

Structure-Activity Relationships of Leu-Enkephalin Analog with (4-Carboxamido)Phenylalanine Substituted for Tyrosine: A Molecular Dynamics Study

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ABSTRACT:

Motivated by recent experimental work on Leu-Enkephalin modification with (4-Carboxamido)phenylalanine (Cpa), we perform MD simulations to study the structure-activity relationships of the [Cpa¹, Leu⁵]-enkephalin (Cpa-LE) for better understandings of the binding affinity in δ -selective opioid ligands. Recently, Tyr¹ substituted into Cpa¹ form was experimentally found to be the first example of an amino acid that acts as a surrogate for Tyr¹ in opioid peptide ligands, which challenges a long-standing belief that a phenolic residue is required for high affinity binding. Our simulations show the Cpa-LE structure in aqueous solution revealed that the occurrence of single-bend packed state can be stabilized by an intramolecular hydrogen bond from Leu⁵-NH to Gly²-CO (5 $\rightarrow$ 2). In addition, an intramolecular sidechain to backbone hydrogen bond, i.e., hydrogen bond binding between the sidechain carbonyl CO group of the Cpa residue and backbone amide NH group of the Phe residue was examined. Furthermore, the hydration effects of carboxamido group (CONH₂) for Cpa residue

and 5 $\rightarrow$ 2 hydrogen bond were calculated via the solute-solvent radial distribution functions $g_{\alpha-\beta}(r)$, providing direct evidence of strong hydrogen bond interactions. Our simulation results further reveal the χ^1 rotamers of the Cpa¹ and Phe⁴ that show preferences for trans and gauche (–), respectively. Finally, we elucidate the probability distributions of two aromatic rings among the Cpa-LE, Leu-enkephalin, and δ pharmacophore model. The results show that modified the Tyr¹ to Cpa¹ can lead to increase the potency and selectivity for δ -opioid receptor (DOR), consistent with experimental findings. © 2007 Wiley Periodicals, Inc. *Biopolymers* 86: 231–239, 2007.

Keywords: molecular dynamics; radial distribution functions; deltaparmacophore model; delta-opioid receptor

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INTRODUCTION

There are three well defined types of opioid receptors, the δ , μ , and κ receptors,^{1–3} all of which belong to the G-protein-coupled receptor (GPCR) family, have been provided a source for speculation on the primary events that lead to each of the multiple in vivo effects in opioid ligands. Endogenous opioid peptides have been studied extensively since their discovery,^{4–7} aiming at

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discovering a perfect analgesic and devoiding side effects. Further investigations of the structural and functional aspects of these bioactive peptides for highly selective agonists and antagonists as pharmacological tools in opioid research are still quite active. Since the endogenous opioid peptides mostly are conformationally flexible leading to loss of specificity in selectivity with respect to their receptors, hence the peptide ligand design has been developed recently in which careful considerations are required for both the structural features of peptide ligands and detailed analyses of their biological activities.⁸

Leu-Enkephalin (Tyr-Gly-Gly-Phe-Leu) is one of endogenous opioid peptides with morphine-like activity,⁴ which possess a slight preference for δ -opioid receptor (DOR) over μ -opioid receptor (MOR). Structural studies indicate that Leu-enkephalin exhibits many different conformations. In the crystalline state, Leu-Enkephalin has been shown to exist in only three conformations, i.e. the single β -bend,⁹ double β -bend,¹⁰ and extended.¹¹ On the other hand, numerous experimental studies have been made concerning the structure of Leu-Enkephalin in aqueous solution.^{12–14} The results of ¹H NMR, ¹³C NMR, and CD spectroscopy show that Leu-Enkephalin possesses folded forms in different solvents. Although some of the structural studies for Leu-Enkephalin have focused on nonaqueous environments that permit the minimized energy characterization of a single conformation, it is well known that the nature of the solvent encasing the peptide is important.^{15,16} Furthermore, it is believed that the solvents have a sizable effect on the conformational switch of the peptides.¹⁷ In theoretical calculations, MD simulations have been performed to analyze the structural features of Leu-Enkephalin in different circumstances, such as aqueous or nonaqueous environments. van der Spoel et al. have performed the Leu-Enkephalin in water and dimethyl sulfoxide (dimethylsulfoxide, DMSO).¹⁸ Their results show the peptide seems to be a preference for a bend structure in water despite its structure is very flexible. In DMSO, the peptide forms a clear salt bridge in the zwitterionic form, but has no preferred conformation in the neutral form. By analyzing the statistics of the resulting clusters, Nielsen et al. examine the probability distributions of the sidechain conformations with Leu-Enkephalin and Met-Enkephalin.¹⁹ These probabilities give rise to reasonable speculation for the selectivity depending on the ratio of δ to μ receptors. Recently, by means of MD simulations, Karvounis et al. use the hydration effect to investigate the structural feature of Leu-Enkephalin.²⁰ Their results demonstrate how the specific water network rearrangements can lead to the formation of critical water bridges that define the structure of Leu-Enkephalin. Nevertheless, the structure–activity studies of the aforementioned

treatments by and large focus on the endogenous enkephalins, i.e. Met- and Leu-Enkephalin.

It is known that changing a few atoms, bonds, or torsional angles in a peptide ligand can have a sizable effect on the binding and transduction properties with their receptors, and this in turn can result in dramatically different biological behaviors. Therefore, based on the peptide ligand design, Dolle et al.²¹ proposed the use of (4-Carboxamido)phenylalanine (Cpa) as a bioisosteric replacement for the terminal tyrosine residue to test a series of opioid peptide ligands. For example, Leu-Enkephalin, Endomorphin-1, and Dynorphin(1-11) were examined for their increasing or decreasing binding affinity to δ , μ , and κ -opioid receptor (KOR), respectively. Consequent experimental results show that the Cpa analogs of the δ -selective peptides have an increase in δ selectivity, in contrast to the responses of the μ -selective receptor. Owing to the Cpa is the first example of an amino acid that acts as a surrogate for Tyr in opioid peptide ligands, challenging the long-standing belief that a phenolic residue is required for high affinity binding, in this article we study the hydroxylated phenyl ring (Tyr¹) modified with Cpa for Leu-Enkephalin to investigate the structure–activity relationships of DOR with the intention of developing peptides with high selectivity ratios.

MD simulations are an important tool for understanding the atomic-resolution structural and dynamic information of biomolecules. Surprisingly, to the best of the authors' knowledge, none of the opioid peptide ligands with Cpa modification is explored for their biological conformations using MD simulations. Therefore, the present study attempts to investigate the structure–activity relationships of the pentapeptide [Cpa¹, Leu⁵¹]-enkephalin (Cpa-LE) in aqueous solution using MD simulations. Moreover, the direct evidence in relation to altering the Tyr to Cpa residue, which can lead to enhancement in the potency and selectivity for DOR via examining the probability distributions between two aromatic rings, is presented. The results of our present work are compared to the Leu-Enkephalin¹⁹ and δ pharmacophore model.²² Our finding show that modified the Tyr¹ to Cpa¹ can lead to increase the potency and selectivity for DOR, consistent with experimental findings.

MATERIALS AND METHODS

Structural Model

Figure 1 depicts the geometric structure of Cpa-LE and major structural parameters, including the rotamers of the sidechain, are labeled. The structural important feature is substituted the OH group with CONH₂ group at hydroxylated phenyl ring (Tyr¹). Moreover, it

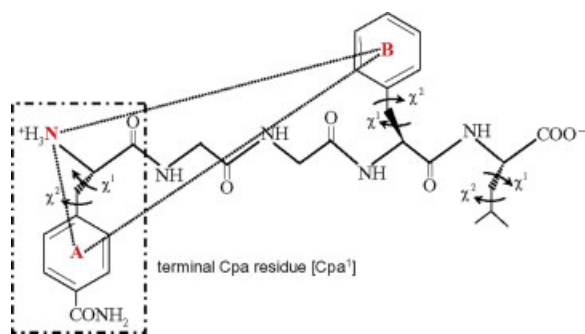


FIGURE 1 Schematic of the model of Cpa-LE structure. The pharmacophoric elements (shown in red) include protonated nitrogen (N), centroid of the carboxamido group (A), and centroid of hydrophobic group (B).

is generally accepted that the most significant pharmacophoric parameters in opioid peptides are (1) the distance from the protonated amine (N) to the Tyr aromatic ring (A), (2) the distance from the protonated amine to a second hydrophobic center (B), and (3) the distance between the Tyr ring and the hydrophobic center (AB).²³ These distances determine the bioactivity of opioid peptide ligands in relation to potency and selectivity to different opioid receptors.

Molecular Dynamics Simulations

Computational techniques are generally used in various ways in drug discovery.²⁴ However, MD simulations have become an established and efficient tool in the study of biomolecules, complementary to experimental techniques. The MD simulations are utilized to bring biomolecular structures visualizable, providing insights into the nature of the dynamics of biomolecules in solution on different timescales.^{25,26} The current MD simulations were performed using the GROMACS simulation program (Version 3.2) with the GROMOS96 (ffG43a1) force field.^{27,28} The starting structures of Cpa-LE were selected to be the double β -bend and extended conformations of Leu-enkephalin, in accordance with X-ray crystallographic studies.^{10,11} The Cpa-Leu was solvated in a box of SPC water,²⁹ with a minimum distance of 10 Å from any peptide atom to the edge of the box. This resulted in ~ 1365 water molecules in an initial box of length 35 Å and 2774 water molecules in a box of length 44 Å for the double β -bend and extended Cpa-Leu, respectively. Periodic boundary conditions were implemented. Energy minimization techniques were then used to refine the starting structure of Cpa-LE in aqueous solution. We built the Cpa-Leu opioid ligand using the small-molecule topology generator PRODRG.³⁰ This PRODRG program converts coordinates and topologies of Cpa-Leu for GROMACS package.

The MD simulations assumed the atoms to have a random velocity consistent with a Maxwellian distribution. Furthermore, the positions and velocities of the atoms were integrated using the standard Verlet algorithm with a time step of 2 fs and SHAKE³¹ to constrain all bond lengths. The simulated system was performed under conditions of constant temperature of $T = 300$ K and a constant pressure of $P = 1$ atm (the *NPT* ensemble) using the Berendsen coupling scheme³² with a dimensionless time constant of 0.2. The time evolutions of the quantities of interest, such as RMSDs (RMSD), radius of gyration (Rg), and solute-solvent radial distribu-

tion functions (RDF), were recorded over duration of 10 ns. In the simulations, the Particle Mesh Ewald method (PME) was adopted to calculate the electrostatic forces,^{33,34} and the non-bonded potentials were truncated using a cutoff radius of 14 Å. The 14 Å cutoff used herein is reasonable and appropriate in avoiding severe artifacts in the simulations since, as pointed out by Darden et al.³⁰ the threshold cutoff radius for the PME method should be at least 9 Å. In our study, the formation of a hydrogen bond between an atomic pair was determined by a geometrical criterion between the pair of the atoms. In other words, a hydrogen bond forms if bond length (r_{HB}) ≤ 3.5 Å and the angle (θ_{HB}) $\leq 30^\circ$ are fulfilled.

RESULTS

This section is organized as follows. First, the structural stability of Cpa-LE in aqueous solution is presented. Then, effects on biological activity due to the 4-carboxamido group are summarized. Finally, results on enhancing the potency and selectivity of Cpa-LE for DOR are presented.

Stability of Cpa-LE Structure

In Figure 1, special attention is drawn to the hydroxyl group for the sidechain of tyrosine residue being substituted as CONH₂ group that referred to as the Cpa residue,²¹ shown in the dashed-line box. The symbols of A and B indicate the center of the aromatic rings for Cpa¹ and Phe⁴, respectively. To elucidate likely binding activity between Cpa-LE and DOR, the sidechain orientations of all residues, i.e., Cpa¹, Phe⁴, and Leu⁵ are marked as χ^1 and χ^2 , and analyzed.

The physical meaning of the RMSD value can be considered as a reasonable measure of structural stability of biomolecules. Here, analysis of the structural stability of Cpa-LE in aqueous solution is through the RMSD value of the backbone atoms in the Cpa-LE corresponding to double-bend initial structure (Figure 2A). It can be seen that, during the simulating time interval, the RMSD value rose to about 2.0 Å and became steady soon after 3.8 ns. Meanwhile, to have a rough measure for the compactness of a structure, we calculate the distribution of Rg of Cpa-LE, as shown in the inset of Figure 2A. Obviously, during the simulation time period of 10 ns, the Rg value of the double-bend initial structure (black colored) started from 3.5 Å, and after several hundred ps, it rapidly rose, and finally reduced to 3.7 Å at about 3.8 ns and remained that value to the end of simulation. This dynamic process infers that the initial double β -bend conformation first changed to the extended-like state, and then reveals a stable compact structure at about 3.8 ns until the end of simulation. In contrast, the packed state is not observed from Rg value in the case corresponding to initial extended structure (red colored) (see Figure 2A). This result has been observed in the work by Aburi and Smith³⁵ for

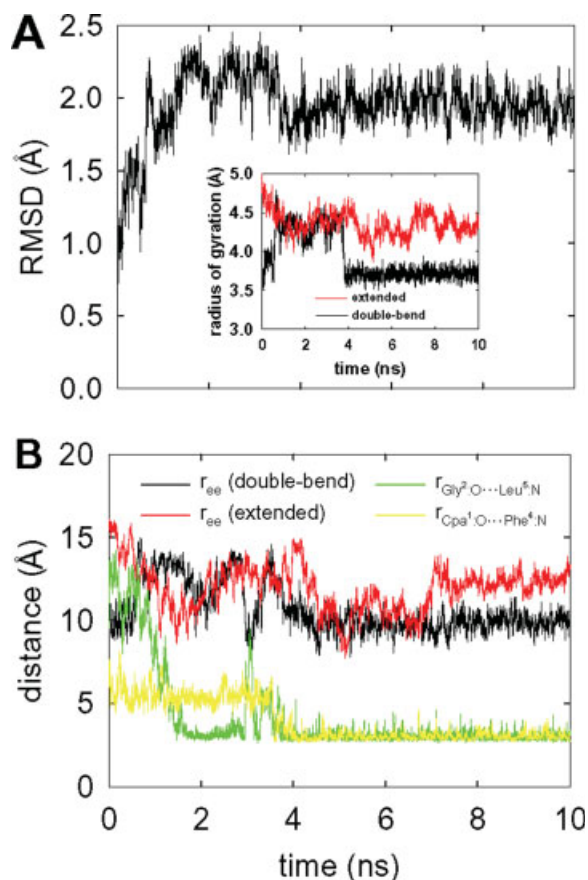


FIGURE 2 The structural stability of Cpa-LE in aqueous solution. (A) RMSD value of the backbone atoms corresponding to the initial structure of double-bend conformation. The inset plots the time profile of the Rg values of C α atoms regarding to the initial structures of extended (red colored) and double-bend (black colored) conformation, respectively. (B) end-to-end distances, r_{ee} , are included for the initial structures of extended (red colored) and double-bend (black colored) conformation; backbone-to-backbone hydrogen bond binding, $r_{Gly^2:O \cdots Leu^5:N}$ (green colored); and side-chain-to-backbone binding hydrogen bond binding, $r_{Cpa^1:O \cdots Phe^4:N}$ (yellow colored).

regular Leu-Enkephalin, but some differences are present in the dynamical behavior of conformations. In their work, during the 10 ns simulation, the Leu-Enkephalin changed from an initially extended conformation, to a folded state, and then back to an extended conformation. In addition, we further study the structural stability via the end-to-end distances, r_{ee} , and two probable hydrogen bonds in Cpa-LE are inspected (Figure 2B). In this figure, two probable intramolecular hydrogen bonds are shown. One is a sidechain to backbone hydrogen bond between the carbonyl oxygen atom of the sidechain of Cpa¹ and amide nitrogen atom of the backbone of Phe⁴ (yellow colored), and the other is formed from backbone to backbone binding (green colored), i.e. from Leu⁵-NH to Gly²-CO (5→2). The latter intramolecular hydrogen bond

leads to the Cpa-LE form a single-bend packed state. The intramolecular hydrogen bond binding behaviors are monitored as a function of time during entire MD simulation. The results of these analyses for structurally stable features of Cpa-LE are almost identical until the end of the simulations after reaching stability at about 3.8 ns. Besides, in the case with initial extended structure of Cpa-LE, the r_{ee} distance was also calculated (red colored), but it seems to prefer the extended-like conformation. Since numerous experimental and theoretical studies have shown that the enkephalins prefer the packed states,^{12–14,18–20} we analyze the Cpa-LE with the double-bend structure as an initial in the following.

To elucidate the CONH₂ group of Cpa¹, that yielded the intramolecular hydrogen bond, the hydration effect of this group was analyzed in our study by calculating the solute-solvent RDF $g_{\alpha-\beta}(r)$, where α and β represent atoms of carboxamido group and water, respectively. These results are plotted as the solute-solvent RDF $g_{\alpha-O}(r)$ and $g_{\alpha-H}(r)$ for the carboxamido group atoms of Cpa¹, including carbonyl oxygen and amino nitrogen atoms (Figure 3). As can be seen the high peaks for both $g_{\alpha-O}(r)$ and $g_{\alpha-H}(r)$ of the amino nitrogen atom are greater than one. Thus provide direct evidence of the high hydrophilicity and its strong interactions with water molecules. On the other hand, it also can be observed from Figure 3 that the carbonyl oxygen atom is hydrophobicity. That is, the carbonyl oxygen atom did not exist very strong attractive interactions with water molecules, but, instead, is strongly interacting with backbone amide NH group of Phe⁴. Furthermore, Figure 4 shows the hydration effect between amide nitrogen atom for Leu⁵ and carbonyl oxygen atom for Gly². Although it seems to bear attractive interactions with water molecules for carbonyl oxygen atom, but the peaks for both $g_{\alpha-O}(r)$ and $g_{\alpha-H}(r)$ are less than one. As a consequence, the intramolecular hydrogen bond can nearly be observed in the stable structure of Cpa-LE (see Figure 2B).

Effects on Biological Activity

Effects on the biological activity can be obtained from the structural features of Cpa-LE, that contrast to endogenous Leu-Enkephalin. For the opioid peptide ligands, it is known that the bioactive structural features are potent and selective for DOR, based on the χ^1 torsional angles of the sidechain, particularly in aromatic rings, such as Tyr¹ and Phe³⁽⁴⁾. In our simulations, to further understanding the structure–activity relationships of Cpa-LE, the sidechain rotamers of Cpa-LE in aqueous solution were analyzed as a function of time, as shown in Figure 5. In this figure, the horizontal lines indicate the corresponding values derived from the X-ray

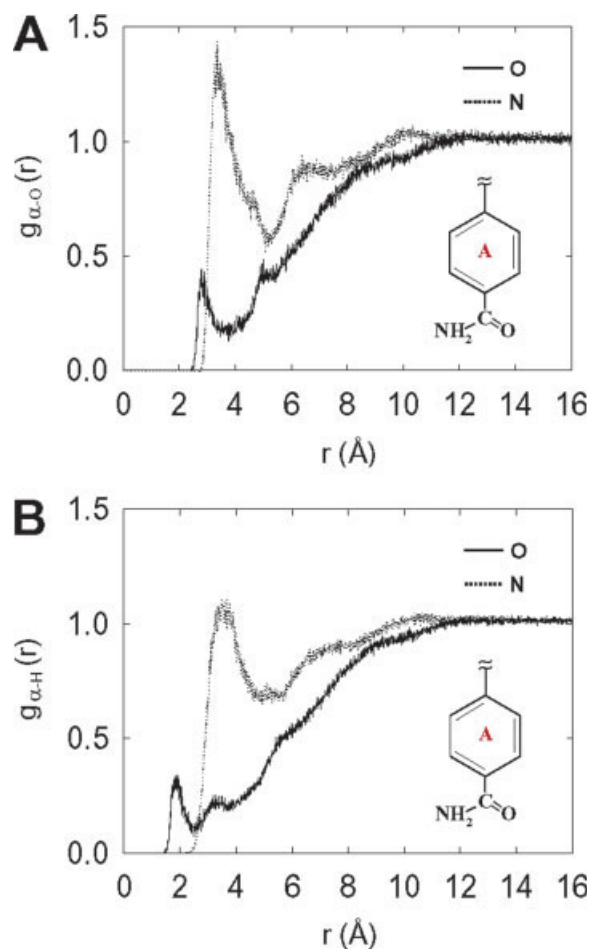


FIGURE 3 The solute-solvent RDF. (A) $g_{\alpha-O}(r)$, and (B) $g_{\alpha-H}(r)$, for the carboxamido group atoms of Cpa residue, including carbonyl oxygen atom (C=O) and amino nitrogen atom (NH₂).

data of Leu-Enkephalin.¹⁰ It is clear known that the rotamer χ^1 of the sidechains, i.e. Cpa¹ and Phe⁴, favor the trans (approximately $\pm 180^\circ$) and gauche (–) (approximately -60°), respectively, throughout almost our entire simulation period, consistent with studies by experimental approaches.¹⁰ Further discussions regarding this issue can be found in the Discussion section.

Enhancing the Potency and Selectivity for δ -Opioid Receptor

The hypothesis,²² which states that potent peptide and non-peptide agonists of the DOR may have a common 3D arrangement of pharmacophore groups upon binding to the DOR, is studied here. Thus, our simulation results were compared with the δ opioid pharmacophore model determined from the best fit conformations of the non-peptide ligands SIOM, TAN-67, and OMI.²² The data of the computational

experiment were obtained by measuring the most important distance relevant to centroids of the hydrophobic rings of Cpa¹ and Phe⁴, as shown in Figure 6. The inset of the Figure 6 shows the time profile of the corresponding ring-to-ring distance for the structure of Cpa-LE during the entire simulation period. The two horizontal lines indicate the limit of ring-to-ring distance for δ -selective opioid peptide ligands. The space enclosed by the two lines denotes the potent and selective DOR in the non-peptide pharmacophore model. As a result of the structural stability of Cpa-LE starting at ca. 3.8 ns and continue to the end of simulation, Figure 6 also shows the probability distribution of stable conformations in terms of their end-to-end and ring-to-ring distances. Our results also show the distance between two rings in the range of 5.7–8.3 Å has a probability of about 48.4% for DOR, which is different from previous study by Nielsen et al. (roughly 39.0%),¹⁹ and will be discussed later.

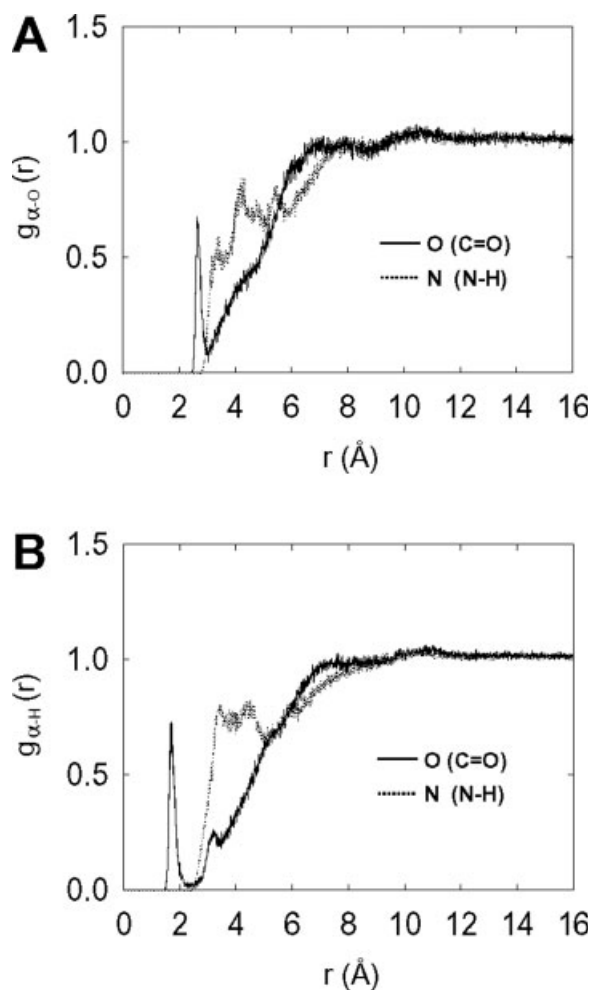


FIGURE 4 The solute-solvent RDF. (A) $g_{\alpha-O}(r)$, and (B) $g_{\alpha-H}(r)$, for the amide nitrogen atom of the Leu residue and carbonyl oxygen atom of the Gly residue.

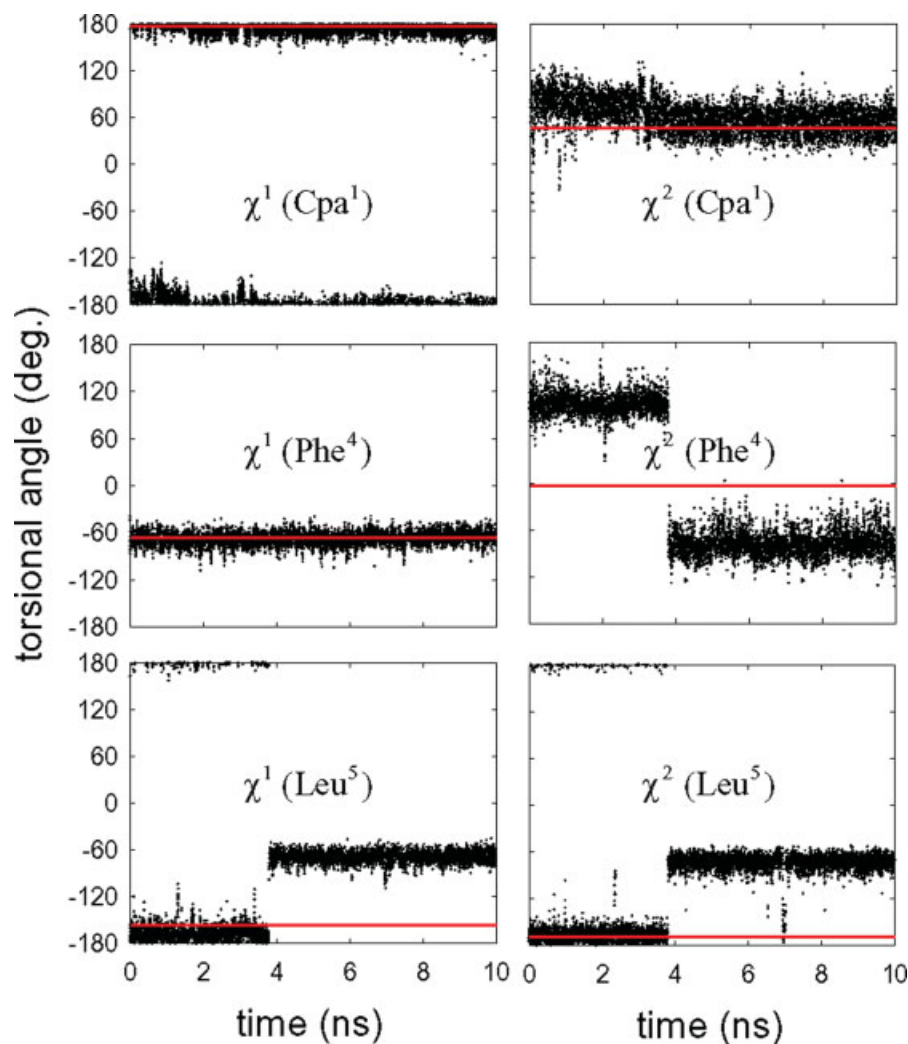


FIGURE 5 Time evolutions of sidechain orientations expressed in terms of χ^1 and χ^2 angles for selected residues. Horizontal lines indicate corresponding values from the experimental x-ray studies on the structure of Leu-Enkephalin.

DISCUSSION

As shown in Figure 1, the major difference between Leu-Enkephalin and Cpa-LE is phenolic OH being substituted by the carboxamido group (CONH_2) at the tyrosine ring's 4'-position. Bioisosteric CONH_2 substitution has been expanded to include morphine and naltrexone,³⁶ and norphone derivatives³⁷ with similar results. The success of the carboxamido residue as a surrogate for the phenolic OH in these non-peptide ligands is attributed to its ability to act as a hydrogen-bond donor.^{32,33,38} Based on this result, Dolle et al.²¹ proposed the use of (4-Carboxamido)phenylalanine (Cpa) as a bioisosteric replacement for the terminal tyrosine residue to test a series of opioid peptide ligands. Their experimental results showed that the Cpa analogs of the δ -selective peptides have an increase in δ selectivity, in contrast to the

responses of the μ -selective receptor. Therefore, to understand the fundamental mechanisms of the δ potency and selectivity for modifying the tyrosine residue, we chose the Leu-Enkephalin as a template, which possesses a slight preference for DOR over MOR, and then implement the MD simulation to depict the structure–activity relationships.

The Binding Behaviors of Intramolecular Hydrogen Bonds

The Leu-Enkephalin (Tyr-Gly-Gly-Phe-Leu) is a well-known endogenous opioid peptide involved in analgesia and having a biological activity resembling that of morphine.⁴ Many structural analyses under various conditions have demonstrated the flexibility of this pentapeptide.³⁹ Therefore, large

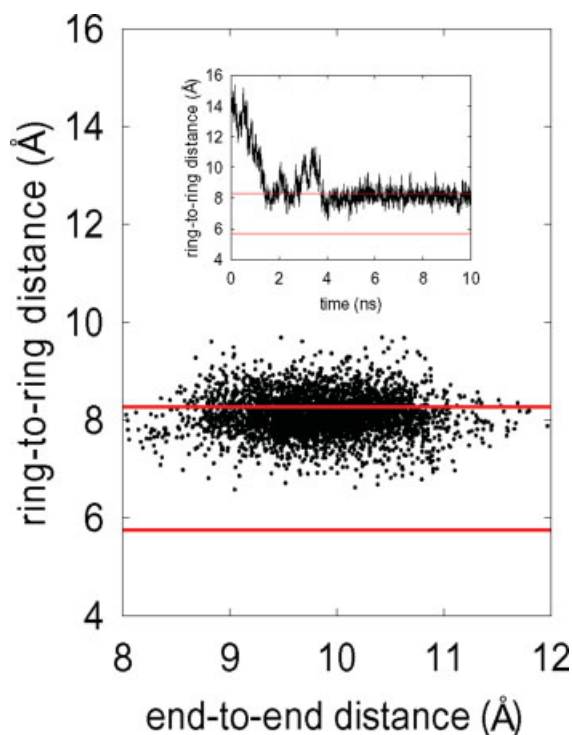


FIGURE 6 The probability distributions of the ring-to-ring distance against the end-to-end distance. The inset plots the time profile of the corresponding ring-to-ring distance. The two horizontal lines represent the limits given in the non-peptide pharmacophore query.²²

number of previous researches was focused on conformational analyses by experiment and theoretical calculations for exploring structure–activity relationships. Here, an important finding in the present work shows that there exists a strong hydrogen bond between the sidechain and backbone, i.e., carbonyl CO group for sidechain of Cpa¹ residue attached to the amide NH group for backbone of Phe⁴ residue (see Figure 2B). It is known that the intramolecular sidechain to backbone hydrogen bond binding of Cpa-LE is similar to crystal structure of deltaxephalin.⁴⁰ Nevertheless, such binding behavior has not been detected to date for prior studies of Leu-Enkephalin in aqueous solution. In our study, the specific effect of carboxamido group of Cpa residue induces the aromatic rings of Cpa¹ and Phe⁴, noticeably closer to each other, so that the end-to-end/ring-to-ring distances exhibit a higher probability of residing in the δ -selective pharmacophoric range (see Figure 6).

On the other hand, the conformational feature of Leu-Enkephalin was first determined from X-ray diffraction by Smith and Griffin,⁹ they have speculated that existence of the single β -bend conformation, with its two intramolecular hydrogen bonds, i.e. 1 $\rightarrow$ 4 and 4 $\rightarrow$ 1, provides the rigid frame

to which sidechains are attached in a specific spatial relationship required for biological activity. Subsequently, numerous experimental studies have been made concerning the structure of Leu-Enkephalin in different environments. Stimson et al.⁴¹ combined the use of ¹³C and ¹H nuclear magnetic resonance experiments and led to the conclusion that Leu-Enkephalin contained a type I β -bend at residues Gly³-Phe⁴ in dimethyl-*d*₆ sulfoxide (Me₂SO-*d*₆) solution. Picone et al.⁴² have been studied by ¹H NMR spectroscopy in media more like the actual environment. Their results suggested the Leu-Enkephalin revealed a 4 $\rightarrow$ 1 β -turn type conformation in organic solvents and cryoprotective mixtures. Furthermore, in membrane-mimetic environments like SDS micelles a 5 $\rightarrow$ 2 β -turn type I' was reported as the preferred conformation. Milon et al.⁴³ implemented the transfer Nuclear overhauser effect (NOE) experiments on Leu-enkephalin bound to lipid bilayers revealed a conformation characterized by a type II' β -turn around Gly³ and Phe⁴ and a γ -turn centered on Gly². From the theoretical study, recently, Aburi and Smith³⁵ have also been performed the conformational studies of Leu-Enkephalin in aqueous solution as a function of pH using MD simulations. During the 10 ns simulation of Leu-Enkephalin at neutral pH, their results showed that the folded conformation was stable for $\sim$ 5 ns, and involved a 2 $\rightarrow$ 5 hydrogen bond and a close arrangement of the terminal groups. However, we have observed the structural feature of Cpa-LE forming a hydrogen bond from Leu⁵-NH to Gly²-CO (5 $\rightarrow$ 2), as well as the aforementioned sidechain-to-backbone interaction (see Figure 2B). Despite our results are different from prior studies, the structural features of Cpa-LE that exist two intramolecular hydrogen bonds in aqueous solution have been examined by means of MD simulations, and identified for the first time.

Hydration Effects

Dudowicz et al.⁴⁴ have studied the solvent structure surrounding the hydrated Met-Enkephalin in aqueous solution using MD simulations. Stimulated by their work, we here attempt to analyze the hydration effects concerning the intramolecular hydrogen bonds. Because of the carboxamido group interacting to backbone amide nitrogen atom of Phe residue, that may induce the different bioactivities for potent and selective relevant opioid receptors. The principal reason lies in ring-to-ring pharmacophoric distances, causing different bioactivities for selectivity to μ -, δ -, and κ -opioid receptors. Therefore, to verify the hydrogen bond that occurs between the sidechain and backbone, we use the solute-solvent RDF, i.e. $g_{\alpha-\beta}(r)$, where α and β represent atoms of CONH₂ group and water, respectively, to examine the hydra-

tion effect. Meanwhile, the hydrogen bond interaction between amide nitrogen atom for Leu⁵ and carbonyl oxygen atom for Gly² was also examined per RDF. Figures 3 and 4 present the distribution functions $g_{\alpha-O}(r)$ and $g_{\alpha-H}(r)$ for the specific atoms. These results provide direct evidence that the carbonyl oxygen atom of carboxamido group is hydrophobic and forms a hydrogen bond binding to backbone amide nitrogen atom of Phe residue, as well as the 5→2 hydrogen bond interactions.

Bioactivity and Pharmacophore Analysis

Studies in the literature^{45–47} have reported that restricting the Tyr¹ sidechain in the trans rotamer is favorable in achieving a high binding affinity to both the DOR and MOR, e.g. c[D-Pen², D-Pen⁵]enkephalin (DPDPE) and Tyr-c[D-Cys-Phe-D-Pen]OH (JOM-13). Meanwhile, restricting the Phe³⁽⁴⁾ sidechain in the gauche (–) rotamer is favorable only in achieving a high binding affinity to the DOR. In general, the χ^1 rotamers of aromatic rings, e.g. Tyr and Phe, play an essential role in determining the pharmacological activity, in particular the selectivity for DOR. As a result, we examine all the rotamers of the Cpa¹ and Phe⁴ sidechains of Cpa-LE. Interestingly, in Figure 5, we find the Cpa- and Phe χ^1 rotamers are rather different when compared with the work by Nielsen et al.¹⁹ Our results show the χ^1 rotamers of Cpa¹ and Phe⁴ exhibit the trans and gauche (–), respectively, during our entire MD simulations through time. Nevertheless, Nielsen et al.¹⁹ reported the χ^1 rotamer of Tyr in Leu-Enkephalin under structural stability, lie in trans and gauche (+), whereas those of Phe χ^1 are almost in trans. The substitution of Tyr residue to Cpa residue may be responsible for these discrepancies. Therefore, this study has shown the Cpa-LE structure possesses the high selectivity for DOR.

Furthermore, in connection with Figure 6, Nielsen et al.¹⁹ found that there are three groups of conformations, contrary to the present results on the Cpa-LE that show only one group of conformation when stability is achieved. However, further analysis shows that the ratio of the present result of the percentage (48.4%) of the conformation in the range of 5.7–8.3 Å to the percentage (39.0%) of the conformation in a pharmacophoric distance defined by Nielsen et al., is 1.24, in good agreement with experimental findings by Dolle et al.²¹ studies. Their result obviously shows the ratio of μ/δ in Cpa-LE to that in Leu-Enkephalin is 1.28, indicating the increase in potency and selectivity for DOR. Although it is generally accepted that bioactivity of peptides is primarily dominated by their conformations, in our simulation work we study only a few structural factors, and therefore other structural

factors may influence bioactivity. However, the fact that bioactive conformation of Cpa-LE should be confirmed with careful experimental work.

CONCLUSIONS

We have examined the structure–activity relationships of the [Cpa¹, Leu⁵]enkephalin (Cpa-LE), and our results show noticeable enhancement in its pharmacological activity. Through this article, we identify the stable conformation of Cpa-LE with two different intramolecular hydrogen bond bindings, which include sidechain-to-backbone and backbone-to-backbone interactions. These binding behaviors cause the Cpa-LE to form a stable conformation and induce the aromatic rings of Cpa¹ and Phe⁴, noticeably closer to each other. Furthermore, our simulation results reveal that the χ_1 rotamers of the Cpa¹ and Phe⁴ show preferences for trans and gauche (–) orientations, respectively. The significance of our simulation results correlate well with recent experimental observations on Tyr¹ substituted into Cpa¹ form acting as a surrogate for Tyr in opioid peptide ligands. Hence, that a phenolic residue is required to achieve high affinity binding is no longer necessary. Therefore, our MD simulations have provided the better understandings of the binding affinity in δ -selective opioid ligands whose tyrosine residue is substituted with the Cpa residue.

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