Pergamon

Tetrahedron Letters 43 (2002) 2653–2656

TETRAHEDRON LETTERS

First crystallographic signature of the highly ordered supramolecular helical assemblage from a tripeptide containing a non-coded amino acid

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Received 3 January 2002; revised 25 January 2002; accepted 6 February 2002

Abstract—The model tripeptide Boc-Leu(1)-Aib(2)-Phe(3)-OMe **1** containing a non-coded amino acid residue (Aib: α-amino isobutyric acid) forms a supramolecular helical assemblage via non-covalent interactions in single crystals. The SEM image of the peptide **1** in the solid state shows the ribbon like fibrillar morphology, a characteristic feature of highly ordered aggregated fibrils like amyloid fibrils. © 2002 Published by Elsevier Science Ltd.

Highly ordered, self-assembled structures of synthetic peptide oligomers can display structural and functional capabilities that can be directed by design and that have enabled their utility in biological science as ion channels to be developed.[1] While the self-assembly of peptides into hollow nanotubes,[1] supramolecular β-sheets[2,3] and tapes[4] has been widely studied due to their many applications in biological systems, the supramolecular peptide helix has been paid little attention due to the lack of an appropriate model system. However, the supramolecular helical structure is interesting, as it occurs in nature.[5] Goldsbury and co-workers have suggested that not only β-sheet structures but also helices may have a role in highly ordered self-aggregated amyloid plaque formation in amyloid diseases.[6] So, it is necessary to construct the supramolecular helical assemblage. Previously a number of different approaches have been pursued to create non-peptide

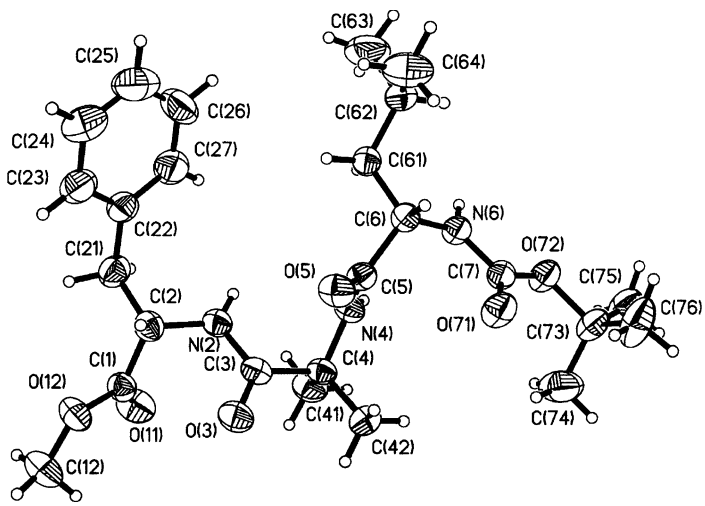

Figure 1. The ORTEP diagram of peptide **1** including the atom numbering scheme. Thermal ellipsoids are shown at the level of 30% probability.

Keywords: supramolecular helix; Aib; amyloid fibrils.
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0040-4039/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0040-4039(02)00283-6

supramolecular helical structures.[7] One such approach is metal-ion directed supramolecular helix formation.[8] Lehn and his co-workers have successfully designed, synthesized and characterized single stranded self-organized helices based on an oligo-amide[9a] strand composed of 2,6-diaminopyridine and 2,6-pyridine dicarboxylic acid. These compounds not only form single helical conformers, but also self-assemble to form reversible double helical dimers.[9b,c] There are several other examples of self-assembled, hydrogen bond-mediated supramolecular helical structures from small organic molecules.[10] However, all previously reported supramolecular helices were constructed using either rigid organic templates or through metal co-ordination. Herein we report the formation of a self-assembled, supramolecular helix in crystals from a terminally blocked synthetic tripeptide[11] Boc-Leu(1)-Aib(2)-Phe(3)-OMe **1** and the formation of amyloid-like fibrils in the solid state. This is the first evidence of a supramolecular peptide helix, which is constructed by purely non-covalent interactions.

The ORTEP diagram of the peptide **1** with the atom-numbering scheme is presented in Fig. 1. The most interesting feature of the peptide **1** in crystals[12] reveals that the compound is unable to form any intramolecular hydrogen bonded folded structures even though the ϕ and ψ values of the constituent amino acid residues fall within the helical region of the Ramachandran map. The reported compound forms an intermolecular hydrogen bonded column along the crystallographic *a* axis (Fig. 2). There are two intermolecular hydrogen bonds N4–H4···O3 and N6–H62···O5 (Table 2) connecting individual peptide molecules to form individual columns. The C=O and NH groups of Leu(1) and Aib(2) are engaged in intermolecular hydrogen bonding leaving the same hydrogen bonding functionalities of the Phe(3) residue uninvolved. The torsion angles of all the amino acid residues of the peptide **1** are listed in Table 1. It is evident from Table 1 that except ψ_3 of Phe(3), all the ϕ,ψ values lie within the α-helical portion of the Ramachandran plot. This might be helpful for the formation of a supramolecular helical assemblage. It was found that the torsion angles ϕ_1 (−62.91) and ψ_1 (−41.20) are in the right handed helical region whereas the ϕ_2 (58.15) and ψ_2 (46.24) are in the left handed helical region. This is due to the presence of the achiral Aib(2) residue. The individual columns are then stacked via van der Waals' interactions along the *c* direction to form highly ordered supramolecular helical assemblies (Figs. 3a and 3b). The stacking of the molecules are generated by the crystallographic 2_1 screw axis, as prescribed by the $P2_12_12_1$ space group of the crystal structure.

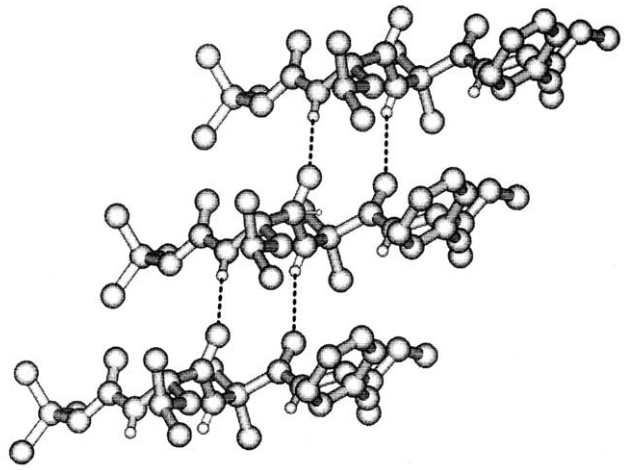


Figure 2. Packing diagram of peptide **1** along crystallographic *a* axis showing intermolecular hydrogen bonding in solid state and the formation of individual columns.

The fibrillar morphology of the reported peptide **1** has been studied using scanning electron microscopy (SEM). The scanning electron micrographs (Figs. 4a and 4b) of the dried fibrous material (slowly growing from ethyl acetate solution) clearly show that the aggregate in the solid state has a filamentous ribbon like fibrillar morphology which is reminiscent of neurodegenerative disease causing amyloid fibrills.[6]

The present result provides an efficient approach to the creation of a helical superstructure through molecular self-assembly directed by the conformational features of the peptide **1** and induced by the non-covalent interactions operating among the molecules. Although, most of the backbone torsion angles lie within the α-helical region, the peptide **1** fails to form any intramolecularly hydrogen-bonded folded conformations. This is due to the absence of the requisite number of amino acid residues in the reported peptide to form a full turn of an α-helix and this assists the peptide **1** molecules to

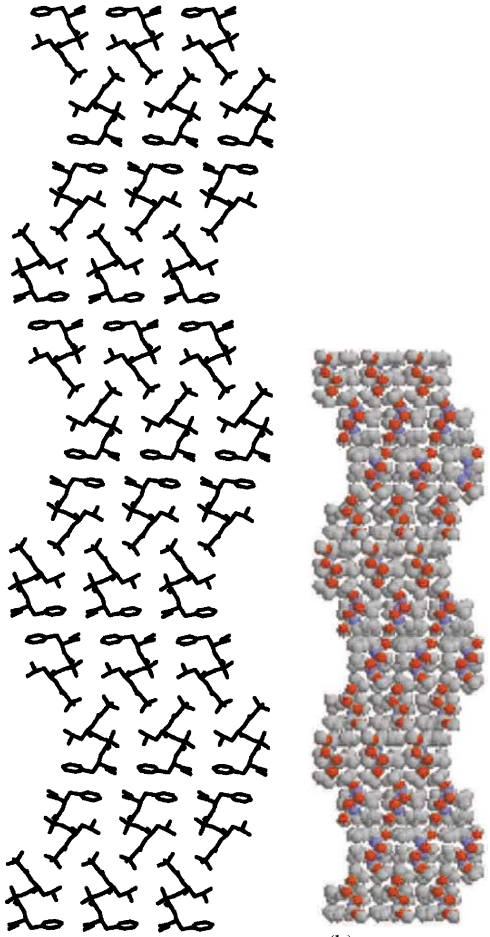
Table 2. Intermolecular hydrogen bonding parameters of peptide **1**

D–H···A	H···A (Å)	D···A (Å)	D–H···A (°)
N6–H62···O5[a]	2.10	2.980	174
N4–H4···O3[a]	2.27	3.083	158

[a] Symmetry equivalent 1+*x*, *y*, *z*.

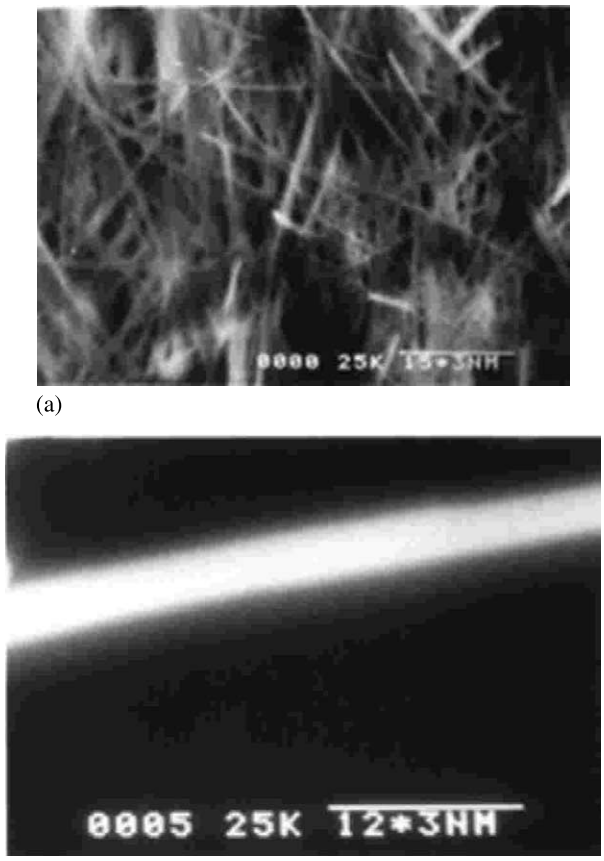
Table 1. Selected torsion angles (°) for peptide **1**

C6–N6–C7–O72	−175.5(3)	ω_0	N4–C4–C3–N2	46.2(4)	ψ_2
C7–N6–C6–C5	−62.8(4)	ϕ_1	C4–C3–N2–C2	166.7(3)	ω_2
N6–C6–C5–N4	−41.5(4)	ψ_1	C3–N2–C2–C1	−55.3(4)	ϕ_3
C6–C5–N4–C4	165.9(3)	ω_1	N2–C2–C1–O12	141.4(3)	ψ_3
C5–N4–C4–C3	58.6(4)	ϕ_2			



(a) (b)

Figure 3. (a) Packing of individual columns in the *c* projection illustrating the formation of a highly ordered supramolecular helical assembly via van der Waals' interactions. (b) Space-filling model showing higher-ordered supramolecular helical assembly via van der Waals' interactions in the solid state.



(a) (b)

Figure 4. (a) SEM image of the peptide **1** showing filamentous fibrillar morphology in the solid state. (b) SEM image of peptide **1** showing a single filament in the solid state.

self-organize and then self assemble to form a supramolecular helical structure by purely noncovalent interactions. Another noteworthy feature of the reported compound is the formation of a fibrillar structure in solid state, as reminiscent of amyloid fibrils. Goldsbury and his co-workers previously reported that amyloid fibrils of human amylin and human calcitonin contain not only β-sheet structures but also significant amounts of helices.[6] Thus, fibrillar morphology with supramolecular helical assemblage of the peptide **1** may be used as a model system[3] to study the fibrillation process of many neurotoxic disease causing amyloid fibrils.

Acknowledgements

We acknowledge the Department of Science and Technology, New Delhi, India for financial support. S.K. Maji wishes to acknowledge the Council for Scientific & Industrial Research (CSIR), New Delhi, India for financial assistance. We are grateful to Dr. Dhananjoy Bhattacharya, Biophysics Department, Saha Institute of Nuclear Physics, Calcutta, India for helpful comments and advice.

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11. The peptide Boc-Leu(1)-Aib(2)-Phe(3)-OMe($C_{25}H_{39}N_3O_6$) was synthesized by conventional solution phase methodology (Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer: New York, 1984; pp. 1–282). Coupling of Boc-Leu-OH with H-Aib-OMe was followed by saponification yielding the dipeptide acid Boc-Leu-Aib-OH which was further coupled to H-Phe-OMe using *N*,*N*′-dicyclohexylcarbodiimde (DCC) and 1-hydroxybenzotriazole mediated condensation. The final compound was purified on a silica gel column (100–200 mesh size) using ethyl acetate and toluene mixture (1:2) as eluent. Yield = 81% 300 MHz ^{1}H NMR ($CDCl_3$, δ ppm): 7.22–7.30 [phenyl ring protons]; 7.13 [Phe NH, 1H, d]; 6.89 [Leu NH, 1H, d]; 6.61 [Aib NH, 1H, s]; 4.81 [$C^{\alpha}H$ of Phe, 1H, m]; 3.98 [$C^{\alpha}H$ of Leu, 1H, m]; 3.70 [-OCH_3, 3H, s]; 3.11 [$C^{\beta}H$ of Phe, 2H, m]; 1.60 [$C^{\beta}Hs$ of Leu, 2H, m]; 1.49 [Boc-CH_3s, 9H, s]; 1.44 [$C^{\beta}H_3$ of Aib, 6H, s]; 1.25 [$C^{\gamma}H$ of Leu, 1H, m]; 0.94 [$C^{\delta}H$ of Leu, 6H, m]. MALDI-MS [M+Na^+ = 500, M_{calcd} = 477]. Anal. calcd for $C_{25}H_{39}N_3O_6$ (477): C, 62.89; N, 8.8; H, 8.17. Found: C, 63.06; N, 8.87; H, 7.9.
12. Single crystals suitable for X-ray diffraction of peptide **1** were grown from dimethyl sulphoxide (DMSO) solution by slow evaporation. Crystal data for peptide **1**, $C_{25}H_{39}N_3O_6$, M_w = 477.59, Colourless tablet 0.48×0.52×0.65 mm, orthorhombic, space group $P2_12_12_1$ (no. 19), a = 6.023(3), b = 10.311(3), c = 43.051(7) Å, U = 2673(15) Å^3, T = 293 K, Z = 4, D_c = 1.186 g cm^{-3}, λ = 0.71073 Å μ = 0.85 cm^{-1}. No absorption correction, 4179 unique reflections ($2\theta_{max}$ = 60°) of which 2887 had $I>2\sigma(I)$. Siemens P4 diffractometer, graphite-monochromated Mo Kα radiation, ω scans. The structure was solved by direct methods (SHELXS-97)[13] and refined against F(obs)×2 by full-matrix least squares (SHELXL-97).[14] Hydrogen atoms were placed at calculated positions and allowed to ride on their parent atoms. Terminal reliability indices were R_1 = 0.058 [$I>2\sigma(I)$], wR_2 = 0.198 for 340 refined parameters, S = 1.21, min./max. res. 0.19/–0.19 e Å^{-3}. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-176329. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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