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Determining the unbinding events and conserved motions associated with the pyrazinamide release due to resistance mutations of *Mycobacterium tuberculosis* pyrazinamidase

Olivier Sheik Amamuddy [1], Thommas Mutemi Musyoka [1], Rita Afriyie Boateng, Sylvia Nakama Zabo, Özlem Tastan Bishop *

Research Unit in Bioinformatics (RUBi), Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, South Africa

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ABSTRACT

Pyrazinamide (PZA) is the only first-line antimolecular drug active against latent *Mycobacterium tuberculosis* (Mtb). It is activated to pyrazinoic acid by the *pncA*-encoded pyrazinamidase enzyme (PZase). Despite the emergence of PZA drug resistance, the underlying mechanisms of resistance remain unclear. This study investigated part of these mechanisms by modelling a PZA-bound wild type and 82 mutant PZase structures before applying molecular dynamics (MD) with an accurate Fe^{2+} cofactor coordination geometry. After observing rapid-scale PZA unbinding from several PZase mutants, an algorithm was developed to systematically detect ligand release via centre of mass distances (COM) and ligand average speed calculations. When applying the statistically guided network analysis (SGNA) method to investigate conserved dynamic motions associated with ligand unbinding, Ligand and cofactor perspectives were also investigated. A conserved pair of lid-destabilising motions was found. These consisted of (1) antiparallel lid and side flap motions; (2) the contractions of a flanking region within the same flap and residue 70 towards the core. Mutations affecting the hinge residues (H51 and H71), nearby residues or L19 were found to destabilise the lid. Additionally, other metal binding site (MBS) mutations dislocated the Fe^{2+} cofactor, also facilitating lid opening. In the early stages of unbinding, a wider variety of PZA poses were observed, suggesting multiple exit pathways. These findings provide insights into the MD events preceding PZA unbinding, which we found to occur in some resistant PZase mutants. Further, the algorithm developed here to identify unbinding events coupled with SGNA can be applicable to other similar problems.

1. Introduction

Tuberculosis (TB) due to *Mycobacterium tuberculosis* (*Mtb*) infection remains a global health concern with recent reports indicating high morbidity and mortality [1]. The World Health Organization (WHO) reported approximately 1.6 million TB-related deaths in 2018 [1] despite the availability of therapeutic options. Approximately one third of the global population is latently infected with *Mtb* [2]. Since its discovery, the pyrazinamide (PZA) drug has become an essential component in first-line TB treatment [3], showing activity against primary TB, multidrug-resistant TB (MDR-TB), and even preventing relapse of the disease [4]. It is frequently combined with isoniazid (INH) and rifampicin (RIF) during the initial phase of therapy [4].

1.1. Mechanism of action of PZA

PZA is a prodrug that requires intracellular activation by the *pncA*-encoded bacterial enzyme pyrazinamidase (PZase) [4] to

Abbreviations: 3D, Three-dimensional; ACPYPE, AnteChamber PYthon Parser interface; CHPC, Centre for High Performance Computing; COM, Centre of mass; FDA, Food and Drug Administration; HTMD, High throughput molecular dynamics; INH, Isoniazid; MBS, Metal binding site; MCBP, Metal Center Parameter Builder; MD, Molecular dynamics; MDR-TB, Multidrug-resistant tuberculosis; PBC, Periodic boundary conditions; PDB, Protein Data bank; POA, Pyrazinoic acid; PZA, Pyrazinamide; PZase, Pyrazinamidase; QM, Quantum Mechanics; RIF, Rifampicin; SGNA, Statistically guided network analysis; TB, Tuberculosis; VAPOR, Variant Analysis Portal; WHO, World Health Organization; WT, Wild type.

* Corresponding author.
E-mail address: O.TastanBishop@ru.ac.za (Ö. Tastan Bishop).
[1] These authors equally contributed to the work.

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