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Prolinoamino acids as a tool to stabilize β-turns with the side chain of natural amino acids

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Abstract—Molecular mechanics calculations, X-ray, FT-IR, and NMR analysis performed on Piv-D-Pro$^{c}_{L}$-L-Pro-NHMe (D-Pro$^{c}_{L}$ = a proline/leucine chimera) show that it possesses a water stable type II′ β-turn structure. The isopropyl side chain of D-Pro$^{c}_{L}$ compares with the leucine side chain of a typical type I β-turn found in a protein (taken from the PDB). Thus *cis*-3-substituted prolines with the appropriate side chain can be used to mimic type I, II or II′ β-turns and incorporate the side chain functionalities on both the $i+1$ and $i+2$ positions of β-turns.

The synthesis of peptides or peptide mimics in which noncovalent forces stabilize the secondary structure is an important goal to generate specific molecules able to inhibit protein/ligand interactions. We have focused our studies on β-turn secondary structures, which are implicated in numerous recognition processes. The β-turn foldings (types I, I′, II, II′, II, etc.) are defined by the arrangement of the four consecutive Cα carbons of the peptide backbone (C^{α}_{i}, C^{α}_{i+1}, C^{α}_{i+2}, C^{α}_{i+3}), which are classified according to the four torsion angles (ϕ, ψ) of the $i+1$ and $i+2$ residues and the distance between C^{α}_{i} and C^{α}_{i+3} ($d < 7$ Å). In the past 20 years, a large number of compounds have been developed as β-turn mimics.[1] Two general approaches have been used to stabilize these β-turn structures. First, the Φ, Ψ torsion angles have been restricted by introducing a proline residue in the $i+1$ position, two successive heterochiral prolines or a proline and an *N*-methylated amino acid, a bicyclic fused ring or a spiro bicyclic ring. The second approach is based on the restriction of the distance $d_{C_i-C_{i+3}}$ to around 7 Å by bridging the N- and C-terminal residues of the β-turns, via peptide cyclization or incorporation of bifunctional templates. However, to our knowledge, there is no general method for keeping all the side chain functions of the natural β-turns, which limits the ability of these motifs to mimic natural turns.

The simplest strategy to induce a β-turn remains the incorporation of the heterochiral sequence D-Pro-NMeAA or Pro-D-NMeAA, which allows the retention of all side chain functionalities of the β-turn except for the $i+1$ position.[2] 3-Substituted prolinoamino acids are chimeras between proline and a proteinogenic amino acid. Most *cis*- and *trans*-3-substituted prolinoamino acids bearing a natural amino acid side chain can be prepared (Pro$^{c}_{X}$ and Pro$^{t}_{X}$, respectively, X = code letter for the amino acid).[3] Our goal was to explore the use of such templates to recover the functionality in the $i+1$ position of a β-turn. In this study, we have chosen prolinoleucine for its bulky side chain. All information gained with this analog should enlarge the application of 3-substituted prolinoamino acids to other side chains.

Molecular mechanics calculations of Ac-Pro$^{c}_{L}$-NHMe and Ac-Pro$^{t}_{L}$-NHMe[4] have shown that the puckering (up or Cγ-*exo* and down or Cγ-*endo*) of the pyrrolidine (and consequently the orientation of the side chain) depends on the stereochemistry (*cis* and *trans*) of the C-3 substituent. In both *cis* and *trans* isomers, the χ_1 *trans* orientations are the most stable conformers. The energy

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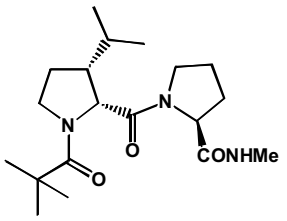

Figure 1. Schematic representation of Piv-D-Pro$^{c}_{L}$-L-Pro-NHMe.

differences between the three (χ_2) rotamers are higher in the *cis* isomers than in the *trans* isomers, indicating stronger side-chain/backbone interaction in the *cis* isomer. With unsubstituted proline, there is a strong interdependence between ring puckering and backbone dihedral angles: the average values of the Ψ dihedral angles are smaller for up-puckering than for down-puckering.[5] The same tendency was observed for Pro$^{t}_{L}$. In contrast, the average value of the Ψ dihedral angles of Pro$^{c}_{L}$ is strongly reduced (below 130°) for the down-puckering, while the up-puckering allows higher values of Ψ (around 150°). These results highlight the importance of the side chain in the *cis* isomer for the orientation of the backbone. While the side chain introduced on C-3 of proline should not alter the induction of a β-turn for Pro$^{t}_{L}$, the conformational effects for the *cis* diastereoisomer are more difficult to predict, due to the influence of the side chain on the backbone conformation. Thus, we have studied the conformational preferences of the heterochiral dipeptide model Piv-D-Pro$^{c}_{L}$-Pro-NHMe (Fig. 1) in reference to the unsubstituted model whose crystallographic structure has been previously solved.[6]

Solid state: The X-ray structure of Piv-D-Pro$^{c}_{L}$-L-Pro-NHMe is reported in Figure 2A.† This peptide is folded with an $(i+3) \rightarrow i$ hydrogen bond typical of a β-turn structure (hydrogen bond distance: 2.14 Å). The set of (ϕ,ψ) torsion angles $[\phi_{i+1},\psi_{i+1} = 67.8(4)°, -124.0(4)°;$ $\phi_{i+2},\psi_{i+2} = -73.7(4)°, -12.8(4)°]$ corresponds to a type II′ β-turn differing slightly from values for Piv-L-Pro-D-Pro-NHMe structured in a type II β-turn $[\phi_{i+1},\psi_{i+1} = -57.7°, -134.4°;\ \phi_{i+2},\psi_{i+2} = 83.2°, -6.5°]$. The up–down puckering of unsubstituted Pro–Pro is shifted towards down–down by the introduction of the isopropyl group. The orientation of the isopropyl side chain is *trans* about the χ_1 and χ_2 torsion angles. The FT-IR spectrum of these crystals in KBr presents four absorption bands at 3312, 1672, 1651, and 1591 cm^{-1}. The bands at 3312 and 1591 cm^{-1} were assigned to the bonded NH(Me) and CO (Piv), respectively.[7] Bands at 1672 and 1651 cm^{-1} were assigned to the carbonyl stretching of CONHMe and Pro$^{c}_{L}$, respectively.

† Crystallographic data (excluding structure factors) for the structure in this paper, have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 222073. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

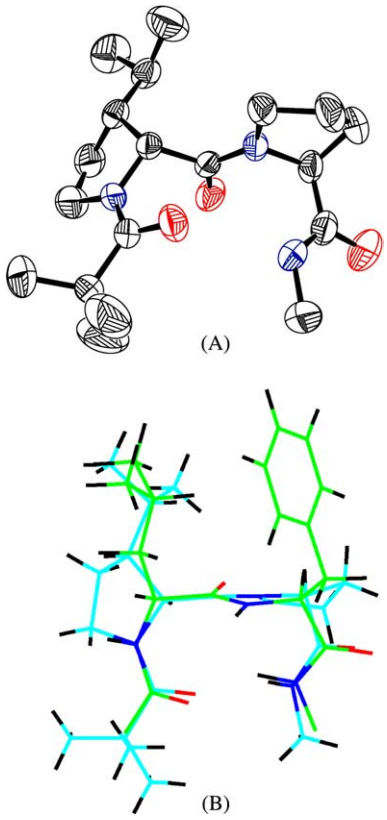

Figure 2. (A) X-ray structure of Piv-D-Pro$^{c}_{L}$-L-Pro-NHMe. (B) Superimposition of the X-ray structures of Piv-D-Pro$^{c}_{L}$-L-Pro-NHMe (blue) and type I β-turn Leu-Tyr from protein 3gpb (green). The RMSD for backbone and Leu side chain atoms is 0.7 Å.

Solution state: The FT-IR spectrum recorded in CH_2Cl_2 presents strong absorption bands near 3338 and 1596 cm^{-1} characteristic of bonded NH(Me) and CO (Piv).[7] The two other absorptions at 1663 and 1649 cm^{-1} correspond to the carbonyl stretching of CONH(Me) and Pro$^{c}_{L}$, respectively. The absence of absorption bands around 3450 cm^{-1} (free NH) and 1620 cm^{-1} (free CO, Piv) indicates a single structure corresponding to a β-turn and the absence of a γ-turn (C_7 conformation). The shifts of IR absorptions compared with the solid state are due to carbonyl solvation by CH_2Cl_2. The CD spectrum (Fig. 3) taken in methanol presents a strong

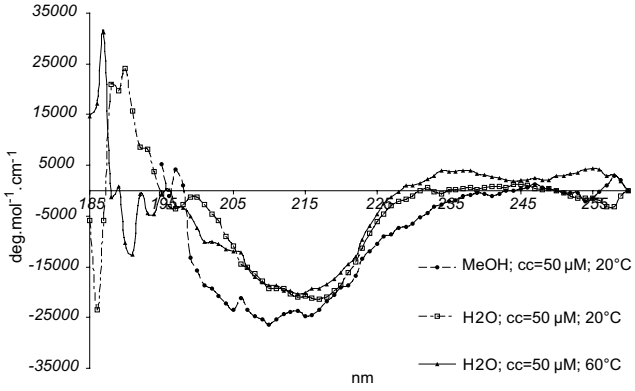

Figure 3. CD spectra of Piv-D-Pro$^{c}_{L}$-L-Pro-NHMe in methanol and in water.

absorption at 212 nm with a molar ellipticity of 22,000 deg mol^{-1} cm^{-1}. The addition of water up to 100% does not significantly affect the frequency of absorption or the molar ellipticity indicating that the type II′ β-turn remains present even in aqueous solution. Values for the molar ellipticities observed in water and methanol are similar to those reported for other type II β-turn models Ac-Pro-D-Xaa-NHCH$_3$ (Xaa = Phe, Val, Leu, Abu, and Ala).[8]

The heterochiral peptide Piv-D-Pro^c$_L$-L-Pro-NHMe has been further analyzed by NMR spectroscopy in methanol and chloroform. A single set of resonances was observed indicating the absence of *cis*/*trans* isomerization of the peptide bond in both solvents, as observed for other heterochiral sequences. In $CHCl_3$, the chemical shifts are similar to those obtained for nonsubstituted L-Pro-D-Pro analogs.[7] The amide proton (δ_{NH} =6.92 ppm) is shifted downfield compared to the free amide proton of Ac-Pro(3,3-dimethyl)-CONHMe, (δ_{NH} = 6.17 ppm),[9] indicating the presence of a hydrogen bond. This hydrogen bond remains present in methanol as shown by the weak temperature coefficient $\Delta\delta_{NH}/\Delta T$ of the amide proton (−3.3 ppb/°C). In methanol, the conformation of the pyrrolidine ring of Piv-D-Pro^c$_L$-L-Pro-NHMe was analyzed using $^3J_{H\alpha-H\beta}$ coupling constants and intraresidual NOEs. NMR parameters match with a down puckering of both prolines. The 9.6 Hz value for the $^3J_{H\beta-H\gamma}$ coupling constant is indicative of a χ_2 *trans* orientation for the isopropyl group. The structure of Piv-D-Pro^c$_L$-L-Pro-NHMe obtained after restrained molecular dynamics and minimization fits perfectly with that observed in a crystal corresponding to a type II′ β-turn with a successive down/down puckering.

Introduction of a methyl group in the β-position of $i+1$ Pro has been previously analyzed on two models: Tos-L-*trans*-Pro(3Me)-D-Pro-NH$_2$ and Tos-L-*cis*-Pro(3Me)-D-Pro-NH$_2$.[10] On the basis of FT-IR data, it was suggested that the heterochiral sequence adopts a type II β-turn in CH_2Cl_2 by forming a hydrogen bond between the amide proton of NH$_2$ and one of the two oxygens of the sulfonamide group. Our results agree with this suggestion on a more relevant dipeptide model and even for a bulkier substituent, which could have destabilized the β-turn. This β-turn is the unique structure in solution whatever the dielectric constant from chloroform to water. Compensation of steric interactions due to the isopropyl group occurs via modification of the pyrrolidine puckering without disturbing the β-turn folding. Thus, it is possible to introduce a side chain functionality in position $i+1$ of the type II′ β-turn using a heterochiral sequence of β-substituted proline. Since the $i+2$ side chain can be recovered by introducing *N*-methylated amino acid[2] or prolino amino acid instead of proline, both the $i+1$ and $i+2$ positions of the type II′ β-turn can be functionalized. In proteins, the type I′ and II′ β-turns are rare, comprising only 4% and 3% of collected structures whereas their mirror image type I and II are strongly favored.[11] Type II β-turns functionalized in the $i+1$ position can be obtained by inverting the stereochemistry of our model peptide, that is with Piv-L-Pro^c$_L$-D-Pro-NHMe.

We have analyzed by molecular modeling the fitting of the type II′ β-turn obtained with Piv-D-Pro^c$_L$-L-Pro-NHMe with the different types of canonic β-turns. We found that a side chain in position $i+1$ of proline having a *cis* orientation in this type II′ β-turn overlaps with the side chain amino acid of a typical type I β-turn provided that its χ_1 is *trans*. Superimposition of our model peptide with a type I β-turn containing a leucine (χ_1 *trans*) in the $i+1$ position is shown in Figure 2B (protein taken from the PDB:3gpb).[12] In the superimposition, the isopropyl substituent of D-Pro^c$_L$ occupies the same position as the leucine side chain. Modeled β-turn structures of Piv-D-Pro^t$_L$-L-Pro-NHMe also fit with the type I β-turn, albeit with a larger root mean square deviation.

Thus 3-substituted prolines and particularly the *cis* isomers with the appropriate side chain represent valuable tools to mimic natural β-turn types I, II, and II′ found in proteins.

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