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Binding of the tautomeric forms of isoniazid-NAD adducts to the active site of the *Mycobacterium tuberculosis* enoyl-ACP reductase (InhA): A theoretical approach

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ABSTRACT

The front-line antituberculosis drug isoniazid (INH) inhibits InhA, the NADH-dependent fatty acid biosynthesis enoyl ACP-reductase from *Mycobacterium tuberculosis*, via formation of covalent adducts with NAD (INH-NAD adducts). While ring tautomers were found the main species formed in solution, only the 4*S* chain INH-NAD tautomer was evidenced in the crystallized InhA:INH-NAD complex. In this study we attempted to explore the modes of interaction and energy binding of the different isomers placed in the active site of InhA with the help of various molecular modelling techniques. Ligand and enzyme models were generated with the help of the Vega ZZ program package. Resulting ligands were then docked into the InhA active site individually using computational automated docking package AUTODOCK 3.0.5. The more relevant docked conformations were then used to compute the interaction energy between the ligands and the InhA cavity. The AM1 Hamiltonian and the QM/MM ONIOM methodologies were used and the results compared. The various tautomers were found docked in almost the same place where INH-NAD was present as predicted by earlier X-ray crystallographic studies. However, some changes of ligand conformation and of the interactions ligand–protein were evidenced. The lower binding energy was observed for the 4*S* chain adduct that probably represents the effective active form of the INH-NAD adducts, as compared to the 4*R* epimer. The two 4*S*,7*R* and 4*R*,7*S* ring tautomers show intermediate and similar binding energies contrasting with their different experimental inhibitory potency on InhA. As a possible explanation based on calculated conformations, we formulated the hypothesis of an initial binding of the two ring tautomers to InhA followed by opening of only the ring hemiamidal 4*S*,7*R* tautomer (possibly catalyzed by Tyr158 phenolate basic group) to give the 4*S* chain INH-NAD tight-binding inhibitor. The predictions of ligand–protein interactions at the molecular level can be of primary importance in elucidating the mechanisms of action of isoniazid and InhA-related resistances, in identifying the effective mycobactericidal entities and, in further step, in the design of a new generation of antitubercular drugs.

1. Introduction

Isoniazid (INH) (Fig. 1) is one of the most common and efficient drugs used in treatment of tuberculosis [1,2]. After activation by the catalase-peroxydase KatG [3–7], it inhibits enoyl-acyl carrier protein reductase InhA, an enzyme involved in the fatty acid biosynthesis (FAS II system), which is an essential process to elaborate important cellular components of *Mycobacterium tuberculosis* [8,9].

Data from X-ray crystallography [10], mass spectrometry [11] and isotopic experiments [12] reveal that the mechanism of the inhibition involves a covalent attachment of the activated form of the drug (isonicotinoyl radical) to the nicotinamide ring of nicotinamide adenine dinucleotide NAD(H) modifying thereby the nature of the coenzyme and its interaction with the target enzyme InhA. Recent studies have suggested that the 1,4-dihydropyridine INH-NAD adduct exhibits the keto-carboxamide structure **3** (Fig. 2) [10,13,14]. On the other hand, our group has reported that the biomimetic activation of INH with manganese(III) pyrophosphate in the presence of the coenzyme NAD^+ **1** (Fig. 1) results mainly in formation of the ring hemiamidal dihydropyridines **6** and **7** (with creation of two new chiral centers

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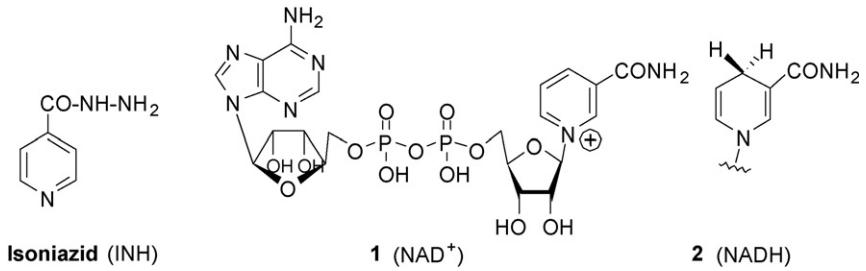

Fig. 1. Structures of isoniazid and of the two redox cofactors NAD^+ and NADH.

at C-4 and C-7 to give four diastereoisomers) along with the chain keto-amide structures **3** and **4** as minor compounds (two epimers 4*S* and 4*R*) [15,16]. It is interesting to note that a purified pool of all these adducts have been shown to be effective inhibitors of InhA [17]. The key structural factors involved in the ring–chain tautomerism equilibrium has been discussed on a series of simplified analogues of isoniazid-NAD adducts [18]. Rawat et al. [19] have reported that the analogue of INH-NAD adduct derived from metabolic activation of benzoylhydrazine (BH-NAD **5**; Fig. 2), with a proposed chain (keto-amide) structure, behaves also as a potent inhibitor of InhA. On the other hand, slow oxidation of the dihydropyridine INH-NAD adducts can afford oxidized derivatives of a pyridinium type which structure was initially proposed to be **10** [13]. However, our recent results show unambiguously their existence under only the two epimeric ring structures **8** and **9** [16].

Recently, it was suggested that the difference between bactericidal (FAS I and FAS II systems) and mammalian (FAS I system) fatty acid biosynthesis makes InhA an attractive molecular target whose selective inhibition is sought in the development of antibiotics with new mechanism of action. In addition, it was observed that the high prevalence of resistances to INH was mainly due to KatG mutants that could not activate isoniazid. Hence, direct inhibitors of InhA not KatG-dependent have been considered as promising antitubercular agents.

Computer-aided drug design approaches are a powerful tool for a better knowledge of the biological effects of molecules. In a recent study [20], Pasqualoto et al. performed a 4D-QSAR analysis of a set of INH analogues hydrazides, which led the authors to develop a 3D pharmacophore model. The resulting hypothesized active conformations were then used as point of departure in molecular dynamics simulations to generate a 3D-QSAR model [21], which allowed them to identify the critical thermodynamic descriptors. In another recent article [22], Bonnac et al. carried out comparative docking experiments of chain INH-NAD adducts and the 4-phenoxybenzamide adenine dinucleotide analogue in interaction with InhA. As part of our effort to develop direct inhibitors of InhA based on the understanding of isoniazid mechanism [23–25] and since computer-aided drug design approaches are widely used to simulate molecular interactions of large systems, we envision in this work to compute the molecular interactions associated with the binding of the chain and ring INH-NAD tautomers to InhA. These studies can be of primary importance in to elucidate the mechanism of action of isoniazid and to better understand the isoniazid-dependent resistances. They can also prove useful in the design of a new generation of antitubercular drugs.

2. Materials and methods

All calculations were performed on a SGI Altix 3700 cluster at Toulouse University Computer Center (France) and locally on PC workstations.

2.1. Protein and ligands structures

The coordinates of INH-NAD adduct complexed to InhA were taken from the Brookhaven Protein Data Bank (PDB code 1ZID) [10]. The adduct was extracted from the PDB file and hydrogen atoms were added to the protein and oriented using the MolProbity software [26]. Hydrogens were added on the crystal structure of INH-NAD adduct and energy-minimized using the AMMP [27] force field implemented on the VEGA ZZ [28] molecular modelling package. The coordinates of the other studied adducts were built from the NADH moiety of the INH-NAD which was kept unchanged. Only the variable part of the molecules was energy-minimized.

2.2. Autodock docking study

The docking studies were performed with the program Autodock version 3.0.5 [29]. Water molecules were discarded, except those surrounding the binding site (i.e. water molecules

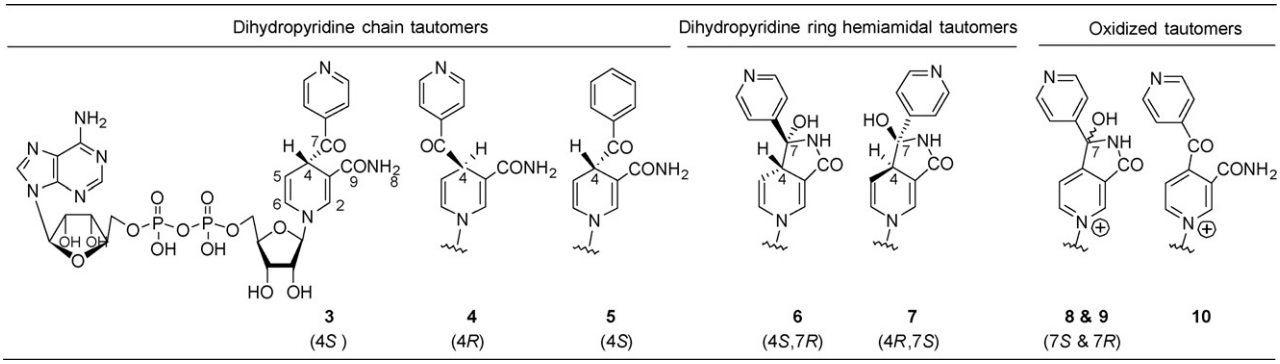

Fig. 2. Structures of compounds **3**–**10** with mention of the proposed stereochemistry of new chiral centers. Dihydropyridine ring hemiamidal tautomers 4*S*,7*S* and 4*R*,7*R* were only detected as traces and are not studied here.