PREFACE

N-terminal methionine excision is the process of co-translational removal of initiator methionine from the N-terminus of the elongating polypeptide chain during protein synthesis. It is a highly conserved and ubiquitous process. In prokaryotes, sequential action of two metalloproteases is required for the methionine removal. The first enzyme of this pathway is peptide deformylase (PDF). It cleaves the formyl group attached to the N-terminus of the initiator methionine. Once the N-terminus of the methionine is exposed another enzymes methionine aminopeptidase (MetAP or MAP) removes the initiator methionine. These two enzymes of the N-terminal methionine excision pathway are considered to be targets for development of new/novel antibacterials considering the emergence of drug resistant strains of different pathogenic bacteria which caused resurgence of diseases that have been thought to be well under control. In this context tuberculosis, the disease caused by *Mycobacterium tuberculosis* is worth mentioning. The work presented here aims to understand the functionality one such enzyme, methionine aminopeptidase of N-terminal methionine excision pathway. Unlike most prokaryotes *M. tuberculosis* has two MetAPs which needs detailed biochemical and structure-function analysis.

The study on the MetAPs from *M. tuberculosis* embodied in this thesis has been divided into six chapters. In chapter 1, the information available regarding the MetAPs from various organisms has been presented in a concise manner, followed by the introduction to the topic of research and main objectives of the study. Chapter 2 deals with various techniques used and the procedures/protocols followed during the study.

In Chapter 3, the distribution as well as organization of MetAPs in different mycobacterial species has been surveyed. The phylogenetic analysis of the MetAPs has also been carried out. Through *in silico* approach, promoters of the two genes were identified and further validated by monitoring green fluorescent protein in Western blotting and fluorescence experiments under different conditions of environmental stress. The hallmark of this chapter is the difference in the behaviour of the two promoters.
In chapter 4, the biochemical comparison of the two metalloproteases has been performed. For this, the recombinant MetAPs were cloned and expressed in *E. coli*. The comparison of the recombinant proteins revealed that both the enzymes have significant differences in their biochemical nature *in vitro*. They not only have different substrate preferences but also differ in the retention of activity at higher temperature. In fact, the kinetic analysis revealed that they differ by ~180 fold in their catalytic efficiency with the same substrate. The active site residues identified on the basis of sequence analysis were validated by mutating them employing PCR based site directed mutagenesis approach. All the residues identified were critical for activity since the mutants were inactive. This indicates that the residues required for the activity are essentially same in both the metalloproteases.

Among the two *M. tuberculosis* proteins one of them has a short unstructured N-terminal extension. The contribution of this extension towards the functionality of the enzyme has not been explored yet. The chapter 5 of this thesis deals with elucidating the role of this N-terminal extension in the activity of the enzyme. Two amino acid residues (Val-18 and Pro-19) have been pin-pointed to be crucial for the activity of the enzyme, though they are not the part of the active site. Besides this, the role of extension in interaction with the ribosomal protein L24 has been studied by employing *in vitro* pull down and *in vivo* expression pull down assays. The results indicate that the residues Pro-Thr-Arg-Pro which have long been postulated to be mediating this interaction do not play a significant role.

Chapter 6 summarises the work done in this thesis and also highlights the future implications of this work.