Volume 213, number 2, 265–268 FEB 04522 March 1987

The three-dimensional similarity between a dimeric antiparallel extended structure and a β-turn folded form of enkephalin

Mitsunobu Doi, Masayuki Tanaka, Toshimasa Ishida and Masatoshi Inoue

Osaka University of Pharmaceutical Sciences, 2-10-65 Kawai, Matsubara-City, Osaka 580, Japan

Received 13 January 1987

The three-dimensional similarity between two fundamental conformations, a dimeric antiparallel extended structure and a type I′ β-turn folded form, of enkephalin was examined by computer graphic and empirical energy calculation methods. By the rotation of Tyr and Phe side chains, one half of the former structure could mimic the three-dimensional form of the latter without a large loss of conformational energy. This result provides a new idea for considering the conformation of enkephalin suitable for the multiple opioid receptors. The active conformation of enkephalin for μ- and δ-opioid receptors is discussed in the light of the present study.

Enkephalin; β-Turn conformation; Extended dimeric conformation; Energy calculation; Conformational similarity; Selectivity

1. INTRODUCTION

Pharmacological and biochemical studies of opioid peptides have revealed the existence of several subclasses of opioid receptors [1–4]. Among them, the μ- and δ-opioid receptors have been most widely discussed. Despite numerous efforts, however, the structural characteristics of opioids suitable for the respective receptors remain to be elucidated.

Recently, the crystal structures of two endogenous opioid pentapeptides, Met^5- and Leu^5-enkephalins, have been analyzed by X-ray diffraction methods [5–12], and two different forms have been characterized as the fundamental conformations of enkephalin: a type I′ β-turn folded form and a dimeric antiparallel extended structure (see fig.1). These two conformations

Correspondence address: M. Doi or T. Ishida, Osaka University of Pharmaceutical Sciences, 2-10-65 Kawai, Matsubara-City, Osaka 580, Japan

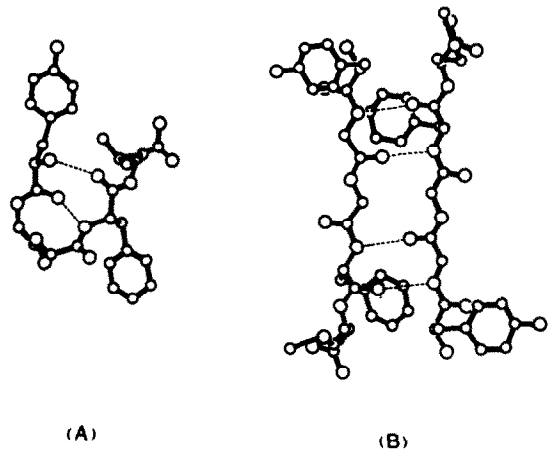

Fig.1. Two fundamental conformations of enkephalin. (A) Type I′ β-turn folded form which has two intramolecular hydrogen bonds (from [7]); (B) a dimeric antiparallel extended structure which is stabilized by four intermolecular hydrogen bonds (from [12]). Structures A and B are denoted as forms I and II in the text, respectively.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

(henceforth denoted as forms I and II, respectively) have also been suggested to be predominant in a solution with a conformational equilibrium [13].

Although it is well known that enkephalin binds to both μ- and δ-receptors [14], it is at present unclear whether the same or a different conformation of enkephalin binds to these receptors. The present report suggests the possibility that either form I or II can simultaneously bind to both receptors.

2. METHODS AND PROCEDURES

Atomic coordinates obtained by X-ray crystal analyses were used for this study: form I was built from Leu^5-enkephalin [7] and form II from Met^5-enkephalin [12]. As is obvious from fig.1, the width between the antiparallel-running backbone chain of form I is almost the same as that of form II (4.5 Å). To inspect the three-dimensional similarity between both forms, therefore, the relative dispositions of Tyr^1, Phe^4 and Met^5 or Leu^5 side chains were considered. The similarity was monitored by the three distances O(Tyr^1)–C$_\alpha$(Gly^3), O(Tyr^1)–C$_\gamma$(Phe^4) and C$_\alpha$-(Gly^3)–C$_\gamma$(Phe^4), where O(Tyr^1), C$_\alpha$(Gly^3) and C$_\gamma$(Phe^4) denote the phenolic oxygen atoms of Tyr^1, C$_\alpha$ atom of Gly^3 and C$_\gamma$ atom of Phe^4, respectively. These amino acid residues are absolutely necessary for the activity emergence of enkephalin [15].

We attempted to fit the conformation of form II to form I by rotation of the Tyr^1 and Phe^4 side chains. Both conformations were then superimposed by the shift of form I so as to minimize the value of $(\Sigma_{i=1}^{N} di)/N$, where di is the distance between the C$_\alpha$ atoms of the i-th residues in both forms, and N is the number of residues (= 5).

The energy change accompanying the rotation was calculated by the empirical PPF (partitioned potential function) method. The energy functions involved in the calculations were nonbonded, electrostatic and torsional energies, respectively. Details of the calculation procedure and the parameters used for the calculations have been presented in [16].

3. RESULTS AND DISCUSSION

In fig.2 is shown the energy variation accompanying the rotation (χ^1) about the C$_\alpha$–C$_\beta$ bond of the Tyr^1 or Phe^4 side chain in form II; the conformation about the C$_\beta$–C$_\gamma$ bond was optimized only within ± 4° of the value observed in the crystal structure, because of the values being nearly equal (−89 and 95° for Tyr^1 and Phe^4 of form I and −89 and −87° for those of form II, respectively) and being in an energetically stable region [17,18]. Fig.2 shows that the rotation about the C$_\alpha$–C$_\beta$ bond does not significantly make the extended dimer structure unstable. A maximum energy gap of 1.81 kcal/mol would be compensated by the proper external factors such as the interaction with

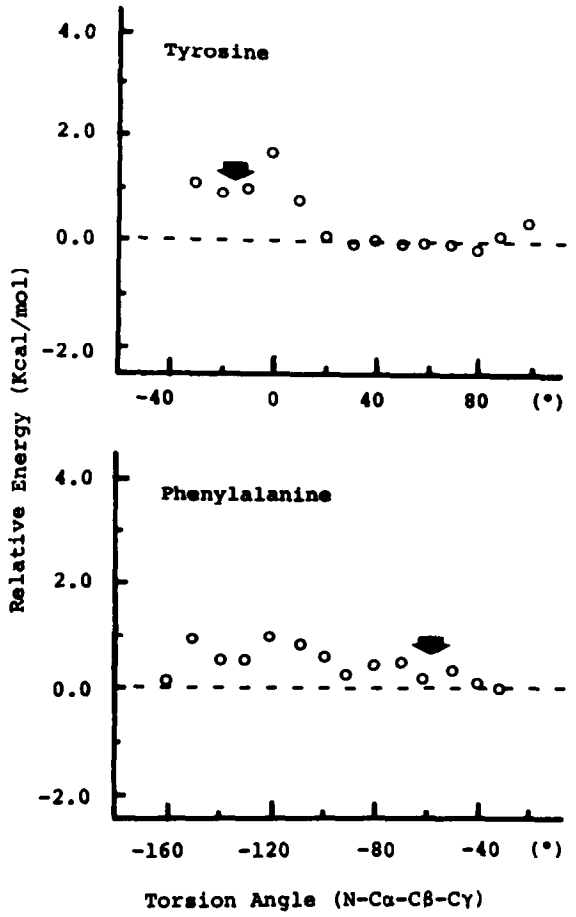

Fig.2. Relative energy of profiles of form II accompanying rotation of angle χ^1 of Tyr or Phe side chains. The profile is represented as the energy difference from the energy at χ^1 = 60° for Tyr, or χ^1 = −40° for Phe, respectively. The arrow indicates the torsion angle used for the superimposition.

opioid receptors ('Zipper' models for ligand binding [19]). The high flexibilities of Tyr^1 and Phe^4 side chains, along with those of Met^5 or Leu^5, have also been supposed from the crystal structures; these side chains are in the various conformation states.

Fig.3 shows the three-dimensional similarity between forms I and II, where the former was superimposed on the latter. One half of form II is nicely fitted to form I of enkephalin. The distances $O(Tyr^1)$–$C_\alpha(Gly^3)$, $O(Tyr^1)$–$C_\gamma(Phe^4)$ and $C_\alpha(Gly^3)$–$C_\gamma(Phe^4)$ are 11.28, 11.75 and 4.60 Å for form I and 11.80, 10.93 and 5.04 Å for form II, respectively, and $(\Sigma_{i=1}^{N} d_i)/N$ is 1.20 Å. Although the relative orientation of each Tyr^1 aromatic ring with respect to its backbone chain is different, the positions of phenolic oxygen atoms are almost in the same site (the deviation between their oxygen atoms is 1.30 Å).

The opioid selectivity for the μ-receptor has been well discussed, based on the conformational similarity between form I of enkephalin and μ-specific morphine [5,7,13]. On the other hand, the conformation of enkephalin suitable for δ-opioid receptors could be supposed to be form II, as judged from the various data; for example, the dimerization of μ-specific des-Leu^5-[D-Ala^2,Phe-NH_2^4]enkephalin is converted into the δ-specific form [20,21], and the stable conformation of δ-specific deltakephalin is predominantly in an extended structure (Ishida, T. et al., in preparation). Provided that forms I and II of enkephalin are the active conformations for μ- and δ-receptors, respectively, the present results imply that form II can also interact with the μ-receptor, and alternately, two enkephalins of form I, which are nearly related by a diad symmetry, with the δ-receptor; in other words, both forms of enkephalin can bind simultaneously to μ- and δ-receptors. This hypothesis can account well for the fact that enkephalin analogues with pure μ or δ activity have not yet been synthesized and, further, why intact enkephalin shows activity with μ- and δ-receptors.

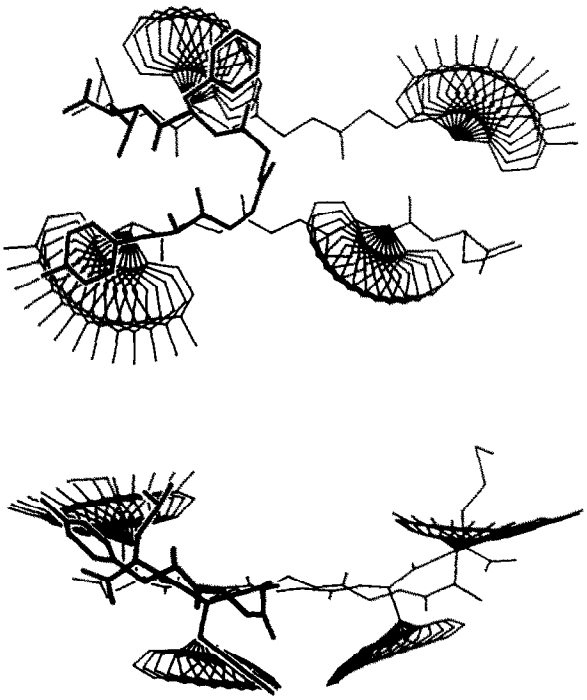

Fig.3. Conformational similarity between forms I and II. Form I was superimposed on form II. The Tyr and Phe side chains of form II were rotated at 10° intervals to fit three relative positions of Tyr, Gly and Phe to those of form I (see text).

REFERENCES

[1] Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E. and Gilbert, P.E. (1976) J. Pharmacol. Exp. Ther. 197, 517–532.

[2] Lord, J.A.H., Waterfield, A.A., Hughes, J. and Kosterlitz, H.W. (1977) Nature 267, 495–499.

[3] Chang, K.J. and Cuatrecasas, P. (1979) J. Biol. Chem. 254, 2610–2618.

[4] Corbett, A.D., Paterson, S.J., McKnight, A.T., Magran, J. and Kosterlitz, H.W. (1982) Nature 299, 79–81.

[5] Smith, G.D. and Griffin, J.F. (1978) Science 199, 1214–1216.

[6] Karle, I.L., Karle, J., Mastropaolo, D., Camerman, A. and Camerman, N. (1983) Acta Crystallogr. B 39, 625–637.

[7] Ishida, T., Kenmotsu, M., Mino, Y., Inoue, M., Fujiwara, T., Tomita, K., Kimura, T. and Sakakibara, S. (1984) Biochem. J. 218, 677–689.

[8] Doi, M., Ishida, T., Inoue, M., Fujiwara, T., Tomita, K., Kimura, T. and Sakakibara, S. (1984) FEBS Lett. 170, 229–231.

[9] Mastropaolo, D., Camerman, A. and Camerman, N. (1986) Biochem. Biophys. Res. Commun. 134, 698–703.

[10] Stezowski, J.J., Eckle, E. and Bajusz, S. (1985) J. Chem. Soc. Chem. Commun. 1985, 681–682.

[11] Griffin, J.F., Langs, D.A., Smith, G.D., Blundell, T.L., Tickle, I.J. and Bedarkar, S. (1986) Proc. Natl. Acad. Sci. USA 83, 3272–3276.
[12] Doi, M., Tanaka, M., Ishida, T., Inoue, M., Fujiwara, T., Tomita, K., Kimura, T., Sakakibara, S. and Sheldrick, G.M. (1987) J. Biochem., in press.
[13] Schiller, P.W. (1984) in: The Peptides – Analysis, Synthesis, Biology (Udenfriend, S. and Meienhofer, J. eds) vol.6, pp.219–268, Academic Press, London.
[14] Paterson, S.J., Robson, L.E. and Kosterlitz, H.W. (1984) in: The Peptides –Analysis, Synthesis, Biology (Udenfriend, S. and Meienhofer, J. eds) vol.6, pp.147–189, Academic Press, London.
[15] Morley, J.S. (1980) Annu. Rev. Pharmacol. Toxicol. 20, 81–110.
[16] Miyamoto, Y., Ishida, T. and Inoue, M. (1981) Chem. Pharm. Bull. 29, 3427–3432.
[17] Manavalan, P. and Momany, P.A. (1981) Int. J. Peptide Protein Res. 18, 256–275.
[18] Cody, V. (1985) in: Chemistry and Biochemistry of the Amino Acids (Barrett, G.C. ed.) pp.625–653, Chapman and Hall, London.
[19] Burgen, A.S.V., Roberts, G.C.K. and Freeney, J. (1975) Nature 253, 753–755.
[20] Shimohigashi, Y., Costa, T., Chen, H.C. and Rodbard, D. (1982) Nature 297, 333–335.
[21] Shimohigashi, Y., English, M.L., Stammer, C.H. and Costa, T. (1982) Biochem. Biophys. Res. Commun. 104, 583–590.