Amplification and cloning of guanosine 5’-triphosphate, 3’-diphosphate phosphohydrolase enzyme from *Mycobacterium tuberculosis*

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Abstract

*Mycobacterium tuberculosis*, the etiological agent of human tuberculosis (TB), is one of the most successful bacterial pathogens, remaining a leading cause of morbidity and mortality. One-third of the world’s population is infected with *M. tuberculosis* in a condition known as latency (without clinical manifestations) which facilitates transmission and complicates treatment. Thus, there is a continuous necessity of studies on mycobacterial metabolism. Guanosine 5’-triphosphate, 3’-diphosphate phosphohydrolase from *Mycobacterium tuberculosis* (MtGpp) is a conserved hypothetical protein similar to Gpp from *Escherichia coli* and is a potential target for rational drugs design, since it may participate in the guanosine 5’-diphosphate, 3’-diphosphate (ppGpp) biosynthesis, an important regulatory nucleotide for prokaryotic organisms. In this work, we report amplification, cloning, subcloning and overexpression for a Gpp from *M. tuberculosis* H37Rv. This proves the knowledge about enzymes involved in *Mycobacterium* metabolism to pave the way the design of new antituberculosis agents and vaccines development.

Introduction

Tuberculosis (TB) is an infectious disease mainly/primarily caused by *Mycobacterium tuberculosis* and that presents a major challenge to public health systems worldwide, especially in poor and developing countries. Over 9 million people develop TB each year, and it is reported that approximately 2 million deaths annually [Dye, 1999]. Furthermore, an estimated 1.4 million were HIV positive, showing the coinfection of these diseases in humans. Based on TB skin tests, the World Health Organization estimates that one-third of the world’s population is currently infected with *M. tuberculosis* in a latent form and therefore at risk of
developing the active disease [WHO, 2009]. This problem is further complicated by a dramatic increase in multidrug-resistant (MDR-TB, resistant to at least isoniazid and rifampicin, the most potent anti-TB drugs) and extensively drug-resistant (XDR-TB, resistant to first- and second-line anti-TB drugs) strains of *M. tuberculosis*.

In most bacteria, nutrient limitations provoke the stringent control through the rapid synthesis of the alarmones known as GDP 3’-diphosphate or GTP 3’-diphosphate, also called (p)ppGpp [Chatterji, 2006]. In *M. tuberculosis*, the failure of frontline anti-TB drugs implies the operation of alternative tolerance mechanisms. Specifically, it is proposed that the emergence of resistant subpopulations might depend on the switch to an altered metabolic state mediated by the stringent response alarmonone, (p)ppGpp, possibly involving some or all of the many toxin-antitoxin modules identified in the *M. tuberculosis* genome [Chatterji, 2006]. The MtGpp functions is probably related with the stringent response, since the pppGpp-5’-phosphohydrolase from *E. coli* converts pppGpp to ppGpp, which increase in the stringent response [Hara, 1993, Keasling, 1993]. Furthermore, based in mutagenesis studies of *M. tuberculosis* the MtGpp hypothetical protein has been classified as an essential gene [Srivastava, 2007].

The objective of this work is amplifying the *gpp* gene (*Rv1026*), clone the DNA fragment into pCR®-Blunt and subclone into pET-23a(+).

**Methods**

Based on the published genome sequence from *M. tuberculosis* H37Rv [S.T. Cole 1998], two oligonucleotide primers, Gpp1 (forward: 5’-tgg *cat atg* gcg cta acc cgg gtc gcc gcg-3’) and Gpp2 (reverse: 5’-ga *aag ctt* tta tcc ggc cag tga caa cgc gat gc-3’), complementary to the 5’ N-terminal and the 3’ C-terminal ends of the hypothetical *gpp* (*Rv1026*) structural gene were synthesized to contain, respectively, *NdeI* and *HindIII* restriction sites (bold). These primers were used to amplify the *gpp* coding sequence (960 bp) from *M. tuberculosis* H37Rv genomic DNA. Using standard Polymerase Chain Reaction (PCR) conditions and *Pfu* DNA polymerase for high-fidelity amplification (Stratagene), the amplified product was purified by electrophoresis on a low-melting agarose gel, and cloned into the PCR-Blunt cloning vector (Invitrogen) and then ligated into the pET-23a(+) expression vector (Novagen). Nucleotide sequence of the *M. tuberculosis gpp* gene will be determinate by automated DNA sequencing to confirm the identity, integrity, and absence of PCR-introduced mutations in the cloned
gene. The recombinant plasmid pET-23a(+)::gpp will be transformed into electrocompetent *E. coli* cells (Novagen).

**Results**

The *gpp* gene (Rv1026), amplified from *M. tuberculosis* H37Rv genomic DNA by PCR, showed a band of 960 bp approximately in agreement with the expected for Gpp of MTB [S.T. Cole 1998]. The PCR product was purified and cloned into pCR®-Blunt vector (Invitrogen), transformed into electrocompetent *E. coli* DH10B and extracted the plasmidial DNA. The insertion of fragment into cloning vector was confirmed for the cleaved with the EcoRI restriction enzyme. Plasmidial DNA was cleaved with NdeI and HindIII (New England Biolabs), and ligated into the pET-23a(+) expression vector (Novagen). The overexpression tests of MtGpp are currently underway in our laboratory. The present results obtained with guanosine-5’-triphosphate, 3’diphosphate phosphohydrolase from *M. tuberculosis* are the first steps to increase knowledge of this enzyme and their importance in one of the important pathways responsible for the maintenance of latency in mycobacterium, as too an attempt to identify potential targets for drug and vaccine development.

**References**