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Three-dimensional structure of monoanionic methionine-enkephalin: X-ray structure of *tert*-butyloxycarbonyl-Tyr-Gly-Gly-(4-bromo)Phe-Met-OH

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The conformation of *tert*-butyloxycarbonyl-Tyr-Gly-Gly-(4-bromo)Phe-Met-OH, as a monoanionic derivative of Met-enkephalin, was elucidated by X-ray crystal analysis. The molecule took an extended conformation which was bended at the Phe residue. The implication of the dimer formation caused by 4 intermolecular hydrogen bonds was discussed in the relation with the opiate receptor.

Met-enkephalin analog *Anionic form* *X-ray analysis* *Solid state* *Extended conformation* *β-Sheet*

1. INTRODUCTION

The elucidation of the stereo-specificities of opiate peptides is of great significance for understanding the structure–function relationship between opiate receptors and their analgetic mechanisms. Therefore, studies on the three-dimensional structure of enkephalins (Met-enkephalin and Leu-enkephalin) and their derivatives have been performed in the solution state [1–7] as well as the crystalline one [8–10]. On the other hand, it could be thought that the monoionic form at the amino- or carboxyl-terminal group of enkephalins, which depends largely on the physiological circumstance in living cells, could change their flexible molecular conformations and consequently affect their analgetic activities [4,6,7]. The solid state conformational analysis of *tert*-butyloxycarbonyl-Tyr-Gly-Gly-(4-bromo)Phe-Met-OH (Boc-Enke), which is also a N^{α}-protected analogue of the COOH-terminal peptides of β-endorphine, has been carried out by X-ray diffraction methods. We here wish to report a unique stereo-structure of monoanionic Met-enkephalin and discuss its structural significance at binding to opiate receptors.

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Abbreviations: Boc-Enke, *tert*-butyloxycarbonyl-Tyr-Gly-Gly-(4-bromo)Phe-Met-OH; Met-enkephalin, H-Tyr-Gly-Gly-Phe-Met-OH; Leu-enkephalin, H-Tyr-Gly-Gly-Phe-Leu-OH; Boc, *tert*-butyloxycarbonyl

2. MATERIALS AND METHODS

Boc-Enke was synthesized with the conventional liquid method and its single crystals were obtained from a methanol–ethyl acetate mixture. Crystal data are as follows: tetragonal, $P4_12_12$ $a = b =$

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9.5589(2), c = 79.904(2) Å, V = 7301(3) Å^3, Z = 8, D_x = 1.370, D_m = 1.378(1) Mg·m^{-3}. Intensity data were measured with a Rigaku Roter AFC-5 diffractometer with graphite-monochromator CuKα radiation (λ = 1.5418Å). The structure determination was performed by a combination of the heavy atom method and the successive Fourier procedure, and the refinement of nonhydrogen atoms with the anisotropic thermal parameters was converged to R-factor to 14.18% for 6053 non-zero[$Fo > \sigma(Fo)$] reflections considering the anomalous dispersion terms.

3. RESULTS AND DISCUSSION

The molecular conformation of Boc-Enke is shown in fig.1 and the conformational torsion angles are listed in table 1. Although the molecule, as a whole, took an extended form, it was bended at Phe residue. While the Phe side chain involving the benzene ring was just extended in the same direction as the backbone chain, the Met side chain was almost perpendicular to this chain.

Boc-Enke molecules were found to be composed of the antiparallel β-sheets, and their three-dimensional molecular packings were mainly stabilized by the many hydrogen bond networks as shown in fig.2. The adjacent extended molecules were mutually linked to one another by the 4 hydrogen bond formations [O(Boc)-N(Met) = 2.83(1), N(Gly^2)-O(Gly^3) = 3.17(1), O(Gly^2)-N(Gly^3) = 2.89(1), N(Phe)-O(Tyr) = 2.95(1) Å]. Such a β-pleated sheet structure and molecular packing are also visible in the crystal structure of Leu-enkephalin [9]. The fundamental pattern characterizing the extended enkephalin conformation is the hydrogen bonded dimer formation, and this was already supposed in the solution state [5–7].

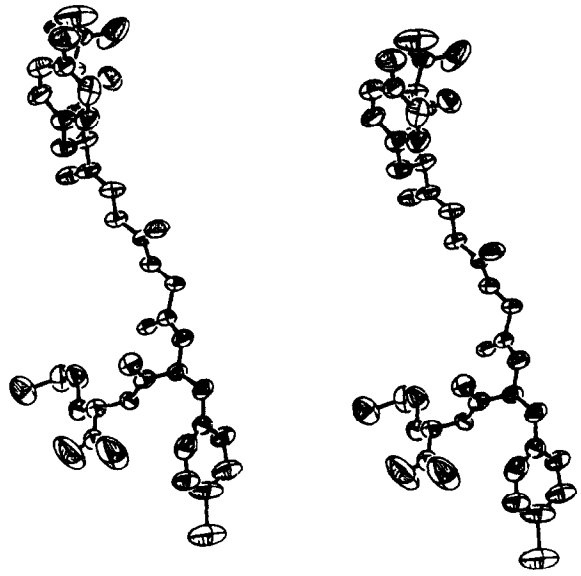

Fig.1. Stereoscopic view of Boc-Enke.

We now solved two enkephalin structures and found that two kinds of conformations could exist as the energetically stable form of enkephalin: folded and extended forms. While the former form would be characterized by the β-turn conformation stabilized by the intramolecular hydrogen bonds [N(Tyr)-O(Phe) and O(Tyr)-N(Phe)] [8,10], the latter one would be largely stabilized by the hydrogen bonded dimer formation. As well as the relationship between the β-turn conformation of enkephalin and its analgetic activity already discussed in [8] and [10], it is very interesting to elucidate the structure–function correlation of the dimeric conformer based on the following aspects:

Table 1

Conformational angles of Boc-Enke

	Tyr^1	Gly^2	Gly^3	Phe^4	Met^5
ϕ	−126.4(8)	−174.7(9)	143.4(11)	−63.7(11)	104.0(10)
ψ	149.1(8)	−174.7(9)	−156.9(10)	141.5(8)	−169.8(14)
ω	176.0(8)	−178.0(10)	176.1(9)	−176.5(8)	–
χ_1	−70.4(10)	–	–	−171.4(8)	−68.8(12)
χ_2	−86.2(13)	–	–	92.2(11)	175.4(8)
χ_3	–	–	–	–	66.1(11)

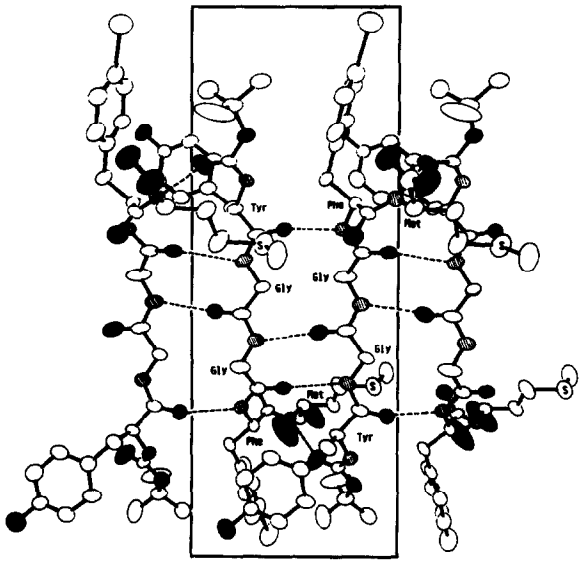

Fig.2. The β-pleated sheet structure of Boc-Enke shown in the crystal. The broken lines represent possible intermolecular hydrogen bonds, and two molecules surrounded by the box is the dimeric form stated in the text, where amino acid residues are labelled. (●) Oxygen atoms, (◍) nitrogen atoms.

(i) the anionic form of enkephalin, as well as cationic form, takes an extended conformation in the solution state [6,7], (ii) our experimental studies using rats showed that Boc-Enke had significant analgetic activities, although its degree was somewhat less than the native zwitterionic enkephalins (unpublished).

It is well known that two or three different opiate receptors participate in the interactions with enkephalins. Among them, two kinds of receptors named μ and δ are well characterized [11]. Based on the structural comparison of the morphine-like opiates selectively bound to the μ-receptor with enkephalin, it was suggested that the β-turn conformation is the most favorable form for the binding to the μ-receptor [8,10]. We might propose that the dimeric extended conformation which is stabilized by the intermolecular hydrogen bonding is the most probable form of enkephalin for the effective binding to the δ-receptor, in contrast to the binding to the μ-receptor proposed in [9]. Our hypothesis would be supported by the findings that the δ-specific binding was enhanced by the dimerization of enkephalins via the covalent bonds [12].

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