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Comparative conformational analysis of [D-Pen^2, D-Pen^5]enkephalin (DPDPE): A molecular mechanics study*

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SUMMARY

A theoretical conformational analysis (molecular mechanics study) of the δ opioid receptor-selective enkephalin analog H-Tyr-D-Pen-Gly-Phe-D-Pen-OH (DPDPE) was performed, based on the use of the SYBYL software. The study led to the identification of several conformers that were significantly lower in energy than previously reported candidate conformers of DPDPE which, for comparative purposes, were also minimized by using the standard SYBYL force field. The results revealed a considerable degree of conformational flexibility of the DPDPE molecule, and suggested that incorporation of further conformational constraints into this enkephalin analog will be necessary in order to elucidate its receptor-bound conformation.

INTRODUCTION

Since the discovery of the opioid peptides Met- and Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Met[or Leu]-OH), the conformations of these two pentapeptides have been studied extensively by means of nearly all theoretical and experimental methods available to date. The results of these numerous studies indicate that the enkephalins are highly flexible molecules capable of assuming a number of both folded and extended conformations of comparably low energy [1]. In fact, there is convincing evidence that in solution the enkephalins exist in a conformational equilibrium [2].

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Abbreviations: Symbols and abbreviations are in accordance with the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature: Biochem. J., 219 (1984) 345. The following other abbreviations were used: A_2bu, α,γ-diaminobutyric acid; DPDPE, [D-Pen^2, D-Pen^5]enkephalin; DPLPE, [D-Pen^2, Pen^5]enkephalin; Orn, ornithine; Pen, penicillamine; NMR, nuclear magnetic resonance; VDW, van der Waals.

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Furthermore, the demonstrated conformational flexibility of these two opioid peptides may explain their lack of receptor specificity, as indicated by the fact that they show only a slight preference for δ receptors over μ receptors.

During the past decade, various attempts have been made to reduce the conformational flexibility of the enkephalins through incorporation of conformational constraints. In particular, conformational restriction through peptide cyclization (side chain-to-end group or side chain-to-side chain) has proven to be very successful in obtaining enkephalin analogs which show not only improved conformational integrity but also high selectivity for either μ- or δ-opioid receptors. The compounds H-Tyr-cyclo[-D-A_2bu-Gly-Phe-Leu-] [3] and H-Tyr-D-Orn-Phe-Asp-NH_2 [4] are examples of μ-selective cyclic opioid peptide analogs, whereas the cyclic enkephalin analogs H-Tyr-D-Pen-Gly-Phe-Pen-OH (DPLPE) and H-Tyr-D-Pen-Gly-Phe-D-Pen-OH (DPDPE) show pronounced δ selectivity [5]. Conformational analysis of these analogs is meaningful because they are unlikely to undergo major conformational changes upon binding to the receptor and, therefore, information about the distinct bioactive conformations at the μ and the δ receptor might be obtained. A review of conformational studies performed with cyclic opioid peptide analogs has recently been published [6].

The δ-selective cyclic enkephalin analog DPDPE has been the subject of several theoretical conformational analyses which were based on the use of various different approaches and programs, including CHARMm [7], AMBER [8–10] and ECEPP [11,12]. In the present paper, we describe a systematic conformational search and energy minimization of DPDPE, using the SYBYL software. The primary goal of this comparative study was an assessment of differences in conformational parameters and energy content between various proposed models of DPDPE.

METHODS

All calculations were performed using the molecular-modelling software SYBYL (Tripos Associates, St. Louis, MO) on a VAX 11/750 mainframe (VMS version 4.6). Molecules were viewed with an Evans & Sutherland PS330 computer-graphics display terminal, and a Hewlett Packard HP7475 plotter was used for the preparation of the figures. To determine the low-energy conformations of DPDPE, we used a previously described stepwise approach [13,14], with some modifications. An incorrect torsional parameter for the disulfide dihedral angle in the original version of the SYBYL force field was corrected by using a twist constant of 7 and a periodicity of 2, as recommended by G. Marshall, Washington University, St. Louis, MO (personal communication).

The first step in this procedure was to construct the 'bare' 14-membered ring structure of DPDPE. Only atoms directly attached to the ring, with their associated hydrogen atoms, were included in this part of the analysis. Thus, the phenylalanine side chain was replaced by a methyl group, and the tyrosine residue was completely omitted. This approach has been used previously [15,16] to allow for the identification of the greatest number of solutions in the conformational search that constitutes the second step of the analysis procedure. Prior to the conformational search the ring structure was energy-minimized using the program MAXIMIN. The conjugate-gradient approach was used, and the potential energies were calculated from

$$E = W_{str}E_{str} + W_{ang}E_{ang} + W_{tor}E_{tor} + W_{vdw}E_{vdw}$$

where the Ws represent weight constants, and the Es are the energy terms for the bond stretching (str) energy, angle bending (ang) energy, torsional (tor) energy and van der Waals (vdw) contact energy (including hydrogen-bonding energy). The standard SYBYL force field [17] was employed. A distance-dependent dielectric constant of 78 was used to simulate an aqueous environment. Minimization was allowed to proceed until the energy change per step was less than 0.0001 kcal/mol. Once a reasonable starting geometry was obtained, the conformational search of the 14-membered ring structure could begin.

The systematic conformational grid search was performed using the software routine SEARCH [18]. This program systematically checks for unfavorable VDW contacts around the non-bonded atoms by scanning all possible torsional angles around all the rotatable bonds. A VDW scaling factor of 0.80 was used for non-bonded atoms. For 1,4-interactions we used a VDW scaling factor of 0.70 [19], and the VDW scaling factor used for hydrogen bonding interactions was 0.50. All amide bonds were held trans and planar. The disulfide bond was chosen as the ring closure bond and the remaining 10 bonds were surveyed over a 30° grid over all space. An allowed conformation was obtained if no unfavorable VDW contacts were found and ring closure could be achieved by allowing for a 0.3 Å variance of the length of a normal disulfide bond and a 30° variance of the valence angles about the ring closure bond.

This search procedure generated 99 distinct ring conformations. Each of these allowed ring structures was then minimized as described above, allowing all the atoms to relax, including the atoms contained in the amide bonds. The minimized ring structures were ranked in order of increasing energy. There were 23 ring conformations within 2 kcal/mol of the minimum energy conformation, and these were retained for further study.

In the third step, the exocyclic tyrosine residue and the phenylalanine side chain were attached to each of the 23 ring conformers obtained above. The exocyclic amide bond was held trans and planar and the six remaining exocyclic bonds were surveyed over a 30° grid in order to determine the allowed configurations for the Tyr^1 residue and Phe^3 side chain. In each case, the program scanned approximately 10^6 possible conformations, and about 10 000 solutions were obtained. After calculation of the energies of these conformers they were grouped into low-energy families, with each family consisting of conformers showing similarity in all torsion angles ($\pm$ 30°) and having energies not more than 2 kcal/mol higher than that of the lowest-energy conformer.

In the final step, the lowest-energy conformer in each low-energy conformational family was subjected to extensive energy minimization. In all, over 160 conformations were generated and finally minimized. These conformers were then ranked in order of increasing energy.

Using published torsion angles, we constructed the various candidate conformers that had been proposed for DPDPE by others [7–12]. Minimization of these conformers using the SYBYL force field permitted a direct comparison of their energies with those of the lowest-energy conformers obtained in the present study. The configuration of the original conformers did not change significantly after minimization.

RESULTS

Of the 160 low-energy conformations of the complete DPDPE molecule obtained in the final step of the analysis, five were found to be within 2 kcal/mol of the minimum energy conformer. The five lowest-energy conformers are depicted in Figs. 1 and 2, and their energies and various

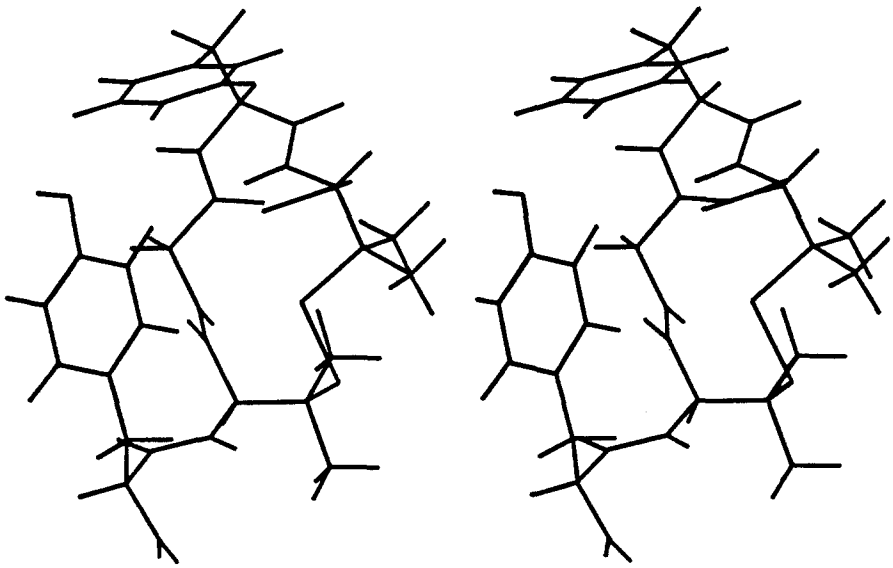

Fig. 1. Lowest-energy conformer (DK11.85) of DPDPE (stereo presentation).

structural parameters are listed in Table 1. None of these conformations contained strong (linear) hydrogen bonds.

In the lowest-energy conformer (DK11.85), the side chain of the Tyr^1 residue is in the t configuration and the Phe^4 side chain is in the g^+ configuration. The Tyr^1 aromatic ring is positioned over the peptide ring structure in relatively close proximity to the Gly^3 residue, and the Phe^4 side chain

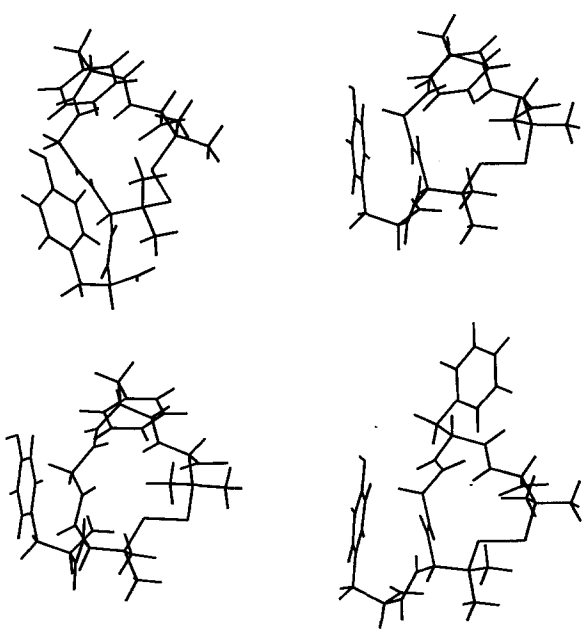

Fig. 2. Low-energy conformations of DPDPE. Clockwise from upper left: DK11.1, DK8.201, DK8.101, DG9.7.

TABLE 1
VARIOUS PARAMETERS OF LOW-ENERGY CONFORMERS OF DPDPE

	Conformers				
	DK11.85	DK11.1	DK8.201	DK8.101	DG9.7
Torsion angles (degrees)					
ψ_1	−55.3	−43.8	−39.0	−43.9	−42.9
ω_1	−175.5	178.6	−179.0	−179.8	178.5
φ_2	63.4	135.5	143.3	138.4	138.4
ψ_2	−150.3	−154.0	−152.3	−152.2	59.8
ω_2	179.3	178.3	179.9	179.4	173.2
φ_3	85.2	75.5	61.3	59.4	−152.5
ψ_3	−141.7	−134.0	−86.4	−84.0	−65.1
ω_3	−176.7	−175.7	−171.0	−173.6	175.5
φ_4	−65.1	−66.5	−70.4	−64.9	−65.2
ψ_4	−39.1	−40.1	−51.8	−62.2	−45.1
ω_4	−176.3	−175.1	−177.3	−175.9	−177.4
φ_5	141.8	141.3	140.3	141.6	138.0
$\chi_1(1)$	164.2	71.4	68.4	70.3	62.5
$\chi_2(1)$	44.9	97.6	87.5	97.4	87.7
$\chi_1(2)$	−48.3	−40.8	−63.2	−63.3	−47.5
$\chi_2(2)$	−54.8	−49.6	−167.8	−168.2	−168.7
$\chi_1(4)$	66.8	70.4	74.2	175.9	67.9
$\chi_2(4)$	82.7	85.0	90.0	82.9	87.7
$\chi_1(5)$	−54.7	−58.4	−87.7	−84.6	−94.3
$\chi_2(5)$	−175.6	−179.4	56.7	59.5	62.8
$\chi_3(5)$	−117.4	−116.7	116.5	115.5	117.7
Intramolecular distance (Å)					
Ring/ring	5.5	6.1	5.7	8.3	5.8
Tyr ring/NH_2	5.1	4.2	4.1	4.1	4.0
Tyr-OH/NH_2	7.8	6.6	6.5	6.6	6.4
Tyr ring/S_2	5.8	5.6	5.6	5.4	5.6
Tyr ring/S_5	4.7	4.8	7.6	7.4	7.6
Tyr ring/Gly-αH	3.6	4.3	4.3	4.3	4.0
Energy (kcal/mol)	−0.86	−0.57	−0.55	0.89	1.14

is also folded over the peptide ring structure. The intramolecular distance between the two aromatic rings in this conformer is 5.5 Å. In all four of the other low-energy conformers, the Tyr^1 side chain assumes the g^+ conformation (Table 1, Fig. 2), resulting in a proximity relationship between the Tyr^1 aromatic ring and the Gly^3 residue similar to that seen in the lowest-energy conformer. In three of these conformers, the Phe^4 side chain is also in the g^+ configuration which, in each case, allows for a number of energetically favorable VDW contacts between the Phe aromatic ring and part of the peptide ring structure. However, in one of the low-energy conformers (DK8.101) the Phe^4 side chain assumes the t configuration and points away from the rest of the

peptide molecule. In all five lowest-energy conformers, the disulfide dihedral angle is limited to values of around either $-115°$ or $+115°$. The observed intramolecular distance between the Tyr[1] and the Phe[3] aromatic rings ranges from 5.5 to 8.3 Å (Table 1).

Three of the conformers of DPDPE identified in the present study are lower in energy than any of the candidate conformers that have been proposed by others [7–12]. The comparatively lowest-energy structure was reported in a study using the AMBER program [10], and was found to be only 0.66 kcal/mol higher in energy than the lowest-energy conformation (DK11.85) obtained in this study.

DISCUSSION

In comparison with the linear enkephalins, the conformational flexibility of DPDPE is considerably reduced not only because it is a cyclic peptide but also because the presence of the geminal dimethyl groups in the Pen[2] and Pen[5] side chains introduce further conformational constraints. However, our grid search of the 'bare' 14-membered ring structure of DPDPE led to the identification of 23 conformers with energies less than 2 kcal/mol higher than that of the lowest-energy conformer found, indicating a still considerable degree of structural flexibility.

Several of the various proposed candidate conformers of DPDPE are characterized by standard turns in their ring structure. One study [8] focused on ring conformations containing a γ-turn or an inverse γ-turn centered around the Gly[3] residue only, and thus was not comprehensive, since a systematic conformational search of the exocyclic Tyr and Phe segments was not performed with the other low-energy ring conformers obtained. Another theoretical analysis resulted in a model characterized by a 'γ-like' turn without a hydrogen bond, also centered around Gly[3] [12]. The results of a further study led to the proposal of an inverse γ-turn centered around the Phe[4] residue [11]. Finally, a low-energy conformation characterized by a type IV β-like turn with no hydrogen bonds has also been proposed on the basis of a theoretical conformational analysis in conjunction with an NMR study [7]. However, none of the lowest-energy conformers identified in the present study contained any of the standard turn structures, and transannular hydrogen bonds did not exist in any of them. There is no compelling reason to assume, a priori, that in this unusual peptide ring structure, obtained through cyclization between the side chains of two D-amino acids, one of the standard turns must be present.

An important structural parameter of the 14-membered ring structure in DPDPE is the dihedral angle of the disulfide bond. Interestingly, values around $+115°$ or $-115°$ for this angle were observed in all five low-energy conformers reported here. In agreement with these results, the lowest-energy conformer obtained in the recent study by Froimowitz [10] showed a dihedral angle about the disulfide bond of 112°. On the other hand, in one of the previously proposed candidate conformers of DPDPE the disulfide dihedral angle was in the vicinity of 180° [11]. It has been shown that the dihedral angle of the disulfide bond of cystines in crystal structures of proteins tends to be close to $+90°$ or $-90°$ [20]. However, the assumption that this is also the case for DPDPE may not be appropriate, for two reasons. First, because of the conformational constraints existing in the relatively small ring structure of DPDPE, a disulfide dihedral angle deviating from $\pm 90°$ may be energetically more favorable despite any barrier of rotation around that bond. Second, the conformational preference of cystine may be different from that of penicillamine. In fact, the dihedral angle of the disulfide bond determined in the crystal structure of 3,3,3′,3′-tetramethyl-D-cystine (D-penicillamine disulfide) dihydrochloride is 115° [21].

The results of the conformational search and energy minimization of the exocylic Tyr^1 residue and the Phe^3 side chain indicated that these moieties have considerable orientational freedom, and that rotation about the exocyclic bonds is hindered only by steric effects due to the presence of the peptide ring structure. The interaction of the Tyr^1 aromatic ring with the peptide ring structure in the Gly^3 region must be energetically favorable, since it is seen in all five lowest-energy conformers. The short intramolecular distance (3.6–4.3 Å) between the center of the Tyr^1 aromatic ring and one of the α-protons of Gly^3 seen in these conformers might be the reason for the large chemical-shift difference between the two glycine α-hydrogens which has been observed in several NMR studies [7,22,23]. Furthermore, the Tyr^1 aromatic ring is also positioned fairly close ($\sim$ 6 Å) to the disulfide bond in these lowest-energy conformers, in agreement with experimental evidence obtained from fluorescence and NMR experiments [24,25]. In the comparison of the various proposed candidate conformers of DPDPE, the Tyr^1 side-chain configuration represents an important conformational parameter. In our lowest-energy conformer the Tyr^1 side chain assumes the t configuration, as was also the case in conformational models proposed in other studies [7,8,11,12]. Our other four low-energy conformers showed a χ_1 angle around $+60°$ for Tyr^1, as also observed in low-energy conformers obtained in one previous study [8], whereas the results of other conformational analyses indicated no significant population of the g^+ configuration [7,9]. In the low-energy conformers most recently described by Froimowitz [10], the Tyr^1 side chain is either in the g^+ or t configuration. The lowest-energy conformer obtained in the latter study, and our lowest-energy conformer, show some overall topographical similarities, insofar as the two aromatic rings are located on the same side of the peptide ring structure and the ring-to-ring distance is similar (4.9 Å vs. 5.5 Å). However, in comparison with our lowest-energy conformer, the Tyr^1 side chain in the lowest-energy conformer reported in the Froimowitz study is oriented somewhat closer to the disulfide bridge and further away from the Gly^3 region. The fact that our search strategy did not produce the latter conformation is difficult to explain. Possibly, the dihedral angle increment (30°) used was still not small enough to permit a totally comprehensive search.

In the lowest-energy conformers described here, the distance between the center of the Tyr^1 aromatic ring and the N-terminal amino group is between 4.0 and 5.1 Å, and the distance between the hydroxyl oxygen and the α amino group of Tyr^1 ranges from 6.4 to 7.8 Å. These distances are similar to the distances between corresponding moieties in morphine, where the distance between the center of the aromatic A ring and the nitrogen atom is 4.5 Å, and the distance between the hydroxyl oxygen of the A ring and the nitrogen atom is 7.1 Å [26]. These results support the notion that the tyramine moiety of morphine and the Tyr^1 tyramine portion of the enkephalins may interact with the same opioid receptor subsites.

The intramolecular distance between the Tyr and Phe aromatic rings of opioid peptides is generally considered an important structural parameter for specific recognition at a distinct receptor type. The ring-to-ring distances determined in our five low-energy conformers range from 5.5 to 8.3 Å. Considerable variation in this distance is seen between the various candidate conformers. Whereas in one model, the Tyr^1 and Phe^4 aromatic rings are in close proximity to one another [12], they appear to be far apart in another proposed low-energy conformer [11]. Obviously, it is still unclear what the requirements of the δ receptor are with regard to this conformational parameter. The recently discovered δ-selective antagonist naltrindole [27] contains an indole moiety fused to the C_{6-7} position of the morphinan skeleton, and it has been speculated that the 6-mem-

bered ring of the indole moiety of this compound may correspond to the Phe^4 aromatic ring in enkephalin. The intramolecular distance between the two 6-membered rings determined in an energy-minimized structure of naltrindole (6.4 Å) is close to the distance between the Tyr^1 and Phe^4 aromatic rings (6.1 Å) seen in one of our lowest-energy conformers of DPDPE (DK11.1) (see Table 1). While this comparison is interesting, it is, of course, by no means certain that the Phe^4 side chain of DPDPE and the indole aromatic ring of naltrindole do indeed interact with the same receptor subsite.

In a recent study [9], a set of 18 distance constraints were derived from NMR data and used in conjunction with distance geometry calculations and energy minimization studies to construct candidate conformers of DPDPE. Low-energy conformers were obtained which, according to the results of the present study, are between 2.6 and 13.9 kcal/mol higher in energy than the lowest-energy conformer obtained by us (see Table 2). Each of the candidate conformers described by Mosberg et al. [9] gave rise to two or three violations of the distance constraints, whereas the low-energy conformations obtained in the present study violated either five or six of these same distance constraints.

TABLE 2
VARIOUS PROPOSED LOW-ENERGY CANDIDATE CONFORMERS OF DPDPE

Reference	Conformer	Energy (kcal/mol)
Wilkes and Schiller (this study)	DK11.85	−0.9
	DK11.1	−0.6
	DK 8.201	−0.6
	DK 8.101	0.9
	DG 9.7	1.1
Hruby et al. [7]	1	10.9
	1P	11.6
	2	5.9
	2P	8.1
Froimowitz and Hruby [8]	2A	5.5
	2B	6.5
	2C	6.8
	2D	7.2
Mosberg et al. [9]	I	13.0
	II	3.9
	III	1.8
	IIIP	4.0
Keys et al. [11]	5	9.9
Nikiforovich and Balodis [12]	1	6.6
Froimowitz [10]	1	−0.2

CONCLUSIONS

Our theoretical analysis, performed without imposing any constraints based on NMR or other spectroscopic data, has led to the identification of conformers of DPDPE that are between 0.7 and 13.9 kcal/mol lower in energy than all the other candidate conformers reported to date. It should, of course, be realized that the lowest-energy conformer may not necessarily be identical with the receptor-bound conformation, and that a conformer a few kcal/mol higher in energy might well represent the bioactive conformation, since the relative increase in the energy content of the molecule could be compensated for by part of the receptor interaction energy. An important aspect of the present study is the demonstration that DPDPE is still a somewhat conformationally flexible molecule. In view of this demonstrated structural flexibility, it is not surprising that no consensus has been reached as yet with regard to the bioactive conformation of this analog. Further conformational restriction of DPDPE (e.g. side chain conformational constraints) will be necessary to elucidate the receptor-bound conformation and to identify the distinct conformational requirements of the δ receptor as opposed to the μ receptor.

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