MOL2NET, International Conference Series on Multidisciplinary Sciences
http://sciforum.net/conference/mol2net-03

Protein model built through molecular modeling by homology of a potential target of anti-leishmania drugs

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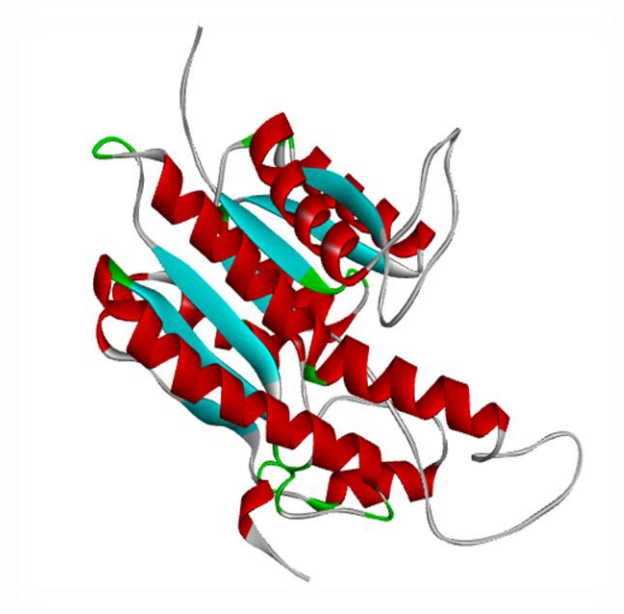
Graphical Abstract



Abstract

Molecular modeling by homology is a methodology widely used for the construction of protein structures that have not yet been crystallized. The constructed models can be used for the identification of inhibitors, representing a great method for the rational planning of drugs. Thus, the objective of the study was to construct the three-dimensional model of the protein structure of the enzyme Pteridine reductase 1 from *Leishmania donovani* (LdPTR1). PTR1 is an enzyme used in the metabolism of pterin from GTP, being considered an excellent specific target of drugs of Leishmania. The target protein and template sequences were obtained from the National Center for Biotechnology Information database and the 3D template structures through the Protein Data Bank (PDB). Sequence alignment was performed on the FASTA, yielding 91.0% identity and 97.2% similarity to the *Leishmania major* template protein (LmPtr1). The LdPtr1 model was constructed using MODELLER software 9.18. The stereochemical quality was evaluated in PROCHECK and the structural quality in VERIFY 3D and WHAT IF software. The Discovery Studio Visualizer software was used for graphical visualization of the modeled protein. Due to the high level of identity and similarity of the target enzymes and template, the results revealed that a

	good model was constructed. The Ramachandran graph showed that the conformations of the main chain of amino acids are in allowed and favored regions. Besides that; 85.07% of the residues have the protein sequence compatible with their 3D structure and do not have unusual atomic contacts.

Introduction

The development of a three-dimensional structural model of protein homologues can be used to identify inhibitors for specific targets by modeling homology and molecular dynamics. Homology modeling is currently the most accurate method, able to predict the structure of a protein based on the general observation that proteins with similar sequences possess. The computational methods for protein structure modeling play an important role in homology modeling [1].

Homology modeling requires the structure of an established protein (template) generated by a homologue containing the target sequence, provided that it shares approximately 30% or more similarity in the sequence or structure of the template [2].

Pteridine Reductase 1 (PTR1) is an enzyme used in the metabolism of pterin and necessary for the survival of the parasite [3]. Previous studies have reported as the PTR1 as an important target chemotherapeutic [3,4]. Inhibitors of Pteridine Reductase 1 (PTR1) proved to be lethal to the parasites in Leishmania *spp*. and *Trypanosoma brucei*, but not in human cells [4].

Thus, the objective of the study was to construct the three-dimensional model of the structure of the enzyme Pteridine reductase 1 from *Leishmania donovani* (LdPTR1) to contribute to the study of anti-leishmania inhibitors.

Materials and Methods

Identification of target sequences and selection of protein template

The sequence of the target protein was obtained from the National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/pubmed) and the identification of the resolved structures in 3D model candidates was done through a [5] and to obtain the structure, the RCSB Protein Data Bank (https : //www.rcsb.org/pdb/home/home.do) [6]. The template protein selected was LmPtr1 (PDB ID: 1E7W).

Alignment of template and target sequences

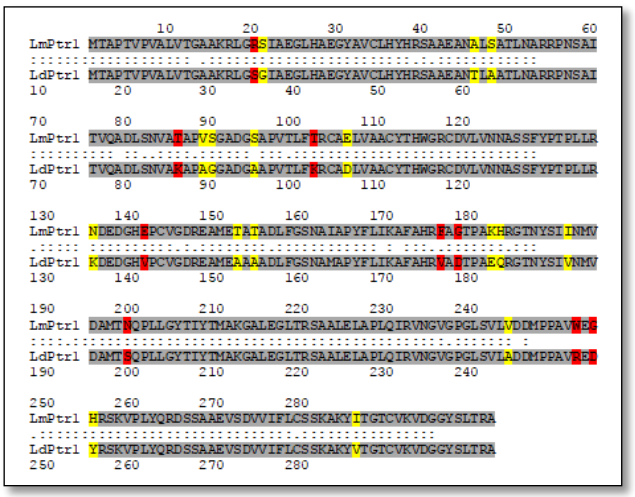
Alignment of multiple sequences was performed using FASTA (http://www.ebi.ac.uk/Tools/sss/fasta/) and obtained the following values for *Leishmania donovani* (LdPtr1): 91.0% identity and 97 , 2% similarity to *Leishmania major* Ptr1 (LmPtr1) (**Figure 1**). This was not the best score alignment in the FASTA, but it is the target organism of the study.

Construction and validation of the model

The LdPtr1 model was constructed using the homology molecular modeling method through MODELLER 9.18 software [7], which is based on the spatial constraints satisfaction modeling. Five models were generated and the lowest energy model was chosen. The stereochemical quality ofthe model was evaluated in the PROCHECK [8], which

evaluates several stereochemical parameters, such as torsional angles of the main chain, torsional angles of the side chain, bad contacts or steric impediments, flatness, among others. PROCHECK generates the Ramachandran graph [9] that verifies allowed and unallowed regions of the main amino acid chain. The structural quality was evaluated in VERIFY 3D software (http://services.mbi.ucla.edu/SAVES/) that analyzes the compatibility of the protein sequence with its 3D structure according to its chemical environments and WHAT IF (http: //swift.cmbi.ru.nl/servers/html/index.html) that analyzes various structural parameters, such as the atomic contacts between the residuals. The software Discovery Studio Visualizer [10] was used for graphic visualization of the modeled protein.

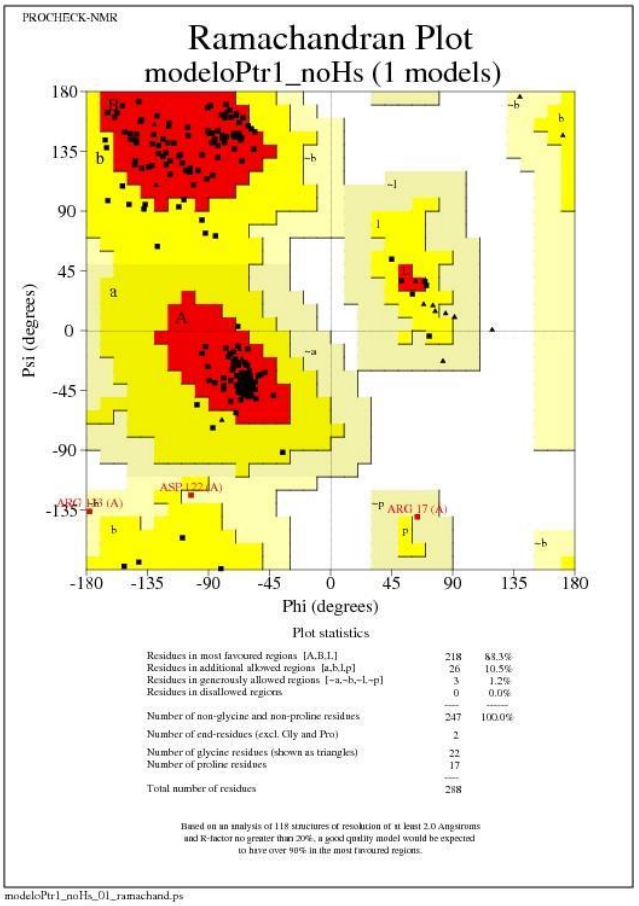
Figure 1 - Alignment between the template sequence (LmPtr1) and the target sequence (LdPtr1). The regions highlighted in gray correspond to the identical amino acids in the two sequences, in yellow are the similar residues and in red the non-identical and non-similar residues.



Results and Discussion

Molecular Modeling by Homology of the enzyme Ptr1 of *Leishmania donovani* was performed using the Ptr1 enzyme of *Leishmania major* as template. A good LdPtr1 template was obtained because of the high level of similarity between the target sequence (LdPtr1) and the template sequence (LmPtr1). To verify and validate the reliability and stereochemical quality of the modeled protein, data from the Ramachandran, VERIFY 3D and WHAT IF graph were considered. Analysis of the Ramachandran graph shows that the main chain conformations are 88.3% of the residues in the most favored regions, 11.7% allowed and 0% outlier (**Figure 2**). In this analysis, since there was no residue in the outlier region, the generated model was considered satisfactory. The G factors, which indicate the quality of covalent distance and bond angle, were 0.15 for dihedrons and 0.09 for phi / psi. Positive or slightly negative values indicate a model of good stereochemistry.

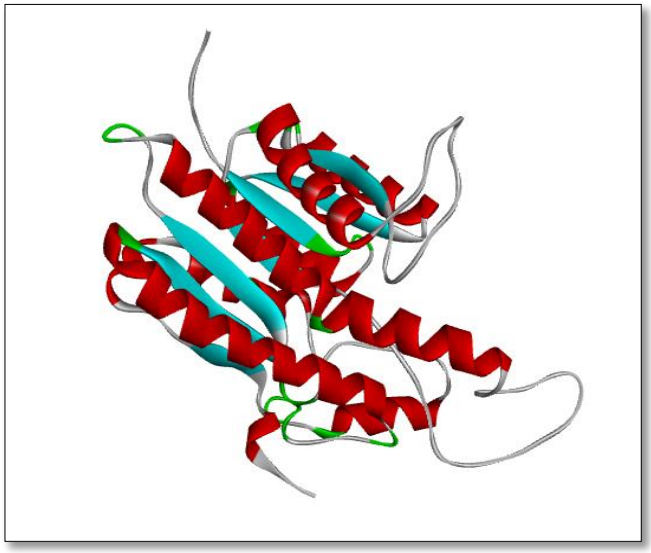
Figure 2 - Ramachandran plot of LdPtr1 using Procheck.



No VERIFY 3D; 85.07% of the residues had a mean 3D-1D score> = 0.2, which indicates

a reliable model, since it is superior to 80% of the amino acids that marked = 0.2 in the 3D / 1D profile. The quality of the atomic contacts involving the atoms of each residue was analyzed using the Fine Packing Quality Control module of the WHAT IF, which compares the distribution of atom positions around each residue. The mean score of all wastes is -0.687. A score of less than -5.0 for a residue means bad or unusual atomic contacts. The graphical visualization of the modeled protein is observed in figure 3.

Figure 3 - Graphical view of LdPtr1 in Discovery Studio Visualizer.



Conclusions

Molecular homology modeling is an excellent computational tool that performs the prediction of protein structures not yet crystallized, contributing to the rational planning of anti-leishmania inhibitors. The LdPTR1 model was considered satisfactory as observed in the validation results.

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