Molecular conformation of achatin-I, an endogenous neuropeptide containing D-amino acid residue

X-Ray crystal structure of its neutral form

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The molecular conformation of achatin-I neutral form (H-Gly-D-Phe-Ala-Asp-OH), an endogenous neuropeptide, was elucidated by X-ray crystal analysis. The molecule has a type II′ β-turn structure with the D-Phe-Ala residues at the corner of the bend, which is further stabilized by two NH(Gly)...C^{γ}=O^{δ}(Asp) and NH(Asp)...C^{γ}=O^{δ}(Asp) intramolecular hydrogen bonds. This turn conformation may be an important feature of achatin-I related to its neuroexcitatory activity.

Achatin-I; Neurotransmitter; Tetrapeptide; Molecular conformation; Crystal structure

1. INTRODUCTION

Recently a neuroexcitatory tetrapeptide termed as achatin-I was isolated from the ganglia of an African giant snail (*Achatina fulica* Férussac) [1]. Achatin-I consists of H-Gly-D-Phe-Ala-Asp-OH and is the first example of the endogenous neuropeptide having a D-amino acid residue.

Achatin-I induces a voltage-dependent inward current due to sodium ions on the identifiable neurons [1,2], and is likely to play an important role as an excitatory neurotransmitter in the heart regulation of *A. fulica* [3]. The neuroactivity of achatin-I is stereospecific, and no other stereoisomers which are replaced with L- or D-amino acid residues show such a biological effect [1,2]. This means that a strict stereospecificity is required for the interaction with the receptor on Achatina neurons.

The elucidation of the three-dimensional structure of the bioactive molecule is important for the understanding of structure-activity relationships and the substrate-specificity of the receptor. The study of the stable conformation of achatin-I would further provide a reason why the D-configuration of Phe at the second position is necessary for the activity. In this paper we report the conformational characteristics of the achatin-I-free form, based on its X-ray crystal analysis.

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2. EXPERIMENTAL

Achatin-I was synthesized with the conventional solid-phase method, and purified by gel filtration (Sephadex G-10) and reversed-phase HPLC. Many attempts to crystallize the molecule finally led to transparent needle crystals, which were grown from the methanol/dioxane mixture by slow evaporation in the open atmosphere at room temperature. Crystal data are as follows: $C_{18}H_{24}N_4O_7$, M_r = 408.41, monoclinic, space group $P2_1$, a = 5.083 (1), b = 9.125 (1), c = 20.939 (3) Å, β = 94.73 (1)°, V = 967.9 (3) Å^3, Z = 2, D_m = 1.400 (3) g · cm^{-3}, D_x = 1.401 g · cm^{-3} and μ (Cu Kα) = 9.77 cm^{-1}.

A total of 1748 intensity data ($2\theta \leqq 130°$) were collected on a Rigaku AFC-5 diffractometer with graphite-monochromated Cu Kα radiation using ω–2θ scan technique (resolution = 0.85 Å), and were corrected for the usual Lorentz and polarization effects. The crystal structure was solved by the direct method with a program MULTAN87 [4]. The refinement of non-hydrogen atoms with anisotropic thermal parameters and of hydrogen atoms with isotropic ones converged to the discrepancy R and R_w values of 0.051 and 0.072, respectively. The crystallographic details will be reported elsewhere, and atomic coordinates at this stage are available from one of authors (T.I.) on request.

3. RESULTS AND DISCUSSION

A stereoscopic view of achatin-I molecular conformation is shown in Fig. 1. The conformational torsion angles are given in Table I. No noticeable abnormality was observed for the bond lengths and angles, and the side chains of the Phe and Asp residues also belong to one of the most frequently observed conformations in peptides and proteins [5,6]. Two C-O bond lengths of the Asp α-carboxyl group are significantly different from each other (1.205 (5) and 1.312 (5) Å), while those

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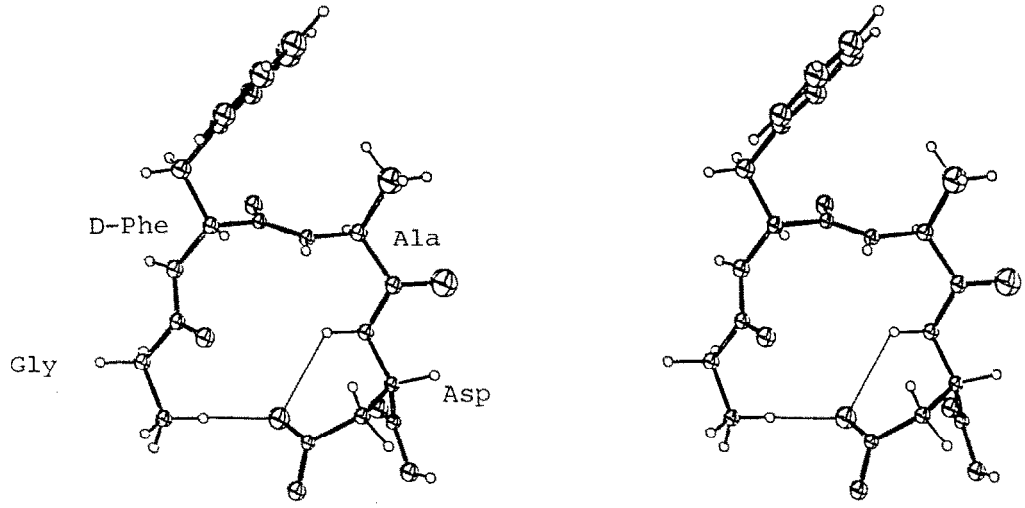

Fig. 1. Stereoscopic view of achatin-I. Thin lines represent possible intramolecular hydrogen bonds.

of the Asp β-carboxyl group are almost the same (1.233 (5) and 1.265 (5) Å). The amino terminal of the Gly residue, on the other hand, participates in the formation of an intramolecular and 2–3 intermolecular hydrogen bonds. Although hydrogen atoms could not be clearly observed on the electron density map, such bond lengths and hydrogen bonding mode indicate that the molecule exists as a zwitterion with the N-terminal end protonated and the carboxyl of the Asp side chain deprotonated; the C-terminal is in a neutral state.

As a whole, achatin-I adopts a turn conformation stabilized by two intramolecular hydrogen bonds of NH(Gly)...$C^{\gamma}=O^{\delta}$(Asp) (2.816 (5) Å) and NH(Asp)...$C^{\gamma}=O^{\delta}$(Asp) (2.950 (5) Å). Although an intramolecular hydrogen bond is not directly formed between the C=O(Gly) and NH(Asp) groups, the molecule shows a type II′ β-turn structure with the D-Phe-Ala residues at the corner of the bend. A similar conformation has also been observed in D-Phe-Ala residues of cyclo(Gly-Pro-D-Phe-Ala-Pro) [7], although the chemical structures are largely different from each other. Thus, this turn conformation corresponds to a favored conformation for the peptide having D-L residues in positions $i+1$ and $i+2$ of the turn [8]. Further the Gly-D-Phe-Ala fragment shows the characteristic of the γ-turn conformation, and a weak interaction is observed between the NH(Ala) and C=O(Gly) groups [N...O = 3.689 (5) Å].

Table I

Conformational angles of achatin-I

	Gly	D-Phe	Ala	Asp
φ	-	95.2 (3)	-138.3 (4)	-111.2 (4)
ψ	157.8 (4)	-111.7 (4)	37.1 (4)	-170.9 (3)
ω	-172.0 (4)	-175.4 (4)	-171.3 (4)	-
χ_1	-	-179.2 (4)	-	76.4 (3)
χ_2	-	89.9 (5)	-	-37.7 (4)

A significant characteristic of achatin-I conformation is the participation of the Asp β-carboxyl O^{δ} atom in two intramolecular hydrogen bonds, thus forming a plane consisting of the Gly-D-Phe-Ala backbone and Asp side chains. This conformation appears to be stable and mostly exists in the solution state; a similar turn conformation has also been derived from the NMR conformational analyses (Iwashita et al., unpublished).

A small peptide that elicits a particular biological response must maintain a suitable orientation of binding groups for productive interaction with a receptor. A turn conformation observed, which is stabilized by the β- and/or γ-turn structure and the intramolecular hydrogen bonds, guarantees the proper arrangement of groups essential for receptor binding. The backbone conformation itself may not be essential to the activity of achatin-I. Instead, optimal placement of D-Phe, Ala and Asp side chains seems to be the requirement for its bioactivity. The replacement of D-Phe with L-Phe would cause severe steric hindrance between the Phe benzene ring and the backbone chain, and thus lead to another type of molecular conformation which is significantly different from the present conformation of achatin-I. Similar steric effects could also be accompanied by the configurational change of Ala and Asp residues. The detailed conformational analyses of all achatin stereoisomers would make the active conformation of achatin-I more clear.

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