Characterization of Water Wires inside Hydrophobic Tubular Peptide Structures

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The organization of water in channels in biological membranes[1,2] and the interior of carbon nanotubes is of immense current interest.[3,4] A single-file arrangement of water molecules in confined environments has been postulated to explain proton conduction based on the "Grotthuss mechanism" which is now two centuries old.[5] Much of our understanding of the structure and dynamics of single-file water chains (wires) is based on modeling and molecular dynamics simulations.[6−11] While the channels in biological membranes can have an inner polar lining which may interact with water,[12] the interior of carbon nanotubes is necessarily apolar precluding any hydrogen bond interaction between the walls of the tube and the confined water. Experimental structural characterization of water wires has not been reported thus far. The formation of hollow tubes in the crystal structures of hydrophobic acyclic[13] and cyclic peptides[14−16] has been previously described. But the characterization of solvent structures trapped inside the tubes has been limited. Cyclic peptide nanotubes based on the alternating L- and D-amino acid architecture assemble in a manner that the flat rings of peptide backbone stack along the tube axis leading to the formation of pores.[14−16] Linear hydrophobic dipeptides, consisting of pairs of Ala, Val, Leu, Ile, and Phe, form nanotubes producing a repertoire of nanostructures.[17] Pore diameters vary with the side chains trapping diverse solvents.[13,17] The diphenylalanine motif of the Alzheimer's β-amyloid peptide also forms discrete and hollow nanotubes in solution.[18] However, these tubular structures have not yielded a structural view for water columns localized inside a hydrophobic channel. We describe the characterization of two distinct structural states of a water wire localized inside a completely hydrophobic tubular structure formed by the self-assembly in single crystals of an apolar synthetic pentapeptide.

We serendipitously observed that the peptide Boc-^{D}Pro-Aib-**Leu**-Aib-Val-OMe (**1**) formed a tubular structure in crystals, belonging to the space group $P6_5$, with a narrow pore devoid of solvent (diameter ~5.2 Å) (Figure 1a). Inspection of the structure revealed that the isobutyl side chain of Leu(3) points into the interior of the pore, sterically impeding entry of water molecules. This prompted us to examine the analogue peptide Boc-^{D}Pro-Aib-**Val**-Aib-Val-OMe (**2**) in which the reduction of the size of the side chain at position 3 to an isopropyl group should enhance pore diameter. As anticipated, **2** also formed a tubular structure which was now occupied by a file of water molecules (pore diameter ~7.5 Å) (Figure 1a). Both the peptide molecules adopt similar molecular conformations with consecutive β-turns (type II′ followed by two successive type I/III) and stabilized by three strong intramolecular C_{10} hydrogen bonds (N3···O0, N4···O1, and N5···O2) (Figure 1b). Such folded structures containing multiple β-turns are commonly observed in sequences containing Aib residues.[19] The ^{D}Pro

residue constrains the N-terminus segment to the type II′ conformation.[20] The assembly of the molecules around the *c*-axis is further stabilized by a single intermolecular hydrogen bond between the screw related symmetry pairs (N2···O3($x − y + 1, x + 1, z − \frac{1}{6}$)) (Figure 1a). In both the peptides, the internal surface of the nanotube is composed of hydrocarbon groups (*tert*- butyl groups from the Boc moiety and CH_3 groups of Val/Leu) with a rugged surface of the pore at the atomic scale (Figure 1c). In **2**, the water molecule was observed to be disordered over two positions with an occupancy ratio of 0.5:0.5 and slightly off the 6-fold screw axis (*Form A*). We recollected the X-ray diffraction data for the crystal of **2** after a span of 8 months, at room temperature as well as at 5 °C, reexamining the status of water and also peptide with the intention of resolving the disorder observed for the entrapped solvent. This
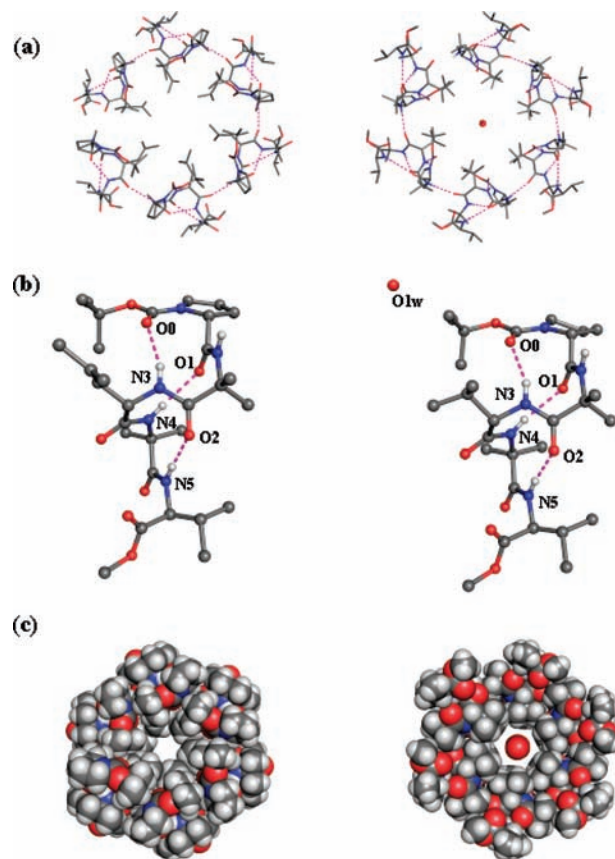

Figure 1. (a) Projection of the crystal structure of peptide **1** (left) and **2** (right) viewed down the 6-fold axis (*c*-axis). (b) Molecular conformation of peptide **1** (left) and **2** (right) indicating the three intramolecular 4→1 hydrogen bonds (dashed lines). (c) Space filling models for the structures of peptides **1** (left) and **2** (right). The view shown is identical to (a). In peptide **1** the central pore (diameter ~5.2 Å) is unoccupied. In peptide **2** the central pore (diameter ~7.5 Å) accommodates water.

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resulted in a structure solution for peptide **2** with identical molecular conformation, but an important difference was observed for the water molecule, which was now ordered and positioned exactly on the 6-fold screw axis, restricting the occupancy to 1/6 (*Form B*).

We develop models for water wires based on the observation of oxygen atoms of water in the pores of **2** (Figure 2). The *Form B* crystal structure revealed a single water molecule occupying a position on the 6-fold axis with an occupancy of 1/6. The water molecule is ordered, and the occupancy of the adjacent sites leads to an unfavorable steric clash (1.75 Å) between the water molecules related by 6_5 screw symmetry (5/6 translation along *c*-axis). This short contact may be avoided by considering a model in which water molecules occupy alternate sites along the *c*-axis (Figure 2a). The relative distance between the water molecules (O···O) is then 3.5 Å. The crystal structure is formed by self-assembly of the tubular peptide columns. The water wires in the independent nanotubes are related by a displacement of the wire by 1.75 Å along the *c*-axis. The water molecules not shown in Figure 2a will form water wires in the independent nanotubes which run through the crystal.
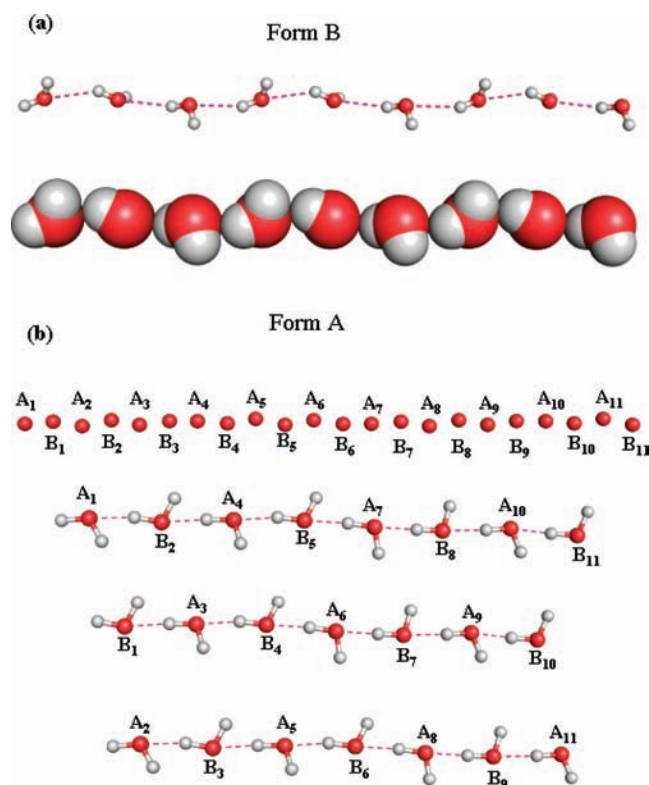

Figure 2. Water wire models for peptide **2**, (a) Form B: O···O = 3.5 Å, H···O = 2.6 Å and (b) Form A: O···O = 2.6 Å, H···O = 1.6 Å.

In *Form A* the water molecule in the asymmetric unit is positionally disordered occupying two sites (A and B, Figure 2b) with an occupancy ratio of 0.5:0.5. Simultaneous occupancy of the sites A1, B1 and A1, A2 leads to short O···O contacts of 0.9 and 1.8 Å, respectively. Therefore, the single-file water wire is built by taking those positions of the water molecules along the *c*-axis of the nanotube which avoid steric clashes. The crystallographic observation of the water molecules leads to three possible water wires as shown in Figure 2b. The distance between the adjacent water molecules in the model is ~2.6 Å. All the wires are identical and a relative displacement of the water wires, of the order of 0.9 Å, will be found in the independent nanotubes, accounting for the spatial disorder observed for the water molecule. The mean square

atomic displacements for the water oxygen atoms after the refinement are as follows: Form A, $U_{iso}(A) = 0.25$ Å^2, $U_{iso}(B) = 0.27$ Å^2 (isotropic) and Form B, $U_{eq} = 0.24$ Å^2 (anisotropic).

Since hydrogen atoms are not directly located from the electron density it is necessary to spatially fix them in a meaningful manner. Hydrogen atoms were arbitrarily fixed using the parameters,[21] $d_{O-H} = 1.0$ Å and ∠H−O−H = 109.5°. The water molecule was oriented with one of the O−H bonds coincident with the crystallographic 6-fold axis, so that the hydrogen bond interaction between the neighboring molecules in the wire is introduced. The water molecule was then rotated about the 6-fold axis, and the distances of the non-hydrogen bonded O−H group from the projecting atoms of the walls of the pore were examined. The final positions were chosen such that the H···H distances between the water molecules and the pore walls were minimal. The closest contacts between the water molecules and the methyl hydrogens of the Val isopropyl groups lining the pore wall are 2.9 and 3.4 Å in *Form B*. In *Form A* the shortest distances from the water hydrogen to the pore walls are to the methyl hydrogen of the Val isopropyl groups and the methyl groups of the *tert*-butyl moiety of the N-terminus protecting group (3.6 and 3.2 Å for A_1 and A_4 and 3.2 and 4.2 Å for B_1 and B_5) (Figure 3). These H···H distances are appreciably larger than van der Waals limit for the H···H interactions (~2.4 Å).[22] In *Form B* there are three water molecules in the unit cell yielding a peptide water ratio of 2:1. The C_3 axis relating the three water molecules is coincident with the crystallographic 6-fold axis. In the *Form A* water wire there are four water molecules in the unit cell. Adjacent water molecules are related by a 2-fold rotation with the C_2 axis coincident with the 6-fold axis of the crystal.
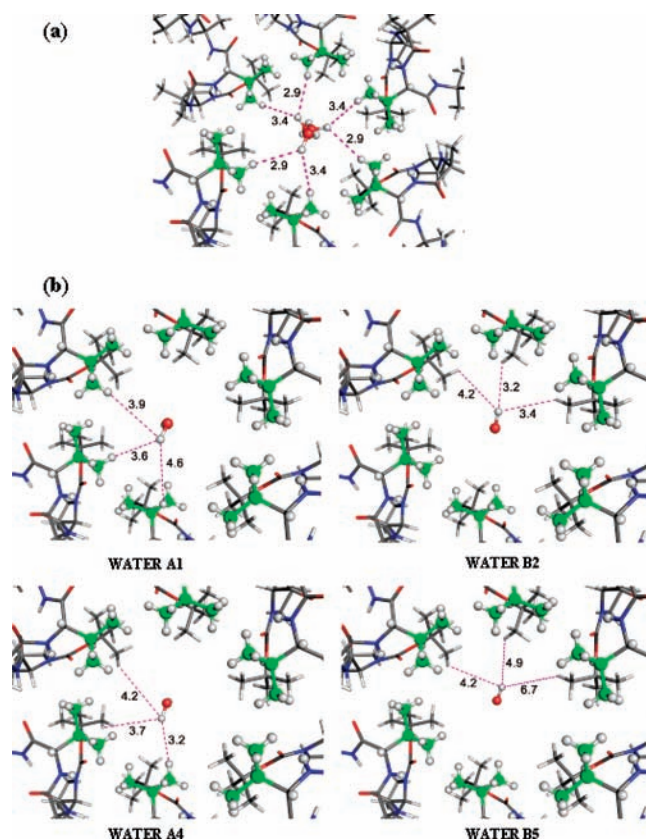

Figure 3. Environment for water molecule within the tubular peptide as observed in the pore of **2**. (a) Form B and (b) Form A. The atoms of Val(3) C$^\gamma$H$_3$ of the walls of the tube which are at the shortest distance (marked in Å) from the water hydrogen are shown as balls.

In *Form B*, the closest H···H distances result in the proximity of each water molecule to two peptide molecules. In *Form A*, two water molecules closely approach three peptide molecules resulting in the 6 peptide/4 water stoichiometry observed in the unit cell. The O···O distances between the adjacent water molecules in the water wires in *Form B* (3.5 Å) and in *Form A* (2.6 Å) result in the accommodation of three and four water molecules in the unit cell along the *c*-axis (*c* = 10.48 Å).

The question arises as to whether the O···O distances are in one case too far for an optimal hydrogen bond and in the other case uncomfortably close. An analysis of the Cambridge Structural Database (CSD),[23] limited to organic structures, was carried out using a search fragment of a pair of water molecules, applying the constraints O···O (2.3−3.6 Å) and O···H (1.5−2.7 Å). The results summarized in Figure 4 reveal that the peak of the distribution is centered at ∼2.8 Å, but the tails stretch out beyond the limits of 2.6 and 3.5 Å. In the *Form A* water wire, the distance to the wall of the hydrophobic nanotube suggests that any nonbonded interactions may be extremely weak. In the case of *Form B*, water molecules display a close approach to the wall suggesting that any decrease in the water−water hydrogen bond energies due to the longer O···O distances is offset by contributions from nonbonded interactions. The transformation of *Form A* into *Form B* over a period of time in the crystalline state and the subsequent stability of the *Form B* structure suggest that *Form A* may be a metastable state, kinetically trapped in crystals during the process of nucleation.

The characterization of narrow wires of water reported here are of relevance to studies of single-file water in hydrophobic channels
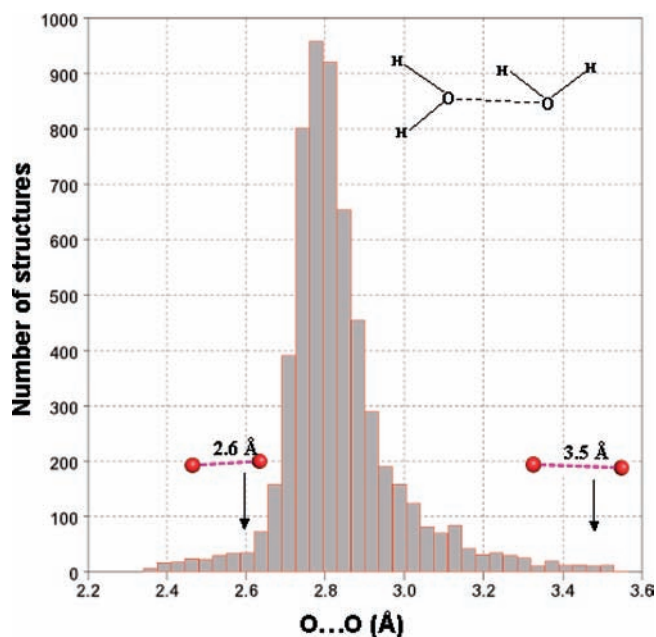

Figure 4. Distribution of O···O distances in 2499 organic structures, from an analysis of Cambridge Structural Database. Search fragment used is shown in the inset.

in biological membranes and carbon nanotubes (CNTs). In large diameter CNTs, computer simulations suggest that internal water configurations can undergo changes from a liquidlike structure to an ordered solidlike arrangement.[3,4] The dimensions of such CNTs (11−14 Å) are significantly larger than those of the hydrophobic peptide nanotubes described above. Single-file arrangement of the water molecules (wires) has been considered in computer simulations when the diameter of the tube is reduced. The structure of water wires inside the peptide nanotubes described in the present report constitutes a direct experimental view of a single-file water arrangement. In principle, rotational motion of the water molecule with alternate hydrogen bond cleavage and formation along the wire forms an efficient "Grotthuss chain" for proton conduction. The stability of the water wire arrangements inside peptide nanotubes is undoubtedly a result of hydrogen bond contributions, dipole−dipole interactions, contributions from rotational entropy, and very weak nonbonded interactions with the hydrocarbon side chains lining the walls. The experimentally derived structures may serve to facilitate the rational design of tubular peptide structures and aid in simulations of the structure and dynamics of the water wires in confined environments.

Supporting Information Available: X-ray crystallographic characterization details and the CIF files are provided. CCDC deposition numbers for **1** and **2** are 728333 and 728334. The X-ray diffraction data collected on peptide **2** after 8 months has been deposited with the CCDC reference number 728335. This material is available free of charge via the Internet at http://pubs.acs.org.

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