J Mol Model (2007) 13:171–177
DOI 10.1007/s00894-006-0139-6

ORIGINAL PAPER

Conformational stability and three-dimensional model of the δ-opioid pharmacophore for the extended antiparallel dimer structure of Met-enkephalin in water

Yng-Ching Wu · Jin-Yuan Hsieh · Hong-Chang Lin · Chi-Chuan Hwang

Received: 6 April 2006 / Accepted: 4 July 2006 / Published online: 14 September 2006

Abstract The conformational stability of the extended antiparallel dimer structure of Met-enkephalin in water was analyzed by examining the hydration structure of enkephalin using molecular dynamics simulations. The result shows that, despite of the hydrophicility of the terminal atoms in the pentapeptide, the main contributor for the stability of the dimer in water is the four intermolecular hydrogen bonds between the Gly^2 and Phe^4 groups. The three-dimensional model of the δ-opioid pharmacophore for this dimer structure was also established. Such a model was demonstrated to match the δ-opioid pharmacophore query derived from the non-peptides SIOM, TAN-67, and OMI perfectly. This result thus strongly supports the assumption that the dimer structure of Met-enkephalin is a possible δ-receptor binding conformation.

Keywords Extended antiparallel dimer · Molecular dynamics · δ-opioid pharmacophore

Introduction

Met-enkephalin and Leu-enkephalin are two endogenous opioid peptides that exhibit analgesic activity [1]. Their physiological effects derive from their interactions with the opioid receptor, which is grouped into three subclasses, namely, μ-, δ-, and κ-receptors [2]. Therefore, it is of particular importance to consider in terms of stereochemistry the reasons why enkephalin binds to these receptors.

It is known that the crystal structures of Met- and Leu-enkephalin include a β-bend monomeric structure and an extended antiparallel dimer structure from both X-ray crystallographic studies [3–10] and molecular dynamics (MD) simulations [11–13]. The monomeric structure was found to be the active conformation responsible for binding with the μ-receptor, while the dimer structure of the enkephalins, although not found to exist so far at physiological concentrations, was suggested to be the active conformation suitable for binding with the δ-receptor [3, 9, 14].

That the dimer structure of enkephalin could be responsible for binding with the δ-receptor is of particular interest in the attempt to design new and potent analgesics because the analgesia mediated through the δ-receptor does not seem to induce side effects, such as drug dependence, respiratory depression, and others [15]. Shenderovich et al. [16] have proposed a δ-opioid pharmacophore query for determining whether a conformation of an opioid peptide is suitable for binding with the δ-receptor. They presented a three-dimensional (3D) pharmacophore model derived from the non-peptide opiates SIOM, TAN-67, and OMI, which bind to a common site at the δ-receptor, and hypothesized that opioid peptides might bind to the same site of the δ-receptor as the non-peptide agonists do if their conformers fit the non-peptide pharmacophore.

In this article, MD simulations were performed in order first to study the stable state of the dimer structure of Met-enkephalin in aqueous solution. Since the dimer structure of Met-enkephalin has two peptide molecules positioned in antiparallel configuration and is simulated as a solute surrounded by water molecules, the stability mechanism

Y.-C. Wu · H.-C. Lin · C.-C. Hwang (✉)
Department of Engineering Science,
National Cheng Kung University,
Tainan 701, Taiwan
e-mail: chchwang@mail.ncku.edu.tw

J.-Y. Hsieh
Department of Mechanical Engineering,
Ming Hsin University of Science and Technology,
Hsinchu 304, Taiwan

for its existence should be strongly relevant to the interactions between the two peptides and those between the enkephalin and water atoms. Therefore, the stability mechanism for the present case, which is somewhat different from the one explained by Yoneda et al. [13], will be complementarily explained by examining the intermolecular interactions occurring in the dimer structure, which are attributed to the hydration structure of the Met-enkephalin dimer.

Furthermore, the 3D δ-opioid pharmacophore model for the dimer structure of Met-enkephalin was derived and compared to the non-peptide pharmacophore query presented by Shenderovich et al. [16] to show that with high probability, the dimer conformation of Met-enkephalin does bind with the δ-opioid receptor.

Materials and methods

The MD simulations performed in this study were carried out using the GROMACS version 3.2.1 simulation program with the GROMOS96 (ffG43a1) force field [17, 18]. An extended antiparallel dimer structure of met-enkephalin was simulated to be surrounded by a total of 4,605 water molecules. The initial structure of the dimer was prepared according to the one constructed using the X-ray crystal analysis of [(4-brome) Phe[4], Met[5]] enkephalin [6].

The whole system of the aqueous solution was contained within a cubic box with a side of ca. 52 Å and periodic boundary conditions, so the average density of the solution was kept at ca. 1 g/cm^3. The MD simulations were begun assuming random velocity of the atoms that followed a Maxwellian distribution at 300 K. The atom positions and velocities were integrated according to the standard Verlet algorithm. The integration time step was chosen as Δt=2 fs. The system was first equilibrated at 300 K and the constant pressure relevant to the density of 1 g/cm^3, using the Berendsen algorithm [19]. After the equilibrium was achieved, the time evolutions of all quantities considered in this study were recorded, and the MD simulations were continued for a simulating time domain of 10 ns. In the MD simulations, long-range Coulomb interactions were calculated using the Particle Mesh Ewald (PME) method, and all the non-bonded potentials were truncated with a cutoff radius of 12 Å. The 12 Å cutoff used herein is appropriate in avoiding severe artifacts in the simulations because, as pointed out by Darden et al. [20], the threshold cutoff for the PME method should be of 9 Å.

Results and discussion

Figure 1 schematically plots the extended antiparallel dimer structure of Met-enkephalin. In this figure, *a* and *b* represent the intramolecular distances of $Tyr^1_A(NH_3^+)\ldots Met^5_A(COO^-)$ and $Tyr^1_B(NH_3^+)\ldots Met^5_B\ (COO^-)$ atomic pairs and *c* and *d* denote the intermolecular distances of $Tyr^1_A(NH_3^+)\ldots Met^5_B\ (COO^-)$ and $Tyr^1_B(NH_3^+)\ldots Met^5_A(COO^-)$ atomic pairs, respectively, where the subscripts **a** and **b** each denotes an enkephalin molecule in the dimer structure. In Fig. 1, HB_c and HB_d represent the two possible intermolecular hydrogen bonds between the terminal NH_3^+ and COO^- groups, with lengths *c* and *d*, respectively. In addition, in the same figure, $HB_1 \sim HB_4$ represent the other

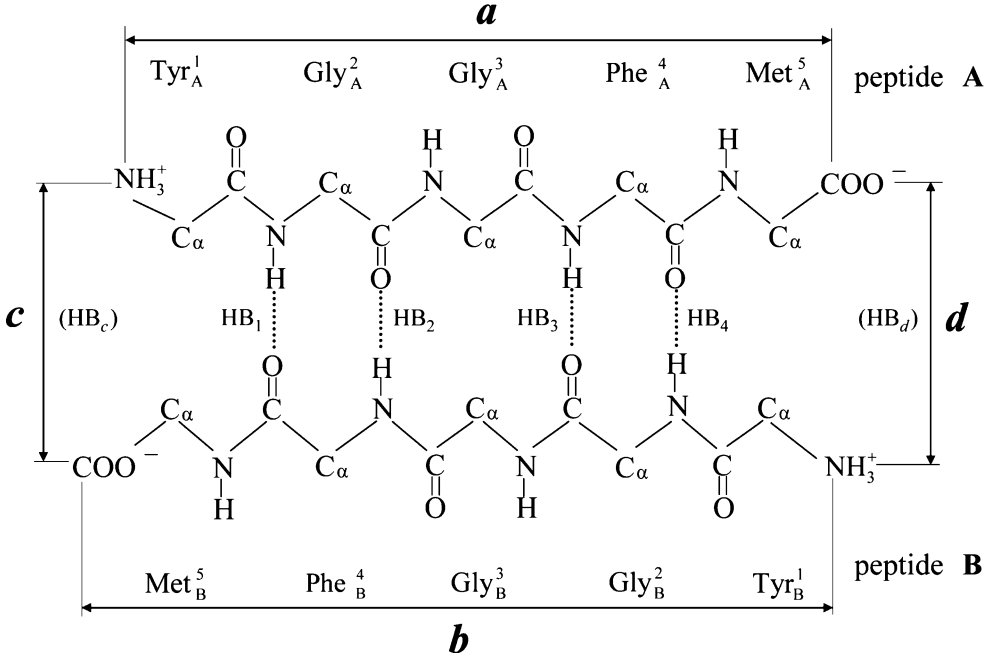

Fig. 1 Schematic model of the extended antiparallel dimer structure of Met-enkephalin

four possible intermolecular hydrogen bonds between $Gly_A^2(N)\ldots Phe_B^4(O)$, $Gly_A^2(O)\ldots Phe_B^4(N)$, $Phe_A^4(N)\ldots Gly_B^2(O)$, and $Phe_A^4(O)\ldots Gly_B^2(N)$ atomic pairs, respectively. In this study, the existence of a hydrogen bond between an atomic pair was determined by the geometric criterion relevant to that atomic pair, i.e., a hydrogen bond exists if the criterion that the length of the bond $r_{HB}< 3.5$ Å and the angle $\alpha_{HB}< 30°$ is fulfilled.

The time evolution of the distances *a*, *b*, *c*, and *d*, together with the time profile of the root mean square deviation (RMSD) value of the C_α atoms in the dimer structure, is shown in Fig. 2. It is clear that, during the simulating time interval, all the distances *a*, *b*, *c*, and *d* maintained approximately constant and the RMSD value rose to about 1.7 nm and became steady soon after 2 ns, implying the stable existence of the dimer structure of Met-enkephalin in water. The existence of hydrogen bonds was recorded after each time step. The amount of the possible intermolecular hydrogen bonds (HB1–HB4) as a function of simulation time are examined and displayed in Fig. 3. The present result reveals that the hydrogen bonds HB_1–HB_4 indeed exist and survive almost throughout the simulating time interval. However, the present result also shows that the other two intermolecular hydrogen bonds HB_c and HB_d can exist only with probabilities of 8.8 and 6.5% (i.e., they appeared at 8.8 and 6.5% of the simulation time steps), respectively.

Obviously, the phenomenon that the hydrogen bonds HB_1–HB_4 exist almost throughout the simulating period yet the bonds HB_c and HB_d do not is strongly relevant to the hydration structure of Met-enkephalin in water. Recently, Dudowicz et al. [21] have performed all-atom MD simulations to show the hydrophilicity and hydrophobicity

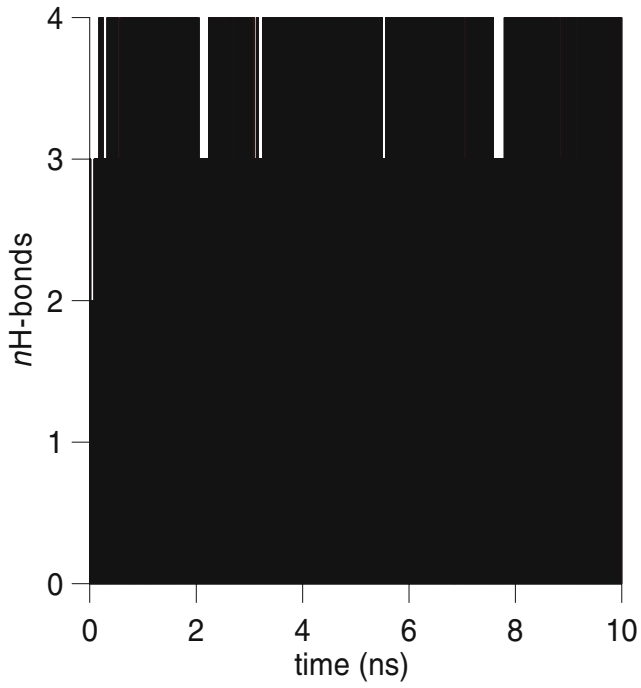

Fig. 3 The amount of the possible intermolecular hydrogen bonds (HB1–HB4) as a function of simulation time. The plotted criterion that the length of the bond $r_{HB}<3.5$ and the angle $\alpha_{HB}<30°$ is fulfilled

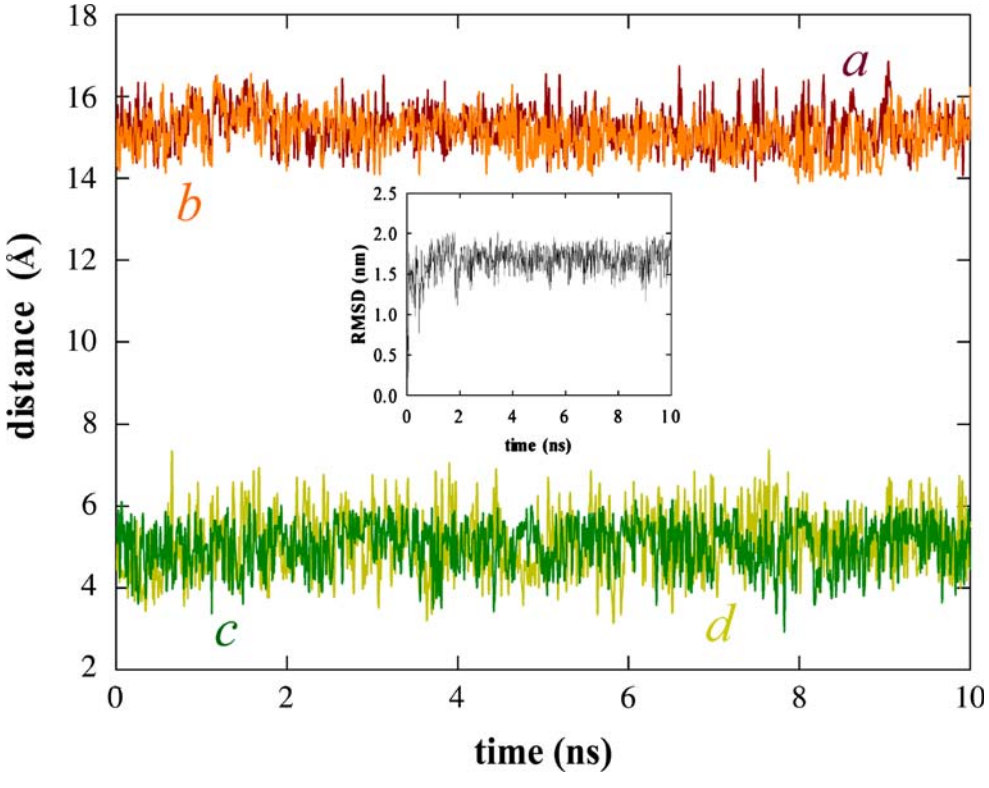

Fig. 2 Time evolutions of the distances *a*, *b*, *c*, and *d*. The inset plots the time profile of the corresponding RMSD value of C_α atoms for the dimer structure of Met-enkephalin

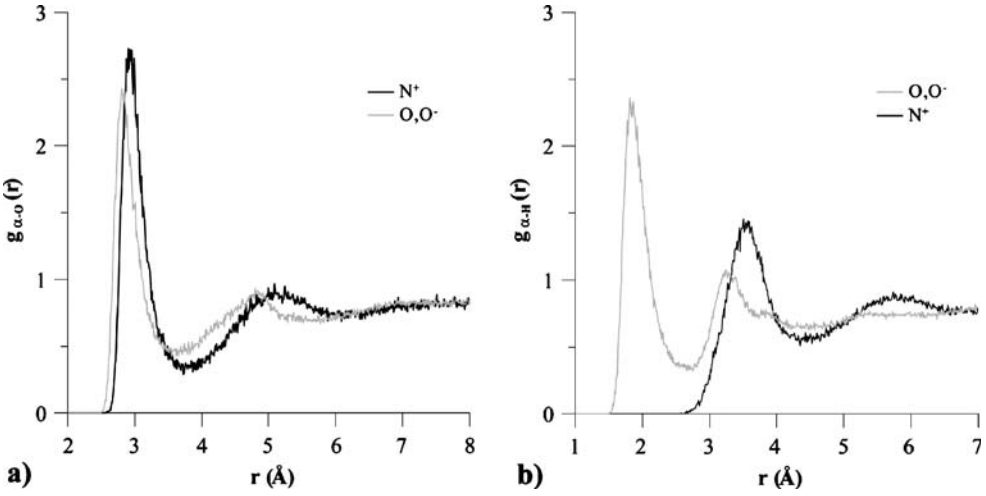

Fig. 4 The solute-solvent radial distribution functions $g_{\alpha-O}(r)$ (**a**) and $g_{\alpha-H}(r)$ (**b**) for the terminal atoms of Met-enkephalin ($\alpha \equiv N^+$, O^-, O). The subscripts O and H represent the oxygen and hydrogen atoms of water

of the individual atoms in a fixed, extended Met-enkephalin. Following Dudowicz et al. [21], in this work, the hydration structure of the dimer of Met-enkephalin was analyzed by calculating the solute-solvent radial distribution functions $g_{\alpha-\beta}(r)$, where α and β represent atoms of Met-enkephalin and water, respectively. Figure 4 plots the solute-solvent radial distribution functions $g_{\alpha-O}(r)$ and $g_{\alpha-H}(r)$ for the charged terminal atoms of Met-enkephalin, including N^+ of the amino group and O^- and O of the carboxyl group. As shown in Fig. 4, the high first neighbor peaks, though somewhat lower than those reported in Ref. [21] for a single Met-enkephalin molecule, are much greater than 1 and thus provide direct evidence of the interactions of the terminal atoms N^+, O^-, and O with water that form strong hydrogen bonds (O^-, O) or strong electrostatic interaction (N^+). As a result, the intermolecular hydrogen bonds HB_c and HB_d therefore rarely occur due to the strong interactions of the terminal atoms with water. Meanwhile, Figs. 5 and 6 plot the solute-solvent radial distribution functions $g_{\alpha-O}(r)$ and $g_{\alpha-H}(r)$ for the nitrogen atom of the amide group and the oxygen atom of the carboxyl group in both the backbone Gly^2 and Phe^4 residues, respectively. It can be clearly observed from Figs. 5 and 6 that the interactions of the nitrogen and oxygen atoms in both the Gly^2 and Phe^4 residues with water are relatively weak. (However, in single Met-enkephalin these oxygen atoms interact strongly with water [21]). Instead, these backbone nitrogen and oxygen atoms have strong interactions with those in the neighboring peptide in the same dimer. As a consequence, the intermolecular hydrogen bonds HB_1–HB_4 can be observed in the stable dimer structure of Met-enkephalin almost in the entire simulation time.

In Ref. [13], Yoneda et al. reported that the cause for the extended antiparallel dimer of Met-enkephalin to exist stably in water is the two intermolecular hydrogen bonds between the terminal NH_3^+ and COO^- groups. However, the simulation time used in the MD computation of Ref. [13] was only 60 ps, while in the present study the time required for the initial structure to become stable in water was shown at least more than 2 ns (as shown in the inset of Fig. 2). As a consequence, the insufficient simulation time employed by Yoneda et al. therefore leads to the

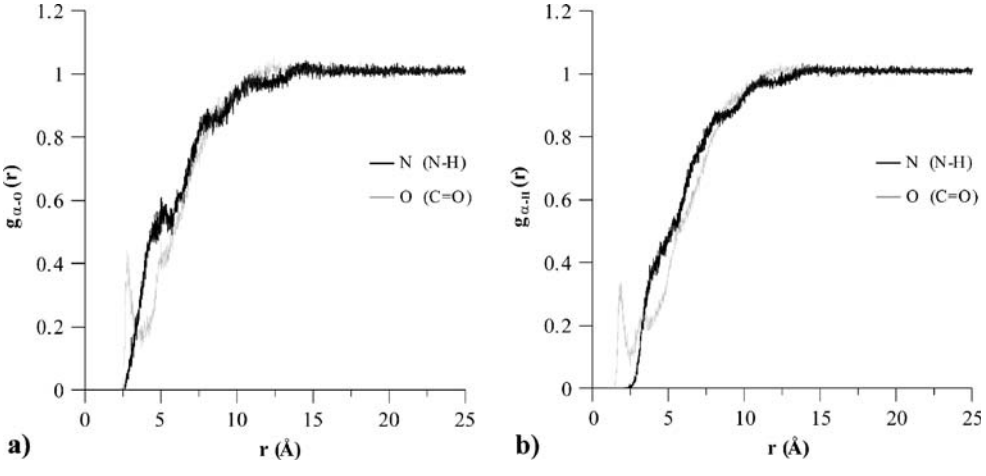

Fig. 5 The solute-solvent radial distribution functions $g_{\alpha-O}(r)$ (**a**) and $g_{\alpha-H}(r)$ (**b**) for the nitrogen atom of the amide group and the oxygen atom of the carboxyl group in the backbone Gly^2 residue ($\alpha \equiv$ N, O)

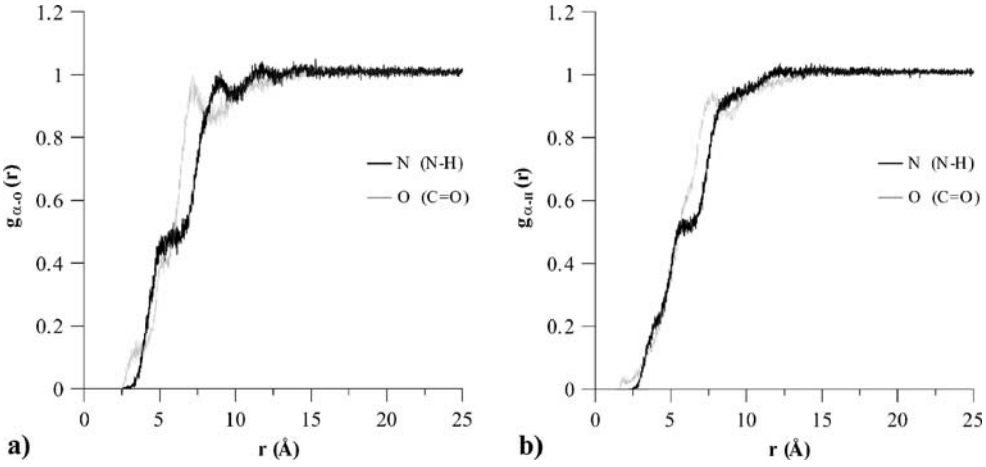

Fig. 6 The solute-solvent radial distribution functions $g_{\alpha-O}(r)$ (**a**) and $g_{\alpha-H}(r)$ (**b**) for the nitrogen atom of the amide group and the oxygen atom of the carboxyl group in the backbone Phe[4] residue ($\alpha \equiv$ N, O)

discrepancy in the stability mechanisms indicated in Ref. [13] and the present study that account for the existence of the extended antiparallel dimer structure of Met-enkephalin in water.

In addition, because of the extended backbone and the constrained conformational mobility of the pentapeptide, the dimer structure of Met-enkephalin was believed to be responsible for binding with the δ-opioid receptor. Therefore, the 3D-arrangements of the δ-opioid pharmacophore groups in the extended antiparallel pentapeptides were studied to determine whether the dimer conformation of Met-enkephalin is a potent and selective ligand for the δ-opioid receptor. The present study followed the hypothesis proposed by Shenderovich et al. [16], which states that potent peptide and non-peptide agonists of the δ-opioid receptor may have a common 3D-arrangement of pharmacophore groups upon binding to the δ-receptor. Accordingly, simulated results were compared with the δ-opioid pharmacophore query determined from the best fit conformations of the non-peptide ligands SIOM, TAN-67, and OMI [16]. The data of the computational experiment were obtained by measuring distances and angles among the specific four points involving the nitrogen atom (N) of the amino group, centroids of the aromatic rings of Tyr[1] and Phe[4] (*R*1 and *R*2), and oxygen of the phenol ring (OH).

From the comparative 3D-model of the δ-opioid pharmacophore for peptide and non-peptide ligands shown in Fig. 7, it is clearly observed that the present results for the peptide pharmacophore perfectly match the non-peptide pharmacophore query. Figure 8 shows the time evolutions of *R*1–*R*2 distance for both peptides *A* and *B* in the dimer structure of Met-enkephalin, respectively. The limits of the ring-to-ring distance defined by the aforementioned non-peptide pharmacophore query are also shown in Fig. 7c for the sake of comparison. From Fig. 8, it can clearly be observed that for most of the time in the computational time domain 95% for peptide *A* and 81% for *B*, respectively, the data were located between the limits for the *R*1–*R*2 distance in the non-peptide pharmacophore query. Furthermore, clear MD trajectories of the *R*1–*R*2, N-*R*1, and N-*R*2 distances for peptide *A* and *B* are presented in Fig. 9, where the colored box in each diagram represents the corresponding limits for the non-peptide pharmacophore query. More than 75% of the trajectory marks for peptide *A* and 71% for peptide *B* were in the boxes, as shown in Fig. 9. The results presented in Figs. 7, 8, and 9 therefore provide strong support for the assumption that the dimer structure of Met-enkephalin is a possible δ-receptor binding conformation.

Conclusions

In conclusion, this study has performed MD simulations for the time evolution of dynamics for the dimer structure of

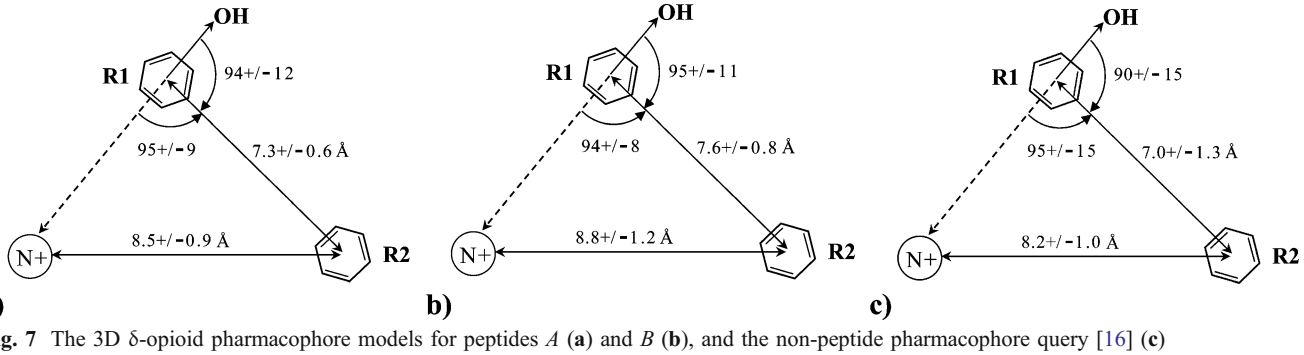

Fig. 7 The 3D δ-opioid pharmacophore models for peptides *A* (**a**) and *B* (**b**), and the non-peptide pharmacophore query [16] (**c**)

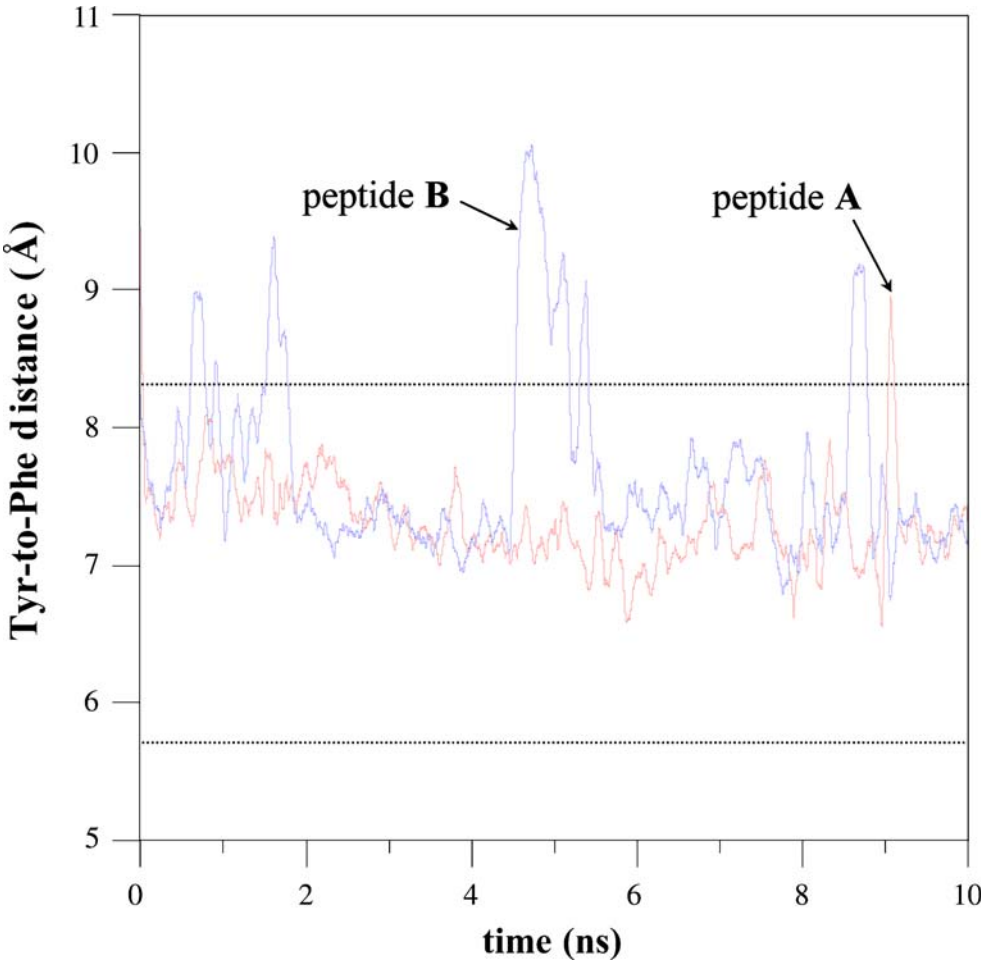

Fig. 8 Time evolutions of *R*1–*R*2 distance for peptides *A* and *B*, respectively. The two horizontal dotted lines represent the limits given in the non-peptide pharmacophore query [16]

Met-enkephalin in water. The results support the proposal that the dimer structure can exist stably in water mainly because of the four strong intermolecular hydrogen bonds between the Gly^2 and Phe^4 groups of the pentapeptides, which are relevant to the hydration structure of the enkephalin dimer. The stability mechanism herein was complementarily explained by examining the intermolecular interactions occurring in the dimer structure, which are attributed to the hydration structure of the Met-enkephalin dimer. In addition, the analysis of 3D-arrangements of the δ-opioid pharmacophore for the pentapeptides in the dimer structure of Met-enkephalin also strongly supports the assumption that the dimer structure of Met-enkephalin is a possible δ-receptor binding conformation. It should be noticed that the key concern herein is the stability mechanism of the extended antiparallel dimer structure of Met-enkephalin in water, so the 10 ns simulation time is appropriate for each simulation performed in this work. If,

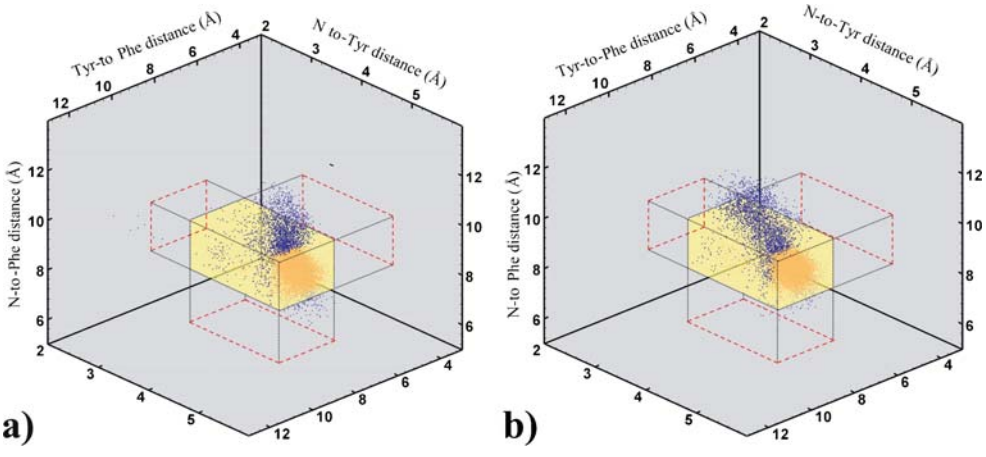

Fig. 9 The MD trajectories representing the *R*1–*R*2, N-*R*1, and N-*R*2 distances for both peptides *A* (**a**) and *B* (**b**) in the dimer. In each diagram the colored box represents the corresponding limits given in the non-peptide pharmacophore query

however, the ligand-receptor binding is to be dealt with, then such a nanosecond time scale could be insufficient. The receptor binding problem is beyond the scope of this work.

Acknowledgment The authors gratefully acknowledge the financial support to this research by the National Science Council, Taiwan, under Grant Nos. NSC 93-2522-S-006-006 and NSC 94-2522-S-006-002.

References

1. Hughes J, Smith TW, Kosterlitz HW, Fothergill LA, Morgan BA, Morris HR (1975) Nature 258:577–579
2. Simon EJ, Gioannini TL (1993) Opioid receptor multiplicity: isolation, purification, and chemical characterization of binding sites. In: Herz A (ed) Opioids I. Springer Berlin Heidelberg New York pp 3–26
3. Griffin JF, Langs DA, Smith GD, Blundell TL, Tickle IJ, Bedarkar S (1986) Proc Natl Acad Sci USA 83:3272–3276
4. Mastropaolo D, Camerman A, Camerman N (1986) Biochem Biophys Res Commun 134:698–703
5. Ishida T, Kenmotsu M, Mino Y, Inoue M, Fujiwara T, Tomita K, Kimura T, Sakakibara S (1984) Biochem J 218:677–689
6. Doi M, Tanaka M, Ishida T, Inoue M, Fujiwara T, Tomita K, Kimura T, Sakakibara S, Sheldrick GM (1987) J Biochem (Tokyo) 101:485–490
7. Mastropaolo D, Camerman A, Ma LY, Camerman N (1987) Life Sci 40:1995–1999
8. Karle IL, Karle J, Mastropaolo D, Camerman A, Camerman N (1983) Acta Crystallogr B39:625–637
9. Aubry A, Birlirakis N, Sakarellos-Daitsiotis M, Sakarellos C, Marraud M (1989) Biopolymers 28:27–40
10. Smith D, Griffin JF (1978) Science 199:1214–1216
11. Shen MY, Freed KF (2002) Biophys J 82:1791–1808
12. van der Spoel D, Berendsen HJ (1997) Biophys J 72:2032–2041
13. Yoneda S, Kitamura K, Doi M, Inoue M, Ishida T (1988) FEBS Lett 239:271–275
14. Hruby VJ, Agnes RS (1999) Biopolymers 51:391–410
15. Porreca F, Bilsky EJ, Raffa RB, Lai J (1995) Pharmacological characterization of delta- and kappa-receptors. In: Tseng LF (ed) The pharmacology of opioid peptides. Harwood Academic Publishers, Germany, pp 219–248
16. Shenderovich MD, Liao S, Qian X, Hruby VJ (2000) Biopolymers 53:565–580
17. Lindahl E, Hess B, van der Spoel D (2001) J Mol Model 7:306–317
18. Scott WRP, Huenenberger P, Tironi I, Mark A, Billeter S, Fennen J, Torda A, Huber T, Krueger P, van Gunsteren W (1999) J Phys Chem A 103:3596–3607
19. Berendsen HJC, Postma JPM, van Gunsteren WF, Dinola A, Haak JR (1984) J Chem Phys 81:3684–3690
20. Darden T, York D, Pedersen L (1993) J Chem Phys 98:10089–10092
21. Dudowicz J, Freed KF, Shen MY (2003) J Chem Phys 118:1989–1995