Biochemical studies of UMP kinase (EC 2.7.4.22) from *Mycobacterium tuberculosis* as a potential antitubercular drug target.

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**Introduction**

Human tuberculosis (TB), mainly caused by *Mycobacterium tuberculosis*, is a major cause of illness and death worldwide, especially in Asia and Africa. Increasing numbers of co-infections with HIV, the widespread emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains have provided a very alarming challenge to global health and led us to focus on the development of new therapeutics against the disease. Strategies based on the discovery of new targets for antimycobacterial agents involve the identification of biochemical pathways that are essential for mycobacterial growth.

Pyrimidine biosynthesis is an essential pathway for mycobacteria viability, therefore, the probable enzyme UMP kinase from *M. tuberculosis* (*Mt*UMPK), encoded by the *pyrH* (Rv2883c) gene is an attractive drug target. Genetic studies have provided evidence that UMP kinase is essential for growth in both Gram-negative (*Escherichia coli*) and Gram-positive bacteria (*Streptococcus pneumoniae*). Essentiality has also been predicted for *M. tuberculosis* in a study which demonstrates the essentiality of the *pyrH* gene, highlighting the relevance of *Mt*UMPK as target for protein inhibitors. These characteristics, and the fact that UMP kinase displays a key role in the metabolism of nucleotides, prompted us to characterize and to
investigate the enzymatic properties of the MtUMPK as a tool to design highly specific inhibitors acting on mycobacteria with little or no effect on host organisms, and which could be active against drug-resistant strains.

**Materials and Methods**

The full-length pyrH coding region (786bp) was PCR amplified from M. tuberculosis H37Rv genomic DNA. The PCR fragment was cloned at the Ndel and HindIII restriction sites of the pET-23a(+) expression vector and the resulting plasmid was transformed into the E. coli BL21(DE3). MtUMPK was overexpressed and the recombinant enzyme was purified using a FPLC system. The N-terminal amino acid residues of homogenous recombinant MtUMPK were determined by automated Edman degradation sequencing and recombinant MtUMPK was analyzed by electrospray ionization mass spectrometry (ESI-MS). Gel filtration chromatography was performed on a Superdex 200 (HR 10/30) column (GE Healthcare) pre-equilibrated with 50 mM Tris HCl pH 7.5 containing 200 mM NaCl in order to determine the oligomeric state of the protein. Kinetic studies were performed to evaluate the steady-state parameters as well as to investigate the allosteric behavior. The kinetic mechanism of MtUMPK and the thermodynamic binding parameters were investigated by isothermal titration calorimetry (ITC) experiments.

**Results and Discussion**

Recombinant MtUMPK was purified to homogeneity by a three-step protocol and was shown to catalyze the phosphorylation of UMP to UDP, using ATP.Mg$^{2+}$ as phosphate donor, using a coupled spectrophotometric assay. The molecular mass of the MtUMPK subunit (27 264.08 Da) was determined by ESI-MS analysis and protein identity was corroborated by N-termini sequencing. Size exclusion chromatography showed that the protein is an oligomer, composed of four subunits. Kinetic studies revealed that MtUMPK exhibits cooperative kinetics towards ATP and, as several bacterial UMPKs, is submitted to allosteric regulation. GTP and UTP are positive and negative effectors, respectively, maintaining the balance of purine versus pyrimidine synthesis.

The ITC results indicate that catalysis proceeds by a sequential ordered mechanism, in which the UMP substrate binds to the enzyme after the addition of ATP molecule, followed
by a random displacement of the products. These characteristics suggest that MtUMPK does not resemble its eukaryotic counterparts, accounting this enzyme for validity as an antitubercular drug target.

**Conclusions**

In summary, we showed that MtUMPK shares several common traits with UMPKs, especially from Gram-positive organisms, which also exhibit cooperative kinetics with ATP as substrate. In addition, thermodynamic studies provided a solid elucidation about the kinetic mechanism and showed that the allosteric effectors bind to the free enzyme.

The results presented in this work provide experimental evidence that the pyrH gene from *M. tuberculosis* encodes a protein which is submitted to allosteric regulation and represent an important step towards the characterization of a specific target for new antitubercular agents.