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An amyloid-like fibril forming antiparallel supramolecular β-sheet from a synthetic tripeptide: a crystallographic signature

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Abstract—Single crystal X-ray diffraction studies of a terminally blocked tripeptide Boc-Leu(1)-Aib(2)-Leu(3)-OMe **1** demonstrates that it adopts a bend structure without any intramolecular hydrogen bond. Peptide **1** self-assembles to form a supramolecular antiparallel β-sheet structure by various non-covalent interactions including intermolecular hydrogen bonds in the crystal and it exhibits amyloid-like fibrillar morphology in the solid state.

The design of short model peptide molecules with a suitable conformation, that is able to self-associate via non-covalent interactions, forming supramolecular β-sheet architectures is particularly important in biological[1] and material sciences.[2] Zhang and co-workers have shown that a self-assembling β-sheet peptide scaffold[1a] can serve as a substrate for neurite outgrowth and synapse formation and this type of biologically compatible scaffold is also very useful for tissue repairing and tissue engineering. Higher order self-association of peptide molecules leads to the formation of fibrils and gels.[3] Understanding the β-sheet aggregation of proteins and peptides is very important for discovering pathway(s) and the mechanism(s) of amyloid[4] aggregation that would lead to drug discovery for neurodegenerative diseases like Alzheimer's disease[5] and Parkinson's disease.[6] Recently, much evidence has suggested that not only disease-related proteins, but also other non-disease related proteins can be induced to form aggregated β-sheet rich amyloid fibrils under appropriate conditions. This suggests that fibril formation is a general property of many unrelated proteins.[7,8] Thorough knowledge of β-sheet aggregation is thus important to probe the mechanism of fibrillogenesis. Unfortunately, amyloid fibrils are non-crystalline and insoluble, and are therefore intractable to conventional tools of structural biology including single crystal X-ray diffraction studies. Recently, significant progress has been made in the establishment of fibrillation pathway(s) and ultimately fibril structures. In 1997, Teplow and co-workers[5d] and Lansbury et al.[9] reported that the protofibril is one of the key intermediates in amyloid fibril formation. Recently, Kirkitadze et al. have demonstrated that the other early stage intermediate, namely the helical structure, is involved during amyloid fibril formation.[10] Significant progress has been made in the understanding of amyloid fibril structure since the establishment of the solid-state structure of amyloid fibrils from Aβ(1-40) (using NMR methods) by Tycko and co-workers.[11] However, we still need a meticulous understanding of the self-assembly of individual components (at atomic resolution) which form aggregated β-sheet structures that are responsible for amyloid fibril formation.

We are actively engaged in developing model peptides, which form supramolecular β-sheets in crystals and amyloid-like fibrils[12] in the solid-state. This work will assist the amyloid-studying community to ascertain the key parameters and other residual interactions in atomic resolution that are involved during amyloid fibril formation.

It is still a controversial issue whether amyloid fibril formation occurs preferentially via the self-assembly of

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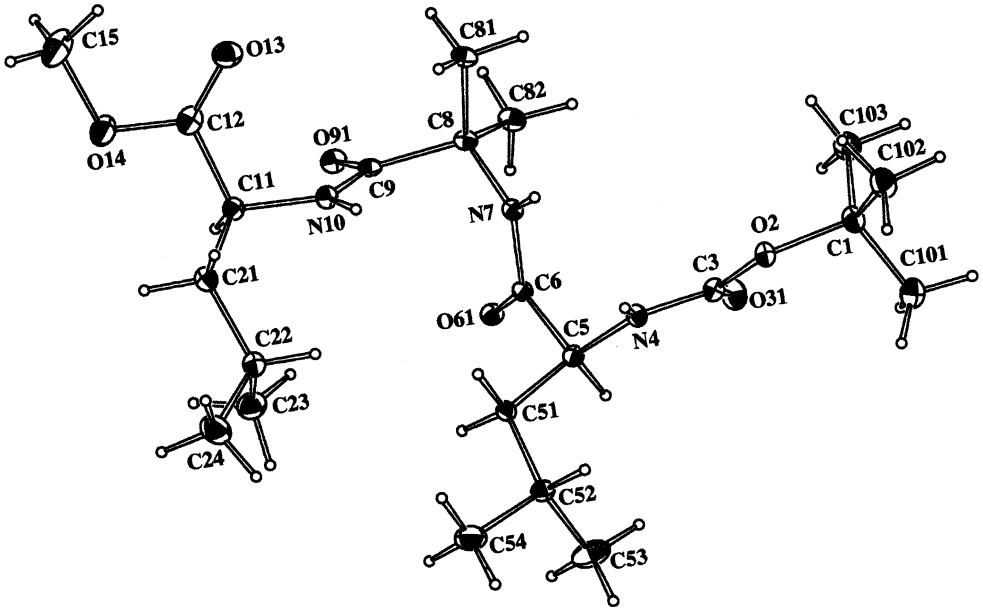

Figure 1. Crystal structure of the peptide **1** showing the atomic numbering scheme. Thermal ellipsoids are shown at the level of 20% probability.

parallel or antiparallel β-sheets. Previous studies have suggested that the full length Aβ peptide and various fragments of the Aβ peptide self-associate via parallel and antiparallel β-sheets, respectively.[13] In our previous communication, we have demonstrated that a short peptide composed of non-coded amino acids self-assembles to form amyloid-like fibril-forming supramolecular parallel β-sheet in the crystals.[12a]

Here, we present the study of a short synthetic peptide[14] with a non-coded amino acid (Aib), Boc-Leu-Aib-Leu-OMe **1** (Leu: Leucine; Aib: α-aminoisobutyric acid), that self-associates via non-covalent interactions to form an antiparallel supramolecular β-sheet structure in crystals and amyloid-like fibrils in the solid-state. The molecular conformation of the peptide **1** in the crystal,[15] presented in Figure 1, reveals that this peptide does not form any intramolecular hydrogen bonded β-turn or γ-turn structures even though the ϕ and ψ values of the majority of the constituent amino acid residues fall within the helical region of the Ramachandran map (Table 2). Despite having a centrally located helix forming Aib residue,[16] this peptide fails to form any conventional turn (β-turn or a γ-turn) structures. Self-assembly of individual monomers leads to the formation of an antiparallel β-sheet ribbon along the crystallographic *a* axis (Fig. 2) stabilized by two intermolecular hydrogen bonds N4–H4···O91 and N10–H10···O61 (Table 1) connecting individual peptide molecules. The C=O and NH groups of Leu(1) and Leu(3) are engaged in intermolecular hydrogen bonding leaving the same hydrogen bonding functionalities of the Aib(2) residue uninvolved. The torsion angles of all the amino acid residues of peptide **1** are listed in Table 2. It is evident that, except for ϕ_3 and ψ_3 of Leu (3), all the ϕ and ψ values lie within the helical portion of the Ramachandran plot. It was found that the torsion angles ϕ_1 (−78.2) and ψ_1 (−27.6) are in the right-handed helical region whereas ϕ_2 (60.5) and ψ_2 (49.5) are in the left-handed helical region. This is due to the presence of the achiral Aib (2) residue.

The individual columns of β-sheets are then stacked via van der Waals' interactions to form a highly ordered supramolecular cross β-sheet structure. The stacking of the molecules is generated by the crystallographic 2_1 screw axis along the *c* axis as shown in Figure 3.

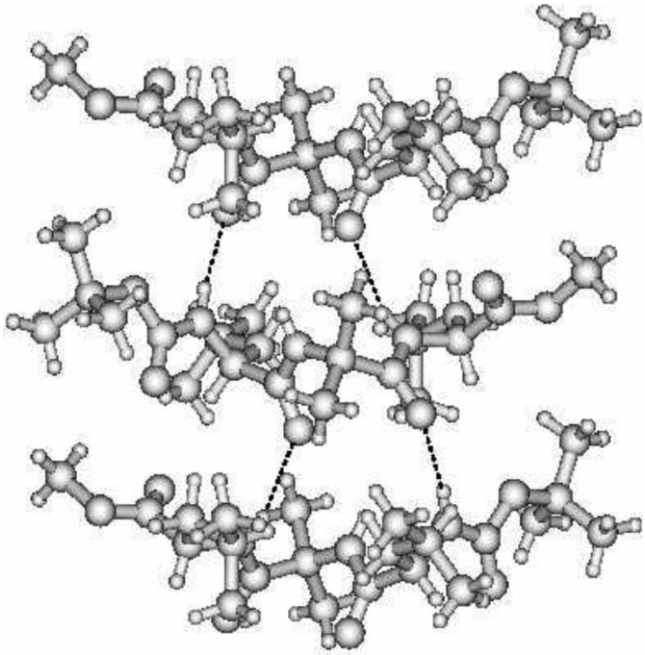

Figure 2. Packing of the peptide **1** showing the intermolecular hydrogen bonded antiparallel β-sheet along the crystallographic *a* direction. Hydrogen bonds are indicated as dotted lines.

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

N–H···O	H···O(Å)	N···O(Å)	N–H···O (°)
N4–H···O91[a]	2.20	3.06	168
N10–H···O61[a]	2.35	3.13	150

[a] Symmetry equivalent $x-0.5$, $y-0.5$, $2-z$.

Table 2. Selected backbone torsional angles (°) of peptide **1**

C5–N4–C3–O2	−179.3(4) (ω_0)	N7–C8–C9–N10	49.5(5) (ψ_2)
C3–N4–C5–C6	−78.2(5) (ϕ_1)	C8–C9–N10–C11	167.3(4) (ω_2)
N4–C5–C6–N7	−27.6(6) (ψ_1)	C9–N10–C11–C12	−81.8(6) (ϕ_3)
C8–N7–C6–C5	−179.7(4) (ω_1)	N10–C11–C12–O14	−176.5(5) (ψ_3)
C6–N7–C8–C9	60.5(6) (ϕ_2)		

The morphological studies of the peptide **1** were carried out using a scanning electron microscope (SEM). The SEM image of the peptide **1** was taken from dried fibrous material obtained from evaporation of ethyl acetate (Fig. 4) and clearly shows that the aggregates in the solid state have amyloid-like fibrillar morphology.[17]

The reported peptide self-associates through non-covalent interactions to form the antiparallel supramolecular β-sheet structure and exhibits amyloid-like fibrillar morphology in the solid state. The subunit of the supramolecular β-sheet of peptide **1** is a new motif, non-intramolecularly hydrogen bonded, bent or folded structure where the majority of the backbone torsion angles adopt helical conformations. Previously, it has been shown that fibril formation of the 34–42 residue of the Aβ peptide [Aβ34-42)] and the Aβ(16-22) fragment proceeds via antiparallel β-sheet formation.[18] Recently, Thirumalai et al. have also shown, using molecular dynamics simulation studies,[19] that the Aβ(16-22) fragment preferentially adopts an antiparallel β-sheet conformation.[9] Hence, this study of the amyloid-like fibril forming model peptide with an antiparallel β-sheet structure at atomic resolution may assist the scientific community studying amyloid diseases in investigating the pathway(s) and self-assembly mechanism during amyloid fibril formation.

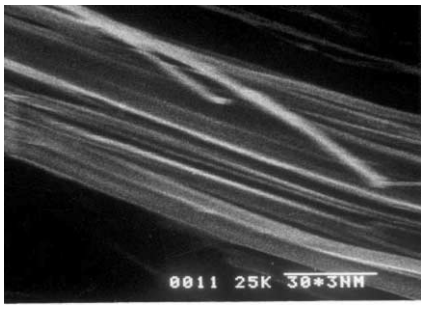

Figure 4. Typical SEM image of the peptide **1** taken from dried fibrous material grown from ethyl acetate solution by slow evaporation exhibiting filamentous fibrillar morphology.

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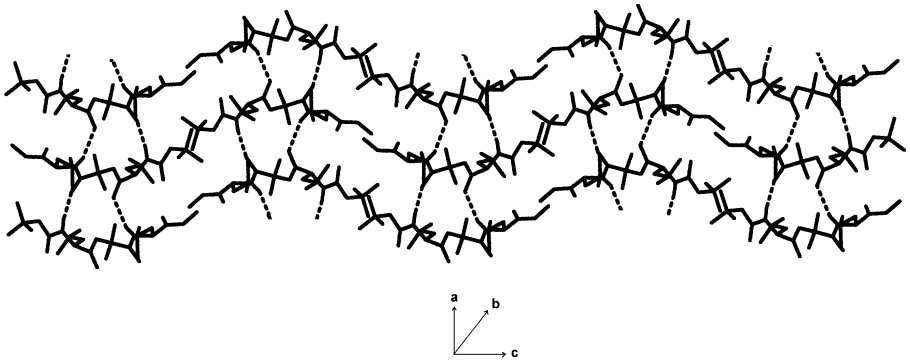

Figure 3. Crystal packing of the peptide **1** where individual columns are stacked via van der Waals' interactions to form a highly ordered supramolecular cross β-sheet structure. The stacking of the molecules is generated by the crystallographic 2_1 screw axis along the *c* axis.

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14. The peptide Boc-Leu(1)-Aib(2)-Leu(3)-OMe ($C_{22}H_{41}N_3O_6$) was synthesized by conventional solution phase methodology (Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: 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-Leu-OMe using *N*,*N*′-dicyclohexylcarbodiimide (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 (3:1) as eluent. Yield = 1.73 g (3.9 mmol, 78%).
300 MHz ^{1}H NMR ($CDCl_3$, δ ppm): 7.05 [Leu(3)NH, 1H, d, J = 8.04]; 6.58 [Aib(2) NH, 1H, s]; 4.87 [Leu(1)NH, 1H, d, J = 5.2]; 4.55–4.59 [$C^{\alpha}H$ of Leu(3), 1H, m]; 3.98 [$C^{\alpha}H$ of Leu(1), 1H, m]; 3.71 [OCH_3, 3H, s]; 1.61–1.67 [$C^{\beta}Hs$ and $C^{\gamma}H$ of Leu(1) and Leu(3), 6H, m]; 1.53–1.56 [$C^{\beta}Hs$ of Aib(2), 6H, s]; 1.44 [Boc-$(CH_3)_3$, 9H, s]; 0.91–0.96 [$C^{\delta}Hs$ of Leu(1) and Leu(3), 12H, m]. Anal. calcd for $C_{22}H_{41}N_3O_6$ (443): C, 59.59; H, 9.25; N, 9.48. Found: C, 59. 54; H, 9.18; N, 9.52%.
15. Single crystals were obtained from methanol–water solution by slow evaporation. Crystal data for **1**, $C_{22}H_{41}N_3O_6$, M = 443.58, orthorhombic, space group $P2_12_12_1$, a = 10.010(14), b = 10.580(14), c = 25.25(3) Å, U = 2674Å^3, D_{calcd} = 1.102 gm cm^{-3}, 4802 independent reflections were collected on a MAR Research Image Plate with MoKα radiation. The crystals were positioned at 70 mm from the Image Plate. 100 Frames were measured at 2° intervals with a counting time of 2 min. Data analysis was carried out with the XDS program.[20] The structure was solved using direct methods with the SHELX86 program.[21] Non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms bonded to carbon 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[22] to R_1 0.0863, wR_2 0.2439 for 2812 reflections with $I > 2\sigma(I)$. Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre, reference CCDC 208708.
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