Characterization of Uracil Phosphoribosyltransferase from *Mycobacterium tuberculosis* H37Rv

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Abstract

The causative agent of tuberculosis (TB), *Mycobacterium tuberculosis*, infects one-third of the world population. The World Health Organization estimates that 9.27 million new TB cases occurred in 2007. Among these cases, 1.37 million were coinfected with HIV, resulting in 2 million deaths annually throughout the world (WHO, 2009). Progression of TB infection may succeed through microbial immediate elimination and/or latency conditioning, or host immune fail resulting in development of active disease (Ducati et al., 2006). There is a critical need for the development of drugs that would reduce progression from latent infection to active TB (Stewart et al., 2003). The pyrimidine salvage pathway is an attractive target for the development of new drugs against TB because it may be involved in latency mechanisms of *M. tuberculosis*.

The gene (*upp, Rv3309c*) encoding uracil phosphoribosyltransferase (UPRTase) was identified by homology in the genome of *M. tuberculosis* H37Rv (Cole et al., 1998). The UPRTase (EC 2.4.2.9) enzyme from *M. tuberculosis* consists of 207 amino acid residues (21.8 kDa). It is part of the pyrimidine salvage pathway, in which pyrimidine bases and nucleosides derived from preformed nucleotides are recycled (Moffatt and Ashihara, 2002). In general, the pyrimidine salvage pathway is preferentially utilized by some bacteria, because it demands less energy than *de novo* biosynthesis (Kadziola et al., 2002). UPRTase catalyzes the conversion of uracil and 5-phosphate-α-1-diphosphate (PRPP) to uridine 5’-monophosphate (UMP) and diphosphate, respectively (Arent et al., 2005). Unlike enzymes in the *de novo* synthesis of UMP, UPRTases have only been characterized in lower organisms and are therefore potential targets for the development of new drugs. Recently, a human UPRTase was isolated from a human fetal brain cDNA library. However, its cloning, expression and
purification resulted in a protein without UPRTase catalytic activity (Li et al., 2007). Then, there is no experimental evidence for the presence of UPRTase in humans.

Thus, UPRTase from *M. tuberculosis* is an attractive target for rational drug design against TB, once it may be possible to find selective inhibitors for this enzyme. Moreover, UPRTase may have an important, though not yet understood, role in the latent stage of the TB bacillus. The goals of this work include PCR amplification and cloning of the *upp* gene, overexpression, purification and characterization of recombinant UPRTase from *M. tuberculosis* to validate its biological role as an UPRTase and its relevancy for the pyrimidine salvage pathway, if any, in the *M. tuberculosis* metabolism.

The *upp* gene (624 bp) was amplified from *M. tuberculosis* H37Rv genomic DNA. Then, it was cloned into the pCR®-Blunt vector (Invitrogen) and subcloned into the pET-23a(+) expression vector (Novagen), which was used to express the recombinant protein in *Escherichia coli* cells. The UPRTase recombinant protein was expressed in the soluble fraction of BL21(DE3) *E. coli* cells, and best expression conditions were growth at 37ºC after 18 hours of incubation without IPTG induction. A FPLC ÆKTA system (GE Healthcare) was utilized for establishing a recombinant protein purification protocol. Three chromatography steps were employed, including an anion exchange (DEAE Sepharose CL6B), size exclusion (Sephacryl S300) and anion exchange (Mono Q) columns. Mass spectrometry analysis and N-terminal amino acid sequencing provided evidence for the identity of the homogeneous recombinant *M. tuberculosis* UPRTase. The molecular mass of native *M. tuberculosis* UPRTase homogeneous protein was determined to be a pentamer by gel filtration chromatography.

The pyrimidine bases cytosine and thymine were evaluated as substrates for *M. tuberculosis* UPRTase using a HPLC ÆKTA system (GE Healthcare), where nucleotides and bases were monitored at 254, 260 and 280nm. Since this analysis resulted in inconsistent data, the nucleotide content was analyzed by liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). These analyses indicated that *M. tuberculosis* UPRTase does not employ thymine and cytosine as enzyme substrates. Recombinant *M. tuberculosis* UPRTase activity was determined spectrophotometrically by measuring the conversion of uracil into UMP at 280nm as previously described (Natalini et al 1979). Then, determination of the apparent steady-state kinetic parameters, $V_{\text{max}}$ and $K_{\text{m}}$, was carried out at varying concentration of one substrate while the concentration of the other substrate was maintained at constant saturation level. Steady-state kinetic analyses (pH-rate
profiles, kinetic constants and product inhibition) will provide evidence for the kinetic mechanism of this enzyme, which may be useful to the rational design of future anti-TB agents.

References


