Engineered Erwinase with possible fewer adverse effects for treatment of acute lymphoblastic leukemia

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Abstract

Acute lymphoblastic leukemia (ALL) is the most frequent neoplasm in children and adolescents.[1-2] Treatment of the disease is performed with L-asparaginase (ASNase), an enzyme obtained from the bacteria *Escherichia coli* and *Erwinia chrysanthemi* (Erwinase).[3] ASNase hydrolyzes L-asparagine and prevents tumor cells from obtaining this amino acid from the bloodstream for protein synthesis, leading to ALL cell death by apoptosis.[3] However, both formulations are associated with a high rate of adverse effects as the production of anti-asparaginase antibodies and hypersensitivity, which compromise the efficacy of the treatment.[4-5] The development of mutant proteoforms from commercially available bacterial enzymes may contribute to the development of an enzyme with lower adverse effects. Therefore, we created a mutant library using the ASNase of *E. chrysanthemi* by error prone PCR, and a double mutant proteoform (DM) presented higher specific activity for L-asparagine (figure 1) and a 30% increase in the k_{cat} in relation to the wild-type (WT) enzyme. In addition, DM enzyme showed less recognition by anti-asparaginase antibodies and is able to kill the same amount of ALL cell line MOLT-4 than WT enzyme, using a smaller amount of protein. The results indicate that the DM enzyme has cytotoxic potential and may have fewer adverse effects.

Image

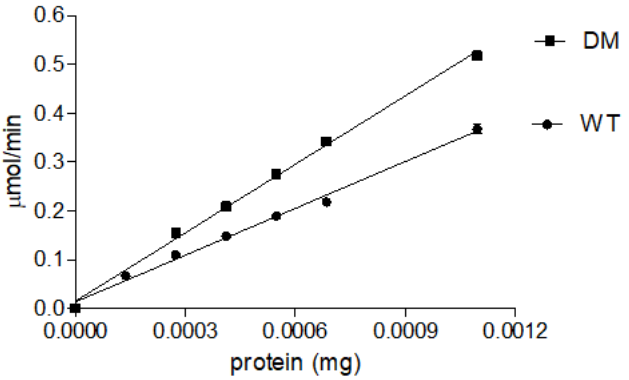


Figure 1: Activity specific for wild-type (WT) and double mutant (DM) proteoforms of *Erwinia chrysanthemi* measured by Nessler reagent. Graph of the speed in µmol of ammonium per minute (µmol/min) as a function of the amount in milligram of protein.

Recent Publications

1. KATZ, A. J. et al. (2015). Acute lymphoblastic leukemia: an assessment of international incidence, survival, and disease burden. Cancer Causes Control, v. 26, p. 1627–1642.
2. PEDIATRIC TREATMENT EDITORIAL BOARD (2018). Childhood Acute Lymphoblastic Leukemia Treatment (PDQ®). NCBI Bookshelf, p. 1-112.
3. SHRIVASTAVA, A. et al. (2015). Recent developments in L-Asparaginase discovery and its potential as anticancer agent. Oncology Hematology, p. 1-12.
4. SALZER, W. et al. (2018). Asparaginase activity levels and monitoring in patients with acute lymphoblastic leukemia. Leuk Lymphoma., v. 59, n.8, p. 1797-1806.
5. DAVIS, A.S.; VIEIRA, A.J.; MEAD, M.D. (2014). Leukemia: An Overview for Primary Care. American Family Physician, v. 89, n. 9, p. 731-738.



Biography

Iris Munhoz Costa is PhD student in the graduate program in Pharmaceutical Technology-Biochemistry, School of Pharmaceutical Sciences, University of São Paulo – USP. She works with biopharmaceutical research for the treatment of acute lymphoblastic leukemia. Master in Pharmaceutical Technology-Biochemistry, School of Pharmaceutical Sciences, University of São Paulo - USP (2015) in the area of biotechnology, biochemistry and molecular biology. Graduation in Pharmacy and Biochemistry at Universidade Paulista - UNIP (2012). She was a student of scientific initiation in the Department of Pharmaceutical Biochemical Technology of the Faculty of Pharmaceutical Sciences of the University of São Paulo - USP (2011), in the area of molecular biology and antioxidant response.

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