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Review article

An overview on crystal structures of InhA protein: Apo-form, in complex with its natural ligands and inhibitors

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ABSTRACT

The enoyl-ACP reductase InhA from the mycobacterial fatty acid biosynthesis pathway has become a target of interest for the development of new anti-tubercular drugs. This protein has been identified as essential for the survival of *Mycobacterium tuberculosis*, the causative agent of tuberculosis, and as the main target of two pro-drugs: isoniazid, the frontline anti-tubercular drug, and ethionamide, a second-line medicine. Since most cases of resistance to isoniazid and ethionamide result from mutations in the mycobacterial activating enzyme (KatG for isoniazid and EthA for ethionamide), research of direct InhA inhibitors, avoiding the activation step, has emerged as a promising strategy for combating tuberculosis. Thereby, InhA is drawing much attention and its three-dimensional structure has been particularly studied. A better understanding of key sites of interactions responsible for InhA inhibition arises thus as an essential tool for the rational design of new potent inhibitors. In this paper, we propose an overview of the 80 available crystal structures of wild-type and mutant InhA, in its *apo* form, in complex with its cofactor, with an analogue of its natural ligands (C16 fatty acid and/or NADH) or with inhibitors. We will first discuss structural and mechanistic aspects in order to highlight key features of the protein before delivering thorough inventory of structures of InhA in the presence of synthetic ligands to underline the key interactions implicated in high affinity inhibition.

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1. Introduction

Tuberculosis (TB) remains one of the leading infectious diseases around the world. The phenomena of co-infection with the Human Immunodeficiency Virus (HIV) and the occurrence of a global bacterial resistance made TB reappear as a re-emerging disease during last decades. According to World Health Organization (WHO) report, in 2015, about 10.4 million persons were newly infected and 1.8 million persons died because of TB infection [1].

Mycobacterium tuberculosis, the bacteria responsible for the infection, possesses an unusual cell wall, particularly rich in lipids (about 60% in weight). Mycolic acids, C_{60}-C_{90} long α-alkyl β-hydroxylated fatty acids (Fig. 1A), are the major components of the mycobacterium cell wall and are essential for its survival. Their precursors are biosynthesized by Fatty Acid Synthesis (FAS) systems of type I and type II (FAS-I and FAS-II, Fig. 1B). It is important to note that the FAS–II pathway is not present in humans and consequently, targeting of the proteins involved in this pathway arises as a hopeful strategy to develop new anti-tubercular drugs.

Among these enzymes, InhA belongs to the short-chain dehydrogenase/reductase (SDR) family of enzyme, more particularly to the NADH-dependent enoyl-ACP reductase family (EC 1.3.1.9). It catalyzes the reduction of the *trans* double bond conjugated to a carbonyl group of an intermediate covalently linked to an acyl-carrier-protein in the FAS-II pathway (Fig. 1C). Residues Phe149 and Lys165 were found to be crucial for the binding of NADH cofactor [2,3], and residue Tyr158 contributes to the stabilization of the enolate intermediate of the reaction [3]. In 2006, Vilchèze et al. [4] identified for the first time the critical role of InhA to the survival of the mycobacteria. They demonstrated that thermal inactivation of InhA in *M. tuberculosis* resulted in the inhibition of mycolic acids biosynthesis, leading to a morphological change of the bacteria and to cell lysis. In addition, InhA protein has been identified as the target of the first-line anti-tuberculosis pro-drug, isoniazid, and also of the second-line pro-drug ethionamide [5]. Furthermore, most cases of *Mycobacterium tuberculosis* strains resistant to isoniazid and ethionamide result from mutations of KatG and EthA, respectively, mycobacterial enzymes responsible for pro-drugs activation. Based on these informations, InhA has become a target of interest and many groups have focused their research on developing new direct anti-InhA compounds.

2. Generalities

Since the early 1990's, the field of structure-based drug design has rapidly grown and medicinal chemists have used tridimensional depiction of protein-ligand complexes to get structural information on their interactions [6]. X-ray crystallography is one of the major techniques developed in structural biology, and the improvement of the technology during the past decades allowed high structural resolution of protein.

With reference to the InhA protein, the first crystal structure was published in 1995 [7] and there are currently 80 structures reported in the Protein Data Bank (PDB). The InhA protein has been crystallized under its *apo* form (without ligands), in complex with NADH/NAD^+ alone and along with its substrate or inhibitors.

All crystallographic structures *of M. tuberculosis* InhA are reported in Table 1. They are sorted according to the chemical structures of the bound compound and corresponding biological activities (IC_{50} (InhA) and MIC (*M. tuberculosis*)) are included as well, when available.

3. APO-FORM of the InhA protein

Although InhA has been known as a potent therapeutic target for a long time, it was only recently that the structure of the *apo* form of wild-type InhA was determined [8]. The 1.8 Å resolution structure (PDB 4TRM) displays six molecules in the asymmetric unit, four of them forming a tetramer that obeys 222 symmetry [8]. Such tetrameric arrangement results in the formation of 2 types of interface. The first type of interface buries approximately 1474 $Å^2$ of accessible surface and allows the formation of an antiparallel 4-