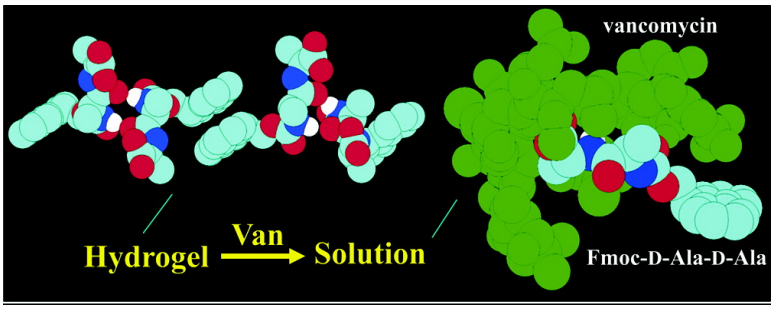
Communication

# Supramolecular Hydrogels Respond to Ligand–Receptor Interaction

Yan Zhang, Hongwei Gu, Zhimou Yang, and Bing Xu

*J. Am. Chem. Soc.*, **2003**, 125 (45), 13680-13681• DOI: 10.1021/ja036817k • Publication Date (Web): 18 October 2003






## More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 32 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 10/18/2003

# Supramolecular Hydrogels Respond to Ligand−Receptor Interaction

Yan Zhang, Hongwei Gu, Zhimou Yang, and Bing Xu*

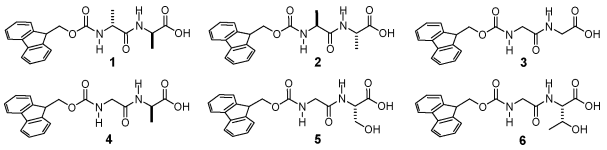
*Department of Chemistry, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong*

Received June 21, 2003; E-mail: chbingxu@ust.hk

This communication describes the first example of supramolecular hydrogels that respond to biological ligand−receptor interaction. As a powerful approach to generate new materials with desired properties, supramolecular interactions[1] have been used to mimic DNA helices or folding of proteins,[2] form liquid crystals,[3] build macromolecules,[4] make chemical sensors,[5] or generate organogels[6] or hydrogels.[7,8] Despite of the report of supramolecular hydrogels that respond to pH or thermal stimuli,[8] a supramolecular hydrogel displaying phase transitions upon ligand−receptor interaction is yet to be designed. In this work, we report a new type of supramolecular hydrogels, based on *N*-(fluorenyl-9-methoxycarbonyl)-D-Ala-D-Ala (**1**), which exhibits gel−sol transition upon binding to its ligand (i.e., vancomycin: Van)[9] via a ligand−receptor interaction that can disturb the delicate balance between hydrophobic interactions and hydrogen bonds and induce a gel−sol transition. In addition, the hydrogel formed by the enantiomer of **1** shows no response to Van. We believe that the ability to control the properties of hydrogels via a biological ligand−receptor interaction or chiral recognition may ultimately lead to a new disease- or tissue-specific drug delivery system.

During the synthesis of pyrene butyryl D-Ala-D-Ala as a short oligopeptidic hydrogelator, we observed that the intermediate, Fmoc-D-Ala-D-Ala (**1**), formed hydrogels with high efficiency (one molecule of **1** can gel ∼15000 $H_2O$ molecules). Since Fmoc protected dipeptides are common intermediates in the synthesis of peptides and some Fmoc-amino acids are antiinflammatory agents,[10] we synthesized a series of Fmoc-dipeptides (Scheme 1) for the

***Scheme 1***




purpose of developing a new class of low-molecular weight hydrogelators that is readily available and may serve as new biomaterials.

Starting from commercially available Fmoc-amino acids, we obtained final products with overall yields of 60−70%.[11] The conditions for the dipeptides to form the hydrogels are listed in Table 1. **1** and **2** show excellent ability to gel water at pH = 3, forming hydrogels with the gelator concentration of ∼4 mM. Substituting the two alanines in **1** with glycines affords **3**, which exhibits behavior of gelation similar to those of **1** and **2** except at higher concentration ([**3**] =11 mM). **4** and **5** form hydrogels at higher gelator concentrations and different pH. **6** fails to gel water in similar conditions probably due to the relatively large side chain at the α-position of threonine. From **3**−**6**, the increase of steric hindrance apparently reduces their ability to form hydrogels. Further study on structure−gelation relationship is underway.

Circular dichorism (CD) of **1** and **2** are nearly mirror images (Figure 1). The Cotton effects at 219 ($n\pi^*$ transition) and 246−304 nm ($\pi\pi^*$ transition) indicate the superhelical arrangements formed by the D-Ala or L-Ala residues and the fluorenyl groups in the hydrogels, respectively.[12] As expected, the hydrogel of **3** gives no CD signal. The peaks of emission at 328 nm in the hydrogels of **1** and **2** suggest that the two fluorenes overlap in an antiparallel mode.[13] The peak at 323 nm and the shoulder at 375 nm of the hydrogel of **3** indicate that a small amount of $\pi-\pi$ overlap is in parallel fashion,[13] likely due to less steric hindrance from **3**.[11] Hydrogels formed by compound **1**, **2**, and **3** exhibit similar responses to thermal and pH changes. The most appropriate pH value for **1**, **2**, and **3** to form gel is 3. The dipeptides precipitate easily at pH lower than 3 and dissolve immediately at pH = 6. The pH response of the gel is completely reversible.[11] Upon heating, the matrix formed by the gelators collapses into a precipitate, and water molecules are expelled. While cooling back to room temperature, the shrunken gel swells to form gel again,[11] similar to the hydrogels reported by Hamachi et al.[8]

***Table 1.*** Conditions for the Sol−Gel or Gel−Sol Transition of **1**−**6**

| | compound | *C* (mM)[a] | *T* (°C)[b] | pH[c] | Van[d] |
|---|---|---|---|---|---|
| **1** | Fmoc-D-Ala-D-Ala | 3.6 | 72 | 3 | 1 |
| **2** | Fmoc-L-Ala-L-Ala | 3.9 | 74 | 3 | − |
| **3** | Fmoc-Gly-Gly | 11 | 68 | 3 | 1 |
| **4** | Fmoc-Gly-D-Ala | 46 | 44 | 5 | 1[f] |
| **5** | Fmoc-Gly-L-Ser | 52 | 53 | 5 | − |
| **6** | Fmoc-Gly-L-Thr | *e* | n/a | n/a | n/a |

[a] The minimum concentrated of the gelator needed for gelation. [b] The temperature at which the gel becomes solution. [c] The pH value for gelation [d] The equivalent of Van needed for the complete gel−sol transition. [e] No gelation (or a negative result). [f] Precipitation.

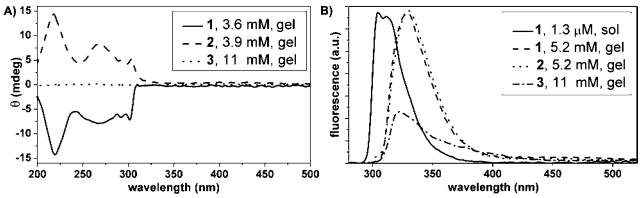



***Figure 1.*** (A) The CD and (B) the emission spectra ($\lambda_{excitation}$ = 266 nm) of the hydrogels of **1**, **2**, and **3**.

The most remarkable feature of the hydrogels is their response to a specific biological ligand−receptor interaction. Since the dipeptides bind to Van with different affinities, the hydrogels formed by them show different responses when Van is added. The hydrogel of **1** is quite sensitive to Van; even the addition of 0.01 equiv (vs **1**) of Van solid to the hydrogel results in ∼40% of water being expelled from the hydrogel, the increase of the amount of Van to 0.1 equiv turns the hydrogel into a milky suspension, and the addition of 1 equiv of Van gives clear solutions (Figure 2, B−D). The hydrogel of **3** shows similar sensitivity to that of the hydrogel of **1**. Upon the addition of 1 equiv of Van, the hydrogel of **4** becomes a suspension. The hydrogel of **2** hardly has any change even after adding 1 equiv of Van, which agrees with the fact that L-Ala-L-Ala essentially does not bind to Van.[14] Since **2** is the



**10.1021/ja036817k CCC: $25.00** 

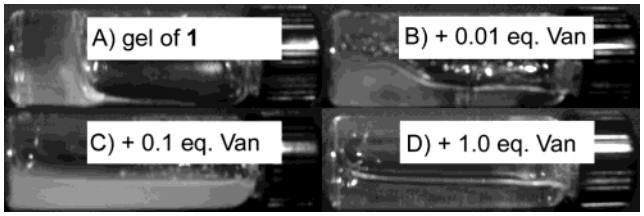



***Figure 2.*** (A) Hydrogel of **1** ([**1**] = 3.6 mM in $H_2O$). The phase transitions of the hydrogel of **1** induced by the addition of (B) 0.01, (C) 0.1, and (D) 1.0 equiv of Van solid into the hydrogel.

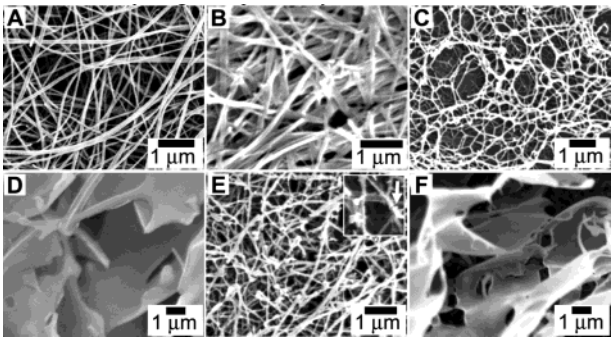



***Figure 3.*** Cryo-SEM images[11] of the nanofibers in the hydrogels of (A) **1**, (B) **2**, and (C) **3**, and the SEM images of the mixtures after 1 equiv of Van solid was added to the hydrogels of **1** (D), **2** (E, inset: arrow indicated the precipitate of Van), or **3** (F).

enantiomer of **1**, this result also represents the first example that a ligand destroys the hydrogel of its receptor only based on stereochemistry.

To elucidate the distribution of the Van in the hydrogels, we performed the morphological study of the hydrogels before and after adding Van. As shown in Figure 3, scanning electron micrographs (SEM) indicate that the nanofibers (~50 nm wide) of the dipeptides constitute the matrices of the corresponding hydrogels, which agree with the transmission electron micrographs (TEM)[11] of the hydrogels. The morphology of the nanofibers formed by **3** differs from that of the nanofibers formed by **1** and **2** in terms of persistent length, which is consistent with their stacking modes and chirality. After adding 1 equiv of Van solid, SEM images show that the network in the hydrogel of **2** remains unaffected, while the matrices in the hydrogels of **1** and **3** are completely destroyed by the ligand−receptor binding between Van and **1** or **3**.[14] The (almost) uniform distribution of the aggregates of Van on the surface of the nanofibers of **2** indicates that Van dissolves homogeneously in the water phase of the hydrogel of **2**. These results agree with the fact that D-Ala-D-Ala has strong ligand−receptor interaction with Van, while L-Ala-L-Ala has no obvious binding.[14] On the basis of CD and emission spectra, we suggest the following plausible mechanism for the response: Van binds to the D-Ala-D-Ala via four to five hydrogen bonds (Figure 4), and the biphenyl moiety on the Van blocks the $\pi-\pi$ interactions between fluorenyl groups, thus preventing the formation of the supramolecular polymers needed for gelation. In addition, one equivalent of teicoplanin solid,[9] an analogue of Van, converts the hydrogels of **1**, **3**, or **4** to suspensions but exerts no influence on the hydrogels of **2** or **5**, which further supports the proposed mechanism. In conclusion, we have shown a new kind of small molecular hydrogelators based on

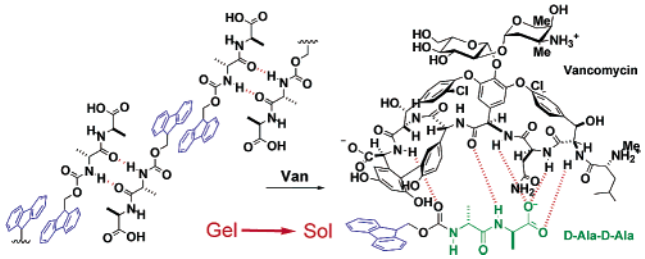



***Figure 4.*** Possible ligand−receptor interactions that induce the gel−sol phase transition.

dipeptides whose hydrogels respond to a ligand−receptor interaction and exhibit chiral recognition. The vast pool of ligand−receptor pairs should offer many useful candidates for the design of supramolecular hydrogels that respond to specific interactions.

**Acknowledgment.** This work was partially supported by RGC (Hong Kong), HIA (HKUST), and a DuPont Young Faculty Grant.

**Supporting Information Available:** Details of the synthesis, the response of the gels to pH and thermal stimuli, and additional electron micrograph (PDF). This material is available free of charge via the Internet at http://pub.acs.org.

## References

(1) Lehn, J.-M. *Supramolecular Chemistry: Concepts and Perspectives*; VCH: Weinheim, 1995.
(2) Hasenknopf, B.; Lehn, J. M.; Boumediene, N.; DupontGervais, A.; VanDorsselaer, A.; Kneisel, B.; Fenske, D. *J. Am. Chem. Soc.* **1997**, *119*, 10956; Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893; Nelson, J. C.; Saven, J. G.; Moore, J. S.; Wolynes, P. G. *Science* **1997**, *277*, 1793.
(3) Kato, T.; Kihara, H.; Kumar, U.; Uryu, T.; Frechet, J. M. J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1644; Ungar, G.; Liu, Y. S.; Zeng, X. B.; Percec, V.; Cho, W. D. *Science* **2003**, *299*, 1208.
(4) Zubarev, E. R.; Pralle, M. U.; Li, L. M.; Stupp, S. I. *Science* **1999**, *283*, 523; Stupp, S. I.; LeBonheur, V.; Walker, K.; Li, L. S.; Huggins, K. E.; Keser, M.; Amstutz, A. *Science* **1997**, *276*, 384.
(5) Zhu, S. S.; Carroll, P. J.; Swager, T. M. *J. Am. Chem. Soc.* **1996**, *118*, 8713.
(6) Murata, K.; Aoki, M.; Suzuki, T.; Harada, T.; Kawabata, H.; Komori, T.; Ohseto, F.; Ueda, K.; Shinkai, S. *J. Am. Chem. Soc.* **1994**, *116*, 6664; Terech, P.; Weiss, R. G. *Chem. Rev.* **1997**, *97*, 3133; Hirst, A. R.; Smith, D. K.; Feiters, M. C.; Geurts, H. P. M.; Wright, A. C. *J. Am. Chem. Soc.* **2003**, *125*, 9011.
(7) Xing, B.; Yu, C.-W.; Chow, K.-H.; Ho, P.-L.; Fu, D.; Xu, B. *J. Am. Chem. Soc.* **2002**, *124*, 14846; Vauthey, S.; Santoso, S.; Gong, H. Y.; Watson, N.; Zhang, S. G. *Proc. Natl Acad. Sci. U.S.A.* **2002**, *99*, 5355; Collier, J. H.; Hu, B. H.; Ruberti, J. W.; Zhang, J.; Shum, P.; Thompson, D. H.; Messersmith, P. B. *J. Am. Chem. Soc.* **2001**, *123*, 9463; Niece, K. L.; Hartgerink, J. D.; Donners, J. J. J. M.; Stupp, S. I. *J. Am. Chem. Soc.* **2003**, *124*, 7146; Holmes, T. C.; de Lacalle, S.; Su, X.; Liu, G. S.; Rich, A.; Zhang, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6728; Menger, F. M.; Caran, K. L. *J. Am. Chem. Soc.* **2000**, *122*, 11679; Estroff, L. A.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2000**, *39*, 3447.
(8) Kiyonaka, S.; Sugiyasu, K.; Shinkai, S.; Hamachi, I. *J. Am. Chem. Soc.* **2002**, *124*, 10954.
(9) Walsh, C. *Antibiotics: Actions, Origins, Resistance*, 1st ed.; ASM Press: Washington, DC, 2003.
(10) Burch, R. M.; Weitzberg, M.; Blok, N.; Muhlhauser, R.; Martin, D.; Farmer, S. G.; Bator, J. M.; Connor, J. R.; Ko, C.; Kuhn, W.; McMillan, B. A.; Raynor, M.; Shearer, B. G.; Tiffany, C.; Wilkins, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 355.
(11) Supporting Information.
(12) Berova, N.; Nakanishi, K.; Woody, R. W., Eds. *Circular Dichroism: Principles and Applications*, 2nd ed.; Wiley-VCH: New York, 2000.
(13) Schweitzer, D.; Hausser, K. H.; Haenel, M. W. *Chem. Phys.* **1978**, *29*, 181.
(14) Jorgensen, T. J. D.; Roepstorff, P.; Heck, A. J. R. *Anal. Chem.* **1998**, *70*, 4427.

JA036817K