CONFORMATIONAL ANALYSIS OF METHIONINE-ENKEPHALIN AND SOME ANALOGS

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Received January 26,1977

SUMMARY - Conformational energy calculations on Methionine-Enkephalin and on several of its analogues indicate that the calculated lowest energy conformation of the native enkephalin may not be the conformer which interacts at the opioid active site. Substitution at the end groups and various D and L-Alanine analogs were examined and a low energy conformation which differs from the native low energy conformer was found for the very active analog, [D-Ala^2]-Met-Enkephalin-NH_2. The stereopositions of side-chain functional groups are discussed and compared to the structure of morphine.

Recently, a conformational energy calculation was carried out to find the lowest energy conformations of the molecules methionine- and leucine-enkephalins (1), H-Tyr-Gly-Gly-Phe-Met-OH and H-Tyr-Gly-Gly-Phe-Leu-OH, respectively. The results of Isogai et al. (1) indicated that the lowest energy conformer for these molecules with uncharged end-groups, was the structure (called conformer IIA in this work) whose dihedral angles are similar to those given in Table I for conformer IA. Recent experimental tests of opioid activity (2-4) have shown that analogs with D-Ala substitution in the 2 position have enhanced activities relative to the native enkephalins, while D-Ala substitution at the 3 position showed little or no opioid activity. An examination of the dihedral angles of conformer IA indicates that D-substitution at the 2 position should result in a conformational change, but at the 3 position the conformational stability should be enhanced. Because of this discrepancy between experiment and theory, conformational energy studies have been carried out on a series of analogs of Met-Ek, in an attempt to resolve the conformation of the active molecule. The results of these calculations will be described here.

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ISSN 0006-291

TABLE I

Low energy conformations of analogs of Methionine-Enkephalin.

Residue	Dihedral angles, deg.							
	ϕ	ψ	ω	χ_1	χ_2	χ_3	χ_4	χ_6
Conformer IA								
Amino (NH_3^+)								
1. Tyrosine	-59.5	146.4	177.2	-171.6	92.2			-147.0
2. Glycine	-154.3	90.7	173.6					
3. Glycine	83.0	-78.3	-173.0					
4. Phenylalanine	-93.6	-40.5	-172.5	178.7	-113.4			
5. Methionine	-155.1	151.4	180.0	-170.4	59.7	-178.1	60.1	
Carboxyl ($-COO^-$)								
Conformer VIC								
Amino (NH_3^+)								
1. Tyrosine	-56.0	81.8	-178.1	-61.1	-74.9			-177.7
2. D-Alanine	68.6	-126.0	179.9	178.9				
3. Glycine	-64.9	-55.3	177.1					
4. Phenylalanine	-64.3	114.0	-173.5	177.3	-108.6			
5. Methionine	49.0	56.2	180.0	-61.8	-175.5	177.2	58.0	
Amide ($-NH_2$)								
Conformer IVB								
Amino (NH_3^+)								
1. Tyrosine	-58.8	76.3	-179.1	-62.6	-63.6			179.0
2. D-Alanine	72.2	-122.0	178.3	58.7				
3. Glycine	-69.5	-50.6	177.3					
4. Phenylalanine	-70.4	149.4	179.0	179.5	-113.6			
5. Methionine	-68.0	115.2	180.0	-173.7	175.0	179.8	60.0	
Carboxyl (COO^-)								

TABLE II

Relative energies of conformers of Methionine-Enkephalin

Analog	Conformer[a] (Energy in Kcal/mole)[b] A	B	C
(I) H_3^+N-Met-Ek-COO^-	0.0	2.0	5.5
(II) H_2N-Met-Ek-COOH	0.0	-[c]	4.4
(III) H_3^+N-Met-Ek-NH_2	0.4	1.6	0.0
(IV) H_3^+N-(D-Ala^2)-Met-Ek-COO^-	2.4[d]	0.0	2.4
(V) H_3^+N-(L-Ala^2)-Met-Ek-COO^-	0.0	12.4	15.0
(VI) H_3^+N-(D-Ala^2)-Met-Ek-NH_2	5.8[d]	1.5	0.0

(a) Dihedral angles for selected analogs of conformers A, B, and C are given in Table I.
(b) E_o values in Kcal/mole for each analog are: (I) -8.96, (II) -3.16, (III) -0.71, (IV) -6.06, (V) -8.25, and (VI) +0.22.
(c) This starting conformation converged upon energy minimization to conformer C.
(d) The dihedral angles ϕ and ψ, of the backbone around the D-Ala residue, differ considerably from those of conformer IA, but the overall structure remains similar to that found for IA.

The empirical energy parameters and functions used for this analysis have been documented elsewhere (5-7), and the method of searching for low energy conformers was similar to that used for the molecule, luteinizing hormone-releasing hormone (8,9). Since an exhaustive search of the conformational space of the enkephalins was carried out previously (1), only those structures with different end-groups, and the alanine analogs discussed here were extensively examined.

RESULTS AND DISCUSSION

Table II gives the energy of each analog relative to zero energy for the lowest energy conformer for that particular analog. Energies between different analogs cannot be compared since they are in effect different

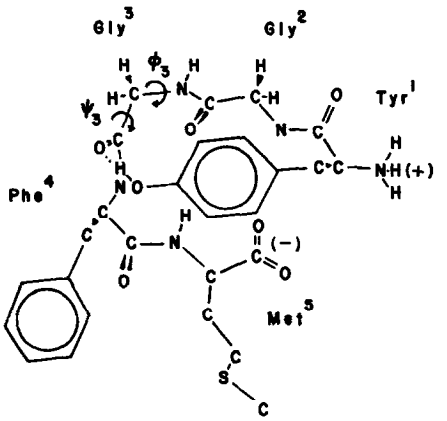

Figure 1. Conformation IA (Table I) of Met-Ek. Some backbone and side-chain hydrogens have been omitted for clarity.

molecules. The results in Table II clearly show the difficulty one encounters in deducing the active conformation of small polypeptides. Analog I differs from analog II, only in the neutralization of the charged end-groups, and in both cases conformer A is of lowest energy. However, upon amidation of the C-terminal (analog III), conformer C becomes lower in energy than A. Upon D-Ala^2 substitution (analog IV), conformer B which is closely similar to C in dihedral angles (see Table I), is of lowest energy. In the case of L-Ala^2 (analog V), conformer A is again lowest in energy. Analog VI, which is the D-Ala^2 amide analog is much lower in energy for the C conformer than the A, and the large energy difference for this very active analog strongly indicates that the active form of the enkephalins must be similar to conformer C.

The structures of conformers IA and VIC are shown in Figures 1 and 2 respectively. These structures clearly differ from one another in the stereo-arrangement of the different side-chains. For example, in structure IA, the tyrosine side-chain ring sits over the backbone of the molecule in such a way that it would be difficult for the ring to insert itself into a receptor pocket. Since it has been shown that the di-iodo derivative of tyrosine is inactive (2), as is also the Phe^1 analog (2), it would appear that the hydroxyl group must fit into the receptor, similarly to the aromatic ring in

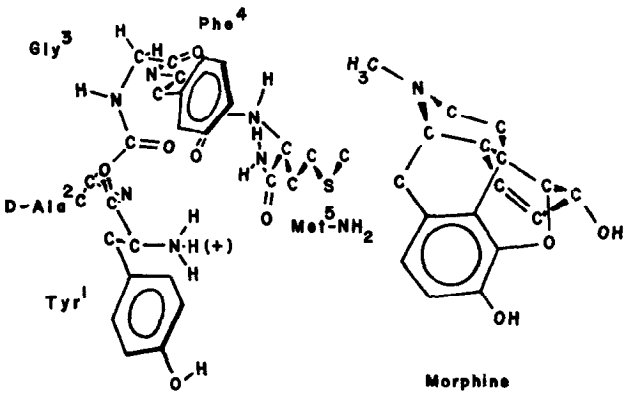

Figure 2. Conformation VIC (Table I) of [D-Ala^2]-Met-Ek-NH_2 and the structure of morphine. Some hydrogens have been omitted for clarity.

morphine (see Figure 2). On the other hand, in structure VIC, the tyrosine ring is extended away from the backbone of the molecule, and it is clearly free to bind at the receptor.

Similarities of structure VIC to the morphine structure are difficult to show. However, the overall shape and size of structure VIC is not too different from morphine (see Figure 2). Since Met-Ek appears to bind at the opioid receptor, better than Leu-Ek (whose conformations are the same as those given here for Met-Ek), it is tempting to speculate that the methionine sulfur fits into a position similar to that taken by the non-aromatic hydroxyl group of morphine.

In light of the results presented here, it is suggested that physical experimental studies should be carried out on the D-Ala^2 amide analog, since the conformation of the native enkephalins most likely to have the highest population in solution is conformer A, not conformer C, the proposed opiate active conformer.

ACKNOWLEDGMENTS

The computations for this work were supported by the Memphis State University Computing Center. The author would like to thank H. A. Scheraga for timely discussions of this work and access to the unpublished data of reference 1.

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