J|A|C|S
COMMUNICATIONS

Published on Web 06/16/2006

Lyotropic Liquid Crystals from Designed Helical β-Peptides

William C. Pomerantz,[†] Nicholas L. Abbott,*[,‡] and Samuel H. Gellman*[,†]

Departments of Chemical and Biological Engineering and Chemistry, University of Wisconsin, Madison, Wisconsin 53706

Received April 7, 2006; E-mail: gellman@chem.wisc.edu; abbott@engr.wisc.edu

Liquid crystals (LCs) have been widely explored for a variety of applications, including display[1] and sensing technologies.[2] Recently, the highly cooperative behaviors of molecules within lyotropic LCs have been exploited to amplify the presence of targeted biological entities, such as proteins,[3] viruses,[4] and microbes[5] for optical sensing applications. Here we introduce a new type of mesogen, helix-forming oligomers of β-amino acids (β-peptides),[6] and we outline relationships between β-peptide sequence and the capacity to form lyotropic liquid crystalline phases in water.

The well-known ability of α-helical poly(α-amino acids) to form lyotropic liquid crystalline phases[7] inspired our efforts. α-Helical polypeptides must be quite long, however, to form LC phases.[8] In general, this length requirement has necessitated the use of materials that are polydisperse in size and limited in sequence, which has hampered exploration of sequence−property correlations. We hypothesized that LC phases would be accessible with relatively short helical β-peptides because these foldamers display higher helix stability, on a per-residue basis, than do α-peptides when cyclically constrained β-amino acids, such as *trans*-2-aminocyclohexanecarboxylic acid (ACHC),[9] are used. If short β-peptide oligomers form LC phases in water, then the perfect control of sequence, composition, and length made possible by solid-phase synthesis can be used to probe relationships between β-peptide structure and liquid crystallinity. These relationships could provide guidance for future mesogen designs directed toward specific applications.

We prepared homologous series **1**−**4** to test our design hypothesis. Extensive structural analysis of closely related β-peptides allows us to predict that **1**−**4** will adopt the 14-helical conformation, a secondary structure that is defined by 14-membered ring C═O(*i*)···H−N(*i*-2) H-bonds between backbone amide groups and that has approximately three β-amino acid residues per turn of helix.[10] In **1**−**4**, the number of ACHC−ACHC−β^3-hLys triads increases from one to four. This sequence design should generate folded conformations displaying a global segregation of hydrophobic ACHC residues on one face of the 14-helix and hydrophilic β^3-hLys residues on the other face (Figure 1, lower left). This global amphiphilicity is intended to promote hydrophobically driven self-assembly in aqueous solution. β-Peptide **5**, a sequence isomer of **3**, does not allow global segregation of ACHC and β^3-hLys residues in the 14-helical state (Figure 1, lower right). Previous studies in dilute solution have shown that the enantiomer of **3** self-associates into small soluble aggregates, while the nonglobally amphiphilic isomer, *ent*-**5**, remains monomeric.[10a]

We used optical microscopy for initial evaluation of aqueous solutions of **1**−**5**. For each β-peptide, aqueous solutions of varying concentration were drawn into microcapillaries and examined between crossed polarizing filters at room temperature (Figure 2). In such experiments, the observation of birefringence is taken as evidence of LC phase formation.[11] No birefringence was observed

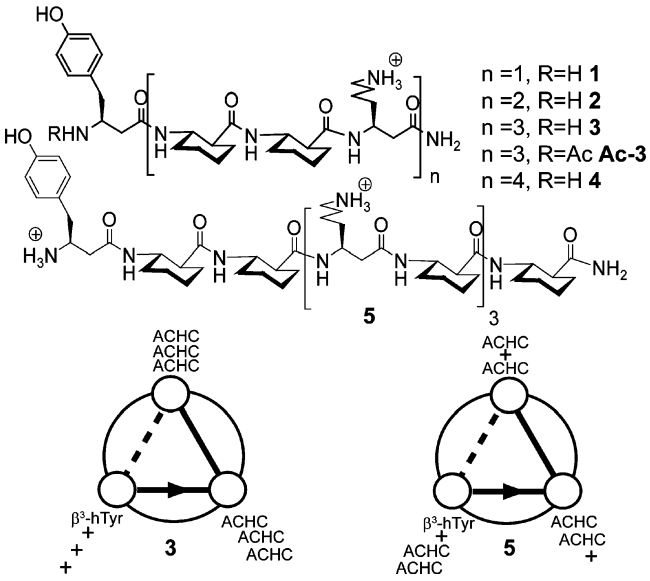

Figure 1. Top: 14-Helical β-peptide sequences **1**−**5**. Bottom: Helical wheel diagrams of **3** and **5**. ACHC = *trans*-2-aminocyclohexane carboxylic acid; "+" = β^3-hLys).

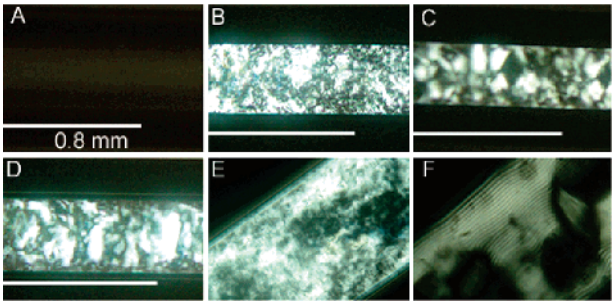

Figure 2. Optical micrographs of aqueous solutions of β-peptides **1**−**3** between crossed polarizing filters. (A) **1**, 25 wt %. (B) **2**, 19 wt %. (C) **3**, 10 wt %. (D) **Ac-3**, 2.5 wt %. (E) 2× **2**, 19 wt %. (F) 2.5× **3**, 10 wt %.

for the shortest β-peptide, tetramer **1**, up to 25 wt % (410 mM, Figure 2A) or upon evaporation to dryness, but heptamer **2** displayed birefringence at concentrations as low as 19 wt % (180 mM, Figure 2B). The marbled optical texture (Figure 2E) suggests that **2** forms a nematic LC phase under these conditions.[12] Increasing β-peptide length by one triad, to generate **3**, leads to LC behavior at lower concentrations, as shown by the image obtained for a 10 wt % sample (61 mM, Figure 2C). In this case, the "fingerprint" pattern[12] evident upon closer inspection (Figure 2F) suggests a cholesteric LC phase. Thus, self-assembly of the chiral helix formed by **3** can induce a helical ordering of molecules in the LC phase, whereas the two-turn, chiral helix formed by **2** is unable to induce this higher ordered phase. This behavior can be rationalized by proposing that **3** displays a greater expanse of hydrophobic surface

[†] Department of Chemical and Biological Engineering.
[‡] Department of Chemistry.

10.1021/ja062412z CCC: $33.50

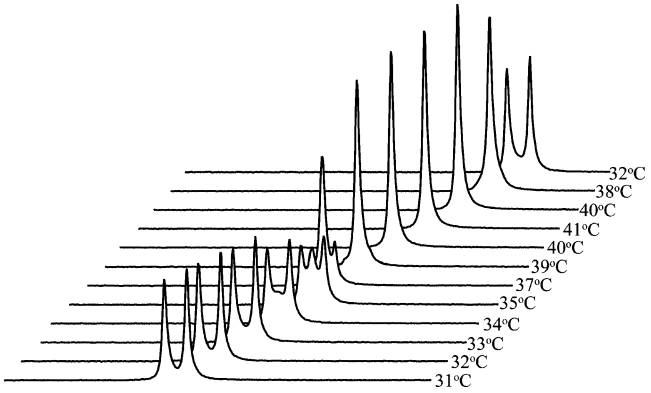

Figure 3. ^{2}H NMR spectra of a 10 wt % solution of **3** in D_2O at various temperatures (10 min equilibration before data acquired).

than does **2**, leading to stronger intermolecular attraction for **3** than for **2**. Further lengthening seems to cause even more avid self-assembly, as aqueous solutions of 2 wt % **4** phase-separate into an isotropic liquid (i.e., nonliquid crystalline) and a gel, which is birefringent.[13] The behavior of **5**, the sequence isomer of **3** that cannot form a globally amphiphilic 14-helix, supports our hypothesis that hydrophobically driven interactions among β-peptides are crucial for LC phase formation because **5** displays no birefringence at the highest concentration tested, 15 wt %.[13] Even upon slowly evaporating the concentrated sample of **5** to dryness, we observed no birefringence.

We explored the behavior of **3** in greater detail by varying concentration as well as net charge. Dilution of a 10 wt % sample of **3** to 8 wt % induced a coexistence state in which both the isotropic and LC phases were observable. This coexistence state was indicated by the presence of nematic droplets.[13] Dilution to 7 wt % completely abolished the LC phase. Acetylation of the N-terminus to generate **Ac-3** reduced the concentration of β-peptide necessary for LC phase formation to as low as 2.5 wt % (15 mM, Figure 2D). This result implies that diminution of electrostatic repulsion can have a profound effect on LC formation. Similar trends have been observed among β-strand-forming α-peptides that display LC behavior.[14]

We turned to NMR measurements for further characterization of the LC phase formed by **3**. If a liquid crystalline phase is formed in D_2O, the quadrupolar coupling between the D atoms gives rise to characteristic D NMR line shapes.[15] Figure 3 shows the effect of temperature on LC phase formation by β-peptide **3** (10 wt %) in D_2O. At or below 31 °C, the D_2O resonance is split, indicating the existence of an LC phase. As the temperature is increased, a third resonance grows between the two branches of the doublet; we interpret this third resonance as arising from D_2O in an isotropic environment. Around 40 °C, only the isotropic resonance is observed, but upon cooling, the LC doublet reappears, which indicates that LC formation is reversible on a time scale of minutes. These observations are consistent with optical microscopy of a 10 wt % sample of **3** at varying temperatures.[13] The NMR approach is superior to microscopy for such studies as NMR allows accurate temperature control and identification of small populations of the LC or isotropic phase.

We have shown that short β-peptides can serve as mesogens for lyotropic LC phase formation in water. The example studied most carefully, deca-β-peptide **3**, forms a cholesteric phase at room temperature. Liquid crystallinity appears to require the adoption of a globally amphiphilic conformation because sequence isomer **5** does not display LC behavior. Although we lack direct evidence for helix formation in concentrated solution, we note that ACHC homooligomers crystallize as 14-helices.[9a] LC phase behavior of **3** is modulated by concentration and temperature; a change of net charge (**Ac-3**) results in a room temperature LC phase at just 2.5 wt %. We are unaware of any LC-forming helical α-peptides that contain so few residues. Nematic LC phases and gels from β-strand-forming α-peptides in this length range are known,[8] but the time scale of formation of these LC phases (weeks) is substantially longer than for the β-peptide LC phases reported here.[8a] Our results suggest that β-peptides offer a tunable scaffold for tailoring LC properties for biomolecular sensing applications.

Acknowledgment. This research was supported by UW−Madison Nanoscale Science and Engineering Center (NSF Grant DMR-0425880). NMR equipment purchase was supported in part by grants from NIH and NSF.

Supporting Information Available: Optical microscopy data and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kelker, H.; Hatz, R. In *Handbook of Liquid Crystals*; Verlag Chemie: Weinheim, Germany, 1980.
(2) (a) Jang, C.-H.; Tingey, M. L.; Korpi, N. L.; Wiepz, G. J.; Schiller, J. H.; Bertics, P. J.; Abbott, N. L. *J. Am. Chem. Soc.* **2005**, *127*, 8912−13. (b) Gupta, V. K.; Skaife, J. J.; Dubrovsky, T. B.; Abbott, N. L. *Science* **1998**, *279*, 2077−80. (c) Tingey, M. L.; Wilyana, S.; Snodgrass, E. J.; Abbott, N. L. *Langmuir* **2004**, *20*, 6818−26. (d) Luk, Y.-Y.; Tingey, M. L.; Hall, D. J.; Israel, B. A.; Murphy, C. J.; Bertics, P. J.; Abbott, N. L. *Langmuir* **2003**, *19*, 1671−80. (e) Tercero Espinoza, L. A.; Schumann, K. R.; Luk, Y.-Y.; Israel, B. A.; Abbott, N. L. *Langmuir* **2004**, *20*, 2375−85.
(3) (a) Van Nelson, J. A.; Kim, S.-R.; Abbott, N. L. *Langmuir* **2002**, *18*, 5031−35. (b) Luk, Y.-Y.; Jang, C.-H.; Cheng, L.-L.; Israel, B. A.; Abbott, N. L. *Chem. Mater.* **2005**, *17*, 4774−82.
(4) Cheng, L.-L.; Luk, Y.-Y.; Murphy, C. J.; Israel, B. A.; Abbott, N. L. *Biomaterials* **2005**, *26*, 7173−82.
(5) Shiyanovskii, S. V.; Schneider, T.; Smalyukh, I. I.; Ishikawa, T.; Niehaus, G. D.; Doane, K. J.; Woolverton, C. J.; Lavrentovitch, O. D. *Phys. Rev. E* **2005**, *71*, 020702-1−5.
(6) (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−80. (b) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219−32. (c) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893−3912.
(7) (a) Robinson, C. *Trans. Faraday Soc.* **1955**, *52*, 571−92. (b) Robinson, C. *Tetrahedron* **1961**, *13*, 219−34. (c) Bellomo, E. G.; Davidson, P.; Impéror-Clerc, M.; Deming, T. J. *J. Am. Chem. Soc.* **2004**, *126*, 9101−5. (d) Yu, S. M.; Conticello, V. P.; Zhang, G.; Kayser, C.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Nature* **1997**, *389*, 167−70.
(8) In contrast, short α-peptides capable of forming β-strands have been shown to form LC phases. (a) Aggeli, A.; Nyrkova, I. A.; Bell, M.; Harding, R.; Carrick, L.; Mcleish, T. C. B.; Semenov, A. N.; Boden, N. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 11857−86. (b) Hartgerink, J. D.; Beniash, E.; Stupp, S. I. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5133−8.
(9) (a) Appella, D. H.; Christianson, L. A.; Karle, I.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 6206−12. (b) Appella, D. H.; Barchi, J. J., Jr.; Durell, S. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 2309−10. (c) Appella, D. H.; Christianson, L. A.; Karle, I.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13071−2.
(10) (a) Raguse, T. L.; Lai, J. R.; LePlae, P. R.; Gellman, S. H. *Org. Lett.* **2001**, *3*, 3963−6. (b) Raguse, T. L.; Lai, J. R.; Gellman, S. H. *J. Am. Chem. Soc.* **2003**, *125*, 5592−3. (c) ACHC homooligomers have been shown to aggregate in water: Martinek, T. A.; Hetényi, A.; Fülöp, L.; István, M.; Mándity, I. M.; Tóth, G. K.; Dekany, I.; Fülöp, F. *Angew. Chem., Int. Ed.* **2006**, *45*, 2396−2400.
(11) Hartshorne, N. H.; Stuart, A. *Crystals and the Polarizing Microscope*; American Elsevier: New York, 1970.
(12) Dierking, I. *Textures of Liquid Crystals*; Wiley: Weinheim, Germany, 2003.
(13) Optical micrographs of β-peptides **1**−**5** are included with Supporting Information.
(14) Aggeli, A.; Bell, M.; Carrick, L. M.; Fishwick, C. W. G.; Harding, R.; Mawer, P. J.; Radford, S. E.; Strong, A. E.; Boden, N. *J. Am. Chem. Soc.* **2003**, *125*, 9619−28.
(15) (a) Blackburn, J. C.; Kilpatrick, P. K. *Langmuir* **1992**, *8*, 1679−87. (b) Boden, N.; Corne, S. A.; Jolley, K. W. *J. Phys. Chem.* **1987**, *91*, 4092−4105.

JA062412Z