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INSILICO STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF MUTATIONS IN PYRAZINAMIDE RESISTANT *MYCOBACTERIUM TUBERCULOSIS*

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Abstract

Background: *Pyrazinamide (PZA), a derivative of nicotinamide is one of the most imperative first-line prodrug activated by pyrazinamidase (PZase) of Mycobacterium tuberculosis. PZase is encoded by 561 bp gene, PncA. Mutations in PncA results in loss of activation of PZA lead to drug-resistant Mycobacterium tuberculosis. In the current study, we have examined the effects of different mutations on the PZase activity.*

Methodology*: We have observed the effect of mutations C138S, C138Y, G132A, G132S, H51Q, H71E, A134V, H82R present in the active site of PZase. The mutated PZase structure was modeled through mutate-model script of Modeller. The functional impact and stability of these mutant structures were also predicted through MuSTAB, Mutations accessor and Provean on line servers. Ligand and protein interaction studies of wild type and mutant structures were recorded through bioinformatics tools. Hydrogen bonding, hydrophobic interactions were recorded with LigPlot+ v.1.4.5.*

Results: *Results showed that the functional effect of C138S, C138Y, G132A, G132S, H51Q mutations and H71E in PZase is high except A134V and H82R which has a medium effect on protein function. The stability of the mutated structure also decreases as a result of mutation except A134V, C138Y and G132S variants that showed increased stability. Hydrogen and hydrophobic interactions between drug and PZase are also significantly different between native and mutated.*

Conclusion: *Mutations not only affect the functional loss but also reduce the stability of the pyrazinamidase (pncA). PZA resistance mutations should be find out in the early stage of TB for better management of treatment. Alternative drugs with better interactions should be designed that possess strong binding affinity with both mutated and native PncA.*

Keywords: *Mutations, Mycobacterium tuberculosis, PncA, Resistant*

INTRODUCTION

To understand the effects of different mutations and drug resistance phenomena, molecular structure and functional characterization providing a better channel towards insight into the effects on molecular structures and activities.

Multidrug resistant tuberculosis (MDR-TB) is one of the major hurdles in the treatment of tuberculosis. One of the drugs that is used for short periods along with combination of isoniazid and rifampicin to treat this MDR_TB is Pyrazinamide (PZA) [1]. The drug in initial stage is converted to active form, pyrazinoic acid by mycobacterial pyrazinamidase (PZase). This enzyme is encoded by 561 nucleotide

gene (PncA) [2]. The conversion property of this enzyme is failed due to mutations in pncA leading to PZA resistance during treatment [3]. These mutations that lead to resistance are not studied at molecular level [4, 5]. The reported pncA mutations are mainly missense mutations, causing amino acid substitutions, while in a few cases, nucleotide insertions or deletions and nonsense mutations in putative promoter or structural region [6]. The most conserved regions of PncA protein obtained from different species reported yet are positions 3–17, 61–76, and 132–142, signifying the importance of these areas in catalytic and structural point of view [7]. Furthermore, previous studies reported the Asp8, Lys96, and Cys138 as key residues during catalytic activity, activating the PZA into active form. Residues Asp49, His51, His57, and His71 are important in metal ion binding [8]. Enlightening the real relationship between natural pncA mutations and their enzyme behavior will be of central importance for the analysis of PncA function as well resistance to PZA. In the study of Yoon et al. [9] on eight clinical isolate of Mycobacterium tuberculosis revealed that PZA resistant conferring mutations in PncA are clustering at specific regions of this gene, showing the significance of these regions during catalytic activity. Here in this study, we have performed a number of analyses to identify the mechanism of resistance including modeling, docking, functional effect and stability of mutations on the function of PZase.

MATERIALS AND METHODS

PROTEIN DATA BANK (PDB) STRUCTURE RETRIEVAL

Crystal structure of PZase (PDB: 3PL1) was retrieved from PDB in PDB format. The pdb structure contained Asp49, His51, His57 and His71 residues in the binding cavity of metal i.e. Fe^{2+} ion.

MUTATIONS SELECTION

Eight mutations reported in the active sites in the literature were selected that were functionally important. As the mutant structure of the pyrazinamidase is not available, we predicted the mutant structure of eight functionally important mutations with mutated_model through Modeller Version 9.15 [10].

IN SILICO ANALYSIS OF MUTATED PROTEIN

The properties of mutants' structures were predicted through Mutation Accesor, Provean online servers, and compared with the native structure. MuSTAB online server was used for the effect of mutations on the stability of pyrazinamidase.

DOCKING AND INTERACTION ANALYSIS OF WILD TYPE AND MUTATED PROTEINS

Original 3D structure of anti-tuberculosis drug molecule, pyrazinamide (PZA) was taken from drug bank in sdf format. CLC Drug Discovery Workbench uses a typical precision approach to establish the good binding poses, which detects various flexible ligand conformations while holding protein as rigid structure during docking. Ligand and protein interaction studies of wild type and mutant structures were carried through CLC Drug Discovery Workbench 2 (CLC Inc, Aarhus, Denmark). Hydrogen bonding, hydrophobic interactions were predicted through LigPlot+ v.1.4.5 [11] and PyMOL [12].

RESULT AND DISCUSSION

Proteins accomplish an extensive range of diverse roles inside the body of organisms. These are mediated by the numerous amino acids residues of functional sites. Mutations in functional sites has a central diverse effect. Regions of proteins where effect is highly notable are active sites and ligand binding site. Enzymes catalytic sites are important for the reaction (catalytic residues) and any change, along with surrounding residues for proper attachment of drugs and receptors will cause the loss of activities leading to drug resistance. The major mechanism of multidrug resistant *Mycobacterium tuberculosis* is mutations in the target genes. In majority of cases the mutations give high functional impact and protein instability as compared to the native proteins.

In the current investigation the functional impact, protein stability and docking studies of the mutated structure of pyrazinamidase were analyzed through bioinformatics tools. The result showed that mutations result a high effect on function of protein i.e. C138S, C138Y, G132A, G132S, H51Q, H71E except A134V and H82R where mutation has a medium effect on PZase function (Table I).

Table I. Effect of mutation on stability and function of PncA

	MuSTAB online server	**Mutation Assessor**	**Provean**
Mutation	Protein Stability	Functional effect	Prediction (cutoff= -2.5)
A134V	Increased	medium	Deleterious
C138S	Decreased	high	Deleterious
C138Y	Increased	high	Deleterious
H51Q	Decreased	high	Deleterious
H71E	Decreased	high	Deleterious
H82R	Decreased	medium	Deleterious
G132A	Increased	high	Deleterious
G132S	Decreased	high	Deleterious

The result indicated the effect of mutations on the function of native PZase. Here the value -3 in subPSEC with -5 of P deleterious or above is considered significant. The stability of mutated structure also decreases as a result of mutations except A134V, C138Y and G132S variants that showed increased stability (Table I).

The docking score used in the Drug Discovery Workbench is the PLANTSPLP score [13] has a good balance between precision and assessment time. Here the score imitates the change in potential energy, when the protein and ligand come in contacts. This means that a very negative score in hydrogen bonding corresponds to a strong binding and a less negative or even positive score corresponds to a weak or non-existing binding. Docking score and steric interaction of native PZase is significantly different from mutated structure (Table 2) (docking score=-24.86, Hydrogen bond Score=-6.0, steric interaction -18.86). The steric interactions score of wild type and mutated structures shows significant difference i.e. -18.86 and between -21 to -25 for mutated structures (Table II).

Table II. Docking score predicted through CLC Drug Discovery Workbench 2.5

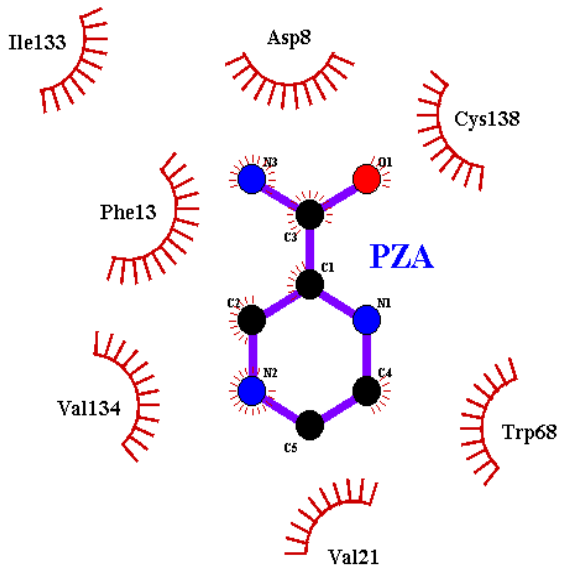
Model	**Docking score**	**H. bond Score**	**Steric interaction**
A134V	-29.04	-4.0	-25.09
C138S	-29.04	-4.0	-25.09
C138Y	-28.43	-6.0	-22.55
G132A	-28.25	-6.0	-22.25
G132S	-28.19	-6.0	-22.19
H51Q	-27.48	-6.0	-21.48
H82R	-28.02	-5.0	-22.56
H71E	-27.48	-6.0	-21.48
Native PncA	-24.86	-6.0	-18.86

Mutated structures of PZase predicted through mutated_model of Modeller have also somewhat different orientation and pocket size

Single hydrogen bond was observed with ligand in mutations Cys138Tyr, Gly132Ala and His51Gln. No significant interactions were found between ligand and mutated structures suggesting that mutations cause loss of function and thus fail to activate drug (Fig. 1).

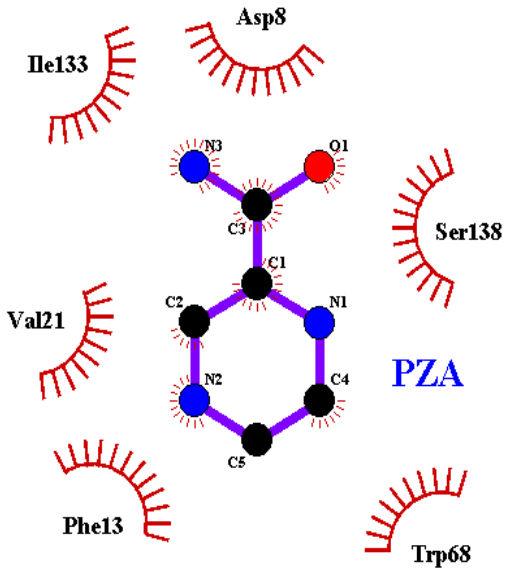
Hydrophobic interaction and hydrogen bond between drug and protein in native and mutated proteins is significantly different. Mutated proteins have less number of hydrophobic interaction compared with native (Fig. 1). Further the effect of mutations was analyzed through Provean online Server revealed the deleterious effect in functions (Table I). Steric interactions and Docking score (Table II) also showed significant differences between native PncA and mutated structures.

(a)



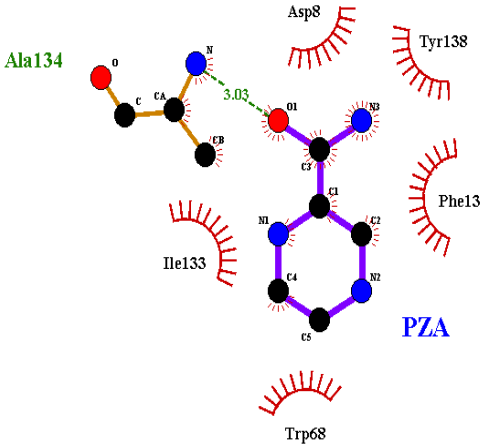
Ala134VAL

(b)



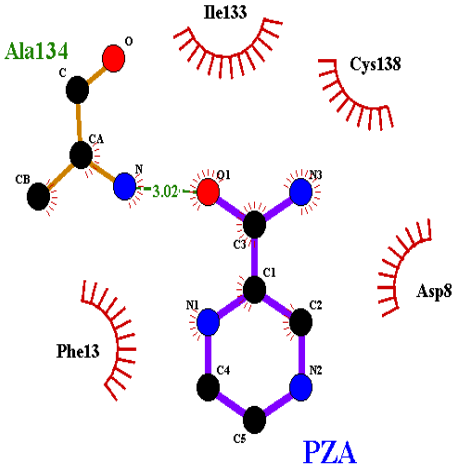
Cys138Ser

(c)



Cys138Tyr

(d)



Gly132Ala

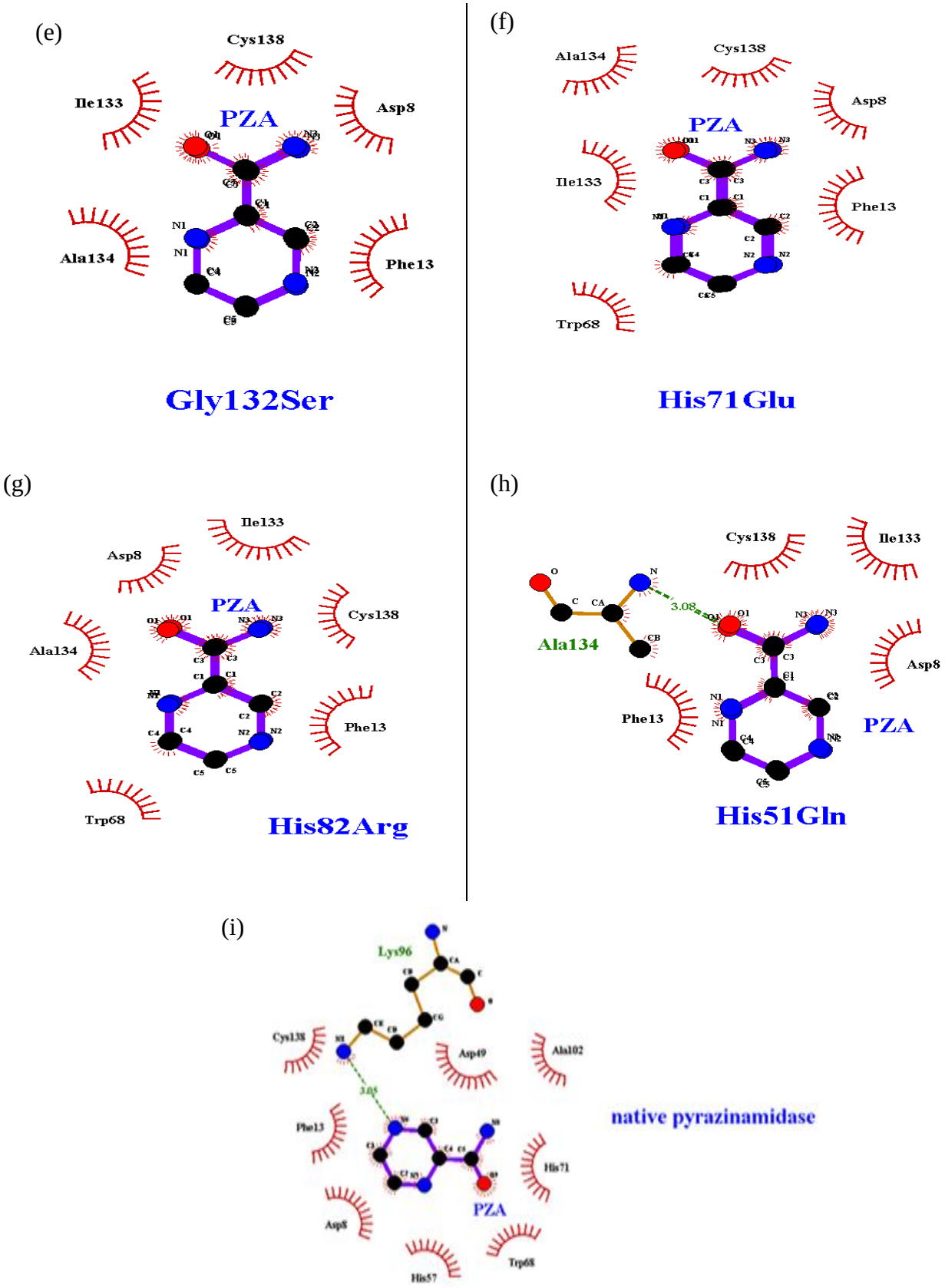


Fig. 1. Hydrogen bonds and hydrophobic interactions of pyrazinamide (PZA) with mutated PZase and native PncA. (a,b,c,d,e,f,g,h, mutated PZase) (i, native PZase)

CONCLUSION

Mutation affects the enzymes function in different ways. These effects not only related to structures but also affect the stability, hydrogen bonding and hydrophobic interactions. The current investigation performed to analyze the PZA resistance by mutations i.e. A134V, C138S, C138Y, G132A, G132S, H51Q, H82R and H71E in pyrazinamidase enzyme of Mycobacterium tuberculosis. We analyzed the hydrogen bonding, hydrophobic interactions, functional effects and stability of native and mutated protein. There

should be alternative drugs for better management of TB. In silico methods are easy and not too much time consuming and can also be compared with in vitro methods of drug resistance results of wet laboratories.

Conflict of Interest:

Authors have no conflict of interest.

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