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Similarity of Ca^{2+}-bound conformations of morphine and Met-enkephalin: a computational study

Boris S. Zhorov**, Vettai S. Ananthanarayanan*

Department of Biochemistry, McMaster University, Hamilton, Ont., L8N 3Z5, Canada

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Abstract The conformations of the free and Ca^{2+}-bound forms of morphine and Met-enkephalin were compared based on an earlier proposal that extracellular Ca^{2+} may dictate the bioactive conformations of peptide hormones and drugs. A Monte Carlo with energy minimization method was used to calculate Met-enkephalin in the absence and presence of Ca^{2+}. The Ca^{2+}-bound conformation of Met-enkephalin was found to have an overall shape that matched well with that of morphine. In contrast, the uncomplexed Met-enkephalin did not have such a match. The data suggest that a ternary association of the μ-receptor, its ligands and Ca^{2+} may be an initial process in the signal transduction mechanism of opioid peptides.

Key words: Opioid peptide; Calcium–hormone interaction; Bioactive conformation; Computer simulation

1. Introduction

The conformationally flexible peptide hormone Met-enkephalin (EK) and the rigid alkaloid morphine (see Fig. 1 for the structural formulae of the compounds) are two classical ligands of the μ-opioid receptor. Following the suggestion of structural similarity between these ligands by Bradbury et al. [1], there have been many attempts to obtain conformations of EK and its analogues that would resemble morphine [2–8]. While a certain degree of similarity between the ligands was demonstrated in these studies, the results and conclusions drawn have been diverse.

We have suggested earlier that peptide hormones [9] and drugs [9–11] may form ternary complexes with Ca^{2+} and corresponding receptors. The fact that enkephalins bind Ca^{2+} in non-polar milieu [12] and Ca^{2+} enhances the binding of opioid peptides to calf brain μ-receptor [13], indicate that this hypothesis may be applicable to μ-opioids. In this work, we calculated many minimum-energy conformations (MECs) of free and Ca^{2+}-bound forms of EK. Using a set of geometrical criteria pertaining to the overall shape of molecules, we demonstrate a striking resemblance between low-energy conformations of the Ca^{2+}-bound forms of morphine and EK.

2. Materials and methods

Ionized forms of morphine and EK which should prevail at physiological pH were considered. To compare our results with those of other authors, the non-ionized form of free EK was also calculated. All calculations were done in vacuum. Conformational analyses were carried out on Iris workstations (Silicon Graphics, USA) using the ZMM package described elsewhere [11,14]. ECEPP/2 force field [15] was used for calculations of peptides. To preserve long-range electrostatic interactions, no cut-off technique was implemented. In the absence of parameters for Ca^{2+} in the ECEPP/2 force field, metal–ligand interactions were approximated by modeling Ca^{2+} as an oxygen atom with a double proton charge [11]. Although this results in an underestimate of the energy of interaction of Ca^{2+} with the oxygen atoms of the peptide (by neglecting the effect of polarization and charge-transfer energy contributions), it helps avoid an over-emphasis of the Ca^{2+}-bound forms of the peptide. Test calculations with this potential yielded distances between Ca^{2+} and oxygen atoms in the MECs of $[Ca(OCH_2)_4]^{2+}$ and $[Ca(cyclo\text{-}(Pro\text{-}Gly)_3)]^{2+}$ to be 2.3–2.5 Å, in good agreement with experimental and theoretical data [16]. The energy of morphine–Ca^{2+} complex was minimized with varied bond and torsional angles using an approach described elsewhere [11].

The Monte Carlo with energy minimization method [17] was used to surmount the multiple-minima problem. Trajectories for free EK in its neutral and zwitterionic forms, as well as for the Ca^{2+}-bound zwitterionic EK, were calculated at $T = 600$ K. An initial point in each trajectory was found by energy minimization from an all-*trans* conformation; Ca^{2+} was positioned near the N-terminal nitrogen atom (where it is least likely for the ion to find oxygen atoms). A subsequent starting point in the trajectory was obtained by changing a randomly selected generalized coordinate G of the preceding point by a random increment D, where $D \varepsilon (-180°, 180°)$ if G was a torsional angle, and $D \varepsilon (-4$ Å, 4 Å) if G was a Cartesian coordinate of Ca^{2+}. From the starting point, energy was minimized until the norm of the gradient became less than 0.1 kcal·mol^{-1}·radian^{-1}. The resulting MEC was accepted in the trajectory if its energy E was less than that of the preceding point of the trajectory E_p, or, if a random number $n \varepsilon (0,1)$ was less than $\exp(-(E - E_p)/RT)$. If, during energy minimization, a Cartesian coordinate of Ca^{2+} deviated by more than 10 Å from its starting value, the minimization was terminated and the corresponding point was not accepted in the trajectory. This ensured that MECs with Ca^{2+} separated from EK were not included in the trajectories. MECs with an energy below 7 kcal/mol from the corresponding apparent global minimum were accumulated in files and sorted in the order of increasing energies. Two MECs were considered to be similar if they had the same backbone letter code [18] and side chain torsions deviated by less than 10°. Trajectories were simulated until the 200 last energy minimizations did not increase the number of accumulated MECs.

3. Results and discussion

The MCM trajectory for the free EK in the non-ionized form comprised 7,800 energy minimizations with 4,338 (56%) MECs accepted into the trajectory. The global minimum corresponding to that reported by Li and Scheraga [17] was found at the 3,388th energy minimization. MCM trajectory for the free EK in the zwitterionic form comprised 13,952 energy minimizations with 9,003 (65%) MECs accepted into the trajectory. The MCM trajectory for the Ca^{2+}-bound zwitterionic EK comprised 9,650

*Corresponding author. Fax: (1) (905) 522-9033.
E-mail: zhorov@fhs.mcmaster.ca

**On leave from Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russian Federation.

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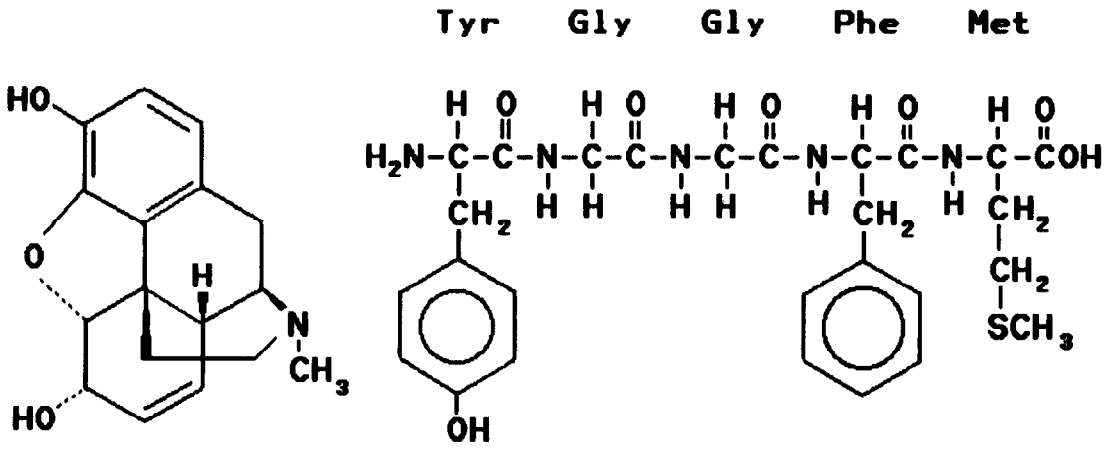

Fig. 1. Structural formulae of morphine (left) and Met-enkephalin in their neutral forms. At physiological pH, morphine is predominantly a cation with a protonated amino group, while Met-enkephalin is predominantly a zwitterion with a protonated amino group at the N-terminal and deprotonated carboxy group at the C-terminal.

energy minimizations with 6,035 (63%) MECs accepted into the trajectory. With reference to the global MEC of the free EK, the global MEC of its Ca^{2+}-bound form had an energy of −79 kcal/mol. This shows that Ca^{2+} association with the zwitterionic EK is energetically favourable. Among the thousands of the MECs calculated for the free and Ca^{2+}-bound forms of the zwitterionic EK, 773 and 328 low-energy MECs, respectively, were accumulated for the comparison with morphine.

The Ca^{2+} complex of EK is structurally very different from free EK. In the former, Ca^{2+} stabilizes conformations which bring together five or six oxygen atoms in close proximity. Such compact structures are energetically unfavourable for the uncomplexed peptide due to electrostatic repulsion between the partial charges of the oxygen atoms.

One might intuitively expect better resemblance of the compact structures of the EK–Ca^{2+} complex to morphine than the relatively more open structures of the free peptide. We therefore formulated certain geometrical criteria of similarity between the EK–Ca^{2+} complexe and the morphine–Ca^{2+} complex. The global MEC of the morphine–Ca^{2+} (which had a geometry

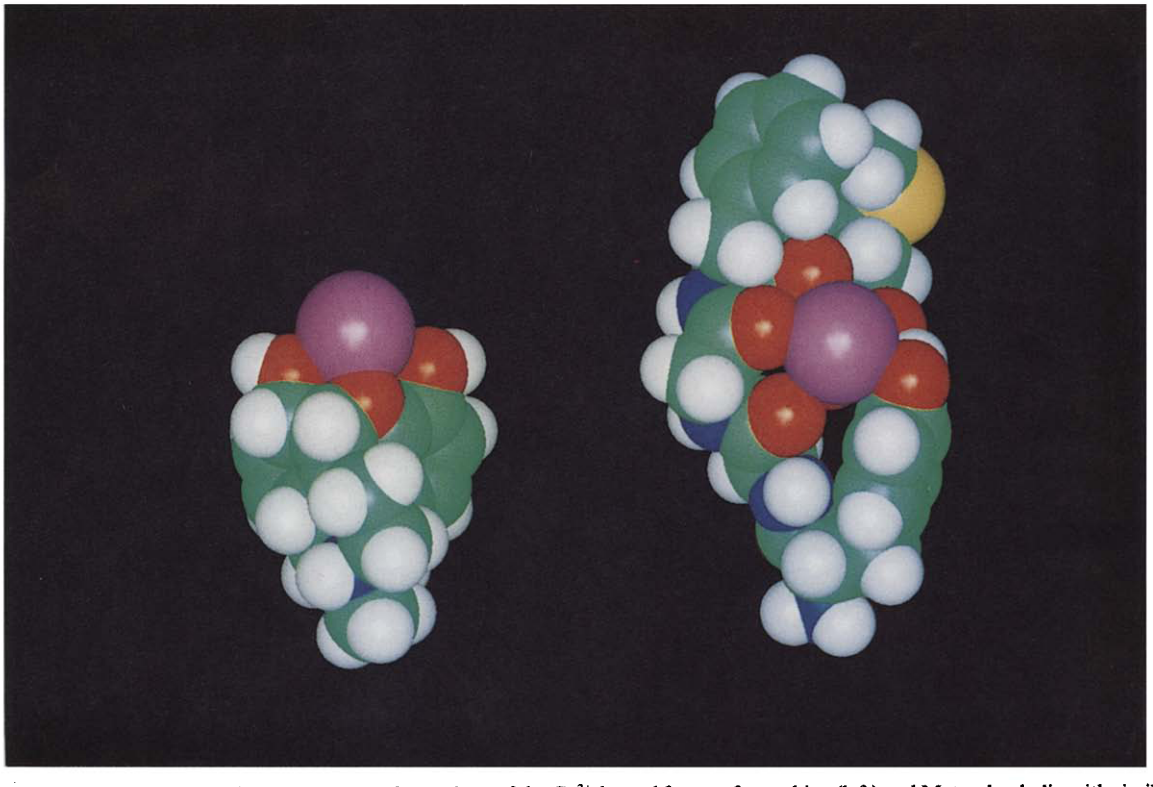

Fig. 2. Space-filling display of the minimum energy conformations of the Ca^{2+}-bound forms of morphine (left) and Met-enkephalin with similar overall shape. Structures were visualized using the INSIGHT II program (Biosym Technologies Inc., USA). The atoms of different type are represented by the following colours: oxygen, red; calcium, purple; carbon, green; hydrogen, white; nitrogen, blue; sulphur, yellow.

close to that of free morphine itself) has a wedge-like shape with N^+ at the sharp end and Ca^{2+} coordinated to three oxygen atoms at the wide end, the vector from N^+ to Ca^{2+} approximately coinciding with the long axis of the wedge. Using a wire-frame molecular model, we calculated the cross-sectional areas of morphine–Ca^{2+} perpendicular to the N^+–Ca^{2+} axis at two levels, 2 and 6 Å from the N^+ (see footnote to Table 1 for more details). These profile areas were 18.4 and 35 Å^2, respectively. To treat the EK–Ca^{2+} complex, we assumed that the conical shape of the morphine–Ca^{2+} complex may be extrapolated, and calculated the extrapolated the cross-sectional area at a level of 9 Å from N^+ along the N^+–Ca^{2+} axis. This was found to be 60 Å^2. To match the overall shape of morphine, the EK–Ca^{2+} complex was required not to exceed the above-calculated dimensions. To ensure the conformational resemblance of the N-C-C-Ph-OH moiety (an essential pharmacophore) in the Ca^{2+} complexes of EK and morphine, we further stipulated that the distance between the N^+ and Ca^{2+} in EK–Ca^{2+} be not less than that in morphine–Ca^{2+} (7.6 Å) and that Ca^{2+} be coordinated to the Tyr^1 hydroxyl group. The last two criteria specify, respectively, the lower and upper limits for the N^+–Ca^{2+} distance.

Twenty-two MECs meeting all the above criteria were found in EK–Ca^{2+} (see Table 1 for the torsional angle data of some of the MECs). Due to the strong electrostatic interaction of Ca^{2+} with several oxygen atoms, not surprisingly some of the backbone torsion angles fall in regions of the Ramachandran plot not usually allowed for l-amino acid residues.

As a control, uncomplexed EK in zwitterionic form was also tested for cross-sectional areas meeting the above criteria. In the absence of Ca^{2+}, any atom of EK was assumed to form an axis with N^+. All the MECs with an energy up to 7 kcal/mol

Table 1
Energy (kcal/mol) and structural parameters of low-energy MECs of Ca^{2+}-bound Met-enkephalin

Parameter	Global MEC	MECs matching morphine:Ca^{2+}				
Energy	−148.1	−145.8	−145,5	−143.3	−143.1	−141.2
Torsional angles (degrees)						
Tyr^1						
Φ	175	55	−64	175	175	−62
Ψ	164	36	155	155	155	158
ω	−176	177	177	177	177	178
χ_1	−178	170	170	170	171	177
χ_2	80	72	−108	−109	−109	−110
χ_3	5	−171	7	10	9	−27
Gly^2						
Φ	−51	179	179	179	179	−160
Ψ	−80	−137	−140	−140	−142	−157
ω	−176	178	178	178	178	−178
Gly^3						
Φ	−63	67	68	67	68	66
Ψ	−100	88	89	88	90	51
ω	174	−173	−174	−173	−174	179
Phe^4						
Φ	−56	45	46	45	46	56
Ψ	163	86	83	86	83	176
ω	−169	−172	−170	−170	−168	167
χ_1	59	−177	−57	−177	−57	−158
χ_2	80	68	100	68	100	66
Met^5						
Φ	42	45	43	44	43	−42
Ψ	76	55	56	−121	−120	118
χ_1	−68	−60	−60	−59	−60	−75
χ_2	174	−179	−176	−68	−178	178
χ_3	−177	84	−179	178	84	−178
Distance (Å) from Ca^{2+}[a]						
N^+	4.7	8.5	8.6	8.5	8.6	9.7
O^1	2.5	7.1	7.1	7.1	7.2	8.5
O^T	9.0	2.6	2.6	2.6	2.6	2.5
O^2	2.5	2.9	3.0	2.9	3.0	4.1
O^3	2.5	2.5	2.5	2.5	2.5	2.6
O^4	3.1	2.6	2.6	2.6	2.6	2.6
O^5	2.7	2.5	2.5	2.5	2.5	2.4
O^5	2.3	2.4	2.4	2.4	2.4	2.5
Minimal profile area (Å^2) at the level:						
2 Å	88.7	9.3	9.3	9.3	9.3	8.7
6 Å	100.2	28.0	27.0	28.2	27.2	25.8
9 Å	100.2	47.1	46.6	47.0	46.6	42.6

[a]Atom designations: O^N, carbonyl oxygen of the Nth residue; O^T, oxygen of the Tyr phenolic hydroxyl.
[b]The profile area of a wire-frame model of a given conformer at a given level was calculated as follows. A plane normal to the N–Ca^{2+} axis and crossing it at this level was drawn. This plane bisected the conformer in two parts. The part comprising N^+ was projected at the plane, and a rectangle was described over the projection. The projection was then rotated around the N–Ca^{2+} axis by 180° in 3° steps. At each step, a new rectangle was described over the rotated projection in such a way that its sides remained parallel to those of the previous rectangle. A rectangle with a minimal area was then selected among the 60 rectangles thus constructed.

above the global minimum were considered. This gave 57,202 combinations of the 773 MECs with 74 axes. None of them had cross-sectional areas at all the three levels below the three specified limits. Thus, binding of Ca^{2+} is crucial for EK to adopt the compact structure matching that of morphine.

Fig. 2 shows the similarity between the MECs of the Ca^{2+}-bound forms of morphine and EK. Both the molecules have a compact shape, with their polar and non-polar moieties identically disposed. The wedge-like lower part of these molecules could fit the bottom of the receptor's ligand-binding pocket, and the hydrophobic residues of EK would extend towards the top of the pocket. This model is compatible with recent data on the funnel-like arrangement of the transmembrane helices of the G-protein coupled receptors [19] where the ligands would fit in. Our data show that Ca^{2+} binding brings a better conformational similarity to EK and morphine than has hitherto been possible in other studies on the free hormone [2–8]. This would imply that the bioactive conformation of EK interacting with the μ-receptor is the Ca^{2+}-bound hormone. We believe that Ca^{2+} links the hormone and the receptor through formation of a ternary complex. This suggestion is compatible with the experimental data on the role of Ca^{2+} in the interaction of EK with μ-receptor [13]. Preliminary calculations [20] demonstrate that many other μ-opioid peptides also have low-energy Ca^{2+}-bound conformations similar to that of EK–Ca^{2+} shown in Fig. 2.

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