Pergamon

Tetrahedron Letters 43 (2002) 5465–5468

TETRAHEDRON LETTERS

Self-assembly of a short peptide monomer into a continuous hydrogen bonded supramolecular helix: the crystallographic signature

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Received 13 May 2002; accepted 31 May 2002

Abstract—The single-crystal X-ray diffraction study of a terminally protected tetrapeptide Boc-Leu-Aib-Phe-Aib-OMe **1** (Aib: α-amino-isobutyric acid) reveals that it forms a continuous hydrogen-bonded supramolecular helix starting from the double bend conformation as an associating sub-unit. The scanning electron micrograph of the peptide **1** exhibits amyloid-like fibrils in the solid state.

The creation of novel supramolecular architectures such as the supramolecular helices and β-sheets is an emerging field of current research due to their many potential applications in biological and material sciences.[1] Supramolecular β-sheets are appropriately studied using a β-strand forming peptide as a subunit. Many research groups are deeply involved in designing supramolecular peptide β-sheets to decipher the self-assembly mechanism and pathway(s) of the β-sheet aggregates which form amyloid fibrils[2] with the aim of developing diagnostics and therapeutics for amyloid diseases. Several approaches have been pursued to construct and study supramolecular helices based on non-peptide systems. This is in contrast to supramolecular β-sheets for which studies are concentrated primarily on peptide systems.[2,3] Most studies of peptide systems have resulted in unimolecular helix formation (3_{10} helix, α helix or π helix) which is mainly stabilized through intramolecular hydrogen bonding interactions.[4] Supramolecular helical architecture can be formed by intermolecular hydrogen bonds,[5] and metal-ion complexation.[6] After its introduction by Lehn and co-workers in 1987, metal-directed supramolecular self-assembly of organo-ligand strands into double and triple-helical arrays (helicates)[7] has become one of the popular routes for designing supramolecular helices.[6] Higher order self-assembly of small poly-functional organic compounds through intermolecular hydrogen bonds and/or van der Waals' interactions to form double and triple helical architectures have been reported in recent years.[5] All previously reported supramolecular helices have, however, been constructed using either rigid organic templates or through metal co-ordination. Much less attention has been paid to supramolecular peptide helices, possibly due to the lack of an appropriate model system. This is despite the fact that supramolecular peptide-helices are particularly important as they occur throughout biological systems with different levels of self-organizations and self-associations such as in collagen,[8] which consists of polypeptide strands that are organized in triple helices (tropocollagen) and subsequently self-assemble to form fibrils of higher order collagen fiber. Again, besides β-sheets, helices also have a role in the formation of amyloid fibrils of human amylin and human calcitonin.[9] So, it is worthwhile to study supramolecular peptide helices. There is only one existing example of the formation of a supramolecular peptide helix and this occurred via the self-assembly of a short peptide monomer.[10] However, this previous study may be of little general significance for supramolecular helix formation because there is a lack of intermolecular hydrogen bonding along the axis of the supramolecular helix.

We have now established and report here the formation of a novel supramolecular helix from a terminally blocked tetrapeptide[11] Boc-Leu(1)-Aib(2)-Phe(3)-

Keywords: supramolecular helix; Aib; amyloid fibrils.
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0040-4039/02/$ - see front matter
PII: S0040-4039(02)01050-X

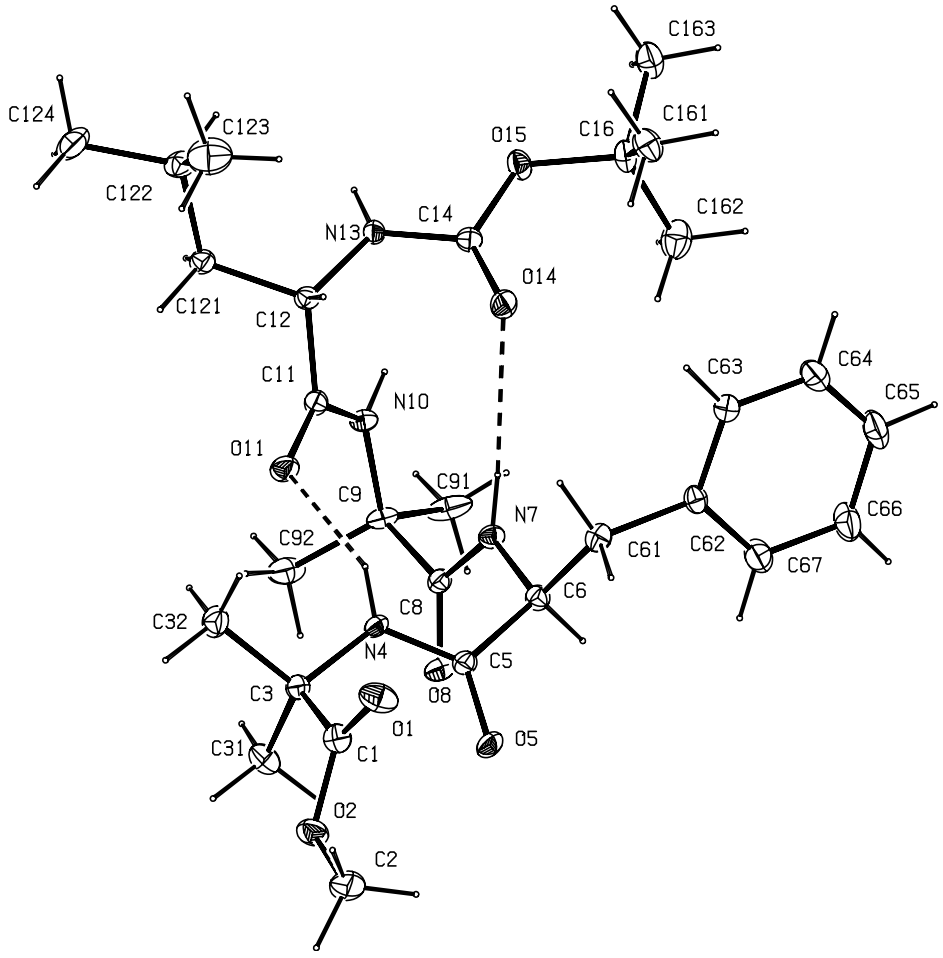

Figure 1. The structure of peptide **1** showing the atomic numbering scheme. Ellipsoids at 20% probability. Intramolecular hydrogen bonds are shown as dotted lines.

Aib(4)-OMe **1** which self-associates, exploits the hydrogen-bonding functionalities of the peptide bonds in crystals[12] and also provides an amyloid-like fibrillar morphology in the solid state.

The crystalline molecular conformation of peptide **1** is represented in Fig. 1. From Fig. 1 it is evident that there are two adjacent intramolecular hydrogen bonds (N4–H4···O11 and N7–H7···O14) resulting in a consecutive double bend (β-turn) conformation in the solid state. The backbone torsion angles of peptide **1** (Table 1) reveal that both these turns in the double bend are a Type I β-turn. The Aib(2) occupies the *i*+2th position of first turn and *i*+1th position of the second turn. 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). Fig. 3 shows a stereo space-filling model of the supramolecular helix formed by peptide **1** in the crystalline form. The hydrogen bonding parameters of peptide **1** are listed in Table 2. There are two intermolecular hydrogen bonds, N10–H10···O5 and N13–H13···O8, which are responsible for connecting individual molecules to create and stabilize the helical self-assembly. Backbone torsions (Table 1) of this peptide are mostly in the right-handed helical region of the Ramachandran diagram [except for the ψ value of Aib(4)]. This might be a prerequisite for supramolecular helix formation.

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

O15–C14–N13–C12	−172.0(4) ω_0	C9–C8–N7–C6	174.1(4) ω_2
C14–N13–C12–C11	−70.2(6) ϕ_1	C8–N7–C6–C5	−56.4(6) ϕ_3
N13–C12–C11–N10	−16.9(7) ψ_1	N7–C6–C5–N4	−35.9(6) ψ_3
C12–C11–N10–C9	177.1(5) ω_1	C6–C5–N4–C3	172.8(5) ω_3
C11–N10–C9–C8	−57.8(7) ϕ_2	C5–N4–C3–C1	51.3(6) ϕ_4
N10–C9–C8–N7	−21.3(6) ψ_2	N4–C3–C1–O2	−135.7(5) ψ_4

A scanning electron microscope was used for the morphological studies of peptide **1**. The scanning electron micrograph (Fig. 4) of the dried fibrous materials (grown slowly from a methanol/water mixture), clearly shows the amyloid-like filament aggregates.[9,13]

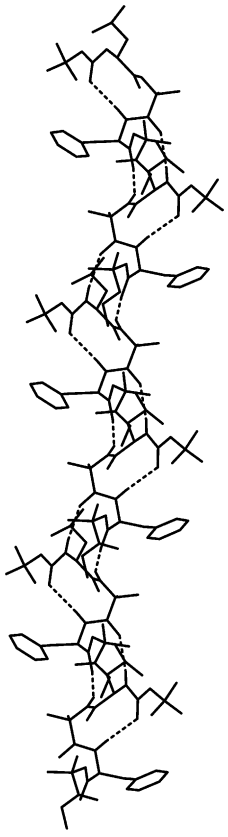

Figure 2. The packing of peptide **1** showing the intermolecular hydrogen-bonded supramolecular helix along the *b* axis. Hydrogen bonds are shown as dotted lines.

The self-assembly of peptide **1**, occurring through multiple hydrogen bonds between peptide linkages of adjacent molecules results in the formation of a supramolecular helical architecture. So, peptide **1** provides a unique conformational subunit of supramolecular peptide helices. Moreover, the hierarchical self-assembly of peptide **1** results in amyloid-like fibril formation in the solid state indicating the mimicry of many naturally occurring macromolecules. Previous results suggested that besides supramolecular sheets, helices also have a role in the formation of amyloid fibrils of human amylin[9a] and calcitonin.[9b] An investigation of the pathway(s) and supramolecular aggregates of amyloid fibril formation have a major role in therapeutics of the amyloid diseases. Hence, the atomic model of peptide **1** significantly increases our understanding of amyloid fibrillogenesis.[9,13]

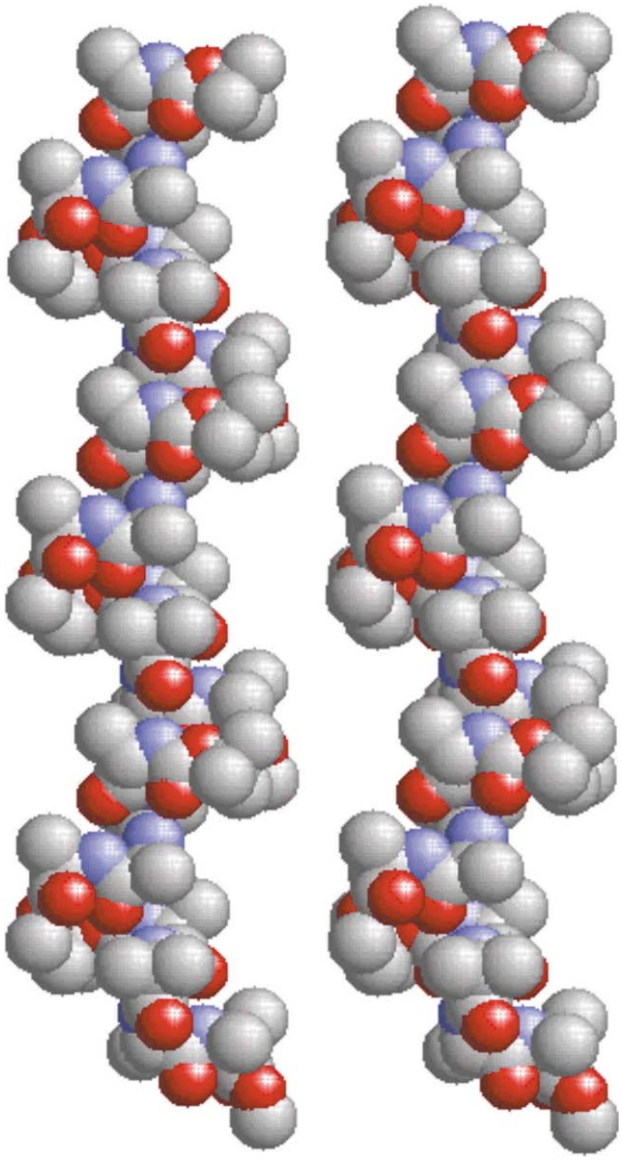

Figure 3. A cross-eye stereo, space-filling representation showing a higher-ordered supramolecular helical assembly as determined by X-ray crystal structure analysis. Nitrogen atoms are blue, oxygen atoms are red and carbon atoms are gray. Side chain of Lue(1), Phe(3) and hydrogen atoms are omitted for clarity.

Acknowledgements

We thank the EPSRC and the University of Reading, UK for funds for the Image Plate System. This research is also supported by a grant from the Department of Science and Technology (DST), India. S. K. Maji wishes to acknowledge the Council for Scientific and Industrial Research (CSIR), New Delhi, India for financial assistance.

Table 2. Hydrogen bonding parameters of peptide **1**

D–H···A	H···A (Å)	D···A (Å)	D–H···A (°)
N4–H4···O11	2.21	2.942	143
N7–H7···O14	2.41	3.263	169
N10–H10···O5[a]	2.20	2.991	152
N13–H13···O8[a]	2.30	3.154	170

[a] Symmetry equivalent $1-x$, $0.5+y$, $-0.5-z$.

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

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11. The peptide Boc-Leu(1)-Aib(2)-Phe(3)-Aib(4)-OMe ($C_{29}H_{46}N_4O_7$) was synthesized by conventional solution phase methodology (Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer: New York, 1984; pp. 1–282).
    ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.19–7.26 [phenyl ring protons of Phe (3)]; 7.17 [Aib(4) NH, 1H, s]; 6.74 [Phe(3) NH, 1H, d]; 6.49 [Aib(2) NH, 1H, s]; 4.79 [Leu(1) NH, 1H, d]; 4.75 [C$^{\alpha}$H of Phe(3), 1H, m]; 3.87 [C$^{\alpha}$H of Leu(1), 1H, m]; 3.72 [-OCH_3, 3H, s]; 3.43 [C$^{\beta}$H of Phe(3), 2H, m]; 1.94 [C$^{\gamma}$H of Leu(1), 1H, m]; 1.60 [C$^{\beta}$Hs of Leu (1), 2H, m]; 1.52 [C$^{\beta}$H of Aib(2), 6H, s]; 1.48 [C$^{\beta}$H of Aib(4), 6H, s]; 1.45 [Boc-CH_3s, 9H, s]; 0.93 [C$^{\delta}$H of Leu(1), 6H, m].
    Anal. calcd for $C_{29}H_{46}N_4O_7$: C, 61.81; H, 8.35; N, 9.95. Found: C, 61.03; H, 8.57; N, 9.19%.
12. Single crystals suitable for X-ray diffraction of peptide **1** were grown from an ethyl acetate solution by slow evaporation.
    Crystal data for peptide **1**, $C_{29}H_{46}N_4O_7$, Mw = 562.70, orthorhombic, space group $P2_12_12_1$, a = 13.253(17), b = 15.694(19), c = 15.768(19) Å, U = 3280 Å^3, Z = 4, dm = 1.137 Mg m^{-3}. Intensity data were collected with Mo Kα radiation using the MARresearch 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 3474 independent reflections. Data analysis was carried out using the XDS program.[14] The structure was solved using direct methods with the SHELX-86 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 = 0.0610 and wR_2 = 0.1779 for 1716 data with $I>2\sigma(I)$. The largest peak and hole in the final difference Fourier were 0.20 and −0.20 e Å^{-3}. The data have been deposited at the Cambridge Crystallographic Data Center with reference number CCDC 184602.
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