

*Discovery of a novel xenobiotics deaminating enzyme from Mycobacterium smegmatis*Vandana Mallinath Gaded¹, Ruchi Anand¹¹Chemistry, Indian Institute Of Technology Bombay, Mumbai, India

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Mycobacterial species pose a global threat with several deaths associated with their infection. This is mostly due to abuse of existing drugs that has led to emergence of several drug resistant pathogenic strains which do not respond to conventional treatments (1). Here with the aim of targeting alternative therapies, we embarked on searching for novel nucleobase pool regulating enzymes. In all organisms, proteins of the nucleobase salvage pathway are essential; hence these pose a great target for design of future drug scaffolds. To identify enzymes unique to the Mycobacterium genus, we selected the ubiquitous nucleobase deaminase family (cluster of orthologous group 0590) with more than 2000 members and performed a sequence similarity search (2). Results revealed a novel sub-group of proteins primarily present in the mycobacterium genus. We selected Msmeg3575 (Msd) from Mycobacterium smegmatis as a representative enzyme from this sub-group which was misannotated as cytidylate/deoxycytidylate deaminase and had no structural or functional information. After extensive insilico and invitro substrate evaluation with a subset of nucleobases, nucleosides and their analogs, the enzyme Msd showed catalytic activity for isoguanine and s-triazine compounds- acetoguanide, 5-azacytosine, ammeline and ammelide. To understand the prerequisite of substrates preferred by Msd, three dimensional structures of native Msd and in complex with ligands (5-azacytosine, ammeline and benzoguanamine) were determined. Due to low sequence similarity of Msd with the known deaminases, X-ray structure of native Msd was determined at 1.89Å resolution using zinc single wavelength anomalous dispersion method. Structural analysis reveals that it displays the typical cytidine deaminase ($\alpha/\beta/\alpha$) core fold with a distinctive active site architecture. The mutagenesis experiments and the structures of Msd-ligand complexes supports the importance of substrate scaffold criterion and also sheds light on the mechanism of catalysis. In this study, we first report the enzymatic deamination of 5-azacytosine and acetoguanide compounds which are extensively used as a core in anti-tumor and herbicide agents respectively (3). Therefore, we believe that this pathway would be the primary means by which mycobacterium species must have evolved to manage and prevent damage to DNA and other cellular processes. This study might help us unravel the mysteries of mycobacterial biology and provide a framework for rational drug design.

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Keywords: [deaminase](#), [sequence similarity network](#), [triazine](#)