Nanoscale Tubular Ensembles with Specified Internal Diameters. Design of a Self-Assembled Nanotube with a 13-Å Pore

Nina Khazanovich,† Juan R. Granja, Duncan E. McRee, Ronald A. Milligan, and M. Reza Ghadiri*,‡

Departments of Chemistry, Molecular Biology, and Cell Biology, The Scripps Research Institute La Jolla, California 92307

Received March 3, 1994

Design of hollow tubular structures has been the subject of considerable research lately.[1-5] The potential utility of tubular objects in a wide variety of chemical, biological,[6] and materials science applications will depend in part on the ability to construct such structures with uniform and specified internal diameters, a task that until now has remained largely unfulfilled.[3e,f] Recently, we have described the design and construction of a new class of tubular structures having a uniform 7–8-Å internal diameter.[1] The strategy, which is based on the self-assembly of ring-shaped cyclic peptide subunits, holds considerable promise for designing nanotubes with tailored pore dimensions. Here we demonstrate, for the first time, that the internal diameter of the peptide nanotubes can be rigorously controlled simply by adjusting the ring size of the peptide subunit employed. In this study we present the design and synthesis of a 12-residue cyclic peptide structure and document its utility in the construction of nanotube ensembles having a uniform 13-Å van der Waals pore diameter. Formation of the tubular structures is supported by high-resolution imaging using cryo electron microscopy, electron diffraction, Fourier-transform infrared spectroscopy, and molecular modeling.

According to our design principles, cyclic peptide structures which are made up of an even number of alternating D- and L-amino acid residues can adopt or sample a flat ring-shaped conformation in which all backbone amide functionalities lie approximately perpendicular to the plane of the ring structure. In this conformation, the peptide subunits can stack, under favorable conditions, to furnish a contiguous hydrogen-bonded hollow tubular structure (Figure 1). The internal diameter of the nanotube ensemble can, in principle, be tailored by adjusting the ring size of the peptide subunit employed. In this study we will describe the largest pore diameter peptide-based nanotube structure thus far constructed by utilizing the following 36-membered-ring peptide subunit *cyclo*[(Gln-D-Ala-Glu-D-Ala)$_3$]. The design principles and the self-assembly strategy employed in this study are similar to those described previously.[1] The requisite peptide subunit was synthesized on a solid support[7] and characterized by mass spectrometry and ^{1}H NMR spectroscopy. Controlled acidification of alkaline solutions of the peptide subunit upon standing afforded rod-shaped crystalline materials.[1] Transmission electron microscopy indicates that each particle is an organized bundle of tightly packed nanotubes (Figure 2). Low-dose cryo microscopy[8] revealed longitudinal striations with spacing

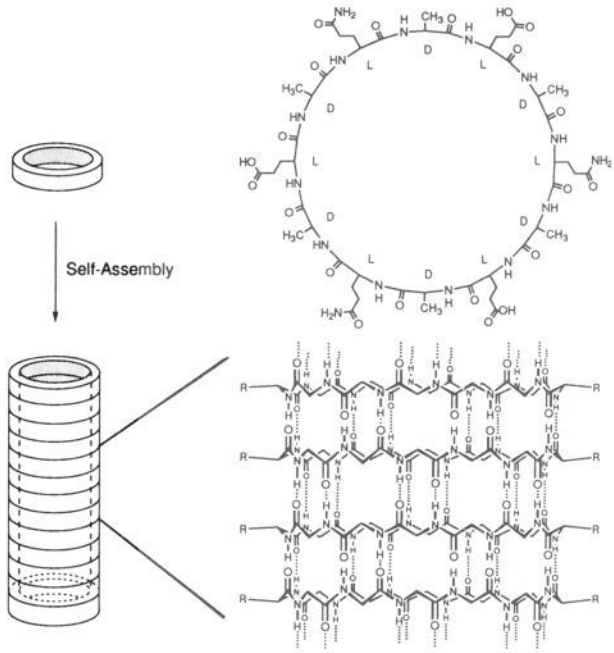

Figure 1. Schematic representation of the strategy employed in the construction of self-assembled nanotubes. Appropriately designed cyclic peptide subunits, under suitable conditions, stack to form hydrogen-bonded tubular structures (for clarity only the backbone structure is represented). The ring size of the subunit sets the internal diameter of the tubular ensemble. The chemical structure of the subunit is shown on the top right (D or L refers to the amino acid chirality).

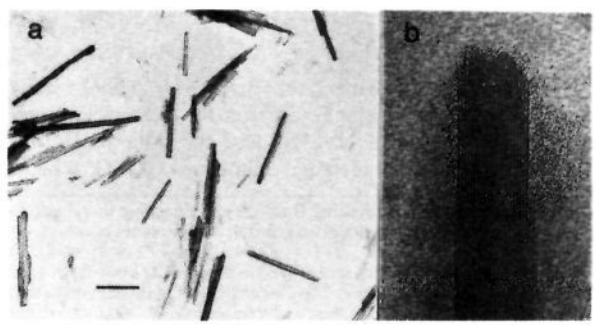

Figure 2. (a) Low-magnification electron micrograph of nanotube suspensions adsorbed on carbon support film (scale bar, 1 μm). (b) Low-dose image of a frozen hydrated single nanotube particle (for methods, see ref 1). Longitudinal striations which are approximately 25 Å apart are due to the side-by-side packing of nanotubes.

† Present address: Department of Biochemistry, University of Alberta, Edmonton, Canada.

‡ Alfred P. Sloan Research Fellow (1993–1995) and Searle Scholar (1991–1994).

(1) Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Kazanovich, N. *Nature* **1993**, *366*, 324–327.

(2) For all-carbon tubular structures see: (a) Iijima, S. *Nature* **1991**, *354*, 56–58. (b) Ebbesen, T. W.; Ajayan, P. M. *Ibid.* **1992**, *358*, 220–222. (c) Iijima, S.; Ichihashi, T. *Ibid.* **1993**, *363*, 603–605. (d) Bethune, D. S.; Kiang, C. H.; de Vries, M. S.; Gorman, G.; Savoy, R.; Vazquez, J.; Beyers, R. *Ibid.* **1993**, *363*, 605–607. (e) Wang, Y. *J. Am. Chem. Soc.* **1994**, *116*, 397–398.

(3) For inorganic tubular and channeled materials, see: (a) *Atlas of Zeolite Structure Types*, 2nd ed.; Meier, W. M., Olson, D. H., Eds.; Butterworths: London, 1988. (b) Davis, M. E.; Saldarriaga, C.; Montes, C.; Garces, J.; Crowder, C. *Nature* **1988**, *331*, 698–699. (c) Dessau, R. M.; Schlenker, J. L.; Higgins, J. B. *Zeolites* **1990**, *10*, 522–524. (d) Estermann, M.; McCusker, L. B.; Baerlocher, C.; Merrouche, A.; Kessler, H. *Nature* **1991**, *352*, 320–323. (e) Kresge, C. T.; Leonowicz, M. E.; Roth, W. J.; Vartuli, J. C.; Beck, J. S. *Nature* **1992**, *359*, 710–712. (f) Monnier, A.; Schüth, F.; Huo, Q.; Kumar, D.; Margolese, D.; Maxwell, R. S.; Stucky, G. D.; Krishnamurty, M.; Petroff, P.; Firouzi, A.; Janicke, M.; Chmelka, B. F. *Science* **1993**, *261*, 1299–1303. (g) Hamilton, E. J. M.; Dolan, S. E.; Mann, C. M.; Colijn, H. O.; McDonald, C. A.; Shore, S. G. *Science* **1993**, *260*, 659–661.

(4) For lipid-based tubular ensembles, see: (a) Georger, J. H.; Singh, A.; Price, R. R.; Schnur, J. M.; Yager, P.; Schoen, P. E. *J. Am. Chem. Soc.* **1987**, *109*, 6169–6175. (b) Fuhrhop, J.-H.; Spiroski, D.; Boettcher, C. *J. Am. Chem. Soc.* **1993**, *115*, 1600–1601. (c) Schnur, J. M. *Science* **1993**, *262*, 1669–1676.

(5) For polymeric tubular systems, see: (a) Lotz, B.; Colonna-Cesari, F.; Heitz, F.; Spach, G. *J. Mol. Biol.* **1976**, *106*, 915–942. (b) Brumlik, C. J.; Martin, C. R. *J. Am. Chem. Soc.* **1991**, *113*, 3174–3175. (c) Roks, M. F. M.; Nolte, R. J. M. *Macromolecules* **1992**, *25*, 5398–5407. (d) Harada, A.; Li, J.; Kamachi, M. *Nature* **1993**, *364*, 516–518.

(6) For the design of membrane ion channels based on self-assembling peptide nanotubes, see: Ghadiri, M. R.; Granja, J. R.; Buehler, L. K. *Nature* **1994**, *369*, 301–304.

(7) Rovero, P.; Quartara, L.; Fabbri, G. *Tetrahedron Lett.* **1991**, *32*, 2639–2642.

(8) (a) Adrian, M.; Dubochet, J.; Lepault, J.; Mc Dowall, A. W. *Nature* **1984**, *308*, 32–36. (b) Milligan, R. A.; Brisson, A.; Unwin, P. N. T. *Ultramicroscopy* **1984**, *13*, 1–10.

0002-7863/94/1516-6011$04.50/0

of approximately 25 Å as expected for the center to center spacing for closely packed nanotubes (Figure 2b). Electron diffraction patterns display axial spacing of 4.80 Å, which is in agreement with the peptide stacking and the formation of a tight network of hydrogen-bonded β-sheet type structure. The electron diffraction patterns display meridional spacings of 12.67 ± 0.06 Å and 21.94 ± 0.05 Å, characteristic of a hexagonal body centered packing of nanotubes.[9] The diffraction patterns also showed a unit cell with an angle of 99° and no symmetry other than the center of symmetry due to Friedel's law. In a fashion analogous to our previous study,[1] using the parameters obtained from the electron diffraction patterns—unit cell with a = 9.6 Å (2 × 4.80 Å for the antiparallel dimer), $b = c$ = 25.66 Å (2 × 12.67/(sin 99)), α = 120°, and $\beta = \gamma$ = 99°—a three-dimensional model of the nanotube structure was built (Figure 3). The model shows structure factors similar to the patterns observed in the electron diffraction, thus supporting the proposed three-dimensional model. Involvement of an intermolecular hydrogen-bonding network in the tube assembly is also supported by FT-IR spectroscopic analysis.[10] Nanotubes display characteristic IR features of a β-sheet structure signified not only by the *amide I* bands at 1626 and 1674 cm^{-1} and an *amide II* band at 1526 cm^{-1} but also by the observed NH stretching frequency at 3291 cm^{-1} supporting formation of a tight network of hydrogen bonds.[11] The observed frequency of the NH stretching mode correlates to an average intersubunit distance of 4.76 Å, which is in close agreement with the value of 4.80 Å obtained independently from the electron diffraction patterns.

In summary, we have demonstrated that the pore size of the self-assembled organic nanotubes can be simply adjusted by the ring size of the peptide subunit employed. The ability to design specifically sized tubular nanostructures is expected to have important applications in catalysis, inclusion chemistry, and molecular electronics.

Acknowledgment. We thank colleagues D. Hecht for assistance in FT-IR spectroscopy and G. Siuzdak for mass spectroscopic analyses. N.K. thanks NSERC and the Alberta Heritage Scholarship Fund for predoctoral fellowships. J.R.G. acknowledges the fellowship from the Consellería de Educación y Ordenación Universitaria (Xunta de Galicia, Spain). Financial support for this work was provided by the Arnold and Mabel Beckman Foundation (Beckman Young Investigator Fellowship 1991–1993 to M.R.G.).

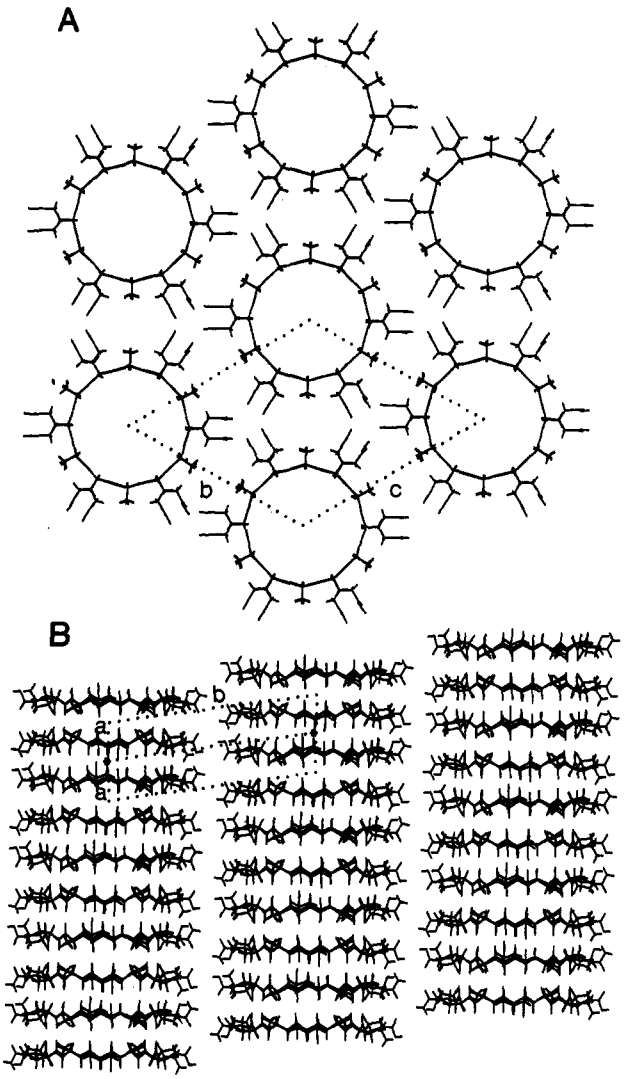

Figure 3. Three-dimensional model of the self-assembled nanotubes (see text and ref 1 for more detail about model building). Views of the crystal packing are shown along (A) the a-axis (for clarity, each nanotube is represented only by the local 2-fold dimer) and (B) the c-axis. The unit cell is indicated by the dashed lines and the local 2-fold axis by the asterisk. The unit cell has the dimensions a = 9.6 Å, $b = c$ = 25.66 Å, α = 120°, and $\beta = \gamma$ = 99°. The positions of the side chains in this model are arbitrary. The tube axis is along a.

(9) A hexagonal lattice resulting from the close packing of cylinders of radius r displays the characteristic two principle lattice planes of radius r and $3^{1/2}r$ such as the one observed here (r = 12.67 Å, and $3^{1/2}r$ = 21.94 Å). The periodicity in this packing produces diffraction spots at $1/3^{1/2}r$, $2/3^{1/2}r$, and so on, and at $1/r$, $2/r$, and so on. The observed electron diffraction patterns on the meridional axes extend to third-order reflections (4.1 Å) signifying the ordered and crystalline state of the nanotube particles.

(10) Krimm, S.; Bandekar, J. In *Advances in Protein Chemistry*; Anfinsen, C. B., Edsall, J. T., Richards, F. M., Eds.; Academic Press: Orlando, 1986; pp 181–364.

(11) The IR spectrum not only is very similar to those of previously characterized nanotubes (ref 1) but also closely resembles that of crystalline gramicidin A, which is known to form dimeric β-helical structures. Gramicidin A ($\uparrow\downarrow\beta^{6,4}$) has *amide I* bands at 1630 and 1685 cm^{-1}, an *amide II* band at 1539 cm^{-1}, and an NH stretching frequency at 3285 cm^{-1}. See: Naik, V. M.; Krimm, S. *Biophys. J.* **1986**, *49*, 1147–1154.