Pergamon

Tetrahedron Letters 43 (2002) 6759–6762

TETRAHEDRON LETTERS

Self-assembly of a tetrapeptide in which a unique supramolecular helical structure is formed via intermolecular hydrogen bonding in the solid state

Samir Kumar Maji,[a] Arijit Banerjee,[a] Michael G. B. Drew,[b] Debasish Haldar[a] and Arindam Banerjee[a,*]

[a]*Department of Biological Chemistry, Indian Association for the Cultivation of Science, Jadavpur, Calcutta 700 032, India*
[b]*Department of Chemistry, The University of Reading, Whiteknights, Reading RG6 6AD, UK*

Received 27 June 2002; accepted 19 July 2002

Abstract—A single crystal X-ray diffraction study of the tetrapeptide Boc-Ala[1]-Aib[2]-Leu[3]-Aib[4]-OMe **1** (Aib: α-aminoisobutyric acid) reveals that it forms a supramolecular helix through continuous intermolecular hydrogen bonds. Scanning electron microscopic studies show that this peptide exhibits amyloid-like fibrillar morphology in the solid state.

The creation of supramolecular β-sheet structures from self-assembling oligopeptides is very common and frequently studied.[1] The design and construction of supramolecular β-sheets is important due to their many potential applications in biological and material sciences.[2] Unimolecular helix design from peptides with appropriate amino acid residues has also been thoroughly studied.[3] However, much less attention has been paid to the construction of a peptide supramolecular helix. The majority of supramolecular helices have been designed and constructed by exploiting the metal-mediated self-assembly of organo-ligand strands (helicates)[4] and intermolecular hydrogen bonding of poly-functional organic compounds.[5] In helicates, co-ordinate bonds are used instead of hydrogen bond interactions to stabilize the supramolecular helical structure.[4] Hanessian et al. have introduced a novel type of metal-free-helicate exploiting the complementary hydrogen bond functionalities of diamine-diol adducts whose helical sense completely depends on the chirality of 1,2-diamines.[5c] They have also shown that, not only neutral adducts of diamine-diols, but also the salts of diamines and carboxylic acids are capable of forming metal-free helicates through intermolecular hydrogen bonds. However, supramolecular helices with different levels of self-organization and self-assembly of the peptide backbone are very common in biological systems including collagen,[6] and the tobacco mosaic virus coat protein.[7] In collagen, polypeptide chains self-associate to form the triple helix which further self-assembles to form highly ordered collagen fibers. Self-assembly of the heavy chain of myosin generates an extended helical coiled coil structure composed of six polypeptide chains.[8] Previous studies have suggested that not only β-sheets, but also the helix has a definite role in the formation of amyloid-fibrils.[9] Recently, Blanch et al. have suggested that polyproline II (PPII) helix might be the precursor conformation in amyloid formation.[10] So, hierarchical self-assembly of a peptide into a supramolecular helical structure and further self-assembly to form fibrils is an important aspect in biology. Our previous studies have suggested that peptide containing noncoded amino acids with appropriate conformation can act as a subunit for fibril forming supramolecular helical architectures.[11] Here we report our study of the terminally-blocked tetrapeptide[12] Boc-Ala[1]-Aib[2]-Leu[3]-Aib[4]-OMe **1** which forms a supramolecular helix, by exploiting its hydrogen bonding potential, and also possesses an amyloid-like fibrillar morphology, in the solid state.

The ORTEP diagram of the crystal structure[13] of the title peptide **1** with the atomic numbering scheme is shown in Fig. 1. Hydrogen bonding dimensions and backbone torsion angles for peptide **1** are listed in Tables 1 and 2, respectively. The molecular structure of

Keywords: supramolecular helix; intermolecular hydrogen bonds; Aib; amyloid fibrils.

* Corresponding author. Fax: +91-33-4732805; e-mail: bcab@mahendra.iacs.res.in

0040-4039/02/$ - see front matter
PII: S0040-4039(02)01521-6

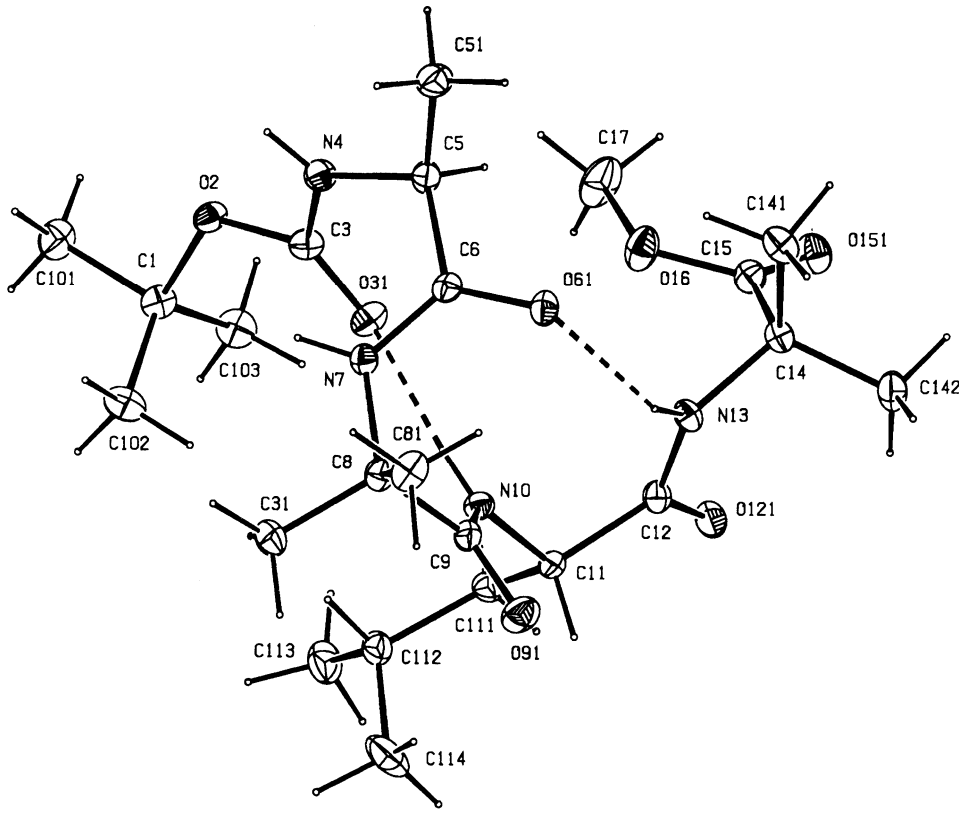

Figure 1. The structure of the peptide **1** showing the atomic numbering scheme. Ellipsoids at 20% probability.

Table 1. Intra- and intermolecular hydrogen-bond parameters for peptide **1**

Donor	Acceptor	Distance (Å)		Angle (°)
D–H	A	H···A	D···A	D–H···A
N10–H	O31	2.12	2.97	166.4
N13–H	O61	2.26	3.04	150.2
N4–H[a]	O121	2.21	3.03	159.5
N7–H[a]	O151	2.50	3.32	161.9

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

Table 2. Selected torsion angles (°) with estimated standard deviations for peptide **1**

Residue	ϕ (°)	ψ (°)	ω (°)
Ala[1]	−68.1(8)	−14.9 (9)	−169.5 (6)
Aib[2]	−52.4 (8)	−29.0 (8)	171.9 (6)
Leu[3]	−105.7 (6)	19.2 (8)	−176.2 (6)
Aib[4]	57.8 (8)	41.6 (8)	−171.1 (5)

the peptide **1** reveals the presence of two consecutive intramolecular hydrogen bonds. The amide nitrogen N(10) of Leu[3] is hydrogen bonded to O(31) of the Boc-protecting group and N(13) of Aib[4] is hydrogen bonded to O(61) of the carbonyl group of Ala[1] thereby forming two overlapping β-turns (Fig. 1). Moreover, the individual molecules are packed to form infinite one-dimensional helical columns along the b screw axis via the intermolecular hydrogen bonds formed between the Ala[1] and Aib[2] NH groups and the Leu[3] and Aib[4] CO groups (Table 1) of symmetry related molecules. Fig. 2a and b clearly show the formation of a supramolecular helix through the self-assembly of peptide **1**.

From Table 2, it is evident that the ϕ and ψ values for Ala[1] and Aib[2] are close to those observed in the right-handed 3_{10}-helix (−60±10°, −30±15°), whereas the ϕ and ψ values of the Leu[3] residue deviate considerably from the helical region. The observed ϕ, ψ values of Leu[3] (−105.7, 19.2°) indicate that there is a distortion from the ideal helical (3_{10} or α) conformation at Leu[3]. Aib[2] simultaneously occupies the i+2th position of the first β-turn as well as the i+1th position of the second β-turn. Theoretical studies show that Aib residues occupy a restricted region of conformational space ($\phi = \pm 60 \pm 20°$, $\psi = \pm 30 \pm 20°$) corresponding to the values found in the right-handed or left-handed α or 3_{10}-helical regions.[17] The experimentally determined dihedral angles of Aib residues in the present peptide are in reasonable agreement with these theoretical results. Values for Aib[2] ($\phi = -52.4$, $\psi = -29.0°$) fall into the right-handed helical region whereas those for Aib[4] ($\phi = 57.8$, $\psi = 41.6°$) fall into the left-handed helical region. Apart from the ϕ value of the Leu[3] residue all other ϕ and ψ values lie within the helical region of the Ramachandran map.

The morphology of the reported peptide **1** has been studied using a scanning electron microscope (SEM). The SEM image (Fig. 3) of the dried fibrous material

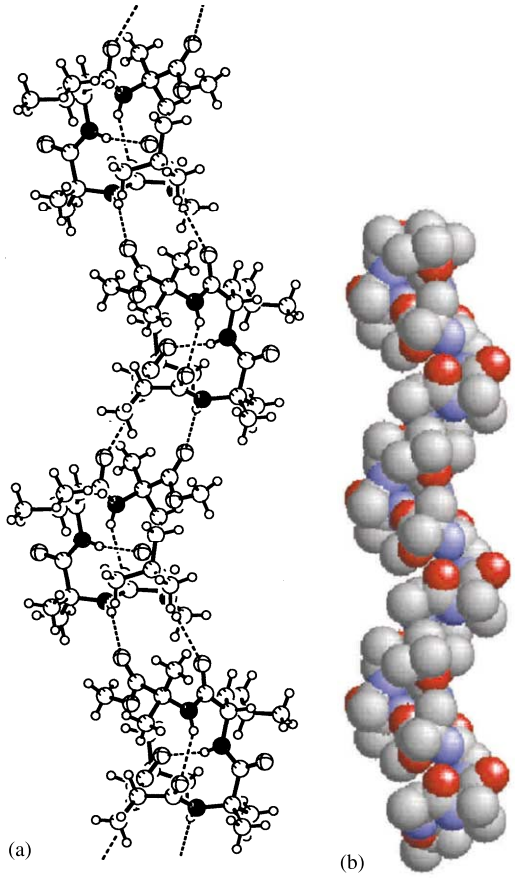

Figure 2. (a) The packing of the peptide **1** illustrating the intermolecular hydrogen-bonded supramolecular helix along the b axis. Hydrogen bonds are shown as dotted lines. Carbon and hydrogen atoms as open circles, oxygen as multilined circles, nitrogen as black circles. (b) Space-filling representation of 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 and the side chain of Leu (3) are omitted for clarity.

Figure 3. SEM image of the peptide **1** showing amyloid-like fibrillar morphology in the solid state.

clearly shows that the filamentous aggregate resembles the morphology of neurodegenerative disease-causing amyloid fibrils.[9,18]

The peptide **1** having double turn conformation represents a new class of peptide subunit, in which the first turn is a Type III β-turn and the second one is an unusual type of β-turn. The hierarchical self-assembly of this peptide subunit through multiple hydrogen bonds forms a supramolecular helical architecture in the solid. Moreover, the amyloid-like fibril forming character of this peptide in the solid state adds a new dimension to this study. The fibril-forming supramolecular helix of the reported peptide **1** mimics some characteristics of naturally occurring macromolecules.[6–8] This result suggests that not only helices[9a] and PPII helices,[10] but amyloid-like fibril formation may occur through supramolecular helix formation. Thus, fibrillar morphology with supramolecular helical assembly of this compound may be used as a model system to study the fibrillation process of many neurotoxic disease causing amyloid fibrils.[9,13]

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.

References

1. (a) Bong, D. T.; Clark, T. D.; Granja, J. R.; Ghadiri, M. R. *Angew. Chem., Int. Ed.* **2001**, *40*, 988–1011; (b) Balbach, J. J.; Ishii, Y.; Antzutkin, O. N.; Leapman, R. D.; Rizzo, N. W.; Dyda, F.; Reed, J.; Tycko, R. *Biochemistry* **2000**, *39*, 13748–13759; (c) Maji, S. K.; Drew, M. G. B.; Banerjee, A. *Chem. Commun.* **2001**, 1946–1947; (d) Fabiola, G. F.; Bobde, V.; Damodharan, L.; Pattabhi, V.; Durani, S. *J. Biomol. Struct. Dyn.* **2001**, *18*, 579–594; (e) Seebach, D.; Abele, S.; Gademann, K.; Jaun, B. *Angew. Chem., Int. Ed.* **1999**, *38*, 1595–1597; (f) Aggeli, A.; Nyrkova, I. A.; Bell, M.; Harding, R.; Carrick, L.; McLeish, T. C. B.; Semenov, A. N.; Boden, N. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 11857–11862.
2. (a) Holmes, T. C.; de Lacalee, S.; Su, X.; Liu, G.; Rich, A.; Zhang, S. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 6728–6733; (b) Krejchi, M. T.; Atkins, E. D. T.; Waddon, A. J.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Science* **1994**, *265*, 1427–1432; (c) Aggeli, A.; Boden, N.; Cheng, Y.; Findlay, J. B. C.; Knowles, P. F.; Kovatchev, P.; Turnbull, P. J. H. *Biochemistry* **1996**, *35*, 16213–16221; (d) Wang, W.; Hecht, M. H. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 2760–2765; (e) Otzen, D. E.; Kristensen, O.; Oliveberg, M. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 9907–9912.
3. (a) Venkatraman, J.; Shankaramma, S. C.; Balaram, P. *Chem. Rev.* **2001**, *101*, 3131–3152; (b) Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747–6756; (c) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219–3232.
4. (a) Lehn, J. M.; Rigault, A.; Siegel, J.; Harrowfield, J.; Chevrier, B.; Moras, D. *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 2565–2569; (b) Berl, V.; Huc, I.; Khoury, R. G.; Lehn, J. M. *Chem. Eur. J.* **2001**, *7*, 2810–2820; (c) Albrecht, M. *Chem. Rev.* **2001**, *101*, 3457–3497; (d) Norsten, T. B.; McDonald, R.; Branda, N. R. *Chem. Commun.* **1999**, 719–720.
5. (a) Moriuchi, T.; Nishiyama, M.; Yoshida, K.; Ishikawa, T.; Hirao, T. *Org. Lett.* **2001**, *3*, 1459–1461; (b) Hanessian, S.; Gomtsyan, A.; Simard, M.; Roelens, S. *J. Am. Chem. Soc.* **1994**, *116*, 4495–4496; (c) Hanessian, S.; Simard, M.; Roelens, S. *J. Am. Chem. Soc.* **1995**, *117*, 7630–7645; (d) Brunsveld, L.; Folmer, B. G. B.; Meijer, E. W.; Sijbesma, R. P. *Chem. Rev.* **2001**, *101*, 4071–4091; (e) Geib, S. J.; Vicent, C.; Fan, E.; Hamilton, A. D. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 119–121; (f) Allen, W. E.; Fowler, C. J.; Lynch, V. M.; Sessler, J. L. *Chem. Eur. J.* **2001**, *7*, 721–729.
6. Deyre, D. R. *Science* **1980**, *207*, 1315–1322.
7. Franklin, R. E. *Nature* **1955**, *175*, 379–381.
8. Stryer, L. *Biochemistry*; W. H. Freeman: New York, 1997; pp. 393–396.
9. (a) Goldsbury, C.; Goldie, K.; Pellaud, J.; Seelig, J.; Frey, P.; Müller, S. A.; Kister, J.; Cooper, G. J. S.; Aebi, U. *J. Struct. Biol.* **2000**, *130*, 352–362; (b) Arvinte, T.; Cudd, A.; Drake, A. F. *J. Biol. Chem.* **1993**, *268*, 6415–6422.
10. Blanch, E. W.; Morozova-Roche, L. A.; Cochran, D. A. E.; Doig, A. J.; Hecht, L.; Barron, L. D. *J. Mol. Biol.* **2000**, *301*, 553–563.
11. (a) Haldar, D.; Maji, S. K.; Sheldrick, W. S.; Banerjee, A. *Tetrahedron Lett.* **2002**, *43*, 2653–2656; (b) Haldar, D.; Maji, S. K.; Drew, M. G. B.; Banerjee, A.; Banerjee, A. *Tetrahedron Lett.* **2002**, *43*, 5465–5468.
12. The peptide Boc-Ala^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: New York, 1984; pp. 1–282).
^{1}H NMR ($CDCl_3$, 500 MHz, δ ppm): 7.27 [Aib^4 NH, 1H, s]; 7.04 [Leu^3 NH, 1H, d]; 6.64 [Aib^2 NH, 1H, s]; 5.14 [Ala^1 NH, 1H, d, J 6.25]; 4.46 [C$^\alpha$Hs of Leu^3, 1H, m]; 3.94 [C$^\alpha$Hs of Ala^1, 1H, m]; 3.68 [-OCH_3, 3H, s]; 1.61–1.67 [C$^\beta$Hs and C$^\gamma$Hs of Leu^3 2H and 1H, m]; 1.46 [Boc-CH_3s, 9H, s]; 1.53 [C$^\beta H_3$ of Aib^4, 3H, s]; 1.48 [C$^\beta H_3$s of Aib^2 and Aib^4, 6H, s]; 1.44 [C$^\beta H_3$ of Aib^2, 3H, s]; 1.38 [Ala^1 C$^\beta H_3$, 3H, d]; 0.87–0.94 [C$^\delta$Hs of Leu^3, 6H, m]. Anal. calcd for $C_{23}H_{42}N_4O_7$ (486): C, 56.79; N, 11.52; H, 8.64. Found: C, 56.81; N, 11.5; H, 8.67.
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 $P2_12_12_1$, $a=9.359(14)$, $b=16.86(2)$, $c=17.79(3)$ Å, $U=2808$ Å^3, $Z=4$, dm = 1.151 Mg m^{-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 2557 independent reflections. Data analysis was carried out with 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 $R_1=0.0634$ and $wR_2=0.1555$ for 1165 data with $I>2\sigma(I)$. The largest peak and hole in the final difference Fourier were 0.199 and –0.187 e Å^{-3}. The data have been deposited at the Cambridge Crystallographic Data Centre with reference number CCDC 184436.
14. Kabsch, W. *J. Appl. Crystallogr.* **1988**, *21*, 916.
15. Sheldrick, G. M. *Acta Crystallogr. Sect. A: Fundam. Crystallogr.* **1990**, *46*, 467.
16. Sheldrick, G. M. *Program for Crystal Structure Refinement*; University of Göttingen: Germany, 1993.
17. (a) Marshall, G. R.; Bosshard, H. E. *Cric. Res.* **1972**, *30/31*, 143–150; (b) Burgess, A. W. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 2649–2653.
18. (a) Goldsbury, C. S.; Wirtz, S.; Müller, S. A.; Sunderji, S.; Wicki, P.; Aebi, U.; Frey, P. *J. Struct. Biol.* **2000**, *130*, 217–231; (b) Sipe, J. D.; Cohen, A. S. *J. Struct. Biol.* **2000**, *130*, 88–98; (c) Inoue, S.; Kuroiwa, M.; Saraiva, M. J.; Guimarães, A.; Kisilevsky, R. *J. Struct. Biol.* **1998**, *124*, 1–12; (d) Hartley, D. M.; Walsh, D. M.; Ye, C. P.; Diehl, T.; Vasquez, S.; Vassilev, P. M.; Teplow, D. B.; Selkoe, D. J. *J. Neurosci.* **1999**, *19*, 8876–8884.