A Novel Looping Structure of Linear Hexapeptide Boc-[D-Ala-(*Z*)-β-phenyldehydroalanine-L-Ala]$_2$-OMe

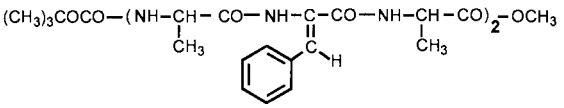
Yoshihito Inai,* Tatsuo Oshikawa,† Mitsuji Yamashita,† Itsuki Kato, and Tadamichi Hirabayashi
Department of Environmental Technology and Urban Planning, Graduate School of Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466-8555
†*Department of Materials Chemistry, Faculty of Engineering, Shizuoka University, Hamamatsu 432-8561*

(Received February 13, 2001; CL-010117)

Hexapeptide Boc-(D-Ala-Δ^ZPhe-L-Ala)$_2$-OCH_3 [Boc = *tert*-butyloxycarbonyl; Δ^ZPhe = (*Z*)-β-phenyldehydroalanine] in the solid state exhibited a novel looping backbone consisting of three partially overlapped β-turns supported by intramolecular (i+3) → i hydrogen bonds. Here the looping backbone also included a β-bend ribbon structure.

To find a novel regular structure other than conventional secondary structures (e.g., α-helix, β-sheet, and β-turn) in peptides and proteins is important when creating a specific molecular shape or a regular arrangement of functional groups, leading to rational design of a wide variety of artificial proteins or supramolecules. Unusual helical structures such as β-helix or γ-helix have been found experimentally and theoretically in periodic oligo- or polypeptides.[1] Herein the repeating units should contain non L-Ala-types of residues such as D-Ala, Gly, and Pro, which essentially differ from L-Ala-type of residues in conformational preference.

We here report the X-ray crystallographic analysis of hexapeptide **1** consisting of L-Ala, (*Z*)-β-phenyldehydroalanine (Δ^ZPhe), and D-Ala residues:



Boc-(D-Ala-Δ^ZPhe-L-Ala)$_2$-OMe **1**
(Boc = *tert*-butyloxycarbonyl; OMe = methoxy)

Herein, the Δ^ZPhe as an unusual residue is shown to provide a specific conformational preference in the main and side chains due to the presence of C^α=C^β double bond.[2] Thus, the introduction of the three different types of amino acids into the same sequence is expected to give a novel regular structure.

Peptide **1** was synthesized by coupling Boc-D-Ala-Δ^ZPhe-L-Ala-OH with H-D-Ala-Δ^ZPhe-L-Ala-OMe using a mixed anhydride method according to reference 3. Both the tripeptides were obtained by deprotection of Boc-D-Ala-Δ^ZPhe-L-Ala-OMe prepared by ring-opening Boc-D-Ala-Δ^ZPhe azlactone with H-L-Ala-OMe. The detailed procedure and characterization will be described elsewhere. Single crystals of peptide **1** ($C_{36}H_{46}N_6O_9\cdot C_2H_3N\cdot H_2O$; fw = 765.86) were grown by slow evaporation from an acetonitrile solution in the monoclinic space group $P2_1$ with a = 10.224(4) Å, b = 16.619(8) Å, c = 12.852(4) Å, β = 92.94(3)°, V = 2180(1) Å^3, Z = 2, and D_{calc} = 1.166 g/cm^3.[4] A perspective view of peptide **1** is shown in Figure 1, and the torsion angles are summarized in Table 1. Interestingly, the backbone, although being an acyclic peptide, exhibits a looping shape. This conformation is a consequence of three partially overlapped β-turns: a type III' β-turn conformation [ϕ_1 = 69(1)°, ψ_1 = 19(1)°, ϕ_2 = 68(1)°, and ψ_2 = 11(1)°] for the Boc(0)-D-Ala(1)-Δ^ZPhe(2)-L-Ala(3) segment, a distorted type II β-turn [ϕ_3 = –62(1)°, ψ_3 = 125.2(10)°, ϕ_4 = 55(1)°, and ψ_4 = 31(1)°] for the Δ^ZPhe(2)-L-Ala(3)-D-Ala(4)-Δ^ZPhe(5) segment, and a type III' β-turn [ϕ_4 = 55(1)°, ψ_4 = 31(1)°, ϕ_5 = 65(1)°, and ψ_5 = 17(1)°] for the L-Ala(3)-D-Ala(4)-Δ^ZPhe(5)-L-Ala(6) segment. Here three intramolecular (i+3) → i hydrogen bonds were observed for the pairs of N3–H···O0', N5–H···O2', and N6–H···O3', as follows.

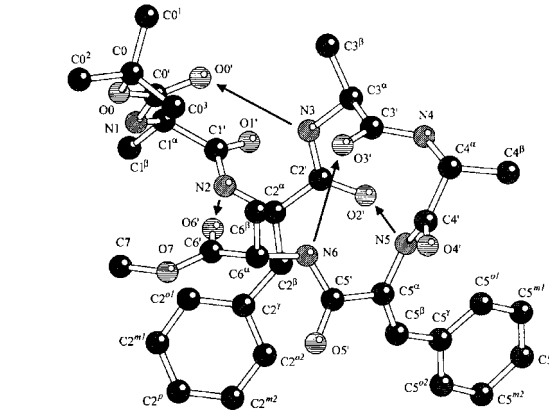
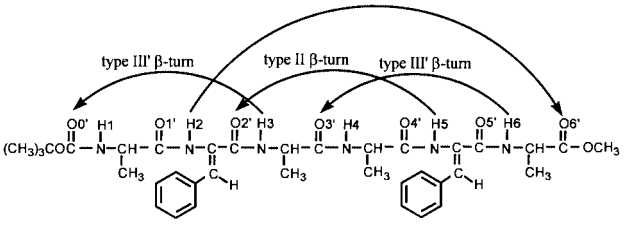


Figure 1. Conformation of peptide **1** obtained from X-ray crystallographic analysis. The arrows indicate the intramolecular hydrogen bonds.

Table 1. Selected torsion angles (°)[a]

Residue (no.)	ϕ_i	ψ_i	ω_i	χ_i^1	χ_i^2
Boc(0)			166.2(8)		
D-Ala(1)	69(1)	19(1)	–177.8(10)		
Δ^ZPhe(2)	68(1)	11(1)	172.2(9)	5(1)	–9(1)
L-Ala(3)	–62(1)	125.2(10)	–175.4(9)		
D-Ala(4)	55(1)	31(1)	–179.8(9)		
Δ^ZPhe(5)	65(1)	17(1)	178.6(10)	5(1)	7(1)
L-Ala(6)	–98(1)	157.3(3)	176.5(5)		

[a]Torsion angles for i th residue are defined as follows: ϕ_i for C_{i-1}'-N_i-C_i^α-C_i'; ψ_i for N_i-C_i^α-C_i'-N_{i+1} (N_i-C_i^α-C_i'-O_{i+1} for C-terminal residue); ω_i for C_i^α-C_i'-N_{i+1}-C_{i+1}^α (C_i^α-C_i'-O_{i-1}-C_{i+1}^α for C-terminal residue); χ_i^1 for N_i-C_i^α-C_i^β-C_i^γ; χ_i^2 for C_i^α-C_i^β-C_i^γ-C_i^{o1}.



Copyright © 2001 The Chemical Society of Japan

The type III' β-turns can be ascribed to the conformational preference in Δ^ZPhe residue, which has been shown to induce a 3_{10}-helix (successive type III β-turns).[2a] The chirality of type III' β-turn should be induced by the chirality of the preceding residue (L-Ala) of Δ^ZPhe, since Δ^ZPhe residue is achiral one. An additional intramolecular hydrogen bond was found between the two remote residues, i.e., N2–H···O6', to complete the looping structure. The intra- and intermolecular hydrogen-bond parameters are summarized in Table 2. All five peptide bonds took *trans* conformations essentially, and the side chains of the two Δ^ZPhe residues adopted a planar conformation.

Table 2. Intra- and intermolecular hydrogen-bond parameters for peptide **1**

Donor D-H[a]	Acceptor A	D···A /Å	H···A /Å	D–H···A /°	Symmetry[b]
N2–H	O6'	2.93(1)	2.19	130	x, y, z
N3–H	O0'	3.084(9)	2.09	172	x, y, z
N5–H	O2'	2.876(8)	2.03	141	x, y, z
N6–H	O3'	2.971(9)	2.01	161	x, y, z
N4–H	O5'	2.786(10)	1.79	177	-x, y+1/2, -z

[a]The hydrogen positions of N-H were based on AM1 semiempirical molecular orbital calculation[5] in MOPAC97.[6] [b]The symmetry operations are applied to the acceptors.

Peptide **1** also includes a 'β-bend ribbon structure' characterized by interesting hydrogen-bonding mode in which alternating (i+3) → i type hydrogen bonds are absent. Namely, in the Boc-D-Ala-Δ^ZPhe-L-Ala-D-Ala-Δ^ZPhe segment, the (i+3) → i type hydrogen bonds are observed for N3–H···O0' and N5–H···O2', but not for N4–H···O1'. The β-bend ribbon conformation was firstly proposed for the sequential peptides –(Aib–Pro)$_n$– (Aib = α-aminoisobutyric acid),[7] and subsequently found in the pentapeptide Boc–Pro–Δ^ZPhe–Ala–Δ^ZPhe–Ala–OMe.[8] The former β-bend ribbon was also called β-bend ribbon spirals,[7b,c] in which the torsion angles are almost the same as those of 3_{10}-helix, but alternative (i+3) → i type hydrogen bonds are absent due to the presence of Pro without N–H. The latter one was referred to as a flat β-bend ribbon including type I and type II β-turns. Obviously, the β-bend ribbon structure in peptide **1** is essentially different from the previous ones: i.e., the present structure includes a type III' β-turn and a distorted type II β-turn, following an additional type III' β-turn to complete a looping structure.

The peptide molecules were linked by intermolecular hydrogen bonds of N4–H···O5' between symmetries (x, y, z) and (-x, y+1/2, –z), as shown in Table 2. This leads to long columns of looping molecules running along the b axis. No marked π–π and CH–π interactions between phenyl groups for neighboring molecules were observed. An oxygen atom of water molecule was found among three peptide molecules, but strongly disordered.

The variation of NH chemical shifts for peptide **1** in $CDCl_3$ with $(CD_3)_2SO$[2b,2c,9] indicated that three NH resonances of L-Ala(3), Δ^ZPhe(5), and L-Ala(6) residues participate in intramolecular hydrogen bonding, but the other NHs do not. The lack of intramolecular hydrogen bonding in Δ^ZPhe(2) NH is inconsistent with the solid-state conformation, suggesting that the terminal ester group in solution fluctuates to prevent the hydrogen bonding with Δ^ZPhe(2) NH. The other five NHs, however, agreed with the hydrogen-bonding mode observed in the solid state, thus indicating that peptide **1** in solution retains the three β-turn structures. Also, the CD spectrum of peptide **1** in chloroform gave marked exciton couplets centered at ca. 280 nm assignable to Δ^ZPhe residue, with a positive peak at longer wavelengths: $\Delta\varepsilon_{261}$= –11.5; $\Delta\varepsilon_{295}$= +7.0. Based on the exciton chirality method,[10] the sign of splitting means a right-handed arrangement of two Δ^ZPhe residues, corresponding to the solid-state conformation shown in Figure 1. The similar CD pattern was observed in acetonitrile, methanol, and tetrahydrofuran. Therefore, the solid-state conformation shown in Figure 1 should be retained in solution essentially.

In conclusion, peptide **1** consisting of three different types of residues adopts a novel looping structure including a β-bend ribbon. Here the two phenyl groups along the backbone are arranged in a head-to-tail manner: the center-to-center (the nearest edge-to-edge) distance is 6.9 (4.6) Å. Thus, a pair of other functional groups will be arranged in a similar manner when the corresponding β-substituted α,β-dehydroalanines are used instead of the Δ^ZPhe residue.

This work was partially supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan under Grant (No. 12650883) to Y. I.

References and Notes

1 a) D. W. Urry, M. C. Goodall, J. D. Glickson, and D. F. Mayers, *Proc. Natl. Acad. Sci. U. S. A.*, **68**, 1907 (1971). b) D. W. Urry, *Proc. Natl. Acad. Sci. U. S. A.*, **69**, 1610 (1972). c) D. U. Römer, E. Fenude-Schoch, G. P. Lorenzi, and H. Rüegger, *Helv. Chim. Acta*, **76**, 451 (1993). d) M. Oka, Y. Baba, A. Kagemoto, and A. Nakajima, *Polym. J.*, **22**, 135 (1990). e) M. Oka and A. Nakajima, *Polym. Bull.*, **33**, 693 (1994).

2 a) R. Jain and V. S. Chauhan, *Biopolymers*, **40**, 105 (1996), and references therein. b) Y. Inai, T. Ito, T. Hirabayashi, and K. Yokota, *Biopolymers*, **33**, 1173 (1993). c) Y. Inai, Y. Kurokawa, and K. Yokota, *Biopolymers*, **49**, 551 (1999). d) Y. Inai, S. Kurashima, T. Hirabayashi, and K. Yokota, *Biopolymers*, **53**, 484 (2000).

3 Y. Inai, T. Oshikawa, M. Yamashita, T. Hirabayashi, and T. Hirako, *Biopolymers*, **58**, 9 (2001).

4 A colorless, prismatic single crystal (0.20 × 0.20 × 0.40 mm^3) was used for collecting three-dimensional X-ray data on a RIGAKU AFC7R diffractometer at 223 K. The structure was solved by direct methods (See ref 4a), and expanded using Fourier techniques (See ref 4b). All the nonhydrogen atoms were refined anisotropically. The final cycle of full-matrix least squares refinement (See ref 4c) on F^2 was based on 2303 observed reflections [$I > 2\sigma(I)$]. The refinement was converged with R = 0.066 (for observed data) and R_w = 0.225 (for all data). Tables of final positional parameters, equivalent thermal factors, bond lengths, bond angles, and van der Waals contacts for peptides **1** are being deposited in the Cambridge Crystallographic Data Bank as a supplementary publication: a) A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori, and M. Camalli, *J. Appl. Crystallogr.*, **27**, 435 (1994). b) P. T. Beurskens, G. Admiraal, G. Beurskens, W. P. Bosman, R. de Gelder, R. Israel and J. M. M. Smits, The DIRDIF-94 program system, technical report of the Crystallography Laboratory, University of Nijmegen, The Netherlands, 1994. c) G. M. Sheldrick, SHELXL-93: Program for the Refinement of Crystal Structures, University of Goettingen, Germany, 1993.

5 M. J. S. Dewar, E. G. Zoebisch, E. F. Healy, and J. J. P. Stewart, *J. Am. Chem. Soc.*, **107**, 3902 (1985).

6 J. J. P. Stewart, MOPAC97, Fujitsu Ltd., Tokyo, Japan, 1998.

7 a) Y. V. Venkatachalapathi and P. Balaram, *Biopolymers*, **20**, 1137 (1981). b) B. Di Blasio, V. Pavone, M. Saviano, A. Lombardi, F. Nastri, C. Pedone, E. Benedetti, M. Crisma, M. Anzolin, and C. Toniolo, *J. Am. Chem. Soc.*, **114**, 6273 (1992). c) M. Crisma, G. Valle, G. M. Bonora, C. Toniolo, and G. Cavicchioni, *Int. J. Pept. Protein Res.*, **41**, 533 (1993).

8 K. R. Rajashankar, S. R. R. Ramakumar, T. K. Mal, and V. S. Chauhan, *Angew. Chem., Int. Ed. Engl.*, **33**, 970 (1994).

9 T. P. Pitner and D. W. Urry, *J. Am. Chem. Soc.*, **95**, 1399 (1972).

10 N. Harada, S. L. Chen, and K. Nakanishi, *J. Am. Chem. Soc.*, **97**, 5345 (1975).