Title: PURIFICATION BY AFFINITY CHROMATOGRAPHY (IMAC-NI) OF L-ASPARAGINASE I OF *Saccharomyces cerevisiae* EXPRESSED IN *Escherichia coli* ORIGAMI

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Abstract:

L-Asparaginase (L-ASNase) is an important antineoplastic agent used to treat acute lymphoblastic leukemia (ALL). Currently there are three clinically approved preparations from bacterial sources, but toxicity and hypersensitivity, including the production of anti-L-Asparaginase antibodies, are common drawbacks. In this context, the search for novel L-ASNases, especially from eukaryotic sources, is important. The yeast *Saccharomyces cerevisiae* has two isoforms of the enzyme: L-ASNase I, a constitutive intracellular enzyme, and L-ASNase II, a cell wall enzyme. In this work, we studied the heterologous production and purification of *S. cerevisiae* L-ASNase I expressed in *E. coli* Origami. The *E. coli* pre-inoculum was cultivated in 2XYT medium (pH 7.0) at 37°C, 180 rpm during 18 hours. The inoculum started with 0.2 of OD_{600nm} and was grown until OD_{600nm} of 0.7 at the same conditions. The culture was induced with 0.1 mM IPTG for 20 hours at 18°C. Following, the culture was centrifuged at 4000rpm for 20 min and the cells collected and cleavage with an ultrasound probe. Cell lysate was centrifuged at 16000xg for 30 min and filtered through a 0.22 µm membrane and purified by Immobilized metal ion affinity chromatography (IMAC-Ni) in an AKTA FPLC. An initial elution gradient from 0 to 100% with 500 mM imidazole was used to determine the imidazole concentration necessary for L-ASNase elution. Two peaks were obtained, only the second corresponding to L-asparaginase eluted with 55% of imidazole. A new elution step with 55% 500mM imidazole was performed and the enzyme purity was confirmed by electrophoresis (SDS-PAGE). The fractions corresponding to the enzyme were submitted to total proteins determinations by the BCA method and enzymatic activity determinations by the Nessler method. Electrophoresis showed only one band corresponding to L-ASNase monomer with molecular weight of 35 kDa approximately. The specific activity in the crude extract was 1.20 U/mg and after purification was 59.02 U/mg, with 48.79 fold purification and a yield of 16.1%. The specific activity of the pure enzyme was similar to previous work, but we only used one step of purification.

Key words: L-Asparaginase, E. coli Origami, Affinity chromatography IMAC, enzymatic activity.

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