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Study of mechanism of interaction of truncated isoniazid–nicotinamide adenine dinucleotide adduct against multiple enzymes of *Mycobacterium tuberculosis* by a computational approach

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ABSTRACT

Objective/Background: Isoniazid (INH) is one of the effective antituberculosis (TB) drugs used for TB treatment. However, most of the drug-resistant *Mycobacterium tuberculosis* (MTB) clinical strains are resistant to INH, a first-line antituberculous drug. Certain metabolic enzymes such as adenosylhomocysteinase (Rv3248c), universal stress protein (Rv2623), nicotinamide adenine dinucleotide (reduced)-dependent enoyl-acyl carrier protein reductase (Rv1484), oxidoreductase (Rv2971), dihydrofolate reductase (Rv2763c), pyrroline-5-carboxylate dehydrogenase (Rv1187) have been identified to bind INH–nicotinamide adenine dinucleotide (INH–NAD) and INH–nicotinamide adenine dinucleotide phosphate adducts coupled to Sepharose resin. These enzymes are reported to be involved in many important biochemical processes of MTB, including cysteine and methionine metabolism, mycobacterial growth regulation, mycolic acid biosynthesis, detoxification of toxic metabolites, folate biosynthesis, etc. The truncated INH–nicotinamide adenine dinucleotide (oxidized) adduct, 4-isonicotinoylnicotinamide, isolated from urine samples of human TB patients treated with INH therapy is proposed to have antimycobacterial activity.

Methods: To understand the mechanism of interaction of the truncated INH–NAD adduct, binding energy studies were carried out on the aforementioned six enzymes with known three-dimensional structures using AutoDock4.2.

Results: *In silico* docking analysis of these MTB enzymes with the truncated INH–NAD adduct showed favorable binding interactions with docking energies ranging from −5.29 to −7.07 kcal/mol.

Conclusion: Thus, *in silico* docking study revealed that the INH–NAD adduct, which is generated *in vivo* after INH activation, may undergo spontaneous hydrolysis to form the

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truncated INH–NAD adduct and further binds and inhibits multiple enzymes of MTB, in addition to InhA, confirming that INH is an effective anti-TB drug acting at multiple enzymes. Further analysis of amino acid residues in the active site of INH–NAD-binding proteins showed the probable presence of catalytic triad in four enzymes possibly involved in INH binding to the enzyme.

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), is a major cause of morbidity and mortality worldwide. Globally, 33% of the population is considered to be infected with MTB, with 9.0 million new patients and 1.5 million deaths in the year 2013, including 360,000 deaths among human immunodeficiency virus (HIV)-positive individuals. India and China had the largest number of TB cases (24% and 11%, respectively) of the total cases identified [1]. In addition, multidrug-resistant strains of MTB in association with HIV have further created a fearsome aspect to the problem [2]. The current epidemics of extensively drug-resistant TB have also been an increasing threat in some regions around the world [3]. Most drug-resistant clinical strains of MTB are resistant to isoniazid (INH, isonicotinic acid hydrazide), a first-line antituberculous drug [4].

INH (Fig. 1) is one of the most effective anti-TB drugs for prevention and treatment of TB. It inhibits the synthesis of mycolic acids, a vital component of the bacterial cell wall. At therapeutic levels, INH is bacteriocidal against actively growing intracellular and extracellular MTB. Under multidrug therapy, it is used in conjunction with other effective anti-TB agents [5].

Mechanisms of action of INH

The mechanism of INH action has been the subject of extensive studies. It is reported to generate a variety of highly reactive compounds, including reactive oxygen species such as superoxide, peroxide, and hydroxyl radical [6], nitric oxide [7], reactive organic species such as isonicotinic acyl radical or anion [8], and certain electrophilic species [9], which then attack multiple targets in MTB cases [10,11]. INH is a prodrug, that is, it requires activation before it becomes therapeutically effective. This process is carried out by the catalase–peroxidase activity of the *katG* gene product, and mutations in the *katG* gene contribute to resistance to INH [12]. INH passively diffuses through the mycobacterial envelope, where it is activated by $MnCl_2$ and catalase–peroxidase of the *katG* possibly into an isonicotinoyl radical or anion. Modified INH appears to covalently attach itself to nicotinamide adenine dinucleotide (oxidized) (NAD^+) to form an INH–NAD adduct, which inhibits the synthesis of mycolic acid. Stigliani et al. [13] studied the binding of the tautomeric forms of INH–NAD adducts to the active site of InhA and noted that the 4S-chain adduct is the effective active form of the INH–NAD adducts. Mahapatra et al. [14] in their mass spectrometry study isolated a novel metabolite—4-isonicotinoylnicotinamide (4-INN), a truncated part of the INH–NAD adduct—from urine samples of human TB patients who received INH therapy. The truncated INH–NAD^+ adduct with some structural similarity to 4-INN was evaluated as an effective inhibitor of InhA. Their test results indicated antimycobacterial activities for the truncated INH–NAD^+ adduct [15]. It has previously been shown that INH is activated to the INH–NAD adduct by *katG* of MTB. This truncated INH–NAD adduct then binds to InhA [2-*trans*-enoyl-acyl carrier protein-(ACP) reductase] and eventually causes inhibition by computational approach [16].

Interestingly, arylamine *N*-acetyltransferase (NAT), a drug-metabolizing enzyme of MTB, can acetylate INH, transferring an acetyl group from acetyl coenzyme A to the terminal nitrogen of the drug, which in its N-acetylated form is therapeutically inactive [12]. The overexpression of NAT in *Mycobacterium smegmatis* showed increased resistance to INH [17]; in addition, when the gene was knocked-out, the bacteria exhibited increased sensitivity to INH [18]. Therefore, it is likely that NAT competes with *katG* for INH [19] (Fig. 2).

High-affinity INH–NAD(P)-binding proteins

Both nicotinamide adenine dinucleotide (reduced)-dependent enoyl-(ACP) reductase [NADH-dependent enoyl-(ACP) reductase; InhA] and the nicotinamide adenine dinucleotide phosphate-dependent dihydrofolate reductase (DHFR; DfrA) have been shown to be inhibited by the INH–NAD(P) adducts with nanomolar affinity. Argyrou et al. [20] have shown that in addition to InhA and DfrA, 15 other proteins bind these adducts coupled to Sepharose resin with high affinity (Table 1).

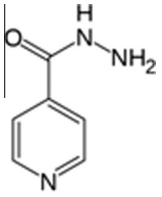

Fig. 1 – Chemical structure of isoniazid (formula: $C_6H_7N_3O$; molecular weight: 137.139 g/mol).

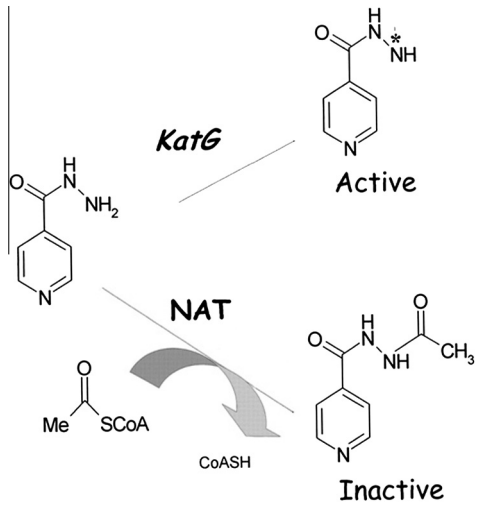

Fig. 2 – Schematic showing the activation and inactivation of INH [12]. INH is a prodrug and requires activation by the catalase–peroxidase protein (the *KatG* gene product). The NAT enzymes can *N*-acetylate INH, rendering the drug therapeutically inactive. The asterisk next to the terminal nitrogen in the active form of INH denotes a range of oxidized species [19]. *Note:* INH = isoniazid; NAT = *N*-acetyltransferase.

Of 17 proteins, six proteins [adenosylhomocysteinase (Rv3248c), universal stress protein (Rv2623), NADH-dependent enoyl-(ACP) reductase (Rv1484), oxidoreductase (Rv2971), DHFR (Rv2763c), and pyrroline-5-carboxylate dehydrogenase (Rv1187)] have known experimentally determined three-dimensional (3D) structure available at the Protein Data Bank (PDB). Mahapatra et al. [14] observed that the formation of 4-INN (a truncated INH–NAD adduct) occurred due to spontaneous hydrolysis of the INH–NAD^+ adduct under *in vitro* INH activation condition, and this compound has potential antimycobacterial activity. In this study, we explored the interaction of the truncated INH–NAD adduct with these six proteins through molecular docking analysis.

Materials and methods

Hardware and software

The study was carried out on a Dell workstation with a 2.26-GHz processor, 6-GB RAM, and 500-GB hard drive running a Windows operating system. Bioinformatics software, such as AutoDock4.2, and online resources were used in this study.

Table 1 – High-affinity INH–NAD(P)-binding proteins from *Mycobacterium tuberculosis*.

Locus	Name	Structure	Function
Rv3248c	S-adenosyl-L-homocysteine hydrolase	3DHY	Thioester hydrolase acting on ether bounds. Could be involved in methionine and selenoamino acid metabolisms
Rv0753c	Methylmalonate-semialdehyde dehydrogenase	—	Plays a role in valine and pyrimidine metabolism
Rv1187	Pyrroline-5-carboxylate dehydrogenase (RocA)	4IHI	Involved in the arginase pathway
Rv0155	NAD(P) transhydrogenase subunit alpha	—	Involved in the transhydrogenation between NADH and NADP, which is coupled to respiration and ATP hydrolysis
Rv2623	Universal stress protein	3CIS	May play a role in the establishment of a persistent infection (latency) in the host, as strains without this gene are hypervirulent.
Rv1996	Hypothetical protein	—	Function unknown
Rv0468	3-Hydroxybutyryl-CoA dehydrogenase	—	Butyrate/butanol-producing pathway
Rv1484	Enoyl-ACP reductase	1P44	This isozyme is involved in mycolic acid biosynthesis
Rv2691	TRK system potassium-uptake protein CEOB	—	Part of a potassium-transport system
Rv0091	Bifunctional 5-methylthioadenosine nucleosidase/S-adenosylhomocysteine nucleosidase	—	Responsible for cleavage of the glycosidic bond in both 5′-methylthioadenosine and S-adenosylhomocysteine
Rv2858c	Aldehyde dehydrogenase	—	Oxidizes a wide variety of aldehydes
Rv1059	Hypothetical protein	—	Function unknown
Rv3777	Oxidoreductase	—	Function unknown; probably involved in cellular metabolism
Rv2971	Oxidoreductase	4OTK	Function unknown; probably involved in cellular metabolism
Rv2766c	3-Ketoacyl-ACP reductase	—	Function unknown; possibly involved in cellular metabolism
Rv2671	Hypothetical protein	—	Involved in riboflavin biosynthesis
Rv2763c	Dihydrofolate reductase	4KL9	Involved in an essential step for *de novo* glycine and purine synthesis

Note: ACP = acyl carrier protein; ATP = adenosine triphosphate; CoA = coenzyme A; INH = isoniazid; NADH = nicotinamide adenine dinucleotide (reduced); NADP = nicotinamide adenine dinucleotide phosphate; CEOB = complement the E. coli OxyR-knockout mutant type B; TRK = K+ Transporter.

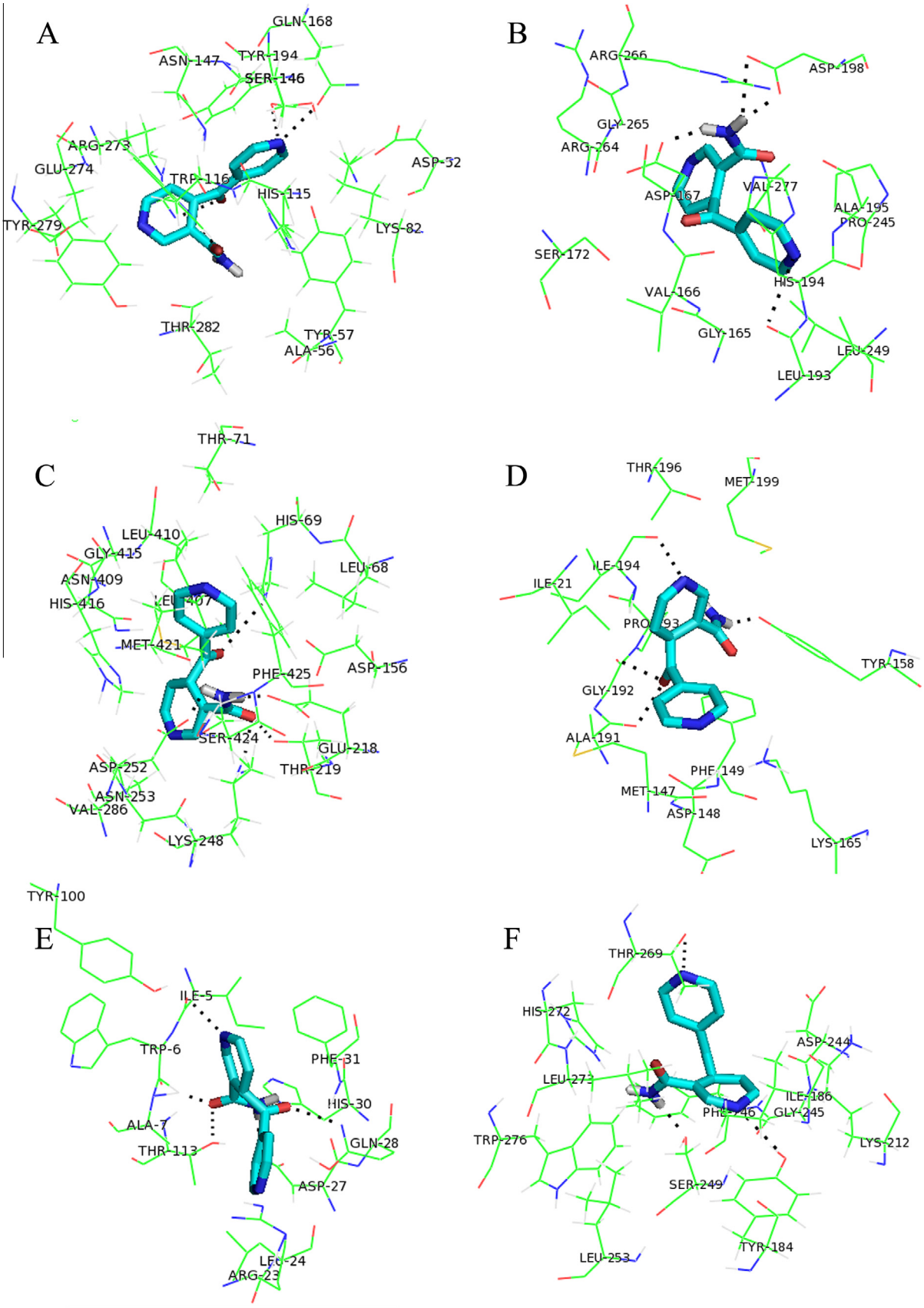

Fig. 3 – Docking interaction of truncated INH–NAD adduct with (A) Rv2971, (B) Rv2623, (C) Rv3248c, (D) Rv1484, (E) Rv2763c, and (F) Rv1187 showing hydrogen bonds in dotted lines. *Note*: INH = isoniazid; NAD = nicotinamide adenine dinucleotide.

High-affinity INH–NAD(P)-binding proteins of MTB

High-affinity INH–NAD(P)-binding proteins of MTB (Rv2623, Rv3248c, Rv2971, Rv2763c, Rv1484, and Rv1187) are reported to have high binding affinity for INH–NAD(P) [20]. The experimentally determined structures of these proteins obtained through X-ray diffraction experiment are available at the PDB. The 3D structures of Rv2623 (PDB ID 3CIS), Rv3248c (PDB ID 3DHY), Rv2971 (PDB ID 40TK), Rv2763c (PDB ID 4KL9), Rv1484 (PDB ID 1P44), and Rv1187 (PDB ID 4IHI) were retrieved from PDB for the docking study.

Ligand preparation

Because the 3D conformer generation of the INH–NAD adduct is disallowed due to too many atoms [21], the PRODRG2 server [22] was used to obtain the chemical structure of the truncated INH–NAD adduct in PDB format.

Protein–ligand docking

Protein–ligand docking studies were performed using AutoDock4.2 [23]. This is one of the most widely used methods for protein–ligand docking. All the preprocessing steps for ligand and protein files were performed using the AutoDock Tools 1.5.4 program (ADT), which had been released as an extension suite to the Python Molecular Viewer [23]. The ADT program was used to prepare receptor molecules [high-affinity INH–NAD(P)-binding proteins of MTB] by adding all hydrogen atoms to carbon atoms of the receptor; in addition, Kollman charges were also assigned. For docked ligands, nonpolar hydrogens were also added. Gasteiger charges were assigned and torsions degrees of freedom were allocated by the ADT program.

The Lamarckian genetic algorithm (LGA) was applied to model the interaction pattern between receptors and ligand. The grid maps representing the receptor proteins in the docking process were prepared using AutoGrid (part of the AutoDock package). A grid of 40, 40, and 40 points in the x, y, and z directions was centered on the known active-site residues of each protein. For all docking procedures, 10 independent genetic algorithm (GA) runs with a population size of 150 were considered for each molecule studied. A maximum number of 25×10^5 energy evaluations; 27,000 maximum generations; a gene mutation rate of 0.02, and a crossover rate of 0.8 were used for LGA [16]. The AutoDock program was run to prepare the corresponding docking log (.dlg) file for further analysis.

Visualization

The visualization of structure files was carried out using the graphical interface of the ADT program and PyMol molecular graphics system (www.pymol.org).

Results

Docking analysis of high-affinity INH–NAD(P)-binding proteins of MTB and truncated INH–NAD adduct

In this study, docking analysis was performed between selected INH–NAD(P)-binding proteins and truncated INH–NAD adduct around known active-site residues of respective proteins. Among the six selected proteins, the truncated INH–NAD adduct binds to Rv2971 (Fig. 3A) with lowest binding energy of −7.07 kcal/mol and with inhibition constant of 6.62 μM (Table 2). The docking of Rv2623 with the truncated INH–NAD adduct was performed around reported substrate-binding-site residues Asp167 and Asp198. The adduct binds to Rv2623 with binding energy of −7.06 kcal/mol and inhibition constant of 6.72 μM. It forms three hydrogen bonds with these binding-site residues (Fig. 3B). Glu218, Lys248, and Asp252 are known binding-site residues of Rv3248c. Rv3248c binds to the truncated INH–NAD adduct (Fig. 3C) with binding energy of −7.05 kcal/mol and inhibition constant of 6.83 μM. We also performed a docking analysis between InhA and truncated INH–NAD adduct around known substrate-binding residue Tyr158 [24]. The truncated INH–NAD adduct binds to InhA with binding energy of –6.86 kcal/mol and with inhibition constant of 9.30 μM. It also formed one hydrogen bond with known binding-site residue Tyr158 (Fig. 3D).

The other two proteins (Rv2763c and Rv1187) interact with the truncated INH–NAD adduct (Fig. 3E and F) with binding energy of −6.42 and −5.29 kcal/mol, respectively, and inhibition constant of 19.57 and 132.67 μM, respectively.

Discussion

Mahapatra et al. [14] observed the formation of a truncated INH–NAD adduct due to spontaneous hydrolysis of the INH–NAD^+ adduct under *in vitro* INH activation condition and stated that there was a possibility of enzymatic hydrolysis of the INH–NAD^+ adduct *in vivo*, because of the presence of several NAD-degrading enzymes in mammals. Thus, to study the potential antimycobacterial activity of the truncated INH–NAD adduct, we applied the same principle for the docking analysis of MTB enzymes with the reported INH–NAD-binding proteins using AutoDock4.2. The scoring algorithm of AutoDock signifies that lower the binding energy (binding energy is given in the negative sign) better the binding affinity [25]. AutoDock is tested as reliable software to precisely predict the binding affinity of a ligand to a protein [23,25]. Of the six selected proteins, three proteins, namely, Rv2971 (oxidoreductase), Rv2623 (universal stress protein), and Rv3248c (S-adenosyl-L-homocysteine hydrolase), were found to interact with the truncated INH–NAD adduct with almost similar binding energies (Table 2).

The protein Rv2971 belongs to the aldo–keto reductases family of MTB and plays an important role in the detoxification of toxic metabolites. Recently, it has been reported as a new target of INH [26], which correlates with our docking study results, which showed inhibition of its activity by the truncated INH–NAD adduct with minimum binding energy of −7.07 kcal/mol. A universal stress protein encoded by MTB Rv2623 helps in regulation of mycobacterial growth both *in vitro* and *in vivo* [24]. It also binds adenosine triphosphate (ATP) and may function as an ATP-dependent signaling intermediate in a pathway that promotes persistent infection [24]. Jain et al. [27] also suggested Rv2623 as a potential biomarker for the diagnosis of the latent as well as active MTB

Table 2 – Docking analysis of the truncated INH–NAD adduct with MTB Proteins.

Locus	Protein	Known active-site residues	Binding energy (kcal/mol)	Inhibition constant (μM)
Rv2971	Oxidoreductase	Tyr57 His115	−7.07	6.62
Rv2623	Universal stress protein	Asp167, Asp198	−7.06	6.72
Rv3248c	S-adenosyl-L-homocysteine hydrolase	Thr71, Asp156, Glu218, Lys248, Asp252, Asn253, Glu305, Asn340, Asn409	−7.05	6.83
Rv1484	NADH-dependent enoyl-(ACP) reductase	Tyr158	−6.86	9.30
Rv2763c	Dihydrofolate reductase	Asp27, Arg32, Arg60, Gly80, Tyr100, Thr113	−6.42	19.57
Rv1187	Pyrroline-5-carboxylate dehydrogenase	Lys212, Ser249, Cys327, Phe427	−5.29	132.67

Note: ACP = acyl carrier protein; INH = isoniazid; MTB = *Mycobacterium tuberculosis*; NAD = nicotinamide adenine dinucleotide; NADH = nicotinamide adenine dinucleotide (reduced).

Table 3 – Amino acid residues in the active site of INH–NAD-binding proteins.

Protein	Active-site residues					Known catalytic triad
	Acidic	Basic	Nucleophilic Neutral (polar) Uncharged	Aromatic	Aliphatic (nonpolar) neutral	
Rv2971	Asp52 Glu274	Arg273, Lys82, His115	Tyr57, Tyr194, Tyr279, Gln168, Ser146, Asn147, Trp116, Thr282	Trp116	Ala56	Tyr, Lys, Asp (Reported in [32])
Rv2623	Asp198, Asp167	Arg266, Arg264, His194	Ser172, Pro245		Gly165, Gly265, Val166, Val277, Leu193, Leu249, Ala195	
Rv3248c	Asp156, Asp252, Glu218	His69, His416, Lys248	Thr219, Asn409, Asn253, Ser424, Thr71	Phe425	Val286, Met421, Gly415, Leu68, Leu410, Leu407	Lys248, Asp252, Asn253 (Probable)
Rv1484	Asp148	Lys165	Tyr158, Pro193, Thr196	Tyr158, Phe149	Met147, Met199, Ala191, Ile194, Gly192	Tyr158, Lys165, Phe149 (Reported in [33])
Rv2763c	Asp27, Glu111	His30, Arg23	Thr113, Gln28, Tyr100, Trp6	Phe31	Lue24, Ile5, Ala7	His30, Asp27 (Probable)
Rv1187	Asp244	His272, Lys212	Thr269, Trp276, Tyr184, Ser249	Phe246	Ile186, Gly245, Leu273, Leu253	

infections. *In silico* inhibition of Rv2623 by the truncated INH–NAD adduct with formation of H-bond with the ATP-binding-site residues of the Rv2623 suggests the truncated adduct as possible inhibitor of the protein. Rv3248c codes S-adenosyl-L-homocysteine hydrolase (Mtb-SahH) in MTB, which is an essential enzyme for optimal growth of the pathogen and is involved in intermediary metabolism. Singhal et al. [28] also revealed its involvement in regulating homocysteine concentration in surrogate host *M. smegmatis* [28]. The truncated adduct also observed to inhibit Rv3248c. INH was reported to act on MTB by inhibiting a 2-*trans*-enoyl-(ACP) reductase (InhA), which is encoded by Rv1484 [4]. The INH–NAD adduct was also demonstrated to be a capable InhA inhibitor [29,30]. This *in silico* docking study correlates with an earlier *in vitro* study by Nguyen et al. [30], who reported the INH–NAD adduct as a powerful inhibitor of InhA. MTB DHFR encoded by Rv2763c was reported as an essential enzyme for nucleic acid synthesis. Argyrou et al. [20] observed the inhibition of MTB DHFR by acyclic 4*R* isomer of the INH–nicotinamide adenine dinucleotide phosphate adduct. The pyrroline-5-carboxylate dehydrogenase encoded by Rv1187 was shown to be involved in the proline-utilization pathway in MTB and this pathway could be a valuable therapeutic target against TB [31]. All the six proteins of MTB are found to be essential for the pathogenic organism and our *in silico* docking study also revealed the effective inhibition of these proteins by the truncated INH–NAD adduct, which can explain the effectiveness of INH as an anti-TB drug.

Further, upon active-site analysis of these six proteins, we observed that of the six proteins, two proteins (Rv2971 and Rv1484) have known catalytic triad in their active site (Table 3). Three amino acids (Tyr57, Lys82, and Asp52) were reported as catalytic triad in Rv2971 [32], whereas Tyr158, Lys165, and Phe149 act as catalytic triad in Rv1484 [33]. A catalytic triad usually refers to the three amino acid residues that function together at the center of the active site of certain hydrolases and transferases (e.g., proteases, amidases, esterases, acylases, lipases, and β-lactamases). Gupta et al. [34] observed that in case of catalytic triad, the hydrogen bond distance between the H^{γ} atom nucleophilic residue (such as Ser H^{γ}) and $N^{\varepsilon 2}$ atom of basic residue (such as His) is more flexible in nature and varied from 2.0 to 2.7 Å, whereas the distance between $H^{\delta 1}$ of basic (such as His) and $O^{\delta 1}$ of acidic residues (e.g., Asp) varied from 1.6 to 2.0 Å. Based on the H-bond distance criteria, two other proteins, namely, Rv3248c and Rv2763c, are proposed to have probable catalytic triad (Table 3). The catalytic triad may have some important role in INH binding and needs to be further investigated.

Conclusion

We have applied the computational approach to study the interaction between truncated INH–NAD adduct and INH–NAD(P)-binding proteins. The *in silico* docking study revealed that the truncated adduct might indeed bind to the selected proteins. Further, the interaction between the truncated adduct and these proteins indicates the presence of a catalytic triad in the active site of some of these proteins, which may possibly play a role in INH binding, but this needs further investigation. In this study, we have included only those proteins with known experimentally determined 3D structure. The interaction of other INH–NAD(P)-binding proteins, whose 3D structures are not available, needs to be explored further to understand the mechanism of action of INH as an anti-TB drug acting on multiple enzymes.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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REFERENCES

[1] World Health Organization, WHO Global Tuberculosis Report 2014, World Health Organization, Geneva, Switzerland, 2014.
[2] M.C. Raviglione, D.E. Snider Jr., A. Kochi, Global epidemiology of tuberculosis. Morbidity and mortality of a worldwide epidemic, JAMA 273 (1995) 220–266.
[3] N.S. Shah, A. Wright, G.H. Bai, et al, Worldwide emergence of extensively drug-resistant tuberculosis, Emerg. Infect. Dis. 13 (2007) 380–387.
[4] H. Marrakchi, G. Lanéelle, A. Quémard, InhA, a target of the antituberculous drug isoniazid, is involved in a mycobacterial fatty acid elongation system, FAS-II, Microbiology 146 (2000) 289–296.
[5] Isoniazid Tablets. Available from: <http://www.rxlist.com/isoniazid-drug/clinical-pharmacology.htm> (last accessed 15.07.2014).
[6] H.A. Shoeb, B.U. Bowman Jr., A.C. Ottolenghi, et al, Peroxidase-mediated oxidation of isoniazid, Antimicrob. Agents Chemother. 27 (1985) 399–403.
[7] G.S. Timmins, S. Master, F. Rusnak, et al, Nitric oxide generated from isoniazid activation by KatG: source of nitric oxide and activity against *Mycobacterium tuberculosis*, Antimicrob. Agents Chemother. 48 (2004) 3006–3009.
[8] R. Rawat, A. Whitty, P.J. Tonge, The isoniazid-NAD adduct is a slow, tight-binding inhibitor of InhA, the *Mycobacterium tuberculosis* enoyl reductase: adduct affinity and drug resistance, Proc. Natl. Acad. Sci. USA 100 (2003) 13881–13886.
[9] K. Johnsson, D. King, P. Schultz, Studies on the mechanism of action of isoniazid and ethionamide in the chemotherapy of tuberculosis, J. Am. Chem. Soc. 117 (1995) 5009–5010.
[10] L.C. Santos, Review: the molecular basis of resistance in *Mycobaterium tuberculosis*, Open J. Med. Microbiol. 2 (2012) 24–36.
[11] V. Bernardes-Génisson, C. Deraeve, A. Chollet, et al, Isoniazid: an update on the multiple mechanisms for a singular action, Curr. Med. Chem. 20 (2013) 4370–4385.
[12] J. Sandy, S. Holton, E. Fullam, et al, Binding of the anti-tubercular drug isoniazid to the arylamine *N*-

acetyltransferase protein from *Mycobacterium smegmatis*, Protein Sci. 14 (2005) 775–782.
[13] J.L. Stigliani, P. Arnaud, T. Delaine, et al, Binding of the tautomeric forms of isoniazid-NAD adducts to the active site of the *Mycobacterium tuberculosis* enoyl-ACP reductase (InhA): a theoretical approach, J. Mol. Graph. Model. 27 (2008) 536–545.
[14] S. Mahapatra, L.K. Woolhiser, A.J. Lenaerts, et al, A novel metabolite of antituberculosis therapy demonstrates host activation of isoniazid and formation of the isoniazid-NAD^+ adduct, Antimicrob. Agents Chemother. 56 (2012) 28–35.
[15] T. Delaine, V. Bernardes-Génisson, A. Quémard, et al, Development of isoniazid-NAD truncated adducts embedding a lipophilic fragment as potential bi-substrate InhA inhibitors and antimycobacterial agents, Eur. J. Med. Chem. 45 (2010) 4554–4561.
[16] L. Jena, P. Waghmare, S. Kashikar, et al, Computational approach to understanding the mechanism of action of isoniazid, an anti-TB drug, Int. J. Mycobacteriol. 3 (2014) 276–282.
[17] M. Payton, R. Auty, R. Delgoda, et al, Cloning and characterization of arylamine *N*-acetyltransferase genes from *Mycobacterium smegmatis* and *Mycobacterium tuberculosis*: increased expression results in isoniazid resistance, J. Bacteriol. 181 (1999) 1343–1347.
[18] M. Payton, C. Gifford, P. Schartau, et al, Evidence towards the role of arylamine *N* acetyltransferase in *Mycobacterium smegmatis* and development of a specific antiserum against the homologous enzyme of *Mycobacterium tuberculosis*, Microbiology 147 (2001) 3295–3302.
[19] J. Bodiguel, J.M. Nagy, K.A. Brown, et al, Oxidation of isoniazid by manganese and *Mycobacterium tuberculosis* catalase peroxidase yields a new mechanism of activation, J. Am. Chem. Soc. 123 (2001) 3832–3833.
[20] A. Argyrou, L. Jin, L. Siconilfi-Baez, et al, Proteome-wide profiling of isoniazid targets in *Mycobacterium tuberculosis*, Biochemistry 45 (2006) 13947–13953.
[21] INH-NAD Adduct. Available from: <https://pubchem.ncbi.nlm.nih.gov/compound/449263> (last accessed 18.05.2015).
[22] A.W. Schüttelkopf, D.M. van Aalten, PRODRG: a tool for high-throughput crystallography of protein-ligand complexes, Acta Crystallogr. D Biol. Crystallogr. 60 (2004) 1355–1363.
[23] G.M. Morris, R. Huey, W. Lindstrom, et al, AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility, J. Comput. Chem. 30 (2009) 2785–2791.
[24] J.E. Drumm, K. Mi, P. Bilder, et al, *Mycobacterium tuberculosis* universal stress protein Rv2623 regulates bacillary growth by ATP-binding: requirement for establishing chronic persistent infection, PLoS Pathog. 5 (2009) e1000460.
[25] A.D. Hill, P.J. Reilly, Scoring functions for AutoDock, Methods Mol. Biol. 1273 (2015) 467–474.
[26] A. Shahine, A. Prasetyoputri, J. Rossjohn, et al, A structural characterization of the isoniazid *Mycobacterium tuberculosis* drug target, Rv2971, in its unliganded form, Acta Crystallogr. F Struct. Biol. Commun. 70 (2014) 572–577.
[27] R.K. Jain, A.R. Nayak, A.A. Husain, et al, Mycobacterial dormancy regulon protein Rv2623 as a novel biomarker for the diagnosis of latent and active tuberculous meningitis, Dis. Markers 35 (2013) 311–316.
[28] A. Singhal, G. Arora, A. Sajid, et al, Regulation of homocysteine metabolism by *Mycobacterium tuberculosis* S-adenosylhomocysteine hydrolase, Sci. Rep. 3 (2013) 2264.
[29] D.A. Rozwarski, G.A. Grant, D.H. Barton, et al, Modification of the NADH of the isoniazid target (InhA) from *Mycobacterium tuberculosis*, Science 279 (1998) 98–102.
[30] M. Nguyen, A. Quemard, S. Broussy, et al, Mn(III) pyrophosphate as an efficient tool for studying the mode of action of isoniazid on the InhA protein of *Mycobacterium tuberculosis*, Antimicrob. Agents Chemother. 46 (2002) 2137–2144.
[31] T. Lagautriere, G. Bashiri, N.G. Paterson, et al, Characterization of the proline-utilization pathway in *Mycobacterium tuberculosis* through structural and functional studies, Acta Crystallogr. D Biol. Crystallogr. 70 (2014) 968–980.
[32] A.H. Ehrensberger, D.K. Wilson, Structural and catalytic diversity in the two family 11 aldo–keto reductases, J. Mol. Biol. 337 (2004) 661–673.
[33] D.A. Rozwarski, C. Vilchèze, M. Sugantino, et al, Crystal structure of the *Mycobacterium tuberculosis* enoyl-ACP reductase, InhA, in complex with NAD^+ and a C16 fatty acyl substrate, J. Biol. Chem. 274 (1999) 15582–15589.
[34] V. Gupta, N.A. Prakash, V. Lakshmi, et al, Recognition of active and inactive catalytic triads: a template based approach, Int. J. Biol. Macromol. 46 (2010) 317–323.