

© Copyright 2000 by the American Chemical Society

VOLUME 104, NUMBER 15, APRIL 20, 2000

LETTERS

Crystalline Glycylglycine Bolaamphiphile Tubules and Their pH-Sensitive Structural Transformation

Hiroshi Matsui* and Bogdan Gologan

University of Central Florida, Department of Chemistry, Orlando, Florida 32816 Received: November 18, 1999; In Final Form: February 11, 2000

The assembled structure of the heptane bolaamphiphile, bis(N- α -amido-glycylglycine)-1,7-heptane dicarboxylate, displays a sensitivity to the acidity of a solution. At pH 4, the heptane bolaamphiphile grows to a crystalline tubule in two weeks. At pH 8, a helical ribbon structure is formed in one week. The degree of carboxylic acid protonation was used to control the final assembled structures since the structures are determined by the strengths of the amide—amide and carboxylic acid dimer hydrogen bonds. Direct structural transformation between tubules and helical ribbons was also confirmed as a function of pH using optical microscopy and Raman microscopy. Conversion from the helical ribbons to the tubules occurs within one day, while the reverse conversion, from the tubules to the helical ribbons, is ten times slower.

In order to design nanometer-scale devices, understanding the rules of molecular self-organization has been an important challenge.^{1,2} We have been able to learn assembly mechanisms from a variety of biological systems that build functional nanoscale structures.³ To match the ability of self-organization in biological systems, peptide assemblies have been synthesized that mimic natural systems.⁴⁻⁶ Peptide assemblies may also have potential application as delivery vehicles into living cells.

It has been observed that crystalline microsphere assemblies are triggered by protonation and that the microspheres are formed instantaneously.^{7,8} These results indicate that protonation of carboxylic groups within molecular assemblies can change their structures dramatically.^{9,10} Recently, a family of bolaamphiphiles was described whose protonation induced tubule and vesicle formations.¹¹ Our bolaamphiphiles have two carboxylic acid headgroups and the assembled structures are expected to be sensitive to solution pH.

In this work, one of the bolaamphiphiles, bis(N- α -amidoglycylglycine)-1,7-heptane dicarboxylate (Figure 1), was dispersed in solutions of various pH. Synthesis of the heptane bolaamphiphile is described elsewhere. Assemblies in citric

acid solutions appeared after one to three weeks at room temperature. At pH 8, the heptane bolaamphiphiles assembled into a helical ribbon in one week (Figure 1a). At pH 4, the bolaamphiphiles assembled into a tubule in two weeks (Figure 1b). The tubule diameter is much smaller than the width of the helical structure. Figure 1b suggests that the tubules are hollow structures.

Raman spectra of the helical ribbon, tubule, and heptane bolaamphiphile crystal phases (Figure 2) were obtained using a Raman microscope (Jobin Yuon/Horiba, LabRam). The crystalline spectrum (c) and the tubule spectrum (b) are almost identical. Thus, the assembled bolaamphiphile tubules have a crystalline structure. Discrepancies of peak positions between the helical ribbon spectrum (a) and tubule spectrum (b) occur in the region from 1600 to 1700 cm⁻¹. This region represents C=O stretches in amide groups. Observed C=O stretch frequency shifts depend on the strengths of the intermolecular hydrogen bonds between C=O and HN. Raman spectra show that the tubule and crystal forms have two C=O peaks at 1637 and 1660 cm⁻¹ while the helical ribbon phase has a peak for the C=O stretch at 1644 cm⁻¹. The C=O stretch with the

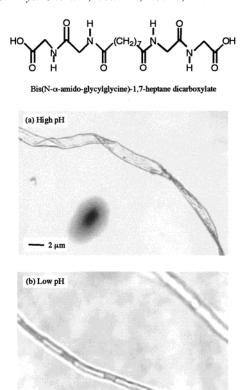


Figure 1. Heptane bolaamphiphile assemblies by light microscopy. (a) In a pH = 8 solution, the heptane bolaamphiphile assembled into helical ribbons. The average width of the helices is about 2 μ m and the length varies from 10 μ m to 40 μ m. The helical structures appeared after one week. (b) In a pH = 4 solution, the bolaamphiphile monomers assembled into tubule structures. The average diameter of the tubules is 500 nm. The tubules with hollow structures appeared after two weeks.

- 1 μm

highest frequency is assigned as the free C=O of the amide group. The C=O frequencies at 1637 and 1644 cm⁻¹ are red shifted from the free C=O stretch since these C=O stretches bind the amide NH group via intermolecular hydrogen bonds. These assignments suggest that the C=O group in the tubule and crystal phases binds to NH stronger than to the C=O group in the helical ribbon form due to a red shift of the vibrational frequency. FTIR microscopy probes a carboxylic acid vibrational mode in the helical ribbon at 1739 cm⁻¹, while a carboxylic acid peak in the tubule appears at 1720 cm⁻¹.8 The blue shifted carboxylic acid frequency in the helical ribbon indicates that the heptane bolaamphiphile molecule assembles with anionacid hydrogen bonds (COO-H+-OOC) at pH 8. Carboxylic acid vibrations in the acid-anion dimers have been observed around 1740 cm⁻¹ in carboxylic acids, 12 anionic lipids on membranes, ¹³ amphiphiles with a glyconamide headgroup, ¹⁴ and various bolaamphiphile assemblies. 11 The red-shifted carboxylic acid frequency at 1720 cm⁻¹ in the tubule suggests that stronger acid-acid hydrogen bonds (COOH-HOOC) induce the tubule formation.¹⁵

The heptane bolaamphiphile structure transformation is modeled based on the single-crystal bolaamphiphile study. Lengths and conformations of amide—amide hydrogen bonds are determined by the C=O frequency shifts in the Raman spectra. Figure 3 illustrates that a unit cell of heptane bolaamphiphile assembly has a hexagonal symmetry in the yz plane. A molecular picture of the transformation from helical ribbon to tubule (Figure 3, (a) \rightarrow (b)), suggests that a bolaamphiphile molecule rearranges to form a strong intermolecular amide—

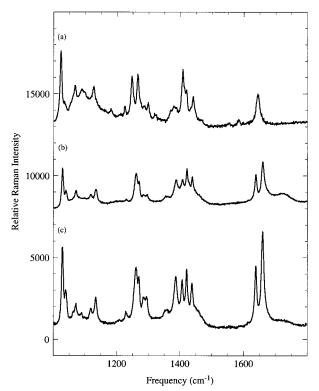


Figure 2. Raman spectra of (a) helical ribbon, (b) tubule, (c) heptane bolaamphiphile crystal.

amide hydrogen bond with another molecule in the xz plane as the pH increases. Another amide also forms the hydrogen bond in the (1/2y + 1/2z,x) plane. A pair of heptane carboxylic bolaamphiphiles is also connected via carboxylic dimer hydrogen bonds along the x direction. Although the configuration in the tubule contains unbound C=O groups, the stronger amide—amide hydrogen bonds seem to pack bolaamphiphiles closer than the configuration in the helical ribbon (Figure 3b). This efficient packing conformation stabilizes the assembly in forming the crystalline structure. Hydrophobic and electrostatic interactions may also contribute to the stability of the tubule assembly. 17

In a macroscopic picture, the heptane bolaamphiphiles assemble as a planar multilayer and then COO⁻—H⁺—OOC and amide—amide interactions induce a twist in the bolaamphiphile layer to produce the helical structure at pH 8. At pH 4, the bolaamphiphile layer curls tighter due to shortening COOH dimer and amide—amide hydrogen bonds. In other words, shortening hydrogen bonds induces further tilt of the heptane bolaamphiphile arrangement and makes the assembly surface more convex. This greater molecular tilting leads the assembled structure to the tubule form. This proposed scheme is summarized in Figure 4. A section of the heptane bolaamphiphile scroll was observed as screw-like stripes in a large tubule grown at pH 5 (Figure 5). This is direct evidence that the heptane bolaamphiphile assembly undergoes the proposed wrapping assembly scheme to form the tubule structure.

One indirect observation supports the molecular tilt effect in these bolaamphiphile assemblies. Hexane bolaamphiphiles, which have six carbons linking two glyclyglycine headgroups, assembled into tubules at pH 3 but did not show structural transformation at higher pH values. X-ray analysis shows that a bolaamphiphile with even carbon numbers uses all of the amide and carboxylic groups to bind to other bolaamphiphile molecules via eight amide—amide hydrogen bonds and two acid—acid hydrogen bonds. ¹⁶ In contrast, the heptane bolaamphiphile assemblies are formed via either eight weak amide—

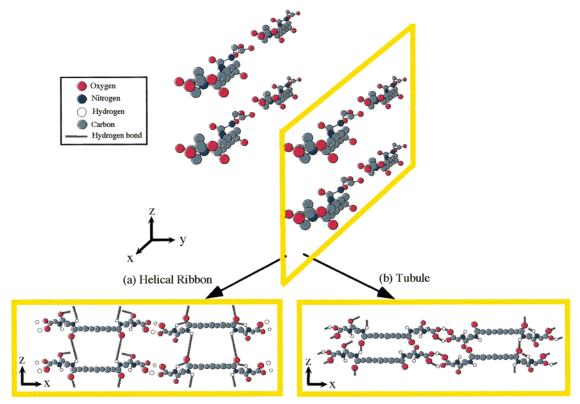


Figure 3. Assembled structures of the heptane bolaamphiphile in (a) the helical ribbon: A pair of the heptane bolaamphiphiles is connected by two amide—amide hydrogen bonds along the z direction and along the 1/2y + 1/2z direction, respectively, while a pair of heptane bolaamphiphiles is connected via acid—anion interactions (COO⁻—H⁺—OOC) along the x direction. (b) The tubule: A pair of heptane bolaamphiphiles is connected by hydrogen bonds between two COOH groups via acid—acid dimer interactions in the x direction. An intermolecular amide—amide hydrogen bond is formed along the z direction and along the 1/2y + 1/2z directions, respectively.

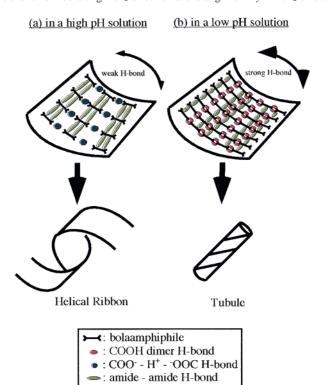


Figure 4. Proposed heptane bolaamphiphile assembly mechanism: (a) the helical ribbon (b) the tubule.

amide and two weak anion—acid hydrogen bonds (helical ribbon), or four strong amide—amide and two acid—acid hydrogen bonds (tubule). The helical ribbons have the same number of hydrogen bonds as the hexane bolaamphiphile assembly, but

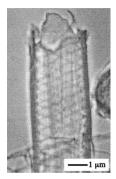


Figure 5. Tubule assembly of the heptane bolaamphiphile in a pH = 5 solution by light microscopy.

the hydrogen bonds in the helical ribbons are weaker. Although the hydrogen bonds in the tubules are stronger, more hydrogen bonds are formed in the hexane bolaamphiphile assembly. Therefore, the heptane bolaamphiphiles seem to have more freedom in the assemblies for molecular tilt to develop. The degree of freedom in self-assemblies, therefore, may play an important role in giving structural transformations with pH change.

Direct structural transformation between the tubules and helical ribbons was also confirmed as a function of pH. Conversion from the helical ribbons to the tubules occurs within one day, while the reverse conversion, from the tubules to the helical ribbons, is ten times slower. Yield of the helical ribbons from tubules under basic conditions is much lower than the yield of tubules from the helical ribbons under acidic conditions. This observation is reasonable since the crystallinity of the tubule is higher than the helical ribbon probed by Raman microscopy.

More flexible helical ribbon structures seem to undergo structural transformations relatively easily.

The heptane bolaamphiphile results presented here may have potential in controlled release applications. Carriers with simple release mechanisms may be more effective if they can be activated at the desired target area. Releasing via structural transformation is one of the simplest mechanisms. The rigid heptane bolaamphiphile tubule structure may also have an advantage for holding molecules until they reach the target location. Optimization of the bolaamphiphile configurations and experimental conditions is proceeding to increase the yield of tubules from the helical ribbon for these applications.

Acknowledgment. This work was supported by University of Central Florida, Office of the Vice President for Research and Graduate Studies, I-4 Matching Fund. We thank Dr. Howard Schaffer at Jobin Yvon/Horiba for Raman microscopic studies. H.M. also acknowledges Professor B. Fookes for assistance with FTIR microscopy and light microscopy. B.G. acknowledges Professor O. Phanstiel IV for the assistance in bolaamphiphile synthesis.

References and Notes

(1) Alivisatos, A. P.; Johnsson, K. P.; Peng, X.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P., Jr.; Schultz, P. G. *Nature* **1996**, *382*, 609.

- (2) Jenekhe, S. A.; Chen, X. L. Science 1999, 283, 372.
- (3) Philip, D.; Stoddart, J. F. Angew. Chem., Int. Ed. Engl. 1996, 35, 154
- (4) Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. *Nature* **1993**, *366*, 324.
 - (5) Fuhrhop, J.-H.; Helfrich, W. Chem. Rev. 1993, 93, 1565.
- (6) Barklis, E.; McDermott, J.; Wilkens, S.; Fuller, S.; Thompson, D. J. Biol. Chem. 1998, 273, 7177.
- (7) Phanstiel, O., IV.; Matsui, H.; Erdos, G.; Lachicotte, R.; Torres, D.; Richardson, M.; Seconi, D. C.; Schaffer, H.; Adar, F., in preparation.
- (8) Matsui, H.; Gologan, B.; Schaffer, H.; Adar, F.; Seconi, D.; Phanstiel, O., IV. *Langmuir* **2000**, *16*, in print.
- (9) Bergeron, R. J.; Phanstiel, O., IV.; Yao, G. W.; Milstein, S.; Weimar, W. R. J. Am. Chem. Soc. 1994, 116, 8479.
- (10) Bergeron, R. J.; Yao, G. W.; Erdos, G. W.; Milstein, S.; Gao, F.; Weimar, W. R.; Phanstiel, O., IV. J. Am. Chem. Soc. 1995, 117, 6658.
- (11) Kogiso, M.; Ohnishi, S.; Yase, K.; Masuda, M.; Shimizu, T. Langmuir 1998, 14, 4978.
- (12) Smith, J. G.; Walzen, R. L.; German, J. B. *Biochim. Biophys. Acta* **1993**, *1154*, 327.
 - (13) Haines, T. H. Proc. Natl. Acad. Sci. U.S.A. 1983, 80, 160.
- (14) Fuhrhop, J.-H.; Demoulin, C.; Rosenberg, J.; Boettcher, C. J. Am. Chem. Soc. 1990, 112, 2827.
- (15) Lin-Vien, D.; Colthup, N. B.; Fateley, W. G.; Grasselli, J. G. *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*; Academic Press: New York, 1991.
- (16) Kogiso, M.; Masuda, M.; Shimizu, T. Supramol. Chem 1998, 9, 183
- (17) Thompson, D. H.; Wong, K. F.; Humphry-Baker, R.; Wheeler, J. J.; Kim, J.-M.; Rananavare, B. J. Am. Chem. Soc. 1992, 114, 9035.