In silico Studies on Modeling of Wild Type and Mutants of Pyrazinamidase and Docking with Pyrazinamide from Mycobacterium tuberculosis

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Abstract

Pyrazinamidase (PZase) plays a key role in activating the prodrug Pyrazinamide (PZA) - an important drug in the anti tuberculosis therapy. Mutation in *pncA* gene coding for PZase is a major mechanism of PZA resistance in *Mycobacterium tuberculosis*. PZase was modeled using software Discovery Studio (DS) 1.7 based on the crystal structure of the PZase from *Pyrococcus horikoshii* (PDB code 1im5), in this study. The model was assessed by PROCHECK and VERIFY-3D graph. It comprises of one sheet with 6 parallel strands and 7 helices. The amino acids analyzed at the active site were Asp8, Asp49, Trp68, Lys96, Ala134, Thr135 and Cys138. Two mutants were generated with Tyr and Ser at position 138. A flexible docking study with PZA was performed with all the three models and the results indicate that Cys138Tyr substitution may lead to higher degree of drug resistance than Cys138Ser. The study provides details about the structural prediction of PZase from *M. tuberculosis*. The predicted model will enable us to explore more about the drug-target interaction and giving idea relating to the resistance mechanism, occurring due to mutations at the active site of the target protein.

Key words: *M. tuberculosis*, PZase, PZA, drug resistance, mutants, docking.

I. INTRODUCTION

Tuberculosis (TB) still remains as a major health problem and a leading cause for mortality. Pyrazinamide (PZA) is an important sterilizing drug and a principle component in the current six-month short course TB-chemotherapy. PZA plays a unique role in shortening the therapy from a period of 9 to 12 months down to 6 months, because PZA kills a population of semi-dormant tubercle bacilli, residing in an acidic environment, which cannot be killed by other TB drugs [1].

Genetic and molecular analysis of drug resistance in *M. tuberculosis* suggests that the bacilli usually acquire resistance either by alteration of the drug target through mutation or by titration of the drug through overproduction of the target. PZA-resistant (PZA^r) *M. tuberculosis* clinical isolates are usually defective for PZase activity, and there is a very good correlation between PZA resistance and loss of this enzyme. Scorpio and Zhang in 1996 had identified the PZase gene (*pncA*) from *M. tuberculosis* and had shown that *pncA* mutations are a major mechanism of PZA resistance [2]. The identified *pncA* mutations are largely missense mutations causing amino acid substitutions, and in some cases nucleotide insertions or deletions and nonsense mutations in the *pncA* structural gene or in the putative promoter region of *pncA* [3]. The uniqueness in the mutations of *pncA* gene is its diversity and scattering along the whole gene though there does appear to be some degree of clustering at three regions of *pncA* (3 to 17, 61 to 85, and 132 to 142). These regions are likely to contain catalytic sites for the PZase enzyme [4].

A Report [5] emphasized the need for further structural studies of *pncA* protein to understand the kinetic property of mutant proteins. In the light of which we predicted a three dimensional (3D) model of PZase and generated two mutant models with Cys138Ser and Cys138Tyr to identify and examine the involvement of key active site residues contributing to the extent of drug resistance.

II. MATERIALS AND METHODS

A. Homology modeling

Comparative modeling or Knowledgebased prediction exploits the fact that evolutionary related proteins with similar sequences have similar structures. The degree of similarity is very high in the so called "core regions" comprising of secondary structural elements (-helices and -sheets) whereas the degree of similarity is usually low in loop regions connecting the secondary structures. In Homology modeling, prediction is based on information derived from known protein 3D structures.

B. Steps involved in Homology Modeling of Pzase

The target protein was submitted to BLASTp from NCBI website and was searched against PDB. The template was chosen based on the percentage identity of greater than 35%, thus the protein pyrazinamidase from the organism *Pyrococcus hirokoshi*, which had a percentage identity of 37.4 %, was taken as the template

for having highest identity score with the target protein. Secondary structure of PZase was predicted using JPRED. Amino acid sequence of the target was aligned to the template sequence based on the secondary structure information for building an accurate model. Structurally conserved regions (SCRs) for the target sequence and the templates were determined by superimposition of two structures and multiple sequence alignment. Coordinates from the reference protein (1im5) to the SCRs, structurally variable regions (SVRs), N-termini and C-termini were assigned to the target sequence based on the satisfaction of spatial restraints. The stereochemical quality of the model structure was analyzed using PROCHECK [6], environment profile using VERIFY-3D graph (structural evaluation server) and Ramachandran plot.

C. Docking of PZA-PZase

Docking was performed using CDOCKER. The ligand, including all hydrogen atoms, were built and minimized using DS. The PZase model was applied with CHARMm force field before performing docking. The CDOCKER protocol is an implementation of the CDOCKER algorithm [7] in the DS environment. It allows running a refinement docking of any number of ligands with a single protein receptor. CDOCKER is a grid-based molecular docking method that employs CHARMm. The receptor is held rigid while the ligands are allowed to flex during the refinement. Ten docking poses were obtained for each ligand. Poses with highest CDOCKER score was used for further analysis. Docking was done using the default settings. The default speed selection was used to avoid a potential reduction in docking accuracy.

III. RESULTS

A. Validation of Pzase

Validation of the model was carried out using Ramachandran plot calculations computed with the PROCHECK program. Altogether 98.8 % of the residues was in favored and allowed regions. No residue was found in the disallowed regions of the Ramachandran diagram. The total quality G-factor was -0.1, which is indicative of a good quality model (acceptable values of the G-factor in PROCHECK are between 0 and -0.5, with the best models displaying values close to zero). The compatibility score above zero in the VERIFY-3D graph corresponded to acceptable side chain environments.

B. Docking

To understand the interaction between PZA-PZase the complex was visualized using DS. It was found that PZA was located in the center of the active site, and was stabilized by Hydrogen (H) bonding interactions. The drug is bound between □1- □4 alpha helices and □A □D beta strands. Three H bonds were identified to form with Asp49 and one H bond with Ala102.

Interaction of the drug molecule with mutated models was also studied. In this study, it was found that mutated model with Tyr138 forms five H bonds, wild type model with Cys138 forms four H bonds, and mutated model with Ser138 forms three H bonds that are important for strong H bonding interactions with the drug. the mutated model with Ser substitution at position 138 forms three H bonds with Asp49. Although, the model structure with Tyr 138 having more (five) H bonds: 3 with Asp49, 1 with His71 and 1 with Ala102, but has low CDOCKER score compared to other model.

IV. DISCUSSION

PZase is involved in the activation of the PZA. Its molecular weight is 19Kda and has got 186 aa. Previously, a study by Lemaitre *et al* 2001 provided structural details about the of PZase on the basis of model obtained from *Arthrobacter CSHase* as template having sequence homology of only about 26% by using SPDV software, whereas in the present study, the chosen template had 37% identity; indicating a greater degree of reliability. PZase was modeled using the DS 1.7 and the models were built based on the sequence of *pncA* gene for wild type with Cys and for mutants with Tyr and Ser at position 138. All the three models were docked with PZA using CDOCKER. The conformational changes occurring due to mutations at the active site of the target protein, causes alteration in the drug binding ability of the protein, at instances when the mutations are severe the binding ability gets diminished leading to the survival of the organism in the presence of drug, hence resulting in drug resistance. The generated wild type and mutant models during its interaction with the drug indicates differences in the binding ability. The mutated model with substitution of Cys to Tyr at position 138 has produced more conformational changes at the active site compared to Cys to Ser model. Hence, Cys to Tyr substitution may lead to higher degree of PZA resistance than Cys to Ser. The predicted model will be useful to determine the conformational changes occurring at active site due to mutation. Such studies will enables to gain an insight into the drug-target interactions leading to the rational design of more efficacious drugs.

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