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# Assessment of the Bioactive Conformation of the Vasoactive Intestinal Peptide by Computational Methods

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**ABSTRACT:** In the present work, we report the results of a computational study aimed at assessing the structural features of the bioactive conformation of the vasoactive intestinal peptide (VIP). Based on previous structure–activity studies the present work reports the results of a comparative analysis of the conformational profiles of the segments 1–11 of the native peptide, two VIP antagonists and two inactive analogs. All these analogs exhibit the same sequence as VIP at C-terminus and only differ in some of the amino acids of the N-terminus. Analogs selected for the present study include VIP(7–28) fused to neurotensin (6–11) (Met-hybrid) and [Ac-Tyr<sup>1</sup>, D-Phe<sup>2</sup>]-VIP (both antagonists) and [Ala<sup>3</sup>]-VIP and [Ala<sup>6</sup>]-VIP (two inactive analogs). The conformational space of the five peptides was thoroughly explored using simulated annealing in an iterative fashion as sampling technique. The bioactive conformation was selected from pairwise cross-comparisons between each of the unique low energy conformations found for VIP(1–11) and each of the different analogs, within a 3 kcal/mol threshold in regard of the respective lowest energy conformation characterized. © 2002 Wiley Periodicals, Inc. *Int J Quantum Chem* 88: 201–210, 2002

**Key words:** vasoactive intestinal peptide; VIP; VIP analogs; conformational analysis; bioactive conformation; AMBER force field

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## Introduction

Vasoactive intestinal peptide (VIP) is a 28 amino acid residue peptide of sequence His<sup>1</sup>-Ser<sup>2</sup>-Asp<sup>3</sup>-Ala<sup>4</sup>-Val<sup>5</sup>-Phe<sup>6</sup>-Thr<sup>7</sup>-Asp<sup>8</sup>-Asn<sup>9</sup>-Tyr<sup>10</sup>-Thr<sup>11</sup>-Arg<sup>12</sup>-Leu<sup>13</sup>-Arg<sup>14</sup>-Lys<sup>15</sup>-Gln<sup>16</sup>-Met<sup>17</sup>-Ala<sup>18</sup>-Val<sup>19</sup>-Lys<sup>20</sup>-Lys<sup>21</sup>-Tyr<sup>22</sup>-Leu<sup>23</sup>-Asn<sup>24</sup>-Ser<sup>25</sup>-Ile<sup>26</sup>-Leu<sup>27</sup>-Asn<sup>28</sup>-NH<sub>2</sub>, first isolated from intestinal porcine extracts [1]. VIP is a member of the secretin-glucagon family and its sequence shares high identity with this class of peptide hormones [2, 3]. However, more than a hormone, VIP can better be considered as a neuromodulator or neurotransmitter [4, 5] since it is released by electrical stimulation of extrinsic nerves to various organs, including the pancreas and gastrointestinal tract. Thus, VIP plays a physiological role [6] in pancreatic and intestinal secretion, regulation of smooth muscle motility, gastrointestinal blood flow, mucosal immune function, etc. On the other hand, it produces anti-inflammatory effects, with demonstrated actions in alleviating the symptoms of rheumatoid arthritis [7] as well as functions as neurotransmitter of the nonadrenergic–noncholinergic inhibitory nervous system [8]. There is evidence suggesting that VIP is the endogenous mediator of bronchial smooth muscle relaxation [9] and functions as a modulator in T-cell and B-cell proliferation [10], which inhibits human natural killer cell activity [11].

It is difficult to justify the wide spectrum of activity exhibited by VIP in physiologic terms. It is well established that its actions are mediated through at least two G-protein coupled receptors (VIP-1 and VIP-2) that were cloned and expressed a few years ago [12], although the peptide is also known to interact with the CD4 receptor of the T lymphocytes [13] or calmodulin [14]. Accordingly, the development of selective analogs that preferentially interact with one of the different receptors is a necessary requirement to understand the different activities exerted by VIP both in basic research and for the development of new therapeutic agents.

Structure–activity relationship studies carried out on VIP, its fragments, and synthetic analogs are in many cases difficult to interpret, since most of them have been carried out on a wide variety of tissues, where the presence of VIP-1 or VIP-2 is not yet well established. However, the studies carried out so far suggest that the integrity of VIP is necessary to exert its full biological activity [15]. More specifically, the peptide C-terminal fragment has been shown to be required for high affinity

binding to the receptor, whereas the N-terminus (residues 1–11) can be considered the locus of its biological activity. Indeed, His<sup>1</sup> is critical for receptor activation [15], the fragment VIP(2–28) being an antagonist. An alanine scan on the peptide shows that residues Asp<sup>3</sup>, Phe<sup>6</sup>, Thr<sup>7</sup>, Tyr<sup>10</sup>, Tyr<sup>22</sup>, and Leu<sup>23</sup> are critical for the receptor binding affinity and for its biological potency in vivo [16]. Similar loss of binding affinity is observed on VIP analogs with D-replacements at positions Ala<sup>4</sup>, Val<sup>5</sup>, Phe<sup>6</sup>, Asp<sup>8</sup>, Thr<sup>11</sup>, Val<sup>19</sup>, and Tyr<sup>22</sup> [17]. Different synthetic analogs of VIP have also been reported in the literature. However, only a restricted number of them show acceptable antagonistic effects with respect to their precursor molecule. A classic competitive antagonist is a hybrid peptide known as Met-hybrid [18] consisting of the fragment VIP(7–28) fused to the fragment 6–11 of neurotensin. Other VIP antagonists like [Ac-Tyr<sup>1</sup>, D-Phe<sup>6</sup>] growth-factor releasing hormone [19], [4-Cl-Phe<sup>6</sup>, Leu<sup>17</sup>]VIP [20], L-8-K [21], VIP<sub>10–28</sub> [22], and peptide T [23] are less effective. Recently, combination of best multiple-substitution sites with potent cyclic analogs has permitted the identification of the exceptionally highly potent compound Ro25-1553 [17], which happened to be a promising therapeutic agent for the treatment of bronchospastic diseases. Although a variety of synthetic VIP analogs have been synthesized and tested, selective analogs that preferentially interact with one of the different receptors with affinity similar to VIP have not yet been described. To date, secretin is the only known peptide that is able to differentiate between the two VIP receptors [24], exhibiting a higher affinity for VIP-1 than for VIP-2.

In order to design new analogs, proposals need to be more substantiated on the structural information of the preferred conformations of the peptide. Two-dimensional nuclear magnetic resonance (NMR) studies on VIP [25] in a solution of 40% of trifluoroethanol suggest that the peptide consists of two amphipatic helices (residues 7–15 and 19–27, respectively) connected by a region (residues 16–18) with an undefined structure. On the other hand, two-dimensional NMR performed on some VIP fragments [26] suggests that the N-terminal region (residues 1–6) exhibits a folded conformation. In order to get new insights into VIP structure–activity relationships, we recently characterized the conformational profile of this peptide using computational methods [27]. Specifically, a detailed analysis of the conformational preferences of VIP(1–11), responsible for the biological activity of VIP, was carried out. The lowest energy conformations of this

fragment were subsequently fused to the remainder of the VIP chain that had been set in the form of two  $\alpha$ -helices according to the peptide model proposed from NMR studies [25]. This study permitted demonstration of the tendency of the peptide N-terminus to adopt  $\beta$ -turn conformations.

Combination of structure–activity relationships and structural studies together with a few site directed mutagenesis studies performed on the receptors has suggested a picture of the peptide–receptor interaction. Accordingly, the N-terminus of VIP interacts with the transmembrane domain of the receptors, whereas the C-terminus interacts with the long N-terminus domain of these receptors [28].

In order to move a step further in the assessment of the structural features that characterize VIP–receptor interaction, we report in the present work the results of a computational study aimed at assessing the bioactive conformation adopted by the N-terminal segment of VIP when it interacts with its receptors. Specifically, the conformational profiles of VIP(1–11) and those of the fragments 1–11 of two VIP antagonists and two inactive analogs were compared. VIP analogs selected for the present study exhibit the same C-terminus as the native peptide but differ in the amino acid sequence at their respective N-terminus. This set includes two antagonists, Met-hybrid [18] and [Ac-Tyr<sup>1</sup>, D-Phe<sup>2</sup>]-VIP [29], and two inactive analogs, [Ala<sup>3</sup>]-VIP [16] and (Ala<sup>6</sup>)-VIP [16].

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## Methods

Peptides were studied with their N-terminus charged and the C-terminus blocked with N-methylamide groups. Calculations were carried out using the all-atom parm94 AMBER force field [30]. The unnatural amino acid residue D-Phe that occurs in the VIP antagonist [Ac-Tyr<sup>1</sup>, D-Phe<sup>2</sup>]-VIP was constructed using the PREP module of AMBER. Partial charges were generated by fitting the molecular electrostatic potential produced with a STO-3G basis set using the GAUSSIAN94 suite of programs [31]. No explicit solvent was included in the calculations, although an effective dielectric constant of 80 was used to screen the electrostatic interactions and no cutoff was used.

The conformational space of the peptides was sampled using simulated annealing (SA) in an iterative fashion [32, 33]. For every peptide, the starting extended structure was energy minimized using the conjugate gradient algorithm until convergence, set

to 0.001 kcal/mol Å. Then, the structure was quickly heated up to 900 K at a rate of 100 K/ps, in order to force the molecule to jump to a different region of the conformational space. Subsequently, the 900 K structure was slowly cooled up to 200 K at a rate of 7 K/ps and then minimized. This final structure was stored on a file and used as the starting conformation for a new cycle of SA. The library of low energy conformations generated at this way was rank ordered by energy. Every 100 cycles low energy conformations were checked for uniqueness. A conformation was considered unique when at least one of its backbone dihedral angles, excluding those of both termini, was different by 60° with regard to any of the previous conformations already stored on the library. The procedure was repeated 5900 cycles where the criterion for convergence of the sampling procedure was achieved for all the peptides.

The convergence criterion on the sampling procedure was established for VIP and subsequently used with the rest of analogs. In our previous conformational study of VIP(1–11) [27] exploration of the conformational space was carried out following the same protocol as explained above. However, the sampling process was considered finished when no new conformations appeared after 50 cycles within a 5 kcal/mol energy range with respect to the lowest energy structures already found. In the present work we have revised that conformational analysis, establishing a convergence criterion on a different basis. Accordingly, in the present work calculations were discontinued when the number of unique conformations within a 3 kcal/mol energy range was constant for at least 500 cycles.

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## Results and Discussion

Table I lists the details of the conformational search performed on VIP and the different analogs studied in the present work. In order to characterize in qualitative terms the conformational tendencies observed in each of the analogs studied, the sets of unique structures obtained were analyzed in terms of the secondary structure motifs they exhibit. Motifs considered for this classification were standard ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -turns, characterized by C13, C10, and C7 cycles, respectively), being closed by a hydrogen bond that connects their extremes. Tolerance limits considered in the present work for hydrogen bond occurrence were set to a donor–acceptor distance not longer than 3.5 Å and a N–H–O angle between 120° and 180°. Conformations with the

**TABLE I**  
**Summary of the computational study performed on VIP and the set of selected analogs.**

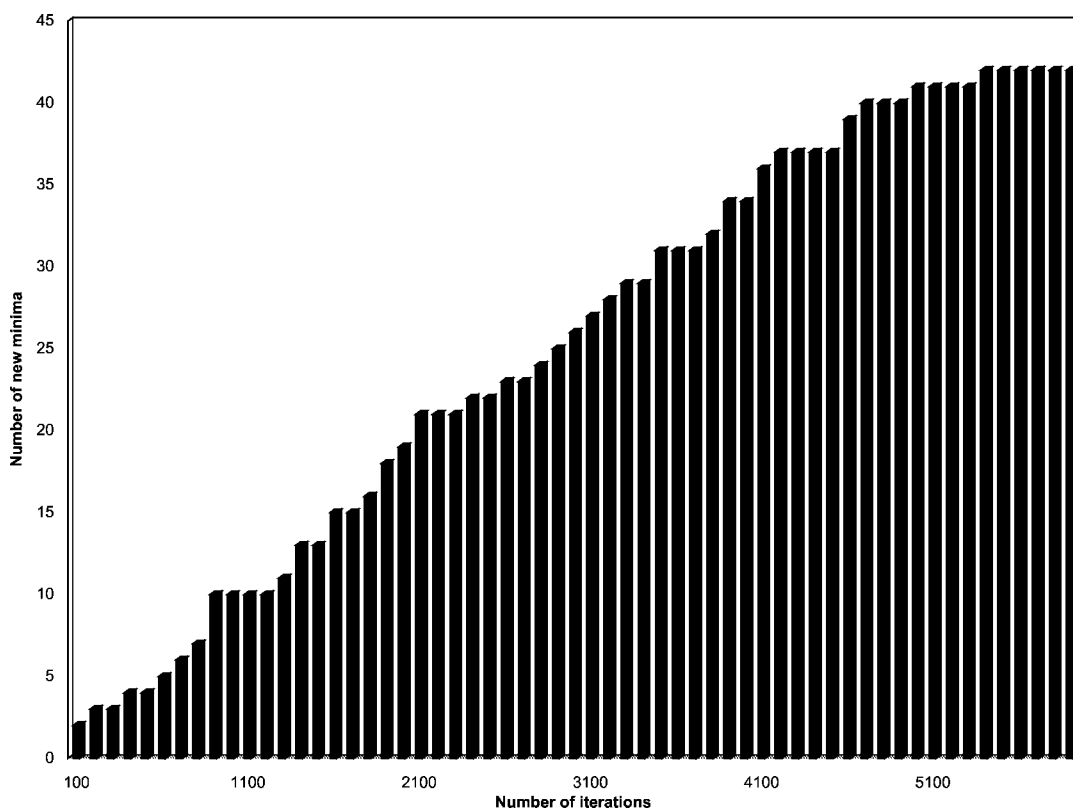
Analog	# Unique conformations (angles)	# Unique conformations in 0–3 kcal/mol	# Unique conformations ( $\alpha$ , $\beta$ , and $\gamma$ motives)	Global minimum
VIP	5607	43	31	3446
Met-hybrid	5858	15	10	1046
TyrDPheVIP	5856	51	40	3069
Ala3VIP	5858	32	27	3088
Ala6VIP	5862	59	46	1835

same structural motifs were subsequently clustered into classes. Detailed descriptions of these results for each of the peptides studied are given individually below.

### VIP

After 5900 cycles of iterative SA, the peptide showed 5607 unique conformations according to the criterion described in the Methods section. Figure 1

shows schematically the evolution of unique conformations within a 3 kcal/mol energy range sampled after every 100 cycles and how convergence is achieved after 5900 cycles of iterative simulated annealing. The lowest energy conformation was found after iteration number 3446. In the set of low energy conformations generated, only 43 conformations resulted with energies within a 3 kcal/mol above the lowest energy minimum. After identification of the



**FIGURE 1.** Evolution of the number of conformations found every 100 cycles for VIP along the iterative simulated annealing procedure.

standard  $\alpha$ -,  $\beta$ -, and  $\gamma$ -motifs in this low energy conformations subset, 31 different classes were obtained.

Table II lists the different classes found as well as the conformational motifs defining each of them for VIP as well as for the rest of analogs studied. The lowest energy conformation of VIP (class # 1) only exhibits a  $\gamma$ -turn between the amide hydrogen of Tyr<sup>10</sup> and the carbonyl oxygen of Asp<sup>8</sup>. The class with the higher number of members (class # 5) is the class containing conformations with no standard turns. There is a great diversity of secondary motifs found in the different low energy structures. Indeed, considering all the possible secondary motifs that a 11 amino acid residue peptide can exhibit, only the  $\alpha$ -turns between the residues Phe<sup>6</sup> and Ser<sup>2</sup>, between Val<sup>5</sup> and His<sup>1</sup>, the  $\beta$ -turns between the residues Tyr<sup>10</sup> and Thr<sup>7</sup>, and between Val<sup>5</sup> and Ser<sup>2</sup> were not found in any of the low energy conformations of VIP within the 3 kcal/mol subset.

### Met-HYBRID

After 5900 cycles of iterative SA, 5858 unique conformations for Met-hybrid were found. The lowest energy conformation was found after iteration 1046. Of the unique structures, only 15 lie below a 3 kcal/mol threshold of the lowest energy conformation. Classification of this low energy subset of structures based on standard secondary motifs led to 10 different classes, as shown in Table II. No structures with  $\alpha$ -turns between the residues Thr<sup>11</sup> and Thr<sup>7</sup>, Thr<sup>7</sup> and Arg<sup>3</sup>, Tyr<sup>6</sup> and Pro<sup>2</sup>, Pro<sup>5</sup> and Lys<sup>1</sup> were found. Similarly, there are no structures with  $\beta$ -turns between residues Tyr<sup>10</sup> and Thr<sup>7</sup>, Asn<sup>9</sup> and Tyr<sup>6</sup>, Tyr<sup>6</sup> and Arg<sup>3</sup>, Pro<sup>5</sup> and Pro<sup>2</sup>, and between Arg<sup>4</sup> and Lys<sup>1</sup>, as well as no structures with  $\gamma$ -turns between the residues Asp<sup>8</sup> and Tyr<sup>6</sup> and between Pro<sup>5</sup> and Arg<sup>3</sup> for this peptide analog. It is important to note that the lack of occurrence of  $\alpha$ -turns between residues Pro<sup>5</sup> and Lys<sup>1</sup>,  $\beta$ -turns between residues Pro<sup>5</sup> and Pro<sup>2</sup>, or  $\gamma$ -turns between residues Pro<sup>5</sup> and Arg<sup>3</sup> is due to the lack of the amide hydrogen on prolines. The lowest energy conformation characterized of Met-hybrid (class # 1), shows a  $\beta$ -turn between the amide hydrogen of Thr<sup>7</sup> and the carbonyl oxygen of Arg<sup>4</sup> as well as three  $\gamma$ -turns between the amide hydrogens of Thr<sup>11</sup>, Asn<sup>9</sup>, and Tyr<sup>6</sup> and the carbonyl oxygens of Asn<sup>9</sup>, Thr<sup>7</sup>, and Arg<sup>4</sup>, respectively. Also in this case, the class with a larger number of structures (class # 2) is represented by bent conformations with no standard secondary motifs.

### [Tyr<sup>1</sup>, DPhe<sup>6</sup>]VIP

After 5900 cycles of iterative SA, 5856 unique conformations for this peptide were found. The lowest energy minimum was obtained after iteration 3069. Of the unique conformations 51 lie within the 0–3 kcal/mol energy range in regard to the lowest energy conformation found. Consideration of the standard secondary motifs present on them resulted in 40 classes. The lowest energy structure exhibits two  $\gamma$ -turns involving the residues Thr<sup>11</sup>–Asn<sup>9</sup> and Phe<sup>6</sup>–Ala<sup>4</sup>, respectively. No structures with  $\alpha$ -turns between the amide hydrogen of Val<sup>5</sup> and the carbonyl oxygen of Tyr<sup>1</sup>, as well as no structures with  $\beta$ -turns involving the residues Ala<sup>4</sup> and Tyr<sup>1</sup>, are found in the 3 kcal/mol threshold subset of conformations. As in the previous analogs, the class of conformations with no standard motifs (class # 17) contains the larger number of structures, although with the same number of conformations of class # 3, characterized by a  $\gamma$ -turn between residues Thr<sup>7</sup> and Val<sup>5</sup>.

### [Ala<sup>3</sup>]VIP

The conformational search of this inactive VIP analog yielded 5858 unique conformations. Of these, 32 were within 3 kcal/mol energy range. The lowest energy minimum was found after iteration number 3088. As shown in Table II no conformations with  $\beta$ -turns between Thr<sup>11</sup>–Asp<sup>8</sup> and Ala<sup>4</sup>–His<sup>1</sup> as well as no structures with  $\beta$ -turns between Asp<sup>8</sup>–Phe<sup>6</sup> are found in the subset of low energy conformations within 3 kcal/mol threshold in respect to its global minimum. As previously found, the class with a larger number of structures (class # 17) contains bent conformations with no standard hydrogen bonds within them.

### [Ala<sup>6</sup>]VIP

The conformational search of this peptide yielded 5862 unique conformations. The lowest energy conformation was obtained after iteration number 1835. Of the unique conformations found, 59 lie within the 0–3 kcal/mol energetic range that are classified into 46 classes. Of these classes, class # 1 that contains the lowest energy conformation is representative of structures with no standard secondary motifs. For this inactive VIP analog, the only standard turn missing with respect to the all those that are possible for an undecapeptide is the  $\beta$ -turn involving the residues Asp<sup>8</sup> and Val<sup>5</sup>.

**TABLE II**  
**Secondary motifs ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -turns) of the representative conformations of each of the different classes found by iterative SA calculations of the peptides studied in this work.**

Motives	H-bonds (N-H-O)	# VIP class	# Met-hybrid class	# TyrDPheVIP class	# Ala3VIP class	# Ala6VIP class
$\alpha$ -turns	11-7	7, 26	—	24, 27, 31, 39	3, 5, 11, 15	3, 6, 10, 11, 21, 24, 33, 34, 38, 39
	10-6	11, 31	3	2, 9, 11, 12, 13, 37, 39	3, 10, 11, 15, 22	3, 10, 11, 21, 24, 28, 30, 32, 33, 34, 35, 38, 39
	9-5	13, 25	3, 5	2, 6, 7, 13, 16, 22	2, 3, 5, 6, 9, 10, 11, 13, 15, 22, 23, 25	3, 4, 11, 21, 28, 33, 34, 37
	8-4	3, 4, 14, 22	7, 8	6, 13, 16, 19, 22, 27, 39	1, 5, 6, 7, 9, 10, 13, 15	2, 8, 9, 11, 12, 13, 17, 21, 28, 33, 42
	7-3	31	—	6, 13, 16, 19, 21, 22, 36, 39	9, 13, 14, 15	6, 16, 21, 28, 33, 40, 41
6-2	—	—	8, 30	9, 15, 16, 27	21, 32, 35, 43, 46	
5-1	—	—	—	11, 15, 19, 23, 24, 25	26, 29, 38	
$\beta$ -turns	11-8	24, 25, 29	9	25, 34, 37	—	13, 25, 42
	10-7	—	—	39	5, 26	3, 38
	9-6	16, 18, 20	—	9, 23, 35	5, 11, 20	11, 24, 25, 26, 27, 36, 37, 38, 44
	8-5	13	1, 8, 10	2, 5, 29	2, 10, 11, 16, 23	—
	7-4	14, 22, 24	—	13, 22	1, 9	2, 13, 20, 33, 34
	6-3	11, 26	—	6, 19, 26	13, 14, 26	4, 9, 40, 41, 45
	5-2	—	—	33	16	30, 32, 33, 43, 46
	4-1	15	—	—	—	35
	11-9	15, 27, 28	1	1, 9, 11, 15, 18, 21, 26, 28, 33, 37	1, 6, 9, 10, 12, 19, 22	18, 27, 32, 33, 43
	10-8	1, 14, 17, 19, 23, 24, 25, 28, 29	10	5, 14, 25, 29, 30, 40	14, 18, 23	12, 19, 27, 29, 31, 35, 37, 42, 46
9-7	6, 10, 28, 29, 30	1, 6, 10	5, 10, 21, 25	21	5, 7, 9, 13, 14, 17, 20, 22	
8-6	12, 19, 23, 24	—	4, 9, 10, 31, 35, 36	—	6, 14, 18, 20, 22, 23	
7-5	13, 16, 17	4	3, 24, 34	16, 23, 24	15, 39, 44, 45	
6-4	4, 21, 23, 25	1, 6, 7, 9	1, 12, 20, 33, 35, 39	8, 23	4, 22, 31, 34, 39	
5-3	4, 6, 8, 9, 20, 27, 28, 30	—	40	21, 22	25, 36, 40	
4-2	23	8	20, 28, 32	3, 4, 5, 23, 24	22, 29, 30	
3-1	2, 7, 9, 15, 25, 30	8	8, 15, 38	14	7, 15, 27, 32, 33, 37, 40, 44	
No H-bonds	5	2	17	17	1	1

## CHARACTERIZATION OF THE BIOACTIVE CONFORMATION

In order to assess the bioactive conformation of the N-terminal fragment of VIP, cross-comparisons of the different sets of unique low energy conformations of VIP and the analogs studied were performed. Selection of the set of analogs for the present study included two antagonists and two inactive analogs of VIP. Their conformational profiles were independently computed and assessment of VIP bioactive conformation was performed by pairwise cross-comparisons between each of the unique low energy conformations found for VIP and each of the different peptide analogs. Common conformations among binders and at the same time not found in any of the inactive peptide sets were identified as candidates for the bioactive conformation. According to the hypothesis that the peptide C-terminal segment is only responsible of receptor recognition whereas the N-terminal fragment may be considered as the locus of its biological activity, peptides were selected for the present study in such a way that their C-terminal fragment was identical to that of VIP and differed in some of the amino acid residues on their N-terminal region (residues 1–11).

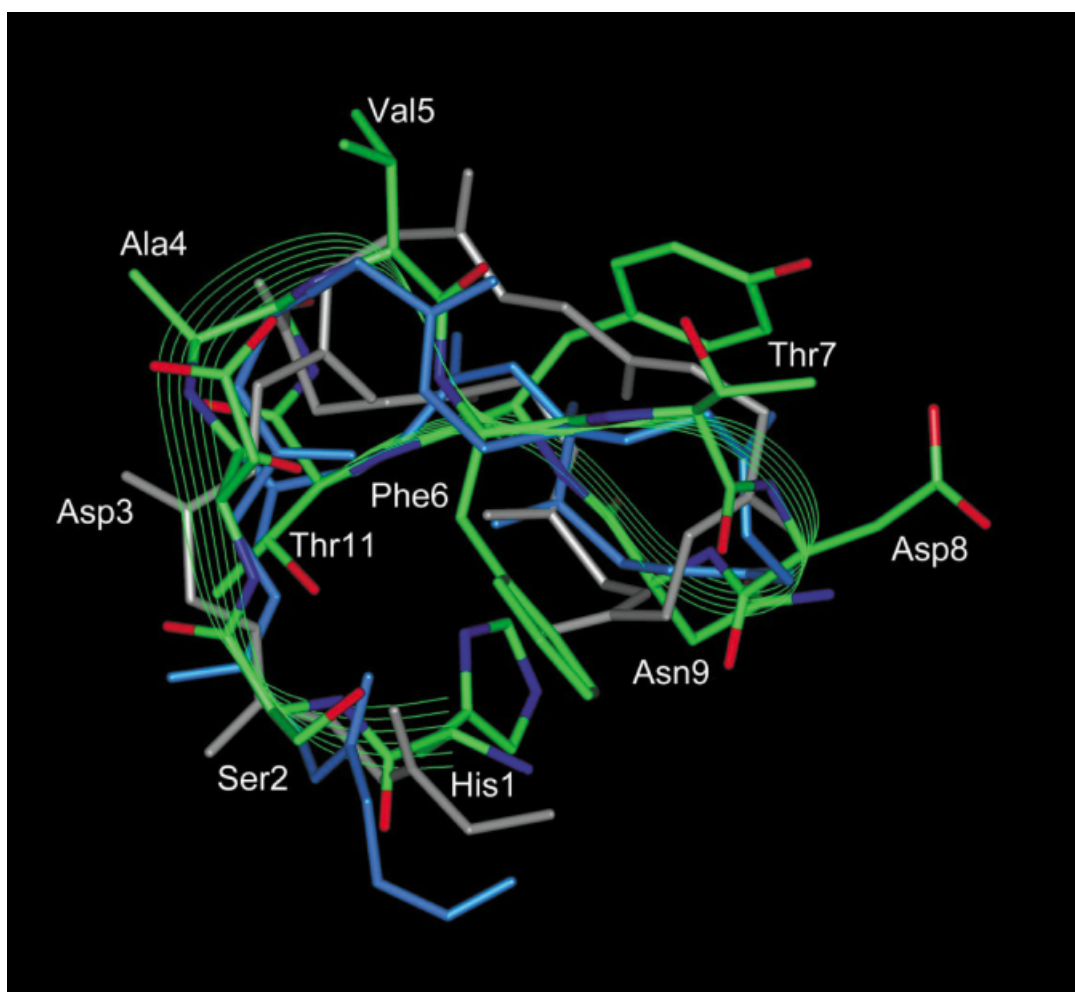
Pairwise cross-comparisons between all conformations within the 0–3 kcal/mol energy range of the different analogs studied were carried out by computing the root mean square deviation (rmsd) between the backbone atom coordinates of every pair of structures, after optimal superimposition. After testing different threshold values by visual inspection, two conformations were considered similar when the rmsd was smaller than or equal to 1.95 Å. Pairwise comparisons of the low energy conformations of the three binders (VIP, Met-hybrid, and [Tyr<sup>1</sup>DPhe<sup>6</sup>]VIP) and the two inactive analogs ([Ala<sup>3</sup>]VIP and [Ala<sup>6</sup>]VIP) permitted identification of only one conformation that is common to all the binders and not found within the low energy conformations of the two nonbinders.

The putative bioactive conformation found in VIP is 2.2 kcal/mol above the lowest energy minimum found and is the representative conformation of class # 11. The corresponding conformation in Met-hybrid lies 2.4 kcal/mol above its lowest energy minimum and in [Tyr<sup>1</sup>, DPhe<sup>6</sup>]VIP 2.6 kcal/mol. The secondary motifs that characterize this conformation in VIP are (Table II) a  $\alpha$ -turn between the amide hydrogen of residue Tyr<sup>10</sup> and the carbonyl oxygen of residue Phe<sup>6</sup> as well as a  $\beta$ -turn between the amide hydrogen of residue Phe<sup>6</sup>

and the carbonyl oxygen of residue Asp<sup>3</sup>. Interestingly, this conformation is similar in qualitative terms to the solution structure of VIP studied by NMR [26]. In this case the structure is described as exhibiting an  $\alpha$ -turn involving Thr<sup>7</sup> and Thr<sup>11</sup>, in addition to two  $\beta$ -turns between Ser<sup>2</sup> and Val<sup>5</sup> and between Thr<sup>7</sup> and Tyr<sup>10</sup>, respectively. However, it is not expected that the bioactive conformation coincides with the structure found in solution. Moreover, the qualitative description by secondary motifs can be misleading. For example, the overall shapes of the corresponding putative bioactive conformation of VIP and those of the other two antagonists are practically identical (Fig. 2). However, the secondary structure motifs they exhibit are different. Specifically, in the case of the Met-hybrid, the conformation with lowest rmsd (less than 1.95 Å) belongs to class # 6 and is characterized by two  $\gamma$ -turns between the amide hydrogen of residue Asn<sup>9</sup> and the carbonyl oxygen of residue Thr<sup>7</sup> and between the amide hydrogen of residue Tyr<sup>6</sup> and the carbonyl oxygen of residue Arg<sup>4</sup>, respectively. Conversely, the corresponding conformation of [Tyr<sup>1</sup>DPhe<sup>6</sup>]VIP belongs to class # 26 and is characterized by a  $\beta$ -turn between the amide hydrogen of residue Phe<sup>6</sup> and the carbonyl oxygen of residue Asp<sup>3</sup> and by a  $\gamma$ -turn between Thr<sup>11</sup> and Asn<sup>9</sup>.

The conformations of the inactive analogs [Ala<sup>3</sup>]VIP and [Ala<sup>6</sup>]VIP that show the lowest rmsd value (higher than 1.95 Å) with the putative VIP bioactive conformation occur at relative energies of 1.3 and 3.0 kcal/mol of their respective lowest energy conformations, respectively. The [Ala<sup>3</sup>]VIP conformation belonging to class # 4 (rmsd of 2.1 Å with the putative VIP bioactive conformation) is characterized only by a  $\gamma$ -turn between the amide hydrogen of residue Ala<sup>4</sup> and the carbonyl oxygen of residue Ser<sup>2</sup>. In contrast, the corresponding conformation of the [Ala<sup>6</sup>]VIP belonging to class # 44 (rmsd of 2.1 Å with the putative VIP bioactive conformation) is characterized by a  $\beta$ -turn involving the residues Asn<sup>9</sup> and Ala<sup>6</sup> and by two  $\gamma$ -turns between Thr<sup>7</sup> and Val<sup>5</sup> and between Asp<sup>3</sup> and His<sup>1</sup>, respectively.

The putative bioactive conformation proposed in this work was found by a procedure that only takes into account features of peptide backbones. It could be argued that structural comparisons should be carried out using the side chains of the different residues, since they are ultimately responsible for ligand–receptor interactions. However, comparative conformational analysis based only on side



**FIGURE 2.** Backbone representation of the the putative bioactive conformation of VIP(1–11) (color by atom) superimposed with the closest structure found for Met-hybrid (grey) and [Ac-Tyr<sup>1</sup>Dphe<sup>2</sup>]VIP (blue). Labels refer to the VIP(1–11) sequence.

chains should be avoided since these exhibit low energy torsion barriers and, consequently, they may easily sample all the conformational space accessible to them [34]. Thus, in the case of comparisons between peptides exhibiting a high sequence identity as in the present case, it is expected that when two analogs adopt the same backbone conformation, their side chains will exhibit accessibility to the same conformational space and make unnecessary comparisons between side chains. However, when the analogs studied exhibit low sequence identity, a procedure that explicitly includes comparison of side chains seems to be necessary [35]. In this case the conformational analysis requires consideration of expected flexibility of the side chains.

Figure 2 shows the superimposition of the putative VIP bioactive conformation and those of the two antagonists also studied in the present work. The bioactive form exhibits a hairpin turn involving the last six residues of the C-terminus. Assuming that this region of the peptide interacts with the transmembrane domains of its G-protein coupled receptors, there are different residues of the three binding analogs whose side chains have access to the same regions of the space. Thus, the superimposition of the three analogs together with the closest conformation of the two nonbinders (not shown) suggests that residues Val<sup>5</sup>, Phe<sup>6</sup>, Asn<sup>9</sup>, and Tyr<sup>10</sup> may be important for the interaction between the N-terminal fragment (residues 1–11) of VIP and the seven transmembrane domain of the VIP receptor.

On the other hand, Asp<sup>3</sup> as shown in the ala scan must also participate in the interaction of VIP with its receptor. Met-hybrid instead has two consecutive arginines. This difference suggests that these residues may interact with complementary charged residues in a region rich in residues of this kind most likely located on the loop connecting the transmembrane helices. These results provide complementary information to the known structure-activity studies reported in the literature and it is expected that they are helpful for designing new peptide and non-peptide antagonists of VIP. Work in this direction is presently being undertaken in our laboratory.

## Conclusions

The conformational profiles of the N-terminal fragments (residues 1–11) of VIP and four VIP analogs were performed by computational methods. The selected analogs included two antagonists and two nonbinders. The conformational energy was computed using molecular mechanics and the exploration of the conformational space was performed using an iterative simulated annealing protocol. VIP bioactive conformation was assessed by pairwise cross-comparisons between each of the unique low energy conformations found for VIP(1–11) and each of its different analogs characterized within a 3 kcal/mol threshold with respect to the respective lowest energy conformation. Present results suggest that residues Val<sup>5</sup>, Phe<sup>6</sup>, Asn<sup>9</sup>, and Tyr<sup>10</sup> may be involved in the interaction with the receptor. Knowledge of the chemical groups putatively involved in the interaction with the receptor, together with their three-dimensional arrangements, can be helpful for designing VIP antagonists with higher affinity.

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