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The Molecular Structure of Ornithine Acetyltransferase from *Mycobacterium tuberculosis* Bound to Ornithine, a Competitive Inhibitor

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Mycobacterium tuberculosis ornithine acetyltransferase (*Mtb* OAT; E.C. 2.3.1.35) is a key enzyme of the acetyl recycling pathway during arginine biosynthesis. It reversibly catalyzes the transfer of the acetyl group from *N*-acetylornithine (NAORN) to L-glutamate. *Mtb* OAT is a member of the N-terminal nucleophile fold family of enzymes. The crystal structures of *Mtb* OAT in native form and in its complex with ornithine (ORN) have been determined at 1.7 and 2.4 Å resolutions, respectively. ORN is a competitive inhibitor of this enzyme against L-glutamate as substrate. Although the acyl–enzyme complex of *Streptomyces clavuligerus* ornithine acetyltransferase has been determined, ours is the first crystal structure to be reported of an ornithine acetyltransferase in complex with an inhibitor. ORN binding does not alter the structure of *Mtb* OAT globally. However, its presence stabilizes the three C-terminal residues that are disordered and not observed in the native structure. Also, stabilization of the C-terminal residues by ORN reduces the size of the active-site pocket volume in the structure of the ORN complex. The interactions of ORN and the protein residues of *Mtb* OAT unambiguously delineate the active-site residues of this enzyme in *Mtb*. Moreover, modeling studies carried out with NAORN based on the structure of the ORN–*Mtb* OAT complex reveal important interactions of the carbonyl oxygen of the acetyl group of NAORN with the main-chain nitrogen atom of Gly128 and with the side-chain oxygen of Thr127. These interactions likely help in the stabilization of oxyanion formation during enzymatic reaction and also will polarize the carbonyl carbon–oxygen bond, thereby enabling the side-chain atom $O^{\gamma 1}$ of Thr200 to launch a nucleophilic attack on the carbonyl-carbon atom of the acetyl group of NAORN.

Keywords: *Mycobacterium tuberculosis*; ornithine acetyltransferase; X-ray crystal structure; ornithine; N-terminal nucleophile

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Abbreviations used: *Mtb* OAT, *Mycobacterium tuberculosis* ornithine acetyltransferase; NAORN, *N*-acetylornithine; ORN, ornithine; *Mtb*, *Mycobacterium tuberculosis*; TB, tuberculosis; 3D, three-dimensional; OAT, ornithine acetyltransferase; ORF, open reading frame; GLU, L-glutamate; *Sclav* OAT, *Streptomyces clavuligerus* ornithine acetyltransferase; *Bhalo* OAT, *Bacillus halodurans* ornithine acetyltransferase; PDB, Protein Data Bank; Ntn, N-terminal nucleophile; GOL, glycerol; ALS, Advanced Light Source; SSRL, Stanford Synchrotron Radiation Laboratory; CCP4, Collaborative Computational Project No. 4; BEZ, benzoic acid.

Introduction

Although more than 100 years have elapsed since the discovery that *Mycobacterium tuberculosis* (*Mtb*) causes the debilitating disease tuberculosis (TB), TB still claims the lives of millions of people worldwide. Approximately one third of the 1.4 million people who died in 2007 were positive for human immunodeficiency virus†. The coexistence of TB and

† http://www.who.int/entity/tb/publications/global_report/2009/pdf/full_report.pdf

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human immunodeficiency virus is growing at an alarming rate with increasing world human population; it has been termed as "double trouble." Also, the noncompliant use of improper drug regimens adopted during TB treatment has led to the emergence of multidrug-resistant TB and extensive drug-resistant TB. The latter form of this disease is not curable with any of the currently available drugs on the market.[1] These alarming statistics necessitate the urgent development of new anti-bacterial drugs to combat this disease. The TB Structural Genomics Consortium was formed in 2000 with the view of establishing a structural proteome of *Mtb* that will eventually help in the development of structure-based antibiotic drugs against this pathogenic bacterium.[2–4] As members of the TB Structural Genomics Consortium, one of our present objectives is to determine the three-dimensional (3D) structures of all of the enzymes involved in arginine biosynthesis in *Mtb* in order to help in understanding the function of these enzymes at the molecular level.[5–8] In the extension of this work, we report the crystal structures of ornithine acetyltransferase (OAT) in unbound native form and in its bound form with the competitive inhibitor ornithine (ORN).

The *argJ* gene corresponding to the open reading frame (ORF) Rv1653 encodes *Mtb* OAT (E.C. 2.3.1.35).[9] OAT in this microorganism reversibly catalyzes the transfer of the acetyl group from *N*-acetylornithine (NAORN) to L-glutamate (GLU), thereby producing ORN and *N*-acetyl-L-glutamate (Fig. 1).[10] It is worth mentioning here that the two enzymes *N*-acetylglutamate synthase (E.C. 2.3.1.1; *argA* gene) and *N*-acetylornithine deacetylase (E.C. 3.5.1.16; *argE* gene), which are present in the linear pathway of arginine biosynthesis in *Mtb*, also carry out the parallel functions of acetylating GLU and deacetylating NAORN, respectively. OAT in bacteria exists either as a monofunctional enzyme or as a bifunctional enzyme.[11,12] In the bifunctional enzyme, the transfer of the acetyl group to GLU is accomplished from both NAORN and acetylcoenzyme A, whereas the monofunctional enzyme transfers the acetyl group only from NAORN. It has also been pointed out that those microorganisms with a monofunctional OAT have a short form of the *argA* gene.[12] In *Mtb*, the ORF Rv2747 has been identified as the *argA* gene.[13] This gene encodes a small protein with a molecular mass of 19 kDa; however, structural characterization of this protein has not been carried out so far. Biochemical analyses of OAT from other bacteria and yeast have been studied thoroughly.[11,14–17] However, the first crystal structure of an OAT was reported from *Streptomyces clavuligerus* [*S. clavuligerus* ornithine acetyltransferase (*Sclav* OAT)], an organism that is involved in the biosynthesis of clavulanic acid, followed by recent work on the structure of the acyl–enzyme intermediate of this enzyme from the same bacterium.[18,19] Also, the coordinates of the structure from *Bacillus halodurans* [*B. halodurans* ornithine acetyltransferase (*Bhalo* OAT)] have been deposited in the Protein Data Bank (PDB) (PDB code 1VRA). However, the details of the structural work on *Bhalo* OAT have not been reported so far. Although recent detailed structural studies on the acyl–enzyme complex of *Sclav* OAT have discussed a possible catalytic mechanism of OAT during the formation of the acyl–enzyme intermediate,[19] the crystal structures of OAT bound to a substrate analogue or an inhibitor have not been determined so far. With this in view, we have carried out the structure determination of *Mycobacterium tuberculosis* ornithine acetyltransferase (*Mtb* OAT) and its complex with ORN in order to understand the details of the catalytic reaction of this enzyme in *Mtb*. It has been demonstrated from a biochemical study on thermophilic organisms that ORN is a competitive inhibitor of this enzyme *versus* GLU.[11] It has also been established from the same study that the substrate GLU binds to the active-site pocket of OAT only when the product molecule ORN has been completely released from the enzyme. The present study on *Mtb* OAT is the first crystal structure of OAT to be reported bound to a product molecule ORN that also acts as an inhibitor.

Results and Discussion

The overall structure of *Mtb* OAT

The crystals of native *Mtb* OAT and the crystals soaked in ORN diffracted to resolutions of 1.7 and 2.4 Å, respectively. They both belong to the orthorhombic space group $P2_12_12_1$ and have similar cell dimensions and solvent contents (Table 1). Two molecules of OAT occupy the asymmetric unit in both crystal forms. The monomer of *Mtb* OAT consists of 404 amino acid residues and has a molecular mass of 41 kDa. Figure 2 shows a sequence alignment of *Mtb* OAT with the sequences of *Sclav* OAT and *Bhalo* OAT. The sequence alignment was performed using CLUSTAL W;[20] it shows that *Mtb* OAT shares identity scores of 32%

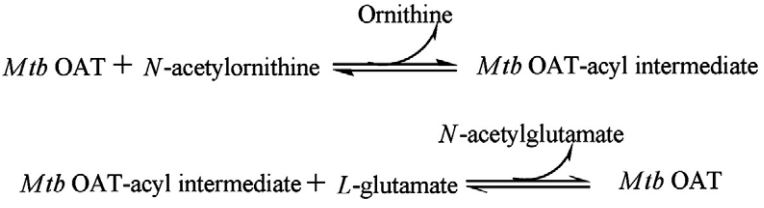

Fig. 1. The reaction catalyzed by *Mtb* OAT.