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Conformational modulation of peptides using β-amino benzenesulfonic acid (S-Ant):‡


Accepted Manuscript

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

This communication describes on the utility of a conformationally restricted aromatic β-amino acid (2-amino benzene sulfonic acid, S-Ant) in inducing various folding interactions in short peptides. Sandwiching S-Ant between diverse amino acid residues was shown to form robust folded architectures featuring a variety of H-bonded networks, suggesting its utility in inducing peptide folding.

Introduction

Nature rivets peptides to be the basic structural entities for numerous biological phenomena. Peptides feature complex folded structures as prerequisite criterion to exhibit their function. Understanding the folding phenomenon is a complex task. Conformational investigation using unnatural amino acid building blocks would aid in unveiling the mysterious mechanisms employed by nature in accomplishing the biological functions.

Recent times have witnessed an increased attention for development of unnatural amino acid building blocks which can induce folding in peptide molecules. Unnatural amino acids play a crucial role in inducing turn formation in synthetic peptides. An excellent example of an unnatural α-amino acid that has been used to induce turns resulting in 3_{10} helical architectures in synthetic peptides is 2-aminooisobutyric acid (Aib). The torsional constraints of Aib impart conformational rigidity to peptide sequences. Similarly, gabapentin (Gpn) is another unnatural amino acid with four degrees of torsional freedom that has become popular in inducing robust turns in peptides. Conformationally restricted aromatic amino acids have also been shown to be useful in the de novo design of foldamers. Herein, we report on our observations that substantiate the turn inducing ability of orthanilic acid (S-Ant) in synthetic peptides. When sandwiched between various amino acid residues, S-Ant has been shown induce folding affording various H-bonded networks. The conformational features of the synthetic peptide backbones containing orthanilic acid has been studied in solid as well as solution states (Fig. 1).

Results and Discussion

Synthesis

Compounds 1-5, required for the present study, were synthesized using multi-step synthetic strategies and 6 and 7 were synthesized by segment doubling strategy, as depicted in schemes 1-3 (ESI, page S3-S5).
Conformational Analysis

We synthesized peptides 1-5 wherein orthanilic acid is sandwiched by a combination of α and β amino acid residues (Fig. 1, vide supra). The idea of altering various amino acid residues around 5′Ant was to investigate its tolerance limit in promoting folding. The peptides 1 and 2 possess orthanilic acid on their backbone surrounded by two α-amino acids: Aib and Gly. The peptide 2 is C-terminus amidated analogue of 1. The peptide 3 was designed in such a way that orthanilic acid is sandwiched between cyclic α-amino acid Pro at the N-terminus and acyclic α-amino acid Gly at the C-terminus. Peptide 4 was designed and synthesized as a reversed sequence of peptide 2, thus the position of Pro and Ant was interchanged. Since anthrancic acid (Ant) is known to cause unexpected conformational changes when introduced at the N-terminus, peptide 5 was also made in order to evaluate the conformational outcome.

Solid-State X-ray Studies of 1-5

Extensive efforts for crystallization led to the crystal formation of peptides 1-5. It is evident from all the crystal structures that the peptides containing 5′Ant feature folded architectures with a variety of inter-residual hydrogen-bonded networks, in addition to the intra-residual 6-membered H-bonding present within the orthanilic acid residue, depending upon the amino acid to which 5′Ant is linked in. The peptides exhibit folding mainly due to the conformational restriction imposed by 5′Ant having the closely positioned amino and sulfonamide groups – separated by a sp2 bond which is part of an aromatic ring. The peptides 1 and 3 adopt 11-membered H-bonded folding and the peptide 2, although a C-terminus amidated analogue of peptide 1, adopts a 14-membered H-bonded folding. It was observed that the crystal lattice of peptide 1 contained two molecules wherein one of the molecules exhibits a 11-membered inter-residual H-bond and another one with an almost similar folded architecture, but devoid of 11-membered inter-residual H-bond (ESI S15). The crystal structures of the peptides 4 and 5 clearly revealed a folded conformation featuring an inter-residual 9-membered H-bonding. The fold adopted by the peptide 4 remains intact even after acetylating the N-terminus of the peptide as in 5, without disturbing the H-bonding pattern on the folded backbone. It is clearly evident from the crystal structures of the peptides that the orthanilic acid containing peptides adopt rigid folded architectures featuring inter-residual H-bonds, which might be attributed to the torsional flexibility of the sulfonamide group varying from -88.9° (as in peptide 5) to 99.9° (as in peptide 2) present on the peptide backbones. The inter-residual H-bonding distance [d(H…A)\text{av}] observed in the peptides is 2.38 Å. The hydrogen-bonding angle [Δ(D-H…A)] varies from 138° (as in peptide 3) to 173° (as in peptide 2). Although folding is prevalent in all the structures 1-5, the structural disparity in their H-bonded network is evident from the overlaid crystal structures. Whereas peptides 1-3 form an extended β-turn-like structure featuring 11-membered H-bonding, peptides 4 and 5 form a pseudo β-turn structure featuring 9-membered H-bonding (Fig. 2).

Solution-State NMR Studies of 1-5

Conformational investigation of the peptides in solution-state was studied using 2D NMR experiments. The characteristic inter-residual NOEs clearly revealed the folded conformations of the peptides, as seen in the solid-state. All the compounds were readily soluble in non-polar organic solvents (>> 100 mM in CDCl3) at room temperature suggesting the hydrogen bonding groups to be strongly shielded, preventing the formation of molecular aggregates. The presence of the inter-residual H-bonding was substantiated by the [D6]-DMSO titration studies of 1, 2, 3, and 5 (Δδ < 0.2 ppm) (ESI S49-S54).

Fig. 2: Overlayed crystal structures of peptides 1-3 (left), featuring a C21 H-bonding and 4 and 5 (right), featuring a C12 H-bonding. Note: The peptides 1-5 are highlighted as cyan, magenta, light green, purple and dark green, respectively. All hydrogens, except the polar ones, have been deleted for clarity and the acceptor and donor atoms involved in hydrogen-bonding are highlighted.

Fig. 3: A) Crystal structure of 2 and its selected 2D NOESY excerpts supporting folded conformation. B) Crystal structure of 5 and its selected 2D NOESY excerpts supporting folded conformation (500 MHz, CDCl3).
and variable temperature studies of the peptides 1, 2, 3 and 5 (268-323° K; Δδ/ΔT < -2 ppb/°K) (ESI S55-S60). The peptides 1, 2, 3 and 5 showed sharp signals rendering their conformational analysis easy. The diagnostic long range inter-residual nOes that supported the folded conformations of the peptides in solution state for 2 are: C13H vs. NH1, C14H vs NH2, 'Boc(H) vs NH4, 'Boc(H) vs NH2 and 'Boc(H) vs C10H (Fig. 3A) and for 5 are: C13H vs C17H and C13H vs C14H (Fig. 3B).

**MD Simulation Studies of 7 and 8**

We also synthesised higher order oligomers 6 and 7 (Fig. 4) corresponding to the folded peptides 2 and 3 to gain insight into their conformational features. All efforts to crystallize the oligomers 6 and 7 went in vain. Thus, the solution-state conformational investigation of these oligomers was done using NOE-based MD simulation studies employing the distance constraints (ESI S81-S85). The signal assignments were done using a combination of 2D NOESY, COSY, TOCSY, HMBC and HSQC experiments. The inter-residual nOes observed in the 2D solution-state NMR experiments supported the 11-membered H-bonding on their backbones and revealed helically folded architectures for the oligomers 6 (Fig. 3A) and 7 (Fig. 4B).

The conformation observed in the crystal structures of 2 and 3 is perfectly reproduced by MD simulation studies as shown by the overlay of the crystal structures and their respective minimum energy structures obtained from the NOE-based simulation studies (ESI S81-S83). The good agreement between the simulated structures and experimental structural data of 2 (RMSD < 0.2) and 3 (RMSD < 0.1), validates the reliability of MD simulation methods for accurate prediction of the solution-state conformation of peptides as illustrated in case of several peptide oligomers in the literature.9

**CD Studies of 3 and 7**

The circular dichroism spectra provided the characteristic signature supporting the helical conformations of the peptides 3 and 7 (Fig. 5).

**Conclusions**

In conclusion, this work provides insight into the folding interactions caused by orthanilic acid in peptides. When sandwiched between amino acids, this conformationally rigid β-amino sulfonic acid has been shown to induce folding featuring a variety of hydrogen-bonded networks, as evident from crystal structure6 and NMR studies. The results described herein suggests that orthanilic acid, a commercially easily available and inexpensive unnatural amino acid, offers good promise of inducing folding interactions in peptides.
Acknowledgement

GP and ASK thank CSIR for fellowship. This work was funded by NCL-IGIB, New Delhi.

Experimental procedures

Crystallographic Data for the compounds 1, 2, 3, 4 and 5 were collected on SMART APEX-II CCD using Mo-Kα radiation (λ = 0.7107 Å) to a maximum θ range of 25.00°. Crystal to detector distance 5.00 cm, 512 x 512 pixels / frame, Oscillation / frame -0.5º, maximum detector swing angle = – 30.0º, beam center = (260.2, 252.5), in plane spot width = 1.24, SAINTEST integration with different exposure per frame and SADABS correction applied. All the structures were solved by direct methods using SHELXTL. All the data were corrected for Lorentzian, polarization and absorption effects. SHELX-97 (ShexTL) was used for structure solution and full matrix least squares refinement on F². Hydrogen atoms were included in the refinement as per the riding model.

Crystal data for 1

Single crystals of 1 were grown by slow evaporation of its solution in ethyl acetate and DCM. Colorless cube like crystal of approximate size 0.31 x 0.12 x 0.07 mm, was used for data collection. Multi-run data acquisition. Total scans = 4, total frames = 1271, Oscillation / frame -0.3º, exposure / frame = 30.00 º is 99.9 %. C₂H₁₂N₂O₂S, MW = 429.49, Crystals belong to Triclinic, space group P-1, a = 9.2146(8) Å, b = 11.0127(3) Å, c = 17.4395(7) Å, V = 1979.93(15) Å³, Z = 4, Dc = 1.447 g/cc, (Mo-Kα) = 0.208 mm⁻¹, 14978 reflections measured, 6559 unique, (I>2(σ(I))), R1 = 0.0388, wR2 = 0.1001, largest diff. peak and hole 0.244 and -0.274 e.Å⁻³.

Notes and references

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† Electronic Supplementary Information (ESI) available: H, 13C, DEPT-135 NMR, 2D study spectra, ESI mass spectra and theoretical study of new compounds are included. See DOI: 10.1039/b000000x


10 Crystallographic data of 1-5 have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. 978806-978810 (for compounds 1-5, respectively).