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Amplification, cloning and expression of orotidine 5'-monophosphate decarboxylase from *Mycobacterium tuberculosis* H37Rv

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Abstract

Tuberculosis is a disease usually caused by the bacillus *Mycobacterium tuberculosis*, which is considered a global public health threat. The tuberculosis treatment currently recommended is long and involves undesirable side effects, leading patients to give up the chemotherapy. Thus, it is essential the development of new drugs to treat TB. Orotidine 5' – monophosphate decarboxylase is an attractive target for rational drug design. The *pyrF* gene was amplified from *M. tuberculosis* H37Rv genomic DNA, cloned into the pCR-Blunt® vector and subcloned into pET-23a(+) expression vector. The recombinant protein was expressed in *Escherichia coli* cells.

Introduction

Tuberculosis (TB) is an infectious disease caused by the bacillus *M. tuberculosis*, which is considered a global public health threat. The major way of TB transmission occurs by ineffective particles expelled by a patient when he/she coughs, sneezes and even through the speech. According to the World Health Organization, it was estimate that 9.4 million of new cases of TB occurred in 2009, the equivalent of 137 cases of TB per 100.000 population (WHO, 2010). The highest incidence rates of TB are in African countries that are linked to the high rates of HIV coinfection, poverty and lack of medicines (Corbett, 2003). The TB treatment currently recommended is long and involves undesirable side effects to the administered drugs, leading patients to give up the chemotherapy. Thus, the emergence of drug resistance, especially multidrug-resistant (MDR-TB), and current extensively drug-resistant (XDR-TB) strains are usually caused by low patient's adherence to treatment (WHO, 2006).

Therefore, with the increase of TB incidence worldwide, there is the need for development of more effective and less toxic anti-tubercular agents. Likewise, the understanding of essential *M. tuberculosis* metabolic routes, as the pyrimidine biosynthetic pathway and the characterization of the enzymes involved in this pathway, are an important step toward the development of new drugs and vaccines to treat TB in order to improve the current treatment. Orotidine 5'-monophosphate decarboxylase (ODCase) catalyses the final step of the *de novo* synthesis of pyrimidines. This enzyme catalyzes the conversion of uridine 5'- monophosphate (UMP) to orotidine 5'- monophosphate (OMP) in the absence of cofactors (Cui, 1999; Lee, 1997). In most prokaryotes, ODCase is a dimer of identical subunits, whereas in higher organisms (Appleby, 1999), it is part of a bifunctional enzyme, uridine 5'-monophosphate synthase (UMPS) that catalyzes the final two steps of the *de novo* pyrimidine biosynthetic pathway. The first reaction of UMPS is carried out by the enzyme orotate phosphoribosyltransferase which converts orotic acid to orotidine 5'-monophosphate. The terminal reaction of UMPS is carried out by ODCase, which converts orotidine 5'-monophosphate to uridine monophosphate (NCBI). ODCase is one of the most proficient enzymes known. Proficiency is the second-order rate constant for the enzymatic reaction divided by the nonenzymatic rate constant ($k_{cat}/k_m/k_{non}$). For ODCase, the proficiency is 2.0 x $10^{23}M^{-1}$ (Lee, 1997). ODCase from *M. tuberculosis* is therefore an attractive target for drug design due to the possibility to find a selective inhibitor to be used as anti-tubercular agent.

Metodology

The *pyrF* gene (Rv1385) encoding *M. tuberculosis* ODCase (E.C. 4.1.1.23) was identified by sequence homology in the genome of *M. tuberculosis* H37Rv. Two synthetic oligonucleotides complementary to regions 5' and 3' of the *pyrF* gene, were designed to contain *Nde*I and *Hind*III restriction sites, respectively. The *pyrF* gene was amplified from *M. tuberculosis* H37Rv genomic DNA by Polymerase Chain Reaction (PCR). The PCR product, in agreement with the expected size (825 pb), was cloned into pCR-Blunt® vector (Invitrogen). Then, the *pyrF* gene was extracted from pCR-Blunt® vector using *Nde*I and *Hind*III restriction enzymes and subcloned into pET-23a(+) expression vector (Novagen). Automatic DNA sequencing was performed to confirm the identity of the gene. Electrocompetent *E.coli* strains were transformed with the recombinant plasmid (pET 23a(+)::*pyrF*) by electroporation. Different expression tests using Luria-Bertani (LB) or Terrific Broth (TB) medium containing the required antibiotics, with or without induction with isopropyl-β-D-tiogalactopiranoside (IPTG), and different temperatures of incubation

(30° or 37°C) were also tested. During incubation, samples (1.0 mL) were collected at various time points, centrifuged and the cells were disrupted by sonication. Soluble and insoluble protein fractions were analyzed by SDS-PAGE.

Results

Automatic sequencing confirmed both identity and integrity of the *pyrF* gene. of *M. tuberculosis* H37Rv and the absence of PCR introduced mutations. The expression of ODCase was observed in insoluble fraction of *E.coli* C41(DE3) strains at 37°C, in TB medium, with IPTG induction. The protein was also expressed in the insoluble fraction of BL21 STAR (DE3), BL21 NH (DE3), ROSETTA (DE3) *E.coli* strains at 37°C at the same conditions. However, no protein with the expected size corresponding to ODcase, was found in the soluble fraction.

Conclusion

The next step of this work will be the optimization of the ODCase expression in the soluble fraction and the purification of the protein. Availability of homogeneous *M. tuberculosis* ODCase protein will allow the biochemical and kinetic characterization of the enzyme, which is an important step toward the development of new drugs to treat TB.

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