BIO_16 - Development of a biopharmaceutical derived from human L-asparaginase through *in silico* and *in vitro* evolution techniques

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Introduction: L-asparaginase, vital in treating Acute Lymphoblastic Leukemia (ALL), originates from bacteria in commercial products. Despite efficacy, these products are immunogenic, posing risks. A promising perspective arises with hASNase1, a human-origin enzyme, potentially reducing immunogenicity. However, hASNase1's catalytic efficiency is lower than commercial variants, requiring protein engineering for viable ALL therapy. Through rational design technique, a hybrid variant (HERA) of hASNase1 with Cavia porcellus (gpASNase1) regions was proposed, as well as variants containing point mutations to enhance enzymatic activity.

Objectives: The goal is to develop human L-asparaginase variants, analyze enzymatic activity, and establish correlations with structural data through Molecular Dynamics (MD) simulations.

Methodology: The gene that codifies HERA was synthesized, and site-directed mutagenesis produced the other variants from HERA. Enzymes were expressed in *E. coli* Rosetta (TB medium, 0.3 mM IPTG, 16 °C, 17 h, 200 rpm), purified via IMAC using His-link resin (Promega), and quantified by Qubit™ fluorimetry. The Nessler colorimetric assay gauged catalytic activity. The most promising variants underwent MD simulations. Systems were set up with the GROMACS 2018.3 package in a dodecahedral box, aqueous system, and Asn insertions at catalytic and allosteric sites. Parameters included GROMOS54a7 force field, SPC water, temperature of 310K, and ions at a 0.15M concentration. Following a 240 ps equilibration, using a canonical and isothermal-isobaric ensemble protocol, the trajectory acquisition phase extended for 300 ns. Subsequently, thermodynamic and structural properties were assessed by calculating RMSD, Interatomic Interaction Potential (IIP), and Radius of Gyration (RG).

Results: Results reveal variants with activity up to two times higher than HERA and 46 times higher than hASNase1. MD results demonstrate that mutations improved catalytic site stability. IIPs between catalytic site residues and Asn are generally more stable and attractive than in hASNase1. IIP analyses between allosteric site residues and Asn suggest that, in the variant with a mutation in this region, the enzyme possibly lost allosterism, expelling Asn and enhancing stability. In HERA, gpASNase1 regions benefited from newly inserted point mutations that further stabilized these regions, contributing to the doubling of activity.

Conclusion: In conclusion, MD results align with experimental findings, indicating higher activity variants with more stable movement and IIP, enhancing catalysis.

Keywords: L-asparaginase; Enzymatic Activity; Molecular Dynamics