Molecular Cloning, Overproduction and Characterization of Mycobacterium tuberculosis IMP Dehydrogenase (EC 1.1.1.205)

Thiago Milech de Assunção, Cristopher Schneider, Luis A. Basso, Diógenes S. Santos

Programa de Pós Graduação em Biologia Celular e Molecular, Faculdade de Biociências - PUCRS

Abstract

Mycobacterium tuberculosis is one of the most widespread bacterium causative agent of tuberculosis (TB), it causes about 3 million deaths per year (Dye C et al, 2002). It is estimated by the World Health Organization (WHO) that one-third of the world’s population is infected with latent TB. If new anti-TB treatments are not developed, one billion of people will be newly infected by 2020 (Sánchez-sixto C et al, 2005). Furthermore, the emergence of multidrug-resistant tuberculosis (TB) has complicated the treatment and control of the disease. Thus, the design of novel antibiotics, active against drug-resistant strains, and the development of drugs that act on new targets are urgently needed (Maus, CE et al 2005).

An understanding of the mode of action and the role of purine de novo pathway enzymes in M. tuberculosis, which are required for mycobacterial growth, could reveal new targets for the rational design of potent and selective anti-TB agents that could be active against drug-resistant strains.

Inosine monophosphate dehydrogenase (IMPDH) is a key enzyme of de novo guanine nucleotide biosynthesis. It catalyzes the nicotinamide-adenine dinucleotide (NAD⁺)-dependent oxidation of inosine 5’-monophosphate (IMP) to xanthosine 5’-monophosphate (XMP) (Shu, Q. and Nair, V., 2008). Because this enzyme acts on the synthesis of purine nucleotides at a critical step, it can be essential for mycobacterial growth. IMPDH has been described in prokaryotes and eukaryotes and its sequence is well conserved, with 41% sequence identity between Mycobacterium tuberculosis and human proteins (Sintchak, M.D. and Nimmesgern, E., 2000). All the same, structural and functional studies should be undertaken in order to explore differences in the mode of action and structural features and
thereby promote the opening of new avenues towards the design of specific inhibitors for this enzyme from *M. tuberculosis*.

**Objectives:**

The purpose of this work is to study and understand the kinetics and biochemistry of the recombinant enzyme IMPDH from *M. tuberculosis*, as well as to establish differences between enzymes present in the TB bacillus and its host. These results have important implications for understanding the metabolism of the mycobacterium and will serve for the future rational design of drugs against TB.

**Methods and Results:**

The region of the DNA coding sequence of IMPDH (EC 1.1.1.205) from *Mycobacterium tuberculosis* was obtained from H37Rv strain genomic DNA and amplified through polymerase chain reaction (PCR). The fragment was cloned into a transition vector pCR-Blunt (Invitrogen) and subcloned into the *NdeI/HindIII* polylinker region of a pET-23a (+) prokaryotic expression vector (Novagen). Nucleotide sequence of the cloned fragment was determined by automated DNA sequencing. The recombinant plasmid was then subjected to different expression conditions in different strains of *Escherichia coli*. The enzyme was expressed in *E. coli* BL21 (DE3) cells in the soluble fraction. Optimization of expression in other *E. coli* strains is currently underway to provide sufficient material for purification by FPLC, biochemistry assays, determination of its three-dimentional structure and study of its enzymological properties.

**References:**


