The enzyme Purine Nucleosidase from *Mycobacterium tuberculosis* (EC 3.2.2.-) as a target for the development of new antitubercular drugs and/or vaccines

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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 nucleosidase (IunH) 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 the 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 IunH 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.

Therefore, this work represents an important step in the production of IunH in large quantities for use as an anti-TB target. For this, the objectives of this work are (a) cloning of *iunH* gene from *M. tuberculosis* H37Rv, (b) protein expression in *E. coli* cells, (c) purification of the recombinant enzyme, (d) measurement of IunH activity and kinetic characterization of this enzyme, (e) site-directed mutagenesis of *iunH* from *M. tuberculosis* H37Rv and (f) structural studies of IunH.
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). This product was purified by agarose gel electrophoresis and ligated into the pCR-Blunt vector. Then the recombinant fragment was prepared for subcloning, cleaved with the restriction enzymes $NdeI$ and $HindIII$, 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 IunH were performed using different strains of $E. coli$ and some different experimental conditions. The overexpression of the protein in BL21(DE3) and Rosetta(DE3) $E. coli$ strains was observed (~32.9 KDa) both in the soluble and insoluble fractions without IPTG induction, at 37 ºC and 30 ºC and with LB or TB medium.

Discussion and Perspectives

The optimization of protein expression of IunH is necessary to implement high-yield purification protocols. Recombinant homogeneous protein will provide sufficient material for biological activity determination of the protein.

Our future goals are to determine if in fact there is a preference of the IunH enzyme for substrates such as uridine and inosine, and determine its basic enzymological features ($K_m$, $K_{cat}$, $K_i$, pH dependency, thermal stability). Enzyme kinetics, site-directed mutagenesis and structural studies will provide a framework on which can be based the rational design of new inhibitors against IunH from $M. tuberculosis$. In addition, these data can help us in identifying an attenuated sample that can be used as a vaccine against TB.

References


