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Significance of catalase-peroxidase (KatG) mutations in mediating isoniazid resistance in clinical strains of *Mycobacterium tuberculosis*

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ABSTRACT

Objectives: Isoniazid (INH) is still the most important first-line antitubercular drug. INH resistance is regarded as a major impediment to the tuberculosis (TB) control programme and contributes to the emergence of multidrug-resistant strains. Mutation at position 315 in the *katG* gene, encoding the catalase-peroxidase (KatG) enzyme, is the major cause of INH resistance in *Mycobacterium tuberculosis*. Therefore, investigation of the molecular mechanisms of INH resistance is the need of the hour.
Methods: To understand the clinical importance of KatG mutants (MTs) leading to INH resistance, in this study five MTs (S315T, S315I, S315R, S315N and S315G) were modelled, docked and interacted with INH in dynamic state.
Results: The binding affinity based on docking was found to be higher for MTs than for wild-type (WT) isolates, except for MT-S315R, indicating rigid binding of INH with MT proteins compared with the flexible binding seen in the WT. Analysis of molecular dynamics (MD) experiments suggested that fluctuations and deviations were higher at the INH binding residues for MTs than for the WT. Reduction in the hydrogen bond network after MD in all KatG enzymes implies an increase in the flexibility and stability of protein structures. Superimposition of MTs upon the WT structure showed a significant deviation that varies for the different MTs.
Conclusions: It can be inferred that the five KatG MTs affect enzyme activity in different ways, which could be attributed to conformational changes in MT KatG that result in altered binding affinity to INH and eventually to INH resistance.

1. Introduction

Isoniazid (INH) (chemical name, isonicotinic acid hydrazide; IUPAC name, pyridine-4-carbohydrazide) is a major component in the current 6-month short-course chemotherapy for tuberculosis (TB). Use of INH as an effective antitubercular drug began in 1952 [1] and INH remains one of the most active drugs for treating both active and latent TB infection as well as acting as a prophylactic agent to prevent TB worldwide.

The prodrug INH is transformed into its active counterpart (isonicotinic acyl radical) by the bacterial catalase-peroxidase (KatG) enzyme following entry into *Mycobacterium tuberculosis* (MTB) (Fig. 1) [2,3]. Interestingly, the association of resistance and virulence has been reported solely for INH compared with other drugs used in TB therapy [4]. The major cause of INH resistance in MTB is due to mutations in the *katG* gene encoding the KatG enzyme [5]. However, the molecular cause of resistance to INH remains ambiguous and mutations in multiple genes [3,6] are reported to be associated with resistance.

The most prevalent mutation in the *katG* gene is a substitution at codon 315 (Ser315→Thr; S315T), which is found to occur in 40–90% of INH-resistant clinical MTB isolates and is therefore considered as a reliable marker for the detection of INH resistance [6]. More importantly, this substitution is commonly observed amongst multidrug-resistant and extensively drug-resistant MTB strains [7] and is associated with intermediate or high levels of resistance to INH [8]. Furthermore, MTB strains with mutations [Ser(AGC)→Thr(ACC)] at codon 315 in the *katG* gene remain virulent [9] and are frequent amongst Beijing strains [10].

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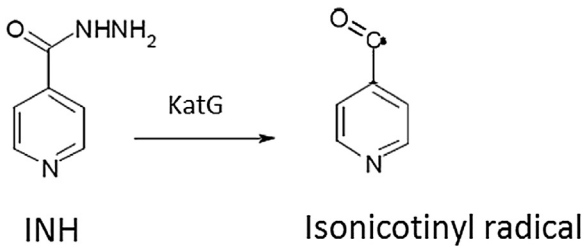

Fig. 1. Activation of isoniazid (INH) by KatG.

Many studies based on S315T [11–13] as well as other mutants (MTs) of KatG [14–17] have demonstrated changes in the KatG activity of MT proteins that corresponded well with alterations in protein conformations. Studies [18–20] on molecular modelling, including ours, have reported on the unstable conformation of MT-S315T and its rigid interaction with INH. Alterations in INH and heme binding, or other structural changes due to amino acid substitutions, have been mostly discussed in relation to KatG MT-S315T and not other MTs at codon 315.

Despite the fact that several studies have provided details on the biochemical properties and structural characteristics of KatG and its MTs, principally S315T as mentioned above, data on all clinical MTs with other substitutions at position 315 leading to INH resistance have not been described. Thus, it is of interest to analyse the interactions between KatG MTs and INH, which will enable us to gain insights into the role of the various mutations at codon 315. Therefore, in this study models for five MTs were developed and docked with INH, followed by simulation to understand their behaviour in the dynamic state.

2. Materials and methods

2.1. Wild-type (WT) and mutant proteins

Five essential clinical MTs of KatG (S315T, S315I, S315R, S315N and S315G) were used previously to investigate interactions with INH derivatives [20], however in this study MTs were explored as opposed to the parent drug, INH. MTs were created from the WT sequence using MODELLER 9.14 software [21].

2.1.1. Template selection and model building

In the present study, the target KatG protein sequence (Rv1908c) obtained from the TubercuList database (http://genolist.pasteur.fr/TubercuList/) was submitted to the alignment program BLASTp [22] and was searched against the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/home/home.do). The WT KatG protein [23] (PDB code 1SJ2) was considered as the template as it displayed maximum sequence identity with the WT KatG protein of MTB. Residues at position 315 of the KatG protein were substituted with indicated amino acids to generate five different MT proteins. In the template 1SJ2, A chain was used and heteroatoms such as heme, glycerol and water were removed. Command line options were provided for sequence alignment between WT and MTs, and commands were provided for model building using MODELLER 9.14. The number difference between the WT and MT models was 23 owing to the fact that the crystal structure of 1SJ2 begins on the 24th residue and therefore the 24th residue of 1SJ2 corresponds to the first residue of the modelled protein.

2.1.2. Model evaluation

To determine the quality of the generated models, validation of the models was done by Ramachandran plot [24] and ERRAT score [25]. Furthermore, deviation between the WT (1SJ2) and the models upon structural superimposition was determined using PDBeFOLD [26].

2.2. Ligand

The ligand (INH) was obtained from the PubChem database in the mol format and was converted to PDB using Discovery Studio v.2.0 [27].

2.3. Docking software

The software GOLD (licensed software v.5.2) is based on the concept of genetic algorithm, with protein as the rigid and ligand as

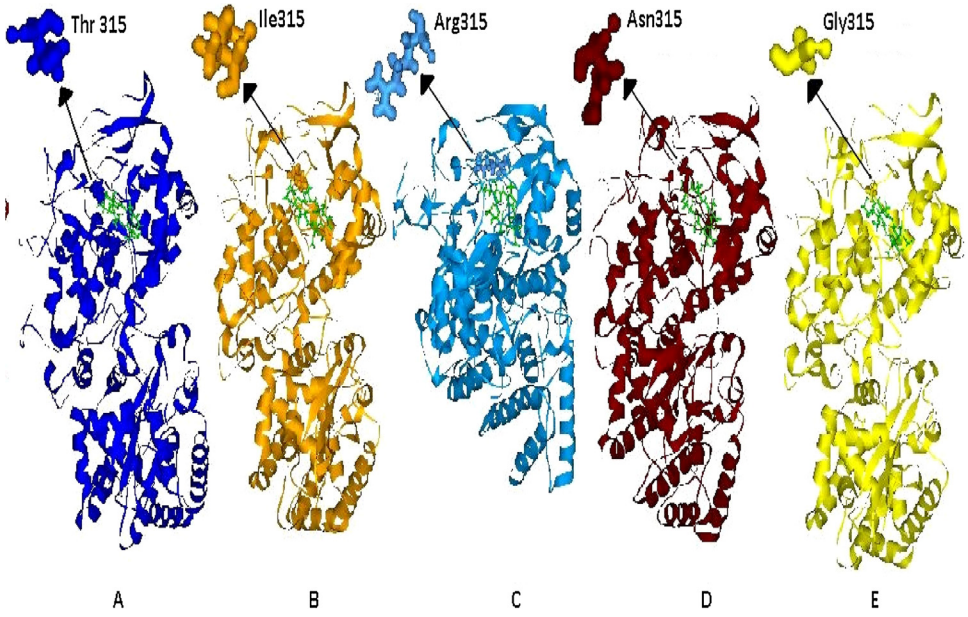

Fig. 2. Modelling of KatG mutants (MTs): (A) MT-S315T; (B) MT-S315I; (C) MT-S315R; (D) MT-S315N; and (E) MT-S315G.