Chinese Chemical Letters 28 (2017) 2125–2128

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Communication

An amino acid-based gelator for injectable and multi-responsive hydrogel

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ARTICLE INFO

Article history:
Received 24 June 2017
Received in revised form 5 September 2017
Accepted 5 September 2017
Available online 6 September 2017

Keywords:
Multi-responsive
Injectable
Amino acid
Gelator
Hydrogel

ABSTRACT

Hydrogels formed by gelators have attracted growing attention for their promising application in biomaterials and biotechnology. We describe in this paper the generation and characterization of a novel photo-, thermal- and pH-responsive hydrogel based on an amino acid gelator AA-Azo-EG_6. Specifically, the gelator bears an amino acid head, an azobenzene (Azo) linker, and a short oligoethylene glycol tail (EG_6). The resulting AA-Azo-EG_6 hydrogel is injectable and exhibits interesting helical self-assembled structures. Meanwhile, the hydrogel is able to experience a gel-sol or gel-precipitate phase transition responding to external stimuli. Thus, this AA-Azo-EG_6 gelator is a promising building block for intelligent materials and drug delivery.

Hydrogels are a kind of soft materials containing large amount of water within small-molecular or polymeric networks that are crosslinked by either physical association or chemical bonding. Because of their unique viscoelastic and biomimetic properties, hydrogels are promising biomaterials [1] for applications such as tissue engineering, regenerative medicine [2], drug delivery [3–7], catalysis [8,9] and separation [10]. Gelators, on the other hand, are generally referred to those monomeric sub-units that self-assemble into cross-linked networks and immobilize solvents within the gels. Particularly, low-molecular weight gelators (LMWGs) are attracting growing attention for their accurate molecular weight, easy modification, abundance, and relative regular nanostructures [11,12]. Generally, LMWGs rely on various supramolecular force such as hydrogen bonding, electrostatic interaction, π–π stacking, and hydrophobic forces [13,14] to form hydrogels. Typical LMWGs include amino-acid derivatives [15], peptides [16,17], urea [18,19], carbohydrates [20,21], and steroid derivatives [22,23].

Recently, stimuli-responsive (*e.g.*, heat [24–26], pH [27,28], light [29–32] and enzyme [33,34]) hydrogels exhibiting tunable, reversible and dynamic gel-sol transitions become particularly desirable for applications such as smart materials and on-demand release. For instance, light-sensitive hydrogels hold tremendous promises for stem cell reprogramming for their attractive ability allowing noninvasive and precise spatiotemporal control over a broad range of parameters [35–37]. Shear-thinning and temperature-responsive materials can be used as injectable hydrogels to encapsulate small-molecular, macromolecular or cellular cargos *in vivo* [38–41]. Ultra pH-responsive hydrogels can deliver and release payloads in tumor microenvironment [42]. Moreover, LMWGs can be designed to form hydrogels inside the malignant cells or at the pericellular tissues in responding to biomolecule cues such as enzymes and glutathione (GSH) [43–47]. Despite all those advances, however, most LMWGs possess only one or two stimuli-responsiveness and the generation of multiple trigger-responsive LMWGs remains a considerable challenge.

Herein, we design and synthesize an amino acid-based LMWG, denoted as AA-Azo-EG_6, that can be used to generate hydrogels possessing responsiveness to multiple triggers including light, temperature and pH (Scheme 1). Briefly, this gelator is composed of three modules and each module can offer at least one type of supramolecular interaction for gelation and a particular type of stimuli-responsiveness. The amino acid (AA) head can form hydrogen bonding and salt bridge, and also respond to pH changes in a reversible fashion. The azobenezene (Azo) module is selected to offer rigidity, hydrophobic π–π interaction and photo-responsiveness. The last module is a thermo-responsive oligoehtylene-glycol (EG_6), which is designed to afford balanced hydrophilicity and flexibility.

^{1}H NMR spectra were recorded on a 400 MHz Bruker ARX400FT-NMR spectrometer. Circular dichroism (CD) spectra

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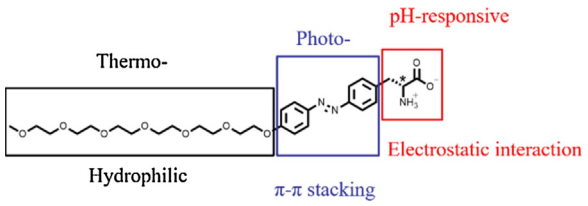
http://dx.doi.org/10.1016/j.cclet.2017.09.019



Scheme 1. Molecular structure of AA-Azo-EG_6 gelator.

were recorded on a CD Spectrometer with a 0.1 cm path length quartz cell. UV–vis spectra were taken on a Perkin-Elmer Lambda 35 UV–vis spectrophotometer. The rheological behavior of the hydrogels was investigated by a Malvern rheometer using a 40 mm parallel-plate geometry, cone-plate geometry or cup-bob at 25 °C. The hydrogelator AA-Azo-EG_6 was synthesized in several steps and confirmed by mass spectrometry, ^{1}H NMR and ^{13}C NMR spectroscopies, which was reported in previous work [48].

The hydrogel was constructed by simply adding AA-Azo-EG_6 to ultrapure water at room temperature, with a critical gelation concentration of ~5.8% (Fig. 1A). The gel could be easily loaded into and extruded out from a 0.45 mm-gauged syringe, indicating shear-thinning property (Fig. 1B). Viscoelastic properties of the gel were characterized by oscillatory rheology experiments including frequency and strain sweep (Figs. 1C-D). As shown in Fig. 1C, the storage modulus (G') of the gel was much higher than the loss modulus (G") over the whole range of frequency swept, which was a characteristic feature of gelation. Strain sweep measurement showed that both the storage and loss moduli of the gel stayed constant until the strain reached ~2%, the so-called linear viscoelastic region (LVER); beyond this region, the storage modulus began to plunge down as the strain increased. The gel eventually collapsed when the strain was over ~25%, as shown by the crossover of the storage and loss moduli.

To investigate the gelation process and morphology of the gel, we studied the self-assembly behavior of AA-Azo-EG_6 in ultrapure water at varied concentrations by using atomic force microscope (AFM) and transmission electron microscopy (TEM) (Fig. 2). As shown in Fig. 2A, spherical micelles with an average diameter of ~120 nm appeared as the only structure observed at a low concentration of 0.1 mg/mL. When the concentration of AA-Azo-EG_6 was increased to 0.5 mg/mL, entangled nanofibers of several micrometers in length and ~120–200 nm in width became the dominating morphology (Fig. 2B). Moreover, the nanofibers seemed to undergo further self-assembly to form densely cross-linked lamellar sheets when the concentration of the gelator was increased to 1.0 mg/mL (Fig. 2C). Similar self-assembled structures were confirmed by TEM observation (Fig. 2D). These lamellar sheets, in our opinion, were likely responsible for the gelation.

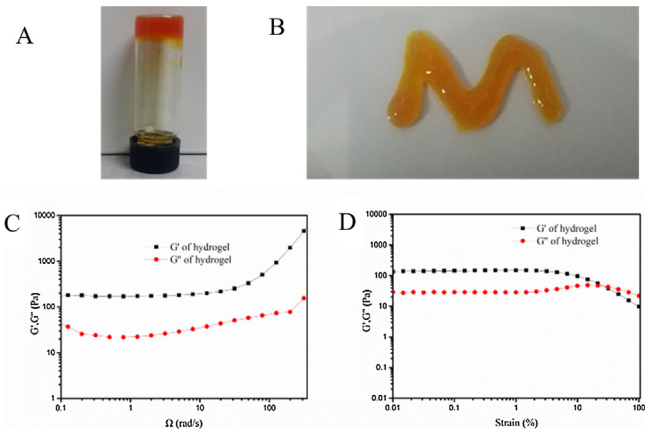


Fig. 1. Gelation property of AA-Azo-EG_6. A) A photograph showing the gel formation in an inverted tube. B) A M-shaped hydrogel produced by syringe injection. C) Oscillatory rheology frequency sweep of the gel at a 0.1% strain. D) Oscillatory rheology strain sweep of the gel at a 10 rad/s frequency.

Interestingly, circular dichroism (CD) spectroscopy examination of AA-Azo-EG_6 at 0.5 mg/mL showed characteristic couplet of bands at 320 nm and 370 nm due to Cotton effect, which was attributed to the π–π^* transition of the azo component (Fig. 2E). The strong CD signal implied that the Azo moiety adopted an ordered arrangement in the gel. Indeed, a helical superstructure was clearly seen in the magnified nanofiber under AFM (Fig. 2F). The height profile of the fiber (red line in Fig. 2F) showed that the super-helices had a pitch of ~50 nm (Fig. 2G.). This superstructure was likely derived from the chiral AA structure, and the resultant hydrogel may hold promises for various applications such as chiroptical switches, chiral recognition and asymmetric catalysis [49–51].

Next, we examined the stimuli-responsiveness of the AA-Azo-EG_6 gel. The azo moiety was known to undergo *trans-cis* isomerization under UV irradiation, which can largely disrupt the π–π stacking; moreover, the *cis*-azo isomer was more polar and aqueous soluble than its *trans*-azo counterpart [52]. As such, both factors would favor a gel-sol transition when treating the gel with UV light. Indeed, UV irradiation (intensity: 1 W/cm^2; the gelator solution temperature was maintained at 25 °C) of the gel at 365 nm for 30 min successfully transformed the hydrogel to a deep red solution (Fig. 3). This light-responsive gel-sol transition can be reversibly repeated for at least 5 cycles within 6 h. AFM study of the UV-irradiated AA-Azo-EG_6 solution at 1.0 mg/mL showed aggregated or isolated globular micellar structure with an average diameter of ~150 nm (Fig. 3). Similarly, TEM study of the same solution gave a globular structure with an average particle size of ~130 nm (Fig. 3). Furthermore, both ^{1}H NMR (Fig. 3) and UV–vis (Fig. 3) spectra of AA-Azo-EG_6 exhibited characteristic reversible switches corresponding to the *trans-cis* isomerization of azo upon UV and visible light irradiation, which verified the pivotal role of the Azo component during the gel-sol transition process.

Interestingly, the hydrogel also showed a reversible responsiveness to both base and acid. By adding 10 equiv. NaOH on the top of gel, the gel transformed to fluid solution gradually and the color turned to dark brown; adding 10 equiv. HCl to gel rapidly (Fig. 4A). On the other hand, similar gel-sol-gel transition was observed by adding HCl first followed by NaOH. This phenomenon implied that it is essential to keep the AA component in a zwitterionic status in order to form the gel. We provisionally propose that the two AA groups might form intermolecular salt bridge or hydrogen bonding, which generates part of the physical crosslinking network together with the hydrophobic azo-azo interaction.

At last, we investigated the thermal responsive behavior of our AA-Azo-EG_6 gel. Because of the lower critical solution temperature (LCST) property of EG_6, it was expected the gel could undergo heat-induced transition to a collapsed state due to dehydration. Indeed, upon heating up the gel at ~70 °C, the solute precipitated out immediately which could be easily separated from the solution by centrifugation (Fig. 4B). This result also implied that at room temperature, the role of EG_6 was to provide enough hydrophilicity to prevent precipitation and hold water for gelation.

In conclusion, we designed and synthesized a novel gelator AA-Azo-EG_6 which possesses photo-, pH- and thermo-responsive properties attributable to its azo, AA and EG_6 domain, respectively. Moreover, the gelator can self-assemble to generate physically cross-linked multi-responsive hydrogel under a combined interaction of salt-bridge, π–π stacking/hydrophobic interactions. The responsiveness of the gel at macroscopic scale was characterized

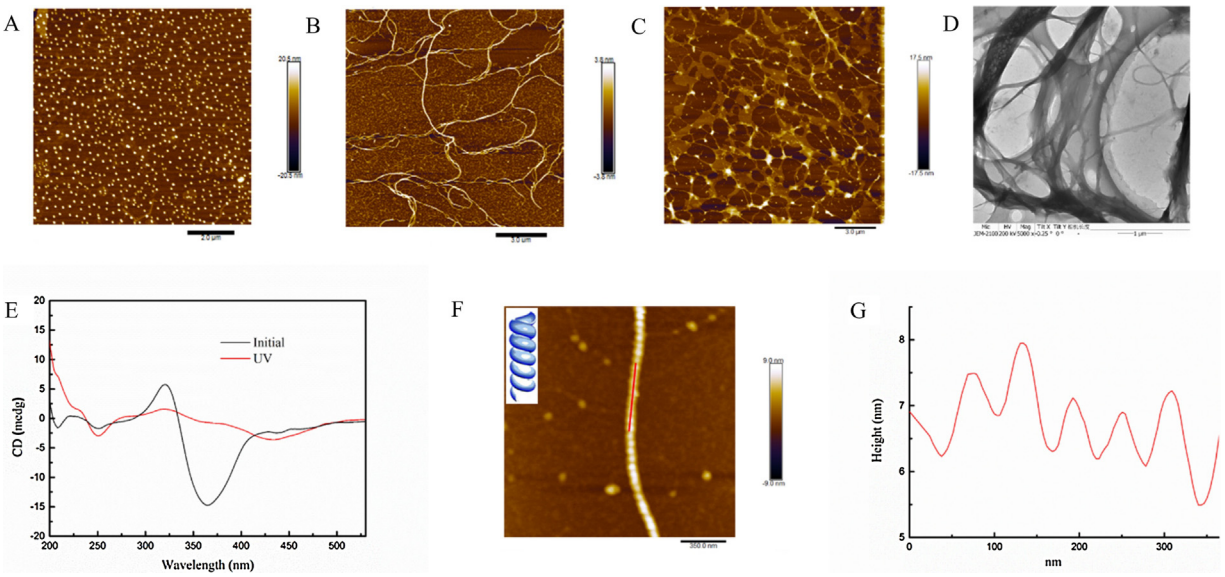


Fig. 2. Self-assembly of AA-Azo-EG_6. A-C) AFM images of AA-Azo-EG_6 at 0.1 (A), 0.5 (B) and 1.0 (C) mg/mL in ultrapure water. D) TEM image of AA-Azo-EG_6 at 1.0 mg/mL. (E) CD spectroscopy of AA-Azo-EG_6 at 0.5 mg/mL in degassed ultrapure water. F-G) Magnified AFM image (F) and height profile (G) of the superhelical nanofiber; sample was prepared at 0.5 mg/mL and scale bar = 350 nm.

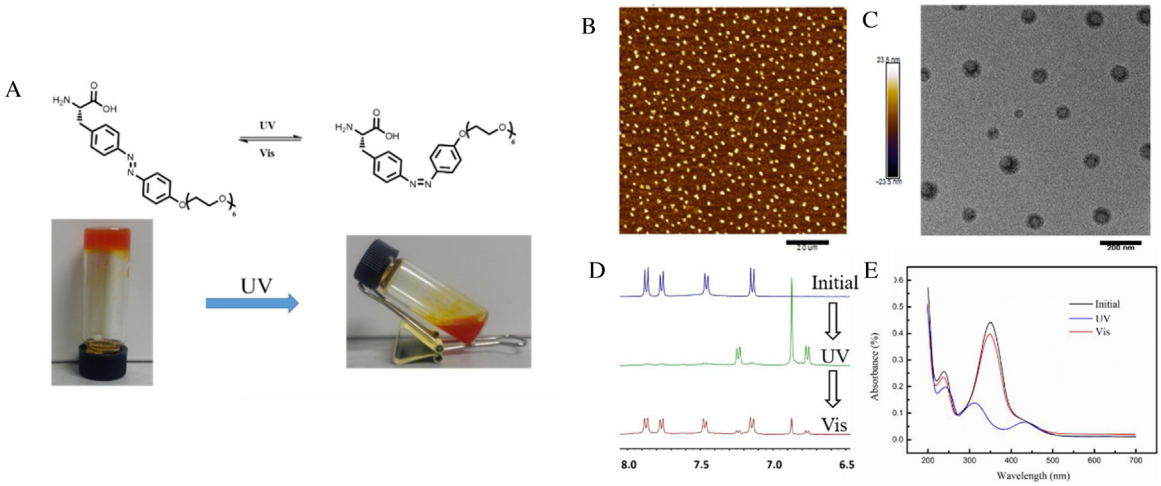


Fig. 3. The photo-responsiveness of molecular structure. A) A photograph show gel-sol transition under UV irradiation (intensity: 1 W/cm^2). AFM (B) and TEM (C) images of AA-Azo-EG_6 under UV irradiation at 1.0 mg/mL. D) ^{1}H NMR spectra of AA-Azo-EG_6 in DMSO-d_6 treated with UV and vis irradiation. E) UV–vis spectra of AA-Azo-EG_6 solution at 0.01 mg/mL treated with UV and vis irradiation.

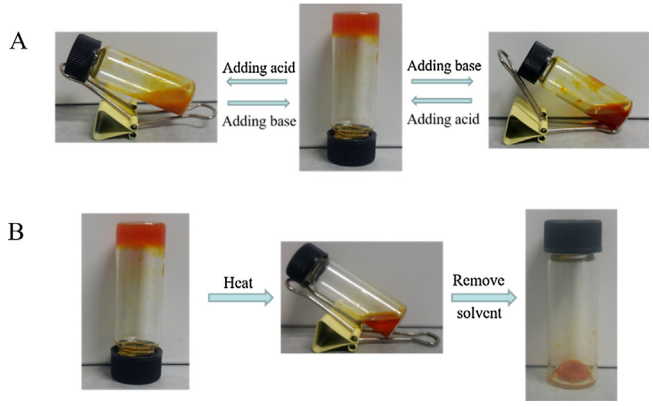


Fig. 4. Photographs of AA-Azo-EG_6. A) The reversible pH-triggered gel-sol transition. B) The thermo-triggered gel-sol transition.

by various spectroscopies and microscopies. This gel could be potentially useful in tissue engineering as well as drug delivery.

Acknowledgments

This work is supported by State High-Tech Development Program of China (863 Program, No. 2015AA020941) and the National Natural Science Foundation of China (Nos. NSFC21474004 and NSFC21434008). H. Lu thanks the Youth Thousand-Talents Program of China for support.

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