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Conformational-Energy Studies of Tetrapeptide Opiates

Candidate Active and Inactive Conformations

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SUMMARY

The conformational behavior of four tetrapeptide enkephalin analogues (Tyr-Gly-Gly-Phe-OH, Tyr-Gly-Gly-Phe-NH_2, Tyr-D-Ala-Gly-Phe-NH_2, and Tyr-D-Ala-Gly-(NMe)Phe-NH_2) was examined to identify conformations that are active and inactive at the opiate analgesic receptor. By using an empirical energy program, conformational energies were obtained for the optimized geometries of each tetrapeptide. Two methods of selecting candidate active conformations from low-energy conformers were used. In the first method, inactive conformers were designated as low-energy conformations of the very weak tetrapeptide, Tyr-Gly-Gly-Phe-OH. These candidate inactive conformers had geometries resembling βV, βI, or "random" peptide conformations. Candidate active conformers selected were low-energy conformations found for both Tyr-D-Ala-Gly-Phe-NH_2 and Tyr-D-Ala-Gly-(NMe)Phe-NH_2 but not low-energy conformers for Tyr-Gly-Gly-Phe-OH. In the second method of selection, conformers with relative energies in the active and inactive peptides that followed the potency order Tyr-Gly-Gly-Phe-OH $\ll$ Tyr-D-Ala-Gly-Phe-NH_2 $\leq$ Tyr-D-Ala-Gly-(NMe)Phe-NH_2 were chosen as candidate active conformers. By using both methods of selection, a βII′ bend geometry was found as the active conformer. This βII′ conformer was not stabilized by a 1—4 hydrogen bond, but instead was stabilized by a hydrogen bond between the tyrosine amine hydrogen atom and the phenylalanine carbonyl oxygen atom. The effect of C-terminal amide derivitization on peptide conformation was also examined by comparing the conformational profiles of Tyr-Gly-Gly-Phe-OH and Tyr-D-Ala-Gly-Phe-OH with their amides Tyr-Gly-Gly-Phe-OH-NH_2 and Tyr-D-Ala-Gly-Phe-NH_2. No significant difference in conformational behavior was found for the Tyr-Gly-Gly-Phe pair; however, a difference in conformational behavior was found between the Tyr-D-Ala-Gly-Phe acid and amide. Thus, on the basis of conformational data, the Tyr-Gly-Gly-Phe-NH_2 analogue is predicted to have very weak opiate activity.

INTRODUCTION

Met-enkephalin and Leu-enkephalin are two endogenous pentapeptides isolated from mammalian brain (1–5) that possess opiate activity. These pentapeptides have the amino acid sequences Tyr-Gly-Gly-Phe-Met-OH and Tyr-Gly-Gly-Phe-Leu-OH, respectively. Enkephalins have been shown to inhibit electrically induced contractions of mouse vas deferens and guinea pig ileum (6, 7), to bind stereospecifically to receptor sites that also bind opiates such as morphine and naloxone (1–7), and to produce transient, naloxone-reversible analgesia when administered intracerebroventricularly (8).

The effect on opiate activity of changes in the structure and sequence of enkephalin has been studied in detail and recently reviewed (9). As shown in Table 1, removal of the terminal methionine or leucine residue results in a drastic reduction in activity of enkephalin. Although relatively inactive, the resulting tetrapeptide, TGGP,[1] is the minimal enkephalin fragment capable of eliciting opioid response. In comparison to one of the parent compounds, Met-enkephalin, the tetrapeptide retains 1%–3% of its ability to inhibit [^{3}H]dihydromorphine binding (10, 14) and 1% of Met-enkephalin's agonist potency in mouse vas deferens and guinea pig ileum (7, 12). Despite its lower activity, the structure-activity profile of this tetrapeptide resembles that of the pentapeptide, Met-enkephalin. Substitution of a D-alanine residue for

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[1] The abbreviations used are: TGGP, Tyr-Gly-Gly-Phe-OH; TGGPA, Tyr-Gly-Gly-Phe-NH_2; TDAGP, Tyr-D-Ala-Gly-Phe-OH; TDAGPA, Tyr-D-Ala-Gly-Phe-NH_2; NMeP, Tyr-D-Ala-Gly-(NMe)Phe-NH_2; ECEPP, empirical conformational energy program for peptides; PCILO, perturbative configuration interaction using localized orbitals; Me_2SO, dimethyl sulfoxide; $(CD_3)SD$, deuterated methyl sulfide; PET, 7-[1-phenyl-3-hydroxybutyl-3]-endoethenotetrahydrothebaine.

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TABLE 1

Relative opiate activities of enkephalins and some tetrapeptide analogues

Compound	Relative receptor affinity (morphine = 1)	Relative agonist potency			
		Whole animal (morphine = 1)		*In vitro* assays (Met-enkephalin = 1)	
		Intravenous	Intraventricular	Guinea pig ileum	Mouse vas deferens
Tyr-Gly-Gly-Phe-Met-OH	0.38[a]	—	0.01[b]	1.0	1.0
Tyr-Gly-Gly-Phe-Leu-OH	0.125[a]	—	—	0.21[c]	1.64[c]
Tyr-Gly-Gly-Phe-OH	<.01[a]	—	—	0.01[c, d]	0.01[c, d]
Tyr-D-Ala-Gly-Phe-OH	0.99[e]	0.1[e]	0.1[e]	—	—
Tyr-D-Ala-Gly-Phe-NH_2	1.14[e]	0.5[e]	2.5[c]	—	—
Tyr-D-Ala-Gly-N(CH_3)Phe-NH_2	1.84[e]	0.5[c]	2.5[c]	—	—
Morphine	1.0	1.0[d]	1.0[d]	1.7[c]	0.026[c]

[a] Terenius *et al.* (10). Binding data from competitive receptor binding assays in rat brain homogenate against [^{3}H]dihydromorphine.
[b] Roemer *et al.* (11).
[c] Waterfield *et al.* (7).
[d] Morgan *et al.* (12).
[e] McGregor *et al.* (13). Binding data from competitive receptor binding assays in rat brain homogenate against [^{3}H]naloxone.

glycine in position 2, the addition of a methyl group to the phenylalanine backbone nitrogen, and amide derivitization of the terminal carboxyl residue lead to increased activity in both pentapeptide (9, 11, 15) and the tetrapeptide (13).

The tetrapeptide enkephalin analogue with a D-Ala^2 substitution, TDAGP, has a receptor affinity 10 times that of TGGP (13). The D-Ala^2 substitution may create an apparent increase in receptor affinity by retarding rapid enzymatic degradation in brain homogenate (16), or it may cause an actual increase in affinity by either inducing a change in the conformational profile of the peptide or interacting directly with sites on the opiate receptor.

The amide tetrapeptide, TDAGPA, has a receptor affinity and analgesic activity 10 times greater than its free acid analogue, TDAGP (13). It is 0.5 and 2.5 times as potent as morphine in intravenous and intraventricular analgesic activity, respectively, and has a somewhat greater receptor affinity. Amide derivitization prevents formation of the zwitterionic form of the peptide that normally exists at physiological pH. The lack of a charged COO^- terminus in the amide could affect observed activity by altering the conformational behavior of the peptide or by changing interactions of the peptide with the receptor.

The addition of a methyl group to the phenylalanine backbone nitrogen, NMeP, results in an analogue with analgesic properties similar to that of TDAGP. NMeP has a greater receptor affinity than TDAGP—almost twice that of morphine—in competitive binding studies with [^{3}H]naloxone (13).

Despite the structural disparity between the many peptide and nonpeptide opiate compounds known, recent experimental evidence suggests that analgesia is produced by binding of all opiate compounds to a common, high-affinity receptor (17). The presence of other receptor populations which discriminate between opiate compounds in their binding affinities is also suggested by these studies, in agreement with previous binding and activity data which demonstrate the existence of multiple opiate receptor populations (18–22), in which analgesia is mediated primarily by one class of receptors (23, 24). Analysis of the binding data from work with the "irreversible" ligand naloxazone (17) indicates that the number of common, high-affinity analgesic sites is small, whereas the number of lower-affinity, discriminating sites is large.

If analgesia is mediated by a single type of opiate receptor, then a similar pharmacophore must be present in all active opiates which enables different compounds to bind and act at the same analgesia-producing receptor.

A chemical moiety found in the tyrosine residue of enkephalins and in most fused-ring opiate compounds such as morphine is a phenolic ring separated from an amine by two methylene groups. This "tyramine" moiety may interact through similar mechanisms in different compounds at a common analgesic receptor site (25–29), thus forming the basis of a shared pharmacophore in all opiates. Substitutions of the tyrosine residue in enkephalin generate analogues with little or no agonist activity (9, 12), giving further support to this proposal.

Much experimental and theoretical effort has been expended to elucidate the conformational and electronic molecular characteristics of the opiate pharmacophore shared by morphine and the enkephalins. Because of the high degree of conformational flexibility in peptides, it is difficult to determine the underlying conformational and electronic features that can explain the relative activity of a series of peptide analogues. Many conformations of a single peptide are energetically possible; hence the relative energy between stable conformers is not a sufficient criterion by which to select candidate conformers for interaction at the receptor. Other criteria such as stereochemical similarity to other classes of opiates must also be used in such a selection process.

In a previous study (29) of Met-enkephalin and D-Ala^2-Met-enkephalin we used the criteria of energy and pharmacophoric overlap with potent oripavine compounds to deduce a candidate for the active form of the pentapeptide opiates. The conformer chosen resembled a βII′ bend between the second and third residue. Although this approximate βII′ bend was not the lowest energy form, it had other features such as accommodation of a D-Ala, but not an L-Ala, as a second residue, and better overlap with oripavines, which made it a likely candidate. A

similar conformer had also been proposed based on model building (25) and resembles the structure of Leu-enkephalin subsequently determined by X-ray crystal structure (26). Subsequent theoretical studies of active enkephalin conformation also led to the proposal of β bend structures, including a βII′ bend between the Gly[2] and Gly[3] residues, as the active form of peptide opiates (30–33). Experimental studies on Met- and Leu-enkephalin using a variety of spectral techniques (34–37) support βI′- and βII′-type bends, but between the Gly[3] and Phe[4] residues rather than Gly[2] and Gly[3] residues as possible active conformations.

In this study we have taken a somewhat different approach to further elucidation of the active form of peptide opiates. The main goal of this study was to determine the type of conformation of peptide opiates corresponding to low and high receptor affinity and analgesic activity. We have investigated the conformational behavior of four related tetrapeptides: TGGP, an almost inactive peptide; TGGPA, not yet tested for activity; TDAGPA, active in opioid assays; and NMeP, a potent opioid agonist despite the presence of an "abnormal" *N*-methylated phenylalanine residue. This *N*—CH_3 group in NMeP should restrict the number of low-energy conformers possible and prevent backbone hydrogen bonding between the tyrosine carbonyl oxygen and phenylalanine amine hydrogen (1 → 4 H-bonding) previously found in β turns. These considerations suggest that this peptide would have an active conformation different from those proposed for the des-methyl analogues. However, the results presented here refute this suggestion. Two sets of mutually exclusive low-energy conformers were found—one common to the highly active peptides, and the other present in the weakly active peptide—that represent candidate conformers of peptide opiates leading to high and low affinity and activity, respectively.

We have also investigated the effect on conformational behavior of changing the C-terminal group from a free carboxylic acid (COOH) to an amide ($CONH_2$). Such derivatization has been found to enhance the activity of many enkephalin analogues (9). The observed enhancement in activity could be due to any one, or a combination, of three factors: (a) changes in the degradation of the peptide, (b) effects on the conformational behavior of the peptide, and (c) interaction of the amide function with a specific site on the opiate receptor upon binding. To address the question of conformational effects of the amide group we have studied the conformational profiles of two tetrapeptide acid/amide pairs: the peptide TDAGP and its more potent amide, TDAGPA, and the nearly inactive peptide, TGGP, along with its yet-untested amide analogue, TGGPA.

METHODS AND PROCEDURES

Conformational energy calculations were performed with the use of a modified version of an empirical energy program called ECEPP,[2] originally developed by Momany and associates (38). This program has been modified in our laboratory to include additional atom types which allow calculations for peptides with unusual amino acid residues such as N(Me)phenylalanine. In this approach the total energy is computed as a sum of contributions of the energy terms:

$$E = E_{el} + E_{rep} + E_{att} + E_{HB} + E_{tor}$$

where E_{el} = electrostatic energy, E_{rep} = repulsive energy between nonbonded atoms, E_{att} = attractive energy between nonbonded atoms such as dispersion, E_{HB} = hydrogen bond energy, and E_{tor} = torsional energy. This program has also been coupled to an energy minimization procedure that employs a gradient search method to optimize backbone and side-chain dihedral angles, leading to conformations which are energy minima. An energy convergence criterion of 0.01 kcal/mole was used in minimizations performed in this study.

In our laboratory, this empirical energy program was found to produce conformational energy profiles with steep potential wells to local minima. In order to compare results obtained by this empirical energy calculation with another method, the semiempirical quantum mechanical method PCILO[3] (39) was also used for single-point energy calculations of energy-optimized geometries of TDAGP obtained by the empirical energy method.

At physiological pH (~7.4) in aqueous media, the tetrapeptide TGGP exists predominantly in its zwitterionic form. The peptides TDAGP and NMeP exist in the charged NH_3^+ form, since the amide function on the carboxyl terminus prevents ionization to the carboxylate anion. Exploratory calculations conducted on the zwitterionic and charged forms of these peptides showed that, *in vacuo*, interactions of the charged groups are the dominant factor affecting conformation (data not shown). Although experimental studies on the effect of pH on the conformation of the Met-enkephalin zwitterion in Me_2SO and $(CD_3)SD$-H_2O solution indicate that such "head-to-tail" intramolecular interactions may contribute to the conformational stability of the peptide (40, 41). The X-ray crystal structure of TGGP (42, 43) indicates that similar interactions do not occur to the same extent in the tetrapeptide. The charged end-groups of the TGGP zwitterion appear to be separated by 4–8 Å (42, 43) in the crystal, and the charged groups are stabilized by intermolecular hydrogen bonds with Me_2SO and water solvent molecules as well as other TGGP molecules. We have interpreted these findings to indicate that the behavior of the zwitterion species exhibited in our laboratory calculations is inappropriate because of the unmitigated interaction between the charged end-groups. In an aqueous physiological environment, these charged end-groups would probably be solvated, resulting in their partial "neutralization." Therefore, for this study, calculations were carried out on the four tetrapeptides with neutral end-groups: a free NH_2 group at the *N*-terminal and a —COOH or —$CONH_2$ group at the *C*-terminal.

Our exclusion of solvent from explicit consideration in our calculations may have a significant effect on the interpretation of our results, depending on the role of solvent at the receptor opiate binding site. Unfortunately, not much is known about the immediate environment of the receptor, e.g., whether the binding site is exposed to

[2] The FORTRAN computer program for ECEPP, its description, and all associated geometric and energy parameters are available on magnetic tape from the Quantum Chemistry Program Exchange. Write to Quantum Chemistry Program Exchange, Chemistry Department, Room 205, Indiana University, Bloomington, Ind. 47401.

[3] The FORTRAN computer program for PCILO and its description can be obtained from the Quantum Chemistry Program Exchange.

TABLE 2

Dihedral angles of initial backbone conformations

	ϕ_1	ψ_1	ϕ_2	ψ_2	ϕ_3	ψ_3	ϕ_4	ψ_4
β Bend I			−60°	−30°	−90°	0°		
β Bend I′			60	30	90	0		
β Bend II			−60	120	80	0		
β Bend II′			60	−120	−80	0		
β Bend III			−60	−30	−60	−30		
β Bend III′			60	30	60	30		
β Bend V			−80	80	80	−80		
Repeating C_5	−154°	153°	−154	153	−154	153	−154°	153°
Repeating C_7	−80	80	−80	80	−80	80	−80	80
Right α-helix	−72	−54	−72	−54	−72	−54	−72	−54
Left α-helix	55	63	55	63	55	63	55	63
γ Turn	172	128	68	−61	−131	162		

aqueous solvent or embedded in the membrane lipid layer. Until more information on the receptor microenvironment is known, the significance of ignoring solvent in these calculations is uncertain.

The following search strategy was employed to determine the energy-conformational profile and particularly the low-energy conformers of the four tetrapeptides investigated, with the exception of TDAGP.

Fifty-two low-energy conformers obtained from previous calculations on Met-enkephalin (30) were employed as starting conformers. To these were added regular repeating C_5 and C_7 conformers and idealized β turns of each type involving the second and third or third and fourth residues, referred to as G—G or G—P bends, respectively. The backbone angles corresponding to these standard conformers are given in Table 2. In all, 98 starting conformations were used. Table 3 identifies these conformers by the closest idealized geometries for Conformers 1–52 and from the actual idealized geometries used for Conformers 54–98.

Total geometry optimization of all dihedral angles was performed by using these starting geometries and peptide bond torsion angles (ω) of 180°. However, when this procedure was followed for the NMeP, either very high-energy conformers were obtained or the optimization procedure failed to converge. One origin of the difficulty in obtaining reasonable low-energy conformers of NMeP is the interference of the N—CH_3 group on phenylalanine with a totally *trans* (ω_i = 180°) backbone configuration. A slightly different search procedure was therefore used for NMeP. With the aid of a computer program that manipulates molecular structure and includes graphics capabilities, starting conformations were chosen which relieved the steric hindrance of the all-*trans* conformation. These structures typically had starting ω angles of ~155°. Torsion angle optimization was then performed with the constraint of keeping the ω angles fixed at their initial values. This was followed by total geometry optimization, including ω angle variation. This procedure yielded totally optimized conformers with significant deviations from ω angles of 180° in some cases, and in others yielded conformers with ω angles close to 180°. A few initial conformers chosen for each tetrapeptide failed to converge to optimal geometries. These were eliminated from further consideration as candidate conformers.

TABLE 3

Initial geometries of conformers[a] chosen

Type	Conformer
Random	15, 37, 38, 40–44, 51
GGβI	32, 33, 36, 45
GGβI′	47
GGβII	27
GGβII′	14, 16, 17, 35, 50, 52
GGβIII	23, 24, 29
GGβIII′	31, 34, 39
GGβV	4–67, 10–13, 18, 19, 46, 48, 49
GPβII′	21
GPβIII	23, 28
GPβIII′	19
Right α-helix	2
Repeating C_5	3
Repeating C_7	1, 22
E_2 structure[b] nearly GGβII′	53
Model building	54
$C_5C_5C_5C_7$	55–63
C_5C_5GPβII′	64
C_5C_7GPβI, . . . βII, . . . βII′, . . . βIII, . . . βIII′, . . . βV	65–71
C_5C_5GPβI, . . . βV	72–28
C_5C_5GPβI . . . βIII (different side-chain angle)	79–84
C_7C_5GPβI . . . GPβV	85–91
C_7C_7GPβI . . . βV	92–98

[a] Conformers 1–52 are optimized geometries from previous studies of Met-enkephalin (29, 30), with conformations labeled to their closest idealized geometry. Conformers 54–98 are initial idealized geometry assignments.

[b] From ref. 30.

This procedure led to more than 90 energy-ordered, optimized conformers for each tetrapeptide studied in this manner.

The next property of these conformations investigated was their ability to overlap with the "tyramine" moiety, i.e., the *p*-hydroxyphenethylamine group common to all fused ring opiates. None of the totally optimized conformers had tyrosine side-chain torsion angles, χ_1 and χ_2, which allowed such overlap. To achieve this overlap with the tyramine conformation as it is found in fused-ring opiates, χ_1 and χ_2 were set to values of 267° and 193°, respectively. The conformational energy of the peptide

was then calculated for each conformer without any optimization. Geometry optimization was subsequently performed with these angles constrained to retain the tyramine overlap. Finally, by using these partially optimized configurations as a start, total optimization was performed. For none of these peptides is a conformation with tyramine overlap with rigid opiates a local minimum. Instead, optimized geometries obtained in this last step were very similar to those obtained by the optimization of the initial geometries.

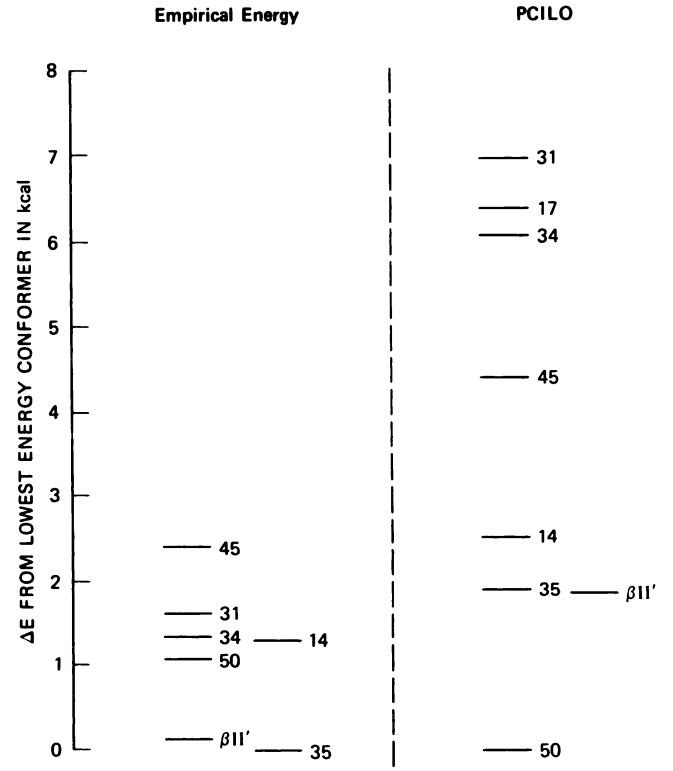
For the acid peptide, TDAGP, the objective of our calculations was to determine whether the absence of a terminal amide group changed the peptide's conformational profile as compared with its amide congener, TDAGPA. To accomplish this, starting geometries that optimized to low-energy conformers of TDAGPA found by the above-described procedure were also used as starting geometries for TDAGP, and total geometry optimization with the empirical energy program was carried out.

RESULTS AND DISCUSSION

In Table 4, relative energies obtained using the empirical energy program are compared with those obtained using the semiempirical quantum mechanical program PCILO for the active tetrapeptide, TDAGPA. The energies are reported relative to the lowest energy conformer obtained by each method. To make this comparison, geometry-optimized conformers with fixed tyramine overlap geometries were obtained by the empirical energy method. These conformers were then used for single-point energy calculations using the semiempirical quantum mechanical method PCILO.

TABLE 4

Comparison of the calculated conformation-energy profiles for TDAGPA[a] by empirical energy and PCILO methods



[a] Total optimization of 12 lowest-energy TDAGPA conformers plus Conformer 53, as described under Methods and Procedures.

Although there are quantitative differences in the energies obtained by these two methods, we see from Table 4 that these methods result in a similar ordering of conformers by their relative conformational energy. The discrepancies between the ECEPP and PCILO results arise largely from the fact that the PCILO energies shown represent single-point calculations of ECEPP-optimized geometries. The geometry at a local minimum from ECEPP may not correspond to an exact energy minimum on the PCILO energy-conformation surface. However, of the nine conformers tested by PCILO and found to have relative energies $\leq$ 7 kcal/mole, all but two also fall within this energy range in the empirical energy calculations. Thus, although candidate conformers of the active and inactive peptides were selected from results of empirical energy calculations, these results indicate that similar conclusions would be drawn from results of at least one other method.

To determine the effect of the amide function on conformation, the results of empirical energy calculations for the tetrapeptides TGGP, TGGPA, TDAGP, and TDAGPA were compared. Tables 5 and 6 show the energy-ordered, optimized conformers for these two acid/amide pairs of tetrapeptides with calculated relative energies (ΔE) $\leq$ 10 kcal/mole.

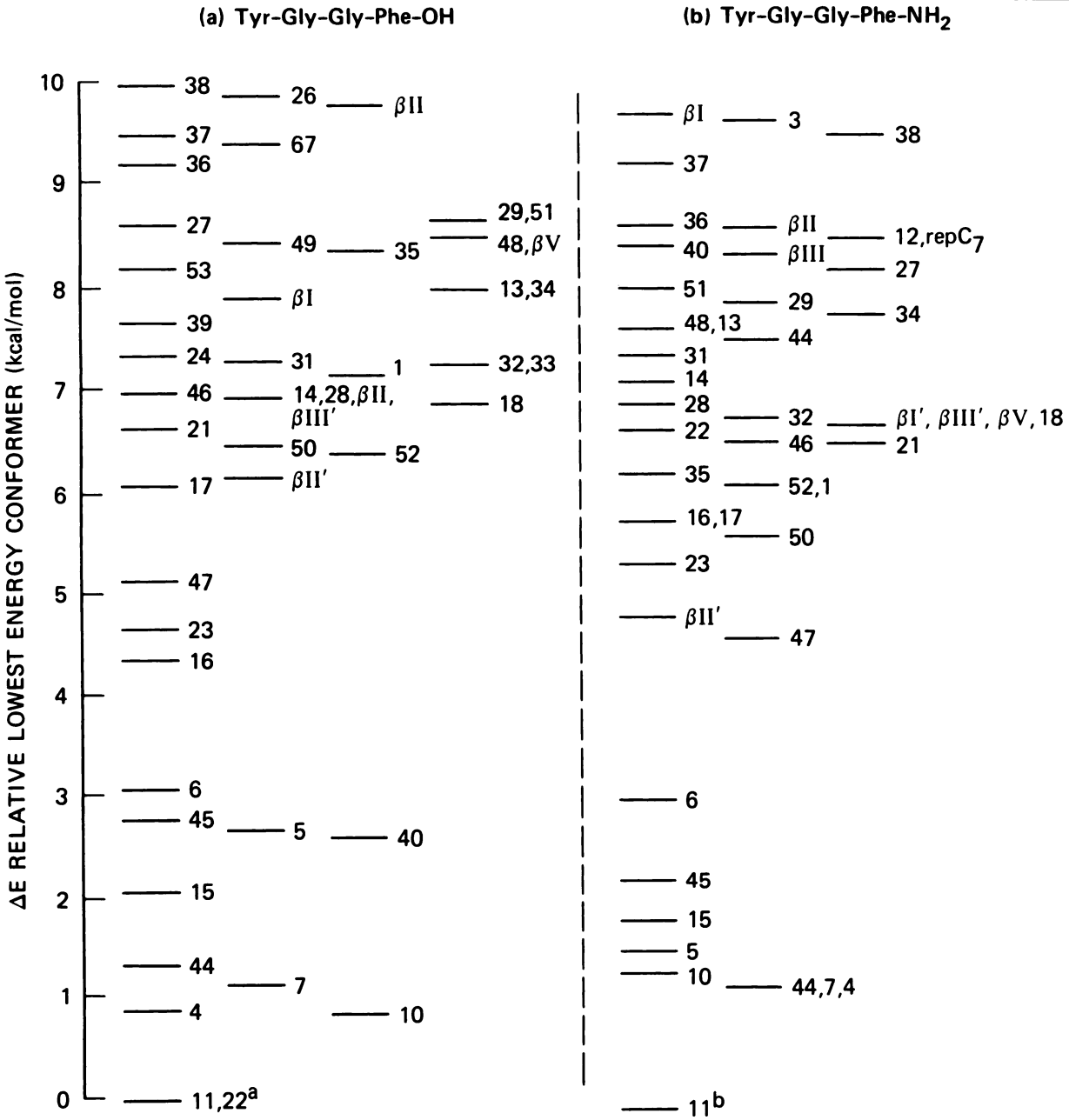
For the pair TGGP and TGGPA, we see in Table 5 that there is a marked similarity in their conformational profile. Both TGGP and TGGPA have the same lowest-energy conformer (Conformer 11), an approximate GGβV turn. Moreover, of the 11 conformers with $\Delta E \leq 3$ kcal/mole in TGGP, all but two, Conformers 22 and 40, are also within the same range in the other peptide, TGGPA, in which the terminal amide group is present. These two conformations of TGGPA have ΔE values of 6.4 and 8.4 kcal/mole, respectively, relative to the energy of Conformer 11. Such parallel conformational behavior holds for both low- and high-energy conformers of both peptides.

As shown in Table 1, TGGP has a very low opiate receptor affinity and weak analgesic activity. The activity of the amide, TGGPA, has not been reported. Our results indicate that the change from a free acid to an amide in this tetrapeptide has little effect on its conformational behavior. On this basis we predict that the amide will have weak opiate activity comparable to that of TGGP. If any significant difference in potency is found between these two related peptides, our results suggest that it would be due to properties other than conformation, e.g., metabolic differences or differences between the interactions of the amide group and ionized free carboxylic acid group with a specific receptor subsite.

In contrast, the results for the pair TDAGP and TDAGPA, shown in Table 6, indicate that the presence or absence of the amide function has a marked effect on the conformational behavior of this tetrapeptide. Although Conformer 15 remains as the lowest-energy con-

TABLE 5

Effect of changing the C-terminal end-group on the conformation-energy behavior of Tyr-Gly-Gly-Phe



[a] Lowest-energy conformers (Conformers 11 and 22) with $E = -3.8$ kcal/mole from total geometry optimization using the empirical energy method.

[b] Lowest-energy conformer (Conformer 11) with $E = -5.04$ kcal/mole from total geometry optimization using the empirical energy method.

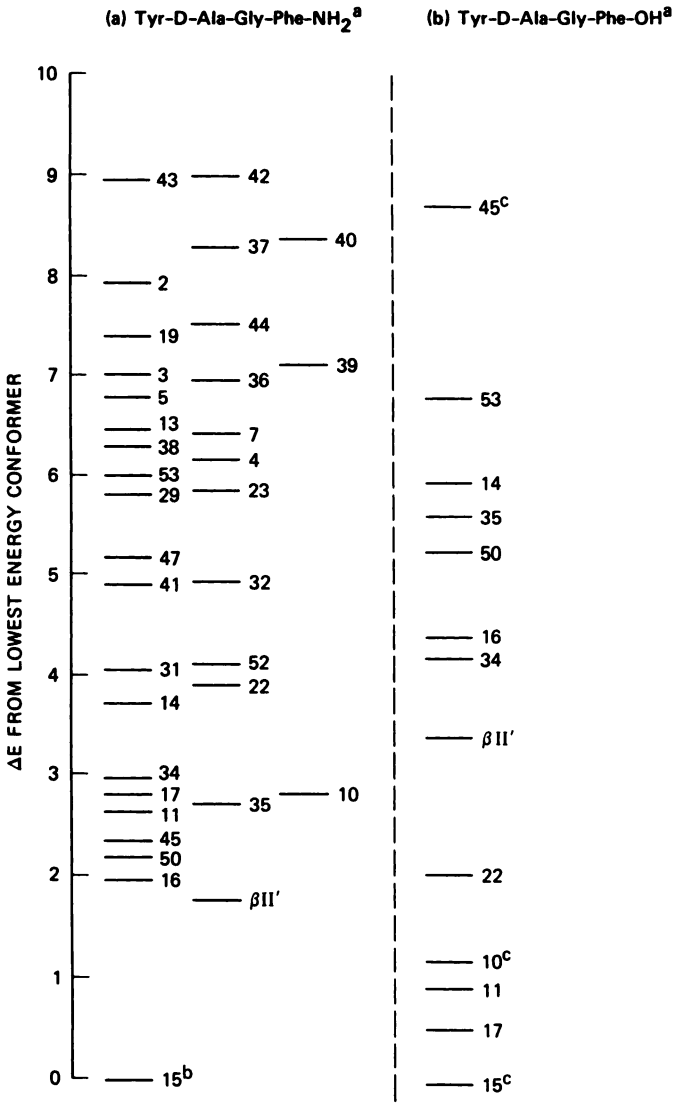
formation for both the amide and acid, the relative energy ordering of the other conformers is different between the two congener peptides. A limited sample and a different sampling technique were used in making this comparison; however, this had no effect on the demonstration by these results that the conformational profile changes dramatically when the C-terminal group of this tetrapeptide is altered.

The activities and receptor affinities of TDAGP and TDAGPA have been determined, as shown in Table 1. The agonist potency and receptor affinity of TDAGP is about one-tenth that of TDAGPA. Our results indicate that these differences in activity are attributable to differences in the conformational profile of the peptide, although other factors, such as differences in receptor site interactions of a charged carboxylate versus a neutral amide, are not necessarily ruled out by these data.

Table 7 summarizes the conformational energy results obtained by the empirical energy method for the three peptides with known activities which were studied: the nearly inactive peptide (TGGP) and the two active peptides (TDAGPA and NMeP). Given in Table 7 are the relative energies of all conformations with $\Delta E \leq 10$ kcal/mole with respect to their lowest-energy conformer.

TABLE 6

Effect of changing the C-terminal end-group on the conformation-energy behavior of Tyr-D-Ala-Gly-Phe



[a] All conformers with $\Delta E \leq 10$ kcal/mole are shown.

[b] Lowest-energy conformer (Conformer 15) with $E = -1.0$ kcal/mole from total geometry optimization.

[c] Lowest-energy conformer (Conformer 15) with $E = 2.05$ kcal/mole from total geometry optimization. Conformers 10 and 45 did not converge to local minima during optimization.

With these data, we have compared the conformational behavior of these peptides for differences that indicate possible inactive and active forms, using two approaches. In the first approach, we propose that "inactive" peptide conformers are defined by low-energy forms found only for the almost inactive peptide, TGGP. We have chosen this compound to delineate possible "inactive" conformations because it is weak but is not completely inactive. The low receptor affinity of this peptide suggests that, although the molecular elements necessary for activity are present in this peptide, it requires a relatively large amount of energy to bind to the receptor. On this basis, as noted in Table 7 and again in Table 8, we have ruled out the 11 conformers of TGGP from Conformers 5–47 with ΔE values ≤ 5 kcal/mole as possible active forms leading to high opiate receptor affinity and analgesic activity. Consistently, as is also shown in Tables 7 and 8, most of these conformers are high-energy forms for the active tetrapeptides. This provides further corroboration that they are not involved in high-affinity receptor binding and activity. The majority of these "inactive" conformers correspond to either GGβV turns (Conformers 5, 6, 7, 10, and 11) or random conformations (Conformers 15, 20, 40, and 44). Typical geometries are shown in Table 9 for four of these conformers, of which two are GGβV turns (Conformers 7 and 11) and two are random geometries Conformers 15 and 22).

The TGGP zwitterion has been examined previously in Me_2SO solution by ^{1}H-NMR and in crystal form using X-ray crystallographic methods by Fournie-Zaluski *et al.* (42) and Prangé and Pascard (43). A GGβI or GGβI′-type conformer was found by both methods with approximate $1 \rightarrow 4$ H-bond stabilization. As shown in Tables 7 and 8, two GGβI conformers, Conformers 45 and 47, were found by our calculations to be low-energy forms of TGGP. The conformations found by Fournie-Zaluski *et al.* (42) and Prangé and Pascard (43) were also examined in this study and optimized using the empirical energy procedures. The final optimized geometries were found to be relatively low-energy forms ($\Delta E \approx 6$–7 kcal/mole), very similar to the original observed β bend conformation.

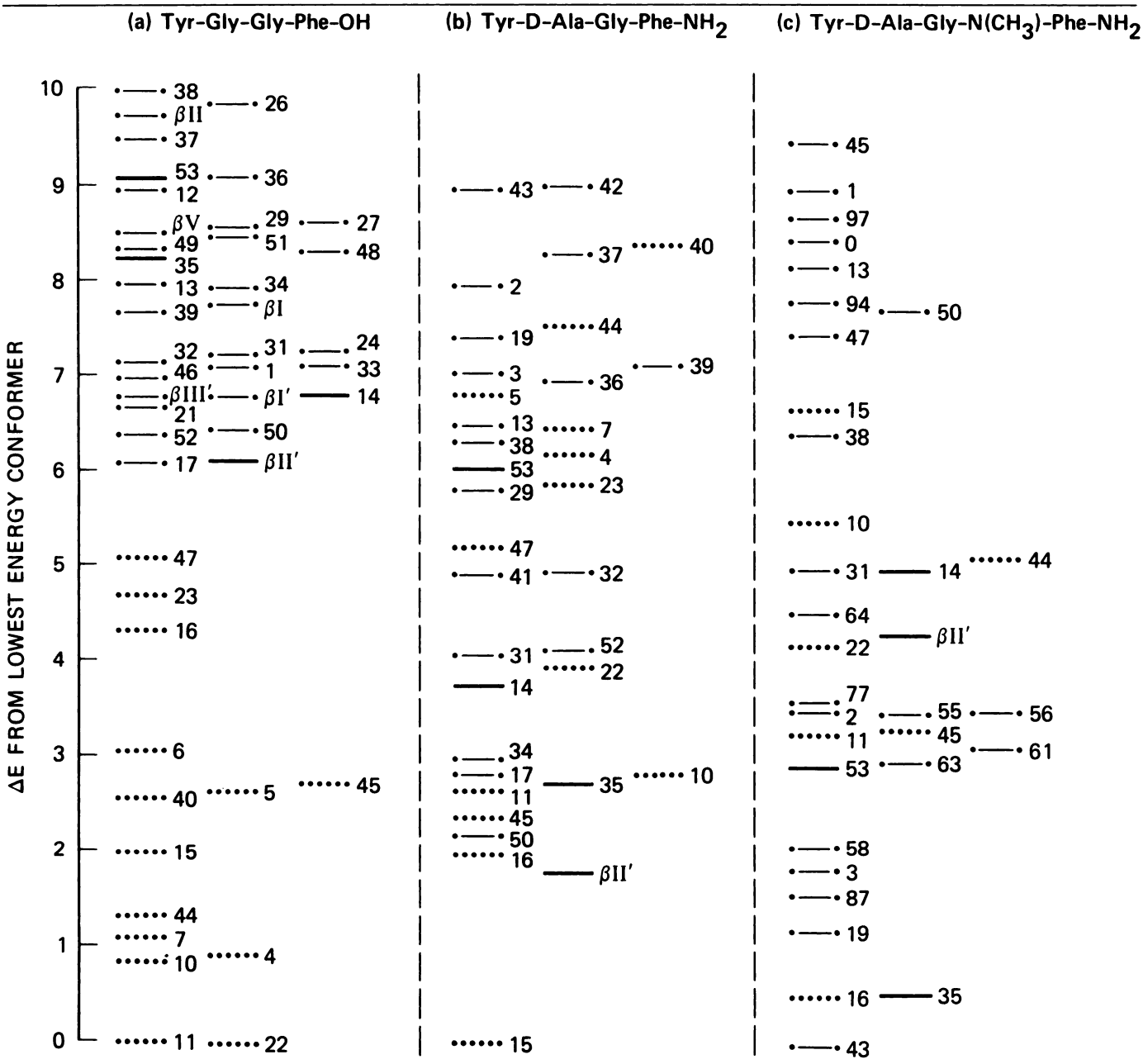
Although low-energy forms of the inactive peptide were chosen by one method of analysis as unfavorable conformations, active forms were chosen as those having low energy in the two potent peptide opiate analgesics and high energy in the inactive peptide. With these criteria, as shown in Tables 7 and 8 (Group IIA conformers), only three candidate geometries remained. All three geometries are approximate βII′ turns between the second and third residues, as shown by the torsion angles given in Table 9. The optimized Conformers 35 and βII′ are identical in TDAGP, but different in NMeP. The βII′ bend is stabilized in these active peptides by a hydrogen bond between the amine hydrogen on the tyrosine residue and the carbonyl oxygen atom on the phenylalanine. This "head-to-tail" interaction replaces the stabilizing effect of a 1—4 hydrogen bond usually found in β turns of this type, and explains in part why a β turn is a possible low-energy form for NMeP, a peptide with an N(CH$_3$)Phe group that interferes with backbone 1—4 H-bonding. In the calculated NMeP low-energy βII′ conformers, the *N*-methyl group twists away from the bend, thereby minimizing repulsive steric interactions, and the head-to-tail hydrogen bond provides the necessary stabilization to maintain the bend.

A second approach to analyzing the results in Table 7 is to look for conformers with relative energies that follow the potency ordering TGGP $\ll$ TDAGPA $\leq$ NMeP. As shown in Table 8 (Group IIB conformers), three conformers meet this criteria: Conformers 16, 35, and 53. As with all conformers selected by the first method, these are also βII′ conformations, as indicated by their torsion angles shown in Table 9.

Our results thus strongly indicate by the two methods of analysis that βII′ turns are the active conformers for these tetrapeptides and that βI turns, βV turns, or random conformations are the inactive forms, and that the

TABLE 7

Calculated energies of optimized conformers[a] for three tetrapeptides by the empirical energy method



····· Candidate inactive conformers obtained from low-energy conformers of weakly active tetrapeptide TGGP.

—— Candidate active conformers obtained from low-energy conformers of both potent active tetrapeptides TDAGP and NMeP.

•—• Remaining conformers.

[a] All conformers with $\Delta E \leq 10$ kcal/mole are shown. Conformer 22 of TGGP is lowest in energy with $E = -3.8$ kcal/mole. Conformer 15 of TDAGPA is lowest in energy with $E = -1.0$ kcal/mole. Conformer 43 of NMeP is lowest in energy with $E = 17.1$ kcal/mole.

differences in activity observed for these peptides *in vitro* are due at least in part to conformational differences.

The ability of the five candidate active conformers to mimic rigid opiates was examined by calculating the energy required to induce a "tyramine overlap" in each. As shown in Table 10, all conformers with the exception of TDAGPA Conformer 53) required similar energy input, ~10 kcal/mole, for such an induced fit. None of them remained in this conformation when total geometry optimization was performed. Thus if tyramine overlap is important at the receptor, binding of the active form of these peptides would involve an induced conformational change at the receptor rather than the binding of a low-energy form of the free peptide already in an "overlap"

TABLE 8

Proposed active and inactive opiate peptide conformers

Conformer	Type	ΔE		
		TGGP[a]	TDAGP[b]	NMeP[c]
		kcal/mole		
Group I: inactive				
5	βV	2.7	6.8	11.4
6	βV	3.1	6.2	17.3
7	βV	1.1	6.3	13.2
10	βV	0.8	2.8	5.5
11	βV	0	2.2	3.4
15	Random	2.0	0	6.7
22	Random	0	3.9	4.3
40	Random	2.7	8.3	9.4
44	Random	1.3	7.5	5.1
45	βI	2.8	2.4	3.4
47	βI′	5.1	5.2	7.3
Group IIA: active, selection method 1				
14	βII′	6.9	3.7	4.4
35	βII′	8.3	2.7	2.4
βII′	βII′	6.1	1.8	2.9
Group IIB: active, selection method 2				
16	βII′	4.3	1.9	0.5
35	βII′	8.3	2.7	2.4
53	βII′	9.0	6.1	2.9

[a] Calculated energy of totally optimized lowest-energy TGPP conformer = −3.8 kcal/mole.

[b] Calculated energy of totally optimized lowest-energy TDAGPA conformer = −1.0 kcal/mole.

[c] Calculated energy of totally optimized lowest-energy NMeP conformer = 17.1 kcal/mole.

conformation. This additional accommodation upon binding of an already favorable form of the peptide to the receptor is in agreement with a proposed "zipper" mode of drug-receptor interaction (44).

In all of these peptides, the amount of energy required to achieve tyramine overlap is comparable for both high- and low-energy conformers. Thus, while such overlap may be necessary, it does not appear to be sufficient for high-affinity receptor binding since, for example, the dipeptide Tyr-Gly is inactive.

TABLE 10

Calculated energy required for tyramine overlap of candidate active opiate peptide conformers with fused-ring opiates

Conformer	E_{tot} (total optimization)	E_{Tyr} (optimized holding overlap fixed)	ΔE_{ind} ($E_{tyr} - E_{tot}$)
Tyr-D-Ala-Gly-Phe-NH_2			
14	2.71	13.27	10.56
16	0.92	12.88	11.96
35	1.69	11.07	9.38
53	4.97	7.67	2.70
βII′	0.78	11.07	10.29
Tyr-D-Ala-Gly-N(CH_3)Phe-NH_2			
14	22.1	32.39	10.29
16	17.6	30.22	12.62
35	17.6	26.47	8.9
53	2.0	28.0	8.0
βII′	21.47	31.22	9.75

To investigate the possibility of additional regions of molecular similarity to fused-ring opiate compounds, the candidate peptide conformers of TDAGPA were superimposed on the potent opioid PET in a manner which included overlap of the tyramine moieties in each compound. PET was chosen for this comparison because of its high opiate activity, because it is a potent binder to μ receptor sites, and because of the presence of two aromatic groups very similar to the tyrosine and phenylalanine phenyl rings. The geometry of the peptides shown is that obtained from optimization while holding the tyramine overlap geometry rigid, as described under Methods and Procedures. These conformations therefore represent the proposed "induced" binding form of the

TABLE 9

Backbone geometry[a] of residues 2 and 3 for proposed active and inactive forms of tetrapeptide opiates

	Inactive			Active					
	Tyr-Gly-Gly-Phe-OH			Tyr-D-Ala-Gly-Phe-NH_2			Tyr-D-Ala-Gly-N(CH_3)Phe-NH_2		
	ϕ_i	ψ_i	ω_i	ϕ_i	ψ_i	ω_i	ϕ_i	ψ_i	ω_i
	7			14			14		
$i = 2$	−143.7	74.02	−167.67	82.65	−80.09	179.80	83.64	−76.33	178.5
$i = 3$	84.1	−68.7	173.10	−69.94	−45.85	179.22	−63.8	−75.4	169.4
	11			35			35		
$i = 2$	175.40	83.9	−165.27	72.09	−109.77	177.37	81.4	−72.9	178.6
$i = 3$	85.37	−65.4	166.65	−67.07	−43.92	−179.48	−160	−63.3	151.0
	15			βII′			βII′		
$i = 2$	−171.68	62.36	179.3	72.1	−109.77	177.37	82.3	−76.5	176.6
$i = 3$	−73.56	−52.4	179.4	−67.05	−43.94	−179.40	−65.2	−78.4	175.0
	22			16			16		
$i = 2$	−83.16	74.86	179.40	76.7	−83.23	175.51	91.7	−75.75	180.38
$i = 3$	−83.24	76.37	−177.97	−143.03	37.10	−179.69	−154.6	90.1	164.58
				53			53		
				65.8	−132.1	175.5	71.5	−125.96	179.52
				−72.8	−66.0	178.6	−155.55	88.04	172.54

[a] Geometrical values observed after optimization with tyrosine side chain held at $\chi_1 = -93$, $\chi_2 = -167$.

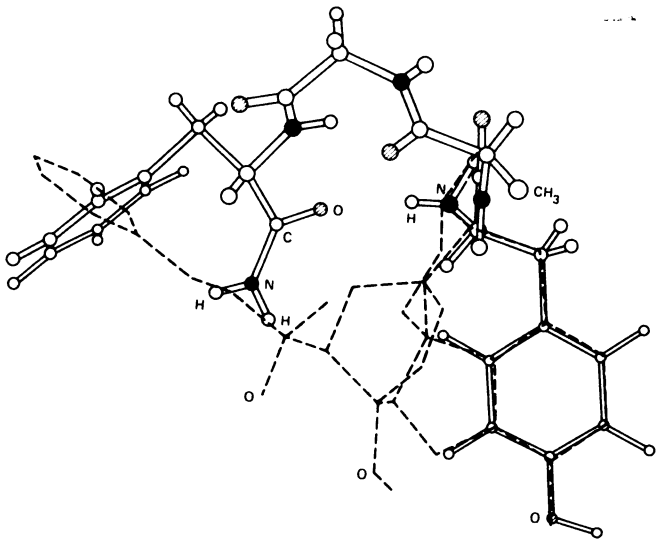


FIG. 1. *Proposed active 1—4 βII′ bend conformation of TDAGPA (Conformer 14) with tyramine overlap geometry, shown superimposed on the structure of PET (- - - in background)*

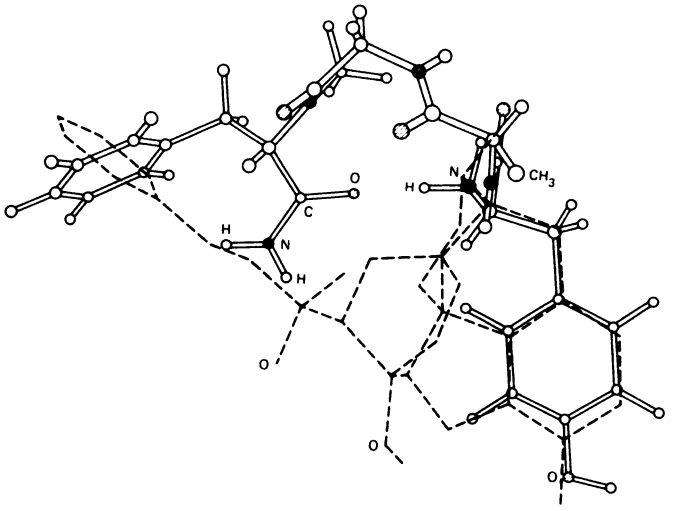


FIG. 2. *Proposed active 1—4 βII′ bend conformation of NMeP (Conformer βII′) with tyramine overlap geometry, shown superimposed on the structure of PET (- - - in background)*

The methyl group of the D-Ala[2] residue has been removed for clarity. Note that the *N*-methyl group of the Phe[4] residue points away from the bend to reduce steric hindrance.

peptides. The geometry of PET used for comparison was an extended-chain conformer obtained by total geometry optimization by an empirical energy program capable of bond length, bond angle, and torsion angle optimization[4] (45). The intramolecular distance between the two aromatic groups in the optimized conformers of PET ranged from roughly 9 to 12 Å, in accord with the distance between the aromatic groups of tyrosine and phenylalanine suggested by Schiller *et al.* (46) as an important parameter in considering peptide active conformations. Figure 1 shows TDAGP Conformer 14 and PET superimposed with tyramine overlap. As can be seen in Fig. 1, approximate overlap of the non-phenolic phenyl ring in PET with the phenyl ring of phenylalanine is obtained simultaneously with tyramine overlap by this conformer. The hydrogen bond between tyrosine and phenylalamine is also shown in Fig. 1.

Another candidate conformer is the βII′ conformer of NMeP shown in Figure 2, again in tyramine overlap with PET. Two features of this conformer discussed above, the N(CH_3) group twisting away from the bend and the hydrogen bond between tyrosine and phenylalanine, are also shown in Fig. 2.

Conformer 35 of NMeP does not show simultaneous tyramine and phenylalanine overlap, suggesting that, while it fulfills our requirements by both methods of selection, it may not be an active conformation. Conformers 16 and 53, chosen as candidate active conformers by the second method of selection, also do not achieve simultaneous tyranine and phenylalanine overlap with PET.

Conformational similarity between PET and candidate inactive tetrapeptide conformers was also examined. When tyramine overlap is superimposed between PET and the low-energy candidate inactive conformers given as Group I in Table 8, the remainder of the tetrapeptide bends away from PET with no phenylalanine aryl ring overlap. This suggests a correlation between activity and similarity to fused-ring opiate structure and further supports the relevance of the method used for sorting active and inactive conformers. However, the actual conformer of PET bound to the receptor is inferred here from only one of its low-energy conformations. Thus the finding of (or lack of) overlap between candidate peptide conformers and PET's aromatic groups should not be taken as a conclusive demonstration that a particular conformation is active or not active at the receptor.

CONCLUSION

With the use of calculated energy-conformational profiles as a guide, a candidate active form of tetrapeptide enkaphalin analogue has been identified as an approximate βII′ turn, whereas a βI turn, a βV turn, or a "random" conformation appears to be an inactive form. The active conformer selected, a βII′ bend between the second and third residues, is the same type previously suggested for Met- and Leu-enkephalin analogues on the basis of theoretical and crystal structure studies. Our results show that such βII′ conformers can exist as relatively low-energy forms for active peptides with an N(CH_3) group on the fourth residues of the bend.

The candidate active forms have a greater degree of spatial overlap with the fused-ring opiate PET than do the inactive ones, suggesting that the extent of this overlap may be important in high-affinity receptor binding and in analgesic activity. The identification of a βI-type conformer as an inactive form of these peptide opiates is in agreement with a proposed inactive conformation of TGGP based on crystallographic and NMR data.

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[4] For further details, see T. Oie, Ph.D. thesis, University of Kansas, 1980.

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