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Short communication

Detection of novel coupled mutations in the *katG* gene (His276Met, Gln295His and Ser315Thr) in a multidrug-resistant *Mycobacterium tuberculosis* strain from Chennai, India, and insight into the molecular mechanism of isoniazid resistance using structural bioinformatics approaches

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ABSTRACT

This study reports on the structural basis of drug resistance targeting the *katG* gene in a multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) strain with two novel mutations (His276Met and Gln295His) in addition to the most commonly reported mutation (Ser315Thr). A structural bioinformatics approach was used to predict the structure of the mutant KatG enzyme (MT). Subsequent molecular dynamics and docking studies were performed to explain the mechanism of isoniazid (INH) resistance. The results show significant conformational changes in the structure of MT leading to a change in INH binding residues at the active site, with a significant increase in the inhibition constant (Ki) of 5.67 μm in the mutant KatG–isoniazid complex (MT-INH) compared with the wild-type KatG–isoniazid complex (WT-INH). In the case of molecular dynamics studies, root mean square deviation (RMSD) analysis of the protein backbone in simulated biological conditions revealed an unstable trajectory with higher deviations in MT throughout the simulation process (1 ns). Moreover, root mean square fluctuation (RMSF) analysis revealed an overall increase in residual fluctuations in MT compared with the wild-type KatG enzyme (WT), whilst the INH binding residues of MT showed a decreased fluctuation that can be observed as peak deviations. Hence, the present study suggests that His276Met, Gln295His and Ser315Thr mutations targeting the *katG* gene result in decreased stability and flexibility of the protein at INH binding residues leading to impaired enzyme function.

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is one of the most deadly diseases of our time. Drug resistance in *M. tuberculosis* is caused by mutations in relatively restricted regions of the genome as well as by overexpression of efflux pumps [1,2]. In 2007 there were an estimated 0.5 million cases of multidrug-resistant TB (MDR-TB), with 131 000 cases reported from India [3]. A recent global surveillance report by the World Health Organization (WHO) estimated that 9.4 million people were affected by TB globally in 2008 [3].

Isoniazid (INH) is a central component of the drug regimen used worldwide to treat TB. INH inhibits biosynthesis of cell wall mycolic acids (long-chain α-branched β-hydroxylated fatty acids), thereby making the mycobacterium susceptible to reactive oxygen radicals and other environmental factors. It is proposed that INH enters *M. tuberculosis* as a prodrug by passive diffusion and is activated by catalase–peroxidase, the enzyme encoded by the *katG* gene, to generate free radicals, which then attack multiple targets in the cell. Intrinsic resistance of mycobacteria to most hydrophilic antibiotics and chemotherapeutic agents is believed to result from a low-efficiency porin pathway in synergy with other resistance mechanisms such as enzymatic inactivation or active efflux of the drugs [4]. It is also assumed that porins are necessary for uptake of the first-line TB drugs INH and ethambutol (ETB), since they are small hydrophilic molecules [4]. KatG couples isonicotinic acyl with NADH to form an isonicotinic acyl–NADH complex. This complex

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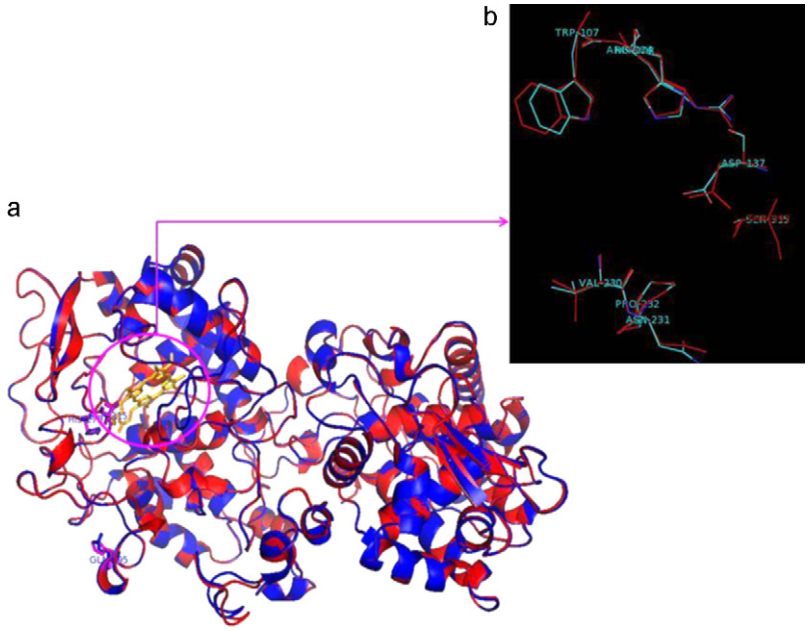

Fig. 1. (a) Superimposition of the three-dimensional structure of *Mycobacterium tuberculosis* wild-type KatG (WT) and mutant KatG (MT). WT is coloured in blue and MT in red. Haem ions are coloured orange in WT and red in MT. The WT hotspots are coloured in blue (His276, Gln295 and Ser315), and in MT (Met276, His295 and Thr315) are coloured in magenta. (b) Superimposition of WT and MT isoniazid (INH) binding residues (WT residues represented in cyan and MT residues in red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

binds tightly to the active site of the enzyme InhA, which is an NADH-dependent enoyl-acyl carrier protein (ACP) reductase, thus blocking the natural enoyl-AcpM substrate and the action of fatty acid synthase. Thus, mycolic acid synthesis is inhibited [5].

KatG is the only peroxide-inducible *M. tuberculosis* protein. It uses one molecule of hydrogen peroxide as an oxidant and an other molecule of hydrogen peroxide as a substrate, ultimately breaking down this toxic substance to water and molecular oxygen. Catalase activity is therefore thought to be essential for the survival of *M. tuberculosis* in highly oxidising environments. It is believed that the catalytic activity in KatG is due to an unusual structural feature, a Met-Tyr-Trp covalent cross-link. *katG* mutant lacks this cross-link, and most mutations are found between codons 138 and 328 [6]. Haem, a cofactor of the wild-type KatG enzyme (WT), is considered to be responsible for its role in the processing and activation of the prodrug INH. The WT crystal structure [Protein Data Bank (PDB) ID 1SJ2; http://www.rcsb.org/pdb/explore/explore.do?structureId=1SJ2] reveals that Arg104, Trp107, His108, Asp137, Val230, Asn231, Pro233 and Ser315 are key residues for binding and activation of INH [7]. Hence, understanding the role of binding site residues in the INH activation process with respect to mutations in the enzyme will enhance the understanding of INH resistance.

The main goal of the present study was to identify the structural changes, binding affinity and functional variation associated with His276Met, Gln295His and Ser315Thr mutations identified in a MDR-TB strain from a sputum specimen. His276Met and Gln295His are novel mutations reported for the first time in this study. The in silico objective of this study was to validate the structural behaviour of WT and mutant KatG enzyme (MT) (His276Met, Gln295His and Ser315Thr). Thus, this study was an attempt to apply molecular modelling approaches, coupled with in silico docking studies of INH binding with WT and MT, to understand the mechanism of antibiotic resistance conferred by the reported mutant enzyme. In addition, explicit molecular dynamics (MD) simulation analysis in solvated conditions have also been integrated in order to demonstrate structural changes using a time-scale of 1 ns to portray the structural changes, binding affinity of INH, and functional variations associated with mutations.

2. Materials and methods

2.1. Phenotypic drug susceptibility testing

Phenotypic susceptibility testing to INH, rifampicin, streptomycin, ethambutol and pyrazinamide was performed using BACTEC MicroMGIT culture system (Becton Dickinson, USA) following the manufacturer's instructions.

2.2. DNA extraction

DNA was extracted from the MDR-TB strain by boiling at 80 °C for 10 min followed by centrifugation at 3000 rpm. Supernatant (5 μL) was used as template DNA.

2.3. Polymerase chain reaction (PCR) targeting the katG gene

Amplification reactions contained 200 μM of each dNTP (dATP, dTTP, dGTP and dCTP; Bangalore Genei, Bengaluru, India), 1 μM of outer and inner set of primers, 1× buffer

Table 1
Molecular interactions of isoniazid (INH) with *Mycobacterium tuberculosis* wild-type KatG (WT) and mutant KatG (MT).

Receptor	Ligand	Binding energy (kcal/mol)	Inhibitory constant, Ki (μm)	Hydrogen bonding residues with INH	Hydrogen bond distance (Å)
WT	INH	−6.59	14.77	His108 Asp137 Val230	2.04 2.09 2.37
MT	INH	−6.40	20.44	Arg104 Trp107 His108 Asp137 Trp229	2.25 2.09 2.31 2.20 1.88