Yeast L-Asn1 Asparaginase: An alternative biopharmaceutical to acute lymphoid leukemia?

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Bacterial L-asparaginases (L-Asn) are used as therapeutic proteins for treatment of acute lymphoid leukemia (ALL), since some tumor cell types are dependent on the availability of extracellular asparagine (Asn). Bacterial L-Asn is able to hydrolyze efficiently Asn into aspartic acid and ammonia, decreasing the source of Asn to tumor cells. International pharmaceutical industries produce L-Asn from *Eschericchia coli* and *Erwinia chrysantemi*, but L-Asn is not produced by the Brazilian pharmaceuticals, and alternative sources of L-Asn are important to avoid treatment failures due to international fluctuations in the production of the biopharmaceutical. The protein sequence analysis of L-Asn from bacteria revealed that *Saccharomyces cerevisiae* presents an enzyme, named L-Asn1, with high homology with the bacterial counterparts (~ 36% identity and ~ 54% similarity) suggesting a potential alternative in the treatment of the ALL. The aim of this work relies on the functional and structural characterization of recombinant L-Asn1 from *S. cerevisiae*. Using bacterial crystallographic coordinates we have constructed a theoretical model of Asn1, which suggest that bacterial and yeast enzyme presents structures. The recombinant enzyme was expressed in *E. coli* BL21 (DE3) strains as histidine tagged protein (6×), and the oligomeric state was determined by size exclusion chromatography under oxidizing and reducing conditions, revealing that the yeast enzyme is a multimeric protein, containing 16 monomers redox insensitive. Since Asn1 posses ten cysteine residues we also investigate by non reducing SDS PAGE, if disulfide bridges account to the quaternary structure, but no disulfide was detected. Crystallization trials and determination of the kinetic parameters are in progress.

Keywords: L-asparagine, L-sparaginase, acute lymphoid leukemia.
Acknowledgment: FAPESP and CNPq.