

Mechanistic studies of Mycobacterium tuberculosis topoisomerase I inhibition by endogenous toxin Rv1495

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Antimicrobial resistance is a worldwide public health concern as existing antibiotics are becoming ineffective. Tuberculosis is the leading infectious cause of death worldwide. According to the World Health Organization, in 2017, there were 558,000 cases of drug resistant tuberculosis (TB), so there is an urgent need of new TB treatment against a novel target. Toxin-antitoxin (TA) systems participate as gene regulators within bacteria. Researchers believe that TA systems contribute to the long-term dormancy of *Mycobacterium tuberculosis* (*Mtb*) within the host-cell environment.

Previous research showed that an *Mtb* toxin Rv1495, a homolog of MazF that is part of the MazEF TA system, has endoribonuclease activity but also functions as an inhibitor against *Mtb*'s essential topoisomerase IA (TopA) by interacting with the 30 kDa C-terminal domain of TopA. We have developed a complementary assay using an *E. coli* strain with temperature-sensitive *topA* mutation to further locate the Rv1495 interaction site to the 24 amino-acid long C-terminal tail of TopA. Site-directed mutagenesis is utilized to identify the critical lysine residues within this C-terminal tail that are responsible for the protein-protein interaction. To further characterize the molecular interactions between TopA and Rv1495, expression and purification of the Rv1495 toxin were conducted under different induction conditions. Thermal shift assays were used to determine if recombinant Rv1495 folding is affected by the growth temperature during induction of expression. TopA relaxation activity was assayed in the presence of recombinant Rv1495. TopA C-terminal domain in mycobacteria are distinct from the C-terminal domain of most other bacteria. The biochemical and biophysical characterization of the mechanism of *Mtb* TopA inhibition will allow the advancement of therapeutic approaches against a new antibacterial target and are selective towards the essential topoisomerase IA enzyme within the pathogenic mycobacteria for treatment of both TB and diseases caused by the non-tuberculosis mycobacteria (NTM).