BIO_07 - Human L-asparaginase engineering for improvement of catalytic activity and application in Acute Lymphoblastic Leukemia therapy

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Introduction: L-asparaginase is a therapeutic enzyme widely used for the treatment of Acute Lymphoblastic Leukemia (ALL). The commercially available formulations present limitations for clinical use due to the bacterial origin of these enzymes, which can trigger adverse reactions in patients. On the other hand, human enzymes stand out as an alternative to immunogenicity. hASNase1 is a human L-asparaginase, derived from the N-terminal domain of the 60kDa-lysophospholipase protein and it is a suitable therapeutic candidate. However, since this enzyme displays low catalytic efficiency (low k_{cat} and millimolar K_m), it requires an engineering approach to enhance its catalytic properties. Our research group uses a rational design strategy to improve the catalytic properties of hASNase1 for applications in ALL therapy, engendering variants from *in silico* approaches.

Objective: The aim of this study is to express nine hASNase1 variants and to evaluate their catalytic activity, comparing them with the native enzyme.

Methodology: Enzyme mutations were suggested through bioinformatics analysis and the variant proteins were obtained following site-directed mutagenesis protocols. The mutations of interest were inserted using pairs of primers through PCR assay. The system used for the expression of hASNase1 and variant proteins was the pET-SUMO vector transformed in *E. coli* (DE3) Rosette. Protein expression was induced by adding 0.25 mM IPTG at 15 °C / overnight and the purification process was carried out by Ni-NTA chromatography. The enzyme activity was determined by Nessler assay.

Results: Sanger sequencing confirmed the mutations of interest. Expression tests showed high production of the enzymes in *E. coli*. Although the predominant protein fraction remains in the insoluble fraction, it was possible to obtain enough soluble and active enzymes for activity assays. The results obtained from Nessler assay showed that all the mutants exhibited higher catalytic activity than the native enzyme (hASNase1), with emphasis on 2 mutants, #4 and #9, which presented a 52-fold and 15-fold better catalytic activity than hASNase1, respectively. The next steps in this study include determining the kinetic parameters of the most promising variants and combining their mutations in order to achieve a synergy between them, which may lead to an even more improved catalytic activity.

Conclusion: In conclusion, all the mutants proposed by *in silico* studies presented superior activities compared to the native one, and the protein engineering approach was effective in reaching catalytic activities close to the commercial ones.

Keywords: L-asparaginase; Immunogenicity; Rational Design