ROLE OF WEAK INTERACTIONS AND FLEXIBLE TETRA-GLYCINE LINKER IN A DE NOVO DESIGNED HELICAL HAIRPIN EICOSAPEPTIDE

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De novo protein design aims to create new sequences with predetermined structure and function. In *de novo* design, helical hairpin is an important super secondary structural motif. Many attempts to design such a motif had failed to realize the desired folded conformation. The situation was changed by the first design of an apolar helical hairpin peptide (HTH1) in our laboratory (PNAS (USA) 98, 870-874, 2001) with sequence Acetyl-Gly-delPhe-(D-Ala-delPhe-delPhe)2-L-Ala-(Gly)4-delPhe-(L-Ala-L-Leu-delPhe)2-L-Ala-NH2. 3(10) helices of opposite handedness in HTH1 are associated through an extensive network of aromatic side-chain to backbone C-H…O interactions. In order to study the consequences of change of chirality, on the association of helices, we have replaced the D-Ala in HTH1 to L-Ala residues giving rise to HTH2. The peptide of length 21 residues was synthesized and crystallized. The synchrotron data (1A) were collected at Brookhaven National Laboratory U.S.A. The structure was solved by Direct Methods using SnB program and refined to R-factor =12.3%. The HTH2 molecule still folds into helical hairpin but both helices are right handed 3(10) helices. Also, the nature of their association is different from that in HTH1. The side chain backbone intramolecular interactions seen in HTH1 are completely absent in HTH2. Instead, HTH2 is stabilized by backbone to backbone Cα-H…O interactions. Both HTH1 and HTH2 are stabilized by weak C-H…O interactions in the interhelical region. The role of the flexible tetra-glycyl linker in facilitating the structural maneuvering necessary to meet the requirements of these two different helical hairpin formations is self-evident. Clear electron densities seen in the tetra-glycyl turn regions of both the molecules indicate that by being flexible Glycine rich segments can first optimize global interactions in a peptide and in the process acquire increased stability themselves. HTH1 and HTH2 define peptide based super secondary structures and can serve as scaffolds for the incorporation of functional sites as in proteins.

Keywords: DE NOVO DESIGN HELICAL HAIRPIN WEAK INTERACTIONS

CRYSTAL STRUCTURES OF O-PHOSPHOSERINE IN DIFFERENT CHEMICAL ENVIRONMENTS

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The structural characterization of phospho- amino acids and their salts is a subject of considerable interest due to the importance of protein phosphorylation in eukaryotic cells. The reversible phosphorylation of proteins is observed mainly for the hydroxyl moiety of serine, threonine or tyrosine.
Our investigations provide information about the interactions between anions of O-phosphoserine (PSer) and cations (K^+, NH_4^+, Na^+ and Mg_2^+) in the solid state. The crystal structures of the potassium salts of O-phospho-L-serine (I) and O-phospho-DL-serine (II) were determined. The asymmetric unit in both crystals consists of one PSer monoanion, potassium cation and water molecule. The overall conformation of the serine moiety in both structures is similar to that found in the zwitterions of O-phosphoserine [1-2].
Three polymorphic forms of the dipotassium salt of O-phospho-L-serine were isolated from the water solutions of different pH. One form (III) crystallizes in the orthorhombic space group $P2_12_12_1$, while others (IV and V) crystallize in the monoclinic space group $P2$. In the crystal structures, all of PSer dianions have similar conformation. In all three crystals, the molecules form different three-dimensional networks via N-H...O hydrogen bonds in which all N-bonded [and O-bonded of water molecules] H atoms are involved.
References
[1] M. Sundaralingam and E. F. Putkey, Acta Cryst. B26 (1970), 790.
[2] F. Putkey and M. Sundaralingam, Acta Cryst. B26 (1970), 782.

Keywords: **X-RAY STRUCTURES O-PHOSPHO-L-SERINE O-PHOSPHO-DL-SERINE**

DESIGN OF A β -TURN-II CONFORMATION WITH α, β DEHYDRO RESIDUES AT (i+1) POSITION

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Highly specific peptide structures can be designed by inserting dehydro-residues into peptide sequences. The conformational preferences of branched β-carbon residues are known to be different than other residues. It has been shown that the non-branched β-carbon dehydro-residues induce β-turn II conformation when placed at (i+2) position while branched β-carbon dehydro-residues induce β-turn III conformation. However, the effects of various dehydro-residues, when substituted at (i+1) position are not yet fully understood. In order to clearly define the role of dehydro-residues for peptide design through their substitutions at (i+1) position, three peptides: (I) Cbz-ΔVal-Ala-Leu-OCH3 (II) Cbz-ΔIle-Ala-Leu-OCH3 and (III) Cbz-ΔLeu-Ala-Leu-OCH3 were synthesized, two with branched β-carbons and the third with a non-branched β-carbon dehydro-residue. Because of the branching at the β -carbon, the bond angles Cα-Cβ-Cγ in (I) and (II) are significantly different [123.3(5)° and 122.6(5)° respectively] than that observed in ΔLeu. Despite this, the structures of the three peptides were found to be identical as all the three adopt β-turn II conformation. All the peptide structures are stabilized by intramolecular 4\\1 hydrogen bonds. Though, the space groups are same, their molecular packings are different as in the former two, there are two intermolecular hydrogen bonds whereas, the latter has only one hydrogen bond indicating dissimilar intermolecular interactions. From these three structures, it can be concluded that a dehydro-residue at (i+1) position induces a type II β-turn conformation irrespective of the nature of branching of its side-chain.

Keywords: **PEPTIDE DESIGN, β-TURN-II, DEHYDRO-RESIDUE**

TOWARDS THE STRUCTURAL DETERMINATION OF THE SHORT CHAIN α-NEUROTOXINS FROM THE ELAPID SNAKES *P. AFFINIS* AND *P. NUCHALIS*

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The protein toxins from the Australian elapid snakes *P. affinis* (dugite) and *P. nuchalis* (gwardar) have not been well characterized structurally, a surprising factor given the potential of structural data to assist with drug and antivenom design. In particular, structures of the short chain alpha-neurotoxins, proteins that mediate paralysis, have yet to be elucidated. The aim of this research was the structural study of previously uncharacterized snake short chain α-neurotoxins by X-ray crystallography and NMR spectroscopy. In order to obtain sufficient quantities of these proteins for characterization, molecular biology techniques were employed to obtain neurotoxin DNA from the snake venom glands. DNA sequencing of the neurotoxin DNA demonstrated that both dugite and gwardar neurotoxins share considerable sequence identity with each other, as well as with other snake short chain α neurotoxins. The DNA was cloned into pGEX expression vectors for the bacterial overexpression of GST-neurotoxin fusion proteins. Trials to optimize toxin production and solubility, such as varying IPTG concentration, temperature, bacterial cells and plasmids, were carried out and are presented here.

Keywords: **SNAKE α NEUROTOXIN, BACTERIAL OVEREXPRESSION**