ORGANIC LETTERS

2005
Vol. 7, No. 9
1741−1744

# Biotin–Amino Acid Conjugates: An Approach Toward Self-Assembled Hydrogelation

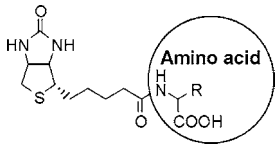
**Sankarprasad Bhuniya, Sun Min Park, and Byeang Hyean Kim***

*Department of Chemistry, Division of Molecular and Life Sciences, Pohang University of Science and Technology, Pohang 790-784, Korea*

*bhkim@postech.ac.kr*

**Received February 14, 2005**

## ABSTRACT




**Amino acid-appended biotin hydrogelators are a new class of low-molecular-weight gelators that display remarkable gelation properties in aqueous media, including buffer solutions with variable pH.**

Although very widespread in nature, for example, in the growth of animal cells, gels are becoming advanced materials for high-technology applications in such fields as drug delivery,[1] tissue engineering, and as scaffolds.[2,3] Recently, supramolecular self-assembly approaches have been used to prepare hydrogels from low-molecular-weight compounds such as simple amphiphiles,[4] bolaamphiphiles,[5] gemini surfactants,[6] and other hydrogelators.[7] These fundamental studies have been of considerable interest for the exploration of supramolecular reaction mechanisms. The supramolecular hydrogels are formed when the monomer units self-assemble into polymerlike fibers that immobilize solvents. A similar approach in which drugs or vitamins are used directly to form hydrogels can lead to new types of biomaterials that may function as "self-delivery" systems.[8] In this paper, we report the examples of low-molecular-weight biotin (vitamin H)-based hydrogels.

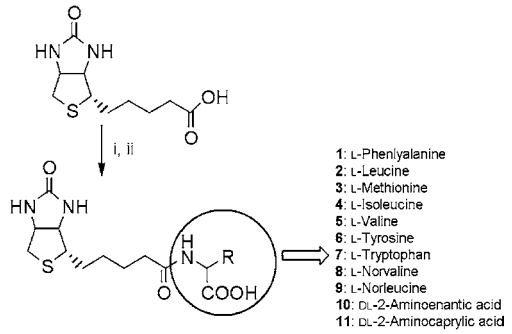
Even though biotin-based organogel[9] had been reported, we chose it to prepare hydrogelators because of its clinical significance,[10] the relative ease of its synthetic modification, and its strong tendency to form receptor−ligand interactions with suitable receptors such as avidin, cyclodextrins, proteins, and insulin. Because of biotin's low solubility in $H_2O$ (1.0−0.8 mmol/L; insoluble in common organic solvents), however, it has a low bioavailability that severely restricts its effectiveness; therefore, it has hitherto been used only to a very limited extent.

(1) Okano, T. *Biorelated Polymers and Gels*; Academic Press: San Diego, 1998.
(2) Dagani, R. *Chem. Eng. News* **1997**, *75*, 26.
(3) (a) Nishikawa, T.; Akiyoshi, K.; Sunamoto, J. *J. Am. Chem. Soc.* **1996**, *118*, 6110. (b) Osada, Y.; Gong, J. P. *Adv. Mater*. **1998**, *10*, 827.
(4) (a) Oda, R.; Huc, I.; Candau, S. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 2689. (b) Oda, R.; Huc, I.; Schmutz, M.; Chandu, S. J.; MacKintosh, F. C. *Nature* **1999**, *399*, 566. (c) Menger, F. M.; Caram, K. L.; Seredyuk, V. A.; Apkarian, R. P. *J. Am. Chem. Soc*. **2002**, *124*, 1140.
(5) (a) Tachibana, T.; Kamabara, H. *J. Am. Chem. Soc*. **1965**, *87*, 3015. (b) Yanagawa, H.; Ogawa, Y. Y.; Furuta, H.; Tsuno, K. *J. Am. Chem. Soc.* **1989**, *111*, 4567. (c) Bhattacharya, S.; Acharya, S. N. G. *Chem. Mater*. **1999**, *11*, 3504.
(6) (a) Shimizu, T.; Masuda, M. *J. Am. Chem. Soc*. **1997**, *119*, 2812. (b) Iwaura, R.; Yoshida, K.; Masuda, U.; Yase, K.; Shimizu, T. *Chem. Mater*. **2002**, *14*, 3047. (c) Iwaura, R.; Yoshida, K.; Masuda, M.; Ohnishi-Kameyama, M.; Yoshida, M.; Shimizu, T. *Angew. Chem., Int. Ed.* **2003**, *42*, 1009.
(7) (a) Hamilton, A. D.; Estroff, L. A. *Angew. Chem., Int. Ed*. **2000**, *39*, 3447. (b) Maitra, U.; Mukhopadhyay, S.; Sarkar, A.; Rao, P.; Indi, S. *Angew. Chem., Int. Ed*. **2001**, *40*, 2281. (c) Hirst, A. R.; Smith, D. K.; Feiters, M. C.; Geurts, H. P. M.; Wright, A. C. *J. Am. Chem. Soc*. **2003**, *125*, 9010. (d) Park, S. M.; Lee, Y. S.; Kim, B. H. *Chem. Commun*. **2003**, 2912. (e) Köhler, K.; Förster, G.; Hauser, A.; Dobner, B.; Heiser, U. F.; Ziethe, F.; Richter, W.; Steniger, F.; Drechsler, M.; Stettin, H.; Blume, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 245. (f) Yang, Z.; Gu, H. W.; Zhang, Y.; Wang, L.; Xu, B. *Chem. Commun*. **2004**, 208. (g) Numata, K. M.; Fujita, N.; Park, S. M., Yun, Y. J.; Kim, B. H.; Shinkai, S. *Chem. Commun*. **2004**, 1996.
(8) Xing, B.; Yu, C.-W.; Chow, K.-H.; Ho, P.-L.; Fu, D.; Xu, B. *J. Am. Chem. Soc.* **2002**, *124*, 14846.
(9) Crisp, G. T.; Gore, J. *Synth. Commun.* **1997**, *27*, 2203.
(10) Friedrich, W. *Vitamins*; Walter de Grueter & Co.: Berlin, 1998; see also references therein.

10.1021/ol050300r CCC: $30.25 
**Published on Web 03/26/2005**

We designed biotin−amino acid conjugates (Scheme 1) such that they would retain the free carboxylic acid unit, as

**Scheme 1.** Synthesis of Gelators[a]




[a] Reagents: (i) methyl ester of amino acids, EDC, DMAP, DMF, rt, 4−6 h; (ii) NaOH, MeOH, rt, 4 h.

in biotin itself, while introducing a new amide bond in conjunction with variable hydrophobicity provided by the amino acid moiety. Notably, the receptor binding site (ureido moiety)[11] remains unaltered.

We examined the gelation ability of gelators **1**−**11** in distilled water, 0.9% aqueous NaCl solution, and buffer solutions with variable pH (Table 1) by adopting the "stable-

**Table 1.** Minimum Gelation Concentrations (MGCs, wt %)[a] of Biotin-Based Hydrogels in Different Solvent Systems

| gelator | $H_2O$ and 0.9% NaCl (aq) | pH 2[b] | pH 4[c] | pH 7[d] | pH 9[e] |
|---|---|---|---|---|---|
| **1** | 2.0 | 1.5 | 2.0 | 3.0 | 4.0 |
| **2** | 1.6 | 1.4 | 1.8 | 2.8 | 3.6 |
| **3** | 1.8 | –[f] | – | – | – |
| **4** | 1.2 | 1.0 | 1.2 | 2.7 | 3.9 |
| **5** | 1.4 | 1.0 | 1.4 | 2.7 | 4.0 |
| **6** | – | – | – | – | – |
| **7** | – | – | – | – | – |
| **8** | 1.2 | 0.9 | 1.2 | 2.5 | 3.8 |
| **9** | 0.3 | 0.2 | 0.5 | 1.2 | 1.5 |
| **10** | 0.8 | 0.6 | 1.0 | 2.0 | 4.0 |
| **11** | 0.6 | 0.8 | 0.8 | 1.7 | 3.5 |

[a] MGC is the lowest gelator concentration at which gelation is observed and restricts flow of medium. [b] Performed with 0.01 M hydrochloric buffer. [c] Performed with 0.05 M phthalate buffer. [d] Performed with 0.08 M MOPSO buffer. [e] Performed with 0.025 M sodium tetraborate buffer. [f] Dashes indicate that no gel formed.

to-inversion of container" method.[12] In this study, weighed quantities of a gelator and a liquid were taken in a sealed glass tube (5 mm i.d.) and then heated at 100 °C until a solution was obtained. The tubes were then maintained at room temperature for 5−10 min. We deemed the samples to be gels when they were not visually phase-separated and did not flow preceptively when the vessels were inverted.

The gelator **9** is the best in this series: its MGC was 0.3% (8 mM) in distilled water, which means that one molecule of **9** can immobilize 6700 molecules of water. The MGC values respond well to the presence of buffer solutions over a range of values of pH. In an acidic milieu, at pH 2, the value of the MGC is even lower than that in distilled water. The hydrogelators having long linear alkyl chains (**9**−**11**) have the lowest values of MGC when compared to branched (**2**, **4**, and **5**) and bulky (**1**) amino acid-appended gelators.

The $\beta$-branched amino acid gelators **4** and **5** and the shorter-alkyl-chain gelator **8** formed opaque gels that had stability lasting for only about 1 week. In contrast, gelators **1**, **2**, and **9**−**11** formed very stable gels that persisted for ca. 6 months in each medium; their clarities ranged from translucent to opaque. Thus, the nature of the hydrophobic residue obviously exerts a strong influence on the stability and the clarity of the gelators. The values of MGC in 0.9% NaCl solution are virtually unchanged relative to those in distilled water, which suggests that these compounds have the potential to be used for in vivo application.

We analyzed the textures of the hydrogels using scanning electron microscopy (SEM). The images of their xerogels[13] (Figure 1) reveal two different types of gels: fibrous and

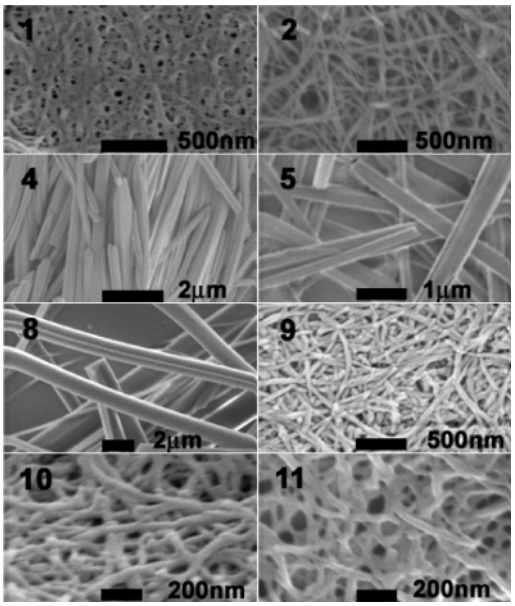



**Figure 1.** SEM images of xerogel of gelators **1**, **2**, **4**, **5**, and **8**−**11**.

lamellar. The hydrogels of **1**, **2**, and **9**−**11** possess fibrous structures that have a range of fiber diameters from 20 to 50 nm. On the other hand, lamellar structures with higher thicknesses existed for the hydrogels of **4**, **5**, and **8**. The gels that had fibrous microscopic structures were either translucent

(11) (a) Hofmann, K.; Titus, G.; Montibeller, J. A. Finn, F. A. *Biochemistry* **1982**, *21*, 978. (b) Weber, P. C.; Ohlendorf, D. H.; Wendoloski, J. J.; Salemme, F. R. *Science* **1989**, *24*, 85.

(12) Menger, F. M.; Caran, K. L. *J. Am. Chem. Soc.* **2000**, *122*, 11679.

(13) Xerogel of hydrogelators was obtained by freezing and pumping the gels of correspoding gelators for 6 h.

or opaque, whereas all of them having lamellar structures were opaque and less stable.

To examine the driving forces behind the self-assembly, we obtained FT-IR[14] and $^1$H NMR spectra. The carbonyl stretching band of the carboxylic acid moiety of each amino acid unit was shifted significantly, to ca. 1699 cm$^{-1}$, in the gel state relative to its location in the solid amorphous state (ca. 1705 cm$^{-1}$). These spectral changes suggest that hydrogen bonding interactions by carboxylic groups provide one of driving forces for gelation. However, the very marginal change of C═O stretching frequency of the amide group in the gel state compared to the solid amorphous state (1645−1650 cm$^{-1}$) indicates that contribution of the amide group toward the self-assembly mode is not appreciable.

We confirmed these findings through $^1$H NMR spectroscopy by monitoring (Figure 2) the changes in the chemical shifts of the protons of the ureido and amido units in solutions of various ratios (v/v) of DMSO-$d_6$ and $H_2O$, as amido and ureido protons do not appear in $^1$H NMR spetra recorded in $D_2O$. We found that when the $H_2O$ content is increased, the amide proton initially shifts to lower field (up to 40% $H_2O$) and then shifts upfield (>40% $H_2O$). This observation indicates a change in the nature of the hydrogen bonding [e.g., from $(CD_3)_3SO\cdots H-N$ to $H_2O\cdots H-N$].[16] Furthermore, the upfield shifts of the amide NH signal at $H_2O$ concentrations > 40% indicate that intermolecular hydrogen bonding occurs among the gelator molecules.[16,17] It has been reported that amide units usually begin to form intermolecular hydrogen bonds at ca. 20−30% $H_2O$;[16] however, in our examples, such formation began at >40% $H_2O$ content. This finding implies that the degree of intermolecular hydrogen bonding of the amide groups is significantly lower than expected, which is consistent with our observations from the FT-IR spectra. A particularly interesting finding we have made is that at an $H_2O$ concentration of only 10%, the resonance of the ureido proton begins to shift upfield; this trend is exactly the same as that observed for biotin itself, which implies that the ureido units form intermolecular hydrogen bonds at this low $H_2O$ concentration and, therefore, that they are responsible for the occurrence of the hydrogelation process. In the crystal structure of biotin, the carboxylic acid unit forms a hydrogen bond with the ureido moiety;[18] thus, we believe that the upfield changes in the chemical shift of the ureido proton are mostly due to intermolecular hydrogen bonding with the carboxylic acid group.

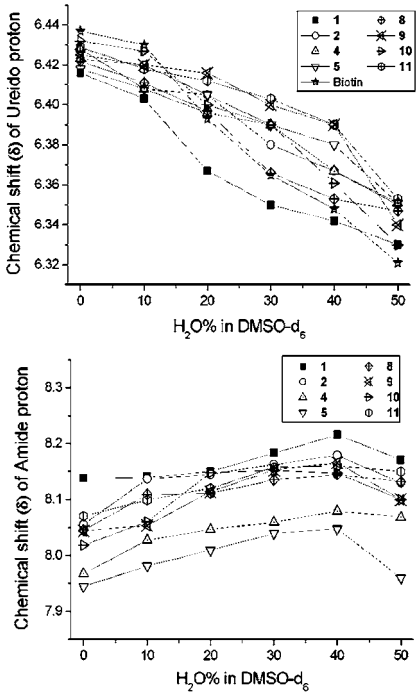



**Figure 2.** Changes in the chemical shifts of protons of the ureido and amido moieties in solutions (5 mg/ mL) containing various ratios (v/v) of DMSO-$d_6$ and $H_2O$.[15]

In addition to these microscopic and spectroscopic studies, we examined the hydrogen bonding interactions between gelator molecules with the help of the AM1 Hamiltonian and considering the MMOK (molecular mechanics corrections to CONH-types of linkages).[19] We found that the most effective hydrogen bonding [lowest heat of formation] occurs between the ureido and carboxylic acid groups of a dimer complex.[14] On the basis of these observations, we anticipate that, in biotin-based hydrogelators, the ureido group of the biotin moiety forms hydrogen bonding intermolecularly to the terminal carboxylic acid unit of another gelator molecule, which leads to the formation of a self-assembled polymer chain. In addition to these hydrogen bonds, hydrophobic interactions and van der Waals forces play important roles in the gelation process as well as in determining the architectural behavior in the gel state.

We added 0.002 equiv[20] of streptavidin to hydrogel in an effort to examine the role of ligand−receptor interaction on the gel state of hydrogelators. It has been observed that the gel state becomes disrupted. In the SEM image of this sample, we observed many cracks on gel fibers (Figure 3), which implies that streptavidin molecules specifically bind with the ureido[11] group of biotin and disrupt the fiber network of the biotin gelator.

Finally, we performed a simple experiment to elucidate the potential for biotinyl hydrogels to act as drug carriers. In this study, gelator **9** was allowed to form a gel in a 50 $\mu$M solution of AZT, and then we monitored the slow release

(14) See Supporting Information.

(15) Maximum $H_2O$ content was 50%. Higher concentration led to solutions too viscous to provide meaningful $^1$H NMR data.

(16) (a) Suzuki, M.; Yumoto, M.; Kimura, M.; Shirai, M.; Hanabusa, K. *Helv. Chim. Acta* **2003**, *86*, 2228. (b) Kogigo, M.; Yumoto, M.; Kimura, M.; Shirai, H.; Hanabusa, K. *Chem. Eur. J.* **2003**, *9*, 348.

(17) (a) Billiot, F. H.; McCarroll, M.; Billiot, E. J.; Rugutt, J. K.; Morris, K.; Warner, I. M. *Langmuir* **2002**, *18*, 2993. (b) Rabenstein, D. L. *J. Am. Chem. Soc.* **1973**, *95*, 2797.

(18) DeTitta, G. T.; Eddmonds, J. W.; Stallings, W.; Donohue, J. *J. Am. Chem. Soc.* **1976**, *98*, 1920.

(19) We used MOPAC6 software (available at http://www.ccl.net) to estimate the hydrogen bonding interactions among these molecules.

(20) We did not extend this experiment further by addition of higher concentration of streptadividin, as its solubility in water is very low (0.1 mg/mL).

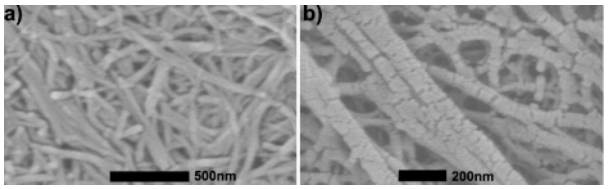



**Figure 3.** SEM image of hydrogel **9**: (a) before (scale bar is 500 nm) and (b) after (scale bar is 200 nm) addtion of streptavidin.

of AZT from the gel phase into water by recording the UV−vis spectroscopic absorption at 266 nm ($\lambda_{max}$ of AZT) with respect to time.[14] It has been found that only ca. 18% of AZT (Figure 4) was released from the gel phase to the water phase after 9 h. In addition, the effect of streptavidin on release of AZT was also investigated. After addition of 0.002 equiv of streptavidin, the amount of released AZT increased by about 1∼7% at each check point. These results indicate that drug release from a biotin hydrogel can be controlled by using streptavidin.

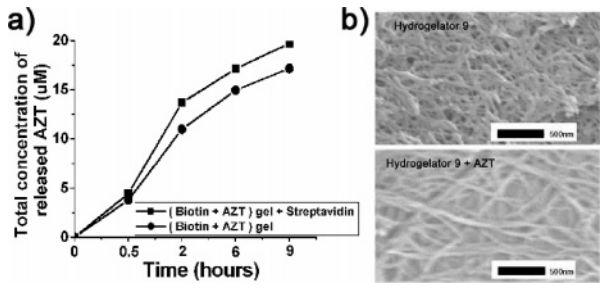



**Figure 4.** (a) Concentration of AZT released from an AZT/**9** hydrogel (0.3 wt %) and an AZT/**9** hydrogel (0.3 wt %) with streptavidin (0.002 equiv) over time. The AZT-containing hydrogel of **9** was prepared in 50 $\mu$M AZT solution. The release of AZT was monitored by UV−vis spectroscopy. (b) SEM images (scale bar is 500 nm) of the gels of **9** and AZT/**9**.

Further evidence that gel contained AZT was provided by its appearance, which changed over time from a homogeneous to a heterogeneous gel, and its microscopic appearance also changed (Figure 4).

In summary, we have synthesized novel and highly efficient hydrogelators using simple amide coupling of amino acids to biotin. The textures of the hydrogels, which reflect their stability, vary depending on the nature of the amino acid moiety's side chain unit. In a preliminary study, we have observed that such hydrogels are efficacious for the slow release of AZT, which suggests that these systems have broad prospects as efficient biomaterials that can play a role as drug carriers.

**Acknowledgment.** We are grateful to MOST for financial support through the NRL program. We also thank the IMT-2000(01-PJ11-PG9-01NT00-0031) and POSCO PBC programs.

**Supporting Information Available:** Experimental procedures and full characterization data of new compounds, FTIR data, calculations of heat of formation data, and process for drug release test. This material is available free of charge via the Internet at http://pubs.acs.org.

OL050300R