Peptide hairpin nucleation with the obligatory Type I' β-turn Aib-^{D}Pro segment†

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The α-aminoisobutyric acid-D-proline (Aib-^{D}Pro) dipeptide is an obligatory Type I' β-turn forming segment that nucleates hairpin formation.

Synthetic peptide hairpins have been successfully constructed using the strategy of placing strong turn forming segments in the center of designed sequences.[1] Prime β-turns (Type I' and Type II') are most frequently found to facilitate hairpin formation in protein structures.[2] Design strategies have therefore employed ^{D}Pro-Xxx segments as the preferred choice for the hairpin nucleating turn.[3] Several examples of crystallographically characterized short peptide hairpins containing central ^{D}Pro-Xxx segments are now available.[4] The overwhelming majority of these structures have a central Type II' β-turn unit which results in the formation of a flattened hairpin structure. In contrast, the Type I' β-turn formed in a central Aib-^{D}Ala segment results in a considerable twisting of the antiparallel strands with a twist angle of −55.8°.[5] The only two examples of Type I' β-turn nucleated hairpins are in the crystal structure of an octapeptide and a decapeptide which contain backbone homologated β-amino acids in the strands.[6] An attempt to generate a mixed hairpin-helix structure in a 19-residue peptide sequence, Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-^{D}Ala-^{D}Leu-Leu-Val-Phe-Val-Aib-^{D}Val-Leu-Phe-Val-Val-OMe, led to the observation of a continuous helix in the crystal structure in which the Aib-^{D}Val segment was accommodated in the center of the helix with ^{D}Val residue adopting a right-handed helical (α_R) conformation.[7] Fig. 1 compares the two examples of Aib-^{D}Xxx (^{D}Xxx = ^{D}Val and ^{D}Ala) sequences adopting two distinct local folds.

In order to develop an obligatory Type I' β-turn unit as a hairpin nucleating segment, we turned to the Aib-^{D}Pro dipeptide. Fig. 2a illustrates the localised region of conformational space accessible to Aib residues as shown by overlap of the allowed region for ^{L}Ala and ^{D}Ala residues. Crystallographically characterized Aib residues cluster closely in the regions which correspond to right-handed (α_R), ϕ negative, and left-handed (α_L), ϕ positive, of the helical conformations.[8] While D-amino acids largely populate conformations with positive (ϕ, ψ) values, several examples of D-amino acids adopting negative ϕ values in the right-handed

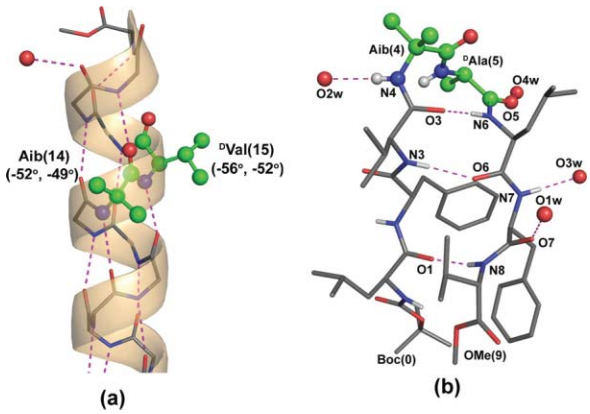

Fig. 1 (a) Aib-^{D}Val segment adopting right-handed alpha-helical (α_R) conformation in a 19 residue peptide.[7] (b) Aib-^{D}Ala segment adopting a Type I' β-turn conformation in an octapeptide β-hairpin.[5]

alpha-helical (α_R) region of the Ramachandran plot are observed (Fig. 2b).[9]

In the case of ^{D}Pro sidechain backbone cyclization limits the value of ϕ to 60 ± 20°. The only 4→1 hydrogen bonded β-turn conformation sterically accessible to the Aib-^{D}Pro segment is the Type I' structure (idealized torsional angles for Type I' β-turn are $\phi_{i+1} = 60°$, $\psi_{i+1} = 30°$, $\phi_{i+2} = 90°$, $\psi_{i+2} = 0°$).

The model octapeptide, Boc-Leu-Val-Val-Aib-^{D}Pro-Leu-Val-Val-OMe (**1**), forms a β-hairpin conformation in solution as established by the presence of cross-strand nuclear Overhauser effect and the presence of four solvent shielded NH groups.[10] Single crystals of the octapeptide **1** were obtained from dimethylformamide(DMF)/water solution.[11] Fig. 3a shows a view of the molecular conformation of **1** in crystals. The observed hairpin conformation is stabilized by three cross-strand hydrogen bonds with the Aib-^{D}Pro segment adopting the anticipated Type I' β-turn conformation (Aib(4) : $\phi = 56.8°$, $\psi = 28.4°$; ^{D}Pro(5) : $\phi = 82.8°$, $\psi = 8.0°$). Fraying of the strands is observed at the termini with the ψ values for Leu(1) ($\phi = -100.3°$, $\psi = -16.8°$) and Val(8) ($\phi = -119.9°$, $\psi = 14.0°$) deviating appreciably from the values expected for a β-strand conformation. Val(2) ($\phi = -136.5°$, $\psi = -126.2°$), Val(3) ($\phi = -140.8°$, $\psi = -134.3°$), Leu(6) ($\phi = -123.1°$, $\psi = -136.0°$) and Val(7) ($\phi = -79.2°$, $\psi = -125.6°$) all adopt (ϕ, ψ) values characteristic of β-strand conformations. The crystal structure compares very well with the structure determined by NMR spectroscopy in solution.

The structure of **1** superposes well with that of the previously characterized hairpin for Boc-Leu-Phe-Val-Aib-^{D}Ala-Leu-Phe-Val-OMe (**2**) sequence (RMSD = 0.122 Å, Fig. 3b).[5] The hairpin

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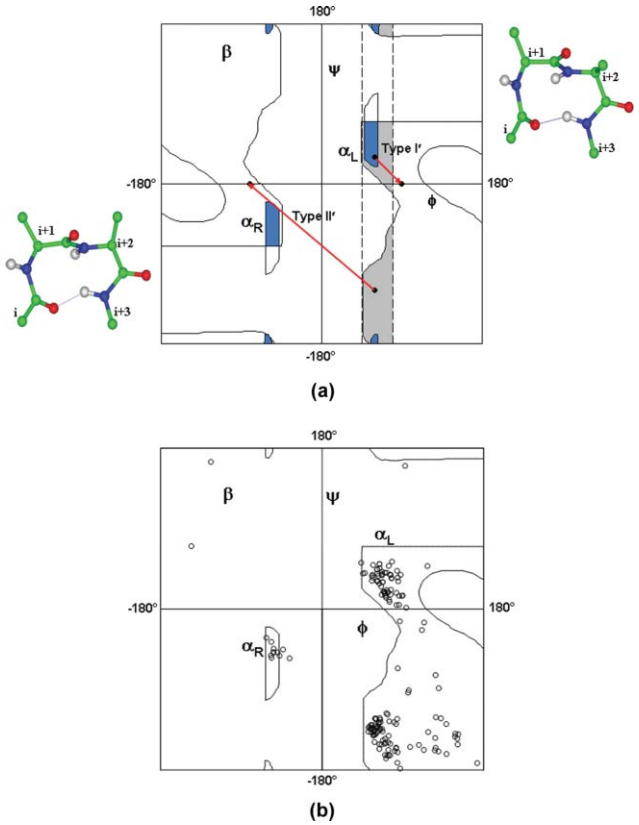

Fig. 2 (a) Ramachandran plot showing the sterically allowed regions for the Aib residue (blue) and the narrow region accessible to the D-Pro residue (gray). The i+1 and i+2 residues of Type I'/II' β-turns are connected by arrows. (b) Distribution of (ϕ, ψ) values of D-amino acids in the Cambridge Structural Database. Data set of 81 linear peptide sequences consisting of 164 D-amino acid residues. 11 residues lie in the α_R region.

has a pronounced right-handed twist angle ($\theta = -55.4°$). Two co-crystallized DMF molecules form hydrogen bonds to the NH groups of Leu(1) and Val(7). Interestingly, if the positions of turn residues are interchanged the resulting sequence, Boc-Leu-Val-Val-D-Pro-Aib-Leu-Val-Val-OMe, forms a considerably flattened β-hairpin (twist angle, $\theta = -3.4°$) nucleated by a Type II' β-turn structure.[4c]

The packing of the flat and twisted hairpins in crystals is somewhat different. In both the cases, the hairpins are arranged in parallel fashion with adjacent molecules held together by hydrogen bonds between the exposed backbone CO and NH groups (Fig. 4). In the case of twisted hairpin, **1** with Aib-D-Pro segment, a single intermolecular hydrogen bond is observed, whereas in the case of flat D-Pro-Aib hairpin two hydrogen bonds hold adjacent molecules.

The present study provides an unambiguous structural characterization of a designed peptide β-hairpin nucleated by a Type I' β-turn. The Aib-D-Pro segment may be used as a conformationally rigid template for the construction of peptide hairpins with a considerable degree of twist between the adjacent antiparallel strands. Such units may be useful in peptidomimetic design[1e,12] and the rational design of multistranded β-sheet structures which mimic those found in the proteins.[13]

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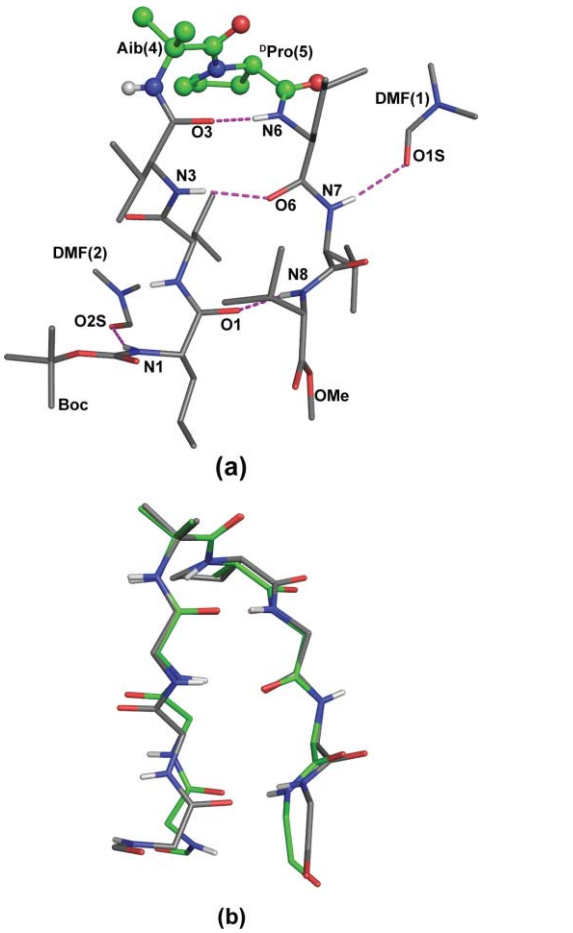

Fig. 3 (a) Molecular conformation of **1**. Intramolecular hydrogen bonds are shown as dashed lines. (b) Superposition of the backbone atoms of residues 2 to 7 of **1** (carbons in gray) and **2** (carbons in green).

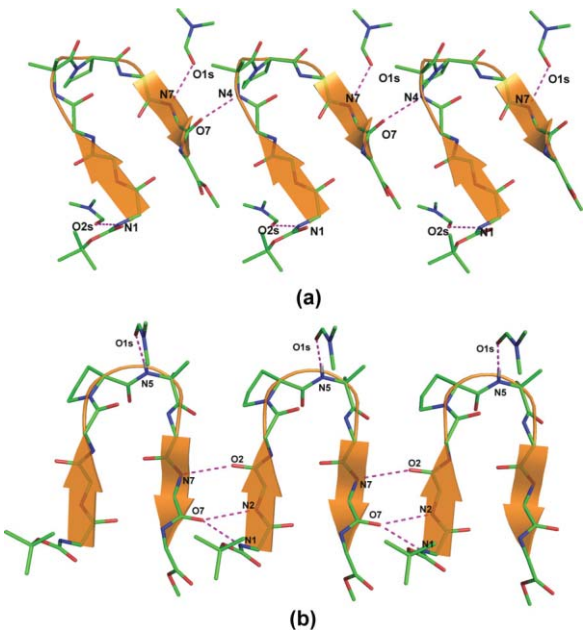

Fig. 4 Packing of the β-hairpins in (a) **1** (Aib-D-Pro), twisted hairpin. (b) **2** (D-Pro-Aib), flat hairpin. Intermolecular hydrogen bonds shown as dashed lines. Side chains of the turn residues shown.

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Notes and references

1 (*a*) J. Venkatraman, S. C. Shankaramma and P. Balaram, *Chem. Rev.*, 2001, **101**, 3131; (*b*) S. Aravinda, N. Shamala, R. S. Roy and P. Balaram, *Proc.–Indian Acad. Sci., Chem. Sci.*, 2003, **115**, 373; (*c*) S. H. Gellman, *Curr. Opin. Chem. Biol.*, 1998, **2**, 717; (*d*) R. M. Hughes and M. L. Waters, *Curr. Opin. Struct. Biol.*, 2006, **16**, 514; (*e*) J. A. Robinson, *Acc. Chem. Res.*, 2008, **41**, 1278; (*f*) M. S. Searle, *Biopolymers*, 2004, **76**, 185.

2 (*a*) B. L. Sibanda and J. M. Thornton, *Nature*, 1985, **316**, 170; (*b*) K. Gunasekharan, C. Ramakrishnan and P. Balaram, *Protein Eng., Des. Sel.*, 1997, **10**, 1131.

3 (*a*) S. K. Awasthi, S. Raghothama and P. Balaram, *Biochem. Biophys. Res. Commun.*, 1995, **216**, 375; (*b*) T. S. Haque, J. C. Little and S. H. Gellman, *J. Am. Chem. Soc.*, 1996, **118**, 6975; (*c*) E. R. Simpson, J. K. Meldrum and M. S. Searle, *Biochemistry*, 2006, **45**, 4220.

4 (*a*) I. L. Karle, S. K. Awasthi and P. Balaram., *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 8189; (*b*) I. L. Karle, H. N. Gopi and P. Balaram, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 5160; (*c*) S. Aravinda, V. V. Harini, N. Shamala, C. Das and P. Balaram, *Biochemistry*, 2004, **43**, 1832; (*d*) V. V. Harini, S. Aravinda, R. Rai, N. Shamala and P. Balaram, *Chem.–Eur. J.*, 2005, **11**, 3609.

5 (*a*) S. Aravinda, N. Shamala, R. Rai, H. N. Gopi and P. Balaram, *Angew. Chem., Int. Ed.*, 2002, **41**, 3863.

6 (*a*) H. N. Gopi, R. S. Roy, S. R. Raghothama, I. L. Karle and P. Balaram, *Helv. Chim. Acta*, 2002, **85**, 3313; (*b*) I. L. Karle, H. N. Gopi and P. Balaram, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 3716.

7 I. L. Karle, H. N. Gopi and P. Balaram, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 13946.

8 (*a*) I. L. Karle and P. Balaram, *Biochemistry*, 1990, **29**, 6747; (*b*) C. Toniolo and E. Benedetti, *Trends Biochem. Sci.*, 1991, **16**, 350.

9 F. H. Allen, *Acta Crystallogr., Sect. B: Struct. Sci.*, 2002, **58**, 380.

10 R. Rai, S. Raghothama, R. Sridharan and P. Balaram, *Biopolymers*, 2007, **88**, 350.

11 Structure of **1** ($C_{53}H_{98}N_{10}O_{13}$) was solved using the dual space recycling method employed in SHELXD (T. R. Schneider, G. M. Sheldrick, *Acta Cryst. D*, 2002, **58**, 1772). Space group $P2_1$, $a = 11.0623$ Å, $b = 18.7635$ Å, $c = 16.6426$ Å, $\beta = 102.369$, Z/Z'=2/1, $V = 3374$ Å^3, $R = 9.47\%$ and w$R_2 = 17.34\%$, for 1432 reflections $|F_o| > 4\sigma(|F_o|)$. The goodness-of-fit is 0.973. CCDC reference number 758802 has crystallographic data for the paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

12 (*a*) C. E. Stotz and E. M. Topp, *J. Pharm. Sci.*, 2004, **93**, 2881; (*b*) J. A. Robinson, *Synlett*, 2000, **4**, 429.

13 (*a*) M. S. Searle and B. Ciani, *Curr. Opin. Struct. Biol.*, 2004, **14**, 458; (*b*) M. Ramirez-Alvarado, F. J. Blanco and L. Serrano, *Nat. Struct. Biol.*, 1996, **3**, 604; (*c*) O. Khakshoor and J. S. Nowick, *Curr. Opin. Chem. Biol.*, 2008, **12**, 722.