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Spatial Structure of Peptide BAM-20P

E.M. Hasanov
Institute of Physics National Academy of Sciences, H.Javid Ave. 131, Baku, Azerbaijan
N.A. Akhmedov
Physics Department, Baku State University, Zahid Khalilov Street, 23, Baku, Azerbaijan

Abstract:- The spatial structure of the molecule Tyr1-Gly2-Gly3-Phe4-Met5-Arg6-Arg7-Val8-Gly9-Arg10Pro11-Glu12-Trp13-Trp14-Met15-Asp16-Tyr17-Gln18-Lys19 Arg20 (BAM 20P - bovin adrenal medulla 20 residue peptide) fragmentally studied by theoretical conformational analysis and it is shown that its spatial structure can be represented by 11 stable conformations falling in the energy interval 0-10 kcal/mol. The values of the dihedral angles of the main and side chains are found, the energy of intra- and interstitial interactions is estimated.

Keywords:-Opioid peptides, BAM–20P, spatial structure, molecule, conformation.

I. INTRODUCTION

The opioid peptide Tyr1-Gly2-Gly3-Phe4-Met5-Arg6-Arg7-Val8-Gly9-Arg10-Pro11-Glu12-Trp13-Trp14-Met15-Asp16-Tyr17-Gln18-Lys19-Arg20 was isolated from the adrenal medulla of bovines, indicated by BAM 20P (bovin adrenal medulla 20 residue peptide). The opiate activity of BAM 20P is several times higher than that of Met-enkephalin and δ-endorphin. The sequence of BAM-20P includes Met-enkephalin (Tyr1-Met5), adrenorphine (Tyr1-Val8), BAM-12P (Tyr1-Gln12), the BAM-20P molecule itself is a part of opioid peptides E and I and therefore of great scientific interest [1-4].

The calculation of the opioid peptide BAM-20P (Tyr1-Gly2-Gly3-Phe4-Met5-Arg6-Arg7-Val8-Gly9-Arg10-Pro11-Glu12-Trp13-Trp14-Met15-Asp16-Tyr17-Gln18-Lys19-Arg20) was performed on based on stable states of overlapping fragments of Tyr1-Asp16 and Asp16-Arg20 over a single residue and is a continuation of our previous studies [5-7]. BAM-20P includes in its sequence a large number of residues with volumetric side chains, five of which carry integer charges. Since the fragments were docked along the negatively charged Asp16 residue, the side chain positions of this residue were taken into account in the most stable states of both the Tyr1-Asp16 and Asp16-Arg20 fragments when constructing the structural variants of the peptide under study. In addition, all structural variants in which there was a potential realization of charged side-chain interactions with each other and their optimal positions were calculated. The problem also consisted in elucidating conformations that were optimal both from the standpoint of intramolecular interactions and the intermolecular interactions of the molecule with water, which could be facilitated by the presence of polar amino acids in the sequence.

The results of minimizing the energy of the initial variants with variation of the dihedral angles $\phi, \psi, \omega, \chi_1, \chi_2, \ldots$ of all the amino acid residues constituting the given molecule are presented in table 1. The optimal conformation of the peptide obtained as a result of minimization is extremely sharply differentiated in energy both in respect of shapes, and forms of the main chain and conformational states. Table 1 clearly shows that minimization of energy leads to a relatively small set of real conformations: only 11 conformations enter the wide energy interval 0-10 kcal/mol, the geometric parameters of which are located in table 2. The values of dihedral angles are given in the following $\phi, \psi, \omega, \chi_1, \chi_2, \ldots$ sequence and correspond to the nomenclature [8].

II. EXPERIMENTAL

The low-energy conformations of this molecule, like the molecules of BAM-12P, are divided into groups A, B and C. The common element in each group is the similarity of their adrenorphine site. Only the conformations of the D group of the BAM-12P molecule under any combinations with the low-energy states of the subsequent section do not form stable structures of the BAM-20P molecule, which was already observed at the previous stage of calculation when considering the conformational capabilities of the Tyr1 -Asp16 fragment. Those chain build-up leads to a limitation of the number of strukure types, moreover, with the transition from the molecule BAM-12P to the molecule BAM-20P, a sharp differentiation of conformations within each group is observed. When comparing the results of the calculation of the peptides BAM-12P and BAM-20P, a certain continuity in the spatial organization of these peptides is observed. For both molecules, the structures of groups A and B are preferable, and the acceptable states of their N-terminal regions correspond to the best states of free Met-enkephalin and adrenorphine molecules. These conformational groups are characterized by the content of regular sections on the Met-enkephalin area-the helix in the conformations of group A and the β-rotation in conformations of group B. As can be seen from the presented results, a number of structures exist in the vicinity of local minima, which explains the wide range of functional activities of this peptide. Table 1 clearly demonstrates the decisive role of electrostatic interactions in the energy differentiation of the structures of the peptide under study. Thus, the structures that are optimal in energy are characterized by the lowest value of the electrostatic contribution.

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Table 1:- Relative Energy and Energy Contributions of Non-Valent, Electrostatic, Torsion, Interactions of Preferred Conformations of the Molecule BAM-20P.

Group	№	Conformations	$E_{non-valent}$	$E_{elec.}$	$E_{tor.}$	$E_{rel.}$
A	1	$B_{211}PRR_{21}B_{332}R_{3222}R_{2222}R_2BL_{22}RR_{32}B_3R_2R_{12}R_2R_2B_{211}B_{22}R_{33}$	-92.8	10.7	12.8	0.0
	2	$B_{211}PRR_{21}B_{332}R_{2222}R_{3222}R_2BL_{22}RR_{32}B_3R_1R_{32}R_2R_2B_{211}R_{32}R_{32}$	-97.7	24.5	11.4	7.5
	3	$B_{211}PRR_{21}B_{332}R_{2222}R_{3222}R_2BL_{22}RR_{32}B_3B_1R_{21}R_1R_2B_{211}B_{32}R_{33}$	-94.3	22.6	10.4	8.0
B	4	$B_{131}BPB_{21}B_{212}B_{1222}B_{2222}R_2BL_{22}RR_{32}R_2B_2R_{33}R_1R_2B_{211}B_{32}R_{33}$	-99.8	15.7	17.5	2.7
	5	$B_{131}BPB_{21}B_{212}B_{1222}B_{2222}R_2BL_{22}RR_{32}R_2B_2R_{33}R_2R_2R_{331}R_{12}R_{32}$	-98.0	20.0	16.7	8.0
	6	$B_{131}BPB_{21}B_{212}B_{1222}B_{2222}R_2BL_{22}RR_{32}R_2B_2R_{33}B_2B_2B_{311}B_{31}B_{32}$	-98.3	20.9	16.8	8.7
	7	$B_{131}BPB_{21}B_{212}B_{1222}B_{2222}R_2BL_{22}RR_{32}R_2B_2R_{33}B_2R_2R_{331}R_{12}R_{32}$	-102.2	23.9	17.9	8.8
	8	$B_{131}BPB_{21}B_{212}B_{1222}B_{2222}R_2BL_{22}RR_{32}R_2B_2R_{33}R_2R_2R_{331}R_{12}R_{32}$	-101.1	21.4	19.4	8.9
	9	$B_{131}BPB_{21}B_{212}B_{1222}B_{2222}R_2BL_{22}RR_{32}R_2B_2R_{33}R_2B_2B_{311}R_{31}R_{32}$	-96.6	19.9	16.7	9.0
	10	$B_{131}BPB_{21}B_{212}B_{1222}B_{2222}R_2BL_{22}RR_{32}B_3R_1R_{32}R_1R_2B_{211}B_{32}R_{33}$	-91.9	21.6	15.9	9.8
C	11	$B_{131}PRB_{33}B_{222}B_{1222}B_{2222}R_2BL_{22}RR_{32}B_3R_1R_{32}B_2B_2B_{311}B_{31}B_{32}$	-93.8	22.2	10.7	8.4

Table 2 :- Geometric Parameters (Degrees) of Optimal Conformations of Various Forms of The Main Chain of the Molecule BAM-202P

The amino acid residue	Conformations		
	$E_{rel.}$= 0.0 kcal/mol	$E_{rel.}$= 2.7 kcal/mol	$E_{rel.}$= 8.4 kcal/mol
Tyr 1	-173, 154, 176, -177, 75, 0	-93, 158, -179, 50, -98	167, 158, 179, 59, -95, 178
Gly 2	80, -83, 180	-86, 92, 75	80, -71, 180
Gly 3	-67, -42, -176	90, -60, 179	-161, -70, 178
Phe 4	-84, -52, 180, 180, 74	-139, 148, -179, 180, 71	-137, 63, 179, -60, -77
Met 5	-148, 126, 174, -75,-60, 180, 180	-73, 109, -179, 174, 58, -179, 180	-155, 146, 181, -167, 177, -177, 180
Arg 6	-96, -55, 177, -64, 180,179, 180	-164, 170, 170, 64, -175, -178, 179	-144, 170, 170, 64, -175, 182, 179
Arg 7	-131, 134, 180, 183,177, 180, 180	-117, 126, -176, -179,, 178, 180, 180	-117, 126, 184, 181, 178, 180, 180
Val 8	-100, -60, -172, 180, 181, 179	-109, 109, 168, 179, -179, 179	-109, -60, 186, 179, 179, 181, 179
Gly 9	-71, 101, 182	-61, 117, 198	-67, 118, 178
Arg 10	58, 84, 175, 183, 180, 178, 180	58, 84, 175, 183, 180, 178, 180	58, 84, 175, 183, 180, 178, 180
Pro 11	-53, 180	-53, 180	-53, 180
Glu 12	-103, -63, 181, -60, 180, 90	-103, -63, 164, -60, 180, 90	-103, -63, 182, -60, 180, 90
Trp13	-143, 163, 182, -61, 92	-114, -60, 163, 174, 72	-141, 167, 179, -61, 94
Trp 14	-61, -35, 189, 180, 90	-155, 136, 181, 186, 92	-61, -36, 190, 62, 84
Met 15	-58, -36, 185, 65, 187, 181, 180	-134, -65, 186, -61, -60, 178, 179	-58, -39, 185, 67, 189, 181, 180
Asp 16	-90, -31, 186, 180, 90	-109, 152, 183, 56, 97	-93, -32, 183, 52, 85
Tyr 17	-80, -62, 178, 179, 83, 0	-84, -41, 197, 175, 83, 0	-81, -63, 178, 177, 82, 0
Gln 18	-94, 107, 180, 181, 65, 71	-72, 129, 177, 185, 68, 74	-96, 107, 179, 181, 64, 71
Lys 19	-96, 124, 178, 180, 60, 180, 90, 179	-79, -63, 178, -60, 174, 180, 176, 179	-96, 125, 178, -58, 179, 180, 180, 179
Arg 20	-125, -50, -61, -66, 183, 183	-133, -52, -60, 180, 179, 180	-124, -50, -61, -67, 184, 183

The conformation of group A differs significantly from the conformations of group B by complete spiralization of the residues of the Met-enkephalin region and the half-curved shape of the main chain of the adjacent fragment Arg6-

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Val8. The spatial structure of all representatives of this group characterizes the fffeffff shape of the Tyr1-Glu12 region, in which the two helical pentapeptide segments are displaced relative to each other due to the unfolded state of the dipeptide site of Met5-Arg6. Due to the tight packing of atoms of the polypeptide backbone and side chains, group A conformation provides the formation of the maximum amount of stabilizing intramolecular contacts, including strong hydrogen bonds between the remnants of the chain. The stereochemical image of the global conformation $B_{211}PRR_{21}B_{332}R_{2222}R_{3222}R_2BL_{22}RR_{32}B_3R_1R_{32}R_1R_2B_{211}B_{32}R_{33}$ of the molecule being investigated, representing the group A, is shown in Fig. 1, a. As we can see, the spatial structure of the molecule is characterized by the content of the three helical pentapeptide regions Tyr1-Met5, Arg6-Arg10 and Trp14-Gln18 separated by the unfolded and half-curved sections of the polypeptide chain. In this conformation, all three types of basic interactions that determine the spatial organization of a given amino acid sequence, i.e. dispersion, electrostatic and torsion ones find the greatest agreement. Note that, due to the high percentage of spiral regions contributing to the interaction of peptide skeleton atoms, this structure is stabilized additionally by establishing hydrogen bonds between the atoms of the main chain.

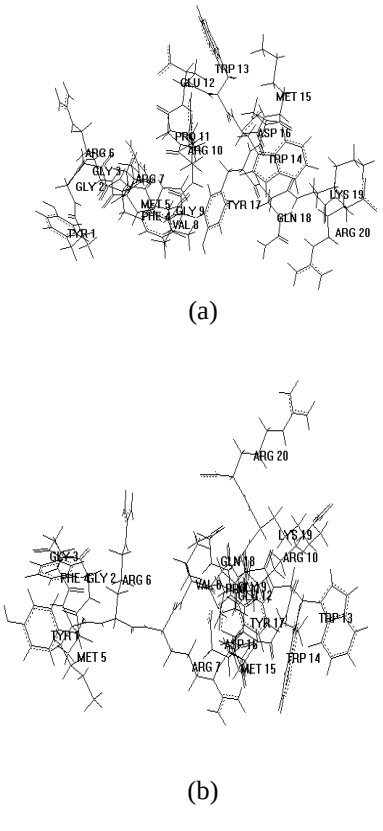

Fig.1:- Low-energy conformations of the molecule BAM-20P with a relative energy of 0 kcal/mol (a) and 2.7 kcal/mol (b)

III. RESULTS AND DISCUSSION

The idea of the length and depth of hydrogen bonds in the global structure can be obtained from table 3. As can be seen from the table, the total stabilizing effect of hydrogen bonds in this conformation was -6.0 kcal/mol. It is obvious that the hydrogen bonds realized in the given structure are due to the complex of many interstitial interactions, among which hydrogen bonds play an additional role. Although most of the residues in this conformation have a coiled form of the main chain, it is optimal not by the contribution of dispersion interactions, but by electrostatic contacts and is also characterized by a low value of torsion contributions. Because of the presence of three pentapeptide spiral segments, brought together in space by flexural regions, the role in the stabilization of this structure is played by both dispersive and electrostatic contacts of oppositely charged remnants that are close in space but are removed the chain. The stacking of the polypeptide chain, characteristic of the global conformation, leads to a steric approach to the remnants of the chain, including residues that have massive ionized side chains. This circumstance provides a significant enhancement of the dispersion and Coulomb interactions, which is accompanied by a significant decrease in the total energy of the molecule. In other words, the global conformation corresponding to the most energy-efficient packing of the main and side chains of the peptide under study provides an optimal balance of stabilizing intramolecular interactions.

Table 3. Parameters of Hydrogen Bonds in the Global Structure of the Molecule Вам-20p

Hydrogen bond	Length (A)	Depth (kcal/mol)
Tyr1 CO…NH Gly3	2.25	-0.50
Gly2 NH…OCMet5	1.90	-1.03
Arg6 N^zH^z…O^eC^eAsp16	2.29	-0.44
Arg7 N^zH^z …OCGly9	2.03	-0.83
Arg7 N^zH^z …O^zC^zGlu12	2.17	-0.60
Trp13NH…OCTrp13	2.38	-0.35
Trp12 CO…HN Tyr17	2.41	-0.33
Met15 HN…HN Tyr17	2.43	-0.31
Met15 HN…H^zN^zGln18	1.95	-0.95
Lys19CO…OOC	2.25	-0.49

The high packing density of the polypeptide chain of this structure is also illustrated by the large number of hydrogen bonds formed in this structure, given in table 3. It should be noted that the residues in the central position of the natural sequence, namely, Arg6, Arg7, Arg10, Glu12, chains, and ionogenic atomic groups, are able to enter into energetically favorable dispersive and electrostatic interactions

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both with each other and with the remaining amino acid residues of the preceding and following of the test peptide segments. It can be said that the center of the electrostatic stabilization of this conformation is the negatively charged Glu12, which plays a cementing role in the formation of the spatial structure of the molecule. An important role is played by the interactions of another negatively charged residue of Asp16 of this sequence. Note that the energy of the molecule is sensitive to the value of the angles of the side chain of the given residue. The most suitable is the directivity of its side chain to the C-terminus of the molecule, which ensures the most effective realization of electrostatic contacts of the negative ionogenic group Asp16 with positively charged atomic groups of the side chains of the residues Lys19 and Arg20. Note that, in spite of the adjacent position of the last two positively charged amino acid residues of this sequence, the directionality of the side chain of the Lys19 residue to the C-terminus of the molecule is most beneficial, which is explained by the advantageous electrostatic contacts of the atoms of the guanidine group of its side chain with the negatively charged terminal carboxyl group, accompanied by the establishment of an appropriate hydrogen bond. The formation of such a quasi-cycle at the C-terminal tail of the molecule promotes additional stabilization of its spatial structure.

Group B of the BAM-20P molecule is representative of a large number of low-energy conformations (with a relative energy of less than 10 kcal/mol), although the best conformation breaks away from the other conformations, gaining an average of 8 kcal/mol in energy and yielding a global one of a few kcal/mole. All the conformations of group B on the Met-enkephalin site contain a-rotation of the polypeptide chain. The subsequent part of the molecule acquires the expanded form of the main chain. The common element for the conformations of this group is the shape of the adrenorphine part, namely effeeee. The predominant role in the stabilization of the structures of group B is due to the dispersion interactions of both the main chain atoms and the side chains. The relative energy of the best conformation $B_{131}BPB_{21}B_{212}B_{1222}B_{2222}R_2BL_{22}RR_{32}R_2B_2R_{33}R_1R_2B_{211}B_{32}R_{33}$ of this group is higher than the energy of the global conformation of the molecule by 2.7 kcal/mol. The stereochemical image of this conformation is shown in figure 1, b. Let us consider the main factors stabilizing the given structure. Compared with the global conformation, the contribution of dispersion interactions is significant here. This type of interaction in this conformation reaches almost -100 kcal/mole. An important role in stabilizing this structure, as in the global one, is played by electrostatic interactions, whose contribution is 15.7 kcal/mol. We note that the center of electrostatic stabilization is the positively charged residues of Arg6 and Arg7, neighboring along the chain. Being on the middle portion of the amino acid sequence having an elongated shape of the main chain of the middle part of Phe4-Val8, characterized by the eeee shape of the peptide skeleton, these residues favorably interact with the spatially adjacent residues of both the preceding and subsequent sections. This convergence is due to the folded conformations of the sections Gly2-Phe4, Val8-Arg10, Pro11-Trp14 creating flexural segments of the molecule. The stability of this structure is sensitive to the conformational state of the Tyr1 residue, the unfolded form of its main chain is preferred. Due to the presence of a turn on the site of Tyr1-Met5, the Tyr1 residue advantageously interacts with the residues Gly3, Phe4, Met5, Glu12 and Asp16. The energy of this conformation is sensitive to the spatial arrangement of residues with aromatic rings. For this reason, hydrogen bonds play an important role in stabilizing this structure, as well as global.

We note that the analysis of the contributions of interstitial interactions in both conformations has shown that the dipeptide interaction Arg10-Pro11 contributes the most to the energy of non-valent interactions, which indicates the stability of the bond to the action of peptidases during the metabolism of this peptide.

Group C is represented by a single structural variant $B_{131}PRB_{33}B_{222}B_{1222}B_{2222}R_2BL_{22}RR_{32}B_3R_1R_{32}B_2B_2B_{311}B_{31}B_{32}$ with a relative energy value of 8.4 kcal/mol. A characteristic element here is the shape fffe of the peptide backbone of the Met-enkephalin site. This conformation is characterized by a relatively low value of the torsion contribution, and although the contribution of dispersion interactions is comparable to that in the global conformation, it also loses the best conformations of the previous groups in electrostatic interactions. This circumstance is due to the unfavorable arrangement of side chains carrying ionogenic groups in it, which leads to the loss of many advantageous interstitial electrostatic contacts and explains the relatively high energy value of this conformation. It can be expected that in strongly polar environments, where the role of intramolecular electrostatic interactions is significantly reduced, this conformation will be most preferable and therefore of interest. The geometric parameters of this structure are also given in table 2.

IV. CONCLUSION

Thus, the results of the calculation showed that of many considered combinations of preferred conformational states of fragments modeling possible structures of the molecule BAM-20P, only a limited set of conformations possessing low energy is isolated. The calculation showed that with the extension of the chain (with the transition from adrenorphine to BAM-12P, then to BAM-20P), i.e. with an increase in the number of conformational degrees of freedom, the number of low-energy structural options does not increase, as one would expect. This is due to the high steric selectivity of the sequence being analyzed, namely, only certain conformational states of constituent fragments lead to real structural variants of the dodecapeptide. This fact testifies to the uniqueness of the conditions for the realization of significant stabilizing interactions between distant residues at the maximum tight packing and consistency of interactions at all sites of the natural dodecapeptide sequence. Sterile complementarity of all segments of the molecule is realized by combinations of the energy-preferential conformational states of the constituent fragments.

The calculation revealed the advantage of realization for the molecule BAM-20P of two structural types,

characterized by a content on the Met-enkephalin area, respectively, -the helix and -turn. The large extent, high lability, and the presence of opposite charges in the molecule sequence creates favorable conditions for both effective dispersion interactions and electrostatic contacts of the remnants removed in a chain, but close in space. Therefore, the specific for this molecule is the tendency of the amino acid residues to settle in space so that the uniquely charged side chains of the residues are as close as possible, while the same ones are the most distant from each other. Most of the charged groups automatically appear on the surface of the globule and meet the solvent, but some of the charged side chains also participate in effective dispersion interactions inside the globule. It is the two conformations analyzed above that meet these requirements.

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