SOLUBLE EXPRESSION OF THE *ESCHERICHIA COLI* K12 *GUAC*-ENCODED GMP REDUCTASE

Dockhorn, P.E., Selbach, B.P., Basso, L.A., Santos, D.S.

Centro de Pesquisas em Biologia Molecular e Funcional, PUCRS

Purine and pyrimidine nucleotides play an important role in many critical cellular functions. They regulate biological processes, such as biosyntheses in response to extracellular signals. Since nucleotides are necessary for DNA replication and RNA transcription, drugs that block the *de novo* pathways of nucleotide synthesis have been successfully used as antiviral and antitumor agents counteracting cell division. In *Escherichia coli*, the guanosine monophosphate reductase (GMPR) is encoded by the *guaC* gene; it catalyzes the irreversible NADPH-dependent reductive deamination of GMP to inosine monophosphate. GMPR is also important in the conversion of nucleobase, nucleoside, and nucleotide derivatives of guanine to adenine nucleotides, maintaining the intracellular balance of G and A. This work aims at expressing the *E. coli* GMPR in its soluble form. The *guaC* gene was amplified from *E. coli* K12 genomic DNA by PCR, using *Pfu* DNA polymerase. The PCR product was sub-cloned into the pET-23a(+) expression vector and subsequently sequenced to confirm the gene identity. The recombinant plasmid was used to transform *E. coli* BL21(DE3) cells for expression. SDS-PAGE analysis revealed the superexpression of the GMPR with the expected 38 kDa MW in the soluble form. Perspectives rely on the purification of GMPR by FPLC and on kinetic studies of the enzyme in order to investigate both chemical and kinetic mechanisms of the catalyzed reaction.