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AMYGDALIN BINDS TO THE CD4 RECEPTOR AS SUGGESTED FROM MOLECULAR MODELING STUDIES

Oriol Llorens,[a] Marta Filizola,[a] Susanna Spisani,[b] Mauro Marastoni,[c] Concepcion Herranz,[a] and Juan J. Perez[a,*]

[a]*Dept. d'Enginyeria Quimica, Universitat Politecnica de Catalunya, ETSEIB, Av. Diagonal, 647, 08028 Barcelona, Spain*
[b]*Dept. of Biochemistry and Molecular Biology, Università di Ferrara, 44100 Ferrara, Italy*
[c]*Dept. of Pharmacuetical Sciences, Univerisità di Ferrara, 44100 Ferrara, Italy*

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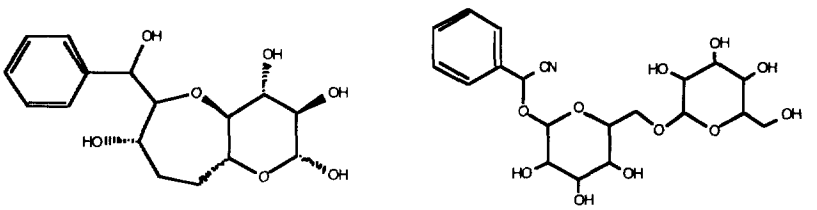
Abstract: The geometrical features of the proposed bioactive conformation of peptide T assessed by computational methods in a previous study, together with available structure–activity studies on peptide T, led us to propose a pharmacophore for the CD4-peptide T interaction. Subsequent, data base searching permitted us to identify amygdalin as a peptide T peptidomimetic.

Introduction

In a recent paper[1] several prospective candidates to bind to the gp120 binding site of the CD4 receptor were described. Ligands were designed by molecular modeling using a de novo approach that requires knowledge of the three-dimensional structure of the receptor. In this procedure, candidates are constructed from molecular fragments, selected to exhibit good binding energy with the receptor. These fragments are subsequently connected to yield actual molecules whose binding energies to the receptor are estimated. Finally, ligands are screened according to various criteria to be proposed for their synthesis. Following this procedure, the best candidate reported in reference 1 to bind the CD4 receptor is the bicyclic molecule **1** (shown in Figure 1). Unfortunately, the authors did not provide ligand affinity data and subsequently, their hypothesis cannot yet be confirmed. However, these results can be indirectly corroborated from knowledge of the binding profile of a similar compound. In the present work we report the moderate affinity exhibited by the natural product amygdalin, **2** (see Figure 1) at the CD4 receptor. Interestingly, this molecule was suggested to bind to CD4 receptor from molecular modeling studies following a completely different approach, as explained below.



1 **2**

Figure 1. (**1**) Best candidate to bind to the CD4 receptor reported in reference 1; (**2**) amygdalin.

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Present results are part of a project aimed at designing non-peptide peptidomimetics of peptide T (Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr) presently being undertaken in our laboratory. This synthetic octapeptide 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[2] and has also proven quite potent in triggering human monocyte chemotaxis through the CD4/T4 antigen[3] Our modeling strategy to design peptidomimetics of peptide T represents an indirect approach that involves the proposal of a pharmacophore for the peptide-receptor interaction. This procedure involves assessment of the bioactive conformation of the peptide by a comparative conformational analysis on carefully selected peptide analogs, together with a careful analysis of the known structure-activity relationship results. The proposed pharmacophore can be subsequently used as query for 3-D data base searches.

Characterization of shortest active fragment of peptide T together with the synthesis of several peptide analogs[3–9] demonstrated the importance of Thr^5, of Asn^6, Tyr^7, and Thr^8 side chains as well as the carbonyl oxygen of the latter for chemotactic activity. After a careful analysis of the binding profiles of different peptide analogs, comparative conformational analysis was carried out on two binders, peptide T and its fragment (4-8)peptide T, and two nonbinders: [Abu^2](4-8)peptide T and (5-8)peptide T. 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 nonbinders, 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[10]

The bioactive conformation was analyzed together with the information available from structure-activity studies on peptide T to propose a pharmacophore for the ligand-receptor interaction. Data base searching methods were used as a source of diverse compounds that fitted the pharmacophore. One of the hits was the natural compound amygdalin that was tested showing moderate monocyte chemotaxis. These results evidence the capabilities of the strategy presented to obtain peptidomimetics.

Methods

Computations

Calculations were carried out within the molecular mechanics framework using the all-atom AMBER 4.0 force field.[11] Peptides were 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. Parameters for the Abu residue were derived following the same protocols as used to generate the parm91 parameter data base in AMBER 4.0.[10,12]

The conformational space was sampled using simulated annealing in an iterative fashion.[13] Comparative conformational analysis was carried out on the subsets of unique conformations within a 5 kcal/mol threshold in respect to the lowest energy conformation found for each of the peptide analogs.

In order to identify common conformations among the different peptide analogs, the root mean square deviation (rmsd) between every pair of structures was computed using all the backbone atomic coordinates after optimal superimposition. Two conformations were automatically considered similar when the rmsd was lower than 0.7 Å and dissimilar when this 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 by means of the program Catalyst[14] using 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.[15]

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. Amygdalin 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 amygdalin have been expressed in terms of the chemotactic index, which is the ratio: migration towards test attractant/migration towards the buffer. Migration in the presence of buffer alone was 35 mm ± 2 SE. Peak response migration for CHO-Met-Leu-Phe-OH (FMLP) occurred at 10^{-8} mol/L and was 68 mm ± 3 SE in these experiments (Chemotactic index 1.94 ± 0.03). In order to confirm that amygdalin bound to 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

The bioactive conformation of peptide T, was assessed in a two step procedure. First, the set of conformations common to the binders was computed. 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. Consequently, both peptides must exhibit a common subset of conformations that include the bioactive conformation. Accordingly, pairwise comparisons of

all the low energy conformations of peptide T and (4-8)peptide T were carried out. This procedure allowed to discard 33 conformations of peptide T as prospective candidates to be the bioactive conformation.

In the subsequent step, the structures belonging set of conformations common to the two active peptides were compared for similarity in a pairwise fashion with all the low energy conformations of the nonbinders. Comparison with the low energy conformations of (5-8)peptide T, showed that every conformation of this analog had a corresponding conformation in the subset of common conformations of the analogs exhibiting chemotactic activity. 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 Thr^4. Structure-activity relationship studies suggest that this moiety can be the carbonyl oxygen of the peptide backbone, since N-methylation of the amide group[4] and replacement of the side chain by Abu yields active analogs. On the other hand, comparison with the low energy conformations of [Abu^5](4-8)peptide T, revealed 15 conformations in the former set with rmsds ranging from 0.7 to 1.0 Å that were inspected for similarity using computer graphics. Fourteen out of the fifteen were discarded since the structural differences found (all of them occurring at both peptide termini) were not considered significant to explain the decrease of chemotactic activity observed. The remaining conformation, although with a 0.89 Å rmsd in respect to the most similar structure in the subset 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 Tyr^7 and the hydroxyl group of the Thr^5 side chain. This conformation is not attainable by the nonactive 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.[10]

Combining the geometrical information derived from the assessment of the bioactive conformation of peptide T and the results of systematic structure-activity relationship studies, a pharmacophore compiling the chemical and geometrical requirements of the peptide T-CD4 receptor interaction can be proposed. This is schematically shown in Figure 2.

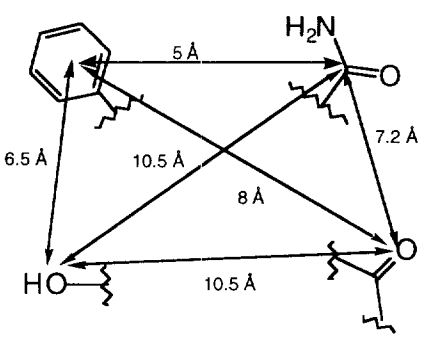

Figure 2. Proposed pharmacophore of peptide T- CD4 receptor interaction.

This hypothesis was subsequently used as query for 3-D data base search, obtaining several hits among which, the natural product amygdalin was found. The disaccharide derivative was tested for its ability to produce chemotaxis on human monocytes and 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 (Figure 3).

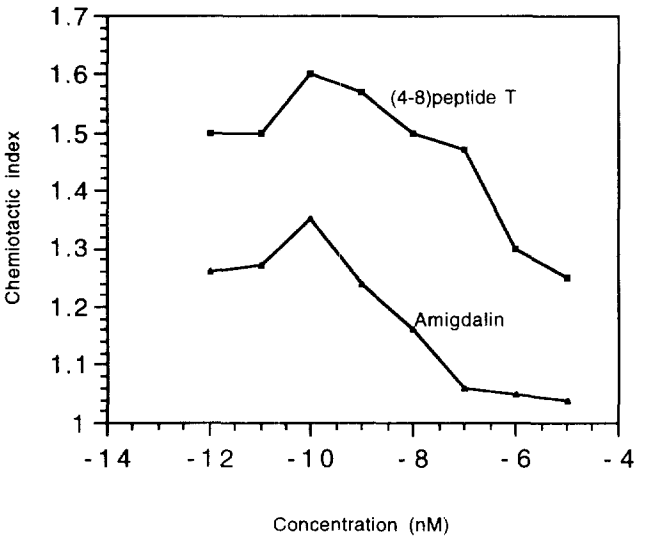

Figure 3. Chemotactic index of amygdalin and (4-8)peptide T versus compound concentration.

However, inspection of its superimposition with the bioactive form of peptide T, suggested simple modifications to improve its activity. Further work is being undertaken at the present time in this direction in our laboratory.

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