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Computational approach to understanding the mechanism of action of isoniazid, an anti-TB drug

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ABSTRACT

Tuberculosis (TB) is an ancient disease caused by *Mycobacterium tuberculosis* (MTB), which remains a major cause for morbidity and mortality in several developing countries. Most drug-resistant MTB clinical strains are resistant to isoniazid (INH), a first-line anti-TB drug. Mutation in KatG, a catalase-peroxidase, of MTB is reported to be a major cause of INH resistance. Normally upon activation by KatG, INH is converted to an active intermediate which has antimycobacterial action in MTB. This INH intermediate in the presence of NADH forms INH-NAD adduct which inhibits inhA (2-trans-enoyl-acyl carrier protein reductase) of MTB, thus blocking the synthesis of mycolic acid, a major lipid of the mycobacterial cell wall. In this docking study, the high binding affinity of INH-NAD adduct towards InhA was observed in comparison with INH alone. In this study, two resistant mutants of KatG (S315T and S315N) were modeled using Modeller9v10 and docking analysis with INH was performed using AutoDock4.2 and the docking results of these mutants were compared with the wild type KatG. Docking results revealed the formation of a single hydrogen (H) bond between the secondary amine nitrogen (–NH) of INH with Thr or Asn residues in place of Serine at 315 position of KatG mutant strains respectively, whereas in the case of the wild type, there was no H-bond formation observed between INH and Ser315. The H-bond formation may prevent free radical formation by KatG in mutant strains thus the development of resistance to the drug. This *in silico* evidence may implicate the basis of INH resistance in KatG mutant strains.

Introduction

Decades after the discovery of the *Mycobacterium tuberculosis* (MTB) organism, tuberculosis (TB) remains a major cause of morbidity and mortality in several developing countries. Nearly 33% of the world's population is considered to be infected with MTB infection, with 8.6 million new patients and 1.3 million deaths in the year 2012, including 320,000

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deaths among HIV-positive individuals. In India alone, there were 2.0 million to 2.4 million infected cases of TB, i.e., 26% of total cases [1]. Multi-drug-resistant strains of this pathogen, emerging in association with HIV, have added a frightening dimension to the problem [2]. Outbreaks of extensively drug-resistant (XDR) tuberculosis have also been an increasing threat in certain regions around the world [3]. Most drug-resistant MTB clinical strains are resistant to isoniazid (INH, isonicotinic acid hydrazine) – a first-line, anti-tuberculous drug [4].

Isoniazid (INH), also known as isonicotinyl hydrazine, is an organic compound used as a first-line drug in the prevention and treatment of TB. It has a simple structure (Fig. 1) containing two essential components required for the high activity against MTB, i.e., a pyridine ring and a hydrazide group [5]. This compound was first synthesized in the early 20th

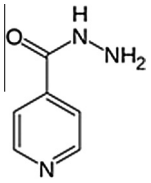

Fig. 1 – Chemical structure of Isoniazid (Formula: $C_6H_7N_3O$; Mol. Wt.: 137.139 g/mol).

Table 1 – Proteins of *Mycobacterium tuberculosis* reported to associate with Isoniazid resistance.

Locus_tag	Name	Protein length	Locus	PDB ID	Mutation
Rv1772	Hypothetical protein Rv1772	103	–	–	Thr4Ala
Rv1909c	Ferric uptake regulation protein furA (fur)	150	furA	–	Ser5Pro
Rv0340	Hypothetical protein Rv0340	179	–	–	Val163Ile
Rv2428	Alkyl hydroperoxide reductase subunit C	195	ahpC	2BMX	Inter-genic region G(−46)A
Rv1483	3-Oxoacyl-[acyl-carrier-protein] reductase	247	fabG1	1UZL	Ala5Pro, Val14Leu, Thr21Ala
Rv1484	Enoyl-(acyl carrier protein) reductase	269	inhA	1P44	Lys8Asn, Ile16Thr, Ile21Val/Thr, Ile47Thr, Val78Ala, Ser94Ala/Leu, Ile95Pro, Ile95Thr, Ile194Thr, Arg202Gly, Glu217Asp, promoter region
Rv3566c	Arylamine n-acetyltransferase nat	283	nat	4BGF	Gly67Arg, Gly207Glu
Rv2243	Acyl-carrier-protein S-malonyltransferase	302	fabD	2QC3	Ser275Asn
Rv0129c	Secreted antigen 85-C FBPC (85C) (antigen 85 complex C) (AG58C) (fibronectin-binding protein C)	340	fbpC	4MQM	Gly158Ser −63(C/T), −23(A/C)
Rv2242	Hypothetical protein Rv2242	414	–	–	Asp3Gly, Met323Thr
Rv2245	3-Oxoacyl-(acyl carrier protein) synthase II	416	kasA	4C6U	Asp66Asn, Met77Ile, Arg121Lys, Gly269Ser,Gly312Ser, Gly387Asp, Phe413Leu
Rv1592c	Hypothetical protein Rv1592c	446	–	–	Pro42Leu, Val430Ala
Rv1854c	NADH dehydrogenase	463	ndh	–	Arg13Cys, Val18Ala, Thr110Ala, Leu239Pro, Arg268His
Rv3139	Acyl-CoA dehydrogenase FADE24	468	fadE24	–	Insertion of 2 base pair (bp) at nucleotide position -64
Rv2247	Acetyl/propionyl-CoA carboxylase beta subunit AccD6	473	accD6	4FB8	Asp229Gly
Rv0341	Isoniazid inducible gene protein INIB	479	iniB	–	Deletion of 12 bp at nucleotide position 665
Rv0343	Isoniazid inducible gene protein INIC	493	iniC	–	Trp83Gly
Rv2846c	Integral membrane efflux protein EfpA	530	efpA	–	Ile73Thr
Rv0342	Isoniazid inducible gene protein INIA	640	iniA	–	Pro3Ala, Arg537His
Rv1908c	Catalase–peroxidase-peroxynitritase T KatG	740	katG	2CCA	Ser315Thr, Ser315Asn, Arg463Leu, Ser17Asn, Gly19Asp, Ser140Asn/Arg, Gly279Asp, Gly285Asp, Gly316Asp, Ser457Ile, Gly593Asp
Rv3795	Integral membrane indolylacetylinositol arabinosyltransferase EMBB	1098	embB	–	Tyr333His
Rv2427a	Transcriptional regulator OxyR′, pseudogene		oxyR′	–	–