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Molecular Nanofibers of Olsalazine Form Supramolecular Hydrogels for Reductive Release of an Anti-inflammatory Agent

Xinming Li, Jiayang Li, Yuan Gao, Yi Kuang, Junfeng Shi, and Bing Xu*

Department of Chemistry, Brandeis University, 415 South Street, Waltham, Massachusetts 02454, United States

Received October 14, 2010; E-mail: bxu@brandeis.edu

Abstract: Conjugation of tripeptide derivatives with olsalazine, a clinically used anti-inflammatory prodrug, yields small molecules that self-assemble in water to form supramolecular hydrogels that undergo a gel-to-sol phase transition upon reduction, resulting in the controlled release of 5-aminosalicylic acid as the anti-inflammatory agent. This methodology will ultimately lead to new biomaterials for site-specific drug delivery.

The paper describes a supramolecular hydrogel as a potential biomaterial for site-specific drug release. Biomaterials derived from synthetic or biological polymeric hydrogels have found widespread applications in biomedical engineering, ranging from tissue repair to regenerative medicine and drug delivery.[1] These polymer-based hydrogels, however, still have several inherent shortcomings, such as relatively slow degradation, unintended immune responses, and the generation of undesirable byproducts.[2] On the other hand, supramolecular hydrogels[3] formed by low-molecular-weight gelators[4] that self-assemble in water through noncovalent interactions have attracted considerable attention because they exhibit several unique merits, such as synthetic economy, biocompatibility, low toxicity, inherent biodegradability, and most importantly, fast thermally reversible formation−dissociation processes.[5] These advantages make supramolecular hydrogels a promising alternative for polymeric hydrogels. Among the molecules that can act as the building blocks for supramolecular hydrogels, peptide-based hydrogelators[6] are the usual candidates because of their biological relevance, the well-established synthetic chemistry (i.e., solid-phase synthesis),[7] and the capability to produce a large set of diverse functional molecules from a small array of residues. There are many examples of peptide-based functionalized building blocks for making nanofibers and generating hydrogels. The nanofibers of peptide amphiphile molecules can display a high density of epitopes for regulating the differentiation of neuron progenitor cells[8] or guiding cartilage regeneration.[9] A supramolecular hydrogel self-assembled from lysine-containing short peptides has been shown to exhibit inherent antibacterial activity.[10] Self-complementary oligopeptides form hydrogels for cell culture and cytokine release.[11] Amino acid-functionalized hydrogel particles release protein when triggered enzymatically.[12] A small peptide conjugated with β-lactam is transformed into a hydrogelator by the catalysis of a β-lactamase.[13] A low-molecular-weight gelator containing amino acid moieties confers liquid-crystalline (LC) gels.[14] Photosensitive spiropyran linking with dipeptide leads to a supramolecular hydrogel that responds to both light and ligand−receptor interaction.[15] Despite these advances, the application of supramolecular hydrogelators in controlled drug release has been less explored,[16] and there has been even less exploration of supramolecular hydrogels for site-specific drug release. It is necessary and important to explore new ways of creating supramolecular hydrogels as smart materials for controlled drug release at specific sites or organs in a biological system.

Because colonic microflora secrete azo reductase to reduce the azo group into the corresponding amine, use of olsalazine (a substrate of azo reductase) as a prodrug achieves colon-specific drug delivery[17] via catalytic generation of mesalazine [or 5-aminosalicylic acid (5-ASA)] inside the colon at the site of inflammation.[18] This opportunity for reductive degradation of azo compounds by colonic microflora has led to the development of a score of polymeric azo compounds that have found application for colon targeting, as the reduction and subsequent splitting of the azo bond occurs only in the large intestine.[19] Encouraged by these results, we developed an olsalazine-containing supramolecular hydrogel as a candidate for a smart biomaterial for controlled release. Specifically, we synthesized hydrogelator **1** by using a tripeptide derivative that consists of a naphthyl group, two phenylalanines, and one modified lysine residue carrying an olsalazine moiety in the side chain. **1** self-assembles to form a hydrogel under mildly acidic conditions. The reduction of olsalazine not only leads to a gel-to-sol phase transition but also releases 5-ASA. Through direct incorporation of the prodrug into the nanofibers, this supramolecular hydrogel demonstrated a new way to encapsulate the prodrug and release the active ingredients. Because a large pool of prodrugs exists, this work contributes to the future design of new smart biomaterials based on supramolecular chemistry[20] and prodrugs.

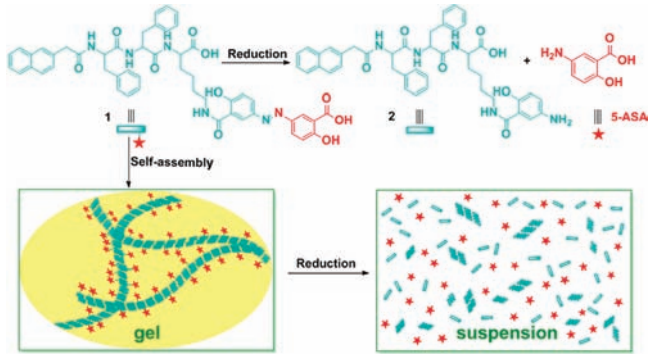
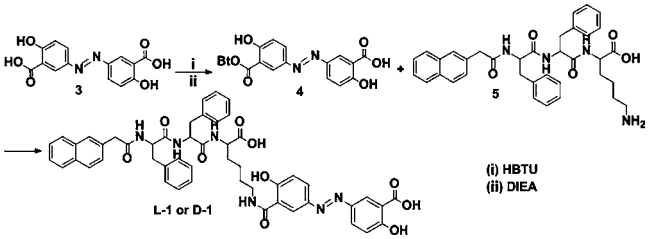


Figure 1. Illustration of drug release from the olsalazine-containing supramolecular hydrogel upon reduction.

Figure 1 illustrates the structure of hydrogelator **1**, which contains a short peptide motif and an olsalazine moiety. We synthesized small-molecule hydrogelator **5**, which is a tripeptide derivative made by conjugating 2-(naphthalen-2-yl)acetic acid with Phe-Phe-Lys. In our recent study,[21] we found that tripeptide derivative **5** forms a hydrogel at a very low critical gelation concentration (0.8 wt %). Conjugation of **5** to an olsalazine moiety through the ε-amino group of the lysine residue afforded **1**, which we expected to form a stable

supramolecular hydrogel as the prodrug reservoir that upon azo reduction would disassemble and release 5-ASA.

Scheme 1. Synthetic Scheme for Olsalazine-Containing Hydrogelator **1**



Scheme 1 shows the synthetic route to **1**. The HBTU-activated compound **3** reacts with **5** to afford hydrogelator **1** in 48% yield after purification by flash column chromatography. After obtaining **1**, we tested its ability to form a hydrogel in water by adjusting the pH. Typically, 6.0 mg of **1** dissolved in 0.50 mL of water to give a clear solution, after which a change in pH to 5.0 resulted in a viscous suspension. Ultrasound sonication of the suspension for 2 min or an increase of its temperature to ~60 °C followed by cooling to ambient temperature afforded a transparent, yellow gel (Figure 2). This experiment demonstrated that **1** is an effective hydrogelator that forms a stable gel in water at a concentration of 1.2 wt %. In order to further confirm that the naphthyl group is necessary for compound **1** to form the hydrogel, we replaced the naphthyl group with an acetyl group. We found that the molecule acetyl-FFK-olsalazine (**7**) failed to form a hydrogel (Scheme S1 and Figure S5 in the Supporting Information). While the hydrogelator L-**1** consists of L-phenylalanine and L-lysine, the hydrogelator D-**1** is made of D-phenylalanine and D-lysine.

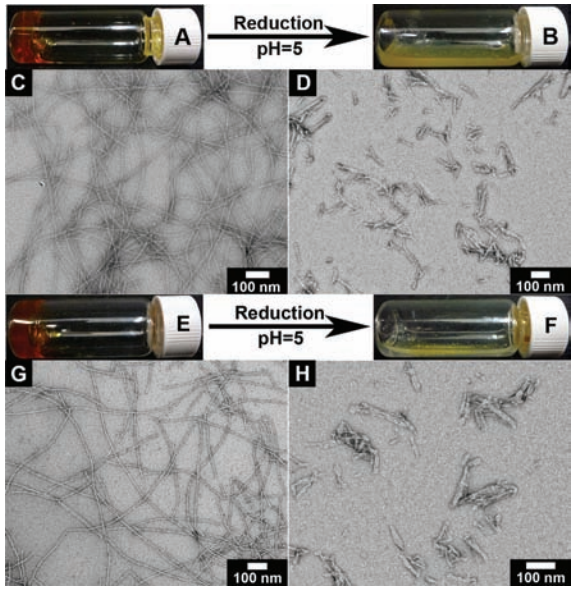


Figure 2. Optical images of (A, E) the hydrogels of (A) L-**1** and (E) D-**1** (1.2 wt %, pH 5.0) and (B, F) the suspensions of (B) L-**1** and (F) D-**1** after the reduction reaction of the hydrogels. TEM images of (C, G) the matrices of the hydrogels of (C) L-**1** and (G) D-**1** and (D, H) the broken fibers in the suspensions of (D) L-**2** and (H) D-**2**.

In order to study reductant-mediated drug release from the hydrogel, we dissolved 11 mg of sodium hydrosulfite in 0.2 mL of pH 5 buffer and injected the reductant over the hydrogel. The final concentration of hydrogelator **1** during the reduction reaction was 0.86 wt %. After incubation at 37 °C for 1 h, the hydrogel of L-**1** or D-**1** was transformed into a light-yellow suspension (Figure 2). HPLC and LC−MS analysis of the suspension (Figures S1 and S2) confirmed the conversion of **1** into the corresponding products **2** (t_R = 18.2 min) and 5-ASA (t_R = 4.1 min). The identification of 5-ASA validates that this supramolecular hydrogel can act as a reservoir of the prodrug and release the 5-ASA upon reduction of the azo bond.

Transmission electron microscopy (TEM) helped to evaluate the extent of the self-assembly of hydrogelator **1** during different stages of the gel-to-sol transition. As shown in Figure 2, the hydrogelators L-**1** and D-**1** self-assemble to afford nanofibers with widths of 11 and 13 nm, respectively, and lengths of more than several micrometers (Figure 2C,G). In addition, the hydrogelator D-**1** shows nanofibers with a right-handed helical structure (Figure S6). These nanofibers constitute the matrices (in the form of bundles or networks) of the hydrogels of **1**. The TEM images of the negative staining suspensions in Figure 2B,F indicate the loss of the long nanofibers after reductive cleavage of the azo bond, in agreement with the observation that **2** failed to act as a hydrogelator. The dissociation of the three-dimensional networks of the nanofibers upon reduction indicates that the hydrogels of **1** should be able to release 5-ASA upon the action of azo reducatase.[17]

Circular dichroism (CD) studies provided further molecular insight into the self-assembly of **1** and the gel-to-sol transition upon reduction. The hydrogelator L-**1** in the gel phase gives a CD spectrum with a β-sheet signature, as evident by negative bands at 205 nm and positive bands at 195 nm (Figure S3).[22] Upon reduction, the gel turns into the sol as a result of the conversion of hydrogelator L-**1** into compound L-**2** accompanied by release of 5-ASA. The CD signal of the β-sheet decreased significantly, indicating that L-**2** self-assembles less efficiently than hydrogelator L-**1** because of the loss of 5-ASA. The reduction of D-**1** generates D-**2** and also exhibits a decrease in the signal between 190 and 204 nm that is similar to the decrease of the β-sheet signal for L-**1** (Figure S3).[22] The hydrogel of D-**1** exhibits a strong CD band around 480 nm that is far from the chromophoric absorption region (~360 nm) of olsalazine. This peak likely originates from a mesophase of D-**1**,[23] which agrees with the birefringence of the hydrogel of D-**1** (Figure S6).

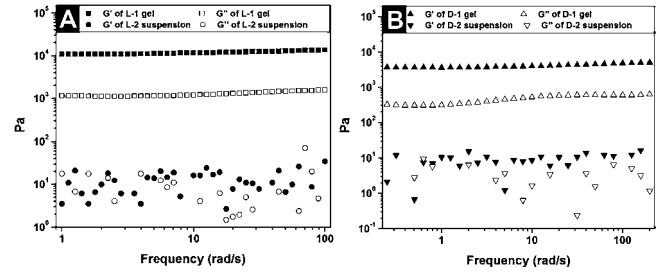


Figure 3. (A) Frequency dependence of the dynamic storage moduli (G') and loss moduli (G'') of the hydrogel of L-**1** and suspension of L-**2**. (B) Frequency dependence of the dynamic storage moduli (G') and loss moduli (G'') of the hydrogel of D-**1** and suspension of D-**2**.

We used oscillatory rheology to examine the viscoelastic properties of the hydrogels before and after reduction. Before the reductive cleavage of the azo bond, the hydrogels of L-**1** and D-**1** both exhibit elastic properties of a solidlike material, as demonstrated by the fact that the storage modulus (G') was almost an order of magnitude higher than the loss modulus (G'') together with the weak frequency dependence of the elasticity (Figure 3). After the addition of the reductant, the value of G' for the sample decreased nearly 3 orders of magnitude, indicating that the material

behaves more like a viscous solution than an elastic gel. The obvious decrease in G' agrees with the gel-to-sol transition upon reduction.

Because site-specific drug delivery also requires that the supramolecular hydrogel be able to resist the attack of proteases in vivo, we synthesized the hydrogelator D-**1** to improve the stability of supramolecular hydrogels in biological environments. In order to evaluate its biostability, we incubated the hydrogel of D-**1** with proteinase K, a powerful enzyme that hydrolyzes a broad spectrum of peptides. The hydrogel of D-**1** remained unchanged (Figure 4) after incubation with proteinase K for 48 h, indicating excellent biostability of D-**1** against proteinase K. The fact that the addition of proteinase K failed to cause the gel-to-sol transition of D-**1** also suggests that the hydrogel of **1** likely is insensitive to impurities.

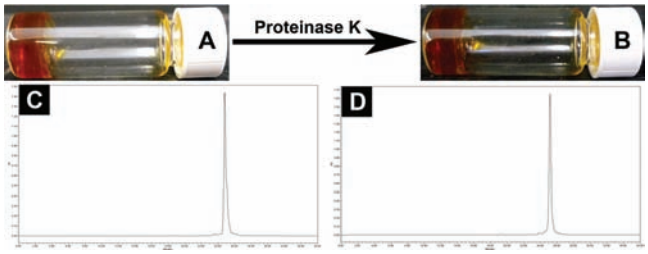


Figure 4. Optical images of the hydrogel D-1 (A) before and (B) after proteinase K treatment. HPLC traces of the hydrogel D-1 (C) before and (D) after proteinase K treatment.

In conclusion, we have demonstrated that tripeptide derivatives conjugated with olsalazine exhibit excellent self-assembly properties and generate prodrug-containing supramolecular hydrogels. We have also shown that reduction of the azo group can disrupt the supramolecular hydrogels and release the active ingredient. The use of D-peptides also should help preserve the stability of the hydrogels against proteases in the upper gastronomical tract. Since it is easy to incorporate therapeutics other than the prodrug in supramolecular hydrogels,[24] this work illustrates a new and facile way to use a prodrug with known metabolic pathways for generating supramolecular hydrogels as smart biomaterials for site-specific drug delivery.

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Supporting Information Available: Synthesis of hydrogelator **1**, HPLC traces and LC−MS data for the reduction of hydrogelator **1**, CD spectra, and rheological data. This material is available free of charge via the Internet at http://pubs.acs.org.

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