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Computational Study of the Conformational Domains of Peptide T

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Abstract: The conformational preferences of peptide T (ASTTTNYT) were analysed by means of computational methods. A thorough exploration of the conformational space was carried out within the framework of the molecular mechanics approach, using simulated annealing as a searching strategy. Specifically, in order to obtain a subset of low-energy conformations with energies close to the global minimum as complete as possible, a simulated annealing protocol was repeated several times in a recursive fashion. The results of the search indicate that the peptide exhibits a α-helical character although most of the conformations characterized, including the global minimum, can be described as bent conformations. Conformations exhibiting β-turn motives previously proposed from NMR studies were also characterized, although they are not very predominant in the set of low-energy conformations. © 1997 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: Peptide T; AMBER force field; conformation; bioactive peptide; molecular mechanics

INTRODUCTION

Peptide T is a synthetic octapeptide of sequence Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr corresponding to a fragment of the envelope glycoprotein gp120 of the human immunodeficiency virus (HIV). The peptide potently inhibits the binding of gp120 to the CD4 receptors expressed on T4 helper/inducer lymphocytes [1]. The peptide has also proven quite potent in triggering human monocyte chemotaxis through the CD4/T4 antigen [2]. On the other hand, pharmacological studies on peptide T and its analogues showed 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].

In vitro experiments show a rapid enzymatic degradation of the peptide by several enzymes [5]. Aimed at enhancing the peptide stability and at the same time characterizing the residues involved in the recognition to the CD4 receptors, several peptide surrogates, including cyclic and linear analogues, have been synthesized in the past. The analogue [DAla[1]]peptide T NH_2 was synthesized early on and proven to be as effective as the native peptide in inhibiting the binding of the gp120 glycoprotein [1]. Furthermore, it was also soon established [2,5] that the five C-terminal residues of the peptide retain high potency. After synthesis and test of several analogues, the importance of residues Thr^5 and Thr^8 was inferred for chemotactic activity. Furthermore, synthesis of cyclic analogues also revealed the involvement of the terminal carboxyl function in biological activity [6, 7]. On the other hand, peptide analogues designed by systematic reduction of the peptide bond revealed the importance of the peptide bond between residues 5 and 6 [8, 9].

These structure–activity relationship studies provide very useful insights into the design of a first generation of peptidomimetics. However, such studies need to be complemented with an adequate knowledge of the conformational preferences of the peptide. Indeed, the fact that cyclic analogues

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exhibit chemotactic activity clearly supports the idea that the ligand interacts with the receptor through a bent conformation. These ideas were based on NMR studies of peptide T [10] in DMSO that suggested that the four C-terminal residues of the peptide might be involved in a type I β-turn. Furthermore, NMR studies of the (4–8) peptide T analogue [11, 12], a fragment that exhibits a similar chemotactic activity, suggest that two separate β-bends between Thr^4 and Tyr^7 and between Thr^5 and Thr^8 may also be possible. However, these results in conjunction with restrained molecular dynamics calculations were reinvestigated to provide an alternative interpretation of the same NMR data [13]. The authors proposed six alternative conformations for peptide T and suggested that the carbonyl oxygen of Thr^4 might be involved in a bifurcated hydrogen bond with amide protons of Tyr^7 and Thr^8.

Despite the work carried out in the past to characterize the conformational profile of peptide T using both theoretical and experimental approaches, little consensus has been reached to date. The aim of the present research is to outline the conformational profile of peptide T as well as assessing the feasibility of finding different structural motives in solution by means of theoretical methods.

METHODS

All the calculations were carried out within the molecular mechanics framework using the all-atom AMBER 4.0 force field [14]. The peptide was studied in its 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.

The strategy used to sample the conformational space was simulated annealing used in an iterative fashion. 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 that were not unique, they were first rank ordered and analysed 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 from 60° with respect to the previous conformations already on the list.

RESULTS

The sampling procedure was completed after 4800 cycles of iterative simulating annealing process. Of these conformations only 1744 were considered unique. The global minimum found was obtained at iteration number 4756, but it is a local rearrangement of the side chains of conformation number 1051. The results, as expected from a simulated annealing procedure, suggest that the method quickly reaches low-energy conformations and in

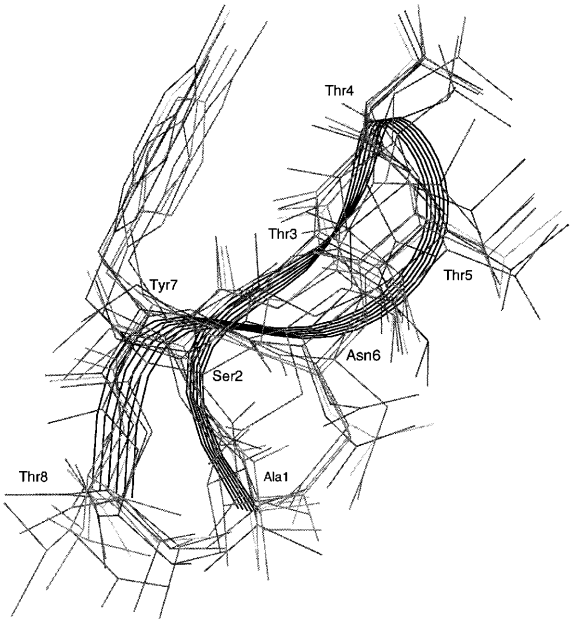

Figure 1 Superimposition of the unique conformations that belong to the same cluster as the global minimum.

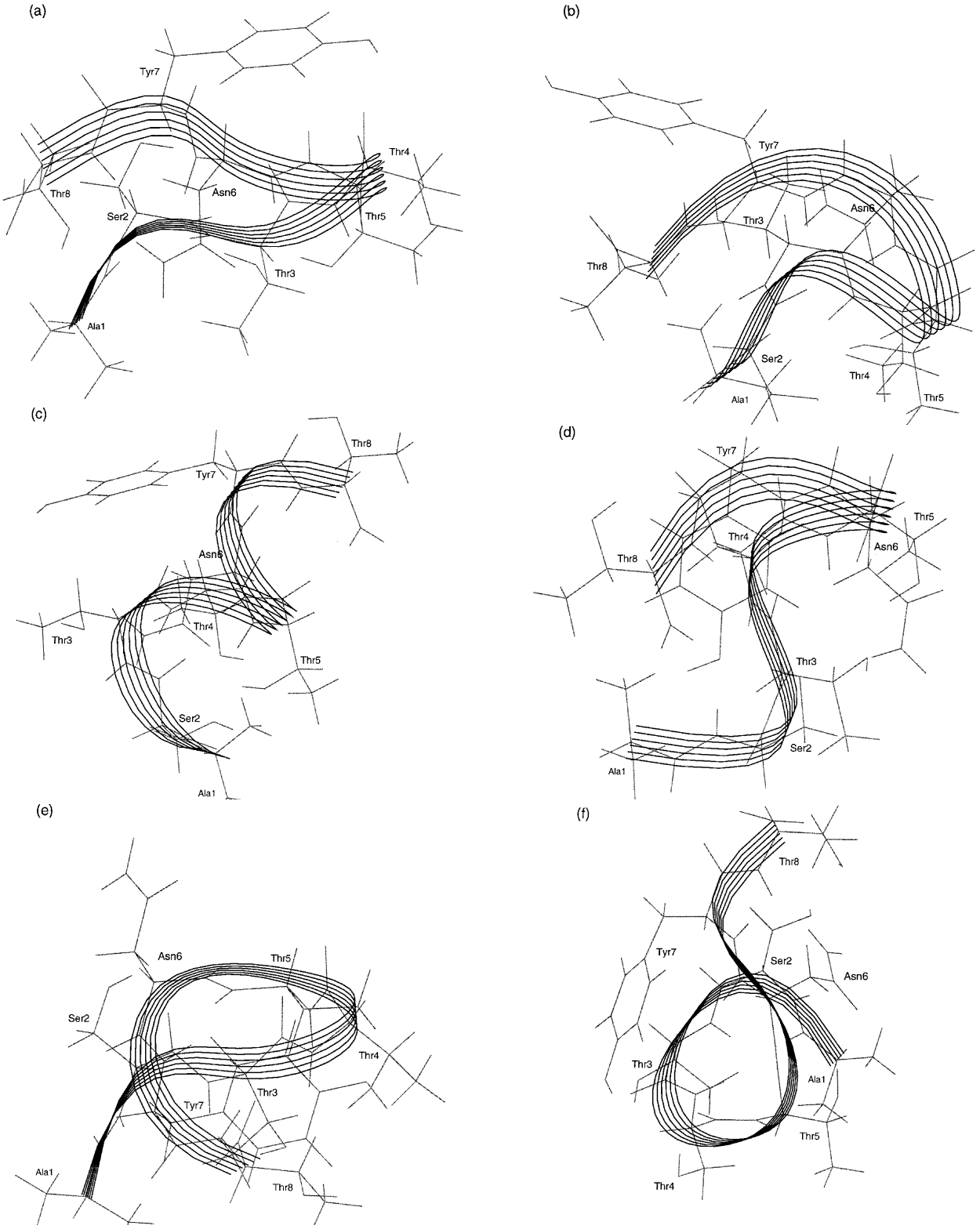

Figure 2 (a) Type I conformation (conformation no. 1); (b) Type II conformation (conformation no. 2) (c) Type III conformation (conformation no. 3); (d) type IV conformation (conformation no. 4); (e) Type V conformation (conformation no. 5); (f) Type VI conformation (conformation no. 22);

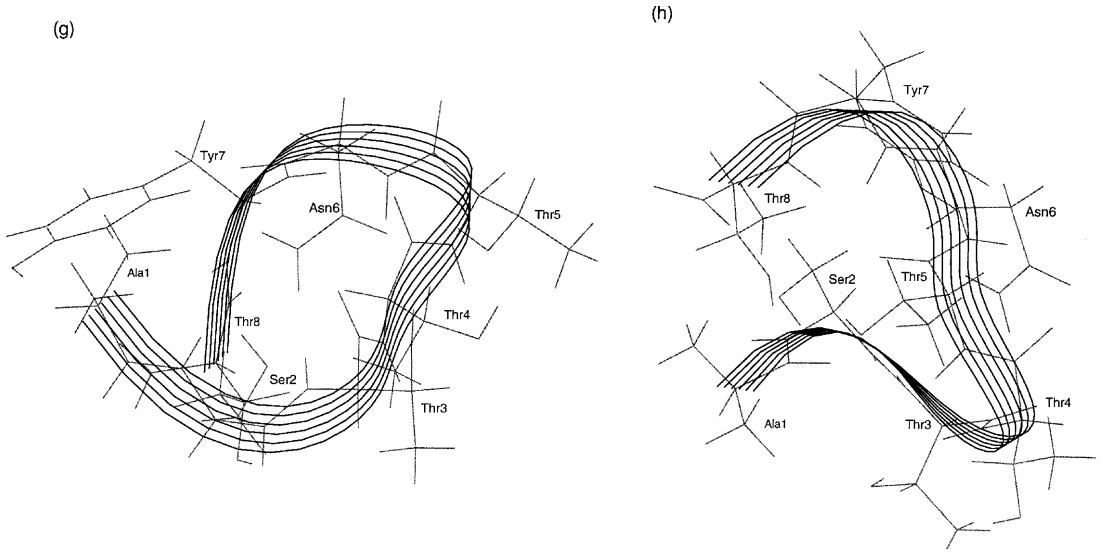

Figure 2 *(continued)* (g) Type VII conformation (conformation no. 28); (h) Type VIII conformation (conformation no. 38).

its recursive search the procedure is robust enough to obtain low-energy structures that may be quasi-degenerate with the global minimum, but situated in different valleys of the peptide landscape.

The analysis of the conformations was carried out on the subset of unique conformations within a 5 kcal/mol threshold with respect to the global minimum. The 130 unique conformations of this resulting subset were clustered into families according to the values of the root mean square difference of the distance between the atoms of the C^{α} trace after an optimal superimposition of the conformations. In addition, the root mean square difference (r.m.s.) between distance matrices of the atoms of the C^{α} trace was also computed. When the threshold imposed to consider two structures as similar is set at 1.1 Å, a total of 74 clusters were obtained. This r.m.s. criterion groups in the same cluster conformations differing slightly in the backbone coordinates although showing an overall identical shape. However, larger differences among the conformations are expected on the side chains. Nevertheless, the similarity of the backbones guarantees a similar space accessibility for the side chains of the different conformations belonging to the same cluster. As an illustration of the similarity of these conformations, Figure 1 shows pictorially the superimposition of the set of unique conformations that belong to the same cluster of the global minimum.

The 74 clusters were visually inspected using molecular graphics in order to identify distinctive secondary structure motives among them. The conformations found to exhibit distinctive motives are referred below by a type number. These structures are depicted pictorially in Figure 2 and their backbone dihedral angles are listed in Table 1. The analysis of the low-energy conformations reveals the high tendency of the peptide to adopt bent structures, some of which exhibit a helical character. Some of the structures characterized exhibit β-turns, although they are not as common as the former.

The lowest energy conformation features a characteristic hairpin turn common to all the structures belonging to the type I class. About 9% of the 129 unique structures with energies within a 5 kcal/mol threshold from the global minimum exhibit this conformation. The conformation is shown pictorially in Figure 2(a). The structure is stabilized by a strong interaction between both peptide termini and by different interactions involving mainly the threonine side chains. In addition, the structure exhibits two γ-turn centred on Ser^2 and Tyr^7 respectively.

Conformation no. 2 is at only 0.9 kcal/mol of the global minimum and represents the type II class of structures. Although this conformation is also a bend, it exhibits a different hydrogen bond scheme from the conformations of the previous group of structures. It might be considered as a bend with a certain helical character at the N-terminus. In these structures the interaction between both peptide termini is preserved but there is a hydrogen bond

Table 1 Dihedral Angles (in degrees) of the Lowest Energy Conformations Representing the Different Conformational Types Shown in Figure 2

Conformation type	I	II	III	IV	V	VI	VII	VIII
ψ_1	152	−73	−65	−49	−59	113	−61	69
ω_1	−179	179	178	−178	178	180	−179	−179
ϕ_2	−83	−159	−72	−71	−158	−81	−60	−89
ψ_2	70	152	160	−92	51	56	−71	72
ω_2	180	178	180	−179	−178	−176	180	180
ϕ_3	−87	−59	−50	−142	−77	−166	−113	−141
ψ_3	169	−40	−50	153	−73	−19	148	−63
ω_3	177	179	180	179	180	−178	−179	175
ϕ_4	−64	−56	−57	−59	−79	−140	−50	−93
ψ_4	−42	−47	−51	−34	−68	−70	−35	−59
ω_4	179	178	−179	178	179	−178	178	176
ϕ_5	−67	−71	−66	−58	−98	−63	−51	−86
ψ_5	−43	−53	−36	−42	−167	−42	−41	80
ω_5	−179	180	178	176	176	179	178	−177
ϕ_6	−143	−162	−62	−71	−75	−142	−122	−76
ψ_6	111	−58	−43	−58	−43	−176	37	69
ω_6	−179	−177	178	179	−178	−178	−175	180
ϕ_7	−83	−162	−67	−166	−81	−147	66	−171
ψ_7	66	144	−54	−55	−47	63	125	−37
ω_7	−179	178	180	−179	178	180	−179	177
ϕ_8	−111	−76	−148	−152	−92	−104	−76	−69

between the carbonyl of Ser^2 and the amide hydrogen of Asn^6, generating a C13 cycle not observed in the previous type class. The structure is shown in Figure 2(b)) and can be considered as a distortion of the type I conformation. This structure and other small variations of it represent about 31% of the unique structures.

The lowest energy conformation of the type III class is conformation no. 3, shown in Figure 2(c), at 1.1 kcal/mol with regard to the global minimum. As can be seen from the figure, the conformation is an α-helical structure, with the N-terminus distorted due to a more favorable interaction with the side chain of Asn^6. These structures represent 5% of the unique conformations.

Conformation no. 4 at 1.3 kcal/mol above the global minimum is the lowest energy structure of the type IV class (Figure 2(d)). The structure exhibits a strong interaction between both termini and a bifurcated hydrogen bond between the carbonyl oxygen of Thr^3 and the amide hydrogens of Asn^6 and Tyr^7. The structure might be seen as a bend with some helical character at the C-terminus, representing about 15% of the structures characterized within 5 kcal/mol above the global minimum.

Conformation no. 5 at 1.6 kcal/mol above the global minimum is the lowest energy structure of the type V class (Figure 2(e)). Although the structure does not exhibit any of the standard secondary structure motives, it can be described as a bend conformation. This type of structures is also stabilized by a strong interaction between both termini and represents about 31% of the unique conformations.

Conformation no. 22 at 2.7 kcal/mol above the global minimum is the lowest energy structure of type VI class (Figure 2(f)). The principal feature exhibited by this structure is a hydrogen bond between the carbonyl oxygen of Ala^1 and the amide hydrogen of both Thr^3 and Thr^4 forming a β-turn between residues Ala^1 and Thr^4 and a γ-turn between residues Ala^1 and Thr^3. This type of structure represents 2% of the structures.

Conformation no. 28 at 3.0 kcal/mol above the global minimum (Figure 2(g) features all the characteristics of type VII structures. The conformation exhibits two consecutive β-turns (a turn of a 3_{10} helix). The first β-turn is between residues Thr^3 and Asn^6 and the second between residues Thr^4 and Tyr^7. In addition, there is a strong interaction

between termini providing a close structure. This structure represents only 1% of the structures.

Finally, conformation no. 38 at only 3.4 kcal/mol of the global minimum represents type VIII of structures. The structure is shown in Figure 2(h). It exhibits a bifurcated hydrogen bond between the carbonyl oxygen of Thr^5 and the amide hydrogens of Tyr^7 and Thr^8, providing a β-turn structure to the C-terminal section of the peptide. In addition, two γ-turns centred on Ser^2 and Thr^5 can be also observed. This type of structure represent 5% of the total.

DISCUSSION

The results reported in this work provide a detailed analysis of the conformational preferences of the peptide. In the conditions used to carry the present computational studies, the most widely observed conformation of the peptide is a bent structure. Indeed, several of the conformational types described above, representing 88% of the total of unique conformations characterized, present this feature. Furthermore, the peptide also exhibits a tendency to adopt α-helical structures, or at least presents the tendency to form hydrogen bonds of this type. β-Turn structures are also observed, more predominantly at the C-terminus of the peptide as in types VII and VIII; however they represent a small percentage of the total number of unique conformations within 5 kcal/mol of the global minimum.

It might be argued that present values are biased due to an artefact of the calculations. However, numerous studies support the methodology used in the present work [15–18] as reliable enough to describe the conformational domains of a peptide in solution. There is the need to use a high dielectric constant to avoid the electrostatic collapse between both peptide termini. On the other hand, the use of this high dielectric constant makes the dispersion terms more important and the structures will be more compact that would be expected.

However, this dispersion collapse will affect the conformation of the side chains, but does not dictate the general shape of the peptide. In the same way it could be argued the electrostatic effects due to the presence of a polar solvent will affect the conformations adopted by the side chains. However, the main features of the backbone will not be altered since they are in the interior of the structure.

Early NMR studies of the peptide in DMSO suggested the existence of a hydrogen bond between the carbonyl oxygen of Thr^5 and the amide hydrogen of Thr^8, proposing the existence of a β-turn between these residues in solution [10]. In a latter study, the same authors studied the solution conformations of peptide T and (4–8)peptide T in DMSO and corroborated the previous findings [11]. In a more recent NMR study, a different group [12] suggested the presence of an additional hydrogen bond between the carbonyl oxygen of Thr^4 and the amide hydrogen of Tyr^7, proposing a 3_{10} helical structure for (4–8)peptide T as the solution conformation of the peptide. However, the conclusions of this study should be taken very cautiously since the authors studied a blocked peptide with all the side chains protected. Conformations of types VII and VIII in this study support the feasibility of a β-turn involving the Thr^4 or Thr^5 carbonyl oxygen and the Tyr^7 and Thr^8 amide hydrogens respectively. However, no structure exhibits simultaneously the two β-turns proposed in [12]. In order to explore the feasibility of this conformation further and to avoid the possibility that the conformations might not have been characterized because of an incomplete sampling procedure, the structure proposed in [12] was generated using molecular graphics and used as a starting conformation for a subsequent minimization. However, the minimizer moved away from the starting point providing a structure already characterized in the subset of low energy conformations.

Based on the sequence homology of peptide T with a fragment of endothiapepsin and bovine pancreatic ribonuclease A [19–21], it was suggested that the conformation of peptide T may exhibit a bent structure. Present studies do support the feasibility of the peptide adopting this type of conformation, although not as the most favorable conformation, if we consider its low percentage in regard with other possible conformational motives. Fragment 19–26 of bovine pancreatic ribonuclease A has the sequence AASSSNYC and exhibits a β-turn between the carbonyl oxygen of Ser^{22} and the amide hydrogen of Tyr^{25}. Due to its sequence homology with peptide T a β-turn between residues Thr^4 and Tyr^7 in peptide T should be expected. This type of conformation is found in our calculations with a small root mean square deviation. Figure 3 shows the overlay between the fragment 19–26 of bovine pancreatic ribonuclease A and conformation no. 28 (type VII), where the match between the atoms involved in the β-turn is very good with an r.m.s. deviation of 0.38, if only the backbone atoms are considered in the comparison.

Exploring further the sequence homology between peptide T and Ribonuclease A, in a recent

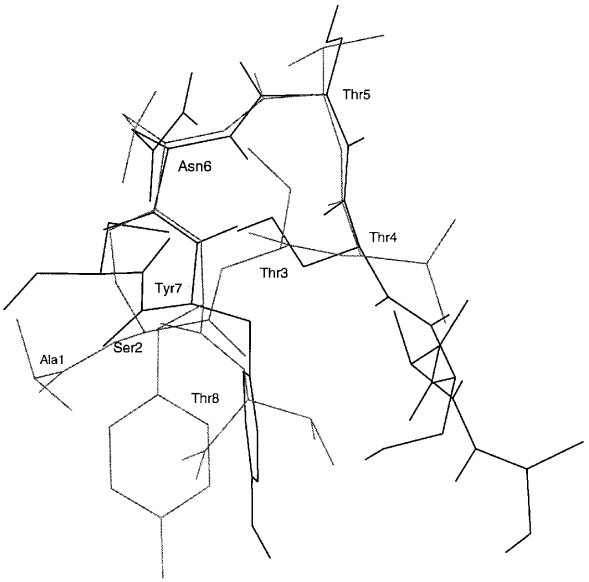

Figure 3 Superimposition of conformation no 28 (grey line) and the crystal structure of the fragment 19–26 of bovine pancreatic ribonuclease A (black line).

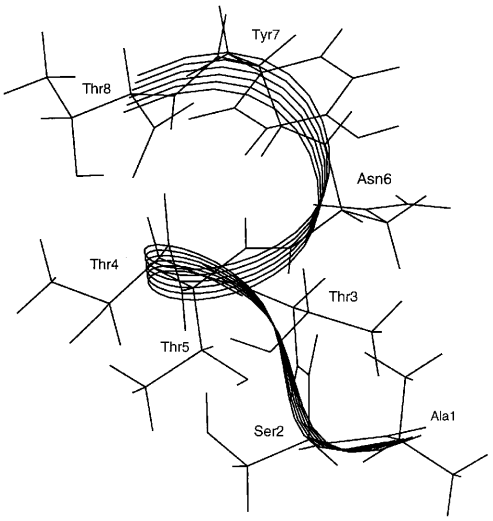

Figure 4 Ribbon presentation of conformation no. 24.

report, NMR data from the latter molecule were used along with restrained simulated annealing computations to study the preferred conformations of peptide T [13]. Since information based on sequence homology allows us only to model the five C-terminal residues of peptide T, the authors first modelled the structure of (4–8) Peptide T for a later use in modelling the preferred conformations of peptide T. The lowest energy structure found compatible with the NMR results exhibits a bifurcated hydrogen bond between the carbonyl oxygen of Thr^4 and the amide hydrogens of Tyr^7 and Thr^8. In order to model peptide T, initial structures of the peptide were constructed following a building up procedure by combining different optimized conformations of the N-terminus with the structure of the C-terminus previously described. After the structures were minimized and rank ordered, the lowest energy structure of peptide T found exhibits a helical character at the N-terminus along with a bifurcated hydrogen bond at the C-terminus. This same structure is found in our library of low-energy conformations included in the type III class set, and corresponds to conformation no. 24 at 2.8 kcal/mol above the global minimum. The structure is depicted pictorially in Figure 4. Interestingly, since the structure exhibits a bifurcated hydrogen bond between the carbonyl oxygen of Thr^4 and the amide hydrogens of Tyr^7 and Thr^8 it indeed represents the lowest energy structure with a β-turn between residues Thr^4 and Tyr^7 of the library of structures. This type of structure may reconcile previous findings indicating the tendency of the peptide to adopt β-turn type conformations and our observation that the peptide also tends to adopt helical structures.

CONCLUSIONS

Present results represent the most thorough computations carried on peptide T to date. The results support previous experimental findings of a β-turn conformation as one of the accessible structures of the peptide. However, present results do not support the hypothesis that this is the most populated conformation. The computational approximations involved in the present simulations are supported by previous studies and represent a careful balance between an adequate extent of the conformational search and meaningful results.

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