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Effect of the D-Phe^2 residue on molecular conformation of an endogenous neuropeptide achatin-I

Comparison of X-ray crystal structures of achatin-I (H-Gly-D-Phe-Ala-Asp-OH) and achatin-II (H-Gly-Phe-Ala-Asp-OH)

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The molecular conformation of achatin-II neutral form (H-Gly-Phe-Ala-Asp-OH), an endogenous peptide from the *Achatina fulica* ganglia, was elucidated by X-ray crystal analysis. The molecule takes an extended β-pleated structure stabilized by 5 intermolecular hydrogen bonds with the antiparallely arranged molecules. This is in contrast with the turn conformation of a neuroactive achatin-I (H-Gly-D-Phe-Ala-Asp-OH) [(1992) FEBS Lett. 276, 95–97]. The conformational comparison of both of the molecules makes clear the structural role which D-Phe residue of achatin-I plays in forming a definite active form.

Achatin-I; Achatin-II; Neuropeptide; Molecular conformation; Crystal structure

1. INTRODUCTION

Since the physiological activity of a naturally occurring biomolecule is intimately related to its 3D structure, it is essential to elucidate the stable conformation and to establish the principles governing it. In order to achieve this end, X-ray single crystal analysis is a desirable method, because it unambiguously provides a complete stereo structure. Previously, we analysed the crystal structure of achatin-I (H-Gly-D-Phe-Ala-Asp-OH) [1,2], a potent neuropeptide isolated from the ganglia of an African giant snail *Achatina fulica* Férussac [3], as a series of elucidating its possible active conformation. An unique turn conformation stabilized by two intramolecular hydrogen bonds of NH (Gly)···$O^{\delta 1}$(Asp) and NH(Asp)···$O^{\delta 1}$(Asp) pairs (Fig. 2a) was observed. Recently, it was reported [4,5] that a structural/physiological feature of achatin-I is dependent on the presence of D-Phe residue at the second position and the substitution of L-Phe (named achatin-II) leads to the drastic decrease or complete loss of the neuroexcitatory activity. In order to examine whether or not the D-Phe plays an important role in forming the unique turn conformation of achatin-I, therefore, the molecular conformation of achatin-II (H-Gly-Phe-Ala-Asp-OH), an endogenous peptide isolated from the same species as achatin-I [3], was elucidated by X-ray single crystal analysis. This paper deals with its results and the conformational change caused by the replacement of D-Phe by L-Phe.

2. EXPERIMENTAL

Crystallization of the achatin-II neutral form, which was chemically synthesized, was carried out by the same procedure as that of achatin-I [1,2]. Transparent needle crystals were grown from the methanol/dioxane (3:1, v/v) mixture by slow evaporation at room temperature. Achatin-II was crystallized as monohydrate, probably due to a small amount of water molecules that were contained in methanol. Crystal data: $C_{18}H_{24}N_4O_7 \cdot H_2O$, $M_r = 426.43$, monoclinic, space group $P2_1$, $a = 13.501(9)$, $b = 9.430(1)$, $c = 8.801(3)$ Å, $\beta = 92.98(6)°$, $V = 1119.0(8)$ Å^3, $Z = 2$, $D_m = 1.270(3)$ g · cm^{-3}, $D_x = 1.266$ g · cm^{-3}, μ(Cu Kα) = 8.12 cm^{-1}, and $F(000) = 452$.

A total of 1944 independent reflection intensities ($2° \leqslant 2\theta \leqslant 130°$) were collected on a Rigaku AFC-5 diffractometer with graphite-monochromated Cu Kα radiation using ω-2θ scan technique (resolution = 0.85 Å). The intensities were corrected for Lorentz and polarization effects, but not for the absorption and extinction factors. The structure was solved by the direct method with a program MULTAN87 [6]. The least squares refinement of non-H atoms with anisotropic thermal parameters and of H atoms with isotropic ones converged to the discrepancy R and R_w indices of 0.059 and 0.085, respectively, for 1833 reflections of $|F_0| > 3\sigma(F_0)$. The values of interatomic distances, bond lengths and torsion angles are available from one of the authors (T.I.) on request. The fractional coordinates and estimated standard deviations are presented in Table I.

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3. RESULTS AND DISCUSSION

Achatin-II molecules are composed of the antiparallel β-sheets, and their 3D molecular packings are mainly stabilized by intermolecular hydrogen-bonding network as shown in Fig. 1. The adjacent extended molecules related by a twofold screw symmetry are mutually linked to one another by five hydrogen bonds of N1(Gly)···$O4^{\delta 1}$(Asp) = 3.178(8) Å, N2(Phe)···O3(Ala) = 2.884(6) Å, O2(Phe)···N3(Ala)=2.900(6) Å, N4(Phe) ···O1 (Gly)=3.090(6) Å and O4′(Asp)···N1(Gly) = 2.753(9) Å. Such an antiparallel β-pleated sheet structure is frequently observed in the crystal structure of linear peptides such as enkephalins [7,8]. The fundamental feature characterizing the achatin-II conformation could be described as the hydrogen-bonded dimer structure.

The molecular conformation of achatin-II is shown in Fig. 2, in which that of achatin-I observed in the crystal structure [1,2] is also given for the comparison. Conformational torsion angles are listed in Table II. The molecular dimensions of achatin-II are all in an accepted region [9]. Judging from the bond lengths and angles of N-terminal amino, C-terminal carboxyl and Asp α-carboxyl groups [N1-C1α = 1.481(8), C4γ–$O4^{\delta 1}$ = 1.300(8), C4γ–$O4^{\delta 2}$ = 1.18(1), C4–O4 = 1.22(1), C4–O4′ = 1.26(1) Å, C4β–C4γ–$O4^{\delta 1}$ = 110.7(4), C4β–C4γ–$O4^{\delta 2}$ = 123.1(4), O4δ1–C4γ–$O4^{\delta 2}$ = 126.2(4), C4α–C4–O4 = 115.6(4), C4α–C4–O4′ = 115.1(4) and O4–C4–O4′ = 128.9(5)°] and (b) the hydrogen bonding pattern observed in the crystal structure, achatin-II appears to exist as a zwitterionic form with the N-terminal end protonated and C-terminal end deprotonated, although three H atoms around Gly N1 atom could not be clearly differentiated in the electron density map because of their relatively weak and broad densities. This chemical state of achatin-II is different from that of achatin-I, where the zwitterion structure occurs between the Gly N-terminal end and the Asp α-carboxyl group, instead of the C-terminal one, as a result of an intramolecular N1(Gly)···$O^{\delta 1}$(Asp) hydrogen bond formation [1,2]. Achatin-II, as a whole, adopts an extended conformation with the Phe, Ala and Asp side chains protruding alternately from the backbone chain; each side chain of Phe and Asp residues belongs to one of the most frequently observed conformations in peptides [10].

Since the crystallization conditions used for achatin-I and -II are quite identical, it would be reasonable to compare the conformational difference of both molecule, in order to estimate the D/L-configurational role which the Phe residue plays in composing three-dimensional structure. The present result indicates that the

Table I

Fractional atomic coordinates with e.s.d.s in parentheses and equivalent isotropic temperature factors

Atom	x	y	z	Beq (Å^2)
N1	0.4044(4)	0.4279(7)	0.7306(7)	4.5(2)
C1α	0.3056(4)	0.4931(7)	0.7459(7)	3.4(2)
C1	0.2551(3)	0.4205(6)	0.8698(6)	2.5(2)
O1	0.2716(3)	0.2959(5)	0.9001(5)	3.9(2)
N2	0.1920(3)	0.5004(5)	0.9394(4)	2.4(2)
C2α	0.1382(3)	0.4520(5)	1.0691(5)	2.1(2)
C2β	0.1990(4)	0.4902(7)	1.2177(6)	3.1(2)
C2γ	0.1497(4)	0.4445(7)	1.3604(5)	2.6(2)
$C2^{\delta 1}$	0.0936(4)	0.5369(8)	1.4411(7)	3.9(3)
$C2^{\delta 2}$	0.1635(5)	0.3051(7)	1.4163(7)	3.8(3)
$C2^{\varepsilon 1}$	0.0495(5)	0.4972(9)	1.5705(8)	4.5(3)
$C2^{\varepsilon 2}$	0.1194(5)	0.2646(8)	1.5484(8)	4.2(3)
C2ζ	0.0622(5)	0.3545(9)	1.6279(7)	4.7(3)
C2	0.0384(3)	0.5251(5)	1.0623(5)	2.1(2)
O2	0.0323(3)	0.6564(4)	1.0605(5)	3.6(2)
N3	−0.0415(3)	0.4416(5)	1.0612(5)	2.4(2)
C3α	−0.1406(4)	0.5006(6)	1.0517(6)	2.6(2)
C3β	−0.1847(5)	0.490(1)	0.8917(8)	4.7(3)
C3	−0.2040(4)	0.4172(6)	1.1591(6)	2.8(2)
O3	−0.1956(3)	0.2872(5)	1.1738(6)	4.1(2)
N4	−0.2693(3)	0.4913(5)	1.2294(6)	3.1(2)
C4α	−0.3381(4)	0.4224(6)	1.3295(6)	2.8(2)
C4β	−0.2853(4)	0.3745(7)	1.4764(8)	3.6(2)
C4γ	−0.3566(4)	0.2908(8)	1.5796(7)	3.7(3)
$O4^{\delta 1}$	−0.3754(4)	0.1654(6)	1.5241(6)	5.0(2)
$O4^{\delta 2}$	−0.3873(4)	0.338(1)	1.6912(8)	7.3(3)
C4	−0.4216(5)	0.5320(8)	1.3536(8)	4.2(3)
O4	−0.5035(4)	0.4817(8)	1.3799(9)	6.9(3)
O4′	−0.3921(4)	0.6573(7)	1.3863(9)	7.3(3)
O(water)	0.4332(8)	0.209(2)	0.282(1)	11.0(6)

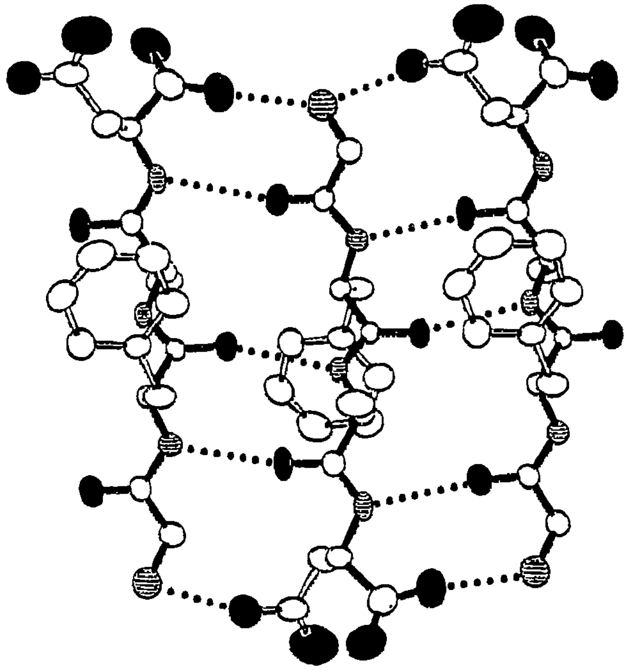

Fig. 1. The β-pleated sheet structure of achatin-II observed in the crystal. The dotted lines represent possible intermolecular hydrogen bonds. Oxygen, nitrogen and carbon atoms are shown with filled, shaded and open ellipsoid circles, respectively. The backbone chains are marked by thick lines.

Table II

Conformational torsion angles (°) of achatin-I and -II

		Achatin-I	Achatin-II
Gly	φ	157.8	151.4(5)
	ω	−172.0	−176.9(5)
Phe	ϕ	95.2	−147.9(5)
	φ	−111.7	124.1(5)
	ω	−175.4	−178.7(5)
	χ^1	−179.2	180.0(5)
	χ^2	89.9	85.3(5)
Ala	ϕ	−138.3	−139.4(5)
	φ	37.1	143.5(5)
	ω	−171.3	176.9(5)
Asp	ϕ	−111.2	−166.6(6)
	φ	−170.9	146.8(7)
	χ^1	76.4	−175.9(5)
	χ^2	141.4	73.1(5)

unique turn conformation in achatin-I, although two intramolecular hydrogen bonds stabilize this structure, importantly, is primarily due to the presence of D-Phe residue at the second position. The replacement of D-Phe by L-Phe causes the steric hindrance between the Phe benzyl group and the backbone chain taking the turn structure (especially, Gly O1 atom and Ala N3H and C3β methyl groups), and this leads to an extended conformation observed in the crystal structure.

It is widely accepted that the low-molecular biopeptides must take the definite 'active' conformations necessary for binding to the receptor, in order to elicit their biological responses. Thus, it may be reasonable to consider the unique turn conformation of achatin-I (Fig. 2a) as a possible active conformation for its neuroactivity. In this sense, the structural importance of D-Phe residue was clearly indicated by the present work.

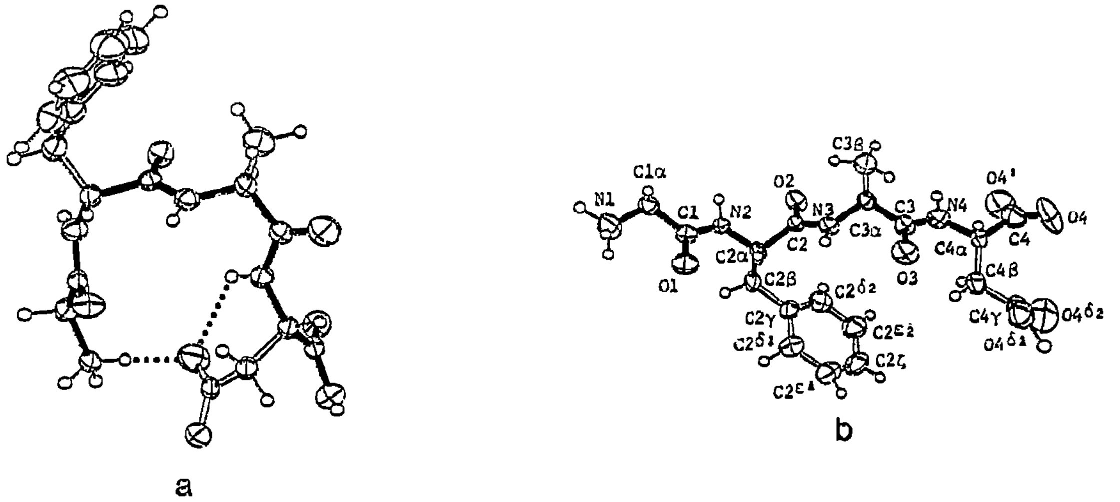

Fig. 2. Conformational comparison of achatin-I (a) and achatin-II (b) molecules observed in their crystal structures. The dotted lines in (a) represent intramolecular hydrogen bonds. The atomic numbering used is given for achatin-II (b).

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