

Isolated and multiple β -turns with proline in the third position

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We report the observation of a number of unexpected isolated β -turns with a proline in the third position. These β -turns are mainly, either type VIa1, type VIb or type IV although 3 examples of type I, 4 examples of type II' and 5 examples of type VIa2 β -turns were also observed in a representative protein data set. Nearly a third of these turns are located in the loop regions and the rest are situated at ends of either helices or β -strands. The formation of double β -turns, where proline in the third position in the first β -turn corresponds to proline in second position in the second overlapping β -turn has earlier been suggested to stabilize the otherwise unfavourable proline in the third position. We observed a number of multiple β -turns, mainly of the type VIb or type IV, with proline in the third position that did not correspond to proline in second position in the overlapping β -turn. Although these β -turns may also be stabilized due to the formation of multiple turns, the isolated β -turns with proline residue in the third position are mainly surrounded by water molecules which are likely to stabilize these turns. The ϕ, ψ dihedral angle values for proline residue in isolated and multiple β -turns show distinct preferences in the Ramachandran plot.

A β -turn consists of four consecutive residues defined by positions $i, i + 1, i + 2, i + 3$ that are not within an α -helix; the distance between $C_{\alpha}(i)$ and $C_{\alpha}(i + 3)$ is less than 7 Å and the turn leads to reversal in the protein chain^{1,2}. β -turns have been classified into 9 different types (I, II, VIII, I', II', VIa1, VIa2, VIb, IV) based on the dihedral angle values (ϕ, ψ) of the residues in the second and third position in the turn^{1,3,4}. Turns are often conserved during evolution and have been implicated in molecular recognition² and protein folding⁵.

It is generally understood that a proline residue in the third (or $i + 2$) position in a β -turn is least favoured, whereas it is most favoured in the second (or $i + 1$) position. However, a number of type VI β -turns with proline residue in the third position observed earlier⁴ were associated with a double turn, in which the proline residue corresponds to the second position in the overlapping β -turn. Formation of double β -turn has therefore been suggested to stabilize the proline in the third position. In this work, we wanted to examine whether β -turns with a proline residue in third position may be isolated.

The representative protein data set for our analysis was obtained from the Protein Data Bank (PDB)⁶⁻⁸ at the Research Collaboratory for Structural Bioinformatics (RCSB) (<http://www.rcsb.org/pdb/>), using the PDB_SELECT program⁹ through ftp (ftp.embl-heidelberg.de/pub/databases/protein_extras/pdb_select). Protein chains with no more than 25% sequence identity, representing crystal structures at 2.0 Å resolution or better and containing at least one β -turn were used in the analysis. The PROMOTIF program¹⁰ that provides details of the location and types of structural motifs in proteins of known three-dimensional structure in the PDB was used to extract the β -turns^{1,3,4,11} classified on the basis of dihedral angles at the second and third positions into the nine known types (I, II, VIII, I', II', VIa1, VIa2, VIb, IV). Computer programs were written to identify the isolated and multiple β -turns with proline in the third position. The PDB code/chain, start and end residues of the β -turn, sequence corresponding to the isolated β -turn and the β -turn types generated by the PROMOTIF program were extracted. The ϕ, ψ Ramachandran plots¹² for the proline residue in the third position in isolated and multiple β -turns were generated. The secondary structure flanking the isolated β -turns with proline in the third position was identified by referring to PDBsum¹³ on the web.

Four hundred and twenty six representative protein chains satisfying the criterion described earlier were selected for the analysis. The PDB codes corresponding to representative protein chains are available from our website at (<http://www.cdfd.org.in/bioinf/res>). 7153 β -turns were identified and classified into the nine different classes by the PROMOTIF program. Proline is the only amino acid observed at the third position in all the 131 type VI β -turns (44 VIa1, 17 VIa2 and 70 VIb). Main-chain hydrogen bonds were not observed for most type VIb β -turns. Ninety-five isolated β -turns were observed with proline in the third position. These included 16 type VIa1 β -turns, 5 type VIa2 β -turns, 41 type VIb β -turns, 26 type IV β -turns, 3 type I β -turns and 4 type II' β -turns. These turns did not form multiple turns with other β or γ -turns. Nearly a third of these isolated β -turns were associated with loop regions in the protein and the remaining were either at the ends of helices or β -strands. Details of the β -turn type, protein PDB code/chain, start and end residue numbers corresponding to the β -turn and the β -turn sequence are available from our website mentioned earlier. The proline residue at the third position in isolated β -turns is mainly surrounded by water molecules which are likely to stabilize these turns. Preliminary analysis did not reveal any specific preference for either the number of water molecules or preferred orientation of water molecules around the proline residue.

We also observed 48 multiple β -turns where the proline residue at the third position in a β -turn did not correspond to the equivalent of a proline residue at the second position in an overlapping β -turn. The β -turn types in

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these multiple turns, start and end residues of the peptide fragment corresponding to the multiple turn and length corresponding to the number of amino acids in the multiple turn are also available from our website. These multiple turns were mainly of the type VIb or type IV β -turns, although 4 examples of type I and 2 examples of type II' β -turns were also observed. The type VIb β -turns were usually preceded either by type I, II, VIII, I', IV β -turns or followed by either type IV, I, II or VIb β -turns. Type IV β -turns with proline in the third position were most often preceded by another type IV β -turn or flanked by type I β -turns. The type IV β -turns unlike other β -turn types are known to have variable conformation.

The representative data set contained 64 multiple β -turns in which the proline residue at the third position corresponds to the equivalent of a proline residue at the second position in the overlapping β -turn. These turns were mainly of type VIa1, VIa2, IV or I. Type VIa1 β -turns often had type IV β -turn as the overlapping turn, type VIa2 β -turns had either type VIII or type IV β -turn as the overlapping turn and type IV β -turns had another type IV or a type I β -turn as the overlapping turn. Nine examples of type I β -turns were observed where the overlapping β -turn was either type I or type IV. The order of the turn types with other relevant details are available from our website.

The Ramachandran plot for the proline residue in isolated β -turns shown in Figure 1 *a* clusters around two regions of the plot, (1) $\phi = (-112.5, -50)$ and $\psi = (-40, 35)$, and (2) $\phi = (-112.5, -50)$ and $\psi = (112.5, 180)$ degrees. Also, the ϕ, ψ dihedral angle values for proline at the third position that does not correspond to proline at the second position in the overlapping β -turn in the multiple turns, shown in Figure 1 *b* cluster around two regions, namely (1) $\phi = (-101.25, -45)$ and $\psi = (-22.5, 22.5)$, and (2) $\phi = (-101.25, -45)$ and $\psi = (112.5, 180)$. However, the ϕ, ψ dihedral angle values for the proline residue in the third position which corresponds to the proline residue at the second position in the overlapping β -turn in the multiple turns, shown in Figure 1 *c*, cluster only around one region of the Ramachandran plot, namely $\phi = (-101.25, -50)$ and $\psi = (-56.25, 35)$ degrees.

Isolated β -turns with the proline residue in the third position are commonly observed in protein structures and the β -turn is mainly of type VIa1, VIb or IV. These turns are located in loop regions or at the ends of helices or β -strands in the protein. The proline residue is mainly surrounded by water molecules which are likely to stabilize these turns. Multiple β -turns, with the proline residue at the third position in a β -turn that does not correspond to a proline residue at second position in the overlapping β -turn were mainly of type VIb or type IV. As the main-chain hydrogen bonds were not observed for type VIb β -turns and the type IV β -turns are known to have variable conformations, these β -turns with the proline residue in

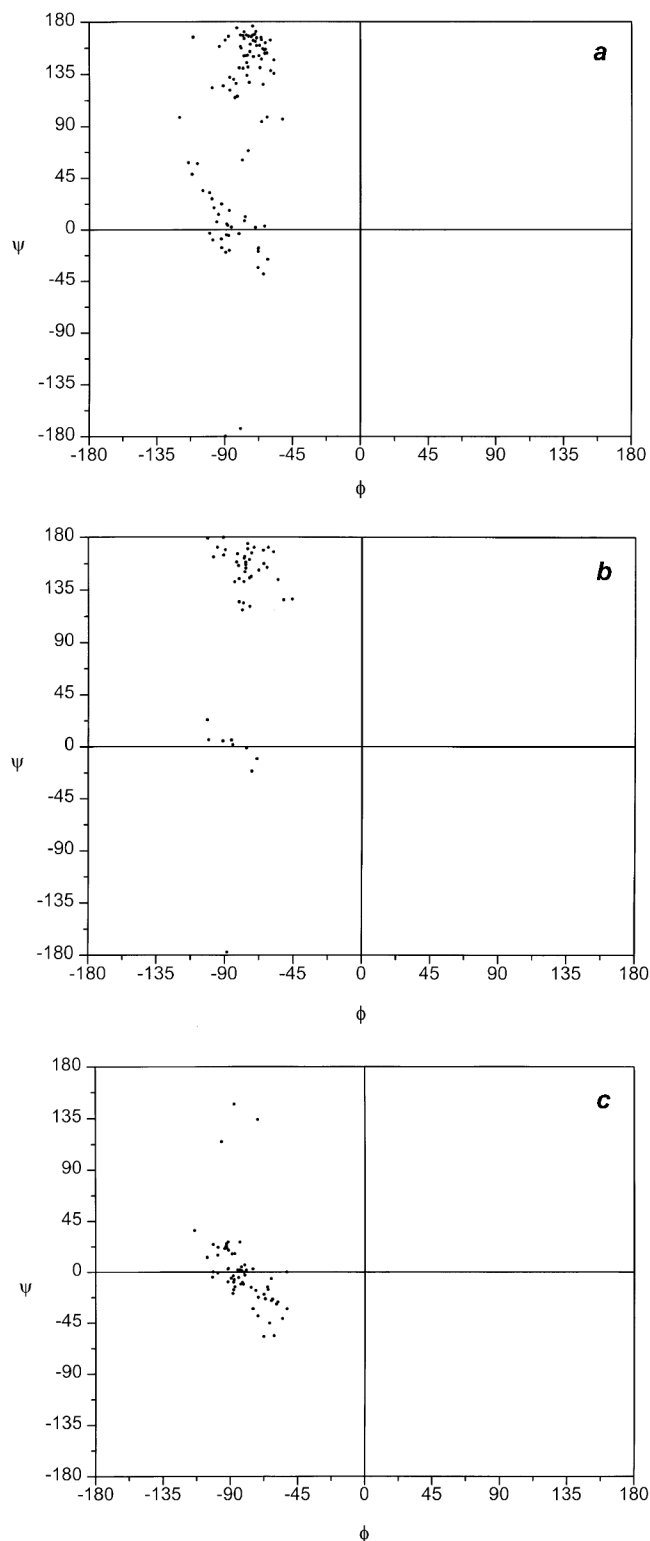


Figure 1. *a*, Ramachandran plot for proline residue in the third position in isolated β -turns. Nine β -turns corresponding to dihedral angle values for proline in the region intermediate to the two clusters are type IV β -turns that may have variable conformation; *b*, Ramachandran plot for proline residue in the third position in multiple β -turns that does not correspond to proline at the second position in an overlapping β -turn; *c*, Ramachandran plot for proline residue in the third position in multiple β -turns that corresponds to the proline residue at the second position in the overlapping β -turn. Three β -turns corresponding to proline with dihedral angle values away from the cluster are type IV β -turns.

the third position are possibly stabilized due to the multiple turns or proline interactions with solvent or form hydrogen bonds or salt bridge interactions with the rest of the protein atoms. Multiple β -turns with the proline residue in the third position in the first β -turn which corresponds to the proline residue in the second position in an overlapping β -turn were mainly of the type VIa1, VIa2, IV or I.

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Received 5 February 2000; revised accepted 12 July 2000
