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Chemistry

Enkephalin: Conformational analysis by means of empirical energy calculations

(peptide with opiate action/peptide conformation/conformational energy/low-energy conformations)

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ABSTRACT **Low-energy conformations of methionine-enkephalin were generated by means of an empirical method of computation. Many compact conformations, including those containing various standard bends, were of comparable energy. However, one conformation was found to have a potential energy about 5 kcal/mol (21×10^3 J/mol) below that of the large group of compact conformations. In this conformation, the 3-glycyl and 4-phenylalanyl residues form a bend of type II′. The conformation is stabilized by a hydrogen bond between the OH group of the 1-tyrosine side chain and the C=O group of 3-glycine or 4-phenylalanine. The phenylalanine and methionine side chains are relatively unrestricted. The conformation is consistent with published nuclear magnetic resonance parameters—coupling constants, temperature dependence of the chemical shift, and spin-lattice relaxation times. It is likely that the molecule undergoes a conformational change when it is bound to the receptor. Leucine-enkephalin appears to have the same conformation as its methionine homolog.**

Enkephalin is an endogenous peptide from mammalian brain with morphine-like activity (1–4). Porcine enkephalin is composed of two pentapeptides (5). The amino acid sequence of the major component, Met-enkephalin, is Tyr-Gly-Gly-Phe-Met. The minor component, Leu-enkephalin, has leucine in place of methionine. In enkephalin extracted from bovine brain, the peptide containing leucine is the major component (4). Enkephalin exhibits an analgesic effect that is similar to that of morphine and its analogs, it competes with them in binding, and it is inhibited by morphine antagonists such as naloxone (1, 4). Thus, it is likely that enkephalin binds to the same stereospecific receptor site as does morphine and the other opiates. In order to clarify the mechanism of action at the opiate receptor site, comparisons between the three-dimensional structure of opiates and of enkephalin are of interest.

Conformational models of Met-enkephalin have been proposed from the results of nuclear magnetic resonance measurements (6–9) and on the basis of presumed structural similarities with morphine and related analgesics (10).

We report the results of a theoretical search for stable low-energy conformations of enkephalin, performed with empirical potential energy functions and energy minimization methods. Computations were carried out on a number of likely stable conformations as well as on several reference structures. A complete search of conformational space was not possible because of the large number of degrees of intramolecular freedom. Even if the bond lengths and bond angles are considered as fixed, there remain 24 single bonds around which rotation is possible (including the four C′–N peptide bonds). This could give rise to 10^6 starting conformations (including all possible side-chain orientations). Therefore, strategies (ref. 11; I. Simon, G. Némethy, and H. A. Scheraga, unpublished data) were used that helped in selecting starting conformations likely to give low-energy structures after minimization. The strategies, to be described in *Methods*, can be summarized as follows. The starting conformations included several regular repeating conformations of the pentapeptide, various forms of chain reversals (bends), combinations of conformations that are of low energy for single amino acid residues or dipeptides, and compact conformations obtained by model building. Energy minimizations were then carried out for these structures. Side-chain orientations were varied systematically to test the effect of possible side chain-backbone interactions.

METHODS

The nomenclature and conventions adopted by an IUPAC-IUB Commission were used (12). The conformational energy calculations were carried out with ECEPP (Empirical Conformational Energy Program for Peptides). ‡ The empirical potential energy functions and energy parameters used are those described by Momany *et al.* (13). Standard residue geometries were used, as supplied in ECEPP. Uncharged NH_2 and COOH groups were used as end groups. Solvent effects were not included. Energy minimization was carried out by using the algorithm of Powell (14) with a convergence criterion of 0.01 kcal/mol (41.8 J/mol). Termination also occurred when each of the variable dihedral angles changed by less than 0.05° between any two iterations. All dihedral angles, including those around peptide bonds (ω), were treated as variables of minimization except the one around the bond between C′ and the OH group of the COOH-terminal residue. This dihedral angle was fixed at 180°.

Selection of Starting Conformations. The selection of starting conformations is explained in strategies *a* to *i*. Except when noted otherwise, the starting dihedral angles for side chains were taken to be those that occur in the lowest energy conformations for any given choice of ϕ and ψ in blocked single residues (15) or in dipeptides. §

Strategy a. Three regular conformations—the extended conformation, the repeating C_7^{eq} bend conformation, and the right-handed α-helix—were chosen as reference conformations. All residues were assigned the same values of ϕ and ψ in each conformation.

Strategy b. Thirteen chain reversals (bends) were tested. These would be expected to minimize to compact conformations in which the two ends of the molecule are near each other.

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‡ 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 QCPE, Chemistry Department, Room 204, Indiana University, Bloomington, Ind. 47401, for standard program request sheets and then order no. QCPE 286.

§ S. S. Zimmerman and H. A. Scheraga, unpublished data; Y. Isogai, unpublished data.

The well-defined standard bend structures listed by Lewis *et al.* (16) were used as starting points. In one set of chain reversals, to be referred to here as the G-G bends, the residues comprising the central portion of the bend were taken to be 2-glycyl and 3-glycyl, respectively. Bend types I, I′, II, II′, III, III′, and V can occur for this sequence. When 3-glycyl and 4-phenylalanyl are taken as the central residues of the bend (to be referred to as the G-P bends), only bend types I, II′, III, and III′ are possible (16). All other types correspond to a high-energy conformation for 4-phenylalanine. The dihedral angles listed by Lewis *et al.* (16) were used for the two central residues. The other three residues were placed in an extended starting conformation, with $(\phi,\psi) = (-154°, 153°)$. In addition, two bends were tested in which the 2-glycyl or 3-glycyl residue is in the C_7^{ax} conformation and the other residues are extended (17).

Strategy c. The conformation of only the two central residues is defined for the various types of bends (16). Therefore, strategy *b* had to be expanded in order to test conformations in which the flexible terminal residue—1-tyrosyl in G-P bends and 5-methionyl in G-G bends—folds against the tetrapeptide portion forming the bend. First, a large number of conformations were generated, starting from the minimized conformations obtained using strategy *b*, by systematic variation of the dihedral angles ψ_1, ϕ_2, χ_1^1, and χ_1^6 in the G-P bends and of ψ_4 and ϕ_5 in the G-G bends. These are the dihedral angles that change the orientation of the terminal residue (or of only the 1-tyrosyl OH group) relative to the tetrapeptide portion. Eighteen of the conformations, in which potential hydrogen bonds were indicated by near approach of polar hydrogens and acceptor atoms, were used as starting points for energy minimization. This is the strategy that yielded the global minimum.

Strategy d. Two starting conformations were generated by combining the lowest energy single-residue conformations (15).

Strategy e. Four starting conformations were generated from low-energy dipeptides. Two successive residues along the sequence were placed in the lowest energy conformation for the corresponding dipeptide,§ and the other three residues were placed in compatible low-energy dipeptide conformations. For example, Gly^3-Phe^4 was assigned the lowest energy Gly-Phe conformation,§ and Gly^2 and Met^5 were placed in that lowest Gly-Gly and Phe-Met conformation, respectively, which contains the previously assigned conformation of Gly^3 or Phe^4; a similar procedure was used for Tyr^1.

Strategy f. Published structural models were also used as a basis to select starting conformations. The structures proposed in nuclear magnetic resonance studies (6–8) are type I bends. Strategy *b* covered these bends. However, two additional starting conformations were constructed by inspection of the photograph of a molecular model published by Bradbury *et al.* (10).

Strategy g. Space-filling molecular models were used to construct compact conformations not tested so far. The aim was to achieve close side-chain interactions, corresponding to either hydrogen bonds involving the tyrosine side chain or to good packing of two or three of the side chains. Fourteen starting conformations were found by this procedure.

Strategy h. In order to test regions of conformational space not covered in the previous strategies, various low-energy conformations of the Tyr-Gly-Gly and the Phe-Met sequences were combined with each other, resulting in eight new starting conformations.

Strategy i. The effect of side-chain rotation in the lowest energy conformation (achieved earlier by minimization) was tested, in addition to the tests in strategy *c*. The three side chains were placed in various low-energy staggered conformations (15) for the same backbone conformation and then the energy was minimized. Seven starting conformations were generated by this strategy. This testing of side-chain rotations was carried out only for the lowest energy backbone conformation. Because the energy was invariably raised by such rotations (see *Results*), it was considered to be unnecessary to use this strategy on other conformations that already had high energy.

Altogether, 71 starting conformations were used in the energy minimizations. In addition, many more conformations were tested and eliminated in various steps of the strategies.

RESULTS

Computations on Met-enkephalin

The results of the calculations are summarized in Fig. 1, which shows all computed conformations with energy less than 11 kcal/mol above the minimum found in this study. The conformations are classified into six groups, according to the starting conformations from which they were obtained (see legend to Fig. 1). The dihedral angles of several low-energy conformations are listed in Table 1.

Reference Conformations (group A of Fig. 1). Three regular (repeating) conformations were selected as reference states. The α-helix (conformation 1 in Fig. 1) is known to be of low energy for oligopeptides. The extended structure (conformation 2) is of low energy for many amino acids (15) and it has the least amount of interaction between the side chains in enkephalin. The C_7^{eq} conformation (conformation 3) is also of low energy for many amino acids (15). The energy of all three conformations is rather high in comparison with that of many compact conformations ($\Delta E \geq 9$ kcal/mol). This suggests that interactions in the latter play a significant stabilizing role and that the selection strategies used in this study were efficient in generating low-energy conformations.

Bend Conformations (groups B and D). The energy of all bend conformations falls into a densely populated band at $\Delta E > 6.0$ kcal/mol, with the exception of variants of the type II′ G-P bend (conformations 4, 5 6 and group C). These low-energy conformations, to be discussed below in detail, are stabilized by a hydrogen bond between the tyrosyl OH and a backbone oxygen. Such a hydrogen bond is not sterically possible in any of the G-G bends, so that no low-energy conformation occurs in group D.

Other Compact Conformations (groups E and F). The various combinations of low-energy single residue and dipeptide conformations (group F) all have $\Delta E > 5.0$ kcal/mol. This indicates that locally favorable—i.e., intraresidue interactions—are not sufficient in this oligopeptide to yield preferred conformations.

The conformations obtained from model building (group E) also have $\Delta E > 5.0$ kcal/mol, even though attempts were made to optimize the side-chain interactions. Close approach between side chains usually requires that dihedral angles in the side chains be varied away from the local minima (or it requires a *trans*-to-*gauche* rotation). The energy gain due to the nonbonded interactions does not necessarily compensate for the torsional energy required in these rotations.

The energies of conformations 16 and 17 were minimized from the model of Bradbury *et al.* (10). Their energy, near 8 kcal/mol, is comparable to that of standard bends. In fact, the gross shape of this model is similar to that of G-G bends. The energy is high even though conformation 16 has a hydrogen bond between the OH of tyrosine and the C=O of methionine.

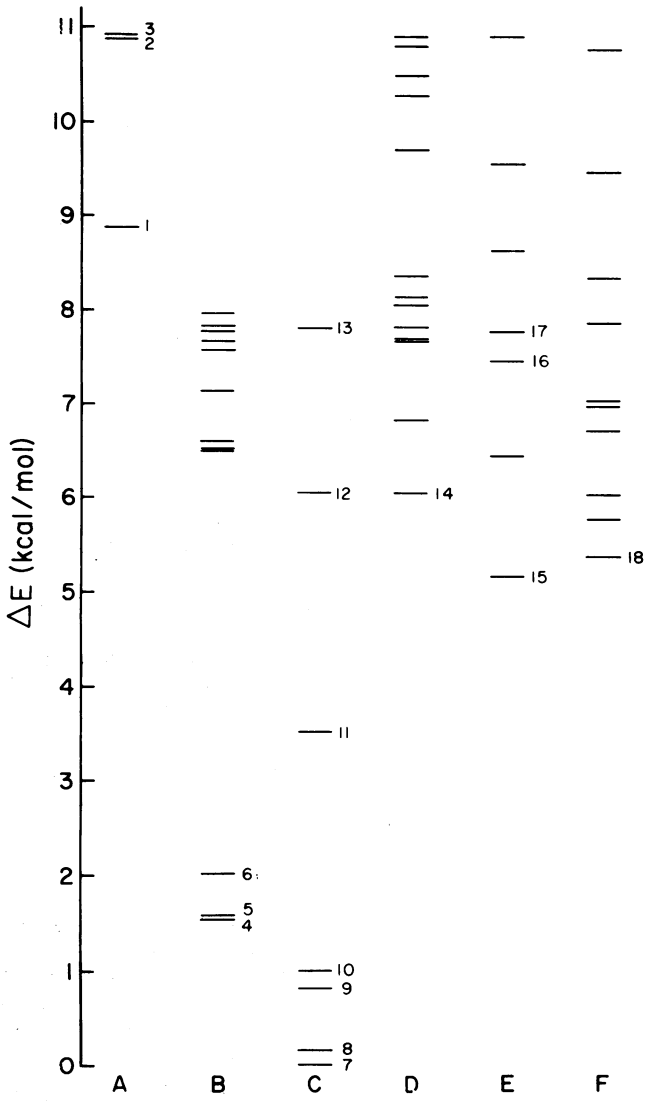

FIG. 1. Low-energy conformations of Met-enkephalin in order of increasing energy. The ordinate is $\Delta E = E - E_0$, with E_0 = energy at the global minimum. Positions along the abscissa are arbitrary. The conformations are grouped as follows: A, reference conformations (obtained from strategy *a*); B and D, conformations in G-P and G-G bends, respectively (strategy *b*) and related conformations derived from them (strategy *c*); C, the lowest energy conformation (obtained in strategy *c*) and conformations derived from it (strategy *i*); E, conformations derived from model building (strategies *f* and *g*); F, combinations of low-energy single-residue and dipeptide conformations (strategies *d*, *e*, and *h*). Numbers placed next to some energy levels refer to the discussion of particular conformations in the *text*.

Table 1. Dihedral angles and energies for global minimum and some representative conformations of Met-enkephalin

Residue		Dihedral angles (degrees) of conformations of Fig. 1*			
		7	14	15	18
Tyr[1]	ϕ	−84	−173	−85	−94
	ψ	155	154	148	158
	ω	−176	178	176	−178
	χ^1	−174	−177	177	47
	χ^2	82	75	81	−98
	χ^6	−147	0	−167	0
Gly[2]	ϕ	−159	80	−177	−86
	ψ	100	−84	121	94
	ω	173	−178	173	174
Gly[3]	ϕ	74	−68	163	91
	ψ	−100	−42	−91	−66
	ω	175	175	−177	180
Phe[4]	ϕ	−85	−150	−77	−139
	ψ	−41	148	145	148
	ω	−179	−179	178	−179
	χ^1	−179	178	179	180
	χ^2	74	79	81	71
Met[5]	ϕ	−165	−152	−137	−73
	ψ	124	136	−32	122
	χ^1	−174	−167	−67	−173
	χ^2	57	61	−173	58
	χ^3	−178	177	−178	−179
	χ^4	60	60	60	60
ΔE (kcal/mol)		0.0	6.0	5.2	5.4

* Columns are as follows: 7, global minimum, G-P bend type II′; 14, lowest G-G minimum, bend type II′; 15, lowest minimum found by starting from molecular models; 18, minimum found by starting from the combination of the lowest energy single-residue conformations.

On the other hand, there is little interaction between the backbones of the terminal residues.

The Lowest Energy Conformation and Related Conformations (group C). The G-P bend type II′ conformation, as generated in the course of strategy *b*, is lower in energy than the other bend conformations (conformation 6). It is stabilized by a hydrogen bond between the tyrosyl OH and the phenylalanyl backbone O=C groups. Conformations 4 and 5 are similar to conformation 6 and were derived from it by variation of ϕ_2 or of ψ_1, respectively (strategy *c*).

Conformation 7 has the lowest energy among all those tested. It was obtained by means of the application of strategy *c* to conformation 6. It differs from conformation 6 mainly by the changed orientation of the tyrosyl side chain (variation of χ_1[6]). In conformation 7, the tyrosyl OH forms a strong hydrogen bond to the O=C group of 3-glycine, with an H···O distance of 1.69 Å. Conformation 7 is characterized further by a type II′ bend in the Gly[3]-Phe[4] position and by unusually favorable backbone-to-backbone interactions of Met[5] with Tyr[1] and Gly[2]. Fig. 2 is a stereoscopic drawing of this conformation. The amide hydrogen of Met[5] is within 2.3 Å of the amide O of Gly[2], and it is partially shielded from the solvent by the latter and by six other atoms ($H_3{}^{\alpha 2}$, amide H_4, $H_4{}^{\beta 2}$, $H_5{}^{\beta 1}$, $H_5{}^{\beta 2}$, and $O_5{}^1$) surrounding it at distances of 2.3–4.0 Å. The phenylalanyl and methionyl side chains point away from the rest of the molecule, but they occupy positions of lowest intraresidue energy for the given backbone conformation.

Conformations 8 to 13 are variants of conformation 7. They were obtained from the latter with strategy *i*, by rotating various side chains, while retaining the type II′ bend conformation of the backbone. In conformations 8 to 11, the tyrosyl side chain-backbone hydrogen bond was retained as well. Conformations 8 to 10 were obtained by rotation of the phenylalanyl or methionyl side chain (or both) into the orientation with the second lowest intraresidue energy (15). This movement of the side chains does not affect the backbone conformation significantly. The starting structure leading to conformation 11 was obtained from a molecular model of conformation 7, in which all dihedral angles of Phe[4] and Met[5] were adjusted visually so as to make good side chain-side chain and side chain-backbone contacts. In spite of these contacts, the minimized energy in-

Table 2. Comparison of calculated and experimentally observed vicinal coupling constants (Hz) in Met-enkephalin

Coupling constant	Residue	Calculated* a	Observed* b	c	d	e
$J_{NH\text{-}C^{\alpha}H}$	Gly^2	4.6† ± 0.4	—	—	4.7	5.4
	Gly^3	5.9† ± 0.4	5.2†	—	4.8	6.1
	Phe^4	8.3 ± 0.4	8.6	8.3	8.8	7.1
	Met^5	6.6 ± 0.4	7.4	7.2	7.8	7.6
$J_{C^{\alpha}H\text{-}C^{\beta}H}$ ‡	Tyr^1	1.9 ± 1.0	2.9	6.2	—	—
		10.4 ± 1.0	5.8	7.5	—	—
	Phe^4	4.3 ± 1.0	3.8	4.0	—	—
		9.0 ± 1.0	9.9	10.1	—	—
	Met^5	2.9 ± 1.0	—	4.5	—	—
		8.3 ± 1.0	7.4	7.3	—	—

* The data in columns d and e were obtained with lower resolution (100 MHz) than those in columns b and c (270 and 300 MHz, respectively). Columns: a, determined from the computed dihedral angles, using the relationships given in refs. 18 and 19; b, in $(C^2H_3)_2SO$, ref. 6; c, in $(C^2H_3)_2SO$, ref. 8; d, in $(C^2H_3)_2SO$, ref. 9; e, in 4:1 $H_2O/^2H_2O$ mixture, ref. 9.

† Mean value.

‡ The two values of J that are listed correspond to the two different protons on the β carbon.

creased to 3.5 kcal/mol, due to the torsional rotations required in the course of the adjustment.

Conformations 12 and 13 were obtained from conformation 6 by rotating the tyrosyl side chain into the other two low-energy positions around the C^{α}-C^{β} bond (15). This breaks the hydrogen bond involving the tyrosyl OH, and ΔE is raised to >6 kcal/mol. This energy becomes comparable to that of the other bends in groups B and D. It is seen that the side chain-backbone hydrogen bond of the tyrosyl OH plays an important role in the stabilization of the preferred conformations with ΔE < 2 kcal/mol.

Comparison with nuclear magnetic resonance studies

Chemical shifts and coupling constants of Met-enkephalin in $(C^2H_3)_2SO$ have been measured in the laboratories of Anteunis (6, 7) and of Gibbons (8). The reported data agree closely. The models proposed by the two groups are similar. Both groups concluded that the molecule has a well-defined conformation. On the basis of $J_{NH-C^{\alpha}H}$ values, both proposed a type I bend with Gly^3 and Phe^4 in the central portion (G-P bend). A G-G bend would not fit the nuclear magnetic resonance data. No stacking of the aromatic side chains was apparent (6), and the spectra in aqueous solution were stated to be similar to those in dimethyl sulfoxide (6, 8). It should be noted that the reported coupling constants are just as consistent with the dihedral angles of a G-P type II′ bend as with those of a type I bend (16). Thus, the experimental data are in complete agreement with the coupling constants computed (18, 19) for the preferred conformation that we propose (Table 2). Agreement is poor only for the $J_{C^{\alpha}H-C^{\beta}H_2}$ of tyrosine, for which more rotational freedom has been suggested (8) than is found in our model. Both groups (6, 8) reported a small temperature coefficient for the chemical shift of the methionyl N-H. This, too, agrees with our model in which accessibility of the NH group of methionine is low (Fig. 2).

Bleich *et al.* (9) measured the ^1H and ^{13}C relaxation behavior of Met-enkephalin both in $(C^2H_3)_2SO$ and in a 4:1 $H_2O/^2H_2O$ mixture. They concluded that the reorientation of the main peptide chain occurs by means of overall molecular tumbling in both solvents, suggesting a well-defined conformation in both. The phenylalanyl and methionyl side chains have considerable rotational freedom, but tyrosine is found to be much more restricted in its reorientational motion. These observations, too, are fully consistent with the predictions of our model. However, Bleich *et al.* (9) suggested tentatively that the conformation of tyrosine might be different in the two solvents, although it is motionally restricted in both. Such an observation would be harder to reconcile with our model in which there is a preferred conformation for only one orientation of the tyrosyl side chain.

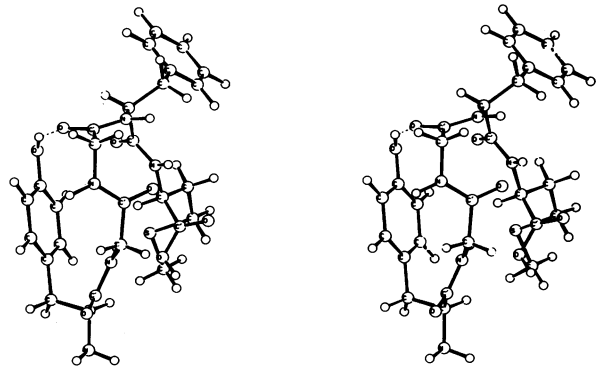

FIG. 2. Stereoscopic illustration of the lowest energy conformation of Met-enkephalin (conformation 7 of Fig. 1).

The model proposed by Bradbury *et al.* (10) does not agree with the nuclear magnetic resonance data (6, 7). It was shown in this study to have a rather high energy.

Computations on Leu-enkephalin

The methionyl side chain in Met-enkephalin interacts very little with the rest of the molecule in most of the low-energy conformations shown in Fig. 1. Therefore, one would expect that substitution of leucine for methionine would change the conformation very little. This hypothesis was confirmed by nine energy minimizations on Leu-enkephalin, with starting conformations selected from various conformational groupings in Fig. 1, including 1, 4, 5, 6, 7, 9, 10, 11, and 14. The relative spacings of the energy minima obtained for Leu-enkephalin were similar to the corresponding ones for Met-enkephalin. The G-P type II′ conformations remained much lower in energy than the others. It seems reasonable to say that the conformational preferences are similar in the two enkephalins and that additional computations on Leu-enkephalin would not yield more information.

CONCLUSIONS

Our computations, in essential agreement with presently available nuclear magnetic resonance data, strongly suggest that, when free in solution, enkephalin exists in a preferred conformation (Fig. 2). This conformation, in the form of a type II′ β-bend centered on Gly^3-Phe^4, is stabilized by noncovalent backbone-backbone interactions and by a hydrogen bond between the OH group of the tyrosyl side chain and the backbone O=C group of either 3-glycine or 4-phenylalanine. The phenylalanyl and methionyl side chains extend away from the backbone and are relatively mobile. In the absence of the hydrogen bond, a large number of conformations can occur, all within a few kcal/mol of each other and none strongly preferred over the others (all conformations in Fig. 2 with ΔE > 5 kcal/mol). Thus, we predict that, in the absence of this hydrogen bond, the molecule would be very flexible.

Solvent effects were not included explicitly in our compu-

tations. It might be argued that the presence of solvent could alter the conformational stabilities. In particular, a polar solvent might destabilize the intramolecular hydrogen bond. We consider it unlikely that the main conclusions would change significently if solvent were considered, because of the following arguments. The lack of flexibility and the general similarity of the structures, observed by nuclear magnetic resonance methods in two solvents, one of them containing water (6–9), coupled with our conclusion about high flexibility in the absence of hydrogen bonding, make it likely that the hydrogen bond persists in polar solvents. Modification of the strength of nonbonded interactions by the solvent—for example, stabilization of hydrophobic interactions between the side chains—can be expected to change free energies by not more than about 2 kcal/mol (20). Changes of this magnitude are not sufficient to bridge the energy gap between conformations of group C and the others (Fig. 2).

Both this theoretical study and the nuclear magnetic resonance measurements refer to the free molecule in solution. No definitive conclusions can be drawn from either study concerning the conformation of the molecule bound to the receptor site, since conformational changes can occur upon binding (cf. refs. 8 and 9). Such a conformational change very likely occurs if our proposed model is correct for the free molecule. It has been proposed, on the basis of structural analogies between morphine and various opiates, that the presence of an aromatic OH group and of an ionizable amino group separated by two carbon atoms from the aromatic ring is essential for activity (10, 21, 22). The NH_2-terminal tyrosine of enkephalin fits this requirement, but only if the side chain points away from the molecule. A rotation of tyrosine could take place upon binding to the receptor. In this case, the unique stabilization of the solution conformation (Fig. 2) would be lost, but several medium-energy conformations would be available to the molecule (Fig. 1). One of these could be the preferred form upon binding. Our computations offer no clue to aid the characterization of such a preferred conformation.

The receptor affinity or the biological activity of several analogs of enkephalin has been studied (23–25). It is tempting to try to correlate the effect of amino acid substitutions with conformational stability. However, substitution may alter affinity or activity because they modify functional groups that are essential either to binding or to action, besides causing changes in conformational preferences. Correlations with the solution conformations are even less feasible, because of the possibility of conformational change upon binding. Such a conformational change is rendered plausible by the observation of the essential retention of activity when Gly^2 is replaced by D-Ala (25) but a large decrease of receptor affinity upon replacement with L-Ala (24). The latter substitution is possible in the conformation of Fig. 2, but the substitution of D-Ala is not because $(\phi_2, \psi_2) = (-159°, 100°)$ in Fig. 2. This conformation is of low energy for L-Ala but of high energy for D-Ala (16). On the other hand, several medium-energy conformations in Fig. 1 (namely, those with $\phi_2 > 0$) permit substitution, without conformational changes, more easily by D-Ala than by L-Ala, so that one of them might be the bound conformation.

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