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A New Motif in the Formation of Peptide Nanotubes: The Crystallographic Signature

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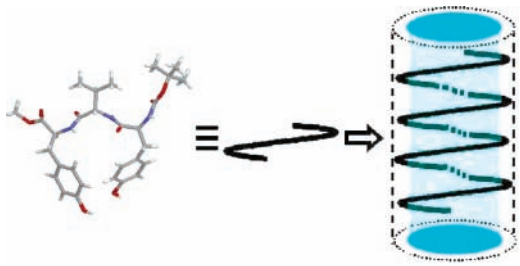
ABSTRACT



Terminally protected acyclic tripeptides Boc-Tyr(1)-Val(2)-Tyr(3)-OMe 1 and Boc-Tyr(1)-Ile(2)-Tyr(3)-OMe 2 self-assemble into nanotubes in crystals through various noncovalent interactions with an average internal diameter of 5 Å (0.5 nm), and the tubular ensemble is developed through the hydrogen-bonded side chains of tyrosine residues. The inside of the hollow nanotubular structures is hydrophilic; however, no solvent molecules have been crystallographically detected.

Construction of nanostructured materials using the self-assembly of oligopeptide-based organic molecules is a rapidly expanding area of current research.[1] During the past several years, considerable attention has been directed to the rational design of peptide-based nanotubular structures, as they can be used in the transport of glucose[2] or in trans-membrane ion channels[3] or even as potential antibiotics against drug-resistant bacteria.[4] Many successful attempts have previously been made to create nanotubular structures using various self-assembling organic compounds including cyclic oligoureas,[5] cyclic peptides,[6] cyclodextrine-based polyionic amino acids,[7] 7-deaza-2-deoxy xanthosine dihydrate,[8] and others.[9] Ghadiri and co-workers have made a

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pioneering contribution in demonstrating the formation of peptide-based hollow nanotubular structures using self-assembling cyclic D,L-α-peptides in which the oligopeptide rings stack one on top of the other mainly through intermolecular hydrogen bonding to form the nanotubes.[6a,b] The internal diameter and the nature of the nanotube can be controlled simply by varying the number of amino acid residues and their nature (hydrophobic/hydrophilic). Whereas the self-association of cyclic peptides or peptide derivatives into hollow nanotubes has been studied in detail, the formation of acyclic peptide nanotubes has been paid relatively little attention, there being only a few examples.[10] A very recent example demonstrates the formation of nanotubes using self-assembly of the dipeptide D-Phe-D-Phe and insertion of platinum nanoparticles inside the tubes.[10d] In this report we present the formation of acyclic peptide nanotubes by self-assembly of two parallel β-sheet-forming terminally protected tripeptides Boc-Tyr(1)-Val(2)-Tyr(3)-OMe **1** and Boc-Tyr(1)-Ile(2)-Tyr(3)-OMe **2**, where the side chain phenolic OH groups from the terminal Tyr residues play a key role in the formation of the nanotubular structure.

Each of the reported tripeptides contains two tyrosine residues in both termini. These uncharged polar side chains of tyrosine residues provide additional hydrogen-bonding functionalities to the peptide NH groups. The tripeptides **1** and **2** were synthesized by conventional solution-phase methods[11] and characterized by ^{1}H NMR spectroscopy and mass spectrometry. Colorless monoclinic crystals suitable for X-ray diffraction of tripeptides **1** and **2** were obtained from methanol−water solutions by slow evaporation. The structures of tripeptides **1** and **2** were determined using single-crystal X-ray diffraction studies (Figure 1a and b).[12] The tripeptide **1** and **2** molecules are lying in opposite directions relative to the *y*-axis. There are no intramolecular hydrogen bonds in these reported peptides in the solid state. Most of the backbone torsion angle values of the constituent amino acid residues of tripeptides **1** and **2** fall within the parallel β-sheet region of the Ramachandran map (Table 1). Both peptides form cylindrical structures containing intermolecular hydrogen bonds involving phenolic OH groups from tyrosine residues. The cylindrical structure is composed of self-assembling flat-ring-shaped acyclic peptide subunits containing backbone amide groups nearly perpendicular to the plane of the ring (Figure 1a and b). These peptide subunits are closely stacked in a parallel orientation around the screw

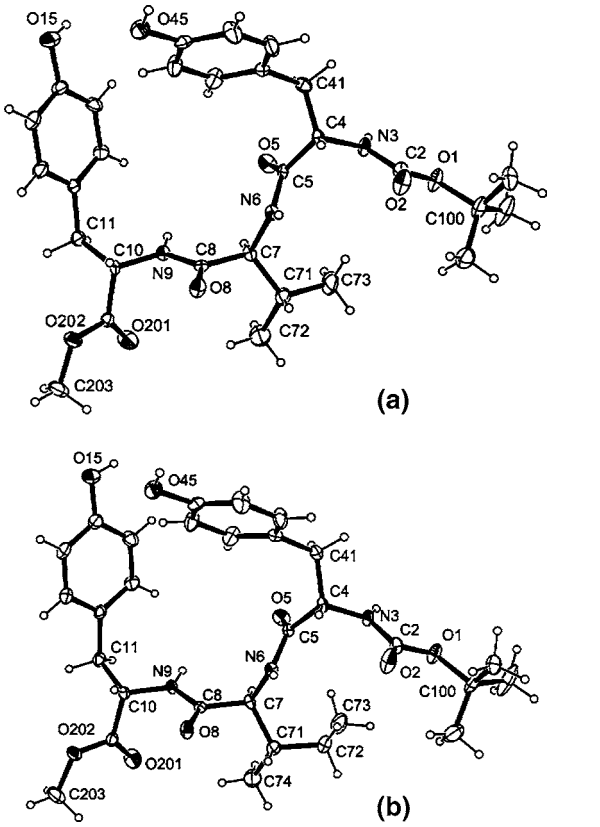

Figure 1. (a) ORTEP diagram with atomic numbering scheme of the peptide **1**. Thermal ellipsoids are shown at 30% probability level. (b) Molecular conformation of the peptide **2** showing the atomic numbering scheme. Ellipsoids at 30% probability.

Table 1. Selected Backbone Torsional Angles (deg) of Peptides **1** and **2**

torsional angles	peptide **1**	peptide **2**
ω_0	172.1(3)	178.3(3)
ϕ_1	−106.6(4)	−110.1(4)
ψ_1	99.7(3)	97.7(4)
ω_1	163.5(3)	−163.1(3)
ϕ_2	−120.6(3)	−116.5(4)
ψ_2	105.9(4)	104.5(3)
$\omega 2$	173.9(3)	174.4(3)
ϕ_3	−74.6(4)	−72.9(4)
$\psi 3$	153.4(3)	153.6(3)

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(12) **Crystal data for peptide 1**: $C_{29}H_{39}N_3O_8$, FW = 557.63, monoclinic, space group *Cc*, a = 24.30(3) Å, b = 5.06(6) Å, c = 25.97(3) Å, β = 102.88(10)°, Z = 4, d_{calc} = 1.189 g cm^{-3}. **Crystal data for peptide 2**: $C_{30}H_{41}N_3O_8$, FW = 571.66, monoclinic, space group *Cc*, a = 24.29(3) Å, b = 5.09(7) Å, c = 26.52(3) Å, β = 103.94(10)°, Z = 4, d_{calc} = 1.194 g cm^{-3} Intensity data were collected with Mo Kα radiation using the MARresearch Image Plate System. The crystals were positioned at 70 mm from the Image Plate, and 100 frames were measured at 2° intervals with a counting time of 2 min to give 5579 and 5582 independent reflections for peptides **1** and **2** respectively. Data analysis was carried out with the XDS program.[13] The structures were solved using direct method with the Shelx86 program.[14] The non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms were included in geometric positions and given thermal parameters equivalent to 1.2 times those of the atom to which they were attached. The structures were refined on F^2 using Shelxl.[15] The final R values were R1 0.0776, 0.0677 and wR2 0.1603, 0.1809 for 4169, 4578 data with $I > 2\sigma(I)$ for peptides **1** and **2**, respectively. The data have been deposited at the Cambridge Crystallographic Data Center with reference number CCDC 242896.

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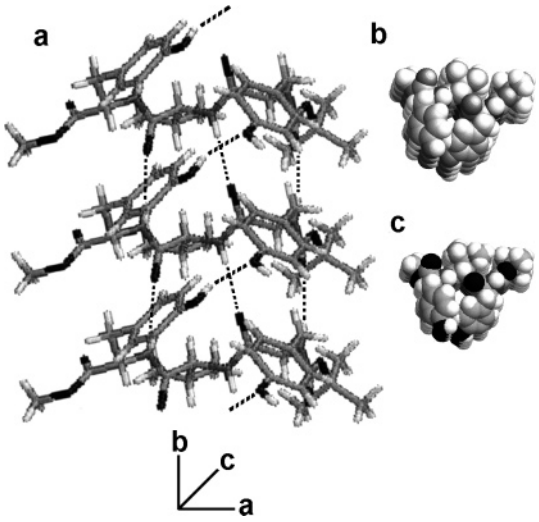

Figure 2. (a) Formation and development of the intermolecularly hydrogen-bonded nanotubular structure along the crystallographic *b*-axis. The tubular structure is composed of the acyclic peptide **1** subunit with extended backbone conformation (which adopts a β-strand-like structure). (b and c) Top view of the nanotubular ensemble obtained from a higher ordered self-assembly of the peptides **1** and **2**, respectively, exhibiting internal tubular diameter of about 5.0 Å in space-filling model.

axis parallel to the crystallographic *b*-axis (Figure 2a). Each cylindrical ensemble is stabilized by four intersubunit hydrogen-bonding interactions (O15···O45 2.86, 2.85 Å; N6···O5 2.98, 2.98 Å; N9···O8 2.98, 2.98 Å; and N3···O2 2.93, 2.95 Å for peptides **1** and **2**, respectively) (Table 2).

Table 2. Intermolecular Hydrogen Bonding Parameters[a]

D−H···A	H···A (Å)	D···A (Å)	D−H···A (deg)
N9−H9···O8 (a)	2.15, 2.16	2.98, 2.98	162, 161
N6−H6···O5 (b)	2.13, 2.13	2.98, 2.98	170, 171
N3−H3···O2 (a)	2.09, 2.11	2.93, 2.95	165, 167
O45−H45···O201 (c)	1.97, 1.94	2.77, 2.76	162, 173
O15−H15···O45 (a)	2.09, 2.04	2.86, 2.85	157, 170

[a] Values for peptides **1** and **2** are provided consecutively in each entry. Symmetry elements: in peptide **1** (a) $x, -1 + y, z$. (b) $x, 1 + y, z$, (c) $0.5 + x, 0.5 + y, z$. In peptide **2** (a) $x, 1 + y, z$. (b) $x, -1 + y, z$. (c) $-0.5 + x, -0.5 + y, z$.

The top view of the supramolecular assemblies in space-filling models (Figure 2b for peptide **1** and 2c for peptide **2**) clearly shows that these peptide molecules stack atop one another, maintaining proper registry between the subunits, generating an open-ended tube with an average internal diameter of 5.0 Å (0.5 nm) including van der Waals contact. The interior of the peptide supramolecular channel is hydrophilic as a result of the presence of the backbone CONH moieties and phenolic OH groups of Tyr residues, whereas the exterior is hydrophobic as it is occupied by the side chains of valine, isolucine, and Boc groups. Though the inside of this hollow nanotube (with an avarage diameter of 5 Å) is hydrophilic, no solvent molecule has been found in the crystal structure. The crystal structures further revealed that the individual peptide cylindrical columns are regularly aligned via intermolecular hydrogen bonds (O45···O201, 2.77 Å for peptide **1** and 2.76 Å for peptide **2**) (Table 2) and other noncovalent interactions to form higher ordered supramolecular arrays along the crystallographic *a*-axis (Figure 3).

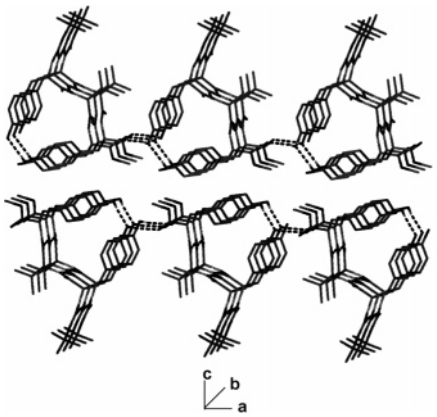

Figure 3. Packing diagram of the peptide **1** showing the formation of tubular arrays along the crystallographic *a*-axis.

In the present study, the reported peptides **1** and **2** have a unique tendency to adopt an extended backbone conformation which self-assembles to form nanotubular structures involving the phenolic OH side chains from the Tyr residues. Actually this result differs from all previous examples of the self-assembling nanotube-forming acyclic peptides, as the method of self-association for the reported nanotube-forming peptides is different. The molecular self-assembly of tripeptides **1** and **2** has created a tubular structure with an average internal diameter of 5.0 Å. The cylindrical assembly is stabilized by directed intermolecular hydrogen-bonding interactions and other noncovalent interactions. This finding indicates that not only cyclic peptides but also acyclic peptides having appropriate amino acid side chain functionalities that can form H-bonds with the main chain or side chain H-bonding group(s) may be used to create nano channels.

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Supporting Information Available: Experimental procedures, spectral characterization, and crystallographic data in CIF format of peptides **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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