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A proposed bioactive conformation of Peptide T

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Summary

The conformational profiles of Peptide T, (5–8)Peptide T, [Abu^5](4–8)Peptide T and (4–8)Peptide T were computed independently to assess the geometrical characteristics of the bioactive conformation of Peptide T. The conformational profiles of the peptides were computed within the molecular mechanics framework using an effective dielectric constant of 80. The conformational space was thoroughly sampled using an iterative simulated annealing protocol. The bioactive conformation was assessed by pairwise cross comparisons of each of the unique low energy conformations found for each of the different analogs studied. After a putative bioactive conformation was selected, in order to further validate our hypothesis the conformational profile of the potent compound cyclo(Thr-Thr-Asn-Tyr-Thr-Asp) was computed and the putative bioactive conformation was found. The conformation exhibits a pseudo β-turn involving the side chain of Thr^5 and the carbonyl oxygen of Tyr^7 forming a C12 ring.

Introduction

The synthetic octapeptide of sequence Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr, known as Peptide T, is a fragment of the envelope glycoprotein gp120 of the human immunodeficiency virus (HIV). The peptide potently inhibits binding of gp120 to the CD4 receptors expressed on T4 helper/inducer lymphocytes [1]. It has also proven quite potent in triggering human monocyte chemotaxis through the CD4/T4 antigen [2]. Furthermore, pharmacological studies on Peptide T and its analogs show them to be very promising for their potential applicability as therapeutic agents for the treatment of neuropsychometric symptoms in AIDS patients [3] and psoriasis [4].

Due to its interesting pharmacological properties and in order to overcome the poor adsorption and pharmacokinetic profile associated to a peptide, efforts were put forward to the design of a Peptide T peptidomimetic. In this direction several peptides, including cyclic and linear analogs were synthesized in the past. The analog [DAla^1]Peptide T-NH_2 was early proven to be as effective as the native peptide in inhibiting the binding of the gp120 glycoprotein to the CD4 receptor [1]. It was also soon established that the five C-terminal residues of the peptide retain high potency [2, 5]. On the other hand, different analogs were synthesized by systematic chemical reduction of the different peptide bonds, in an effort to understand their role on the bioactive conformation of the peptide. These studies demonstrated that specific reduction of peptide bonds 4 and 8 diminished ligand recognition by the receptor [6, 7]. Other studies [5, 8–10] also established the importance of residues Thr^5 and Thr^8 for chemotactic activity.

Experimental and theoretical studies carried out in the past by several groups pointed out the tendency of the peptide to adopt a β-turn at its C-terminus, although with discrepancies about the residues involved. Thus, NMR spectroscopy studies on Peptide T and on its fragment (4–8)Peptide T carried out by Motta and co-workers [11, 12] indicated a predominant β-turn conformation involving backbone atoms of residues Thr^5 and Thr^8. In an independent study, Cotelle at al. [13] carried out NMR studies on blocked (4–8)Peptide T, suggesting that the structure in solution exhibits two consecutive β-turns involving backbone atoms be-

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tween Thr^4-Tyr^7 and Thr^5-Thr^8 respectively. These results were further supported by the observation that two proteins, Ribonuclease A and Pepsin, exhibit a β-turn structure of the type Thr^4-Tyr^7 in segments that exhibit a high sequence homology with Peptide T [14, 15]. Based on these results, NMR data of Ribonuclease A were used to construct a 3D model of the solution conformation of Peptide T. This model exhibits a β-turn at the C-terminus stabilized by a bifurcated hydrogen bond between Thr^4 and both Tyr^7 and Thr^8 simultaneously [16].

Despite the apparent disparity of the results outlined, all this information was very helpful for the design of the peptide analog cyclo(Thr-Thr-Asn-Tyr-Thr-Asp), a first generation peptidomimetic that exhibits a slightly better chemotactic activity than (4–8)Peptide T [8, 9].

In order to put a step forward in the design of second generation peptidomimetics, besides rationalizing a wealth of structure–activity relationship (SAR) studies, it is necessary to assess the characteristics of the bioactive conformation of Peptide T and subsequently, propose a pharmacophore of its interaction to the CD4 receptor. Thus, the goal of the present study is to investigate the geometrical characteristics of the bioactive conformation of Peptide T. For this purpose the conformational profiles of a carefully selected group of analogs were studied and compared pairwisely. Specifically, two active analogs, Peptide T itself and its fragment (4–8)Peptide T, and two non-binders, [Abu^5](4–8)Peptide T and (5–8)Peptide T, were selected for the present study. A putative bioactive conformation of Peptide T was characterized in a two step procedure. First, a subset of conformations containing the low energy structures common to the active peptides was created. Second, structures from this set were systematically compared with the low energy conformations of the non-binders, in order to find those conformations common to the active peptides and at the same time not attainable by the non-binders. Following this procedure only one conformation was finally kept. This structure provides an adequate explanation of the SAR data and was consequently considered as the bioactive conformation. In order to validate this hypothesis, in an independent study the conformational profile of the potent cyclic analog cyclo(Thr-Thr-Asn-Tyr-Thr-Asp) was computed. Analysis of its conformational profile yielded a low energy conformation that matched well with the putative bioactive conformation, providing further support to the proposed hypothesis.

Methods

All the calculations were carried out within the molecular mechanics framework using the all-atom AMBER 4.0 force field [17]. The peptides were studied in their zwitterionic form. 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. Parameters for the Abu residue were assessed following the same protocols as used to generate the parm91 parameter data base in AMBER 4.0 [18].

The strategy used to sample the conformational space was simulated annealing used in an iterative fashion [19]. Starting from the extended conformation, the structure is minimized and subsequently heated to 900 K in a very short time. At this time the structure is cooled slowly to 200 K and then minimized. This structure is the starting conformation for another cycle, creating a library of conformations that are rank ordered by energy every 100 cycles. The procedure is repeated until no new conformations, excluding those that are local reoptimizations of the side chains, appear after a predetermined number of cycles (200 in the present case) within a 5 kcal/mol energy range with respect to the lowest energy structure already found. Heating is fast in order to make the molecule jump to a different region of the space. In our case, the heating rate was constant at 100 K/ps. On the other hand, the cooling rate is slow to get the lowest energy minimum of the region. In the present work the rate was constant at 7 K/ps.

As mentioned before, every 100 cycles the new structures obtained were added to a master list of unique conformations. In order to reject from the list those structures not unique, they were first rank ordered and analyzed for uniqueness in ascending energy order. A conformation was considered unique if at least one of the backbone dihedral angles, excluding those situated at both termini, was different to 60° with respect to the previous conformations already in the list.

The conformational analysis of each peptide was carried out on the subset of unique conformations within a 5 kcal/mol threshold with respect to its global minimum. In order to facilitate the description of the preferred conformational domains exhibited by each peptide, the conformations of each low energy subset were clustered into families according to the values of the root mean square deviation (rmsd) of the distance between the Cα atoms between every pair of structures

after they had been optimally superimposed. The use of the rmsd as similarity criterium requires to select a threshold value that depends on the number of atoms involved in the comparison [20]. In the present work a value of 1.1 Å was used for the octapeptide and this value was scaled linearly for the rest of the analogs depending on the number of atoms considered: 0.5 Å for the pentapeptides and 0.4 Å for the tetrapeptide. In a final step, in order to attain a qualitative picture of each of the conformational domains, families of structures exhibiting the same structural motives were grouped into classes.

Finally, pairwise cross comparisons between all the unique low energy conformations within a 5 kcal/mol threshold of the different analogs were performed. For this purpose, computation of the rmsd of all the backbone atoms of a subset of residues, depending on the peptide analogs compared, was performed to locate common conformations among them. In the case of comparisons involving five residues, two conformations were automatically considered similar if the rmsd value was lower than 0.7 Å and different if the value was higher than 1.25 Å. In the region between these two values, visual inspection was necessary to decide if the structural differences were meaningful or not. Sometimes a large rmsd value between a pair of conformations is due to the atoms of peptide termini, whereas the rest of the peptide chains deviate very little with respect to each other. In these cases the two conformations were considered similar. In other cases, high rmsd values were due to appreciable differences between most of the pairs of atoms in the chain. In this case the two conformations were considered as different. Also, in some cases deviations occur in two consecutive residues. In the present work, it was considered that the two conformations were similar when the side chains of the residues involved were able to sample the same regions of the space.

Results and discussion

Conformational analysis of Peptide T and its analogs

Peptide T

The conformational analysis of Peptide T has been object of a previous report [21] and will be only discussed briefly. After 4800 cycles of simulated annealing, 1744 unique conformations were found. Of these, 130 exhibited conformational energies within 5 kcal/mol above the lowest energy conformation found. Computation of the rmsd involving only the Cα's of the peptide chain allowed the classification of these conformations into 74 families. Bent type conformations represent about 88% of the different motives found in the peptide within a 5 kcal/mol spread of energy from the global minimum. The peptide also shows a tendency to form consecutive α turns, suggesting that the molecule retains a certain helical character. Moreover, β-turn structures are also observed, predominantly at the C-terminus of the peptide; however, they represent a small percentage of the total number of unique conformations.

(4–8)Peptide T

After 3200 cycles of simulated annealing, only 286 conformations were characterized as unique. Their energies ranged up to 12 kcal/mol above the global minimum, 99 of them being within the 5 kcal/mol threshold above the global minimum. After calculation of the pairwise rmsd as explained in the Methods section, the subset of low energy unique structures was reduced to 43 classes. Analysis of the low energy conformations reveals a high tendency of the peptide to adopt bent structures, where both peptide termini are interacting as in the case of the global minimum. Type I β-turns between residues Thr^5 and Thr^8 and sometimes between residues Thr^4 and Thr^7 form the most frequent motif. However, two consecutive γ-turns or a β-turn simultaneously with an α-turn are also observed.

(5–8)Peptide T

The exploration of the conformational space of this tetrapeptide was completed after 2900 cycles of simulated annealing. After completion of this procedure, 142 unique conformations were characterized ranging in a spread of energies up to 11 kcal/mol above the global minimum. Of these, only 44 conformations were in the low energy subset with energies up to 5 kcal/mol above the global minimum. Conformations were clustered by computation of the rmsd of the α-carbon coordinates between every pair of structures as explained in the Methods section. Following this procedure 11 families of structures were characterized. A large number of low energy conformations, including the global minimum, are bent conformations exhibiting interactions between both peptide termini. A second class of conformations can be described as extended and finally, there are few conformations (about 5%) exhibiting a β-turn between residues Thr^5 and Thr^8.

[Abu^5] (4–8)Peptide T

The exploration of the conformational space of the peptide required 4300 cycles of simulated annealing and provided 314 unique conformations. The structures lie in a range of energies of 10 kcal/mol above the global minimum. Of these, 143 were within the threshold of 5 kcal/mol above the global minimum. The structures were subsequently clustered from the values of the rmsd between each pair of structures. In this way the low energy structures were classified into 59 families. The conformational profile of the peptide is similar to that of the analog (4–8)Peptide T, although the presence of the α-aminobutyric acid in position 5 instead of threonine introduced differences in the relative population of the different secondary motives. Indeed, although bent structures with the peptide termini interacting are found, the global minimum exhibits simultaneously an α-turn between residues Thr^4 and Thr^8 and a β-turn between residues Thr^4 and Thr^7.

Characterization of the bioactive conformation

The bioactive conformation of Peptide T was assessed by a comparative conformational analysis. This methodology has been used satisfactorily in the past [22]. It is based on the paradigm that the bioactive conformation is one of low energy conformations found in the set of common structures of all the binders and furthermore, it is not found in the set of low energy conformations of the non-binders. Accordingly, in order to characterize the bioactive conformation of Peptide T, the subsets of unique low energy conformations within 5 kcal/mol above the lowest energy conformation found for both Peptide T and (4–8)Peptide T, were pairwisely compared. Since both peptides exhibit a similar capability to trigger human monocyte chemotaxis through the CD4/T4 antigen [2], it is plausible to assume that both peptides perform their activity exhibiting the same structural domains to the receptor. This conformation must consequently be common to all the low energy subsets of the different active analogs studied. The 99 unique conformations of (4–8)Peptide T were compared with the 130 unique conformations of Peptide T. Rmsds were computed including all the backbone atoms of the former analog and all the backbone atoms of the five C-terminal residues of the latter peptide. Pairwise comparison of the low energy conformations of the two peptides produced a range of rmsd values between 0.08 Å and 1.44 Å. Visual inspection of all pairs of conformations superimposed indicated that pairs of conformations with rmsd values higher than 1.25 Å could be considered as dissimilar, whereas pairs of conformations with an rmsd lower than 0.7 Å could be considered as similar. Since the rmsd is an average value of the distance between atoms, pairs of conformations with rmsd values between 0.7 Å and 1.25 Å have to be inspected visually in order to understand the structural origin for such a deviation and to decide their similarity. Most of the time such rmsd values are due to large deviations of the atoms in the terminal residues characterized by exhibiting a higher flexibility. In these cases the rmsd of the rest of the chain could be computed to decide the degree of similarity between the two molecules compared. On the other hand, the rmsd could reflect deviations in all the atoms compared. In such cases the two conformations are considered as dissimilar. Thus, pairwise comparison of the low energy conformations of the two peptides allowed to discard 33 conformations of Peptide T as prospective candidates to be the bioactive conformation.

In a further step, the subset of conformations common to the two active peptides were pairwisely compared for similarity with the low energy conformations of the non-binders. These peptide analogs are not capable of inducing human monocyte chemotaxis [2, 12]. However, the loss of activity exhibited by these analogs can be due to two different reasons: (i) they may not be capable to attain the bioactive conformation; (ii) they may lack one or more chemical moieties, essential for ligand-receptor interaction. Comparison of the 44 unique low energy conformations of the analog (5–8)Peptide T showed that every conformation of the analog had a corresponding one in the set of common conformations of the active peptides. Accordingly, the loss of activity exhibited by this analog must be due to the absence of a chemical group important for ligand-receptor interaction and located on residue Thr^4. SAR studies suggest that this moiety is likely to be the carbonyl oxygen of the peptide backbone, since N-methylation of the amide group [5] and replacement of the side chain by Abu [23] yields active analogs. On the other hand, comparison of the 143 unique low energy conformations of [Abu^5](4–8)Peptide T with the set of unique low energy conformations common to the active peptides revealed 15 conformations of the former set with rmsd ranging from 0.7 to 1.0 Å that had to be visually inspected for similarity. In this case, similarity was assessed by comparing positions of the backbone atoms and accessibility of side chains to the same regions of the space. Accordingly, 14 out of the 15 conforma-

tions were discarded since the structural differences found, all of them occurring at both peptide termini, were not considered significant. The remaining conformation, although with a 0.89 Å rmsd with respect to the most similar structure in the set of common low energy conformations of the active peptides, is qualitatively different. Indeed, the structure selected among all the common to the active peptides is stabilized by a hydrogen bond between the carbonyl oxygen of the $Tyr^7$and the hydroxyl group of the Thr^5 side chain. This conformation is not attainable by the non-active Abu^5 analog, due to the lack of the hydroxyl group on its side chain. Accordingly, the conformation was considered the bioactive conformation. Figure 1 shows pictorially the bioactive conformation of (4–8)Peptide T superimposed with the most similar conformation of the Abu^5 analog. Figure 1 clearly shows the differential spacial arrangement of residues Tyr^7 and Thr^8 in the active and non-active analog and since Thr^8 is known to be essential for peptide activity, this differential spacial arrangement must be the cause of the lack of activity exhibited by the Abu^5 analog. Dihedral angles of the putative bioactive conformation of the two binders are listed in Table 1.

The putative bioactive conformation is compatible with all SAR studies available. First, it can be explained that substitution of Thr^5 by Ser or Cys yields active analogs [22] because these residues still have the capability to form a hydrogen bond with the backbone carbonyl oxygen. Second, residues Asn^6 and Tyr^7 that had been shown to be important for peptide activity [2, 5, 9] exhibit their side chains directed towards the same face of the peptide, arranged to make appropriate interactions with the receptor. Third, the residues that were recognized as important, Thr^4 and Thr^8, are also exposed. Fourth, it explains the lack of activity exhibited by the [Abu^5] (4–8)Peptide T.

Table 1. Backbone dihedral angles (in degrees) of the putative bioactive conformations of Peptide T, (4–8) Peptide T and cyclo(Thr-Thr-Asn-Tyr-Thr-Asp). Residues are numbered with respect to the Peptide T sequence

	Peptide T	(4–8) Peptide T	cyclo-Asp^9(4–8)Peptide T
ψ_1		158	
ω_1		178	
ϕ_2		–62	
ψ_2		–29	
ω_2		178	
ϕ_3		–72	
ψ_3		–48	
ω_3		–178	
ϕ_4	–71		89
ψ_4	–71	162	–59
ω_4	180	178	–179
ϕ_5	–96	–75	–68
ψ_5	167	173	165
ω_5	178	177	177
ϕ_6	–60	–59	–56
ψ_6	–46	–44	–34
ω_6	178	176	179
ϕ_7	–80	–82	–69
ψ_7	163	162	–66
ω_7	178	180	180
ϕ_8	–79	–73	–168
ψ_8			151
ω_8			177
ϕ_9			57
ψ_9			77
ω_9			179

Further evidence supporting the choice of the putative bioactive conformation

The putative bioactive conformation was searched in the set of low energy conformations of the potent compound cyclo(Thr-Thr-Asn-Tyr-Thr-Asp). Comparisons were carried out superimposing residues Thr^4-$Thr^7$and Thr^4-Thr^8, respectively. Thus, rmsd values of the putative bioactive conformation of Peptide T with all the conformations of the subset of low energy conformations of the cyclic peptide were computed. The dihedral angles of the most similar structure to the putative bioactive conformation found in the set of low energy structures of the cyclic analog are listed in Table 1. The structure exhibits an rmsd between residues 4–7 of 0.17 Å and 0.82 Å when residues 4–8 are compared. Figure 2 shows the superimposition of the bioactive form of Peptide T, (4–8)Peptide T and that of the cyclic analog. This larger rmsd value observed when Thr^8 is included in the comparisons can be easily explained by inspection of Figure 2. Cyclization constraints do not allow the peptide to attain a distance between Thr^4 and Thr^8 of about 7.2 Å as in the case of the linear analogs. This explains the large increase observed in the rmsd value when residue Thr^8 is included in its computa-

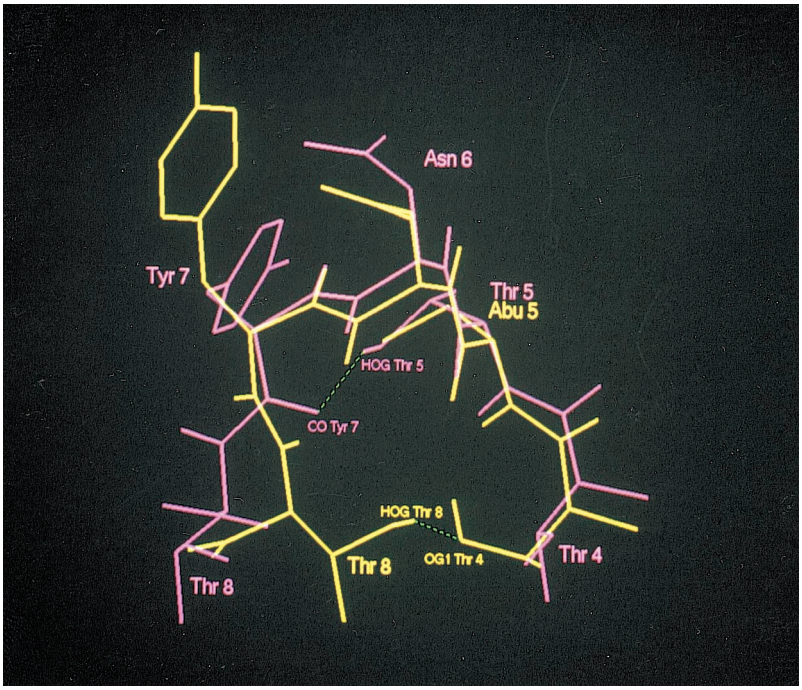

Figure 1. Superimposition of the putative bioactive conformation of Peptide T (in magenta) and the conformation of [Abu^5] (4–8) peptide T (in yellow) that exhibits the smaller rmsd with the previous structure.

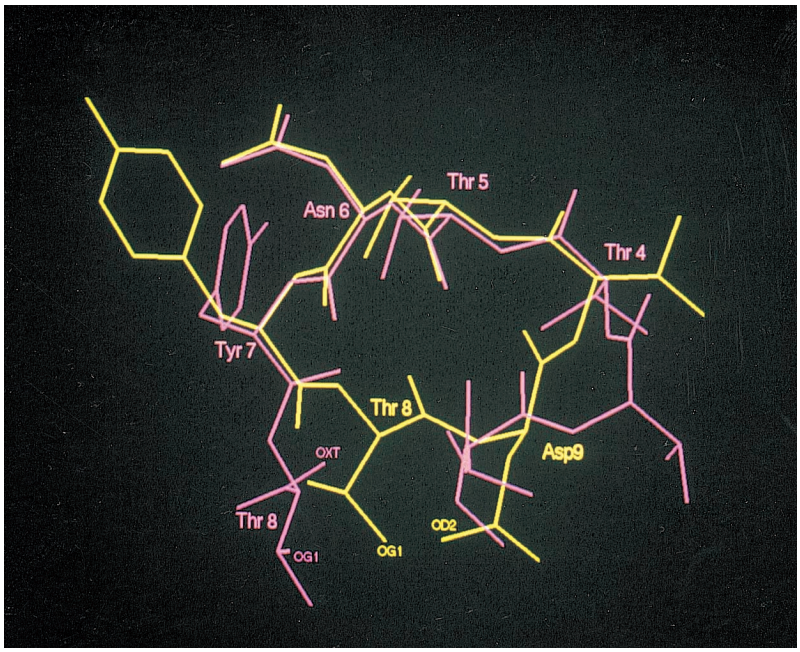

Figure 2. Superimposition of the putative bioactive conformation of Peptide T (in magenta) and the conformation of cyclo(Thr-Thr-Asn-Tyr-Thr-Asp) (in yellow) that exhibits the smaller rmsd with the previous structure.

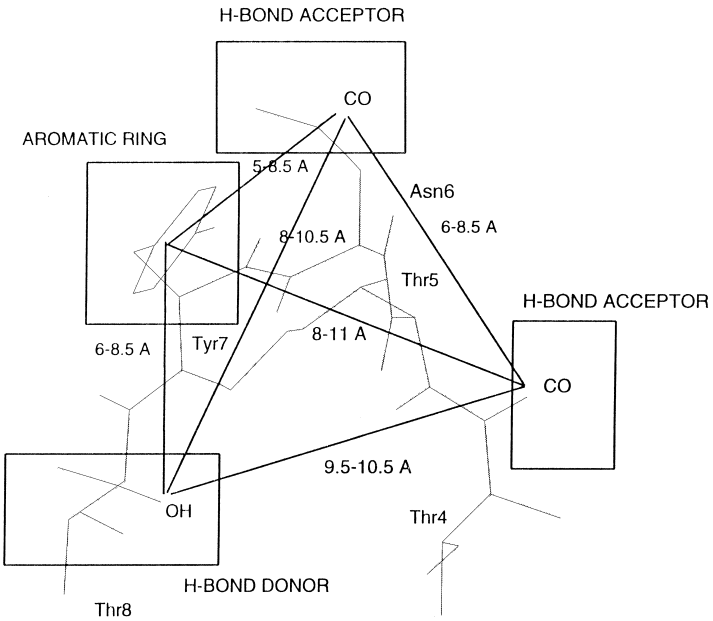

Figure 3. Proposed pharmacophore for the recognition of Peptide T to the CD4 receptor. Tolerances are computed from the maximum and minimum distances between points of the corresponding solid angles generated by rotating the Cα-Cβ bonds (see text).

tion. However, the spacial arrangement of Thr^8 in the cyclic analog has access to a similar region of space as the Thr^8 of the linear peptide. This is a qualitatively different situation to the one observed in the comparison between the Abu^5 analog and the bioactive form (Figure 1). In that case the carboxylate group and not the threonyl side chain is the moiety directly superimposed with the Thr^8 side chain.

Assessment of the bioactive conformation of the peptide together with SAR studies permit the proposal of a pharmacophore for the interaction of Peptide T with the CD4 receptor. Important chemical groups involved in this interaction are: (i) the carbonyl of Thr^4; (ii) the amide located on the side chain of Ans^6; (iii) the aromatic ring of the side chain of Tyr^7 and (iv) the secondary alcohol on the side chain of Thr^8. Figure 3 shows schematically the descriptors of this pharmacophore as well as their respective geometrical arrangement. Distances and angles cannot be very strictly assessed due to the flexible nature of the template and consequently tolerances must be large. As a proof of principle of the validity of the present hypothesis, the pharmacophore was used to search on 3D data bases. One of the hits characterized was the disaccharide derivative amigdalin. This natural product was tested for binding to the CD4 receptor, exhibiting moderate affinity [24].

It is expected that these results are helpful for the design of a second generation of Peptide T peptidomimetics. Further work in this direction is presently being undertaken in our laboratory.

Conclusions

A careful conformational analysis of Peptide T, (4–8)Peptide T, (5–8)Peptide T and [Abu^5](4–8)Peptide T was carried out using the AMBER 4.0 force field along with a simulated annealing protocol. Pairwise comparison between the unique conformations of the four analogs suggested that the low affinity observed by the fragment (5–8)Peptide T is due to the removal of residue Thr^4 and not to conformational factors. Furthermore, the comparative analysis showed one conformation found in Peptide T and (4–8)Peptide T not present in the Abu^5 analog. The most representative characteristic of this conformation is the presence of a hydrogen bond between the hydroxyl group of residue Thr^5 side chain and the carbonyl oxygen of Thr^7. This conformation was also found in the conformational profile of the potent cyclo(Thr-Thr-Asn-Tyr-Thr-Asp) analog. We hope that this work provides new insights into the SAR results presently available and can be helpful for the design of a second generation of Peptide T peptidomimetics.

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