RESEARCH ARTICLE

Probing the architecture of the *Mycobacterium marinum* arylamine N-acetyltransferase active site

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ABSTRACT

Treatment of latent tuberculosis infection remains an important goal of global TB eradication. To this end, targets that are essential for intracellular survival of *Mycobacterium tuberculosis* are particularly attractive. Arylamine N-acetyltransferase (NAT) represents such a target as it is, along with the enzymes encoded by the associated gene cluster, essential for mycobacterial survival inside macrophages and involved in cholesterol degradation. Cholesterol is likely to be the fuel for *M. tuberculosis* inside macrophages. Deleting the *nat* gene and inhibiting the NAT enzyme prevents survival of the microorganism in macrophages and induces cell wall alterations, rendering the mycobacterium sensitive to antibiotics to which it is normally resistant. To date, NAT from *M. marinum* (MMNAT) is considered the best available model for NAT from *M. tuberculosis* (TBNAT). The enzyme catalyses the acetylation and propionylation of arylamines and hydrazines. Hydralazine is a good acetyl and propionyl acceptor for both MMNAT and TBNAT. The MMNAT structure has been solved to 2.1 Å resolution following crystallisation in the presence of hydralazine and is compared to available NAT structures. From the mode of ligand binding, features of the binding pocket can be identified, which point to a novel mechanism for the acetylation reaction that results in a 3-methyltriazolo[3,4-a]phthalazine ring compound as product.

KEYWORDS *Mycobacterium tuberculosis*, *Mycobacterium marinum*, tuberculosis, arylamine N-acetyltransferase, 3D crystal structure, binding pocket

INTRODUCTION

Tuberculosis (TB) remains one of the leading causes of death by bacterial infection. *Mycobacterium tuberculosis*, the etiological agent of TB, infects one-third of the human population and is responsible for approximately 2 million deaths annually (WHO, 2009). *M. tuberculosis* is characterized by its inherent resistance to antibiotics due to its extremely slow growth rate and the complex lipid composition of its cell wall, in particular the mycolic acid constituent (Smith, 2003). These unique properties allow *M. tuberculosis* to survive for long periods of time, and even replicate inside macrophages. Contained within granulomas, and utilizing endogenous compounds as fuel (Pandey and Sassetti, 2008), *M. tuberculosis* can persist for decades without causing any symptoms in a form known as latent infection (Manabe and Bishai, 2000). Latent infection can progress to active TB if the host defenses are perturbed. The best-known factor driving progression of latent TB toward active infection is HIV co-infection (McShane, 2005). Treatment of latent TB is an important TB control strategy but is usually complicated by the insensitivity of the dormant bacilli to antibiotics, the lack of means to ascertain the success of treatment, and the increased risk of drug resistance (Dooley and Sterling, 2005). Treatment of TB with chemotherapeutic agents is likely to remain as the cornerstone of patient management for the foreseeable future, necessitating the development of novel, non-toxic antituberculosis agents with potent sterilizing activity (Rivers and Mancera, 2008; Brown et al., 2009; Gumbo et al., 2009; Makarov et al., 2009).

Arylamine N-acetyltransferase (NAT) is a cytosolic enzyme that is found in *M. tuberculosis* and many other prokaryotes (Sim et al., 2008). This enzyme catalyzes the transfer of an

acetyl group from acetyl-CoA (AcCoA) to an arylamine species through a vital conserved cysteine residue by a 'Ping-Pong' bi-bi mechanism (Sinclair et al., 2000). Within *M. tuberculosis*, the NAT enzyme is transcribed as part of an operon that contains genes encoding a group of enzymes involved in sterol ring degradation, including: HsaA (an oxidoreductase), HsaD (a C-C bond hydrolase), HsaC (an extradiol dioxygenase), HsaB (a hydroxylase) and a pseudogene (Rengarajan et al., 2005; Anderton et al., 2006; Yam et al., 2009). The NAT enzyme was found to be able to utilize the cholesterol metabolite n-propionyl-CoA, linking it to cholesterol catabolism (Flynn and Chan, 2003; Lack et al., 2009).

The products of this operon were shown to be essential for survival of mycobacteria inside macrophages and to be involved in cholesterol degradation, highlighting the importance of these enzymes in latent TB. Cholesterol is likely to be the fuel used by the microorganism inside macrophages (Pandey and Sasseti, 2008).

Deleting the *nat* gene prevents survival of bacilli in macrophages and induces an altered cell wall, rendering the microorganism sensitive to antibiotics to which it is normally resistant (Bhakta et al., 2004). NAT inhibitors were demonstrated to have similar effects to that of deleting the gene (Westwood, 2005; Westwood et al., 2006), suggesting the validity of NAT as a potential anti-tubercular target. Interestingly, as prokaryotic NATs show a different substrate profile compared to eukaryotic enzymes (Westwood et al., 2006), it should be possible to design NAT inhibitors that are selectively toxic to mycobacteria and screen protocols have allowed such specificity to be incorporated (Westwood et al., 2010).

The NAT enzyme from *M. tuberculosis* has been difficult to prepare as a recombinant enzyme, although progress is being made (Sikora et al., 2008; Lack et al., 2009). To date, NAT from *M. marinum* (MMNAT) is considered the best available model for TBNAT. The enzyme is 73% identical to TBNAT (Supplemental Fig. 1) (Fullam et al., 2008, 2009). Both enzymes show comparable substrate specificity profiles, especially in their selectivity to hydralazine (HLZ) as an acetyl group acceptor, with K_m values of 60 μ M in TBNAT (Sikora et al., 2008; Fullam et al., 2009) compared with 36 μ M in MMNAT (Fullam et al., 2009). Recently, the 3D structures of native MMNAT protein and its complex with CoA have been determined (Fullam et al., 2008, 2009). To help elucidate the interactions of the enzyme with ligand, structural changes occurring upon binding of substrate have been investigated by protein crystallography. Here we present the three-dimensional structure of the MMNAT-HLZ complex solved to 2.1 Å resolution and compare it with available NAT structures to facilitate understanding of ligand binding.

RESULTS AND DISCUSSION

Wild-type MMNAT was co-crystallized with HLZ, and crystals of the complex, belonging to space group P4₁2₁2, were

obtained using condition E2 of the customised screen (see Materials and Methods). Numerous other conditions in the same screen yielded crystals that were too small for data collection. The structure was solved by molecular replacement (MR) and the final model contains only one molecule per asymmetric unit with a Matthews coefficient of 1.95 Å³ Da⁻¹, corresponding to a solvent content of ~37% (Matthews, 1968). Upon refinement of the model (Supplemental Table 1), high difference electron density was observed close to the conserved catalytic triad (Cys70, His110 and Asp127) which is consistent with the presence of HLZ (Supplemental Fig. 2A). The quality of the electron-density map allowed HLZ to be unambiguously oriented in the model. An additional relatively weaker difference electron density was observed at another position located in the space between the pairs of β -hairpins of the opposite β -sheet flaps of the protein. Another HLZ molecule was docked into the secondary binding pocket with an alternate conformation to fit the negative peaks in the $F_o - F_c$ electron density maps (Supplemental Fig. 2B).

The structure is similar to a previously determined native MMNAT structure (Fullam et al., 2008, 2009), and consists of three domains: domain I is an α -helical bundle, domain II forms a β -barrel and domain III forms the α/β lid (Fig. 1A). A comparison between the C α backbones of the native MMNAT and MMNAT co-crystallized with HLZ shows that these backbones are almost superimposable and have an RMSD of only 0.281 Å (DS Visualizer <http://accelrys.com>). The backbones do, however, differ in some of the superficial hairpin loops that were reported to be mobile regions (Fullam et al., 2008) (Fig. 1B).

Hydralazine binding pocket

NAT enzymes are characterized by having a wide active site that can accommodate the extended coenzyme A moiety of AcCoA. This site occupies a volume of ~860 Å³ and extends between the flaps of the two β -sheets of the protein, forming an L-shaped cleft (Fig. 1B).

HLZ (IUPAC name: phthalazin-1-ylhydrazine) binds in a plane parallel to the β -sheet lid covering the active-site cleft, where it occupies an inner hydrophobic pouch of about 200 Å³ within the binding pocket (Fig. 2). This sub-pocket consists mainly of aromatic amino acids including Phe38, Tyr69, Val95, Trp97, Phe130, Phe204 and Met209, which form the bottom and the lid of this pocket, while the middle part is composed of hydrogen-bond forming groups of Cys70, Thr109, Gly129 and Gly131 and a single explicit water molecule (Fig. 2B). The water molecule is held in place by forming a net of hydrogen bonds with Val95 and Thr109 and is observed in other MMNAT structures. The residues constituting this binding space are shown in Supplemental Table 2 with their average B-factors. All these amino acids are either conserved within mycobacterial NATs or conservatively

Figure 1. MMNAT-HLZ complex and a comparison of MMNAT, MMNAT-CoA complex and MMNAT-HLZ complex. (A) The schematic presentation of the MMNAT-hydralazine complex showing the secondary structure of the protein and the different subdomains. The structure is colored according to the temperature factor (from red to blue with increasing temperature-factor value). (B) The ribbon representation shows the superposition of the Ca trace of the MMNAT apoprotein (PDB code 2VFB; 2.0 Å; gray), MMNAT-CoA complex (PDB code 2VFC; 2.7 Å resolution; blue), and MMNAT-HLZ complex (in pink). The figure also shows the space available for CoA binding in the enzyme presented by the co-crystallized CoA (2VFC). Residues are highlighted to define the position of the CoA binding site within the enzyme. Hydralazine molecules bound in the active site are shown in pink and those bound to the secondary site in yellow.

Figure 2. Hydralazine binding pocket within MMNAT-HLZ complex. (A) A solvent accessible surface representation of the MMNAT-HLZ complex colored by the electrostatic potential (blue for positive and red for negative) shows the sub-pocket where HLZ binds. In the rectangle, an enlarged cross section shows the orientation of HLZ (in pink) in the pocket and the catalytic Cys70. (B) A surface representation of the residues in the active site binding pocket shows the hydrophobic areas in gray and the hydrogen bond forming area including a water molecule (H₂O10) in orange. The figure was prepared using Discovery Studio Visualizer 2.5.

substituted (Tyr71 and Val196 in MMNAT are Phe and Ala respectively in TBNAT) except for Met209 in MMNAT which is replaced by Thr in TBNAT and in NAT from *Mycobacterium smegmatis* (MSNAT) (Supplemental Fig. 1).

The phthalazine nucleus of HLZ forms strong π - π interactions with Phe130 that involve both the benzene and the pyridazine rings. This interaction is further supported by the π - σ interaction with Phe204 and Val95.

The hydrazine side chain is oriented toward the middle region of the pocket, which is relatively polar and can accommodate the polar part of the HLZ molecule (Fig. 2B). Although HLZ has four basic nitrogen atoms, only two of them (N2 and N4) can become protonated as shown in Fig. 3. In

HLZ the positive charge can be located at the imino nitrogen of the hydrazino group (N4; structure C, Fig. 3) or at the protonated ring nitrogen (N2; structure D, Fig. 3) (Datta et al., 1976).

HLZ can form hydrogen bonds with Cys70 and Thr109 (Fig. 3), depending on which HLZ tautomer or ionization state is in the binding pocket. HLZ can also form a π -cation interaction with Tyr69, Tyr71, His110, Phe130 and Phe204 if it binds in an ionized protonated form (Fig. 3G and 3H). Cation- π interactions between ligands and proteins have been reported in many 3D structures, even in active sites that lack any anionic amino acids to neutralise positive charges (Hendlich, 1998; Wouters, 1998; Gallivan and Dougherty,

Figure 3. The different tautomers and protonation states of hydralazine and their interactions with the main amino acids in the MMNAT binding pocket. (A) and (B) are the non-ionic states of HLZ that are in equilibrium. Protonation of A and B results in the formation of C and D, respectively. Frames E–H show the binding to MMNAT of the different HLZ tautomeric states A–D, respectively. Orange, π – π interactions; blue π – σ interactions; violet: π –cation interactions; green: hydrogen bonds. For clarity, the hydrogens on some carbon atoms are not shown. In frames E–H, the appropriate HLZ tautomeric form is shown in the bottom left hand corner. The figures were prepared using Discovery Studio Visualizer 2.5.

1999) and are considered much more favorable than an analogous interaction involving a neutral amine (Meot-Ner and Deakyne, 1985; Rodham et al., 1993; Gallivan and Dougherty, 1999). This interaction, if present would be expected to contribute to the observed affinity of MMNAT toward HLZ as a favorable acetyl group acceptor (Fullam et al., 2009). The product of the biological acetylation of HLZ is a cyclic metabolite known as 3-methyltriazolo[3,4-a] phthalazine (structure **D**, Fig. 4), while the uncyclized intermediate (compound **B**, Fig. 4) has never been isolated. We propose that tautomer **B** and its protonated form **C** (Fig. 3)

are more likely to be the abundant species in the binding pocket of MMNAT. While the protonated form **C** would enhance the affinity of the enzyme MMNAT for HLZ, the unionized form **B** is likely to be more favorable as an acetyl group acceptor. Both nitrogen atoms of HLZ involved in the cyclic acetylation product (i.e., N2 and N5, Fig. 4) have electronegativity in this tautomer. The fact that the heterocyclic nitrogen can exist in a non-aromatized form, and given its orientation in the binding pocket relative to the catalytic Cys70, raises the possibility that acetylation could take place at the ring nitrogen N2 rather than at the hydrazine nitrogen

Figure 4. Acetylation of hydralazine. Possible acetylation paths of hydralazine (A) by NAT enzymes leads to the formation of 3-methyltriazolo[3,4-a]phthalazine (D). The acetylated intermediate (B) has been suggested but never identified. The intermediate (C) is now proposed from this work.

(N5), especially since this N5 is oriented close to the Cys70 SH, where the acetyl group binds. In support of the idea that a heterocyclic N atom can be acetylated, heterocyclic amines such as pyridine are used as catalysts in acetylation reactions. In these reactions, pyridine functions as a nucleophilic catalyst, forming an N-acylpyridinium compound (Butler and Gold, 1961), which is a more reactive species especially toward intramolecular rearrangement or cyclization. If a similar reaction occurs within the MMNAT binding pocket, this could explain the inability to identify the previously proposed intermediate (Fig. 4B) and the higher efficiency with which HLZ is acetylated by NAT enzymes (Sikora et al., 2008; Fullam et al., 2009) compared with other aromatic hydrazines which have no comparable heterocyclic nitrogen e.g. isoniazid. However, at 2.1 Å resolution it is not possible to determine which of the proposed tautomers or protonation states of HLZ exist in the binding pocket (Fig. 3).

The second HLZ molecule binds to a superficial secondary binding pocket located toward the end of the CoA binding pocket. The binding of the second HLZ molecule is mainly through hydrophilic residues; aromatic hydrophobic interactions are not available. The second HLZ molecule is hydrogen bonded to Arg170 and His229. Amino acid residues within this secondary binding pocket are conserved across NATs (Supplemental Fig. 1). An equivalent binding pocket has been identified in an MMNAT-isoniazid complex (Fullam, 2007), where it helped in identifying the CoA binding site in the MMNAT-CoA complex (PDB code 2VFC; 2.7 Å resolution). However, the biological significance of the secondary binding site has not been established.

Comparison of the hydralazine structure to available NAT structures

To further explore the arylamine substrate binding pocket identified within the MMNAT active site, the interactions formed with isoniazid (INH) with MSNAT (PDB code 1W6F; resolution 2.1 Å; Fig. 5) (Sandy et al., 2005a) and with MMNAT (Fullam, 2007) were compared to that formed with HLZ. INH binds to the pocket in a comparable way to that observed for HLZ (Fig. 5). The hydrazide group of INH is oriented toward the thiol group of Cys70, forming a hydrogen bond network with Cys70 and Thr109. The pyridine ring forms a π - π interaction with the benzene ring of Phe130. The ionization of the hydrazide group of INH is less likely because it is less basic than the hydrazino group of HLZ.

Figure 5. The binding of isoniazid to the arylamine substrate binding pocket. A closer look at the main residues interacting with isoniazid in the MSNAT (PDB code 1W6F; resolution 2.1 Å) binding pocket. Orange: π - π interactions, green: hydrogen bonds. The figure shows an overlay of hydralazine (in yellow) and isoniazid (in gray) in the active site pocket.

To investigate the rigidity of the active site, we compared the HLZ binding pocket with that of the MMNAT-CoA complex (PDB code 2VFC; 2.7 Å resolution) and also of the C70Q mutant of MSNAT (PDB code 1W5R, resolution 1.45 Å) (Fig. 6) which approximates to the acetylated form of the protein (Sandy et al., 2005b).

The-SH group of CoA (that carries the acetyl group in AcCoA) extends to the arylamine pocket and lies within hydrogen bond range of the Cys70 thiol group, which indicates that both the Cys70 and CoA-SH groups are sufficiently close to exchange the acetyl group. The hydrophobic interaction between CoA and Phe204, Val195 and Tyr69 identifies the role of these amino acids in substrate recognition. Fig. 6A shows a large vacant area in the binding pocket. To confirm that there is sufficient space for the acetyl

Figure 6. The binding pocket of hydalazine in different NAT structures. Close-up of the active site with the space filling of the pocket cavity represented as transparent solid surface (pink), showing the shape and volume of the pocket available for binding. The key residues round the binding pocket are labeled. (A) A superimposition of MMNAT-HLZ (white ribbons) and MMNAT-CoA complex ((PDB code 2VFC; 2.7 Å resolution; blue ribbons) shows the space occupied by the CoA mercaptoethylamine group, and in (B) the space-filling shape of the same group in blue. (C) The superimposition of MMNAT-HLZ (white ribbon) and the C70Q mutant of MSNAT (PDB code 1W5R; resolution 1.45 Å; red ribbon) with the pocket cavity represented as a mesh surface in green shows the relative orientation of the thiol and hydalazine (in pink) to the glutamine side chain (shown in black). It also shows the difference between the pocket sizes of the cavities and a considerable vacant area that can accommodate the acetyl group during acetyl-transfer.

group of Ac-CoA, we compared the shape of the mercaptoethylamino group with that of the pocket (Fig. 6B). The vacant area available between Tyr69, Val196 and Phe204 is enough to accommodate the acetyl group. Additional space is likely to be provided by the flexibility of the side chains of these three residues, such that propionyl-CoA could also be accommodated, since propionyl-CoA can also act as an acyl donor to arylamines and HLZ by MMNAT (Fullam et al., 2009). We have also compared the binding pockets of the MMNAT-HLZ complex with that observed in the C70Q MSNAT mutant, which simulates the acetylated form of the enzyme (Fig. 6C and Supplemental Fig. 3). There is slight shift of ~ 0.8 Å of the glutamine moiety compared to Cys70 such that the amido group of the glutamine is directed toward the center of the pocket, creating additional space (Fig. 6C). This can be explained by the hydrophilic nature of the amido group that allows it to form hydrogen bonds with the bulk water molecules filling the pocket. The acetylated Cys is more hydrophobic and is likely to orient preferentially to the hydrophobic area created by the residues Tyr69, Val196 and Phe204. This shows that the active site pocket has only slight mobility, which is also obvious in the isotropic displacement coded visualization of the protein (Fig. 1A).

Summary and implications for drug design

The mycobacterial NAT enzyme has been proposed as a possible target for antitubercular therapy, since deletion of the *nat* gene disrupts cell-wall lipid synthesis, although the precise biochemical pathway has not yet been elucidated. From studies with the Δnat strain of *M. bovis* BCG, it may be

that the decrease in mycolic acid synthesis caused by *nat* deletion is due to a change in the regulation of acetyl-CoA or propionyl-CoA levels.

Small molecule NAT inhibitors are being developed (Westwood et al., 2010), but more understanding of the binding pocket of the protein is needed as a starting point for structure based optimization of the identified inhibitors. Binding of HLZ arises through the cooperative forces of hydrophobic, aromatic, and hydrogen bonds. Some of the key residues involved in binding have been elucidated. Aromatic residues stack over the phthalazine ring, providing a potentially strong binding interaction which is further enhanced by hydrogen bond formation and the possible π -cation interaction. The fact that the HLZ binding pocket within the active site is now clearly defined and relatively immobile are good indicators for iterative structure based medicinal chemistry of inhibitors to improve their potency. A mechanism for cyclization of HLZ in the acetylated form is proposed.

MATERIALS AND METHODS

All chemicals and reagents were purchased from Sigma Aldrich (Poole, Dorset, UK), unless otherwise stated.

Expression and purification

The MMNAT gene was expressed in *E. coli* BL21(DE3)pLysS cells transformed with the pET28b(+) vector containing MMNAT gene insert as described previously (Fullam et al., 2008).

The hexa-histidine tagged protein was purified using Ni^{2+} -ion affinity chromatography to apparent homogeneity and the hexa-histidine tags were cleaved by thrombin digestion. The yield

of the recombinant protein in the present study was 28.8 mg/L culture, which is slightly more than in the previous work (Fullam et al., 2008).

Co-crystallization of MMNAT with hydralazine

Crystals of the MMNAT-HLZ complex were obtained by co-crystallization using the sitting-drop vapor diffusion technique. A preliminary search for suitable crystallization conditions was carried out using the Molecular Dimensions JCSG-plus screen. Drops were prepared by mixing 0.1 μ L protein solution (10 mg/mL, 20 mM Tris-HCl pH 8.0, 1 mM DTT) containing 20 mM HLZ, with 0.1 μ L reservoir solution using a Tecan Crystallization robot (Tecan UK, Theale, UK), and were equilibrated against 100 μ L reservoir solution at 293 K. Initial crystals of MMNAT grew in condition Number 2.2 (E2) of Molecular Dimension JCSG-plus screen (0.2 M NaCl, 0.1 M Na-cacodylate pH 6.5, 2 M $(\text{NH}_4)_2\text{SO}_4$). This was followed by carrying out a series of customized fine grid optimization screens at 277 and 293 K around the favorable conditions known from the initial screen. In the optimisation screens, Na-cacodylate was replaced with MES. Diffraction quality bipyrimal crystals (~100 μ m, Supplemental Fig. 4) formed within 3 weeks at 293K in condition E2 (0.10 M NaCl, 0.10 M MES pH 6.7, 1.65 M $(\text{NH}_4)_2\text{SO}_4$). For cryo-protection, crystals were briefly (10–30 s) washed with a 7 M sodium formate solution containing HLZ (20 mM), and were then flash cryo-cooled into liquid nitrogen (77 K).

Data collection, structure solution and refinement

X-ray diffraction data were collected at 100K remotely using beamline ID23.EH2 at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) with a CCD Quantum 3 $\times$ 3 ADSC detector (Supplemental Table 1). Data were indexed and integrated with MOSFLM5 (Leslie, 2006) and scaled with SCALA (Evans, 2006) within the CCP4 program suite (Collaboration Computational Project, Number 4, 1994) (Collaborative, 1994). The crystal structure was solved by MR using the program MOLREP (Vagin and Teplyakov, 2000) using a previously determined native MMNAT crystal structure, stripped of heteroatoms, as a search model (PDB code: 2VFB, 1.8 Å). Conventional crystallographic refinement (rigid body) of the MR solution and the remaining cycles of restrained refinement were carried out with REFMAC5 (Murshudov et al., 1997). Molecular models of the substrate were constructed using SKETCHER, while model building was performed using COOT (Emsley and Cowtan, 2004) within the CCP4 program suite. An anomalous difference Fourier map was calculated using data to 2.1 Å resolution in the COOT program in order to identify HLZ binding sites. The HLZ monomer and CCP4 library were created with SKETCHER and the HLZ was placed in the appropriate position in the MMNAT model. The model was further refined in REFMAC5 with TLS (Winn et al., 2001) and restrained refinement, and was manually rebuilt utilizing 2 $F_o - F_c$ and $F_o - F_c$ electron-density maps in COOT. The overall isotropic B factor, which was initially set to 100 Å², refined to a value of 20 Å². Negative $F_o - F_c$ density was observed surrounding some side chains, indicating that the occupancy at these positions was < 1. Occupancies were manually adjusted to 0.5, an alternate conformation introduced, or the side chain was deleted until no negative $F_o - F_c$ density (map contoured at 3 σ) was observed in each position. TLS refinement of the B factors in REFMAC5, as described previously (Chaudhry et al., 2004), was beneficial in terms of clarifying the

electron-density maps, reducing R_{free} and minimizing the gap between R_{free} and R_{cryst} (i.e., removing model bias). The refinement converged to values of 0.216 and 0.265 for R_{cryst} and R_{free} respectively, and the model had reasonable stereochemistry (Supplemental Table 2). The model and structure-factor amplitudes have been deposited in the Protein Data Bank under accession code 3LTW. The B-factor analysis of protein residues and ligands was carried out using BAVEAGE within the CCP4 suite, and the surface areas of the pockets and volumes were analyzed using the Computed Atlas of Surface Topography of proteins (CASTp) (Binkowski et al., 2003) online server <http://cast.engr.uic.edu>. Graphics were created by the Discovery Studio Visualizer <http://accelrys.com> and CCP4MG (Potterton et al., 2004).

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ABBREVIATIONS

AcCoA, acetyl-CoA; DTT, 1,4-dithiothreitol; DS, Discovery Studio; ESRF, European Synchrotron Radiation Facility; HLZ, hydralazine; INH, isoniazid; MANAT, NAT from *M. avis*; MES, 2-(N-morpholino) ethanesulfonic acid; MMNAT, NAT from *M. marinum*; MR, molecular replacement; MSNAT, NAT from *M. smegmatis*; NAT, arylamine N-acetyltransferase; PDB, protein data bank; TB, tuberculosis; TBNAT, NAT from *M. tuberculosis*

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