ORGANIC LETTERS

2012
Vol. 14, No. 3
732–735

The Serine-Proline Turn: A Novel Hydrogen-Bonded Template for Designing Peptidomimetics

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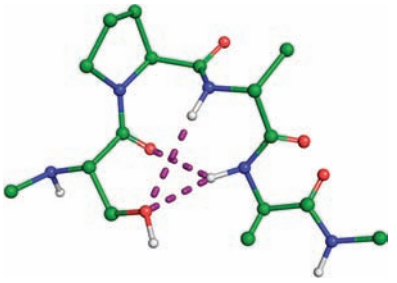
Received December 7, 2011

ABSTRACT



Serine-Proline (SP) dipeptide motifs have been shown to form unique hydrogen-bonding patterns in protein crystal structures. Peptides were designed to mimic these patterns by forming the 6 + 10 and the 9 + 10 hydrogen-bonded rings. Factors that contribute to the formation of SP turns include controlling backbone flexibility and amino acid chirality along with creating a hydrophobic environment around the intramolecular hydrogen bonds.

Serine-Proline (SP) is an important dipeptide motif in proteins. The SP dipeptide sequence is frequently found in gene regulatory and DNA-binding proteins.[1] This motif is also recognized as a substrate by several kinases and forms a preferred site for protein phosphorylation.[2]

In proteins, the SP dipeptide is most likely to induce a type I β-turn. A comprehensive analysis of position potentials of type I β-turns revealed that serine is one of the most frequently occurring residues immediately preceding the proline in a nonhomologous protein data set.[3] However, an analysis of SP motifs in protein structures indicated a unique pattern of hydrogen bonding.[4]

The SP motifs feature a backbone-to-backbone (BB-BB) hydrogen bond between the carbonyl oxygen of Ser (i residue) and the amide proton of the i+3 residue, which is typically seen in β-turns. However, in addition to this classical hydrogen bond, Ser can participate in a side chain-to-backbone (SC-BB) hydrogen bond to form two distinct patterns, hereafter referred to as SP turns (Figure 1). In the 6 + 10 pattern (Figure 1A), the Ser hydroxyl group forms a hydrogen bond with the backbone amide proton of the i+3 residue to create a 6-membered hydrogen-bonded ring adjacent to the classical 10-membered ring. In the 9 + 10 pattern (Figure 1B), the Ser hydroxyl group forms a hydrogen bond with the backbone amide proton of the i+2 residue to create a 9-membered hydrogen-bonded ring that intersects the classical 10-membered ring.

The distinct patterns produced by the SP motifs provide novel structural templates for designing functional peptides and peptidomimetics. While the 6 + 10 and the 9 + 10 patterns have been observed in protein crystal structures,[4] such SP turns have not been engineered in peptides. Accordingly, the objective of this study was to explore the feasibility of creating the unique patterns of SP turns in synthetic peptides. To achieve this goal, we designed and synthesized a series of hexapeptides (Table 1). Conformational features of these peptides were evaluated using two-dimensional nuclear magnetic resonance (2D NMR)

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10.1021/ol203272k
Published on Web 01/18/2012

spectroscopy experiments conducted in deuterated dimethylsulfoxide (DMSO-d_6). Specifically, rotating frame Overhauser effect spectrosopy (ROESY) was used to characterize peptide secondary structures.

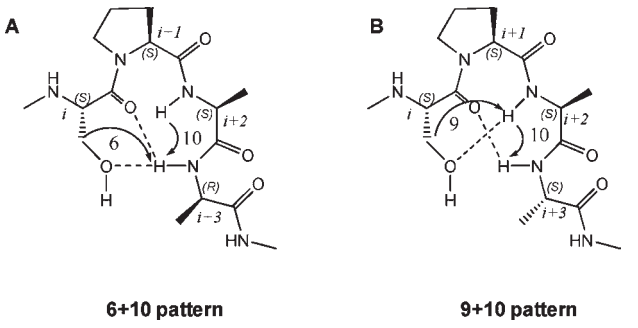

Figure 1. The Ser-Pro dipeptide motif forms a unique turn structure, referred to here as the SP turn. In the SP turn, two distinct patterns of hydrogen bonding are possible. The left panel shows an SP turn with the 6 + 10 hydrogen-bonding pattern and the corresponding diagnostic NOEs (NOE-6 and NOE-10). The right panel shows an SP turn with a 9 + 10 hydrogen-bonding pattern and the corresponding diagnostic NOEs (NOE-9 and NOE-10).

To determine the hydrogen-bonding patterns featured in Figure 1, we monitored the following three diagnostic NOEs: (1) A cross-peak between the i+2 and the i+3 backbone amide protons (NOE-10) was used as evidence for a turn structure in solution.[5] (2) A cross-peak between the backbone amide proton of the i+3 residue and the Ser side chain β-protons (NOE-6) provided evidence for the SP turn with an SC-BB hydrogen bond shown for the 6 + 10 pattern (Figure 1A). (3) A cross-peak between the backbone amide proton of the i+2 residue and the Ser side chain β-protons (NOE-9) was used as evidence for the SP turn with an SC-BB hydrogen bond, as shown for the 9 + 10 pattern (Figure 1B).

Initially, peptide 1 was designed featuring the Ser-Pro-Xaa-Xaa sequence. Ala was chosen as a substituent for the Xaa positions in this motif because the Ala side chain is less likely to interfere sterically or electronically with the intramolecular hydrogen-bonding patterns. This tetrapeptide motif was flanked with two Leu residues on either side to provide a hydrophobic environment. We hypothesized that this type of hydrophobic closure can protect the turn from competitive hydrogen bonding with the solvent molecules, thereby strengthening intramolecular hydrogen bonds and facilitating nucleation of the SP turn structures.[6] In the ROESY experiment, peptide 1 showed a medium NOE between the β-protons of Ser and the i+3 backbone amide proton and between the β-protons of Ser and the i+2 backbone amide proton (Figure 2, Table 1). These diagnostic NOEs indicate that peptide 1 has a mixed population of conformers that show both the 6 + 10 pattern and the 9 + 10 pattern.

Table 1. A List of Synthetic Peptides and Their Corresponding Diagnostic NOEs[a]

	Sequence	NOE-10	NOE-6	NOE-9
1	Ac-Leu-Ser-Pro-Ala-Ala-Leu-NH_2	W	M	M
2	Ac-Leu-Ser-Pro-Ala-D-Ala-Leu-NH_2	M	M	–
3	Ac-Leu-Ala-Pro-Ala-D-Ala-Leu-NH_2	–	–	–
4	Ac-Leu-Ser-Ala-Ala-D-Ala-Leu-NH_2	–	–	–
5	Ac-Ala-Ser-Pro-Ala-D-Ala-Ala-NH_2	–	W	–
6	Ac-Leu-Ser-Pro-Gly-Ala-Leu-NH_2	W	–	–
7	Ac-Leu-Ser-Pro-Ala-Gly-Leu-NH_2	–	–	W
8	Ac-Leu-Thr-Pro-Ala-D-Ala-Leu-NH_2	–	–	W

[a] NOE-10: A cross-peak corresponding to the 10-membered hydrogen bond. NOE-6: A cross-peak corresponding to the 6-membered hydrogen bond in the 6 + 10 pattern of the SP turn. NOE-9: A cross-peak corresponding to the 9-membered hydrogen bond in the 9 + 10 pattern of the SP turn. M is medium, and W is weak. The intensities (medium and weak) are relative to the strong sequential NOEs that were used to establish connectivity.

Next, our goal was to constrain the peptide chain such that only one of the two hydrogen-bonding patterns was predominantly observed. Accordingly, we designed peptide 2 where the configuration of the i+3 residue was changed from L to D. Based on previous experience, we hypothesized that this targeted change in configuration will alter the hydrogen-bonding pattern.[7] As anticipated, peptide 2 did not show NOE-9 but instead showed a medium NOE-6 (Figure 2). These diagnostic NOEs indicated that peptide 2 predominantly adopts the 6 + 10 pattern of the SP turn. Additional support for the 6 + 10 pattern of peptide 2 was provided by another medium diagnostic NOE between the side chain β-protons of Ser and the side chain β-protons of D-Ala in the i+3 position (SI Figure 4). This additional diagnostic NOE was also observed for peptide 1 (SI Figure 2). Because we were able to precisely control the population of solution conformers for peptide 2, we made a series of substitutions in this peptide to determine the contribution of each of the residues toward the 6 + 10 pattern of the SP turn. Initially, we substituted Ser and Pro with Ala in peptides 3 and 4, respectively. As anticipated, these peptides were not able to sustain the 6 + 10 pattern of hydrogen bonding. Moreover, peptides 3 and 4 also did not show any evidence of a turn structure. These results suggest that both Ser and Pro are required to induce the SP turn structures and the presence of either one of these residues in these peptides is not sufficient in inducing SP turns.

To assess the impact of the hydrophobic ends on the conformational behavior of SP turns, we changed the flanking residues from Leu to Ala. These substitutions were expected to decrease the hydrophobicity of the flanking residues, thereby significantly minimizing or eliminating the hydrophobic enclosure of the intramolecular

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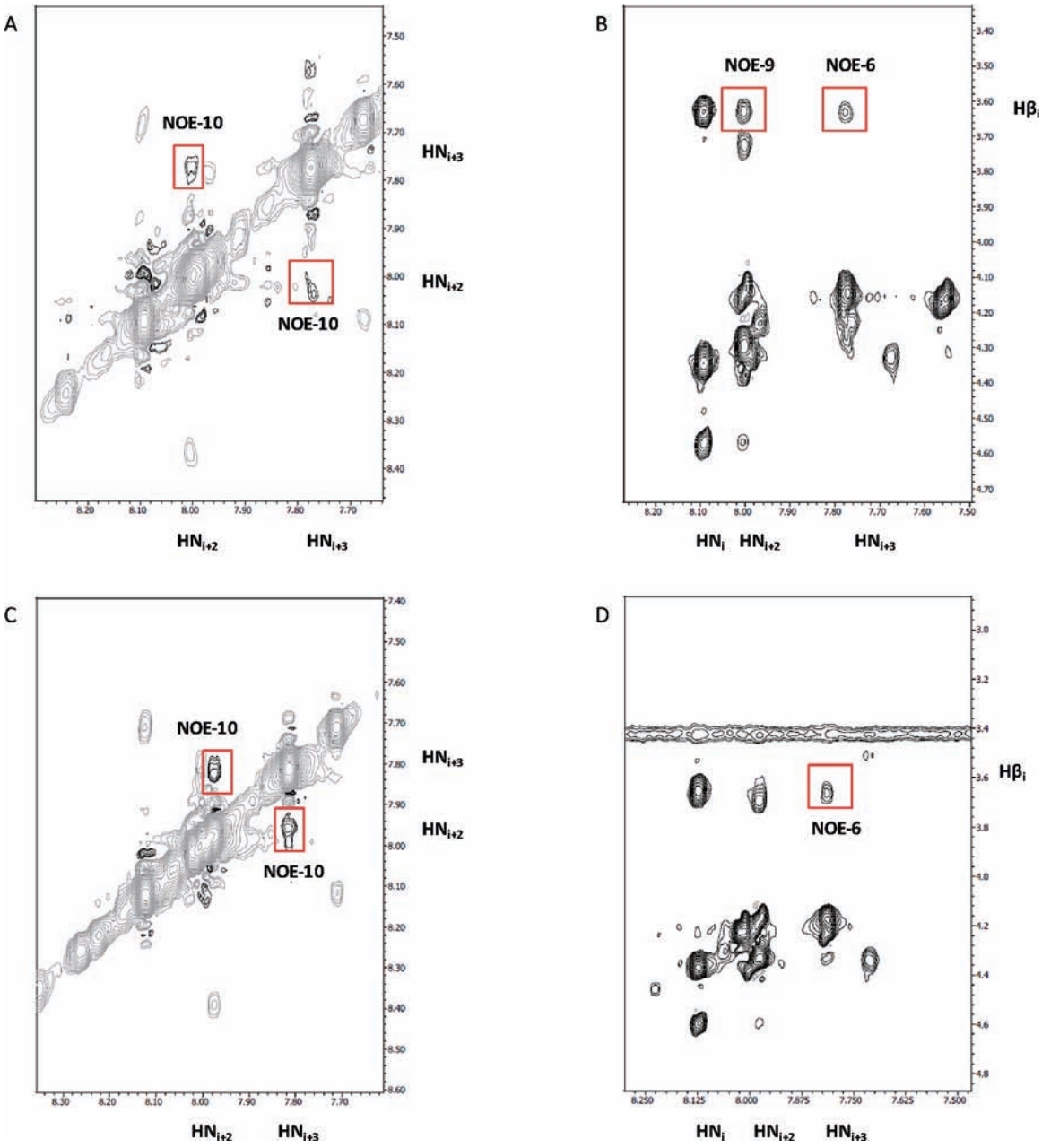

Figure 2. Portions of the ROESY spectra: (A) peptide 1 with NOEs highlighted in red indicating a turn structure with a 10-membered BB-BB hydrogen bond; (B) peptide 1 with NOEs highlighted in red indicating 6-membered and 9-membered hydrogen-bond patterns; (C) peptide 2 with NOEs highlighted in red indicating a 10-membered hydrogen-bond pattern; (D) peptide 2 with NOEs highlighted in red indicating a 6-membered hydrogen-bond pattern.

hydrogen-bonding network. Accordingly, peptide 5 only showed a weak NOE-6, but the diagnostic cross-peak for a turn structure (NOE-10) was missing. Therefore, the flanking Leu residues likely facilitate intramolecular hydrogen bonding by lowering the extent of solvation of the polar side chain of Ser and the backbone.

Next, we analyzed the importance of the Ala residue in positions i+3 and i+4 by substituting them with an achiral Gly in peptides 6 and 7, respectively. None of these peptides showed NOE cross-peaks that were observed for the SP turns in peptides 1 and 2. Only peptide 6 showed any evidence of a turn structure in solution as indicated by the presence of a weak NOE-10. Peptide 6 contains a Pro-Gly dipeptide motif, which is known to induce a β-turn structure,[8] but peptide 7 lacks this motif and consequently did not show any evidence of a turn structure. However, peptide 7 did show a weak NOE-9, which corresponds to the formation of an ST turn. These results indicate that an ST turn can be observed in peptides that predominantly

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adopt an extended backbone conformation.[9] The fact that peptide 6 or 7 did not show SP turn features can be attributed to the two effects that Gly can have within peptide chains. First, Gly is inherently a more flexible amino acid than Ala because it lacks a C_β atom. Second, the Gly residue provides optimal solvation of the peptide backbone.[10] Both of these effects—increased backbone flexibility and increased competition from solvent molecules for hydrogen bonding—individually or in combination could be responsible for the elimination of intramolecular hydrogen-bonding patterns in peptides 6 and 7.

Lastly, we substituted Ser in peptide 2 with Thr to assess the effect of a β-branched side chain on the hydrogen-bonding patterns. Interestingly, peptide 8 did not show any characteristic NOEs for the SP turns. This is likely to be a result of the steric hindrance between the side chain methyl groups of Thr and atoms inside the turn structure. Moreover, none of the peptides in Table 1 showed the classic diagnostic NOE— $d_{\alpha\alpha}(i, i+1)$—for a cis amide bond, which suggests that all the amide bonds in these peptides predominantly adopted the trans configuration. A list of all the observed NOEs for the peptides in Table 1 is provided in the Supporting Information.

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In summary, we were able to design synthetic peptides that featured SP turns. All of the data collectively suggest three requirements for engineering SP turns in synthetic peptides: (1) the presence of the SP dipeptide motif, (2) hydrophobic amino acids such as Leu that flank the SPXX tetrapeptide, and (3) a non-Gly residue at the i+2 and i+3 positions. With a targeted L to D substitution at the i+3 position, peptide 2 was designed to predominantly show the 6 + 10 hydrogen-bonding SP turn pattern. We were able to determine the contribution of each of the residues and to define strategies that lead to the formation of SP turn structures in peptide chains. The synthetic peptides featured here may be valuable as templates for designing structured peptides and peptidomimetics with functional roles as catalysts and therapeutics. Such peptides could also be used as tools to understand the contribution of the 6 + 10 and 9 + 10 hydrogen-bonding patterns in protein phosphorylation.

Acknowledgment. CADRE was established with support from the Commonwealth of Virginia. The authors thank Dr. Richard Foust, and David Brakke at James Madison University for access to the NMR. Dr. Walter Moos at SRI International is gratefully acknowledged.

Supporting Information Available. Experimental details and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.