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SUMMARY OF THE THESIS

Polyphosphates (poly-P) are involved in cellular metabolism and development in bacteria especially during stress. The enzyme principally involved in polyphosphate biosynthesis and its mobilization is known as polyphosphate kinase (PPK). Among two genes of polyphosphate kinases (*ppk1* and *ppk2*) in *Mycobacterium tuberculosis*, the present study is mainly focused on PPK1, the principle poly-P biosynthesis enzyme. The hallmark of mycobacterial life cycle is the ability to undergo persistence especially during stress conditions. Dearth of energy sources particularly during persistence or stationary conditions is met by the utilization of "polyphosphates" for the generation of ATP. Therefore, it is intriguing to have detailed knowledge regarding the structure-function analysis of enzyme which is implicated in both poly-P synthesis and its mobilization.

The study on *M. tuberculosis* PPK1 (mPPK1) embodied in this thesis is divided into six chapters. Chapter 1 introduces the topic of research and reviews various structural and functional aspects of the polyphosphate kinases. Additionally, it defines various objectives of the study. Chapter 2 describes the details of various strains, plasmids, primers, chemicals, media, buffers and methodologies/protocols used in this study.

Chapter 3 deals with the detailed biochemical characterization of mPPK1. The course of reaction (also termed as forward reaction) can be categorized mainly in two steps: firstly, binding of NTP(s) to PPK1, followed by autophosphorylation of the protein and secondly, synthesis of poly-P. All the steps are focused sequentially. mPPK1 is comprised of domain structure where N terminal domain residues important for ATP binding were found to be indispensable for its enzymatic activity. In contrast to the earlier report where His-491 and His-510 were found to be responsible for autophosphorylation of mPPK1, the result presented here highlights the involvement of the former in the process. Mutational analyses were carried out to pin point the residues which are important for mPPK1 mediated forward reaction. The major finding of this study is that the residues which are involved in interaction with either His-491 or ribose sugar of ATP are obligatory for the functionality of the enzyme.

As a part of its reverse reaction, PPK1 mobilizes the inorganic phosphate moiety from poly-P and assists in the generation of various NTPs from the cognate

NDPs/d NDPs. Hence, PPK1 is identified as an enzyme with auxiliary NdK activity. Mutational studies identified the residues from catalytic domains which are dispensable for forward but are crucial for the reverse reaction. In a nutshell, this chapter reveals the ability of mPPK1 to exhibit auxiliary NdK activity. It has been demonstrated that the residues affecting mPPK1 mediated forward reaction would definitely abrogate the reverse reaction (ATP synthesis); however, the reverse is not true.

The enzyme (mPPK1) is active as a dimer. After detailed structure-function analysis of mPPK1 with respect to its poly-P and ATP syntheses, the dimerization of the mPPK1 which is crucial and decisive for all its activities has been focused upon in Chapter 5. Despite the availability of crystal structure of *E. coli* PPK1, no information is available regarding the residues involved in dimerization of the enzyme. Prediction based on *E. coli* structure and mutational studies were used to identify the residues from H and C-1 domains of mPPK1 involved in enzyme dimerization.

Chapter 6 summarizes all the findings and also highlights the implications of the present study. PPK1 is present ubiquitously in prokaryotes but absent in eukaryotes. Therefore, identification of 6 residue stretch in H domain of mPPK1 which is decisive for the enzyme activities could serve as the target for designing and screening of rational inhibitor(s). Therefore, in the present era when the multidrug resistance is the major problem to deal with *M. tuberculosis* infection, details regarding structure-function analysis of this major poly-P biosynthetic enzyme, mPPK1, will open new avenues for the future research.