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MOLECULAR MECHANICS STUDIES OF DERMORPHIN

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SUMMARY: Molecular mechanical simulations have been carried out on dermorphin. Presence of D-Ala$_2$ at the N-terminus and L-Pro$_6$ residue at the C-terminus indicated the probability of β-turns. From the stereochemical considerations, three types- II', III' and V'- for the β-turn at the N-terminus of the peptide and two types-I and III- for the C-terminus side of the peptide are possible. In our molecular mechanics calculations, we considered six folded and one extended conformations for dermorphin to asses the relative stabilities. Three of the six folded conformations are lower in energy and have the following general features- similar in energy, three hydrogen bonds, semirigid β-sheet segment and favorable Tyr$_1$-Tyr$_5$ interaction. The presence of β-sheet structure might play a role in μ-receptor selective interaction of dermorphin. © 1986 Academic Press, Inc.

Dermorphin, H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2, an opioid peptide isolated from an amphibian source is unique in containing D-Ala as the second amino acid residue (1-3). Dermorphin is an extremely potent analgetic (for a recent review see Feuerstein, 4) and in the hot plate test it is 2000-fold more potent than morphine (5-7). To date few conformational studies have appeared on this potent analgetic (8-11). Schiller and DiMaio (12), from their studies on linear enkephalin analogs and their cyclic counterparts, suggested that the opioid receptors differ in their conformational requirements. Later several groups have proposed preferred conformational states for μ- and δ-receptor

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interaction (13-20). As dermorphin is a potent opioid peptide of nonmammalian origin, it will be an excellent model to investigate structure-analgetic activity and structure-conformation selectivity. Furthermore, the superimposition of the three dimensional structure of dermorphin on morphine may give insights into the structural requirements for μ-receptor selectivity and aid in the design of potent analgetic drugs.

FT-IR, Raman and 2D NOE studies of dermorphin in aqueous solution have demonstrated the presence of a manifold of folded conformations of dermorphin (21). The presence of D-Ala and L-Pro are generally conducive to the formation of ß-turn(s)(22). We have therefore considered a combination of the major types of ß-turns, namely types I,II,III and V (23-25) for the two turns: referred as turn I for the N-terminus ß-turn and as turn II for the C-terminus ß-turn. The presence of D-Ala residue and L-Phe as the corner residues at the N-terminus suggested that ß-turn types I', II', III', and V' should also be considered. Further, a completely extended conformation (except for ϕ of Pro_6) was also included in our studies. From the Ramachandran plot for L and D amino acid, type II' is excluded for turn II on steric considerations (26). Therefore for the turn I only types II', III' and V' are favorable while for the turn II only types I and III are favorable. In this paper we report the results of molecular mechanics calculations on II'-I, II'-III, III'-I, III'-III, V'-I, V'-III and extended conformations of dermorphin.

METHODS

An extended conformation for dermorphin was generated and displayed using MIDAS on an Evans and Sutherland PS2 (27). The torsion angles ϕs and ψs were fixed at values suggested by Venkatachalam (23) for the ß-turns. In our models, the remaining ϕs, ψs and χs were rotated until the Tyr_1 has close interaction with Tyr_5 as reported earlier (9). Molecular mechanics calculations were preformed on the generated structures using the program AMBER, Assisted Model Building with Energy Refinement(28). The total energy term consisted of bond length stretching energy, bond angle bending energy, torsional energy, van der Waals, electrostatic, and hydrogen bond energy. The electrostatic interaction energies were calculated using a distance dependent dielectric constant. The partial atomic charges were taken from Singh and Kollman (29), and the various constants to evaluate the energy were from Weiner et al. (30). The structures were refined until the root mean square gradient is less than 0.1 kcal/mol Å.

RESULTS AND DISCUSSIONS

Table I lists the total energies in kcal/mol for the seven energy minimized models of dermorphin. From the results presented in Table I, the extended conformation for dermorphin is less favorable than the folded conformations. From Table I, the energy difference between the most favorable conformation model and the extended conformation model is approximately 24 kcal/mol. Since the energy difference among the folded conformation models, III'-I, II'-III and II'-I is less than 1 kcal/mol, it is likely that these models are equally favorable models for folded conformation of dermorphin. The remaining three folded conformation, models, III'-III, V'-I and V'-III are at least less stable by $\sim$12 kcal/mol than the III'-I, II'-III, and II'-I folded conformation models.

In Table II, the residue interaction energies of each residue with the other residues of dermorphin for the seven minimized models are given. The first column represents the various models and that of the second to eighth column is for each amino acid residue- Tyr_1, D-Ala_2, Phe_3, Gly_4, Tyr_5, Pro_6 and Ser_7 respectively. On comparing the seven residue interaction energies among the seven energy minimized models, it is obvious that for the folded conformations, the residue interaction energies for the other six

Table I. Conformational Energies in kcal/mol of the Seven Models of Dermorphin

Model	β-Turn-I	β-Turn-II	Total Energy
III'-I	III'	I	-70.7
II'-III	II'	III	-69.9
II'-I	II'	I	-69.8
III'-III	II'	III	-57.2
V'-I	V'	I	-56.9
V'-III	V'	III	-46.4
Extended	Trans	Trans	-46.3

TABLE II. Residue Interaction in kcal/mol Energies of Various Models of Dermorphin

Model	Tyr	D-Ala	Phe	Gly	Tyr	Pro	Ser
III'-I	-43.5	-21.2	-22.1	-33.5	-32.0	-20.4	-15.2
II'-III	-48.3	-20.3	-19.4	-33.6	-30.3	-20.8	-14.5
II'-I	-48.5	-21.2	-19.7	-33.3	-31.6	-19.0	-14.1
III'-III	-35.0	-19.7	-22.1	-33.1	-25.2	-18.2	-14.6
V'-I	-32.3	-17.9	-34.1	-22.5	-24.5	-13.8	-14.2
V'-III	-16.6	-20.2	-21.2	-22.2	-24.1	-13.9	-14.2
Extended	-10.7	-18.3	-23.3	-20.7	-18.7	-14.6	-9.7

residues are more favorable than the corresponding residue interaction energies for the extended conformation model. Among the favorable folded conformation models of dermorphin except for Tyr_1 residue, the difference in the residue interaction energies are less than 1 kcal/mol. It may be noted for the Phe residue the residue interaction energy favors the folded conformation model V'-I.

Fig. 1 shows a stereo-view of the skeletal model of one of the favorable energy minimized models, III'-I. As discussed earlier Tyr_1 is interacting favorably with Tyr_5 residue. There is some stacking interaction between Tyr_1 and Tyr_5 residues. There are three favorable hydrogen bonds, the

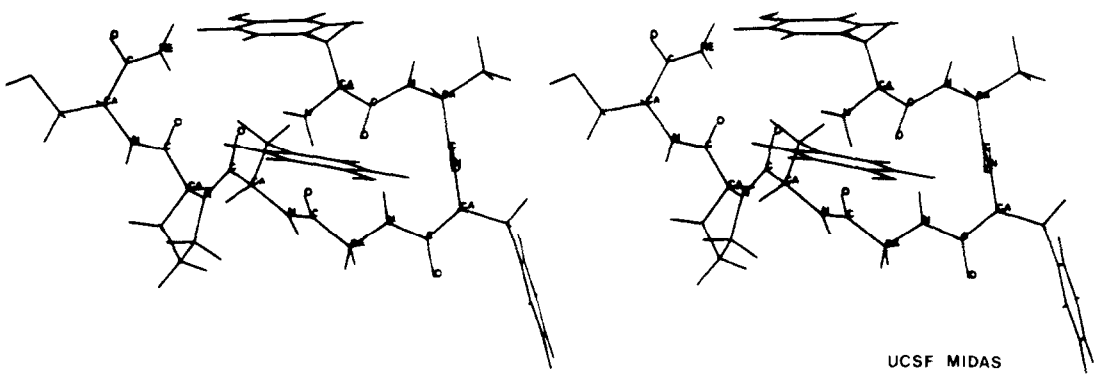

Fig. 1. Stereo-pair of the energy-minimized dermorphin model, III'-I.

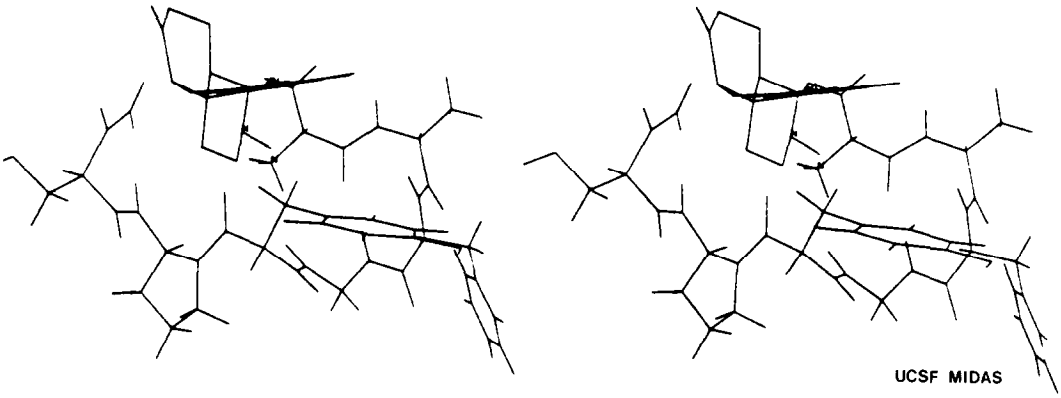

Fig. 2. Stereo-pair of the superposition of morphine with the model of dermorphin.

first between N-H of NH_3^+ at the N-terminal end and C=O of Gly_4, the second between C=O of Tyr_1 and N-H of Gly_4, and the third between N-H of NH_3^+ and C=O of Tyr_5. At the N-terminal end with the formation of the β-turn and the above mentioned three hydrogen bonds, the residues 1 to 5 form a small segment of β-sheet structure. In the turn II, the hydrogen bond between N-H of NH_2 of C-terminal end and C=O of Tyr_5 is not stabilized. This may be due to the favorable Tyr_1 and Tyr_5 interaction and also the hydrogen bond between C=O of Tyr_5 and N-H of NH_3^+ at the N-terminal end. However, the N-H at the C-terminal forms a seven-membered hydrogen bond with C=O of Pro_6, resulting in a C_7 structure. Even though different β-turns were assumed for the turns for the three energetically favorable models, the general features of the three models are the same. On comparing the three models, the following features are observed:

1. The models have similar folded conformations with a rigid β-sheet segment at the N-terminal end, 2. Tyr_1 has favorable interactions with Tyr_5 and 3. The turn at the C-terminal end is flexible. It is likely that there is a competition between N-H of NH_3^+ at the N-terminal end and N-H of C-terminal end to form hydrogen bond with C=O of Tyr_5.

Due to the rigid β-sheet segment at the N-terminal end, the residues 1 to 5 may be important in the pharmacological activity of dermorphin. Fig. 2 shows

a stereoview of the superposition of morphine with the III'-I model of dermorphin. The A-ring of morphine is superimposed on the side chain aromatic ring of Tyr_1 which is crucial to the pharmacological activity of dermorphin. The tertiary nitrogen of morphine and the N of NH_3^+ of dermorphin are within a distance of 1. 5 Å. The results derived here from molecular mechanics are in agreement with detailed experimental studies using Raman, FT-IR, and 2D-NMR spectroscopy (Renugopalakrishnan et al to be submitted). Toma et al (11) concluded from molecular mechanics calculations and ^1H NMR studies that a type I ß-turn was most likely to occur at the C-terminus and our conclusions are similar to theirs, for the C-terminal segment of dermorphin. 2D NOE studies of dermorphin in D_2O provide support for Tyr_1-Tyr_5 interactions which is consistent with a folded conformation at the N-terminus and hence we suggest that at least in aqueous solutions, which is of physiological relevance, dermorphin exists essentially in an ensemble of well defined folded conformations, stabilized by a system of three intramolecular hydrogen bonds as described earlier. It will be interesting to perform molecular dynamics calculations on these models incorporating the solvent molecules in order to understand the flexibility of the folded conformation of dermorphin.

Conformation-receptor selectivity has been described by several groups and the ß-turn and ß-sheet structure are proposed to play a role in determining the μ-and δ-receptor selectivity (13-20, for a review see 31). The lowest energy conformation i.e. type III'-I was superimposed on the molecular structure of morphine. From the superimposition presented in Fig. 2, the topological similarity of the A ring of morphine and Tyr_1 residue, which is critical for for the pharmacological activity can be readily understood. The folded type III'-I conformation presents a flat ß-sheet like structure and this is in agreement with our earlier proposal that the ß-sheet structure might be involved in μ-receptor recognition (21). Furthermore the topological similarities to morphine may also be indicative of its μ-receptor selectivity.

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