Received: 13 January 2016 | Revised: 12 May 2016 | Accepted: 6 August 2016

DOI: 10.1111/cbdd.12840

RESEARCH ARTICLE

WILEY

Characterization of MymA protein as a flavin- containing monooxygenase and as a target of isoniazid

Iti Saraav[1,2] | Kirti Pandey[1,2] | Richa Misra[1,2] | Swati Singh[1,2] | Monika Sharma[1,2] | Sadhna Sharma[1,2]

[1]D S Kothari Centre for Research and Innovation in Science Education, Miranda House, Delhi, India

[2]Department of Zoology, Miranda House, University of Delhi, Delhi, India

Correspondence
Dr. Sadhna Sharma, Department of Zoology, Miranda House, University of Delhi, Delhi, India.
Email: sadhna.sharma@mirandahouse.ac.in

Tuberculosis is a global health problem especially with the emergence of drug-resistant *Mycobacterium tuberculosis* strains, creating an urgent need to identify new drug targets. The mycobacterial cell wall is an attractive target for chemotherapeutic agents. Gene products of *mymA* operon are known to be required for the maintenance of cell wall and play an important role in persistence, thus making them important drug targets. This study was undertaken to biochemically characterize the MymA as a flavin-containing monooxygenase (FMO). Our results established its enzymatic activity *in vitro* and found that the mycobacterial FMO requires NADPH and FAD as cofactors, similar to other characterized bacterial FMOs. The enzyme follows Michaelis–Menten kinetics to catalyze substrates such as trimethylamine and thiourea. We also propose that MymA could be one of the targets of the antituberculosis drug, isoniazid (INH), which is a cell wall inhibitor. Molecular docking studies revealed that INH targeted NADPH-binding site of the MymA. Further, experimental validation revealed that INH inhibits MymA with the IC_{50} value of 4.9 μM. Thus, this study characterizes for the first time that MymA is a mycobacterial FMO, which may be a target of INH.

KEYWORDS
FMO, INH, Molecular docking, *Mycobacterium tuberculosis*, MymA

Mycobacterium tuberculosis (*M. tuberculosis*), the causative agent of tuberculosis (TB), is an ancient but still widespread disease. Around 8 million new cases are reported to emerge each year and cause 3 million deaths annually.[1] Multidrug-resistant (MDR) strains have added more to the menace, and there is an urgent need to identify new drug targets for *M. tuberculosis. M. tuberculosis* resides inside the host macrophages, where it encounters stressful conditions such as changes in pH, exposure to reactive oxygen species, nitrogen intermediates, degradative enzymes, and deprivation of essential nutrients. During these conditions, the unique lipid and mycolic acid-rich cell wall protect it from assault by the host system and play an important role in virulence.[2,3] These long-chain mycolic acids have an alkyl side chain and a hydroxyl group at α and β positions, respectively. They are mainly found attached covalently to the distinctive peptidoglycan–arabinogalactan complex of the mycobacterial cell wall.[4] Proteins encoded by *mymA* operon are required for maintaining the appropriate mycolic acid composition of *M. tuberculosis* when exposed to acidic pH in host environment.[5,6] The fatty acid synthase (FAS) I and FASII system of lipid synthesis are responsible for production of mycolic acids.[7,8] FAS I is responsible for the generation of C_{18} short-chain fatty acids which are then fed into the FAS II system that generates long-chain fatty acids referred to as merochains of length up to C_{60}.[4] When the pathogen is under stressful conditions such as acid shock inside the macrophages, the FAS II system is downregulated and *mymA* operon takes over to produce and process long-chain fatty acids and delivers them to appropriate acceptors for further modification.[5] Thus, *mymA* operon proteins bring about omega oxidation of fatty acids (C_{24}/C_{26}) and condense these oxidized fatty acids to produce meromycolic acids (C_{60}),

Richa Misra