

## Amplification, cloning and expression of human uracil phosphoribosyltransferase.

Gregório H. S. Laseck<sup>1,2</sup>, Daiana Renck<sup>2</sup>, Ana C. O. Dias<sup>2</sup>, Luiz Augusto Basso<sup>1,2</sup>, Diogenes S. Santos<sup>2</sup>

<sup>1</sup> Faculdade de Biociências, PUCRS. <sup>2</sup> Instituto Nacional de Ciência e Tecnologia em Tuberculose, Centro de Pesquisas em Biologia Molecular e Funcional

### Resumo

Pyrimidine nucleotides can be synthesized either through the de novo synthesis from compounds unrelated to nucleotides and by the salvage pathways, which use nucleotides, nucleosides, or nucleobases already present in the cell. In general, the pyrimidine salvage pathway is preferentially utilized by cells, because it demands less energy than de novo biosynthesis. Uracil phosphoribosyltransferase (UPRTase, EC 2.4.2.9), encoded by the *upp* gene, is a key enzyme in the pyrimidine salvage pathway, which is important in nucleotide metabolism. UPRTase catalyzes the conversion of uracil/5-fluorouracil (5-FU) and 5-phosphoribosyl- 1-R-diphosphate to uridine monophosphate (UMP)/ 5-FUMP and pyrophosphate in the presence of Mg<sup>2+</sup>. 5-FU has been used in clinical practice against many types of solid tumors. Clinical studies have demonstrated that uridine can be used to reduce 5-FU toxicity, leading to an increased therapeutic index, and to selectively protect normal tissues from this host toxicity; high doses of exogenous uridine are not well tolerated in humans. In this study, the *upp* gene encoding human UPRTase was obtained from design of two oligonucleotide primers complementary to regions 5' and 3' of *upp* gene contain *Nde*I and *Hind*III restriction sites, respectively. The *upp* gene was amplified from the human blood cDNA by Polymerase Chain Reaction (PCR). The PCR product in agreement with the expected size (927 bp) was cloned into pCR<sup>®</sup>-Blunt vector (Invitrogen). Then, the *upp* gene was extracted from pCR<sup>®</sup>-Blunt vector using *Nde*I and *Hind*III restriction enzymes and subcloned into pET-23a(+) expression vector (Novagen). Automatic DNA sequencing confirmed both identity and integrity of *upp* gene and the absence of PCR introduced mutations. Electrocompetent *E. coli* strains were transformed with the recombinant plasmid

(pET-23a(+):::upp) by electroporation. Different expression conditions were employed in order to obtain UPRTase expression in the soluble fraction. The protein expression was analyzed by SDS-PAGE. The expression of UPRTase was observed in the insoluble fraction of C41 (DE3), BL21 (DE3) and Rosetta (DE3) *E. coli* strains at 37°C, using the culture medium LB, with isopropyl- $\beta$ -D-thiogalactopiranosídeo (IPTG) induction. The protein was also expressed in the insoluble fraction of BL21-NH (DE3) *E. coli* strains at 37°C, using as a culture medium LB and with IPTG induction. However, a small amount of protein could be found in the soluble fraction of BL21-NH (DE3) at 30°C, using as a culture medium TB, LB or 4YT and with IPTG induction, though the major amount of protein is yet expressed in the insoluble fraction. The next step of this work will be the optimization of the UPRTase expression in the soluble fraction and the purification of the protein. Availability of homogeneous protein will allow the biochemical and kinetic characterization of the enzyme which is important step toward the development of new drugs to treat cancer.

## Referências

- Arent, S.; et al., Allosteric Regulation and Communication between Subunits in Uracil Phosphoribosyltransferase from *Sulfolobus solfataricus*. **Biochemistry**. Vol 44 (2005), pp. 883-892.
- LI, J.; et al., Identification and characterization of human uracil phosphoribosyltransferase (UPRTase). **J Hum Genet**. Vol. 52 (2007), pp. 415-22.
- MAGNI G, et al., Bakers' yeast uridine nucleosidase is a regulatory copper containing protein. **Biochem Biophys**. Vol. 69 (1976), pp. 724 –730.
- NATALINI, P.; et al., Baker's yeast MP:pyrophosphate phosphoribosyltransferase. Purification, enzymatic and kinetic properties. **J Biol hem**. Vol 254, N° 5 (1979), pp. 1558-1563.
- SHAMBAUGH G.E., Pyrimidine biosynthesis. **The American Journal of clinical Nutrition**. Vol. 32 (1979), pp. 1290-1297.
- ROOSILD T.P.; et all., Implications of the structure of human uridine phosphorylase 1 on the development of novel inhibitors for improving the therapeutic window of fluoropyrimidine chemotherapy. **BCM Structural Biology**. 9:14 (2009).