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An Observation of a Folded β-Ala Conformation in a Model Peptide: Boc - L-Pip - β-Ala - $NHCH_3$, X - Ray Diffraction Study

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Abstract: An X-ray crystallographic characterization of a 'locally folded' conformation of a β-Ala residue, in a short linear peptide: Boc - L-Pip - β-Ala - $NHCH_3$, has been described. The critical μ torsion angle between the methylene groups of the β-Ala adopts a typical *gauche* (g^-) conformation. The urethane moiety is found in the uncommon type *a*. The influence of the geometrical variation of the Pip residue on β-Ala conformation has been emphasized.

The design and the development of molecular tools that can force flexible amino-acid residues to adopt energetically favourable conformations has attracted considerable attention.[1] We have been investigating the conformational properties of β-Ala peptides by introducing simple stereochemical constraints to limit the available range of backbone conformations and to induce preferred geometry in a flexible unsubstituted β-Ala residue (-NH-$C^{\beta}H_2$-$C^{\alpha}H_2$-CO-).[2] Insertion of a β-Ala residue into a peptide chain comprised of α- amino - acids, introduces an additional backbone torsion angle μ around C^{β}-C^{α} bond.[3] Extensive investigations of short linear- and homodetic cyclic- peptides have revealed the frequent occurrence of two distinctly different conformational characteristics *i.e.* the extended and the folded conformations, of the β-Ala residue (Figure 1). [2-4]

Figure 1 : Schematic presentation of an extended : $\mu \approx \pm 180 \pm 20°$ (left) and a folded : $\mu \approx \pm 60 \pm 20°$ (right) conformations, frequently adopted by β-Ala residue in peptides.

While an observation of a 'folded conformation' of the β-Ala residue is prevalent in cyclic peptides, its occurrence has been rare in short linear peptides.[2a] In this paper we extend our study and wish to describe the first crystallographic characterization of a 'locally folded' β-Ala residue in a model peptide: Boc - L-Pip - β-Ala - $NHCH_3$, **1** (Figure 2).[5] The constrained six-membered piperidine ring of the pipecolic (Pip) residue, in its chair form, expected to exhibit significant restriction about the φ torsion angle, is introduced to observe its influence on the folding behaviour of β-Ala residue.[6]

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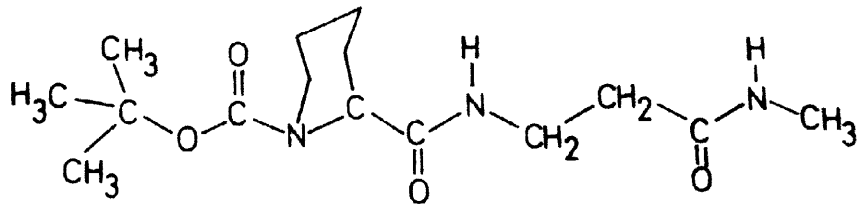

Figure 2 : The chemical structure of Boc - L-Pip - β-Ala - $NHCH_3$

In the crystal structure of **1** the urethane moiety adopts the uncommon conformation, classified as type *a*, the set of torsion angles θ_1 and ω_0 being 178.9° and 14.0°, respectively.[6,7] The pipecolic moiety is able to restrict the rotation of the urethane tertiary amide bond to its *cis* conformation. The observed 2.64Å separation between the urethane O (O1) and Pip C^{α} (C6) atoms, is consistent with the non-planar *cis* urethane ω_0 torsion angle.[1b] This observation is of particular interest since theoretical findings reported for Ac - L-Pip - $NHCH_3$, have indicated that in the chair forms the *cis* conformers are slightly less stable than the *trans* conformers.[1b] The sterically allowed *cis* urethane ω_0 torsion angle in short linear peptides may significantly alter the preferred conformation of the remaining residues.

The most attractive feature of the X-ray structure of **1** is an accommodation of a folded conformation of the β-Ala residue (Figure 1). The backbone torsion angles: ϕ = 123.0°, μ = -60.2° and ψ = 134.7° corresponding to *skew* (+), *gauche* (-) and *skew* (+) conformations respectively, characterize the folded structure of the β-Ala residue (Figure 3). Interestingly, the folded β-Ala moiety in **1** restricted the critical rotation about C^{β}-C^{α} bond to the *gauche* conformation: $\mu \approx \pm 60 \pm 20°$ and precluded from assuming the *trans* geometry.

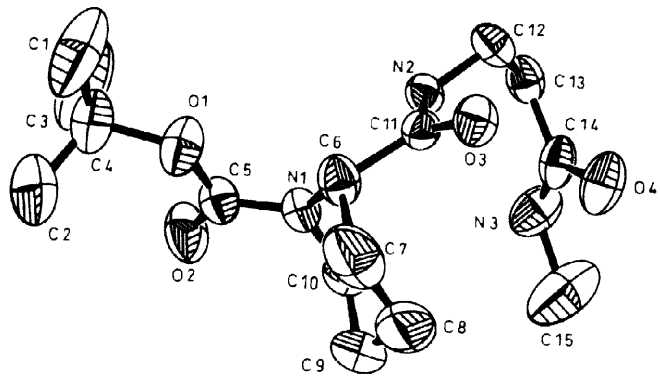

Figure 3 : An ORTEP representation of the molecular structure of Boc - L-Pip - β-Ala - $NHCH_3$, in the solid state. The thermal ellipsoids are shown to the 35% probability level.

Conclusive evidence for the induced folding of the β-Ala residue by the constrained Pip residue was obtained from an analysis of the X-ray diffraction structures of three peptides available to date and offer valuable models for correlation studies (Table 1). From the table it is clear that the molecular conformation of the β-Ala residue in **1** strongly differs from those reported for related model peptides. In short linear peptides, the μ torsion angle of the β - Ala residue usually tends to adopt an extended *trans*

Table 1 : A comparison of the torsion angles (°) of the β-Ala residues in model peptides [a]

Peptides	Torsion angles			References
	ϕ	μ	ψ	
Boc - Pip - β-Ala - $NHCH_3$	123.0	-60.2	134.7	This paper
Boc - Pro - β-Ala - $NHCH_3$ [b]	87.0	-155.0	-159.0	1a
Boc - Aib - β-Ala - $NHCH_3$ [c]	-132.8	165.0	131.0	2a
Boc - Ala - β-Ala - $NHCH_3$	135.9	-175.8	-163.4	2a

a : chiral amino-acids Pip, Pro and Ala are of L configuration. b : $\mu \approx -155.0$ was calculated from molecular structure as described in 1a. c : Aib is an α-aminoisobutyric acid or α,α-dimethylglycine.

conformation: $\mu \approx \pm 180 \pm 20°$. The signs of the torsion angles of the β-Ala residue however, exhibited either positive or negative values. It was expected that the β-Ala residue in **1** would adopt an extended conformation. High propensities of the Boc-Aib or Pro-Xaa -$NHCH_3$ (wherein Xaa = an α - amino acid) sequences, for intramolecularly hydrogen bonded β-turn conformations have been well documented.[1a, 8] Earlier studies have already concluded that the constraints imposed by the Aib and Pro residues in the peptide chains, are not sufficient to induce folding across the β-Ala residue.[1a, 2]

Significantly, the marked differences in the bulkiness, geometrical variation as well as in the conformational restrictions between the Pro and the Pip residue might be responsible for the altered gross conformational differences observed for the β-Ala residue. It is worth underlining that in contrast to the pyrrolidine ring of the Pro residue, the piperidine ring of the Pip residue, in the chair forms, fixes the ϕ torsion angle in the range $\approx -115 \pm 15°$. [1b, 6]

The Pip residue, in **1** assumes a semifolded backbone conformation with ϕ and ψ values of -131.8° and 26.3°, respectively. The six-membered ring adopts the most stable chair conformation and as expected the -CONH - substituent is orientated axially. The observed mean endocyclic torsion angles of the structure is ±56.25°. These values are fully consistent with the theoretical analysis performed on Ac - L-Pip - $NHCH_3$.[1b, 8a] The results indicated that the Pip residue in its perfect chair form adopts the low energy conformation at the values: $\phi \approx -115°$ and $\psi \approx 40°$.

These results are in favour of a largely preferred *cis* tertiary amide bond at the N-terminal and a folded conformation of the β-Ala residue at the C-terminal end. The overall conformation of **1** appears to be 'significantly folded'. Nevertheless, despite the N - and the C - terminal residues being semifolded and folded respectively, the formation of an intramolecular hydrogen bond is disfavoured. So far, we are aware of only one example of a linear tripeptide sequence Boc - Aib - Aib - β-Ala - $NHCH_3$, where the β-Ala moiety adopted a folded conformation and is part of an intramolecularly hydrogen bonded β-turn structure.[2b] The β-Ala residue occupied the right corner of the β-turn and constituted a novel C_{11}-ring structure.

This communication establishes, to the best of our knowledge, the first observation of a 'locally folded' conformation of the β-Ala moiety in a short linear peptide. Attention has been paid towards the implication(s) of restricted conformational flexibility on the β-Ala residue, induced by a constrained

neighboring chiral Pip residue. The induction of a folded conformation around C^{β}-C^{α} torsion angle in a flexible β-Ala residue may find application in the design and construction of novel scaffolds. Currently we are expanding this approach to other molecules and it should be possible to develop potential sites for well defined secondary structure exploiting unsubstituted β-Ala residue. These, in turn, would help us to elucidate the structure-function relationships of complex bioactive pharmaceutical agents. Finally, to conclude the conformational adaptability of the β-Ala residue by specific stereochemical constraints, a sufficient number of X-ray crystal structures of model compounds need to be determined and analyzed.

Acknowledgements : We thank CSIR for partial financial support. Authors are extremely grateful to Prof. T.P. Singh for his help for solving the crystal structure and extending the National Diffractometer Facility (DST) AIIMS, New Delhi, for data collection. R.K. wishes to acknowledge the Director for his consistent encouragements. This is IMTech communication Number 016 / 98.

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5. The peptide was synthesized by conventional peptide synthesis procedure and purified by silica-gel column chromatography. 300 MHz ^{1}H NMR spectrum was in full agreement with the structure. Suitable single crystals were obtained from MeOH-$CHCl_3$ mixtures. The diffraction data were collected on CAD4 Enraf-Nonius 4-circle automatic diffractometer using CuKα radiation (λ = 1.5418 Å) and graphite monochromator.
   Crystal data of **1** : Molecular Formula $C_{15}H_{27}N_3O_4$, Molecular weight = 313.39, Tetragonal, Space group $P4_12_12$, Cell constants : a = b = 9.292Å, c = 42.603Å, α = β = γ = 90°, V = 3676.3Å, Z = 8, Dc = 1.132 mg m^{-3}, T = 293 K, Final R = 0.0708, Final R_w = 0.2185. Details of synthesis, purification and characterization and crystal structure determination by application of direct method programmes (SHELX - 97) shall be reported elsewhere.
6. a) Toniolo,C.; Bardi, R.; Piazzesi, A. M.; Crisma, M.; Balaram, P.; Sukumar, M.; Paul, P. K. C. (Eds. Jung, G. and Bayer, E.). in "*Peptides 1988*", 477. de Gruyter, W. Berlin. **1989**; b) 1b. c)Bardi, R.; Piazzesi, A. M.; Crisma, M.; Toniolo, C. *Acta Crystallogr.*, **1992**, *C48*, 1645.
7. Benedetti, E.; Pedone, C.; Toniolo, C.; Pottle, S. M.; Scheraga, H. A. *Int. J. Peptide Protein Res.*, **1980**, *6*, 156.
8. a) Zimmerman, S. S.; Scheraga, H. A. *Biopolymers*, **1977**, *16*, 811; b) Prasad, B. V. V.; Balaram, P. *CRC Crit. Rev. Biochem.*, **1984**, *16*, 307; c) Toniolo, C. *CRC. Crit. Rev. Biochem.*, **1980**, *9*, 1; It is noteworthy that while the Aib residue restricts the backbone torsion angles in the helical regions ($\phi \approx \pm 60 \pm 20°$, $\psi \approx \pm 30 \pm 20°$) the five membered pyrrolidine ring of the Pro resiue restricts the ϕ values in the range -65±15°, of the conformational space.