THEORETICAL CONFORMATIONAL ANALYSIS OF MET-ENKEPHALIN

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1. Introduction

Two pentapeptides, enkephalins (H–Tyr–Gly–Gly–Phe–X–OH, X = Met or Leu), have recently been isolated from mammalian brain [1]. Subsequent investigations have mainly concentrated on Met-enkephalin which is synonymous with enkephalin in the present paper. It was assumed on the basis of physiological experiments that enkephalin is the endogenous ligand of opiate receptors. It binds to isolated receptors but it is not yet clear whether its affinity for these receptors is higher or lower than that of morphine since affinity ratios ranging from 1/20 [2] to 3 [1] have been reported under similar conditions.

The assumption that enkephalin is the endogenous opiate has found one more justification in the fact that intracranial microinjection of a high dose produces an evanescent analgesia in rats [2]. Note, however, that a different peptide which produces a long lasting analgesia has recently been isolated [3].

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Structure activity studies [2,4] have shown that few structural modifications are allowed. The similarity between enkephalin and morphine has prompted a number of attempts to predict the conformation of the peptide [5] and of its tyramine part [6] from theoretical considerations. Concomitantly with this approach nuclear magnetic resonance (NMR) methods were used to determine the conformation of Met-enkephalin in solution. Two groups [7,8] reported the existence of a folded conformation in water and $DMSOd_6$, which involves an intramolecular hydrogen bond between the NH of Met_5 and the carboxyl of Gly_2 in contradiction with an almost simultaneous NMR study [9] and with the theoretical predictions [5].

We report here the results of a detailed theoretical analysis of the conformational properties of the zwitterionic form of Met-enkephalin which was carried out in order to obtain additional information about the possible conformation of this peptide.

2. Methods and results

Only a brief outline of the method used for the exploration of the conformational space of enkephalin will be given here. It is based on a strategy which has

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been used earlier in a similar analysis of angiotensin [10] and extensively described elsewhere [11]. A standard geometry based on crystallographic data [11] was used for the amino acids. All torsional angles were allowed to vary except those along the amide bonds which were fixed in the *trans*-planar conformation. The conformational energy was calculated according to an empirical scheme as the sum of two terms:

$$E_{tot} = E_{loc} + E_{int}.$$

The first term (E_{loc}) contains all contributions resulting from local interactions in each residue. It was evaluated for all values of the φ and ψ-angles [12] using an increment of 20°, while the side chain angles χ were varied by steps of 30°. When this term only is taken into account the molecule is considered as an unperturbed entity devoid of any interactions between amino acid residues. The results of the calculations at this level of simplification show that this idealized enkephalin has many nearly equiprobable conformations corresponding both to extended ($d(C_1^\alpha - C_5^\alpha) > 10$ Å) and folded forms ($d(C_1^\alpha - C_5^\alpha) < 8$ Å) with a preference for the former type.

The evaluation of the second term (E_{int}) which describes the interactions between residues is of course much more time consuming. For this reason it was only computed, at this stage, for the conformations corresponding to the relative minima of E_{loc}.

The $\varphi.\psi$ energy map of each residue presents several minima: 2 for the Tyr amino terminal, 5 for the Gly, 4 for Phe and 2 for the Met at the carboxyl end. The combination of those minima gives rise to 2X5X5X4X2 = 400 different backbone conformations for which the E_{int} term was calculated.

The first important result of this analysis is that only 15 out of the 400 conformers considered represent 97% of the total probability at 25°C. This is shown in table 1 which gives the probability of existence of each conformer at 25°C taking into account the large number of possible side chain arrangements.

The figures reported in column 5 give an idea of the relative mobility of the side chain for a given backbone conformation. It is interesting to note that the lowest energy models seem to correspond to the smallest side chain flexibility. The conformation of the backbone is described by a stereochemical code in which each letter corresponds to a set of φ and ψ-angles given in table 2. This table contains all the torsional angles of the most probable structures. Both extended and folded structures are found. The latter contain a turn either at the level of Gly_2 Gly_3 (type-II′ β-turns (e*a) for models 3, 9 and 14; e*e- and ee*-turns for models 4, 6 and 10)

Table 1
Conformers of enkephalin in equilibrium at 25°C

	Stereochemical code	$d(C_1^\alpha - C_5^\alpha)$ (Å)	Energy of E_{min} (kcal. m^{-1})	nb of side chain arrangements $E_{tot} \leq E_{min} + 3$ kcal. m^{-1}	Probability at 25°C (%)
1	b e b e b	7.0	−13.5	51	20.4
2	b e e e b	11.4	−13.1	185	25.3
3	b e* a b b	5.0	−13.0	218	19.1
4	b e* e e b	9.2	−12.9	239	18.2
5	b b e e b	11.6	−11.8	241	3.2
6	b e e* b b	7.4	−11.3	277	1.5
7	b e b b b	11.1	−11.0	475	1.4
8	b e e a* b	8.9	−10.9	253	0.7
9	b e* a b a*	5.0	−10.9	560	1.5
10	b e* e b b	9.3	−10.7	865	1.4
11	b e e b b	11.9	−10.7	1162	2.2
12	b e* e* b b	11.1	−10.6	393	0.6
13	b e e e a*	11.4	−10.6	218	0.4
14	b e* a e b	6.3	−10.5	492	0.6
15	b e* e* e b	9.5	−10.4	595	0.6
Total					97 %

The β-turns are underlined

Table 2
Most probable conformers or enkephalin

	Tyr					Gly		Gly		Phe				Met					E_{min}	
	φ_1	ψ_1	χ_1^1	χ_1^2	χ_1^3	φ_2	ψ_2	φ_3	ψ_3	φ_4	ψ_4	χ_4^1	χ_4^2	φ_5	ψ_5	χ_5^1	χ_5^2	χ_5^3	Before min	After min
1	60	140	180	90	−90	−80	80	−180	180	−80	80	−60	−60	−140	140	−60	180	180	−13.5	−14.6
2	60	140	180	90	−90	−80	80	−80	80	−80	80	−60	−60	−140	140	−60	180	−60	−13.1	−14.7
3	60	140	180	90	90	80	−80	−60	−60	−160	160	60	90	−140	140	180	180	−60	−13.0	−14.0
4	60	140	180	90	90	80	−80	−80	80	−80	80	−60	−60	−140	140	−60	180	−60	−12.9	−15.0
5	60	140	180	90	90	−180	180	−80	80	−80	80	−60	−60	−140	140	−60	180	−60	−11.8	−13.2
6	60	140	−60	−60	90	−80	80	80	−80	−160	160	60	90	−140	140	−60	180	−30	−11.3	−13.6
7	60	140	180	90	−90	−80	80	−180	180	−180	180	60	90	−140	140	−60	180	180	−11.0	−14.6
8	60	140	180	90	−90	−80	80	−80	80	60	60	−60	90	−140	140	−60	180	−150	−10.9	−13.2
9	60	140	180	90	90	80	−80	−60	−60	−160	160	30	90	60	40	−60	180	180	−10.9	−14.8
10	60	140	180	90	90	80	−80	−80	80	−160	160	90	90	−140	140	−60	180	−60	−10.7	−13.5
11	60	140	180	90	−90	−80	80	−80	80	−160	160	−60	90	−140	140	−60	180	−60	−10.7	−13.7
12	60	140	180	90	90	80	−80	80	−80	−160	160	60	90	−140	140	−60	180	180	−10.6	−11.7
13	60	140	180	90	−90	−80	80	−80	80	−80	80	−60	−60	60	40	−60	180	−60	−10.6	−11.9
14	60	140	180	90	90	80	−80	−60	−60	−80	80	−60	−60	−140	140	180	180	−60	−10.5	−14.5
15	60	140	180	90	90	80	−80	80	−80	−80	80	−60	−60	−140	140	−60	−60	180	−10.4	−12.7

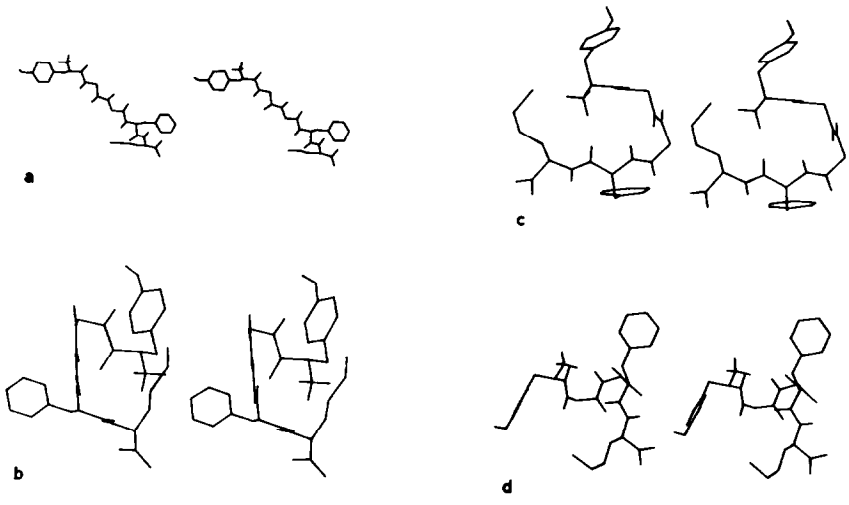

Fig.1. Stereoscopic views of the most characteristic conformers of enkephalin. Fully extended model (a) Models 1 (b), 3 (c) and 14 (d) of table 2 after minimization.

or at the level of Gly_3 Phe_4 (type-II β-turn (ea*) in the case of model 8; type-I β-turn (a e) for model 14).

3. Discussion

The most typical structures are illustrated by the stereopictures in fig.1. Model 14 is related to that proposed on the basis of high resolution NMR data [7,8]. The figures in the last two columns of table 2 represent the values of the energy before and after application of a simplex minimization procedure [13]. The effect of the refinement upon the values of the torsion angles is negligible in most cases ($\Delta < 20°$) and is of the order of the error for this type of calculation. It is clear, however, that the minimization affects models 7, 9 and 14 more than the first ones. As a result the probability of these models will increase compared with the values reported here. Quantitative results for the true statistical weight of the different models cannot be obtained without integrating the energy surface around each minimum. Such a calculation is in progress.

According to crystallographic data the distance between the OH group and nitrogen atom in morphine is 7.07 Å [14]. In the 15 most probable conformers the χ_1 value of the tyrosine residue is always equal to 180°, except in the case of model 6 for which $\chi_1 = -60°$. The corresponding N–O distances are 7.97 Å and 6.18 Å. The weighted mean of the N–O distance taking into account all populated conformers is 7.6 Å.

Since the role of the solvent is usually to select one or more of the minima found in vacuo, the results obtained so far suggest that the conformational state of enkephalin in solution can be described as an equilibrium between extended and folded structures. The present study provides supporting evidence for the existence of a conformer involving a β_1-turn at the level of Gly_3 Phe_4 as a probable structure as indicated by high resolution NMR measurements in DMSO [7,8]. However, since the environment at the receptor is not necessarily well simulated by solution conditions it should be remembered that there are many other conformations which present only minor energy differences when speculating about possible interactions with a receptor and/or similarities with narcotic analgesics.

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