Characterization of Purine Nucleoside Hydrolase from *Mycobacterium tuberculosis* (EC 3.2.2.-) as a target for the development of a new antitubercular drug and/or vaccine

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**Introduction**

*Mycobacterium tuberculosis* is a pathogenic bacterium which infects humans and causes tuberculosis (TB), an important infectious disease (Cole et al, 1998). It has been estimated that about one third of the world population is latently infected with *M. tuberculosis*, and that about 8.8 million new cases and almost 2 million deaths occur each year (Jain e Mondal, 2008; Pieters, 2008). Since currently available anti-TB drugs has became ineffective, new drugs and vaccines are needed to treat and prevent TB, respectively (Jain e Mondal, 2008). Purine nucleoside hydrolase (PNH) is an enzyme involved in the purine salvage pathway which catalyzes the hydrolysis of all the common occurring purine and pyrimidine nucleotides into ribose and its associated base. This enzyme was characterized in other organisms, for example, *Bacillus thuringiensis* (Liang et al, 2008), *Ochrobactrum anthropi* (Ogawa et al, 2001) and *Trypanosoma vivax* (Versées et al, 2001). Interestingly, to date no report using the sequence of PNH from *M. tuberculosis* is available. Moreover, no experimental evidences about the probable role of this enzyme in the purine salvage pathway in the latency mechanism of the bacillus is available.

Therefore, this work represents an important step in the production of an anti-TB drug or as a new vaccine against TB. For this, the objectives of this work are (a) cloning of *iunH* gene from *M. tuberculosis* H37Rv, (b) protein expression and purification, (c) measurement of PNH activity and kinetic characterization of this enzyme, (d) disruption and complementation of the *iunH* gene from *M. tuberculosis* H37Rv and (e) studies *in vitro* and *in vivo* of mutant strains of *M. tuberculosis*. 
Materials, Methods and Results

In this project we have designed synthetic oligonucleotide primers in order to amplify the coding sequence of the *iunH* (*Rv3393*) gene from *M. tuberculosis* H37Rv. After an amplification step using *M. tuberculosis* genomic DNA as a template, we obtained a product with the expected size (927 bp), as observed by agarose gel electrophoresis. The amplicon was then extracted from agarose and ligated into the pCR-Blunt vector. Thus the recombinant fragment was prepared for subcloning, cleaved with the restriction enzymes *Nde*I and *Hind*III, and ligated into the pET-23a(+) expression vector, which was used for the expression tests of the recombinant protein in *E. coli* cells. Expression assays of the PNH were performed using different strains of *E. coli* and varied experimental conditions. The best expression of the protein was observed in C41(DE3) cells in the soluble fraction with 0.5 mM IPTG induction, at 30 ºC using TB medium. The homogeneous protein was obtained through three steps purification protocol, which comprises two anion exchange chromatography columns (HiPrep Q Sepharose FF and Mono Q) and a size exclusion chromatography column (Superdex 200). Protein quantification was made by the Bradford method using serum bovine albumin as a standard (Bradford, 1986). Mass spectrometry and sequencing were carried out, confirming the PNH’s sequence and the removal of the N-terminal methionine. Kinetic parameters are currently under determination.

Discussion and Perspectives

Our future goals are to determine if in fact there is a preference of the PNH enzyme for substrates such as uridine and inosine, and determine its fundamental enzymological features (*K*<sub>m</sub>, *K*<sub>cat</sub>, *K*<sub>i</sub>, pH dependency, thermal stability). Disruption and complementation of the *iunH* gene from *M. tuberculosis* H37Rv by gene replacement (allelic exchange) and studies in vivo of mutant strains of *M. tuberculosis* will provide knowledge of the importance of *iunH* gene in *M. tuberculosis* biology. In addition, these data can help us in identifying an attenuated sample that can be used as a vaccine against TB or to develop a new antitubercular drug.

References


