J|A|C|S
COMMUNICATIONS

Published on Web 11/22/2002

Hydrophobic Interaction and Hydrogen Bonding Cooperatively Confer a Vancomycin Hydrogel: A Potential Candidate for Biomaterials

Bengang Xing,† Chun-Wing Yu,† Kin-Hung Chow,‡ Pak-Leung Ho,‡ Degang Fu,† and Bing Xu*,†

Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, and Center of Infection and Department of Microbiology, Faculty of Medicine, The University of Hong Kong, Pokfulam Road, Hong Kong

Received September 13, 2002

This paper reports the first antibiotic gelator—vancomycin-pyrene (**1**)—that forms hydrogels via hydrophobic interaction and hydrogen-bonding-promoted self-assembly in water. Hydrogels, formed by three-dimensional, elastic networks whose interstitial spaces are filled with water, present many useful properties (e.g., response to the external stimuli) and applications (e.g., gel electrophoresis, chemical sensing, drug delivery, as a biointerface, and as actuators).[1,2] Biopolymers (e.g., collagens,[3] polysaccharide,[4] etc.) and hydrophilic synthetic polymers (e.g., polyacrylamide[2,5] and polypeptides[6]) have been successfully employed to form hydrogels. Nonpolymeric hydrogelators, however, are rare despite that their counterparts—small molecular organogelators[7,8]—have expanded rapidly and received intensive studies in the past decade. Recently, Hamilton,[9] Shinkai,[10] Zhang,[11] and others[12] have reported low molecular weight hydrogelators that form hydrogels via carefully balancing the hydrophobic interactions and hydrogen bonds in water to induce aggregations of those small molecules. Their results inspired us to design and synthesize hydrogelators based on antibiotics—an important class of biomolecules—in the hope of developing biomaterials that can treat infectious wounds, serve as an antiseptic matrix, and provide a new way of drug delivery.[13]

We chose vancomycin (Van), one of the most important antibiotics, as the platform to make the hydrogelators because of (1) its clinical significance in treating Gram-positive bacterial infections;[14] (2) its relatively easy synthetic modifications;[15] and (3) its strong tendency to form multiple hydrogen bonds with suitable substrates or itself in aqueous solution, as revealed by Wash et al. in the decipherment of the molecular logic of vancomycin resistance enterococci (VRE),[16] by Williams et al. in the elucidation of binding mode of Van,[17,18] and by Whitesides et al. in the studies of multivalency of Van.[19] Learning from the principles developed in the study of low molecular weight organogelators,[7,20] we successfully generated a hydrogelator based on Van by introducing a pyrene group to the C-terminal of the backbone of Van. Our results indicate that the $\pi-\pi$ stacking and intermolecular hydrogen bonding in water provide driving forces to form a noncovalent polymer of **1**, which is primarily responsible for the gelation. We believe that such an approach, which eliminates the biologically inactive molecules (e.g., cross linked polyacrylamide, etc.) in conventional hydrogels, could lead to a new kind of biomaterial for useful applications—for example, controlled releases of therapeutics or surface coatings of medical devices.

Figure 1 shows the chemical structure of **1** and the picture of the hydrogel (formed by adding 6.5 mg of **1** into 1.8 mL of water, corresponding to ~0.36 wt % (2.2 mM) of the gelator and ~23 000 water molecules/gelator molecule). Figure 2a shows the emission spectra of the hydrogels of **1** at two different concentrations. The broad band of the emission (λ_{max} = 460 nm), resembling the emission of pyrene excimer (λ_{max} = 480 nm),[21] indicates that the pyrenes of **1** dimerize exclusively via $\pi-\pi$ stacking in the gel. Figure 2b shows the circular dichroism[22] (CD) spectra of Van (2.2 mM) and the hydrogel (2.2 mM). The large Cotton effect at 220 nm in the spectrum of Van originates from the peptidal backbone of Van. The subsequent decrease of its intensity in the gel phase indicates that the peptidal backbones associate in head-to-tail fashion by (possibly) four hydrogen bonds, as indicated in the crystal structure[23] and solution structure[17] of Van. The head-to-tail arrangement of the peptidal leads to meso orientation of the backbones and results in smaller CD signals at 220 nm in the gel. The intensity increases at 285 and 340 nm (the induced circular dichroism) suggest that the biphenyl and pyrene moieties adopt helical arrangements in the polymer of **1**. The helical arrangement was later confirmed by transmission electron micrography (Figure 3c), which reveals that the polymers aggregate into superhelices with the diameter of ~25 nm, similar to the observations in many other systems.[24]

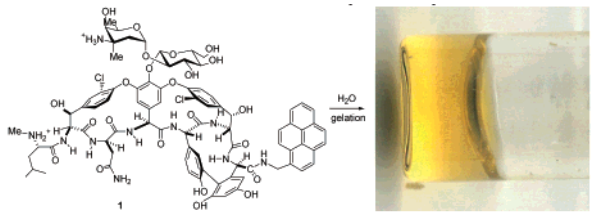

Figure 1. The structure of **1** and the optical image of the hydrogel of **1** (0.36 wt %) (taken by a flatbed scanner when the vial lay horizontally).

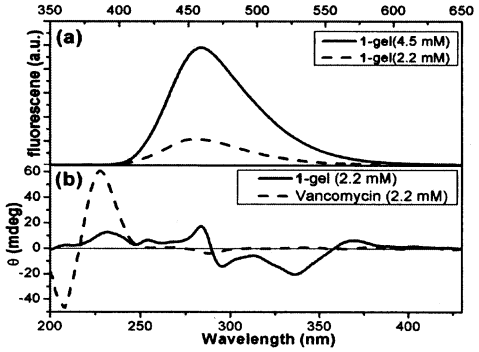

Figure 2. (a) Emission spectra of Van-pyrene (**1**) hydrogels at two different concentrations ($\lambda_{excitation}$ = 330 nm). (b) Circular dichroism spectra of an aqueous solution of Van (2.2 mM) and Van-pyrene (**1**) hydrogel (2.2 mM).

* To whom correspondence should be addressed. E-mail: chbingxu@ust.hk.
† The Honk Kong University of Science and Technology.
‡ The University of Hong Kong.

10.1021/ja028539f CCC: $22.00

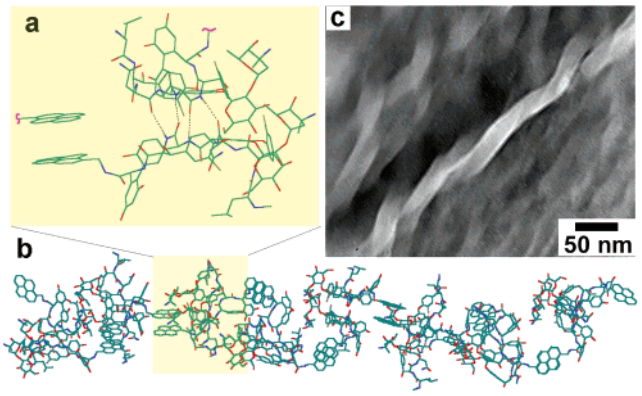

Figure 3. (a) Illustration of $\pi-\pi$ stacking and multiple hydrogen bonding (the dotted lines) in the polymer of **1**, (b) one of the possible conformations of the helix of **1**, and (c) the transmission electron micrograph (TEM) of the helical fibers in the hydrogels.

The differential scanning calorimetry (DSC) measurement indicates that the formation of the gel is thermally reversible with the gel-to-sol transition temperature (T_{GS}) at ~73 °C and ΔH_{GS} of ~7.05 kcal/mol, which corresponds to the entropy of 20.4 cal/mol. The sum of ΔG's, being considered separately, of dimerization of Van (~ −3.54 kcal/mol)[17] and $\pi-\pi$ stacking of pyrenes (~ −2.9 kcal/mol),[25] however, is not enough to compensate for the entropy loss due to immobilization of water. Therefore, cooperative interactions (e.g., ~0.99 kcal/mol in the case of pyrene-peptide conjugates[25]) should exist to provide additional driving force for the gelation. Such an interaction may originate from the conformational change of Van-pyrene upon $\pi-\pi$ stacking of the pyrenes.

On the basis of the CD, fluorescent spectra, and electron microscopy of the gel, we suggest that the Van-pyrene self-assemble to form a helical polymer, whose molecular superstructure is proposed in Figure 3a and b. The small-angle X-ray diffraction of the hydrogel exhibits a broad peak at ~25°, suggesting that the fibers may form random networks rather than an ordered phase. The absence of higher order peaks also suggests that a columnar phase due to $\pi-\pi$ stacking of pyrene is a unlikely scenario, prevented by the steric congestion imposed by Van.

We found, in a separate research, that **1** is unexpectedly potent (0.125−2 μg/mL, being 8- to 11-fold dilutions lower than the corresponding vancomycin) against VRE (2 *vanA*-positive *Enterococcus faecalis*, 4 *vanA*-positive *E. faecium*, 4 *vanB*-positive *E. faecium*).[21] The strong tendency to polymerize and the unexpected potency of **1** also lead us to speculate that **1** might aggregate into polymer-like structures at the cell surface when its local concentration is high, and we are working on confirming this hypothesis.

In summary, we have demonstrated a new kind of small molecular hydrogelators based on an important member of antibiotics. Such antibiotic hydrogels will provide a new kind of biomaterial.

Acknowledgment. This work was partially supported by the Research Grant Council of Hong Kong, a Direct Allocation Grant (HKUST), a DuPont Young Faculty Grant (for B.X.), and a Grant from the University Development Fund (HKU). We thank Dr. Yesha Zheng for the TEM measurement.

Supporting Information Available: Details of the synthesis and the in vitro test of **1** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ilmain, F.; Tanaka, T.; Kokufuta, E. *Nature* **1991**, *349*, 400−401. Holtz, J. H.; Asher, S. A. *Nature* **1997**, *389*, 829−832. Simonsen, L.; Hovgaard, L.; Mortensen, P. B.; Brondsted, H. *Eur. J. Pharm. Sci.* **1995**, *3*, 329−337. Osada, Y.; Gong, J. P. *Adv. Mater.* **1998**, *10*, 827−837. Weissman, J. M.; Sunkara, H. B.; Tse, A. S.; Asher, S. A. *Science* **1996**, *274*, 959−960. Andrianov, A. K.; Cohen, S.; Visscher, K. B.; Payne, L. G.; Allcock, H. R.; Langer, R. *J. Controlled Release* **1993**, *27*, 69−77. Osada, Y., Khokhlov, A. R., Eds. *Polymer Gels and Networks*; Marcel Dekker: New York, 2002.

(2) Chu, Y. H.; Chen, J. K.; Whitesides, G. M. *Anal. Chem.* **1993**, *65*, 1314−1322. Lee, K.; Asher, S. A. *J. Am. Chem. Soc.* **2000**, *122*, 9534−9537.

(3) Chirila, T. V.; Constable, I. J.; Crawford, G. J.; Vijayasekaran, S.; Thompson, D. E.; Chen, Y. C.; Fletcher, W. A.; Griffin, B. J. *Biomaterials* **1993**, *14*, 26−38. Tabata, Y.; Ikada, Y. *Adv. Drug Delivery Rev.* **1998**, *31*, 287−301.

(4) Nishikawa, T.; Akiyoshi, K.; Sunamoto, J. *Macromolecules* **1994**, *27*, 7654−7659. Nishikawa, T.; Akiyoshi, K.; Sunamoto, J. *J. Am. Chem. Soc.* **1996**, *118*, 6110−6115. Akiyoshi, K.; Deguchi, S.; Tajima, H.; Nishikawa, T.; Sunamoto, J. *Macromolecules* **1997**, *30*, 857−861.

(5) Pan, G. S.; Kesavamoorthy, R.; Asher, S. A. *J. Am. Chem. Soc.* **1998**, *120*, 6525−6530. Sano, M.; Okamura, J.; Shinkai, S. *Langmuir* **1999**, *15*, 7890−7893. Philippova, O. E.; Rulkens, R.; Kovtunenko, B. I.; Abramchuk, S. S.; Khokhlov, A. R.; Wegner, G. *Macromolecules* **1998**, *31*, 1168−1179.

(6) Nowak, A. P.; Breedveld, V.; Pakstis, L.; Ozbas, B.; Pine, D. J.; Pochan, D.; Deming, T. J. *Nature* **2002**, *417*, 424−428.

(7) Terech, P.; Weiss, R. G. *Chem. Rev.* **1997**, *97*, 3133−3159.

(8) Weiss, R. G.; Ramamurthy, V.; Hammond, G. S. *Acc. Chem. Res.* **1993**, *26*, 530−536. de Loos, M.; van Esch, J.; Stokroos, I.; Kellogg, R. M.; Feringa, B. L. *J. Am. Chem. Soc.* **1997**, *119*, 12675−12676. Jung, J. H.; Ono, Y.; Hanabusa, K.; Shinkai, S. *J. Am. Chem. Soc.* **2000**, *122*, 5008−5009. Xing, B.; Choi, M.-F.; Xu, B. *Chem. Commun.* **2002**, 362−363. Placin, F.; Desvergne, J.-P.; Lassegues, J.-C. *Chem. Mater.* **2001**, *13*, 117−121. Xing, B.; Choi, M.-F.; Xu, B. *Chem.-Eur. J.* **2002**, *8*, 5028−5032.

(9) Estroff, L. A.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2000**, *39*, 3447−3450.

(10) Jung, J. H.; John, G.; Masuda, M.; Yoshida, K.; Shinkai, S.; Shimizu, T. *Langmuir* **2001**, *17*, 7229−7232.

(11) Vauthey, S.; Santoso, S.; Gong, H. Y.; Watson, N.; Zhang, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5355−5360 and references therein.

(12) Menger, F. M.; Caran, K. L. *J. Am. Chem. Soc.* **2000**, *122*, 11679−11691. Suzuki, M.; Yumoto, M.; Kimura, M.; Shirai, H.; Hanabusa, K. *Chem. Commun.* **2002**, 884−885. Maitra, U.; Mukhopadhyay, S.; Sarkar, A.; Rao, P.; Indi, S. S. *Angew. Chem., Int. Ed.* **2001**, *40*, 2281−2283.

(13) Langer, R. *Nature* **1998**, *392*, 5−10. Langer, R. *Acc. Chem. Res.* **2000**, *33*, 94−101. Ottenbrite, R. M., Huang, S. J., Park, K., Eds. *Hydrogels and Biodegradable Polymers for Bioapplications*; American Chemical Society: Washington, DC, 1996.

(14) Murray, B. E. *N. Engl. J. Med.* **2000**, *342*, 710−721.

(15) Sundram, U. N.; Griffin, J. H.; Nicas, T. I. *J. Am. Chem. Soc.* **1996**, *118*, 13107−13108. Nicolaou, K. C.; Hughes, R.; Cho, S. Y.; Winssinger, N.; Labischinski, H.; Endermann, R. *Chem.-Eur. J.* **2001**, *7*, 3824−3843. Nicolaou, K. C.; Hughes, R.; Cho, S. Y.; Winssinger, N.; Smethurst, C.; Labischinski, H.; Endermann, R. *Angew. Chem., Int. Ed.* **2000**, *39*, 3823−3828.

(16) Walsh, C. *Nature* **2000**, *406*, 775−781. Fan, C.; Moews, P. C.; Walsh, C. T.; Knox, J. R. *Science* **1994**, *266*, 439−443.

(17) Williams, D. H.; Maguire, A. J.; Tsuzuki, W.; Westwell, M. S. *Science* **1998**, *280*, 711−714.

(18) Searle, M. S.; Williams, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 10690−10697.

(19) Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2755−2794. Rao, J. H.; Lahiri, J.; Weis, R. M.; Whitesides, G. M. *J. Am. Chem. Soc.* **2000**, *122*, 2698−2710. Rao, J. H.; Lahiri, J.; Isaacs, L.; Weis, R. M.; Whitesides, G. M. *Science* **1998**, *280*, 708−711. Rao, J.; Yan, L.; Xu, B.; Whitesides, G. M. *J. Am. Chem. Soc.* **1999**, *121*, 2629−2630.

(20) van Esch, J. H.; Feringa, B. L. *Angew. Chem., Int. Ed.* **2000**, *39*, 2263−2266.

(21) Xing, B.; Yu, C.-W.; Chow, K.; Ho, P. L.; Xu, B., Supporting Information.

(22) Nakanishi, K., Berova, N., Woody, R. W., Eds. *Circular Dichroism: Principles and Applications*, 1st ed.; VCH: New York, 1994.

(23) Loll, P. J.; Bevivino, A. E.; Korty, B. D.; Axelsen, P. H. *J. Am. Chem. Soc.* **1997**, *119*, 1516−1522.

(24) Engelkamp, H.; Middelbeek, S.; Nolte, R. J. M. *Science* **1999**, *284*, 785−788 and references therein.

(25) Jones, G.; Vullev, V. I. *Org. Lett.* **2001**, *3*, 2457−2460.

JA028539F