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A proposed bioactive form of peptide T and the design of peptidomimetics

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Summary

Peptide T is a non-natural octapeptide of sequence Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr, taken from the sequence of the protein gp120 of HIV. The peptide has been shown to bind competitively to the CD4 receptors of the helper/inducer lymphocytes T. The peptide is presently used for the treatment of AIDS-associated dementia and has been proven useful for the treatment of psoriasis. Using molecular modeling procedures, we studied the conformational profile of this peptide as well as those of several active and inactive analogs. The analysis of these results gave rise to the proposal of a bioactive conformation of the peptide, which can be described as a pseudo β -turn structure, involving the last four residues at the C-terminus of the peptide. The secondary structure is stabilized by a hydrogen bond between the hydroxyl hydrogen of the side chain of Thr⁵ and the carbonyl oxygen of Tyr⁷. From the bioactive form and different structure–activity relationship studies, a pharmacophore was proposed. This hypothesis was used to search on several 3D data bases. One of the hits obtained was the natural compound amigdaline, which was tested and exhibited moderate activity.

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 peptide T peptidomimetics. Characterization of the shortest fragment of the peptide exhibiting activity and the synthesis of several analogs [2,5–10] demonstrated the importance of Thr⁵, the side chains of Asn⁶, Tyr⁷ and Thr⁸ and the carbonyl oxygen of the latter for chemotactic activity. On the other hand, experimental and theoretical studies pointed out the tendency of the peptide to adopt a β -turn at its C-terminus [11–15], although with discrepancies about the residues involved. 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].

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In order to put a step forward in the design of second-generation peptidomimetics, the geometrical characteristics of the bioactive conformation of peptide T were assessed. For this purpose, the conformational profiles of two active analogs (peptide T itself and its fragment (4–8)peptide T) and two non-binders ([Abu²](4–8)peptide T and (5–8)peptide T) were studied. The 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 not attainable by the non-binders. Following this procedure, only one conformation was finally kept. This structure has been used for new lead discovery.

Methods

Computations

Peptides were studied in the 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. Calculations were carried out within the molecular mechanics framework using the all-atom AMBER 4.0 force field [16]. Parameters for the Abu residue were assessed following the same protocols as used to generate the parm91 parameter data base in AMBER 4.0 [17].

The strategy used to sample the conformational space was simulated annealing used in an iterative fashion. Details of the method are explained elsewhere [18]. The conformational analysis of each peptide was carried out on the subset of unique conformations within a 5 kcal/mol threshold in respect to its global minimum.

Computation of the root mean square (rms) deviation of the distance between the backbone atoms between every pair of structures after they had been optimally superimposed was performed to carry out pairwise cross comparisons between conformations of the low-energy subsets of the different analogs in order to distinguish common conformations among them. Two conformations were automatically considered similar if the rms difference 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.

Data base searching was carried out with the program Catalyst [19] with four different data bases: NCI, Derwent, Maybridge and BioByte. Ligands were allowed for a flexible fitting following the pole function method built in the program [20].

Monocyte chemotaxis

Mononuclear cells were isolated from heparinized blood of normal human volunteers by sedimentation over Ficoll–Paque. Chemotaxis was performed in a modified Boyden chamber, using the leading-front methods utilizing an 8 mm Millipore filter which separated upper and lower compartments. Mononuclear cells (0.5×10^6) in a Krebs–Ringer phosphate buffer (KRP) were placed in upper wells. Amigdaline was dissolved in DMSO at 10^{-2} mol/l, diluted before use with KRP containing 1 mg/ml of bovine serum albumin and tested in the lower compartment at a final concentration of 10^{-12} – 10^{-7} mol/l. To obtain accurate comparison, the results for amigdaline have been expressed in terms of the chemotactic index, which is the following ratio: migration towards test attractant/migration towards the buffer. Migration in the presence of buffer alone was $35 \text{ mm} \pm 2\text{SE}$. Peak response migration for CHO-Met-Leu-Phe-OH (FMLP) occurred at 10^{-8} mol/l and was $68 \text{ mm} \pm 3\text{SE}$ in these experiments (chemotactic index 1.94 ± 0.03). In order to confirm that amigdaline binds to the CD4 receptor, its chemotactic effects were blocked by low doses (0.1–0.2 mg/ml) of OKT4, a specific monoclonal antibody for the CD4 molecules. In this assay, peak response migration for FMLP was not affected by OKT4.

Results and discussion

In order to characterize the bioactive conformation of peptide T, the subsets of low-energy conformations of peptide T and (4–8)peptide T were 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 at the receptor. This conformation must consequently be common to all the low-energy subsets of the different active analogs studied. Pairwise comparison of the low-energy conformations of the two peptides allowed discarding 33 conformations of pep-

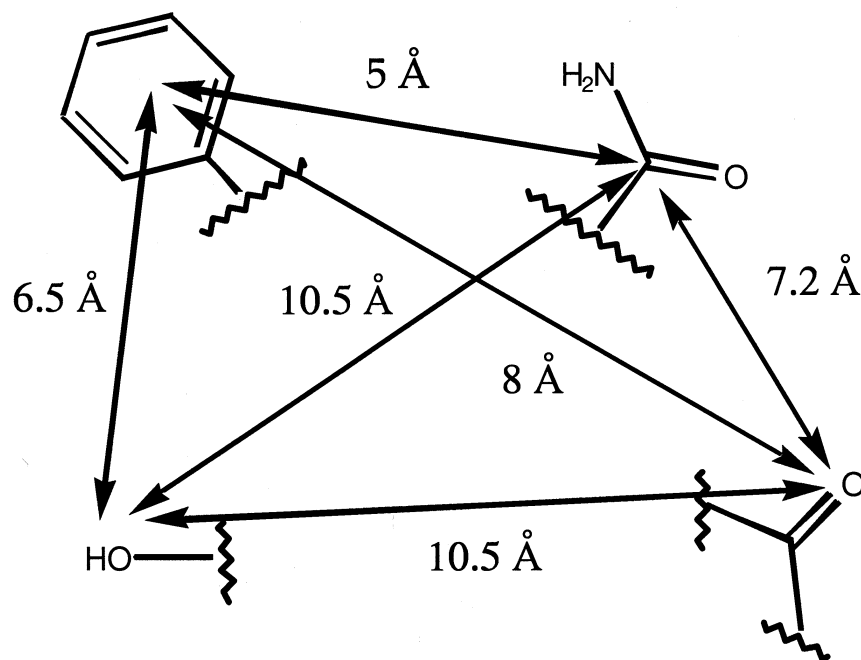


Figure 1. Pharmacophore of peptide T interacting with the CD4 receptor.

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 pairwise compared for similarity with the low-energy conformations of the non-binders. Comparison of the low-energy conformations of the analog (5–8)peptide T showed that every conformation of the analog had a corresponding conformation 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 the ligand–receptor interaction and located in residue Thr⁴. Structure–activity relationship studies suggest that this moiety can be the carbonyl oxygen of the peptide backbone, since N-methylation of the amide group [5] and replacement of the side chain by Abu yield active analogs. On the other hand, comparison of the low-energy conformations of [Abu⁵](4–8)peptide T with the set of low-energy conformations common to the active peptides revealed 15 conformations in the former set with rms differences ranging from 0.7 to 1.0 Å that had to be visually inspected for similarity. Fourteen out of the 15 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 rms

deviation 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 of the active peptides is stabilized by a hydrogen bond between the carbonyl oxygen of the Tyr⁷ and the hydroxyl group of the Thr⁵ side chain. This conformation is not attainable by the non-active Abu analog due to the lack of the hydroxyl group on its side chain. Accordingly, this conformation was considered as the putative bioactive conformation. Further details on the peptide T hypothesis generation will be given elsewhere [21].

Assessment of the bioactive conformation of the peptide together with structure–activity relationship studies permit the suggestion of a pharmacophore to explain the points involved in the recognition between peptide T and the CD4 receptor. This is schematically shown in Figure 1. This hypothesis was subsequently used for 3D data base search, obtaining several hits, among which the natural product amigdaline was found. The disaccharide derivative was tested for its ability to produce chemotaxis on human monocytes and was found to exhibit a maximum chemotactic index of 1.35 at 0.1 nM, compared to a maximum of 1.60 exhibited by (4–8)peptide T at the same concentration. However, an inspection of its superposition with the bioactive form of peptide T suggested simple modifi-

cations to improve its activity. Further work is being undertaken at the present time in this direction in our laboratory.

Conclusions

Comparative analysis of the conformational profiles of peptide T, (4–8) peptide T, (5–8) peptide T and [Abu⁵] (4–8) peptide T was carried out by computational methods. Systematic comparison of the conformations permitted to select a candidate to be the bioactive form. These results together with structure-activity studies published in the literature led us to propose a pharmacophore for the peptide T-CD4 interaction. Data base searches permitted us to identify the natural subsequent product amigdaline as a peptidomimetic of peptide T.

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