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Supramolecular peptide helix from a novel double turn forming peptide containing a β-amino acid

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Abstract—A single-crystal X-ray diffraction study of the terminally protected tetrapeptide Boc-β-Ala-Aib-Leu-Aib-OMe **1** (Aib: α-aminoisobutyric acid; β-Ala: β-Alanine) reveals that it adopts a new type of double turn structure which self-associates to form a unique supramolecular helix through intermolecular hydrogen bonds. Scanning electron microscopic studies show that peptide **1** exhibits amyloid-like fibrillar morphology in the solid state. © 2003 Elsevier Science Ltd. All rights reserved.

Helicity in supramolecular architecture is important and ubiquitous in nature. Self-assembled supramolecular helices can be frequently found in many biologically important macromolecules including collagen[1] and the tobacco mosaic virus coat protein.[2] Helical self-assembly is also an important aspect in material sciences.[3] Recently, Karus and co-workers have demonstrated that helical self-assembly of polyionic amino acids results in nanotubular structures.[4] Significant efforts have been made to construct supramolecular helices in non-natural systems.[5–7] Previously, the main effort in such research has been directed in the creation of self-assembled supramolecular helical architecture through metal ion co-ordination (helicates).[6] However, other approaches include metal free helicate formation through the self-assembly of polyfunctional organic compounds.[7] Parthasarathi and his colleagues have synthesized and characterized a series of tripeptides which produce extended helical structures within the crystal structure.[8] However, these helices include intervening water molecules between two consecutive peptide molecules and show a hydrated helix pattern.[8] Recently, growing evidence has suggested that not only β-sheets but also helices have a significant role in amyloid fibril formation,[9] which is the main cause of many neurodegenerative diseases including Alzheimer's disease, Huntington's disease and prion protein diseases.

We are actively involved in designing fibril forming supramolecular architectures including continuous helices[10] and sheets[11] based on self-assembly of appropriate peptide conformations. We have demonstrated that self-assembly of tetrapeptides having the double turn conformation can lead to the formation of continuous hydrogen bonded supramolecular helices in crys-

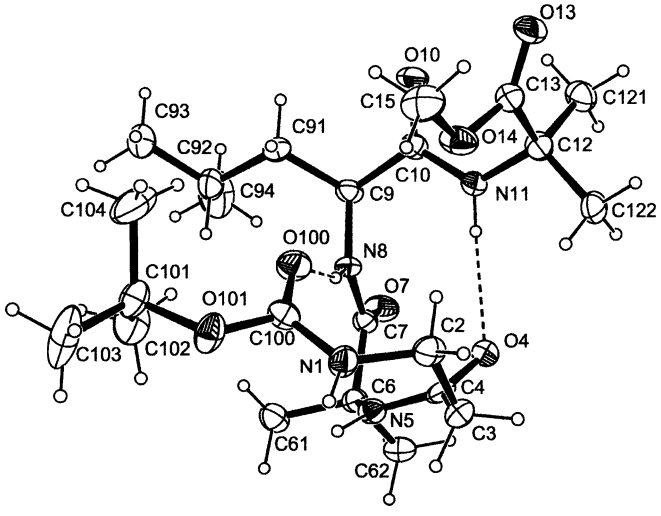

Figure 1. ORTEP diagram of peptide **1** including the atomic numbering schemes. Thermal ellipsoids are shown at the level of 20% probability. Intramolecular hydrogen bonds are shown as dotted lines.

Keywords: supramolecular helix; Aib; β-Ala; fibrils.

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Table 1. Selected torsion angles (°) of peptide **1**

Residue	ϕ	ψ	ω	θ
β-Ala[1]	−103.8(7)	−84.7(9)	−173.8(9)	83.7(9)
Aib[2]	−56.1(9)	−35.2(9)	−173.9(6)	–
Leu[3]	−106.9(8)	19.3(10)	−174.2(6)	–
Aib[4]	54.2(9)	45.8(9)	−170.8(7)	–

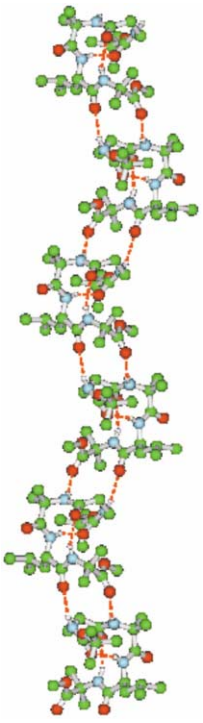

Figure 2. Packing diagram of peptide **1** along the crystallographic *b* axis showing intermolecular hydrogen-bonded supramolecular helix. Nitrogen atoms are blue, oxygen atoms are red, carbon atoms are green and hydrogen atoms are grey. Hydrogen bonds are shown as dotted lines.

tals and exhibit amyloid-like fribillar morphology in the solid state.[10b,c] So, it is worthwhile to study whether a tetrapeptide with an *N*-terminal flexible β-amino acid residue can lead to the formation of a supramolecular helix. In this context, we have designed and synthesized a tetrapeptide[12] Boc-β-Ala(1)-Aib(2)-Leu(3)-Aib(4)-OMe **1** that adopts a new type of double turn conformation and its self-assembly leads to form a unique supramolecular helix in the crystal.[13] A scanning electron microscopic study has revealed that peptide **1** exhibits amyloid-like fibrillar morphology in the solid state.

The molecular conformation of peptide **1** in the crystal is depicted in Figure 1. In the crystal structure there are two adjacent intramolecular hydrogen bonds (N8–H8···O100 and N11–H11···O4) resulting in a consecutive double bend conformation in the solid state. Interestingly, in this reported peptide, the insertion of one CH_2 group into the backbone (β-Ala) causes an unusual, modified 4→1 type hydrogen bond between the CO of the Boc group (O100) and the Leu(3) NH (N8) forming an 11-membered hydrogen bonded ring (Fig. 1). In addition, a 10-membered distorted type I β-turn in peptide **1** adjacent to the 11-membered ring, involving a hydrogen bond between β-Ala(1) CO (O4) and Aib(4) NH (N11) is also formed (Fig. 1). The majority of the ϕ, ψ values of the constituent amino acid residues of peptide **1** are within the helical region of the Ramachandran plot. Only the ϕ values of β-Ala(1) and Leu(3) deviate from the ideal helical ϕ values (Table 1). The torsion angle about the methylene groups of the β-Ala (θ) in peptides **1** is in the *gauche* conformation. This facilitates the easy accommodation of the backbone CH_2 group into the folded structure leading to an unusual 11-atom hydrogen bonded ring motif. This motif has been observed previously in synthetic peptides containing a centrally located -β-Ala-γ-Abu- segment.[17] The Aib(2) of peptide **1** simultaneously occupies the *i*+2th position of the first turn and *i*+1th position of the second turn in a double turn conformation.

The individual sub-units of this double bend peptide are themselves regularly inter-linked via multiple intermolecular hydrogen bonds and thereby form a supramolecular helix along the crystallographic b direction (Fig. 2). Figure 3 shows a space-filling model of the supramolecular helix formed by peptide **1** in the crystal. The hydrogen bonding parameters of peptide **1** are listed in Table 2. There are two intermolecular hydrogen bonds N1–H1···O10 and N5–H5···O13 that are

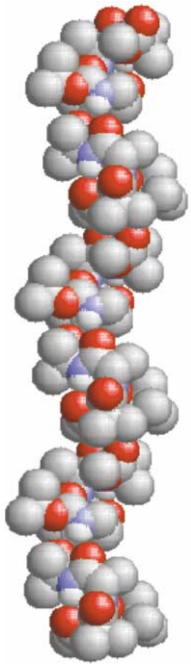

Figure 3. Space-filling representation of the higher-ordered supramolecular helical assembly of peptide **1** via intermolecular hydrogen bonds in the solid state. Nitrogen atoms are blue, oxygen atoms are red and carbon atoms are grey. Hydrogen atoms not involved in hydrogen bonds and the side chain of Leu(3) are omitted for clarity.

Table 2. Intermolecular and intramolecular hydrogen bonding parameters of peptide **1**

D–H···A	H···A (Å)	D···A (Å)	D–H···A (°)
N8–H8···O100	2.19	3.02	161
N11–H11···O4	2.22	3.03	157
N1–H1···O10[a]	2.28	2.97	137
N5–H5···O13[a]	2.31	3.13	158

[a] Symmetry element $x+1$, $y-1/2$, $-z+1/2$.

responsible for connecting individual molecules to create and stabilize the supramolecular helical structure.

The morphological study of the peptide **1** has been performed using a scanning electron microscope (SEM). The SEM image (Fig. 4) of the fibrous material clearly shows that the filamentous aggregate resembles the morphology of neurodegenerative disease-causing amyloid fibrils.[9,18]

Peptide **1** with an *N*-terminal flexible β-Ala residue forms a new type of double turn conformation in which the first turn is an unusual (11-membered) turn and the second one is a distorted type I β-turn. Further self-assembly of this new type of structural subunit with a conformationally flexible β-amino acid leads to the formation of a unique supramolecular helical architecture. Our previous results have also established the self-assembly of double turn conformations into supramolecular helices.[10b,c] So supramolecular helicity can be pre-programmed by judicious selection of peptide sequences that are intended to form the intramolecular hydrogen bonded double turn structures. The hierarchical self-assembly of individual supramolecular helices of peptide **1** ultimately leads to the formation of fibrils in the solid state. So, fibril forming supramolecular helix formation by this model peptide **1** can assist to map out the structure–function relationship of many biological events including amyloidosis.[9,18]

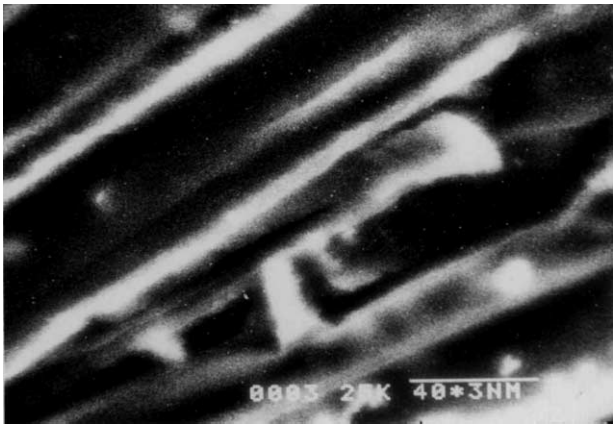

Figure 4. SEM image of the peptide **1** showing amyloid-like fibrillar morphology in the solid state.

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12. The peptide Boc-βAla(1)-Aib(2)-Leu(3)-Aib(4)-OMe ($C_{23}H_{42}N_4O_7$) was synthesized by conventional solution phase methodology (Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: New York, 1984; pp. 1–282).
^{1}H NMR 300 MHz ($CDCl_3$, δ ppm): 7.24 [Aib(4) NH, 1H, s]; 6.60–6.63 [Leu(3) NH, 1H, d, *J* 7.8]; 6.49 [Aib(2) NH, 1H, s]; 5.53 [β-Ala(1) NH, 1H, d, *J* 9.5]; 4.46–4.54 [C^{α}Hs of Leu(3), 1H, m]; 3.72 [-OCH_3, 3H, s]; 3.35–3.41 [CβHs of β-Ala(1) 2H, m]; 2.38–2.43 [C^{α}Hs of β-Ala(1) 2H, m]; 1.6–1.66 [CβHs (2H) and C^{γ}H (1H) of Leu(3), m]; 1.45 [Boc-CH_3, 9H, s]; 1.48–1.54 [CβH_3s of Aib(2) and Aib(4), 6H, s]; 0.9–0.95 [C^{δ}Hs of Leu(3), 6H, m]. MALDI-MS [M+Na^++H^+ = 510.5, M_{calcd} = 486]. Anal. calcd for $C_{23}H_{42}N_4O_7$ (486): C, 56.79; H, 8.64; N, 11.52. Found: C, 56.92; H, 8.67; N, 11.46%.
13. Single crystals were obtained from ethyl acetate solutions by slow evaporation. Crystal data: **1**, $C_{23}H_{42}N_4O_7$, M = 486.61, orthorhombic, space group $P2_12_12_1$, a = 9.342(14), b = 16.638(19), c = 17.82(2) Å, U = 2770 Å^3, Z = 4, dm = 1.167 M gm^{-3}. Intensity data were collected with MoKα radiation using the MAR Research Image Plate System. The crystal was positioned at 70 mm from the Image Plate. 100 frames were measured at 2° intervals with a counting time of 5 min to give 2806 independent reflections. Data analysis was carried out with the XDS program.[14] The structure was solved using direct methods with the Shelx86 program.[15] 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 structure was refined on F^2 using Shelxl.[16] The final R values were R_1 = 0.0924 and wR_2 = 0.2554 for 1554 data with $I>2\sigma(I)$. The largest peak and hole in the final difference Fourier were 0.20 and –0.19 e Å^{-3}. The data have been deposited at the Cambridge Crystallographic Data Center with reference number CCDC 195016.
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