BIO_10 - Development and optimization of the heterologous expression of a human L-asparaginase variant with potential enhanced catalytic activity

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Introduction: L-asparaginase is a therapeutic enzyme widely used for Acute Lymphoblastic Leukemia (ALL) treatment. Since the antileukemic protein drug has a bacterial origin, it may cause several side effects to patients. A human homologous enzyme (hASNase1), which belongs to the N-terminal domain of the 60kDa lysophospholipase protein, stands out as a potential candidate to replace the use of bacterial enzymes and to overcome immunogenicity challenges in chemotherapy. However, hASNase1 displays low catalytic efficiency, which requires an engineering approach to improve its catalytic properties. In this work, a chimera (HERA) was assembled by inserting a loop from *Cavia porcellus* L-asparaginase (gpASNase1) in a corresponding region in hASNase1, as gpASNase1 possesses high catalytic activity.

Objective: The aim of this study focuses on the optimization of bacterial expression, solubilization and purification of hASNase1 and HERA, in order to evaluate and compare their catalytic activities and to relate their functions to structural information obtained in simulations of Molecular Dynamics (MD).

Methodology: GpASNase1 (PDB: 4R8K), a model of hASNase1 and HERA were submitted to MD for 500 ns in aqueous system using GROMACS 2018.3 program. For experimental tests, two expression systems were tested, both using pET-SUMO vector: a) expression of hASNase1 in *E. coli* BL21-DE3 (Star) - evaluation of temperature on protein expression (28?C and 37 ?C, 1mM of IPTG for 4h) and different solubilization buffers were tested. b) hASNase1 and HERA were expressed in *E. coli* Rosetta. IPTG concentration (0.1 mM and 0.3 mM) and induction temperature (15?C), overnight. Solubilization and refolding protocols for insoluble protein aggregates were also tested. The enzymes were purified by Ni matrix.

Results: HERA's structure was maintained during MD and it showed a reduction in the Turning Radius (TR) (from 3.35 nm to 3.20 ? 0.01 nm) due to the approximation between the monomers. HERA's TR is closer to that of gpASNase1 (3.22 ? 0.01 nm) than that of hASNase1 (3.26 ? 0.01 nm). The two protocols yielded high expression level for both proteins, however most of them remained as inclusion bodies. The best condition for solubilization recovery was achieved in protocol b) at IPTG concentration of 0.3 mM, 15?C - overnight. HASNase1 and HERA were fully soluble using urea 2M, which is considered a mild denaturation condition. After purification, both enzymes showed activity, through the Nessler colorimetric assay. Trials are still underway to verify whether HERA's kinetic parameters are improved compared to those of hASNase1.

Conclusion: HERA has not shown loss of structure in MD analyzes, which is in agreement with the preserved catalytic activity. In addition, its structure is more compact compared to hASNase1 and it is closer to gpASNase1. Expression and solubilization assays have been optimized to obtain soluble enzymes for catalytic characterization assays.

Keywords: Human L-asparaginase; Heterologous expression; Catalytic activity