X-Ray crystallographic signature of supramolecular triple helix formation from a water soluble synthetic tetrapeptide†

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Single crystal X-ray diffraction studies on the water soluble, synthetic tetrapeptide Tyr(1)–Aib(2)–Tyr(3)–Val(4) 1 with a non-coded amino acid residue (Aib: α-amino isobutyric acid) reveal that the peptide adopts an "S"-shaped molecular structure which self-assembles to form a supramolecular triple helix using various non-covalent interactions including water mediated hydrogen bonds in the solid state.

Mimicking natural structures and functions of biomacromolecules using self-assembling small molecules is really a challenging task. Triple helical structures are ubiquitous in nature, starting from the collagen triple helix[1] to DNA triple helices.[2] Previously a significant amount of attention has been directed to construct well-structured helical peptides by stepwise covalent synthesis[3] rather than utilizing self-assembly of monomeric small peptide units. However, there are several recent examples of the construction of peptide based supramolecular helices.[4] Different approaches have been pursued to create non-peptide supramolecular triple helical structures. A very common approach is the construction of supramolecular triple helices with the assistance of coordinating metal ions.[5] Capo and his coworkers have reported the formation of a tri-nuclear sodium/lithium triple stranded helicate obtained from a chiral ligand.[5] Perkins *et al.* have reported the formation of a Ni^{2+} assisted supramolecular triple helix from a tris-bipyridyl based ligand.[5] Supramolecular triple helices were also constructed using rigid organic templates without metal ions.[6] Hanessian *et al.* have introduced a metal-free triple stranded helical superstructure by self-assembly of chiral diamine/diol.[6] Roelens and his coworkers have successfully designed, synthesized and characterized triple stranded self-organized helices based on the hydrogen bonding abilities of *trans*-1,2-diaminocyclohexane and chiral diacid salts.[6] A considerable amount of work has been reported in the literature about triple helix formation and its stability in collagen based peptides.[7] However, the formation of a supramolecular triple helix using a non-collagen based short peptide segment is almost unnoticed. In this report, we present the formation of a water mediated supramolecular triple helix through molecular self-assembly of a water-soluble tetrapeptide Tyr(1)–Aib(2)–Tyr(3)–Val(4) (peptide **1**)‡ by various non-covalent interactions.

Peptide **1** has been synthesized using conventional solution phase methodology.[8] We used the Tyr residue to increase the hydrogen bonding ability and the conformationally constrained Aib residue to enhance crystallinity and to increase the helical nature of the peptide backbone.[9] The Boc group was used for N-terminal protection and the C-terminus was protected as a methyl ester. Couplings were mediated by dicyclohexylcarbodiimide-1-hydroxybenzotriazole (DCC/HOBt). Deprotection of methyl ester was performed using saponification method and the Boc group was deprotected by TFA. The final compound was fully characterized by ^{1}H NMR spectroscopy and mass spectrometry (ESI†). A colorless hexagonal crystal, suitable for an X-ray diffraction study was obtained from water–methanol solution by slow evaporation. The structure of the peptide **1** is shown in Fig. 1 with the atomic numbering scheme. The majority of the backbone torsion angles of peptide **1** are in the extended region (ψ_1 142.7(5); ψ_3 139.5(5); ϕ_4 123.2(6); ψ_4 −151.9(4)°) except ϕ_3 −62.5(6)° and the dihedral angles around the C^α of Aib(2) that are in the right handed α-helical region (ϕ_2 62.1(6); ψ_2 32.9(7)°). Thus, the overall molecular backbone of peptide **1** takes an "S"-shaped conformation. Fig. 1 shows that the molecule does not form any intramolecular hydrogen bonds in the "S"-shaped backbone molecular conformation in the crystalline state. There are two water molecules in the asymmetric unit and these waters form two intermolecular hydrogen bonds (O1W–H1W2···O5 and O3N–H3N···O2W) with the tetrapeptide **1** (Table 1). Each peptide **1** molecule is then stacked one on top of the other maintaining the proper registry to form an intermolecular hydrogen bonded helical strand along the crystallographic *c* axis (Fig. 2). There are two intermolecular hydrogen bonds O1N–H1N···O4 and N4–H4···O1N (Table 1) connecting individual peptide molecules to form the right handed supramolecular helical strand.

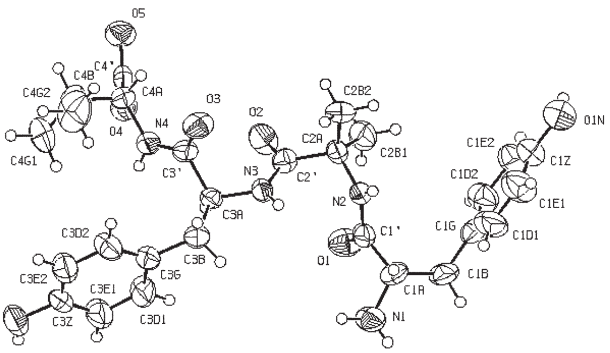

Fig. 1 ORTEP diagram with atomic numbering scheme of the tetrapeptide **1**. Thermal ellipsoids are shown at the 30% probability level. The asymmetric unit also contains two molecules of water that are not included in this figure.

† Electronic supplementary information (ESI) available: synthesis and characterisation details. See http://www.rsc.org/suppdata/cc/b4/b417726f/

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Table 1 Intermolecular hydrogen bonds for peptide **1**

D–H···A[a]	H···A/Å	D···A/Å	D–H···A/°
N1–H1B···O3 (a)	2.00	2.88	169
N1–H1C···O4 (b)	2.00	2.81	149
N1–H1A···O1W (c)	1.87	2.74	164
O1N–H1N···O4 (d)	1.84	2.62	159
N2–H2···O3N (d)	2.52	3.34	159
N3–H3···O5 (b)	2.12	2.94	159
O3N–H3N···O2W	1.93	2.68	153
N4–H4···O1N (e)	2.37	3.16	154
O1W–H1W2···O5	1.88	2.77	153
O2W–H2W1···O2 (f)	2.60	3.01	107
O2W–H2W2···O1 (g)	2.44	3.32	154
O2W–H2W2···O3 (f)	2.56	2.93	103

[a] Symmetry equivalent (a) $-1 + x, -1 + y, z$. (b) $x, -1 + y, z$. (c) $-1 + x, -2 + y, z$. (d) $1 + x - y, x, -1/6 + z$. (e) $y, 1 - x + y, 1/6 + z$. (f) $-1 + y, -x + y, 1/6 + z$. (g) $y, -x + y, 1/6 + z$.

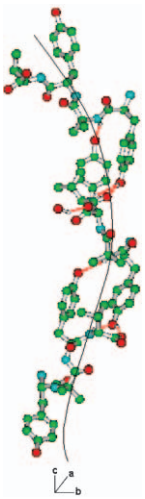

Fig. 2 Packing diagram of peptide **1** molecules maintaining the proper registry to form an intermolecular hydrogen bonded helical strand along the crystallographic *c* axis. Nitrogen atoms are blue, oxygen atoms are red and carbon atoms are green. Non hydrogen bonded hydrogen atoms are omitted for clarity.

The side chain phenolic –OH functionality of Tyr(1) is intermolecularly hydrogen bonded to Val(4) CO and Val(4) NH is also hydrogen bonded to the side chain phenolic O of Tyr(1) to form each supramolecular single helical strand. Three individual helical strands are then connected *via* non-covalent interactions to form the triple helical superstructure along the axis parallel to the crystallographic *c* axis. The space-filling model of the supramolecular helix (Fig. 3) clearly demonstrates that the individual "S"-shaped subunits are stacked by maintaining proper registry between the subunits, generating a right handed supramolecular individual helical strand which forms a triple helical structure upon further self-assembly. The individual helical strands are connected by three intermolecular hydrogen bonds among peptide molecules (N1–H1B···O3, N1–H1C···O4 and N3–H3···O5) and by intervening bridging water molecules through intermolecular hydrogen bonding interactions (O2W–H2W1···O2, O2W–H2W2···O3 and O3N–H3N···O2W, Table 1) (Fig. 4). Previous reports have mentioned the role of water molecules in stabilizing supramolecular single stranded helical structures in short oligopeptides.[10]

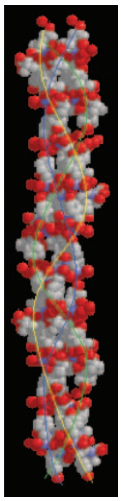

Fig. 3 Space-filling model of a supramolecular triple helical array showing the stacking of subunits maintaining the proper registry. Nitrogen atoms are blue, oxygen atoms are red and carbon atoms are grey. Hydrogen atoms are omitted for clarity.

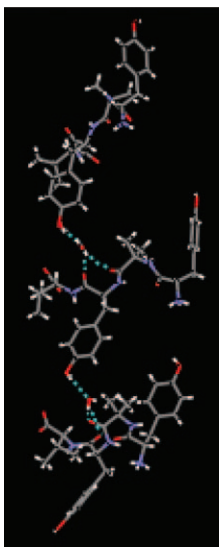

Fig. 4 The intermolecular hydrogen bonding interactions between three individual helical strands and the intervening bridging water molecules. Only one molecule from each helical strand is shown for clarity.

We present here the construction of a supramolecular triple helical structure using molecular self-assembly directed by the conformational features of the tetrapeptide **1** and induced by various non-covalent interactions operating among the peptide molecules and their hydrogen bonding interactions with the bridging water molecules. The remarkable feature is that the reported water-soluble tetrapeptide **1** fails to form any intramolecularly hydrogen bonded turn conformation in spite of having a helix forming Aib residue.[9] The "S"-shaped tetrapeptide **1** subunits are connected by intermolecular hydrogen bonds among themselves to construct a right handed helical strand which on further self-assembly forms a supramolecular triple helix, where the individual strands of the triple helix are connected through intervening water molecules. To the best of our knowledge this

report is the first example of supramolecular triple helix formation from a water-soluble tetrapeptide with an Aib residue.

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Notes and references

‡ Crystallographic data: ($C_{27}H_{36}N_4O_7 \cdot 2(H_2O)$), M = 564.63, hexagonal, space group $P6_5$, a = 8.807(3), b = 8.807(3), c = 64.67(4) Å, V = 4344(3) Å^3, Z = 6, μ = 0.098 mm^{-1}, ρ_{calcd} = 1.295 g cm^{-3}, $F(000)$ = 1812, R_1 = 0.0850, $wR2$ = 0.2360 for 5080 reflections with ($|F_0| > 4\sigma(|F_0|)$). X-Ray crystal data were collected on a Bruker AXS SMART APEX CCD diffractometer with MoKα (λ = 0.71073Å) radiation at 20 °C. The structure was obtained by direct methods using SHELXS-97.[11] The water molecules were located from difference Fourier maps. Refinement was carried out with a full matrix least squares method against F^2 using SHELXL-97.[12] Hydrogen atoms bonded to C1A, C3A, O1W and O2W were located from difference Fourier maps. The OH bond distances and OHO bond angle in O1W and O2W were restrained to 0.96 Å and 104.5° respectively. The remaining hydrogens were fixed geometrically in ideal positions and refined as riding over the non-hydrogen atom to which they are bonded. CCDC 235556. See http://www.rsc.org/suppdata/cc/b4/b417726f/ for crystallographic data in .cif or other electronic format.

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