BIO_05 - Characterization of a recombinant mycobacterial L-asparaginase produced in *Escherichia coli*

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Introduction: Acute lymphoid leukemia (ALL) is the second cause of death worldwide due to cancer among children up to 4 years old. During treatment, the enzyme L-asparaginase (L-asp) type II is used, due to its higher affinity for the substrate L-asparagine over L-glutamine. When administered effectively, it leads to a decrease in the former substrate in the extracellular environment and, consequently, to selective apoptosis of the ALL neoplastic cells. However, in the treatment with L-asp, adverse effects are observed, such as pancreatitis and neurological problems, due to the double recognition of the substrates asparagine and glutamine since glutamine is essential for many biological processes, especially in pancreatic and synaptic functions. The immune response can also cause other adverse effects, such as enzyme inactivation and/or an anaphylactic reaction, with consequent treatment interruption or modification. The use of the mycobacterial L-asp has been identified as a promising strategy, due to its lower glutaminase activity.

Objective: Characterize the asparaginase / glutaminase activity of a recombinant mycobacterial L-asparaginase expressed in *E.coli*.

Methodology: 1) Cloning of the L-asp gene: the mycobacterial L-asp gene was amplified and cloned in a plasmid of the pET family in *E. coli*. 2) Expression: *E.coli* harboring recombinant plasmid was grown in Luria-Bertani (LB) medium under antibiotic selection. After induction, samples were collected to analyze heterologous protein solubility. Total proteins were precipitated with trichloroacetic acid, resolved on SDS-PAGE polyacrylamide gel followed by western blotting with anti-his antibody. 3) L-asp purification: the recombinant protein was purified from the soluble fraction by immobilized metal affinity chromatography. 4) L-asparaginase assay: The activity of the recombinant mycobacterial L-asp was measured for the asparagine and glutamine substrates, and assays were performed to characterize optimal pH and temperature. L-asparaginase / glutaminase activity was measured by ammonium release using Nessler's reagent method.

Results: After electrophoresis and western blotting assays we detected in the intracellular proteome a band corresponding in size to L-asp with a 6-His tag, and the corresponding cellular fraction was used to purify recombinant protein. The recombinant mycobacterial L-asp showed asparaginase activity, but no glutaminase activity under the conditions tested. The optimal pH for asparaginase activity varied between 8.0-9.0 in Tris-HCl buffer and the optimum temperature was 37 °C.

Conclusion: Mycobacterial L-asp was successfully obtained as a heterologous recombinant protein on *E. coli* and it showed a high drug potential due to its higher asparaginase/glutaminase activity ratio when compared to other commercial L-asp (Leuginase).

Keywords: L-asp; mycobacteria; heterologous expression