ABSTRACT

Mycobacterial diseases, especially tuberculosis, remain a cause of massive suffering and death in many parts of the world despite development of diagnostic tests, cheap vaccines, and effective antibiotics. For more than a century, *Mycobacterium tuberculosis* has posed a formidable challenge to biomedical researches owing to its long generation time, fastidious growth requirements, and high risk of contagion. The situation has further worsened with the development of multi-drug resistant TB, the human immunodeficiency virus (HIV) pandemic, and poor tuberculosis control programs. Deciphering the entire genome sequence of the TB bacillus has therefore necessitated a targeted approach to evaluate the dreadfulness of *M. tuberculosis*.

Understanding how latency is established and how the bacilli are able to reactivate is of key importance for identification of new drug targets or development of new vaccines against TB. Among novel virulence factors in the tubercle bacillus that were identified through the genomic sequence, the mammalian cell entry (Mce) proteins are of particular interest. There are 24 proteins that belong to this Mce family and they make up a substantial proportion of the total repertoire of exported proteins. Among the four copies of mce found, in each case the genes preceding mce coded for two integral membrane proteins, YrbE, while the consequent six mce genes were predicted to encode proteins with hydrophobic stretches at the N-terminus. These hydrophobic stretches probably represent signal sequences. Looking into the other aspects of mycobacterial pathogenicity, concentration of iron in the host has been found to be important in *M. tuberculosis* infection. Iron is an essential nutrient for all pathogens, but this element appears to play an especially critical role in the pathogenesis of tuberculosis. Among the few proteins that are expressed in high iron concentration and not in low iron concentration, Peptidyl-prolyl cis-trans isomerase A or PpiA is one of them. In the present study, main emphasis has been given on Mce and PpiA of TB bacilli.

The spontaneous folding of proteins to their native states is the point at which life makes the giant leap from the one-dimensional world of DNA and protein sequences to the three dimensional world we inhabit. Therefore, to predict protein structures from their sequences, this study explores: homology modeling, secondary structure prediction,

fold recognition and subsequently predicting three dimensional structures of the proteins with novel folds. Establishment of structure-function relations for Mce proteins from the background of structure-sequence alignment should improve the understanding of virulence mechanism, especially the cell entry process performed by the bacteria. The study has also aimed at characterizing the epitope region in Mce1A following bioinformatics and immunological replacement studies. Insolubility of Mce proteins has restrained their *in vitro* details.

However, the other mycobacterial protein PpiA has been cloned, overexpressed and purified to homogeneity. To delve out the details of interaction of PpiA with immunosuppressive drug, Cyclosporin A (CsA), docking study has been performed. Apart from *in silico* analysis, various methods viz. X-ray crystallography & NMR (high resolution techniques), CD, FTIR and fluorescence (low resolution techniques) can be exploited for structural investigations of proteins. Thus, secondary as well as tertiary structure analyses in solution have been carried out for PpiA in presence/absence of CsA, following spectroscopic characterization. To meet the intriguing challenge of protein folding, secondary as well as tertiary structure unfolding/refolding of PpiA and PpiA-CsA have been characterized using chemical and thermal denaturation / renaturation experiments. Keeping the significant discovery by Anfinsen in mind that the information determining the 3D structure of a protein resides in the chemistry of the amino acid sequence, site directed mutagenesis has been performed on the active site residues of PpiA. The PpiA mutants have been cloned, expressed and purified to homogeneity. Alteration of the catalytic as well as the cis → trans isomerization activity of PpiA have been discussed using PpiA and its mutants in Human carbonic anhydrase II (HCAII) refolding and peptide cis-trans isomerization assay respectively. In conclusion, this study may contribute in unlocking the esoteric stores of information contained in Mce and PpiA amino acid sequences.