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Conformational studies of γ-turn in pseudopeptides containing α-amino acid and conformationally constrained meta amino benzoic acid/meta nitro aniline

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HIGHLIGHTS

- Pseudopeptides **I** & **II** with appropriate N & C terminal protecting groups adopt γ-turn conformation.
- Pseudopeptides **III**–**V** displaying extended conformation in solid state forms γ-turn in solution.
- Importance of steric interactions amongst amino acid residues is crucial for γ-turn stabilization.
- The work may open a new avenue in introducing γ-turn within the bioactive conformation of peptides.

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ABSTRACT

Reverse turns (commonly β-turns and γ-turns), a common motif in proteins and peptides, have attracted attention due to their relevance in a wide variety of biological processes. In an attempt to artificially imitate and stabilize these turns in short acyclic peptides, a series of *N*-terminally protected pseudopeptides comprising of an α-amino acid and conformationally constrained meta amino benzoic acid (*m*ABA)/meta nitro aniline (*m*NA) (peptides **I**–**VI**) have been synthesized. The molecules were well characterized by various spectroscopic techniques and subjected to a systematic conformational analysis. Our experimental results reveal that only pseudopeptides **I** and **II** with methyl as the sidechain, tertiary butyloxy carbonyl as the *N*-terminal protecting group and (*m*ABA)/(*m*NA) at the C-terminus adopt γ-turn conformations in solid state as well as in solution. Even slight modification of any of the stated conditions donot support the formation of this γ-turn architecture in the solid state. Interestingly, the peptides **III**–**V** which displays extended conformation in solid state forms γ-turn structure in solution. Thus this result reflects the importance of co-operative steric interactions amongst various amino acid residues in stabilizing a particular conformation in peptides in different phases (solid and solution). This report may open a new avenue in introducing γ-turn motifs within the bioactive conformation of selected peptides.

1. Introduction

In proteins, γ-turn consist of three amino acids, which may or may not be stabilized by an intramolecular hydrogen bond between the C=O of the first residue (*i*) and the NH of the third residue (*i* + 2), forming a seven-membered ring [1,2]. Depending on whether the side chain of the residue *i* + 1 is in an equatorial or axial orientation on the seven-membered ring, γ-turns are classified as inverse or classical, respectively giving rise to a kink in the chain or a direction change [3]. Although less recurrent than β-turn, γ-turns play important roles in various biological processes. Structural studies have revealed that naturally occurring peptides such as vasopressin and the related desmopressin, bradykinin, and angiotensin II, that function as hormones or neurotransmitters, or have other regulatory roles in organisms, adopt γ-turn conformations [4–7]. The γ-turn present in vitronectin has been reported to contribute to the specific recognition by integrin receptor αvβ3 playing a role in tumor cell adhesion angiogenesis, and osteoporosis [8,9]. γ-Turn mimetic inhibitor forms complex with HIV-I protease which provides information about structure–activity relationship and helps in rational drug design [10]. It

Abbreviations: *m*ABA, *m*-amino benzoic acid; *m*NA, *m*-nitro aniline; Boc, tertiary butyloxy carbonyl; OMe, methoxy carbonyl; DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxy benzotriazole.

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is also proposed that in proteins, hydrated γ-turns promote helix-coil unfolding [11].

Various investigations suggest that γ-turns seldom exist in short, natural peptides. Initially it was believed that short acyclic peptides do not have the ability to adopt any preferential conformation. Spectroscopic studies indicate that the conformational space of tripeptides is more restricted than originally thought so that structures of limited stability can be formed [12]. Small peptides which are biomedically relevant as protease inhibitors, as taste receptors and for enzyme regulation, should maintain γ-turn conformation to show the activity [13–17]. Therefore designing and stabilizing γ-turn structure in small acyclic peptides is challenging and has been of great interest recently.

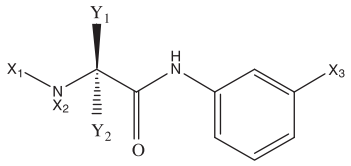
Jimenez et al. have demonstrated the use of D-c_3Dip (*N*′methyl-2,2-diphenyl-1-amino cyclopropane carboxamide), when coupled with acetyl L-Pro to constitute the first unequivocal evidence of the ability of a cyclopropane α-amino acid to adopt a γ-turn disposition in a non-propitious environment [18]. Y. D. Wu and his group has made a seminal contribution in inducing γ-turns in peptides comprising of alternating α-aminoxy acids and α-amino acids [19]. Martinez and his co-workers has made substantial inputs in designing γ-turn mimetics by incorporating 2-alkyl 2-carboxy-azetidine residue in the $i+1$ position of simply modified peptides and showed that these non-protenogenic amino acids possess a tremendous ability to nucleate γ-turns [20,21]. Therefore, it is apparent that various governing factor dictating the overall nucleation of γ-turn formation remains still in its infancy.

To gain further insight regarding the design of γ-turns in small acyclic peptides and contribution of various residues in the induction of this motif, we have synthesized pseudopeptides, **I–VI** (Table 1, Entry a–f), comprising of an α-amino acid and conformationally biased meta amino benzoic acid (*m*ABA)/meta nitro aniline (*m*NA), with an all trans extended configuration. The idea was to introduce some conformational constraint on the adjacent peptide bond incorporating *m*ABA/*m*NA which may compel the carbonyl at the *i*th position and NH of the $i+2$th amino acid to come closer in the same direction and attain the γ-turn conformation. Keeping this view in mind, our design aims in performing a

Table 1
List of pseudopeptides (Entry a–k) with torsion angles (°) of the residues at $i+1$.

Entry	Peptides	φ_1 (°)	ψ_1 (°)	Ref.
	Idealized classical γ-turn	75.0	−64.0	[1–3]
	Idealized inverse γ-turn	−79.0	69.0	[1–3]
a.	Boc-L-Ala-*m*NA[a] (**I**)	−105.9(2)	95.7(2)	This work
b.	Boc-D-Ala-*m*NA[a] (**II**)	107.7(3)	−95.0(3)	This work
c.	Piv-L-Ala-*m*NA[a] (**III**)	−157.3(1)	160.1(1)	This work
d.	Fmoc-L-Ala-*m*ABA-OMe[b] (**IV**)	–	–	This work
e.	Boc-L-β-cyano-Ala-*m*ABA-OMe[b] (**V**)	145.3(2)	−79.5(2)	This work
f.	Boc-Me-Gly-*m*ABA-OMe[b] (**VI**)	−85.4(2)	−160.8(1)	This work
g.	Boc-L-Ala-*m*ABA-OMe[b]	101.66	−96.31	[28]
h.	Boc-Gly-*m*ABA-OMe[b] (Mol.A)	62.6	−130.6	[29]
	(Mol. B)	−65.2	−179.6	
i.	Boc-β-Ala-*m*ABA-OMe[b]	−139.2	142.8	[22]
j.	Boc-γ-Aba-*m*ABA-OMe[b]	155.8	177.6	[22]
k.	Boc-β-Ala-*m*NA[a]	136.4	140.2	[30]
l.	Boc-γ-Aba-*m*NA[a]	–	–	[30]
m.	Boc-Gly-*p*NA[c]	−60.70	143.11	[31]
n.	Boc-β-Ala-*p*NA[c]	95.24	−165.30	[31]
o.	Boc-Pro-*p*NA[c]	55.13	−165.27	[31]
p.	Boc-Pro-*m*NA[a]	−68.02	176.31	[32]
q.	Boc-Pro-*m*ABA-OMe[b]	76.58	−179.62	[32]

Aib = α aminoisobutyric acid, β-Ala = β-Alanine, γ-Aba = γ-amino butyric acid.
[a] *m*NA = meta nitro aniline.
[b] *m*ABA-OMe = meta amino benzoic acid.
[c] *p*NA = para nitro aniline.



Entry	Peptide	Protected group: X_1	X_2	Y_1	Y_2	X_3
1.	**I**	Boc	H	Me	H	NO_2
2.	**II**	Boc	H	H	Me	NO_2
3.	**III**	Piv	H	Me	H	NO_2
4.	**IV**	Fmoc	H	Me	H	COOMe
5.	**V**	Boc	H	Me	H	COOMe
6.	**VI**	Boc	Me	H	H	COOMe

Fig. 1. Schematic representations of Peptides **I–VI**.