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βγ-fused turn structures in sugar amino acid (SAA) containing cyclic tetrapeptides with α3δ architecture

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ABSTRACT

The current manuscript describes conformational analysis of 15-membered cyclic tetrapeptides (CTPs), with α3δ architecture, containing sugar amino acids (SAA) having variation in the stereocenter at C5 carbon. Conformational analyses of both the series, in protected and deprotected forms, were carried out in DMSO-d_6 using various NMR techniques, supported by restrained MD calculations. It was intriguing to notice that the α3δ macrocycles got stabilized by both 10-membered β-turn as well as a seven-membered γ-turn, fused within the same macrocycle. The presence of fused sub-structures within a 15-membered macrocycle is rare to see. Also, the stereocenter variation at C5 did not affect the fused turn structures and exhibited similar conformations in both the series. The design becomes highly advantageous as fused reverse turn structures are occurring in the cyclic structure with minimalistic size macrocycle and this can be applied to develop suitable pharmacophores in the drug development process.

1. Introduction

Small molecule based therapeutics, despite their advantages of being smaller in size, easy to synthesize, lower in cost etc., are not showing the expected level of success in recent years as a large number of them fail in the clinical trials, especially at the late stages, due to their non-specificity and toxicity.[1] Peptide therapeutics have advantages over small molecules as they overcome some of the limitations of the small molecules in modulating cellular processes displaying a diverse range of biological properties.[2] Bioactive peptides isolated from natural resources act as peptide therapeutics, e.g., glucagon-like peptide-1 (GLP-1) for diabetes, ghrelin for obesity control, gastrin-releasing peptide in cancer treatment, defensins as antimicrobial agents and many others.[3] Similarly certain synthetic peptides, e.g., goserelin for treating breast cancer, prostate cancer, glatiramer acetate for multiple sclerosis and exenatide for diabetes are also available.[4] Despite their advantages over small molecules and success of certain blockbuster peptide therapeutics in the market, the growth of peptide therapeutics has been hampered, because peptides are inherently unstable within the body, they have poor pharmacological properties, they will rapidly break down into inactive fragments by proteases and low bio-availability.[5] Peptides, being smaller in size and lacking ordered structures, are quite flexible, thus preventing their specificity. Bioactive peptides and certain synthetic peptides gain the advantage due to their intrinsic stable fold, and suitable orientations of the side-chain residues imparting requisite functions, such as binding to the extracellular receptors and acting as inhibitors and/or modulators of protein–-protein interactions (PPI).[6] Normally only one suitable single conformation is needed for the peptides to interact with their receptors for performing their roles that can be achieved by restricting their intrinsic conformational flexibility by cyclization and incorporating further conformational constraints in the cycle making them even more rigid. Cyclic peptides, resistant to both *exo*- and *endo*proteases, alleviate the drawbacks of their linear precursors and, in addition, generate interesting structural and functional features, which bestow them with better therapeutic properties.[7] It is often noticed that certain constraints are also incorporated in many cyclic peptides, which further limit their conformational degrees of freedom and provide the precise pre-organized conformation necessary to interact with the target receptors. Common amino acids, disulfide bonds, metal ions, aromatic groups, unnatural amino acids, unsaturation in the backbone, triazole ring etc., are noticed normally as constraints in the macrocyclic peptides.[8]

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Developing peptide based macrocycles with pre-organized conformations is often a challenging task. In macrocycles containing four or more residues, the conformational stability is mostly achieved from the intramolecular H-bonding in the backbone resulting into α-, β- or γ-reverse turn structures. Earlier[9] we reported β- or γ-turn structures in a 13-membered cyclic tetrapeptides (CTPs) with α3β architecture and recently, α-turn structures were observed by us in CTPs with 14-membered macrocycle displaying α3γ architecture.[10] Fused turn structures, such as ββ, βγ further restrict the conformational flexibility in a macrocycle and assist in having more stable pre-organized conformations.[11] 15-membered is the minimalistic size that is required for the macrocycle to possess fused β- and γ-turn structures. Introducing a δ-amino acid, a dipeptide isostere, will make the ring size in a CTP, suitable to explore fused β- and γ-turn structures. A sugar amino acid (SAA) containing enkephalin analogue showed earlier an unusual β-turn structure having an intramolecular 10-membered H-bond involving the free OH group of the SAA.[12] In continuation of our work on cyclic peptides, the present article presents the synthesis and conformational analysis of 15-membered CTP with α3δ architecture, containing sugar amino acid (SAA) as a constraint.

2. Results and discussion

2.1. Synthesis of cyclic peptides Ia, IIa, I and II

Peptides **Ia** and **IIa** were synthesized by cyclization of H_2N-SAA-Val-Orn(*Z*)-Leu-OH[13] using FDPP in dry DMF under high dilution following our earlier reported procedure.[14] Cbz deprotection of the resulting *cyclo*-[SAA-Val-Orn(*Z*)-Leu] resulted into **I** and **II**.[15] In **Ia**, **I** and **IIa**, **II**, the stereo centre at C5 position in the sugar ring is changed to verify the effect of sugar puckering on the conformational preferences among these cyclic peptides (Fig. 1).

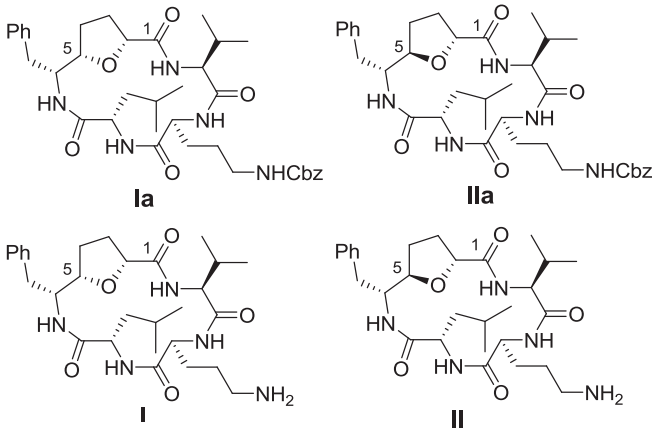

Fig. 1. Structures of cyclic tetrapeptides **Ia**, **I**, **IIa** and **II**.

2.2. Conformational analysis of cyclic peptides Ia, I and IIa, II

Detailed solution conformational analysis of macrocyclic peptides **I**, **Ia**, **II** and **IIa** in DMSO-d_6 with ~5–7 mM in concentration was carried out to investigate their conformational preferences, employing various NMR techniques.[16] ^{1}H NMR spectra of all the peptides show sharp single set of resonances with well dispersed amide protons suggesting that the macrocycles stay in a stable single conformation in solution. Restricted conformational flexibility in these macrocycles can be attributed to some of the amide protons participating in hydrogen bonding. Amide protons being labile and those which are not involved in H-bonding will be sensitive to temperature, which can be detected from Variable Temperature (VT) experiments in DMSO-d_6 as the non-bonded amide protons will tend to show higher deviation in the chemical shifts compare to those which participates in H-bonding.[9,10] VT experiments carried out from 303 to 343 K suggested that in all these peptides the Val and Leu amide protons are participating in H-bonding (Fig. 2).

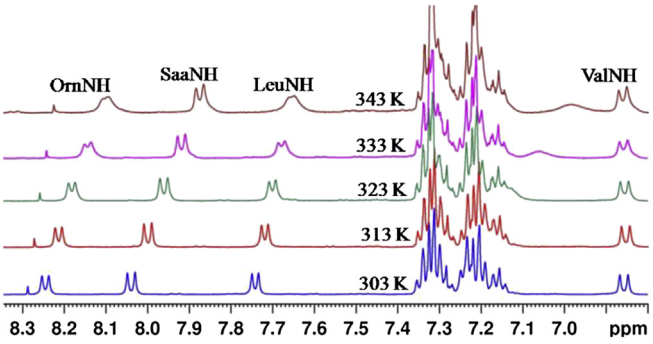

Fig. 2. Variable temperature (VT) studies. Amide protons of Val, Leu show smaller $\Delta\delta/\Delta T$ values of 0.5 and 2.25 ppb/°K compared to that of SAA and Orn suggesting the former amide protons' involvement in H-bonding.

The vicinal coupling constant ^3JHN-CαH values are around 9.0 Hz for all the residues except for Orn, which is around 7.0 Hz, suggesting that for all the residues, except for Orn, the ϕ value is of the order of −120° and for Orn it may be averaging. The ^3JCδH-Cδ′H values of SAA, either <5 Hz or >11 Hz, suggest rigid side-chain conformation for Phe side-chain and similar side-chain preference is noticed for Phe in all the studied cyclic peptides. Further from the 2D-ROESY spectra, strong NOE cross peak between SAANH↔Cδ′H$_{(pro\text{-}R)}$ and weak NOE cross peak between SAANH↔Cδ′H$_{(pro\text{-}S)}$ confirm ~60° (g+) for the dihedral angle (χ1).

2.3. Molecular conformations of cyclic peptides

Conformational features of the macrocycles **I**, **Ia**, **II** and **IIa** were investigated using 2D-ROESY spectra collected at 303 K. NH–NH correlations between ValNH↔LeuNH suggested their close proximity in the space. Further the involvement of these two amide protons in hydrogen bonding suggests their orientation to be towards the inner core of the macrocycle. Interestingly, NOE between ValNH↔SaaCδH, which is equivalent to the NH(i+3) and CαH(i+1) NOE observed in routine β-turns, suggests that ValNH may be H-bonded with Leu carbonyl, resulting into a 10-membered β-turn structure. NOE peak between ValNH↔SaaNH, further confirms their close proximity and possibility for the ValNH participating in hydrogen bonding. Other prominent NOEs include LeuNH↔OrnNH and ValNH↔OrnNH (Fig. 3).

2.4. Restrained molecular dynamic (MD) simulations

MD calculations for the macrocycles were carried out using distance constraints derived from ROESY experiments, applying two-spin approximation, by simulated annealing method, and constraining the backbone dihedral angle ϕ as −120°±10°, for all the residues, except for Orn, which was left out without any restraint throughout the simulation.[10,11] The χ^1 of phenyl side chain of Saa was restricted with a dihedral angle value of about 60°±10° as deduced in all the peptides. The 15 best structures (Fig. 4) obtained from the calculations have a heavy atom RMSD of 0.35 Å and all atoms RMSD of 0.9 Å, with none of the distance and angle constraints deviating by more than 0.25 Å and 15°, respectively.