Pergamon

Tetrahedron Letters, Vol. 38, No. 17, pp. 3039-3042, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00529-7

Conformational Analysis of β-Turn Structure in Tetrapeptides Containing Proline or Proline Analogs

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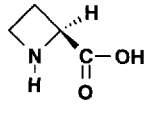
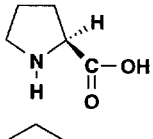
Abstract: In order to evaluate the influence of cyclic secondary amino acids on the stability of β-turn structure, we have prepared Ac-Gly-L-Xxx-L-Leu-Gly-N(CH_3)$_2$ (Xxx = Aze, 4-membered ring: **1**, Xxx = Pro, 5-membered ring: **2**, Xxx = Pip, 6-membered ring: **3**). The NOE cross peaks that support β-turn structure were observed in **1-3**. The NOE cross peak between both terminals of the synthetic peptides, however, was observed only in the NOESY spectra of **2**. This result indicates that 5-membered ring side chain in proline plays a very important role in the formation of β-hairpin structure.

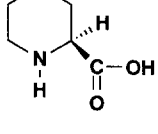
Proline is quite often observed at the (i + 1) position of β-turn structure.[1] In the 20 proteinic amino acids, proline is the only amino acid whose C^{α}-N bond is a part of the pyrrolidine ring. This cyclic side chain imposes strong restraints on peptide conformation. It is known that the role of proline is not only to control the peptide structure but also to expose the recognition site for the protein-protein interaction on the surface of protein.[2,3] Recently, we have prepared the oligopeptides involving nonproteinic amino acid, focusing on the new unique structure of artificial peptide. For example, it is of particular interest to compare the structural effect of nonproteinic cyclic amino acid with that of proteinic proline on the β-turn conformation stabilized by intrinsic hydrogen bonds. In this paper, we wish to present the structural features of three tetrapeptides **1-3** having L-2-azetidinecarboxylic acid (Aze, 4-membered ring) (**4**), L-2-pyrrolydinecarboxylic acid (Pro, 5-membered ring) (**5**), or L-2-piperidinecarboxylic acid (Pip, 6-membered ring) (**6**), respectively, at the (i + 1) position of peptide sequences.



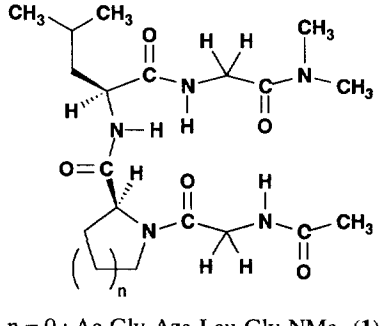
L-2-azetidinecarboxylic acid
Aze, (**4**)



L-2-pyrrolidinecarboxylic acid
Pro, (**5**)



L-2-piperidinecarboxylic acid
Pip, (**6**)

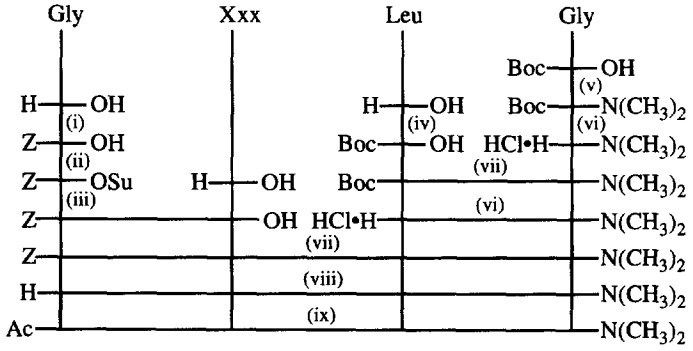


n = 0 : Ac-Gly-Aze-Leu-Gly-NMe_2 (**1**)

n = 1 : Ac-Gly-Pro-Leu-Gly-NMe_2 (**2**)

n = 2 : Ac-Gly-Pip-Leu-Gly-NMe_2 (**3**)

Synthetic routes to the tetrapeptides Ac-Gly-Xxx-Leu-Gly-$N(CH_3)_2$ (Xxx = Aze; **1**, Pro; **2**, Pip; **3**) are shown in Scheme 1. These peptides were designed as follows; (i) Glycine and leucine that have nonpolar side chains were adopted as the components of the tetrapeptide sequences, since these amino acids seem not to interfere with the β-turn conformation induced by cyclic amino acid. (ii) Acetyl and dimethylamino groups were used for *N*- and *C*-terminal protection groups, respectively, to monitor the hydrogen-bonding interaction of amide groups in β-turn structure. All peptides prepared by the conventional solution method in Scheme 1 were characterized by NMR and mass spectroscopic methods.[4]



Scheme 1. Conditions: (i) Z-Cl/2N NaOHaq., (ii) HONSu/DIPCI, (iii) $NaHCO_3$, (iv) Boc_2O/1N NaOHaq, (v) Et_3N/EtOCOCl/-15 °C, then 50% $HN(CH_3)_2$aq., (vi) HCl/dioxane, (vii) WSCI/HOBT, (viii) H_2 gas/10% Pd/C, (ix) Ac_2O, Abbreviations: Z-Cl; carbobenzoxy chloride, HOSu; *N*-hydroxysuccinimide, DIPCI; *N,N'*-diisopropyl-carbodiimide, Boc_2O; di-*tert*-butyl dicarbonate, WSCI; 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, HOBt; 1-hydroxybenzotriazole.

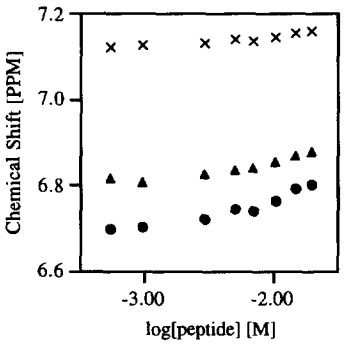


Figure 1. ^1H NMR chemical shifts of the amide protons in peptide **2** at 25 °C in CD_2Cl_2 as a function of the logarithm of peptide concentration. Gly^1 NH (●); Leu^3 NH (▲); Gly^4 NH (×).

To evaluate the aggregation effect of the peptides, ^1H NMR spectra for **2** were recorded over the concentration range of 0.55 to 20 mM in CD_2Cl_2 at 25 °C.[5,6] Figure 1 shows changes in chemical shifts of the amide protons in **2**. Each chemical shift of the three amide protons in **2** was almost independent of concentration at or below 3 mM. Thus the undesired aggregation of the peptide was not observed below 3 mM of **2**. ^1H NMR signals for **1-3** were assigned by DQF-COSY and NOESY spectra in the phase sensitive mode at 2.5 °C in CD_2Cl_2.[7] The ^1H NMR spectra of peptides **1-3** show the presence of a minor component (<15% of the major component) probably due to *cis-trans* isomerization about Gly^1-Xxx^2 peptide bond.[8] The minor component was not analyzed conformationally in this study.

The results of NOESY experiments for **1-3** were summarized in Table 1. The NOESY spectra of peptides **1-3** show NOE cross peaks typical for β-turn conformation (category (i) in Table 1). NOE cross peaks characteristic for a Type II β-turn conformation were also observed (category (ii) in Table 1).[9-11] NOE cross peaks that indicate *trans* conformation with respect to Gly^1-Xxx^2 amide bond were observed for peptide **2** and **3** (category (iii) in Table 1),[12,13] whereas the NOESY spectrum for **1** showed no NOE cross peak indicating *trans* conformation (Gly^1 $C^{\alpha}H$-Aze^2 $C^{\gamma}H$) or the one indicating *cis* conformation (Gly^1 $C^{\alpha}H$-Aze^2 $C^{\alpha}H$). The most significant difference in the NOESY spectra for **1-3** is that NOE cross peak between methyl

Table 1. Selected NOESY Connectivities Observed at 2.5 °C in CD_2Cl_2.[a]

categories		peptide		
		1	**2**	**3**
(i)[b]		Leu^3 N*H* - Gly^4 N*H*	Leu^3 N*H* - Gly^4 N*H*	Leu^3 N*H* - Gly^4 N*H*
		Leu^3 C$^\alpha$*H* - Gly^4 N*H*	Leu^3 C$^\alpha$*H* - Gly^4 N*H*	Leu^3 C$^\alpha$*H* - Gly^4 N*H*
	(ii)[c]	Aze^2 C$^\alpha$*H* - Leu^3 N*H*	Pro^2 C$^\alpha$*H* - Leu^3 N*H*	Pip^2 C$^\alpha$*H* - Leu^3 N*H*
		Leu^3 N*H* - Leu^3 C$^\alpha$*H*	Leu^3 N*H* - Leu^3 C$^\alpha$*H*	Leu^3 N*H* - Leu^3 C$^\alpha$*H*
(iii)[d]		——	Pro^2 C$^\delta$*H* - Gly^1 C$^\alpha$*H*	Pip^2 C$^\varepsilon$*H* - Gly^1 C$^\alpha$*H*
(iv)[e]		——	Acetyl CH_3 - NMe_2 CH_3	——

[a] The NOESY spectra were recorded on a 500 MHz NMR spectrometer. [b] The NOE signals typical for a β-turn conformation. [c] The NOE signals typical for Type II β-turn conformation. [d] The NOE signals typical for Gly^1-Xxx^2 trans amide bond. [e] The NOE signal between *N*- and *C*-terminals.

protons of acetyl group at the *N*-terminal and methyl protons of $N(CH_3)_2$ at the *C*-terminal was observed only in the spectrum for **2** (category (iv) in Table 1 and Figure 2).

It is clear from NOESY experiments that peptides **1**-**3** form β-turn conformation, however, the distances between both terminals of peptides **1** and **3** are longer than that of **2**. This suggests that peptide **2** adopts an unambiguous antiparallel conformation at the *N*- and *C*- terminals, whereas peptides **1** and **3** incompletely adopt β-hairpin structure (strand-loop-strand arrangement).[14] Further work on the analysis of peptides **1**-**3** (VT-NMR, VT-IR, X ray analysis, etc.) is in progress, and the details will appear in future publications.

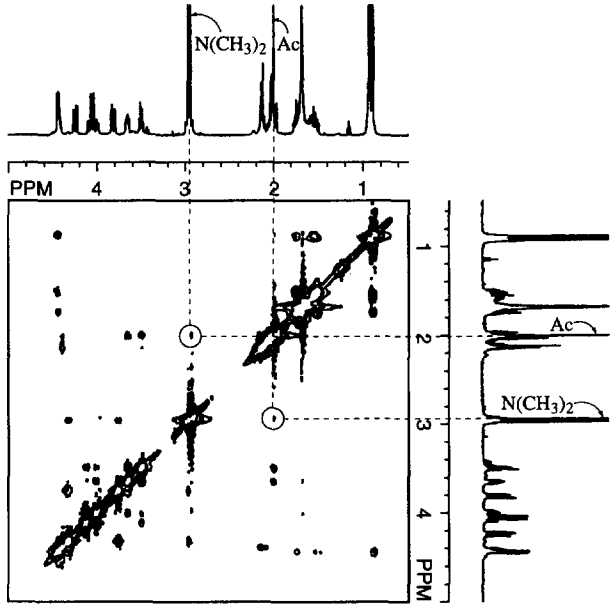


Figure 2. Portion of the 500 MHz NOESY spectrum of **2** in CD_2Cl_2 at 2.5 °C recorded in the phase sensitive mode. The NOE connectivities between acetyl-CH_3 and dimethylamino-CH_3 (Table 1 in category (iv)) are highlighted in curcles.

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4. Spectral data for peptide **1**: ^{1}H NMR (CD_2Cl_2, 25 °C, 500 MHz) δ 0.911 (d, J = 6.4 Hz, 3H), 0.932 (d, J = 6.4 Hz, 3H), 1.5 - 1.7 (m, 2H), 1.695 (ddd, J = 13.1, 7.9 and 5.2 Hz, 1H), 1.998 (s, 3H), 2.5 - 2.7 (m, 2H), 2.941 (s, 3H), 2.950 (s, 3H), 3.8 - 3.9 (m, 2H), 3.932 (ABX, J = 17.2 and 4.2 Hz, 1H), 4.045 (ABX, J = 17.2 and 4.7 Hz, 1H), 4.1 - 4.2 (m, 2H), 4.405 (ddd, J = 9.2, 8.2 and 5.3 Hz, 1H), 4.864 (dd, J = 9.5 and 6.7 Hz, 1H), 6.323 (bs, 1H), 7.014 (bs, 1H), 7.647 (d, J = 7.9 Hz, 1H); HRMS (FAB, NBA) *m/z* 397.2329 [(M)$^+$; calcd. for $C_{18}H_{31}O_5N_5$ 397.2318]. Spectral data for peptide **2**: ^{1}H NMR (CD_2Cl_2, 25 °C, 500 MHz) δ 0.887 (d, J = 6.4 Hz, 3H), 0.923 (d, J = 6.4 Hz, 3H), 1.5 - 1.7 (m, 2H), 1.739 (ddd, J = 13.4, 8.9 and 4.6 Hz, 1H), 1.9 - 2.1 (m, 2H), 2.004 (s, 3H), 2.1 - 2.2 (m, 2H), 2.942 (s, 3H), 2.883 (s, 3H), 3.499 (ddd, J = 10.0, 7.5, 7.4 Hz, 1H), 3.658 (ddd, J = 10.0, 7.2, 4.9 Hz, 1H), 3.623 (ABX, J = 17.1 and 3.7 Hz, 1H), 4.023 (ABX, J = 17.3 and 3.8 Hz, 1H), 4.077 (ABX, J = 17.3 and 5.5 Hz, 1H), 4.431 (ABX, J = 17.1 and 5.8 Hz, 1H), 4.444 (dd, J = 7.6 and 4.6 Hz, 1H), 4.4 - 4.5 (m, 1H), 6.725 (d, J = 8.6 Hz, 1H), 6.852 (bs, 1H), 7.132 (bs, 1H); HRMS (FAB, NBA) *m/z* 412.2553 [(M + H)$^+$; calcd. for $C_{19}H_{34}O_5N_5$ 412.2570]. Spectral data for peptide **3**: ^{1}H NMR (CD_2Cl_2, 25 °C, 500 MHz) δ 0.906 (d, J = 6.4 Hz, 3H), 0.926 (d, J = 6.1 Hz, 3H), 1.4 - 1.7 (m, 8H), 2.002 (s, 3H), 2.1 - 2.3 (m, 1H), 2.948 (s, 6H), 3.144 (ddd, J = 13.0, 13.0 and 2.8 Hz, 1H), 3.6 - 3.7 (m, 1H), 3.949 (ABX, J = 17.3 and 4.0 Hz, 1H), 4.020 (ABX, J = 17.3 and 4.3 Hz, 1H), 4.095 (d, J = 4.3 Hz, 2H), 4.4 - 4.5 (m, 1H), 5.1 - 5.2 (m, 1H), 6.350 (d, J = 8.2 Hz, 1H), 6.5 - 6.7 (m, 1H), 6.8 - 7.0 (m, 1H); HRMS (FAB, NBA) *m/z* 425.2646 [(M)$^+$; calcd. for $C_{20}H_{35}O_5N_5$ 425.2628]
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(*Received in Japan* 21 *February* 1997; *accepted* 14 *March* 1997)