part of the reporting system so that it can serve as a low-cost assay to be used in developing economies.[9]

The above merits motivate us to design a precursor to examine whether a β-lactamase triggers supramolecular hydrogelation. Our results show that the addition of a β-lactamase to the solution containing the precursor (**3**) results in formation of a supramolecular hydrogel. Moreover, **3** shows selective response to the lysates of bacteria (e.g., *E. coli*) containing different types of β-lactamases. As the first β-lactamase-catalyzed hydrogelation, this result has several significant advances. First, it confirms the use of hydrogel to report β-lactamase, thus offering a general platform to design precursors to report a specific subclass of β-lactamases. Second, it provides a simple, low-cost strategy to identify β-lactam antibiotic-resistant pathogens and to screen the inhibitors of β-lactamases. Third, it may lead to the use of self-assembly and hydrogelation to study or modulate biological processes and interactions of interest (including intracellular gel−sol transition related cell adhesion and cell motility[10]) because of the well-established method of controlled expression of β-lactamases in cells.[11,12]

Figure 1 outlines the general principle and molecular design for a β-lactamase-catalyzed hydrogelation. Using the cephem nucleus as the linker, a hydrophilic group connects a hydrogelator to

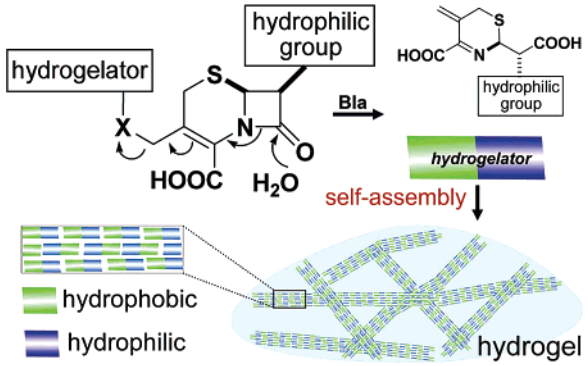
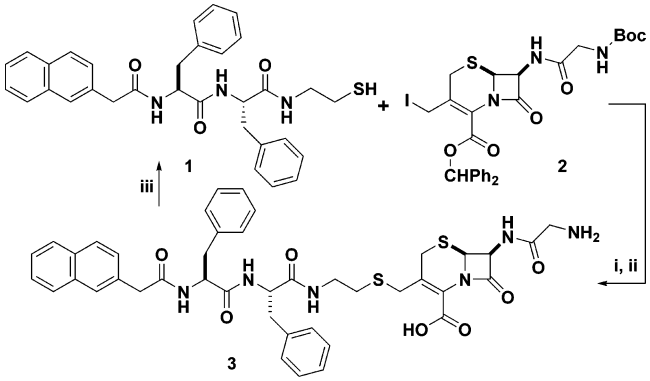


Figure 1. Illustration of the design of a substrate of β-lactamase (Bla) as the precursor of a hydrogelator (X = S or COO); the opening of β-lactam ring catalyzed by Bla; one possible mode of the self-assembly of the hydrogelator and the formation of the hydrogel.

Scheme 1[a]



[a] Conditions: (i) $NaHCO_3$, DMF; (ii) TFA, anisole, CH_2Cl_2; (iii) β-lactamase, pH = 8.0.

constitute the precursor, which is too soluble to form a hydrogel (i.e., the precursor supplies too little hydrophobic interaction to self-assemble into nanofibers that gel water[6]). Upon the action of a β-lactamase, the β-lactam ring opens to release the hydrogelator, which self-assembles in water into nanofibers to afford a hydrogel. The key feature of the design is to use a β-lactamase to generate a hydrogelator. Scheme 1 shows the actual structures and the synthesis of the molecules that employ the design in Figure 1. An *N*-hydroxysuccinimide (NHS)-activated napthalene Phe−Phe (Nap-FF) reacts with 2-aminoethanethiol to yield an effective hydrogelator, **1**, which forms hydrogels at the concentration of 0.3 wt %. Following literature procedure,[11] we converted 7-amino-3-chloromethyl 3-cephem-4-carboxylic acid diphenyl methyl ester hydrochloride (ACLH) into **2**. The nucleophilic substitution between **1** and **2** in a weak basic condition, followed by a simple deprotection (i.e., removal of Boc), creates the precursor (**3**) in a good yield (85.4%).[13]

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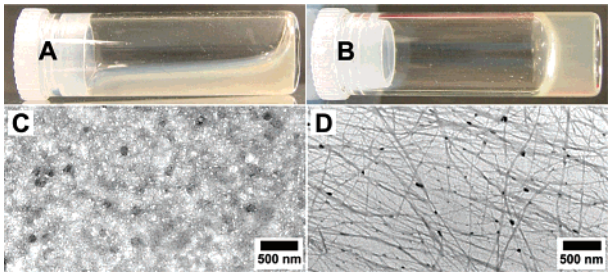

Figure 2. The optical images and the transmission electron microscopy (TEM) images of (A, C) viscous solution of **3** and (B, D) gel **I**.

Table 1. The Results of Adding Different Types of Cell Lysates to the Solutions of **3** (0.35 wt %)[a]

sample	enzyme[b]	gelation[g]	conversion (%)[h]	nitrocefin
A	C600[d]	−	<0.5	−
B	CTX-M13[c]	+	99.7	+
C	CTX-M14[c]	+	99.5	+
D	JP995[d]	−	5.8	+
E	SHV-1[e]	+	65.2	+
F	TEM-1[e]	+	84.3	+
G	none[f]	−	<0.5	−

[a] Conducted as a blind test. [b] Enzyme in 1.0 mL of the lysates of *E. coli* (10E10 cells), except G. [c] Extended-spectrum β-lactamase (ESBL). [d] A and D were β-lactamase negative *E. coli* controls. [e] Broad-spectrum β-lactamase. [f] G contains only water. [g] Gels form in less than 2 h. [h] Percentage of **3** to form **1** after 6 h.

After obtaining the precursor (**3**), we tested if a β-lactamase would trigger hydrogelation. **3** (1.75 mg) dissolves in water (0.50 mL, pH = 8.0) to result in a viscous solution (Figure 2A). A half of an hour after the addition of 0.55 mg of a β-lactamase (15−25 U/mg[13]) to the solution at room temperature the liquid turns into an slightly opaque hydrogel (gel **I**, Figure 2B). HPLC test reveals that 49.0% of **3** transforms to **1** 1 h after addition of the β-lactamase.[13] Rheological experiment (i.e., dynamic time sweep) confirms that the solution of **3** is a Newtonian liquid and indicates that the hydrogelation starts at about 22 min after addition of the β-lactamases.[13] According to the TEM images shown in Figure 2, the cryo-dried solution of **3** is unable to exhibit a well-defined nanostructure (Figure 2C), and the cryo-dried gel **I** shows nanofibrils with the diameters from 30 to 70 nm (Figure 2D). We also found that the addition of **3** into a solution of β-lactamase and its inhibitor (i.e., clavulanic acid) results in only 3.4% conversion of **3** to **1** after 12 h (based on the HPLC test) and fails to yield a hydrogel.[13] This result may lead to a convenient method to screen the inhibitor of β-lactamase by using enzymatic hydrogelation.[8]

To evaluate whether **3** would respond to β-lactamases in bacteria, we used the sonicated lysates of *E. coli* to treat the solution of **3**. As shown in Table 1, samples B, C, E, and F are the lysates containing different kinds of β-lactamases (CTX-M13, CTX-M14, SHV-1, and TEM-1, respectively);[14] others are controls. We observed hydrogelation triggered by the four kinds of β-lactamase.[13] The HPLC traces clearly indicate the effective conversion of **3** to **1** (99.7, 99.5, 65.2, and 84.3% in samples B, C, E, and F, respectively, but 5.8% in sample D and <0.5% on samples A and G) by adding different cell lysates. TEM images[13] also show that the self-assembled nanofibers in those four hydrogels resulted from the hydrolysis of **3** catalyzed by the β-lactamases. No hydrogelation observed for sample D indicates that this gelation-based assay has a higher reporting threshold than the nitrocefin assay does, suggesting that the assay based on enzymatic hydrogelation provides a particularly useful reporting method for systems that have significant background activity, which cause a false positive on nitrocefin assay. More completed conversion in samples B and C than in samples E and F also indicates that CTX-M13 and CTX-M14 are ESBLs.[14] This observation may lead to an alternative approach to assay the β-lactamase (e.g., ESBL) in a more specific way via tailoring the structure of the precursors.

In summary, we demonstrate that a β-lactamase is able to catalyze the formation of a supramolecular hydrogel. This approach, which involves the use of a β-lactamase to control the self-assembly of small molecules, offers an alternative platform to study the inactivation of β-lactam antibiotics for the approaches that counteract antimicrobial drug resistance, provides a unique opportunity to generate nanostructures in regulated biological environment, and may lead to useful practical applications (e.g., selectively detecting ESBL bacteria in a clinical setting).

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Supporting Information Available: Synthesis of **1** and **3**, rheological experiments, HPLC traces, protocol for the preparation of cell lysates, and test on lysates of *E. coli*. This material is available free of charge via the Internet at http://pubs.acs.org.

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