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New Cyclic Peptide Assemblies with Hydrophobic Cavities: The Structural and Thermodynamic Basis of a New Class of Peptide Nanotubes

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In recent years, considerable effort has been devoted to the synthesis of organic and inorganic nanotubes.[1] Self-assembling peptide nanotubes (SPN) made from cyclic D,L-α-peptides[2] or from cyclic β-peptides[3] have structural and functional properties that may be suitable for various applications in biology and materials science.[1b,4−6] Here we describe a new member of self-assembling peptides with novel structural and internal cavity, validating the possibility of expanding the SPN class to other hybrid systems. The present structures are based on a hybrid of α−γ-cyclic peptides[7] subunits and have been characterized by NMR, FT-IR, and X-ray crystallography.

Cyclic peptides in which (1*R*,3*S*)-3-aminocyclohexanecarboxylic acid (γ-Acc-OH) alternates with a D-α-amino acid, such as **1a**, can adopt a conformation in which the peptide backbone is essentially flat and the C═O and N−H groups lie roughly perpendicular to the plane of this cyclic backbone (Figure 1). This flat ring-shaped conformation may facilitate antiparallel β-sheetlike hydrogen bonding between oppositely oriented rings and the formation of hydrogen-bonded nanotubes composed of rings of alternating orientation, in which one face of each ring is hydrogen-bonded via γ-Acc C═O and N−H groups to the similar face of the neighbor (Figure 1, frame A), while the other face is hydrogen-bonded via C═O and N−H groups of the α-amino acid to the similar face of the other neighbor (Figure 1, frame B). In such structures, the β-methylene moiety of each cyclohexane is projected into the lumen of the cylinder, creating a partial hydrophobic cavity.

To establish and evaluate the feasibility and the thermodynamic properties of such nanotubes, we prepared dimers featuring each of the two hydrogen-bonding patterns described above, using cyclic peptides in which hydrogen bond donation from one face of the ring structure was blocked by *N*-methylation (Figure 2).[8] Specifically, cyclopeptide **1b**, in which the α-amino acids are *N*-methylated, was used to prepare dimer **2b**, which features A-type hydrogen-bonding (Figure 1), and cyclopeptide **1c**, in which all γ-Acc are *N*-methylated (^{Me}N-γ-Acc), was used to prepare staked dimeric ensemble **2c**, which features Figure 1B-type hydrogen bonding.

The cyclo peptides *cyclo*-[(1*R*,3*S*)-γ-Acc-D-^{Me}NAla]$_3$ (**1b**, R = Me) and *cyclo*-[D-Phe-(1*R*,3*S*)-^{Me}N-γ-Acc]$_3$ (**1c**, R = Bn) were synthesized by standard procedures and characterized by NMR and MS. The ^{1}H NMR spectra of the peptide **1b**, in both polar and nonpolar solvents (CCl_4, $CDCl_3$, MeOH, or DMSO), are well defined, highly symmetrical, and show a $J_{NH,\gamma H}$ coupling constant of 7.5−8.2 Hz, indications that the peptide exists in an all-trans conformation with a flat-ring-shaped backbone. In nonpolar solvents, the formation of dimers is reflected by the downfield shift upon increasing the concentration of the N−H resonance of γ-Acc from δ 6.6 to 7.8 ppm;[9] the corresponding association constant, determined at 298 K by dilution experiments, is 230 M^{-1} in $CDCl_3$ and 2.5 × 10^4 M^{-1} in 2:3 $CDCl_3/CCl_4$. Van't Hoff plots for the range 273−313 K afford values of −34.1 KJ mol^{-1} for $\Delta H°_{298}$ and −69.8 J/K·mol for $\Delta S°_{298}$ that, like the fall in K_a with increasing solvent polarity, are consistent with dimerization being essentially an enthalpy-driven[10] hydrogen-bonding process.[8a,c] The β-sheet nature of the hydrogen bonding is also supported by the chemical shift of ^{Me}N-Ala C_α undergoing a concentration-dependent downfield shift of > 0.1 ppm[11] and by FT-IR spectra recorded in $CHCl_3$, which show amide I and amide II_{ii} bands at 1626 and 1538 cm^{-1}, respectively (positions that are typical of β-sheets and similar to those found for nanotubes and dimers composed of cyclic D,L-peptides). Hydrogen bonding by N−H is further supported by amide A bands near 3325 cm^{-1}, while a band that appears at 3400 cm^{-1}

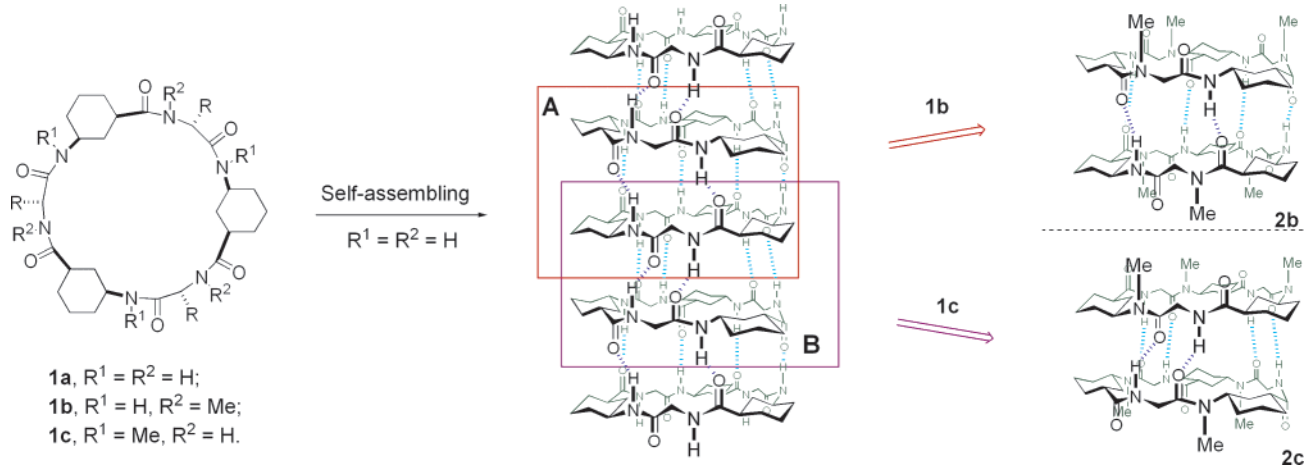

Figure 1. Design for self-assembling peptide nanotubes composed of *cyclo*-[(1*R*,3*S*)-γ-Acc-D-Aa]$_3$ units (for clarity, amino acid side chains have been omitted in the representation of the nanotube and dimers). The two different H-bonding patterns (A and B) are shown in different colors. Dimers used to study the basic parameters of the proposed self-assembling nanotube are shown at the right.

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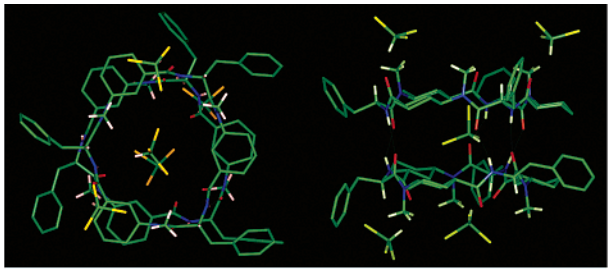

Figure 2. Crystal structures of dimeric assemblies **2c** with five molecules of chloroform, one of which occupies the central cavity of the dimer: (left) top view; (right) side view.

in more dilute (0.5 M) solutions may correspond to the N−H band of the monomer.[8e]

A flat, all-trans conformation is also indicated by the ^{1}H NMR spectra of **1c** in polar and nonpolar solvents ($J_{NH-\alpha H} = 9.0-9.5$ Hz). However, the chemical shift of Phe NH in nonpolar solvents is a constant 8.7 ppm at concentrations down to 2×10^{-4} M and with heating or addition of up to 20% of methanol, showing that if dimers are formed the association constant for dimerization must be at least 10^5 M^{-1} (larger than for any peptide nanotube reported hitherto). Solution ($CHCl_3$) FT-IR studies showed the characteristic features of β-sheet structure of peptide nanotubes and dimers: amide I (1625 cm^{-1}), amide II$_{ii}$ (1523 cm^{-1}), and amide A (3309 cm^{-1}) bands. Dimer formation was also supported by FAB-MS, showing a signal arising from single charged species corresponding to proton dimer **2c** (1718).

Colorless prismatic crystals suitable for X-ray were obtained from the solutions of peptide **1c** in chloroform by vapor-phase equilibration with hexane. The crystal structure characterized by X-ray crystallography shows an unsymmetrical dimeric ensemble that corroborates the nanotube structure. The two peptide subunits are closely stacked in an antiparallel orientation with the β-sheetlike cyclindical ensemble stabilized by six intersubunit hydrogen-bonding interactions with an intersubunit N−O distance ranging between 2.78 and 2.94 Å and distorted by the presence of five chloroform molecules hydrogen-bonded to the peptide backbone. The cyclindical dimer has an approximate van der Waals internal diameter of 5.4 Å and a volume of 165 Å^3, which is filled with one molecule of chloroform, showing the partial hydrophobic character of the inner face of the nanotube which is distinct from previously reported hydrophilic nanotubes.[2,8,12]

In conclusion, NMR, FT-IR, MS, and X-ray diffraction data show conclusively that the cyclic peptides **1b** and **1c** form dimers in which antiparallel peptide rings are linked by a β-sheetlike set of six hydrogen bonds. These dimers (one of which, **2c**, is extremely stable in nonpolar solvents) may be considered as essentially the basic units of a new class of peptide nanotubes, in which the inner-cavity properties are due in part to the cyclohexane C2. Cyclic peptides containing C2-modified γ-Acc should endow SPN with a functionalized inner surface, something that is precluded in the α- or β-nanotubes because all amino acid side chains lie on the exterior of the ensemble and additional modification in C_α or C_β would disrupt the assembling.[2] Appropriate functionalization should lead to nanotubes with greater selectivity as ion channels, catalysts, receptors, or molecule containers. Work is in progress to this end.

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Supporting Information Available: Detailed descriptions of the synthesis and characterization of key compounds and crystal data, atomic coordinates, bond lengths and angles, and anisotropic displacement coefficients of **1c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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