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Nucleation of β-hairpin structure in a pyrrole amino acid containing peptide

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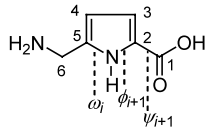
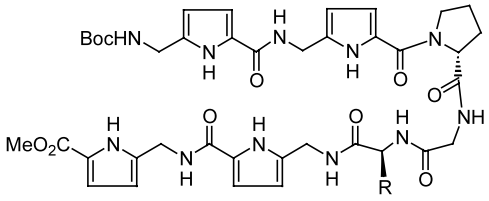
Abstract—Synthesis and conformational studies of two short peptides containing pyrrole amino acids (**1**, Paa), Boc-Paa-Paa-D-Pro-Gly-Xaa-Paa-Paa-OMe (**2**: Xaa = Ala; **3**: Xaa = Val), were carried out in which it was established that replacement of Ala in **2** with a Val residue helps peptide **3** to adopt a well-defined β-hairpin conformation in a nonpolar solvent, like $CDCl_3$. © 2002 Elsevier Science Ltd. All rights reserved.

Several β-hairpin models have been developed in recent years[1] that mimic this widely occurring secondary structure in peptides and proteins.[2] This has led to the design and synthesis of a large number of conformationally constrained scaffolds, which help to induce β-turns in short peptides, often a prerequisite to β-hairpin nucleation.[3] Recently, we have developed a new peptidomimetic scaffold based on a pyrrole amino acid (Paa), 5-(aminomethyl)pyrrole-2-carboxylic acid **1** and used it as a structurally restricted surrogate of the Gly-ΔAla dipeptide isostere in the synthesis of peptides.[4] In this paper, we describe the design, syntheses and detailed conformational studies of two Paa-containing peptides, Boc-Paa-Paa-D-Pro-Gly-Xaa-Paa-Paa-OMe (**2**: Xaa = Ala; **3**: Xaa = Val) having a centrally located type II′ β-turn nucleating D-Pro-Gly motif[5] and repeating units of Paa dimers at both N- and C-termini. It was envisaged that the D-Pro unit with a φ value of +60±20° can induce a reverse turn that can be further stabilized by noncovalent interactions facilitated by the near planar disposition of the Paa-dimers at both ends with fixed ω_i, φ_{i+1} and probably, ψ_{i+1} torsional angles[4] leading to the formation of hairpin architecture.



1 (Paa)



2 [R = Me], **3** [R = $(CH_3)_2CH$]

Synthesis of Paa **1** has already been described by us.[4] The peptides **2** and **3** were synthesized by conventional solution phase methods using 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) as coupling agents and dry CH_2Cl_2 and/or amine-free dry DMF as solvents. Reaction of Boc-Paa-OH with H_2N-Paa-OMe under the conditions mentioned above gave the dimer, Boc-Paa-Paa-OMe. Saponification of the dimer with LiOH in THF–MeOH–H_2O was followed by reaction with the dipeptide D-Pro-Gly-OMe to give Boc-Paa-Paa-D-Pro-Gly-OMe. Next, Boc-deprotection of the protected dimer using TFA–CH_2Cl_2 (1:1) and subsequent reaction of the resulting amine with Boc-Xaa-OH furnished the tripeptide segment Boc-Xaa-Paa-Paa-OMe. Finally, the tetrapeptide Boc-Paa-Paa-D-Pro-Gly-OMe and the tripeptide Boc-Xaa-Paa-Paa-OMe were linked together following steps analogous to those described above to furnish the final products, **2** and **3**. The products were purified by silica gel column chromatography[6,7] and used for conformational studies.

Detailed conformational analysis of peptides **2** and **3** were performed using various NMR techniques. The

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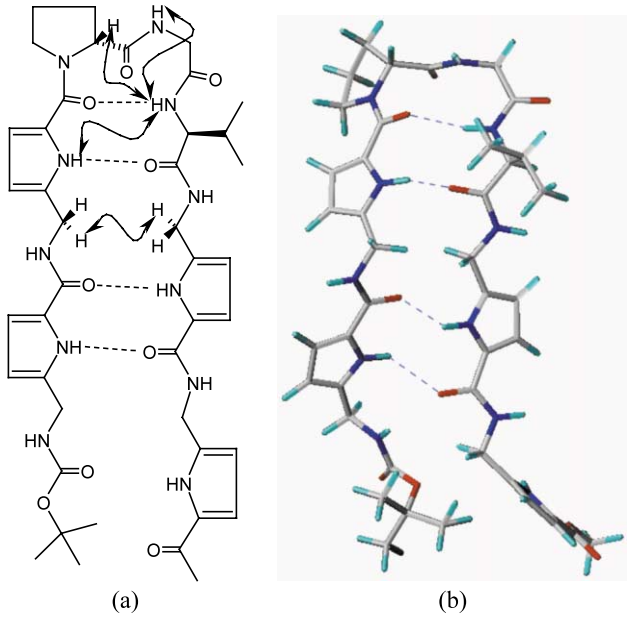
assignments were carried out with the help of two-dimensional total correlation spectroscopy (TOCSY)[8] and were further confirmed by rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments,[8] which, in addition, provided information on the proximity of the protons. All the spectra were recorded in $CDCl_3$ containing 7.7% (v/v) of DMSO-d_6, because of exchange broadening or poor solubility in pure $CDCl_3$ solution.

In peptide **2**, the absence of any long-range ROE cross peak as well as intramolecular hydrogen bondings indicated the possible nonexistence of any well-defined structure in solution. The presence of cross peaks between PaaNH to the preceding PaaC3H and PaaNH to the self PaaC4H and the absence of PaaNH to the self pyrroleNH cross peak strongly supports the view that the Paa dimer moiety is behaving as an ideal β-sheet scaffold.

In a noncompetitive solvent such as $CDCl_3$, intramolecularly hydrogen bonded amide protons resonate downfield.[9] The ^{1}H NMR spectrum of **3** in $CDCl_3$ containing 7.7% (v/v) of DMSO-d_6 showed downfield chemical shifts for all its amide protons, indicating their participation in such intramolecular hydrogen bonds. Especially, three pyrrole NH signals, those of Paa(1), Paa(2) and Paa(6), were approximately 0.4 ppm downfield compared to their chemical shifts in **2**, strongly suggesting that they are in hydrogen bonded states in **3**. This was further proved by solvent titration studies in which the addition of increasing amounts of DMSO-d_6, a strong hydrogen bonding solvent, to a $CDCl_3$ solution of **3** caused very little change in the amide proton chemical shifts of its four constituent amino acids ($\Delta\delta = 0.17$ ppm for Paa(1)pyrroleNH, 0.19 ppm for Paa(2)pyrroleNH, –0.10 ppm for Val(5)NH and 0.18 ppm for Paa(6)pyrroleNH, on addition of 20% v/v of DMSO-d_6), indicating that they are intramolecularly hydrogen bonded.



(a) (b)

Figure 1. (a) Schematic representation of the proposed structure of **3** with the long-range ROEs seen in the ROESY spectrum. (b) One of the 50 energy-minimized structures of **3** sampled during the 300 ps simulated annealing MD studies.

Some of the important ROE cross-peaks seen in the ROESY spectrum of **3** are shown schematically in Figure 1(a). The appearance of ROE cross peaks between Val(5)NH–Gly(4)NH, Val(5)NH–Pro(3)CαH and almost equal intensities of Gly(4)NH–Pro(3)CαH and Gly(4)NH–Gly(4)CαH, as well as the participation of Val(5)NH in intramolecular hydrogen bonding as shown by solvent titration studies, indicate that a well known type-II′ β-turn is nucleating around the D-Pro-Gly residues. This leads to the subsequent stabilization of β-hairpin formation in the molecule that is very clearly evidenced by the interstrand ROE cross peaks, shown in Figure 1(a), between Val(5)NH–Paa(2)-pyrroleNH, Paa(2)C6H–Paa(6)C6H, as well as the interstrand hydrogen bonds of Val(5)NH, Paa(1)-pyrroleNH, Paa(2)pyrroleNH and Paa(6)pyrroleNH, as indicated by solvent titration studies.

The cross-peak intensities in the ROESY spectrum of **3** were used to obtain the restraints in constrained molecular dynamics (MD) simulation studies.[10] Several long-range (more than FOUR bonds) distance constraints from the ROEs shown in Figure 1(a) were used in the energy calculations and MD studies. A 300 ps simulated annealing was run comprising 50 cycles, each with a heating step to 700 K for 1 ps followed by cooling to 300 K in 5 ps. Structures were sampled after each cycle and minimized. One of these structures is shown in Figure 1(b). In this structure, the Val(5)NH is strongly hydrogen bonded to Paa(2)C=O intramolecularly (i+3→i) leading to a 10-membered ring structure of type II′ β-turn, supported by the φ, ψ angles of D-Pro and Gly (70, –108 and –106, 23°, respectively). This induces nucleation of three additional hydrogen bonds, Paa(2)pyrroleNH→Val(5)CO, Paa(6)PyrroleNH→Paa(1)CO and Paa(1)PyrroleNH→Paa(6)CO, leading to the hairpin formation. Comparison of the structures of **2** and **3** makes it evident that the replacement of Ala by Val in the 5-position helps compound **3** to adopt the hairpin conformation due to the restricted rotation about χ_1 of Val, 57 and 181° in Figure 1(b). Further work is under progress.

In conclusion, the known propensity of D-Pro-Gly unit to induce type II′ β-turn coupled with the sheet forming tendency of pyrrole amino acid (Paa) can lead to a very well defined β-hairpin conformation in short peptides.

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6. Selected physical data of **2**: ^{1}H NMR ($CDCl_3$+7.7% v/v DMSO-d_6, 500 MHz): δ 11.08 (bs, 1H, Paa(1)pyrroleN*H*), 11.00 (bs, 1H, Paa(6)pyrroleN*H*), 10.80 (bs, 1H, Paa(7)pyrroleN*H*), 10.68 (bs, 1H, Paa(2)pyrroleN*H*), 8.29 (dd, J=5.7, 7.2 Hz, 1H, Gly(4)N*H*), 8.21 (d, J=8.7 Hz, 1H, Ala(5)N*H*), 7.82 (t, J=6.0 Hz, 1H, Paa(7)N*H*), 7.73 (t, J=5.8 Hz, 1H, Paa(2)N*H*), 7.47 (t, J=5.7 Hz, 1H, Paa(6)N*H*), 6.75 (t, J=3.2 Hz, 1H, Paa(7)C3*H*), 6.68 (t, J=3.2 Hz, 1H, Paa(1)C3*H*), 6.58 (t, J=3.2 Hz, 1H, Paa(6)C3*H*), 6.48 (t, J=3.2 Hz, 1H, Paa(2)C3*H*), 6.07 (t, J=3.2 Hz, 1H, Paa(7)C4*H*), 6.06 (bs, 1H, Paa(1)N*H*), 6.06 (t, J=3.2 Hz, 1H, Paa(2)C4*H*), 6.04 (t, J=3.2 Hz, 1H, Paa(1)C4*H*), 5.98 (t, J=3.2 Hz, 1H, Paa(6)C4*H*), 4.79 (dd, J=6.7, 15.5 Hz, 1H, Paa(2)C6*H*), 4.61 (m, 1H, Ala(5)Cα*H*), 4.49 (dd, J=6.3, 8.4 Hz, 1H, Pro(3)Cα*H*), 4.49 (m, 1H, Paa(7)C6*H*), 4.49 (m, 1H, Paa(7)C6*H*′), 4.40 (dd, J=5.7, 15.2 Hz, 1H, Paa(6)C6*H*), 4.34 (m, 1H, Paa(2)C6*H*′), 4.28 (m, 1H, Paa(1)C6*H*), 4.28 (m, 1H, Paa(1)C6*H*′), 4.23 (dd, J=5.7, 15.2 Hz, 1H, Paa(6)C6*H*′), 4.06 (dt, J=7.2, 16.5, 1H, Gly(4)Cα*H*), 3.95 (m, 1H, Pro(3)Cδ*H*), 3.80 (s, 3H, OCH_3), 3.73 (m, 1H, Pro(3)Cδ*H*′), 3.63 (ddd, J=5.7, 12.8, 16.5 Hz, 1H, Gly(4)Cα*H*′), 2.22–2.02 (m, 4H, Pro(3)Cβ*H*, Cβ*H*′, Cγ*H*, Cγ*H*′), 1.39 (s, 9H, Boc). MS(LSIMS): m/z 846 [M$^+$+H].
7. Selected physical data of **3**: ^{1}H NMR ($CDCl_3$+7.7% v/v DMSO-d_6, 500 MHz): δ 11.52 (bs, 1H, Paa(6)pyrroleN*H*), 11.45 (bs, 1H, Paa(2)pyrroleN*H*), 11.39 (bs, 1H, Paa(1)pyrroleN*H*), 10.86 (bs, 1H, Paa(7)pyrroleN*H*), 8.22 (d, J=9.0 Hz, 1H, Val(5)N*H*), 8.07 (dd, J=4.8, 8.3 Hz, 1H, Gly(4)N*H*), 7.89 (t, J=6.2 Hz, 1H, Paa(7)N*H*), 7.72 (t, J=6.2 Hz, 1H, Paa(2)N*H*), 7.56 (dd, J=4.6, 6.8 Hz, 1H, Paa(6)N*H*), 6.74 (t, J=3.2 Hz, 1H, Paa(7)C3*H*), 6.68 (t, J=3.2 Hz, 1H, Paa(1)C3*H*), 6.67 (t, J=3.2 Hz, 1H, Paa(6)C3*H*), 6.52 (t, J=3.2 Hz, 1H, Paa(2)C3*H*), 6.15 (t, J=3.2 Hz, 1H, Paa(2)C4*H*), 6.10 (t, J=3.2 Hz, 1H, Paa(6)C4*H*), 6.09 (t, J=3.2 Hz, 1H, Paa(7)C4*H*), 6.04 (t, J=3.2 Hz, 1H, Paa(1)C4*H*), 5.96 (t, J=6.6 Hz, 1H, Paa(1)N*H*), 4.95 (dd, J=6.2, 15.6, 1H, Paa(2)C6*H*), 4.76 (dd, J=6.8, 15.2, 1H, Paa(6)C6*H*), 4.51 (m, 1H, Pro(3)Cα*H*), 4.50 (m, 1H, Paa(7)C6*H*), 4.50 (m, 1H, Paa(7)C6*H*′), 4.47 (dd, J=6.2, 15.6 Hz, 1H, Paa(2)C6*H*′), 4.31 (m, 1H, Paa(1)C6*H*), 4.30 (m, 1H, Paa(6)C6*H*′), 4.29 (m, 1H, Gly(4)Cα*H*), 4.24 (m, 1H, Paa(1)C6*H*′), 4.22 (t, J=9.0 Hz, 1H, Val(5)Cα*H*), 4.04 (m, 1H, Pro(3)Cδ*H*), 3.79 (s, 3H, OCH_3), 3.72 (m, 1H, Pro(3)Cδ*H*′), 3.41 (ddd, J=4.8, 7.7, 17.1 Hz, 1H, Gly(4)Cα*H*′), 2.42 (m, 1H, Val(5)Cβ*H*), 2.23–2.04 (m, 4H, Pro(3)Cβ*H*, Cβ*H*′, Cγ*H*, Cγ*H*′), 1.39 (s, 9H, Boc), 0.97 (d, J=6.6 Hz, 3H, Val(5)CH_3), 0.89 (d, J=6.6 Hz, 3H, Val(5)CH'_3). MS(LSIMS): m/z 874 [M$^+$+H], 896 [M$^+$+Na].
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