Abstract

*Mycobacterium tuberculosis* is a highly successful pathogen primarily because of its ability to persist in the human host for many years in a latent/persistent state avoiding the host immune system. The present study mainly involves the *M. tuberculosis* Phosphoserine phosphatase (MtSerB2, Rv3042c). Phosphoserine phosphatases are known to be involved in signal transduction mechanisms in eukaryotes and host invasion in prokaryotes, but not much is known about their roles in bacteria. *M. tuberculosis* harbors two homologs of the enzyme *viz.* MtSerBl and MtSerB2. The former (Rv0505c) is not essential for the pathogen’s viability while the latter (Rv3042c) is known to be essential for the viability of the pathogen, as shown by transposon mutagenesis studies. It also has two amino acid binding domains (ACT domains) at the N-terminus in tandem that is intriguing in terms of their roles in the enzyme’s functions and represents a sub-family of SerB that has not been characterized in detail.

When this work was initiated, Rv3042c had been tentatively annotated in the databases as a putative Phosphoserine phosphatase. The sequence contains the three typical motifs present in these enzymes. In the first instance, we were interested in characterizing the protein biochemically and biophysically. The roles of the individual domains in protein’s function were also planned to be investigated. Additional studies to probe the effects of different amino acids and already known inhibitors in the regulation of protein function were also to be carried out. The reported results are important from the standpoint of deciphering the novel molecular mechanisms of MtSerB2 and the long-term goal of exploiting its potential as a novel therapeutic target.

The first chapter introduces the superfamily of haloacid dehalogenases to which the enzyme belongs. Subsequently the reader is introduced to the sub-family of phosphoserine phosphatases that is the main concern of the thesis. A brief introduction to structure based rational methodologies in new inhibitor identification is also given.

The second chapter details the materials and methods used during the course of the work. The reported work utilized a variety of approaches including molecular biology protocols to clone the genes of interest, spectroscopic methods like Circular dichroism, fluorescence etc., X-ray crystallography, bioinformatics and computational approaches to gain insights to the problems being investigated. The different methods used to perform the experiments are also described here.
The cloning, purification, biochemical and biophysical characterization of MtSerB2 and the cloning of MtSerB1 are reported in detail in the third chapter. This includes the characterization of the activities and novel substrate specificity of MtSerB2. The effects of the different amino acids on the activity of the enzyme are also detailed. The efficacy of several known phosphatase inhibitors on the activity of MtSerB2 is also reported.

In the fourth chapter, the contribution of individual domains and active site residues in modulating the enzyme's activity have been probed by mutagenesis and activity assays. The crystallization attempts of the full-length, mutants and domain-deleted versions of the protein are also detailed. The roles of the ACT domains and also that of the catalytic domain on the protein stability and activity were studied using Isothermal calorimetry and circular dichroism approaches. The results present a picture of a novel phosphoserine phosphatase where the binding of amino acid ligands to the ACT domains modulates the functions of the enzyme.

The author was involved in a collaborative project involving virtual screening and X-ray crystallography studies on small molecules that might bind to the ERα Ligand Binding Domain (ERα LBD). The results of these studies are reported in the fifth chapter. The work resulted in the identification of certain tetrazolyl indoles as new types of antagonists for the ERα LBD. The chapter subsequently reports the crystallization, data collection and the structure solution of two of the best scoring compounds in the study.

The details pertaining to the chemicals, stains, media and buffers composition have been described in the Annexure.

During the course of the work reported in the thesis, the author was also involved in another exciting collaborative project, which involved the structure solution and analysis of the full-length Negative factor (Nef14/7) from a patient infected with HIV-1 (GenBank accession code GQ184340). The crystal structure and the structure factors have been deposited in the Protein Data Bank (http://www.rcsb.org) with accession numbers 2x1l and r2x1sf respectively.

(Continued...)
A part of the results of this thesis have already been reported/will be communicated:


2. Characterisation of a novel phosphoserine phosphatase from *M. tuberculosis* H37Rv. 
Gaya Prasad Yadav & Ravishankar Ramachandran *(Manuscript under communication)*

3. Crystal structure of the full-length HIV-1 Nef protein from a patient’s sample 
Pankaj Singh, Sudipti Gupta Gaya Prasad Yadav*, Anil Kumar Tripathi, Ravishankar Ramachandran* and R. K Tripathi* 
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