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Characterization and protein engineering of *L-asparaginase 1* from *Saccharomyces cerevisiae* to evaluate its use as biopharmaceutical

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Acute lymphoblastic leukemia (ALL) is the most common neoplasia in children. The leukemic cells depend on the presence of L-asparagine (Asn) in the bloodstream for protein synthesis and cell proliferation. L-asparaginase (ASNase) is an important anticancer biopharmaceutical in the treatment of the disease, since it hydrolyzes Asn resulting in ammonia and aspartic acid, preventing tumor cells of using such amino acid for protein synthesis, leading to apoptotic cell death. ASNase is currently obtained from *Escherichia coli* and *Erwinia chrysanthemi* and both formulations are associated with a high rate of adverse effects, mainly drug resistance and severe hypersensitivity, which undermine the progress and effectiveness of the treatment. The yeast *Saccharomyces cerevisiae* has the gene ASP1 responsible for the production of L-asparaginase 1 (ScASNase1) that possesses high similarity in amino acids sequence with the bacterial enzymes used as biopharmaceutical. However, there are few studies about ASNase produced by *S. cerevisiae*. Thus, this work aims to verify the catalytic efficiency of ScASNase1, to characterize the catalytic site and perform site directed mutagenesis to improve the kinetic parameters and compare them with bacterial ones, to assess whether ScASNase1 may represent an interesting therapeutic alternative in the treatment of ALL. The ScASNase1 was isolated from the *S. cerevisiae* genomic DNA and cloned into *NdeI* and *BamHI* restriction sites of pET15b expression vector, which was cloned in *E. coli* (BL21(DE3)) for protein expression. The proteins were purified by metal affinity chromatography. The specific activities and kinetic parameters were obtained using the Nessler's reagent (Merck) colorimetric method for identification of ammonia. The ScASNase1 showed an specific activity of 330 U/mg, K0,5 = 8,9 mM with a Hill coefficient value of 2.005 indicating positive cooperativity of the enzyme with substrate, and catalytic efficiency of $4x10^4$ M^{-1}. The reactions of site directed mutagenesis at residues of predicted catalytic site (T64A, T141A, K215A and Y78A) and at residues S301N, A331D, ΔG77, Y243S and K335E supposed to improve kinetic parameters were performed using the kit QuickChange® (Agilent Technologies). The proteins with mutations T64A, T141A, K215A and Y78A showed 99% loss of activity compared to wild type. The ScASNase1 was characterized in relation to cytotoxic effect in leukemia cells MOLT-4 and presented antitumor activity. The isoforms S301N, A331D, ΔG77, Y243S and K335E were obtained and will be expressed to the determination of their kinetic parameters compared with the wild type and the bacterial enzymes. Our results suggest that ScASNase1 shows allosteric feature and considering the catalytic efficiency and specific activity, it seems to be promising as biopharmaceutical.

Biography

Gisele Monteiro de Souza graduated in Biological Sciences -Modalidade Molecular Biology from the State University of Campinas (Unicamp - 2000) and a PhD in Biological Sciences (Genetics) from the University of São Paulo (USP - 2005). She is currently Professor of FCF / USP. It has experience in Molecular Biology with emphasis on Molecular Function Study Genes and Biological involved in cellular processes such as antioxidant response, cancer, drug resistance. It has experience in the production of heterologous proteins in microorganisms like bacteria, yeasts and protein engineering.

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