Kinetic and Biochemical Characterization of Recombinant Enzyme Adenylosuccinate Synthase (EC 6.3.4.4) from *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis is a pathogenic mycobacterium that is causative agent of tuberculosis (TB) and currently infects one-third of the world's population. It is the leading cause of mortality owing to an infectious agent, claiming 2 million lives annually (Navin T.R. *et al*., 2002). There are effective available drugs for TB treatment (Bass J.B. Jr. *et al*., 1994; Blumberg H.M., 2005), but the disease is not effectively controlled in many poor and developing countries. This situation in part comes from the fact that drugs must be given for a prolonged period of time, and patient's non-compliance with the therapeutic regimen has permitted TB to remain a serious health problem in the world. New anti-TB drugs should ideally shorten duration of treatment, reduce total number of doses, and be active against latent bacteria. Drugs that can act against multi-drug resistant strains of *M. tuberculosis* would also contribute to effective control of this disease. A recent approach to develop these new agents is rational drug design. Therefore, studies of possible new molecular targets are a fundamental condition.

Enzymes of purine biosynthesis in *M. tuberculosis*, which are required for mycobacterial growth, have been extensively studied by our research group and provide many new targets for rational design of more effective antimycobacterial agents that could be active against drug-resistant strains.

Adenylosuccinate synthase governs the committed step in the de novo biosynthesis of AMP, coupling the hydrolysis of GTP with the synthesis of adenylosuccinate from L-aspartate and IMP (Honzatko, R.B., and Fromm H.J., 1999). Because this enzyme acts on the synthesis of purine nucleotides at a critical step, it can be essential for mycobacterial growth. The enzyme is present in all known organisms. Vertebrates have two forms of the enzyme: (i) a basic isozyme which is a component of the purine nucleotide cycle and the de novo pathway for the biosynthesis of AMP and (ii) an acidic isozyme, which functions exclusively in de novo AMP biosynthesis (Iancu, C.V., *et al.*, 2006). However, structural and functional studies should be undertaken in order to explore differences in the mode of action and structural features and thereby promote the development of specific inhibitors for this enzyme in *M. tuberculosis.*

The main goal of this work is to study and understand the kinetics and biochemistry of recombinant enzyme Adenylosuccinate Synthase (EC 6.3.4.4) from

Mycobacterium tuberculosis, as well as to establish differences between enzymes present in the TB bacillus and its host. These studies will serve for the future rational deign of drugs against TB.

The DNA coding sequence of Adenylosuccinate Synthase (EC 6.3.4.4) from M. tuberculosis was obtained from H37Rv strain genomic DNA and specific primers by polymerase chain reaction. The fragment was cloned into the pCR-Blunt vector (Invitrogen) and subcloned into the pET-23a(+) expression vector (Novagen). Nucleotide sequence of the cloned fragment was determined by automated DNA sequencing. Different strains of Escherichia coli were tested for expression of the recombinant enzyme. The enzyme was expressed in *E. coli* BL21 (DE3) cells in the insoluble fraction and solubilization was achieved with a freeze-thaw protocol (Magalhães, M.L.B., *et al.*, 2002). Optimization of expression in other *E. coli* strains and enzyme solubilization are currently being done to provide enough material for purification of the recombinant protein and characterization of its three-dimentional structure and study of enzymological properties.

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