

BIO_14 - Rational design of a human-like L-asparaginase as a strategy to improve catalytic efficiency

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Introduction: Treating Acute Lymphoblastic Leukemia (ALL) involves using the therapeutic enzyme L-asparaginase, whose commercially available formulations are of bacterial origin and can trigger a series of immunogenic reactions in patients. In this context, human-origin enzymes stand out as an alternative to the immunogenic effects in patients. hASNase1 is a human enzyme belonging to the N-terminal domain of the 60kDa lysophospholipase, proposed as a therapeutic candidate to minimize immunogenic reactions. However, hASNase1 displays low catalytic affinity towards L-asparagine and requires protein engineering strategies to improve its catalytic efficiency.

Objectives: Rational design of hASNase1 to generate and select mutants with high affinity for L-asparagine with the prospect of developing a therapeutic product with lower immunogenicity for ALL treatments.

Methodology: The his₆-SUMO-hASNase1 gene construct was subjected to mutagenic PCR using primer pairs with the selected mutations of interest identified through *in silico* analysis for rational design purposes. The system for protein expression was the pET-SUMO vector in *E. coli* (DE3) Roseta. The production of the native and recombinant enzymes was performed in overnight cultivation at 15°C. The isolation of protein combined IMAC and IEC chromatographic techniques. The Nessler assay determined enzyme activity. Molecular Dynamics (MD) analyses were conducted using the GROMACS package.

Results: All mutations were inserted at the positions of interest, confirmed by Sanger sequencing analysis. The expression condition and isolation strategies of hASNase1 and the variants enabled the production of soluble and active enzyme for conducting specific activity assays. All variants exhibited higher activity than the native enzyme (hASNase1), with particular emphasis on mutant 4, which showed a catalytic activity 52x greater than hASNase1. The MD analyses demonstrated how the amino acid substitution in Mutant 4 improved the enzyme's interaction with the substrate.

Conclusion: The rational design strategy was successful and generated variants with superior catalytic activity compared to hASNase1. Mutant 4 has the potential for an innovative therapeutic product with the hypothesis of low immunogenicity effects.

Keywords: hASNase1; Acute Lymphoblastic Leukemia; Protein engineering