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Amyloid-like fibril-forming supramolecular β-sheets from a β-turn forming tripeptide containing non-coded amino acids: the crystallographic signature

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Abstract—The crystal structure of a terminally protected tripeptide Boc-Leu-Aib-β-Ala-OMe **1** containing non-coded amino acids reveals that it adopts a β-turn structure, which self-assembles to form a supramolecular β-sheet via non-covalent interactions. The SEM image of peptide **1** exhibits amyloid-like fibrillar morphology in the solid state.

The design and synthesis of peptides with appropriate conformational features, which act as potential subunits for desired supramolecular architectures is an important area of current research. The most common supramolecular architectures, derived from the self-assembly of peptide monomers are supramolecular helices[1] and sheets.[2] Supramolecular β-sheets have many potential applications in material[3] as well as in biological sciences.[4] The higher order self-assembly of engineered β-sheet-forming peptide molecules leads to the formation of fibrils and gels.[5] Zhang and his co-workers have introduced a series of self-assembled β-sheet-based peptides that can serve as important biomaterials.[6] Recently, they have established that self-assembly of ionic, self-complementary β-sheet oligopeptides can be useful for tissue engineering applications.[7] Moreover, β-sheet based peptide materials are particularly important when they are established as forming amyloids.[8] Several neuro-degenerative diseases including Alzheimer's disease,[9] Huntington's disease[10] and prion protein related encephalopathies[11] appear to be caused by the deposition of aggregated proteins/protein fragments as insoluble amyloid plaques. The correlation between amyloid and a variety of amyloid diseases suggests that fibril formation is pathogenic.[12] Despite the absence of similarity in native structures, sequences and lengths of different amyloidogenic proteins/protein fragments, the ultra-structure and physicochemical properties of all amyloid fibrils are very similar. The X-ray fiber diffraction data reveals that amyloid fibrils are of 'cross-β-structure'.[13] Amyloid fibrils bind to dyes like congo-red.[14] Although amyloid fibrils have been widely studied by different electron microscopic techniques (SEM, TEM, STEM), solid state nuclear magnetic resonance, and X-ray fiber diffraction, finer structural details still remain unclear because of the non-crystallinity and the insolubility of amyloid fibrils. In particular, models of amyloid fibrils proposed thus far have suffered from a lack of critical information about how individual peptide subunits contact with each other in an assembly. Understanding the self-assembly process and the associated non-covalent interactions that connect complementary interacting molecular surfaces in amyloid aggregates are the central issues in pathogenesis of amyloid diseases.

Thus, the design and synthesis of model peptides which form supramolecular β-sheets in the crystal and amyloid-like fibrils in the solid state is one of the convenient approaches to improve our knowledge for understanding the fibrillogenesis process at the atomic level. In our previous communication,[2] we established that a short peptide containing non-coded amino acids can adopt an extended backbone conformation, which further self assembles to form a supramolecular β-sheet structure in the crystal and amyloid-like fibrils in the solid state. Here, we introduce for the first time, a new type of self-assembling β-sheet subunit (β-turn rather than a

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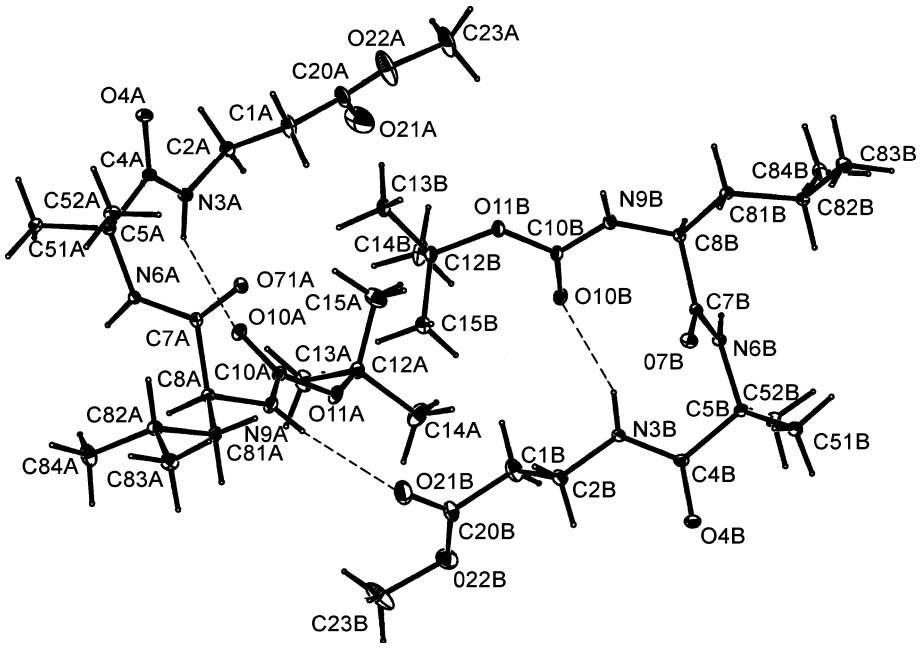

Figure 1. The molecular structure of peptide **1** showing the atomic numbering scheme. There are two molecules in the asymmetric unit of the crystal indicated as A and B. Hydrogen bonds are shown as dotted lines. Ellipsoids at 50% probability.

conventional β-strand conformation) for the synthetic tripeptide[15] Boc-Leu-Aib-β-Ala-OMe **1** which self-assembles to form a supramolecular β-sheet structure in the crystal state[16] and amyloid like fibrils in the solid state.

The ORTEP diagram with atom numbering scheme is represented in Figure 1. Interestingly, Figure 1 shows that there are two molecules A and B in the asymmetric unit which are joined together by one intermolecular hydrogen bond (N9A–H···O21B) to form a molecular dimer of two conformers which are for the most part equivalent but contain some differences. However, both molecules A and B in the asymmetric unit form a 10-membered intramolecular hydrogen bonded β-turn[17] conformation with a hydrogen bond between N3–H (N3A–H for molecule A and N3B–H for molecule B) and O10 (O10A for A and O10B for B). The dimer then self-assembles through two intermolecular hydrogen bonds exploiting the hydrogen bond functionalities of the Aib CO and the Aib NH of each molecule B (N6B–H···O4B) forming a unique β-sheet subunit (Fig. 2). These individual subunits are regularly stacked via van der Waals' interactions of the isobutyl groups of

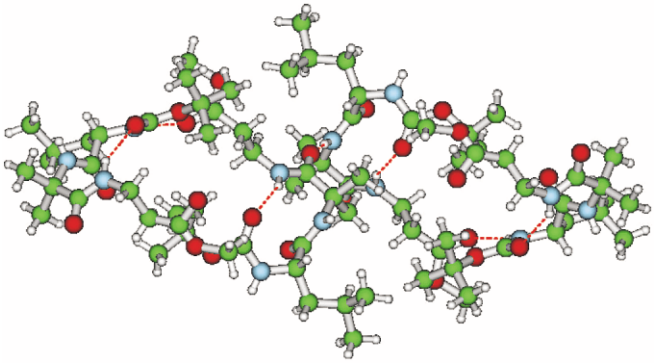

Figure 2. The packing of peptide **1** illustrating a unique type of β-sheet-subunit formation via intermolecular hydrogen bonds between B molecules of the asymmetric units in the crystal. Nitrogen atoms are blue, oxygen atoms are red, carbon atoms are green and hydrogen atoms are gray. Dotted lines indicate hydrogen bonds.

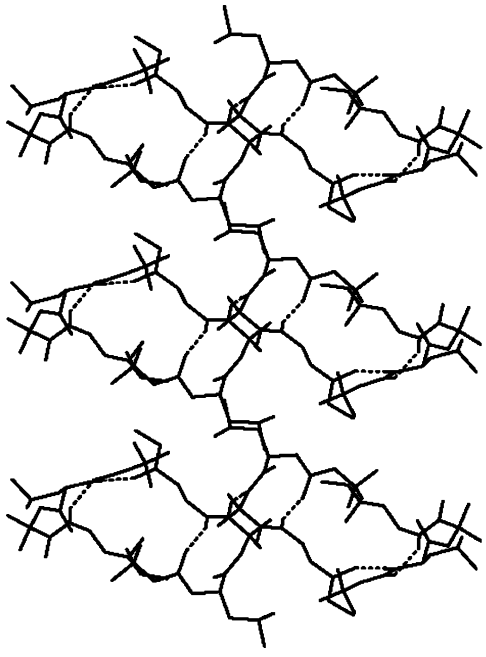

Figure 3. The packing of peptide **1** showing the formation of a continuous β-sheet column along the crystallographic *b* axis via intermolecular hydrogen bonds and van der Waals' interactions. Dotted lines indicate hydrogen bonds.

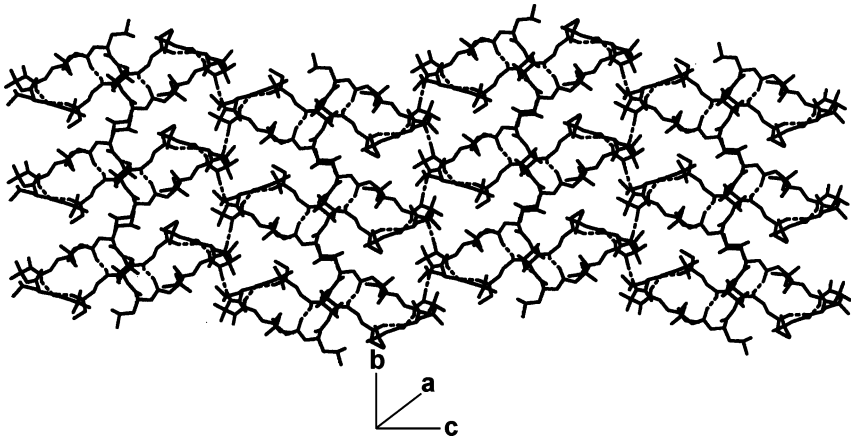

Figure 4. The packing of peptide **1** dictating the packing of individual columns through the hydrogen bonds between A molecules of the asymmetric units into a higher order β-sheet ladder structure in crystal. Dotted lines are indicate hydrogen bonds.

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

D–H···A	H···A (Å)	D···A (Å)	D–H···A (°)
N3A–H3A···O10A	2.24	3.01	148.4
N3B–H3B···O10B	2.17	2.97	153.2
N9A–H9A···O21B	2.37	3.21	167.0
N6A–H6A···O4A[a]	2.00	2.86	171.0
N6B–H6B···O4B[b]	2.02	2.88	175.5

[a] $1-x$, $0.5+y$, $1.5-z$.
[b] $-0.5+x$, $1.5-y$, $2-z$.

the individual leucine side chains to form a continuous β-sheet column along the crystallographic *b* axis (Fig. 3). The individual β-sheet columns are linked to an adjacent column through two intermolecular hydrogen bonds involving the hydrogen bonds functionalities of the Aib CO and the Aib NH of molecule A (N6A–H···O4A) forming a higher order β-sheet ladder structure along the crystallographic *c* axis (Fig. 4). The hydrogen bonding parameters of peptide **1** are listed in Table 1.

For each conformer in the asymmetric unit of peptide **1**, backbone torsions are listed in Table 2. There is a bend at the central position (of each molecule) which is occupied by the helicogenic Aib residue and the corresponding ϕ, ψ values of this residue fall within the helical region; whereas the deviations from helical values of torsion angles occur at the C-terminal β-Ala residue for both molecules A and B, which is the only flexible residue in peptide **1**. The values of ϕ, ψ, ω, θ in both molecules in the asymmetric unit of the peptide are similar (difference $<\pm 10°$), with the exception in the case of C-terminal β-Ala(3) residue (Table 2).

The morphological characteristic of the reported peptide **1** has been studied using a scanning electron microscope (SEM). The SEM image (Fig. 5) of peptide **1** clearly shows that the aggregate in the solid state has an amyloid-like fibrillar morphology.[9d,e,18]

Peptide **1** represents a new class of subunit (β-turn) which self-assembles to form a dimer and then the hierarchical self-assembly of this peptide subunit through multiple hydrogen bonds and hydrophobic interactions offers a supramolecular sheet assemblage in the solid state. The amyloid-like fibril forming character of this peptide in the solid state indicates that it may be used as a model system for studying the amyloid fibrillogenesis process. Recently, Kirschner and co-workers have established on the basis of fiber diffraction patterns of Aβ (31–35) that the β-turn conformation may have a crucial role in amyloid fibrillogenesis.[19] In this report we have demonstrated for the first time that the quaternary structure of a β-sheet formation and amyloid-like fibril formation are mediated by the dimerization of the reported peptide molecules having β-turn conformations rather than the conventional β-strand conformation.

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

Residue		ϕ	ψ	ω	θ
Leu(1)	Mol A	−57.1(7)	128.3(4)	−173.1(5)	–
	Mol B	−57.9(6)	126.5(4)	−175.5(4)	–
Aib(2)	Mol A	66.0(5)	18.1(6)	174.9(4)	–
	Mol B	70.7(5)	13.1(6)	177.5(4)	–
β-Ala(3)	Mol A	69.2(7)	166.7(10)	−176.4(4)	163.3(7)
	Mol B	−125.5(7)	−112.4(10)	−179.7(5)	−177.3(7)

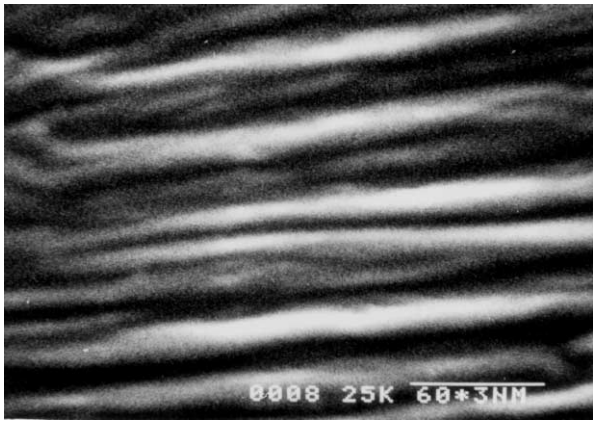

Figure 5. Typical SEM image of peptide **1**, indicating amyloid-like fibrillar morphology in the solid state.

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16. Single crystals were obtained from methanol–water solution by slow evaporation. Crystal data for **1**: $C_{19}H_{35}N_3O_6$, $M=401.50$, orthorhombic, space group $P2_12_12_1$, $a=10.210(14)$, $b=10.373(14)$, $c=44.00(6)$ Å, $U=4660$ Å^3, $D_{calcd}=1.145$ g cm^{-3}, 5936 independent reflections were collected on a MAR research Image Plate with Mo Kα 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 SHELX-86 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 $R_1=0.0860$, $wR_2=0.2011$ for 4795 reflections with $I>2\sigma(I)$. Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre reference CCDC 191375.
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