A STEREOCHEMICALLY-CONSTRAINED ENKEPHALIN ANALOG

α-Aminoisobutyryl[2] methionine[5] enkephalinamide

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1. Introduction

The discovery that the pentapeptides Tyr–Gly–Gly–Phe–Met (Met[5]-enkephalin) and Tyr–Gly–Gly–Phe–Leu (Leu[5]-enkephalin), present in the mammalian central nervous system [1,2], possess opioid activity has stimulated considerable interest in their conformations [3–15]. The enkephalins have been shown to bind to the opiate receptor in brain and displace naloxone, a powerful opiate antagonist [16]. The structures of the enkephalins have been the subject of a number of spectroscopic and theoretical investigations. There have been attempts to build a structural model for the pentapeptides that mimics the essential features of the morphine molecule [8–11]. From these studies conflicting suggestions have emerged about the low energy conformation of enkephalins and also the possible conformation of the pentapeptides at the opiate receptor site. A single crystal X-ray diffraction study of [Leu[5]] enkephalin has been reported [17], in which the molecule has been shown to possess a Gly[2]–Gly[3] β-turn stabilised by two intramolecular hydrogen bonds between the CO and NH groups of Tyr[1] and the NH and CO groups of Phe[4]. This conformation has not been suggested by any of the spectroscopic or theoretical studies, though it was put forward earlier on the basis of empirical predictive procedures [9]. The presence of two glycine residues in enkephalin is likely to result in enhanced flexibility of the peptide backbone in solution. Consequently nuclear magnetic resonance (NMR) investigations may be confronted with the problem of dynamic averaging, between conformers of similar energies. The substitution of the C^{α} hydrogen atoms with alkyl residues in peptides, restricts conformational freedom. Experimental [18–20] and theoretical [21–23] studies have shown that the dimethyl analog of glycine, $H_2N-(CH_3)_2-COOH$, (α-aminoisobutyric acid, Aib) is an extremely sterically hindered amino acid capable of occupying only a small region of conformational space ($\phi \sim \pm 60^{\circ}$, $\psi \sim \pm 30^{\circ}$) in the right- or left-handed 3_{10} or α-helical regions of the conformational map [24]. Substitution of the glycine residues in enkephalin with α-aminoisobutyric acid residues should then lead to analogs with fewer conformational possibilities. We describe here the synthesis of the stereochemically-constrained enkephalin analog, [Aib[2] Met[5]] enkephalinamide (I).

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2. Experimental

Amino acid methyl esters were prepared by the thionyl chloride–methanol procedure [25] while *t*-butyloxycarbonyl (Boc) amino acids were prepared as in [26]. The coupling reactions were carried out using dicyclohexylcarbodiimide (DCC). Removal of the Boc protecting groups were effected using HCl/tetrahydrofuran. The synthetic scheme is outlined in fig.1. [Aib[2] Met[5]] enkephalinamide was obtained as a white solid, thin-layer chromatography on Silica gel, $R_F = 0.57$ (*n*-butanol–acetic acid–water, 4:1:1). A faint ninhydrin negative spot at

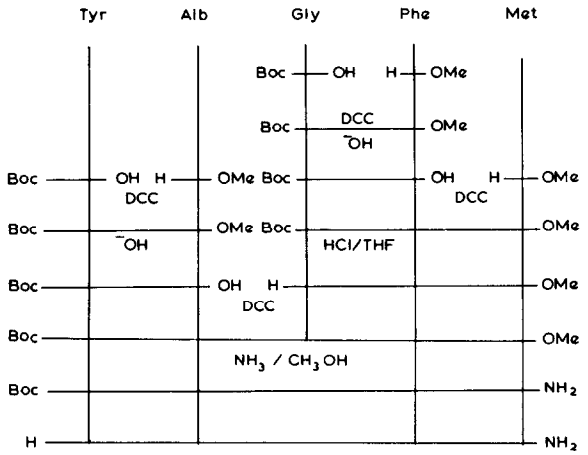

Fig.1. Synthetic scheme for the preparation of [Aib² Met⁵] enkephalinamide.

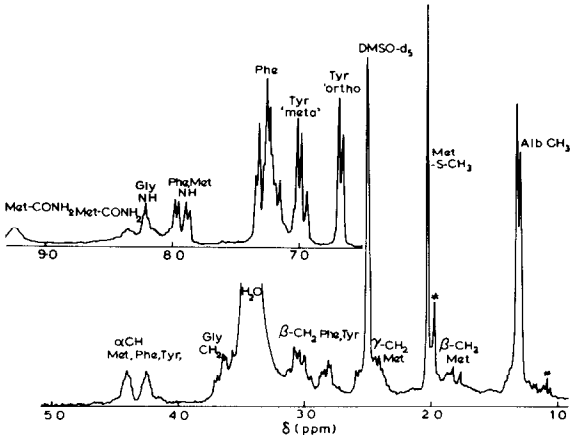

Fig.2. 270 MHz ^{1}H NMR spectrum of I in $(CD_3)_2SO$. Chemical shifts are expressed as δ (ppm) downfield from TMS. Tyr *'ortho'* and *'meta'* are the protons *ortho* and *meta* to the hydroxyl group. Peaks marked with an asterix are due to impurities.

R_F = 0.83 was also noted. Ultraviolet (methanol) λ_{max} = 276 nm. On addition of NaOH λ_{max} was shifted to 296 nm (tyrosine absorption). Satisfactory 270 MHz ^{1}H NMR spectra, in accordance with the structure were obtained on a Brucker WH-270 Fourier Transform NMR Spectrometer, at the Bangalore NMR Facility.

3. Results and discussion

The 270 MHz ^{1}H NMR spectrum of I in $(CD_3)_2SO$ is shown in fig.2. The assignments of the resonances are indicated. It is interesting to note that the $C^{\alpha}H_2$ protons of Gly³ are non-equivalent and appear as the AB part of an ABX spectrum centred at ~3.6 δ. The two methyl groups of Aib² are also non-equivalent as evidenced by the resonances at 1.38 δ and 1.40 δ. Earlier studies have shown that the $C^{\alpha}H_2$ protons of both Gly² and Gly³ in Met⁵-enkephalin are non-equivalent in H_2O and $(CD_3)_2SO$, suggesting that the molecules are inflexible on a chemical shift time scale. Non-equivalence of the $C^{\alpha}H_2$ protons of Gly residues has also been used to support the view that ordered conformations exist in solution, for a tetrapeptide fragment of tropoelastin Boc–Val–Pro–Gly–Gly–OMe [27]. While geminal non-equivalance of the $C^{\alpha}H_2$ protons of Gly³ and the CH_3 groups of Aib², is not conclusive evidence for the presence of ordered structures, it is likely that I adopts fairly well defined conformations in solution.

Preliminary studies indicate that I possesses high analgesic activity in rats, suggesting that the conformations necessary for receptor binding are still accessible in the Aib² analog. D-Ala²-enkephalinamide [28,29] is a potent long lasting analgesic with a high affinity for the opiate receptor whereas the L-Ala analog exhibits only weak receptor binding. These results suggest that the conformations permissible for the D-Ala and Aib residues fit the opiate receptor whereas the conformations adopted by the L-Ala residue are unfavourable for receptor interactions. Theoretical studies [30] have shown that peptide sequences with alternating configurations (DL or LD) have a high probability of forming β-turns [31]. α-Aminoisobutyric acid residues have also been shown to have a very strong tendency to initiate the formation of β-turns, from X-ray diffraction and spectroscopic investigations of Aib-containing peptides [18–20]. It is significant that the solid state conformation of Leu⁵-enkephalin possesses a β-turn with Gly² and Gly³ as the central residues [17]. A conformation that is accessible to all three active enkephalin analogs involves the β-turn with residues 2 and 3 at the corners. It is likely that binding to the

opiate receptor can then be accomplished by reorientation of the Tyr and Phe sidechains.

The torsional angles allowed for residue 2 as judged by the activity of the D-Ala^2 and Aib^2 analogs is restricted to the region $\phi \sim 60^\circ$ and $\psi \sim 30^\circ$, since the Aib residue is energetically confined largely to the regions $\phi \sim \pm 60^\circ$ and $\psi \sim \pm 30^\circ$. It is noteworthy that the values obtained in the X-ray study [17] for Gly^2 are $\phi = 59^\circ$ and $\psi = 25^\circ$. Based on computer modelling studies the D-Ala residue in D-Ala^2-enkephalinamide has been suggested to have $\phi = 160^\circ$ and $\psi = -87^\circ$ in the conformation involved in receptor binding [32]. These values of ϕ and ψ would lead to a very high energy for the Aib^2 analog, which is unlikely to be offset by binding interactions at the opiate receptor. The backbone conformation at the receptor site is therefore unlikely to involve large departures from the β-turn involving residues 2 and 3. The judicious use of α-alkyl amino acid residues in preparing synthetic analogs may allow a better definition of the biologically active conformation. Further studies on the synthesis of the Aib^2 and [Aib^2 Aib^3] enkephalin analogs and studies of their conformations and biological activity are in progress.

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