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Enzyme-Instructed Molecular Self-assembly Confers Nanofibers and a Supramolecular Hydrogel of Taxol Derivative

Yuan Gao,[†] Yi Kuang,[†] Zu-Feng Guo,[‡] Zhihong Guo,[‡] Isaac J. Krauss,[†] and Bing Xu*,[†]

Department of Chemistry, Brandeis University, 415 South Street, Waltham, Massachusetts 02454, and Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China

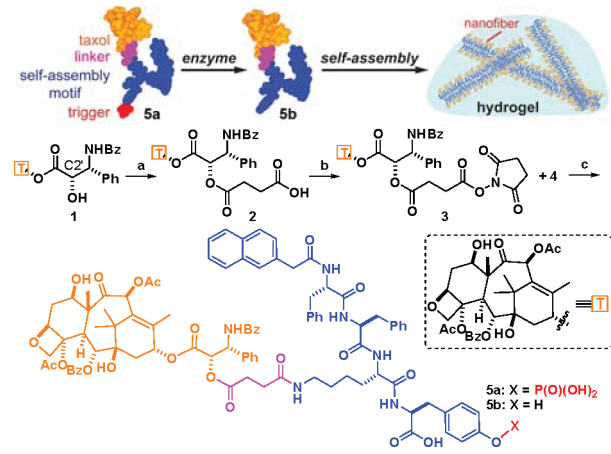
Received May 31, 2009; E-mail: bxu@brandeis.edu

This communication reports the use of an enzymatic reaction to initiate the self-assembly of a derivative of taxol in water to form nanofibers that result in a supramolecular hydrogel. Composed of a three-dimensional, elastic network spanning the volume of aqueous media, hydrogels are an important class of biomaterials.[1] Natural hydrogels exist as components in complex organisms,[2] such as the bodies of jellyfish, connective tissues in joints, the cornea in the eye, and nuclear pore complexes inside cells,[3] and man-made hydrogels continue to find successful applications in biomedicine such as contact lenses,[4] drug delivery,[5] and tissue engineering.[6] Although a majority of hydrogels are polymeric hydrogels whose networks consist of covalently cross-linked natural or synthetic polymers,[6] supramolecular hydrogels,[7,8] whose networks consist of nanofibers formed through the self-assembly of small molecules (i.e., hydrogelators[7]), have emerged as promising biomaterials in the past decade.[9] Usually, changes in temperature, pH, or ionic strength can successfully trigger the formation of supramolecular hydrogels. It is, however, advantageous to use inherent biological processes to create supramolecular hydrogels *in vivo* or *in situ* for certain biomedical applications.[10] By mimicking biomacromolecular self-assembly (e.g., formation of collagen fibrils[11]), the integration of enzymatic reactions with self-assembly of small molecules provides an effective means to form nanofiber networks and results in hydrogels under various conditions.[12−14]

While it is feasible to initiate hydrogelation using an enzyme that converts a precursor into a hydrogelator,[13] most precursors explored so far bear limited biological activities. To create hydrogels with sophisticated biological functions by design, we chose to synthesize and examine a hydrogel precursor (**5a**) based on taxol (**1**), a well-established antineoplastic agent that binds specifically to the β-tubulin subunit of microtubules (MT) to arrest mitosis and result in programmed cell death (i.e., apoptosis) and that has shown remarkable activity in the treatment of breast, lung, ovarian, bladder and head and neck cancers.[15] To connect taxol covalently with a motif that tends to self-assemble and a group that is cleavable by an enzyme, we designed and synthesized a precursor for producing a taxol hydrogel without compromising activity of the taxol. As illustrated in Scheme 1, upon the action of an enzyme, the precursor (**5a**) transforms into a hydrogelator (**5b**), which self-assembles into nanofibers and affords a supramolecular hydrogel of the taxol derivative (**5b**). This hydrogel can slowly release **5b** into an aqueous medium. In addition to representing the first example of enzyme-instructed self-assembly and hydrogelation of complex, bioactive small molecules, this result demonstrates a new, facile way to formulate highly hydrophobic drugs, such as taxol, into an aqueous form (e.g., hydrogel) without comprising their activity and promises a general methodology to create therapeutic molecules that have a dual role[16] as the delivery vehicle and the drug itself.

Scheme 1 shows the synthetic route and the structure of **5a**, which consists of a self-assembly motif,[17] an enzyme-cleavable group,[14] a linker, and a taxol molecule. Based on the study of the structure−activity of taxol derivatives,[18] we connected a simple linker (succinic acid) to the C2′ hydroxyl group of taxol (**1**) and obtained the intermediate (**2**),[19] which can be activated by *N*-hydroxysuccinimide (NHS) to afford **3**. The reaction of **3** with a phosphatase substrate (NapFFKYp, **4**)[20] that consists of the self-assembly motif and the enzyme-cleavable group affords **5a** in an overall yield of 37.1%. Compared to taxols and the pyridinium taxol prodrug,[21] **5a** exhibits better aqueous solubility (7.6 mg/mL or 4.26 mM in a 100 mM phosphate buffered saline solution) and has a distribution coefficient (octanol/water) of 0.61. Compound **5a** has excellent stability in water and shows hardly any dephosphorylation over months in the absence of a phosphatase.[20]

Scheme 1[a]



[a] (a) Succinic anhydride, DIEA, chloroform; (b) NHS, DCC, chloroform; (c) sodium carbonate, acetone, water.

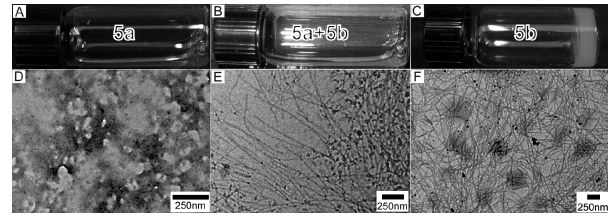


Figure 1. Optical (A−C) and the corresponding transmission electron microscopic (TEM) images (D−F) of the solution of **5a** with [**5a**] = 1.0 wt % (A, D); the solution of **5a** at 5 min after the addition of alkaline phosphatase (ALP) (B, E); and the hydrogel of **5b** overnight after the addition of ALP (C, F).

After dissolving 10 mg of **5a** into 1 mL of water at pH = 7.3 with the aid of sonication (Figure 1A), we added 5 μL of alkaline phosphatase (10 U/μL) into the solution. The solution becomes slightly turbid (Figure 1B) 5 min after the addition of the enzyme

[†] Brandeis University.
[‡] The Hong Kong University of Science and Technology.

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and turns into a translucent hydrogel (Gel_{5b}, Figure 1C) overnight. Mass spectroscopic (MS) and HPLC data (Figure S3) confirm the complete conversion of **5a** to **5b** in the hydrogel.[20] Moreover, MS analysis indicates that **5b** is stable in the gel state over weeks,[20] an important prerequisite for the sustained release of **5b** from its own hydrogel (*vide infra*). As shown in the TEM image (Figure 1D), a solution of **5a** gives featureless aggregates after cryo-drying. According to the TEM in Figure 1E, 5 min after the addition of the enzyme, the mixture already contains the nanofibers with a width of 20 nm, in addition to particle aggregates. The nanofibers appear to stretch out of the amorphous area, suggesting that the nanofiber grows from the enzymes, consistent with an enzyme-catalyzed self-assembly process. While its scanning electron micrograph (SEM) shows lamellar microstructures (Figure S4),[20] the cryo-dried Gel_{5b} exhibits well-dispersed nanofiber networks with a uniform fiber width of 29 nm in its TEM (Figure 1F). These results confirm the self-assembly and formation of the nanofibers upon enzyme catalysis. Circular dichroism (CD) spectra of the solution of **5a** and the corresponding Gel_{5b} (Figure S5)[20] further help elucidate the molecular arrangement of **5b** in gel phase. The spectrum of Gel_{5b} exhibits a positive band near 192 nm ($\pi\pi^*$ transition of the amide bonds) and a broad negative band near 216 nm ($n\pi^*$ transition of the amide bonds and $\pi\pi^*$ of the naphthyl aromatics), coinciding with the CD of NapFFGEY[17] and indicating the existence of β-sheet-like features. Moreover, the intensity of the peak at 298 nm, a characteristic peak of taxol (**1**),[22] decreases dramatically in the CD of Gel_{5b}, compared to that of the solution of **5a**, indicating that **5b** might align in the nanofibers in such a way to force the intrinsic dipole transition moments of the taxols to opposite directions to reduce each other,[23] which agrees with the antiparallel arrangement in a β-sheet-like secondary structure.[17] Collectively, CD, TEM, and SEM indicate that **5b** self-assembles into a β-sheet-like structure to afford nanofibers that reach high density and result in sheet-like matrices in the hydrogel.

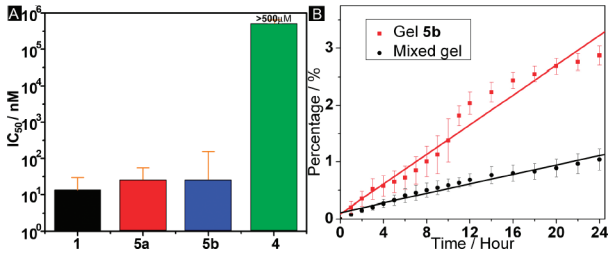

Figure 2. (A) Cytotoxicity (y-axis in $\log_{10}$ scale) of taxol (**1**), **5a**, **5b**, and **4** after incubated with HeLa cells for 48 h and (B) accumulative drug release profile of two kinds of taxol gels in 100 mM PBS buffers.

To evaluate the activity of **5a**, we used it to treat HeLa cells and used taxol (**1**) as the control. As shown in Figure 2A, after 48 h of incubation with HeLa cells, **5a** exhibits an IC_{50} value of 25.2 $\pm$ 2.2 nM, which is comparable to that of **1** (13.5 $\pm$ 2.2 nM). Further examination shows that the phosphatase substrate (**4**) is essentially biocompatible (IC_{50} > 500 μM). **5b** itself exhibits an IC_{50} of 25.2 $\pm$ 6.2 nM, which is also comparable to that of **1** and **5a**. In addition, the shapes of the regression curves of **1**, **5a**, and **5b** are similar.[20] These results indicate that the activity of taxol is conserved successfully in the precursor and the hydrogelator. The poor solubility of **5b** (21.6 μg/mL or 12.66 μM) in water, unfortunately, prevents it from forming a hydrogel directly by changing temperature or pH, but it is easy to generate hydrogels that consist of or contain **5b** by enzymatic dephosphorylation of **5a**, which allows us to evaluate the release of **5b** from the hydrogels. Figure 2B shows the release profiles of **5b** from two kinds of gels—Gel **5b** resulting from the treatment of the solution of **5a** (0.8 wt %) with alkaline phosphatase, and a mixed gel made by adding alkaline phosphatase into the solution of **5a** (0.6 wt %) and **4** (0.6 wt %). Once in contact with a fresh PBS buffer solution,[24] Gel **5b** and the mixed gel release **5b** at rates of 0.13% and 0.046% per hour, respectively. These preliminary results demonstrate the sustained release of **5b** from its own gel and suggest a way for release rate control via the concentration of **5b** in the mixed gel.

In conclusion, we have demonstrated that, with proper molecular design, the integration of enzymatic reaction and self-assembly provides a powerful method to create molecular hydrogels of clinically used therapeutics without compromising their bioactivities. This work also suggests that drug molecules are excellent candidates for engineering functional hydrogels or soft materials for various biomedical applications, including sustained or controlled drug delivery. In addition, this work demonstrates enzyme-instructed self-assembly as a facile strategy for generating the supramolecular hydrogels of molecules that inherently have poor solubility in water.

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Supporting Information Available: The experimental section, MS, NMR, CD, SEM, optical images, HPLC traces, cell viability, drug release procedure, and the structures of **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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