Volume 227, number 2, 127–130 FEB 05499 January 1988

A model for the δ-receptor-bound conformation of enkephalin

G.V. Nikiforovich and J. Balodis

Institute of Organic Synthesis, Latvian SSR Academy of Sciences, Aizkraukles 21, 226006 Riga, USSR

Received 13 October 1987; revised version received 24 November 1987

Sets of low-energy structures were determined by energy calculations for two cyclic analogues of enkephalin (Ek), [D-Pen², D-Pen⁵]-Ek and [D-Pen², L-Pen⁵]-Ek, possessing the highest specificity towards δ-opioid receptors. Comparison of mutual spatial orientations of the α-amino group and aromatic moieties of the Tyr and Phe residues permitted one to suggest a model for the δ-receptor-bound conformation of enkephalin-related peptides. The model involves a pronounced γ-like turn of the peptide backbone centred on the Gly³ residue.

Energy calculation; Enkephalin cycloanalog; Receptor-bound conformation; Receptor selectivity

1. INTRODUCTION

Presently, the existence of at least three subclasses of opioid receptors, designated μ, δ and κ, has been shown quite reliably (e.g. [1]). It has been demonstrated, too, that the μ- and δ-opioid receptors require different receptor-bound conformations of enkephalin and its analogues [2]. For example, the conformationally restricted cyclic analogues Tyr-D-Pen-Gly-Phe-D-Pen ([D-Pen²,D-Pen⁵]-Ek, molecule I; Pen is penicillamine alias β,β-dimethylcysteine) and Tyr-D-Pen-Gly-Phe-Pen ([D-Pen²,L-Pen⁵]-Ek, molecule II) [3] have the highest so far reported selectivity towards δ-opioid receptors among enkephalin-like peptides [1]. Recently, some differences in the 'averaged' conformations of molecules I and II in water solution were determined by NMR spectroscopy [4]. The enkephalin α-amino group and the aromatic moieties of the Tyr and Phe residues are commonly suggested to be the key elements essential for a particular bioactivity of the molecule [1]. Thus, our aim was to search for low-energy three-dimensional structures of both molecules with geometrically similar relative spatial orientation of these key elements in order to propose the model for the δ-receptor-bound conformation of enkephalin.

Correspondence address: G.V. Nikiforovich, Institute of Organic Synthesis, Latvian SSR Academy of Sciences Aizkraukles 21, 226006 Riga, USSR

2. METHOD

Energy calculations were performed using the parameters described in [5,6] (methyl substituents at the C^{β}-atoms were regarded as united centres). All combinations of the local energy minima of peptide backbone for a single residue [7] and those of the side chain rotamers of D-Pen² and D/L-Pen⁵ residues providing correct ring closure were considered as probable conformations for both molecules.

Geometrical similarity shared by the pair of conformations was assessed calculating mean-square deviation D for the best spatial fit of the given atoms [8]. The two conformations were regarded as similar when D was below the chosen level D_0.

3. RESULTS

Energy calculations revealed 19 low-energy structures for molecule I (75 structures for molecule II) with the relative potential energies $\Delta U = U - U_{min} < 6$ kcal/mol allowing disulphide bridge closure without sterical hindrance when energetically optimal Tyr and Phe side chain rotamers were selected (the optimization procedure is described in [9]).

Comparison of the relative spatial arrangement of the α-NH_3 group and C^{α}- and C^{β}-atoms of Tyr

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

and Phe within the calculated conformation sets resulted in several classes of geometrically similar (D_o = 0.1 Å^2) peptide backbone structures for both molecules. Additional energy calculations were performed for conformations with the lowest potential energy within each class taking into account all possible combinations of Tyr and Phe side chain rotamers. The low-energy conformations (ΔU < 6 kcal/mol) selected at this step of calculation (22 structures for molecule I and 36 structures for molecule II) were then subjected to the same kind of geometrical comparison as that used previously, considering the relative spatial arrangement of the α-amino group and C^{α}-, C^{β}-, C^{γ}- and C^{ζ}-atoms of Tyr and Phe (D_o = 1.0 Å^2). Thus the comparison procedure concerned only conformations of the Tyr-D-Pen-Gly-Phe fragment for both molecules.

As a result, four low-energy backbone structures of this fragment in molecule I were shown to share geometrical similarity with one or several of the six low-energy structures of molecule II and vice versa (table 1).

4. DISCUSSION

Despte marked similarity in the overall spatial organization of conformations, listed in table 1, they can be divided into three main types: (i) with the γ-turn centred on the Gly^3 residue and stabilized by the (Phe)NH...OC(D-Pen^2) hydrogen

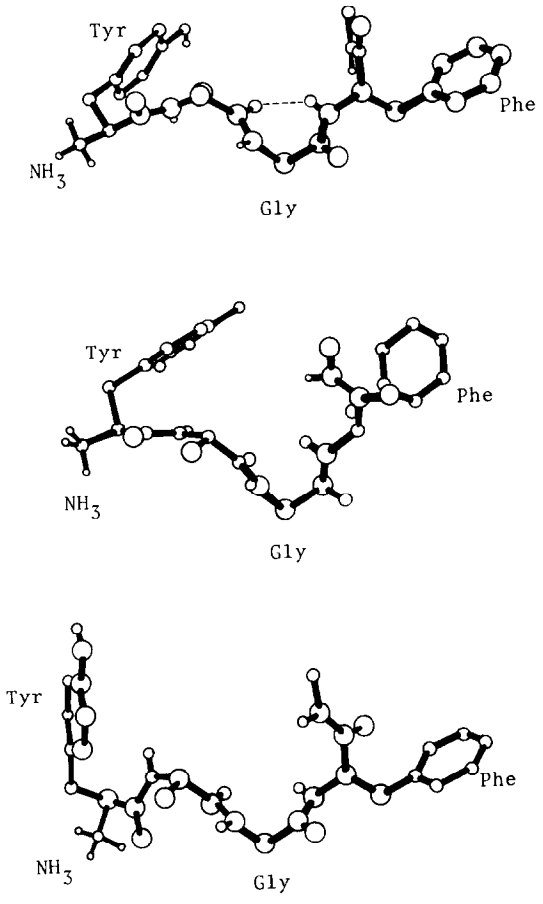

Fig.1. Three types of γ-like turn in the Gly^3 region of peptide backbone inherent to the suggested δ-receptor-bound conformation.

Table 1

Conformations of the peptide backbone fragment 1–4 of molecules I and II sharing geometrical similarity as revealed by intermolecular fitting procedure (angles given in degrees)

Compound	Struct. type	Struct. number	Tyr	D-Pen		Gly		Phe	
			ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ
[D-Pen^2,D-Pen^5]-Ek	I	1	161	75	−139	57	−37	−144	−43
		2	159	144	−98	85	−71	−155	−56
	II	3	158	77	−143	70	27	−165	−60
		4	156	140	−143	68	27	−165	−59
[D-Pen^2,L-Pen^5]-Ek	I	1	161	74	−137	30	−64	−88	3
		2	160	78	−150	77	−78	−76	−37
	II	3	159	77	−143	76	29	−167	−53
		4	161	137	−144	76	29	−167	−52
	III	5	162	69	−141	97	−83	−149	73
		6	161	146	−133	82	−92	−109	67

bond, the NH group of D/L-Pen[5] being directed 'inward' (fig.1a); (ii) with the γ-like turn without hydrogen bonds, the Phe and D/L-Pen[5] amide protons being oriented 'inside' the turn (fig.1b); (iii) with a distorted γ-turn, the Phe NH group being directed 'inward' and the same group in D/L-Pen[5] directed 'outward' (fig.1c). It should be noted that shielding of the D-Pen[5] NH from the solvent has been suggested for molecule I also from the experiment in [4].

Comparison of the structures listed in table 1 with the low-energy conformations of Leu-enkephalin [10,11] performed by the same procedure revealed a certain similarity shared by several structures, the χ_1 (Tyr and Phe) values being nearly 180° (see table 2). The γ-like turn discussed here centred on the residue in position 3 remains in these enkephalin conformations, although the overall shape of the molecular backbone resembles the β-II- or β-II′-turn centred on the Gly[3] and Phe[4] residues (fig.2).

Generally, it can be concluded that the δ-receptor-bound conformations of enkephalin and its analogues should involve a pronounced γ-like turn in the peptide backbone centred on the Gly[3] residue. In such a model the relative spatial orientation of the Tyr and Phe aromatic moieties corresponds to an extended molecular structure rather than to a folded one (see table 2), which does not contradict the conclusions reached in a recent work [12].

Table 2

Geometrically similar conformations of the Leu-enkephalin molecule and its cyclic analogues (example; angles given in degrees)

Compound	Struct. type	Tyr			(D-Pen)/Gly			Gly		Phe			(D/L-Pen)/Leu		
		ϕ	ψ	χ_1	ϕ	ψ	χ_1	ϕ	ψ	ϕ	ψ	χ_1	ϕ	ψ	χ_1
[D-Pen[2],D-Pen[5]]-Ek	II	−64	144	180	70	−143	174	69	27	−165	−58	175	131	−146	−69
[D-Pen[2],L-Pen[5]]-Ek	I	−64	147	180	71	−150	−70	77	−78	−77	−38	180	−83	139	72
	II	−64	144	180	70	−143	174	75	29	−167	−62	180	−70	148	75
Leu-enkephalin	β-II′	−62	121	−177	155	176	–	69	−91	−82	−36	179	−158	111	175
	β-II′	−63	119	180	160	176	–	69	−96	−79	−34	180	−157	106	177
	β-II′	180	139	179	155	−50	–	−154	79	54	31	−162	−161	126	176

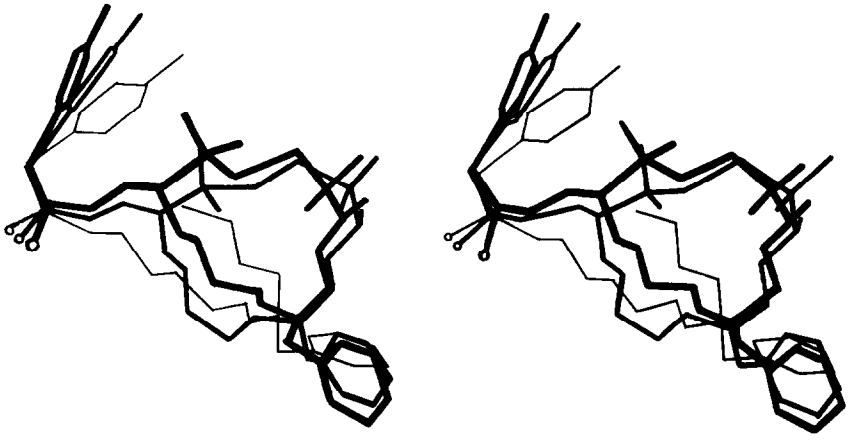

Fig.2. Stereoview of the δ-receptor-bound conformation. The peptide backbone, disulphide bond, aromatic acid side chains and α-amino group (circle) are depicted. [D-Pen[2],D-Pen[5]]-Ek, [D-Pen[2],L-Pen[5]]-Ek, and enkephalin are drawn in thick, normal and thin line, respectively.

REFERENCES

[1] Hansen, P.E. and Morgan, B.A. (1984) in: The Peptides – Analysis, Synthesis, Biology (Udenfriend, S. and Meienhofer, J. eds) vol.8, pp.269–321, Academic Press, London.
[2] Schiller, P.W. (1984) in: The Peptides – Analysis, Synthesis, Biology (Udenfriend, S. and Meienhofer, J. eds) vol.6, pp.219–268, Academic Press, London.
[3] Mossberg, H.I., Hurst, R., Hruby, V.U., Gee, K., Yamamura, H.I., Galligan, J.J. and Burks, T.F. (1983) Proc. Natl. Acad. Sci. USA 80, 5871–5874.
[4] Mossberg, H.I. (1987) Int. J. Peptide Protein Res. 29, 282–288.
[5] Momany, F.A., McGuire, R.F., Burgess, A.W. and Scheraga, H.A. (1975) J. Phys. Chem. 79, 2361–2381.
[6] Dunfield, L.G., Burgess, A.W. and Scheraga, H.A. (1978) J. Phys. Chem. 82, 2609–2616.
[7] Lewis, P.N., Momany, F.A. and Scheraga, H.A. (1973) Isr. J. Chem. 11, 121–152.
[8] Nyburg, S.C. (1974) Acta Cryst. B30, part I, 251–253.
[9] Nikiforovich, G.V., Shenderovich, M.D. and Balodis, J. (1981) Bioorgan. Khim. 7, 179–188.
[10] Balodis, J.J., Nikiforovich, G.B., Grinsteine, I.V., Vegner, R.E. and Chipens, G.I. (1978) FEBS Lett. 86, 239–242.
[11] Betins, J., Nikiforovich, G.V. and Chipens, G.I. (1986) J. Mol. Struct. (Theochem.) 137, 129–132.
[12] Doi, M., Tanaka, M., Ishida, T. and Inoue, M. (1987) FEBS Lett. 213, 265–268.