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Self-Assembling Peptide Nanotubes from Enantiomeric Pairs of Cyclic Peptides with Alternating D and L Amino Acid Residues

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During the past 10 years studies aiming at the design and synthesis of molecules that self-assemble into supramolecular structures have received increased attention.[1] A particular attractive system is cyclic peptides composed by an even number of alternating D- and L-amino acid residues. These can self-assemble into extended hollow tubular structures, so-called peptide nanotubes, by forming antiparallel hydrogen bonds between homochiral amino acid residues of adjacent rings.

The formation of such nanotubes was first proposed by DeSantis in 1974 and later demonstrated experimentally and studied in a series of elegant papers by Ghadiri and co-workers.[3] These studies have also shown that nanotubes with different inner diameters can be obtained by varying the number of amino acid residues in the peptide. Peptides of this class have also been demonstrated to be able to form artificial transmembrane channels for ion and glucose transport, as well as to exert antibacterial activity.[4]

In most of the studies reported thus far, the nanotubes have been assembled in solutions by millimolar concentration of a single peptide. The only example of an ordered assembly of nanotubes from more than one peptide is a salt-bridged nanotube[5] formed in a mixture of *cyclo*-[(L-Glu-D-Leu)$_4$] and *cyclo*-[(L-Lys-D-Leu)$_4$]. However, this approach severely restricts the choice of amino acids to those that are capable of specific electrostatic interactions.

In peptide nanotubes, the relative positions of the side chains are strictly determined by the interactions between peptide backbones. In principle, this allows the formation of molecular surfaces where different functional groups could be assigned an exact relative positions. However, this requires new methods to control the self-assembly of these peptides into nanotubes.

In this communication, we present a first step toward a new strategy for a stereochemical control of peptide nanotube formation by imposing sterical restrictions upon the hydrogen-bonding pattern.

Our model studies of the stacking of the backbone hydrogen bonds in nanotubes by the peptide *cyclo*[-(L-Gln-D-Tle-L-Glu-D-Tle)$_2$-] (**1**, Figure 1) suggested that peptides incorporating the bulky amino acid *tert*-leucine[6] (Tle) in every second position, could not readily be sampled into nanotubes either by formation of parallel or antiparallel hydrogen bonds. This is the result of sterical clashes between near-neighboring homochiral residues of Tle.

However, the interaction of **1** with its enantiomer *cyclo*[-(D-Gln-L-Tle-D-Glu-L-Tle)$_2$-] (**2**) could readily be modeled into nanotubes with close antiparallel hydrogen bonding. To obtain such optimal interaction between the backbone peptide bonds the bulky *tert*-butyl side chains must be positioned above the homochiral Gln and Glu residues of the enantiomeric peptide. This could only be achieved by stacking peptides **1** and **2** on top of one another, forming a repetitive layered pattern of enantiomers (**3**).

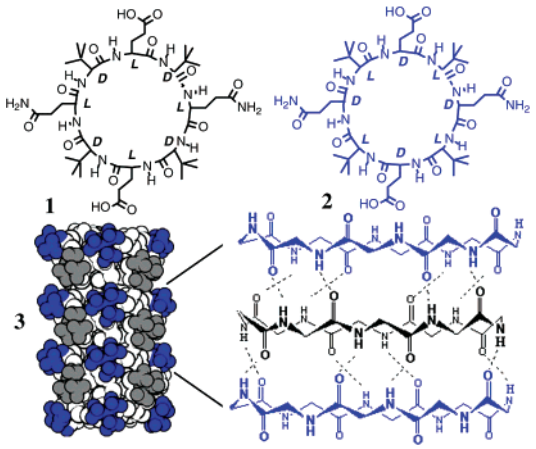

Figure 1. Proposed layered nanotube structure **3** is formed in equimolar mixture of peptides **1** and **2**. Intersubunit hydrogen bonds are formed between homochiral amino acid residues of adjacent enantiomers. Side chains of D-*tert*-leucine (gray) and L-*tert*-leucine (blue) are effectively packed on top of nonsterically hindered side chains of glutamine or glutamic acid (omitted for clarity).

To test these suggestions experimentally, we therefore synthesized enantiomers **1** and **2** by solid-phase peptide synthesis using the Boc/benzyl strategy.

Solutions of the individual enantiomeric peptides were stable up to a concentration of 5 mM in 33% (v/v) acetonitrile-water, 0.25% (v/v) TFA (used for acidification), and no precipitation could be observed even after prolonged storage for several days. At higher concentrations, the peptide immediately precipitated after acidification, but FT-IR analysis did not show any amide frequencies that are characteristic for peptide nanotubes.[7] It can therefore be concluded that Tle residues strongly disfavor both parallel and antiparallel stacking of peptide **1** into nanotubes.

However, if both enantiomers **1** and **2** were mixed in a molar ratio 1/1 in a concentration range between 25 and 500 μM, an increase in the turbidity of the solution could be observed.

These solutions were centrifuged, and the pellets of peptide formed were analyzed with transmission electron microscopy studies (TEM, Figure 2) and Fourier transform infrared spectroscopy (FT-IR) techniques.

The corresponding electron diffraction patterns (Figure 2b) had sharp spots, revealing many of the crystallites to be well ordered. The positions of the reflections in the ED patterns indicated one long unit cell axis with $a \approx 16.5$ Å as expected for closely packed nanotubes and one shorter with $b \approx 4.8$ Å with an interjacent angle of 90°. This axial periodicity of 4.8 Å suggests that each nanotube is made up of stacked rings with an intersubunit distance corresponding to an ideal antiparallel β-sheet structure and is in agreement with the distance found in peptide nanotubes.[3a,b,5] The third axis and remaining two angles could not be determined.

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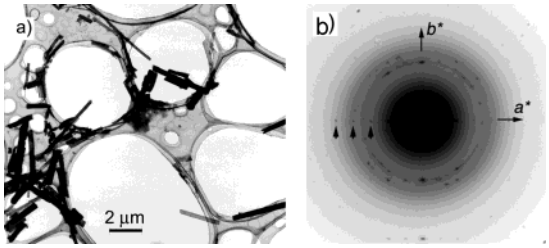

Figure 2. (a) Peptide nanotubes observed with transmission electron microscopy. The rodlike structures with sizes up to 0.5 μm × 10 μm long represent bundles of nanotubes. (b) Electron diffraction pattern of peptide nanotube crystallite.

FT IR studies could further confirm presence of intermolecular hydrogen bonding in the peptide assembly. The presence of amide $I_\perp$ (1631 cm^{-1}) and amide II (1532 cm^{-1}) bands being characteristic of β-sheets[7] could be demonstrated. Furthermore, amide $I_{||}$ frequency at 1687 cm^{-1} is highly characteristic for an antiparallel β-sheet, while amide A band that appears near 3309 cm^{-1} further indicates the formation of a hydrogen-bonding network.

These observations can be explained by assuming that nanotube formation is controlled by the chirality of the Tle residues and proceeds by incorporating a given enantiomer in every second position in the nanotubes position as shown by Figure 1.

For the mixture of peptides **1** and **2** in this study, the formation of nanotubes is very rapid. We found that in the concentration range 0.3−0.5 mM an instantaneous increase of turbidity could be observed upon mixing both enantiomers, indicating a rapid association between peptides **1** and **2**. This can be compared to the analogous peptide *cyclo*[-(L-Gln-D-Ala-L-Glu-D-Ala)$_2$-] initially reported by Ghadiri and co-workers. In that report, peptide nanotube formation was studied at a concentration of approximately 20 mM, and nanotube formation occurred over a period of several hours.[3a]

To characterize the concentration dependence of nanotube formation, the association was studied turbidimetrically by mixing both peptide enantiomers in a molar ratio 1:1 at a set of different total concentrations. The maximally achieved plateau values of turbidity were found to be linearly dependent on total peptide concentration. Extrapolation of the turbidity increment to zero gives an intercept of the abscissa at a nonzero concentration, critical concentration C_r, that has been demonstrated to reflect the apparent dissociation equilibrium constant of the growth reaction.[8a,b] The nanotube formation studied in 33% acetonitrile−water (0.25% TFA) resulted in a C_r value 18 ± 12 μM of total peptide concentration and is demonstrated by amino acid analysis to be C_r < 10 μM. These results strongly indicate that the polymerization is a cooperative process and proceeds by a nucleated assembly model.[8]

Peptide nanotube formation is disfavored in hydrogen-bonding solvents, but even in water solution (0.25% TFA) without organic solvents, peptide nanotubes were formed as confirmed by EM and FT IR. Here we observed significant development of turbidity at concentrations between 50 and 500 μM.

We suggest that the enhanced propensity of peptides **1** and **2** to form nanotubes can be explained by effects of the Tle. It is known that Tle significantly decreases the conformational entropy of a peptide chain and favors extended conformation of the peptide.[6] It can therefore be expected that peptides **1** and **2** have an increased propensity to adopt a fully extended planar conformation that would thereby facilitate intermolecular recognition of the corresponding enantiomer.

The presence of Tle residues in every second position can also be expected to shield the hydrogen bonds between peptide backbones in a nanotube from interaction with water, something that will result in increased stability in aqueous solution.

In summary, we have reported a new strategy for peptide nanotube formation in which the assembly process occurs more rapidly and at significantly lower concentration then previously reported. Association at low concentrations of peptides takes place even in pure water solution, where formation of hydrogen-bonded aggregates is particularly disfavored.

Particularly noteworthy is the possibility to impose stereochemical control of the self-assembly process. We suggest that for peptides with the general structure *cyclo*[-(D-Xaa-L-Tle-D-Xaa-L-Tle)$_2$-] and *cyclo*[-(L-Yaa-D-Tle-L-Yaa-D-Tle)$_2$-] the relative positions of the different functional groups of the side chains of residues Xaa and Yaa in the nanotubes will be determined by sterical control of the self-assembly imposed by the Tle residues. This suggests a general route for the synthesis of macromolecular structures, where in principle any two different functional groups, and possibly molecules conjugated to the side chains, can be assigned exact relative positions as a result of the highly specific intermolecular noncovalent interaction between the peptide backbones.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Desiraju, G. R. *Nature* **2001**, *412*, 397−400.
(2) DeSantis, P.; Morosetti, S.; Rizzo, R. *Macromolecules* **1974**, *7*, 52−58.
(3) (a) Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. *Nature* **1993**, *366*, 324−327. (b) Hartgerink, J. D.; Granja, J. R.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1996**, *118*, 43−50. (c) Clark, T. D.; Buriak, J. M.; Kobayashi, K.; Isler, M. P.; McRee, D. E.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 8949−8962.
(4) (a) Ghadiri, M. R.; Granja, J. R.; Buehler, L. K. *Nature* **1994**, *369*, 301−304. (b) Granja, J. R.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1994**, *116*, 10785−10786. (c) Fernandez-Lopez, S.; Hui-Sun, K.; Choi, E. C.; Delgado, M.; Granja, J. R.; Khasanov, A.; Kraehenbuehl, K.; Long, G.; Weinberger, D. A.; Wilcoxen, K. M.; Ghadiri, M. R. *Nature* **2001**, *412*, 452−455.
(5) Hartgerink, J. D. PhD Thesis, Scripps Research Institute, La Jolla, CA, 1999.
(6) (a) Paterson, Y.; Leach, S. J. *J. Am. Chem. Soc.* **1978**, *11*, 409−415. (b) Conformational studies of *N*-acetyl-*N*′-methyl amide of *tert*-leucin has shown that it has a restricting effect on the number of peptide backbone conformations allowing only the (ϕ,ψ) values in a narrow area around (−130°, 140°).
(7) (a) Miyazawa, T.; Blout, E. R. *J. Am. Chem. Soc.* **1961**, *83*, 712−719. (b) Fraser, R. D. B.; MacRae, T. P. *Conformation in Fibrous Proteins and Related Synthetic Peptides*; Academic Press: New York, 1973; pp 95−125. (c) Krimm, S.; Bandekar, J. In *Advances in Protein Chemistry*; Anfinsen, C. B.; Edsall, J. T.; Richards, F. M., Eds.; Academic Press: Orlando, 1986; Vol. 38, pp 181−364.
(8) (a) Andreu, J. M.; Timasheff, S. N. *Methods Enzymol.* **1986**, 47−59. (b) Timasheff, S. N. In Protein−Protein Interactions; Frieden, C. F., Nichol, L. W., Eds.; Wiley: 1981; pp 315−336. (c) Berne, B. J. *J. Mol. Biol.* **1974**, *89*, 755.

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