Physico-Chemical Characterization of Asparaginase from *Escherichia coli*

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L-Asparaginase (ASNase) from *E. coli* is widely used in acute limphoblastic leukemiain children, presenting high toxicity and low stability in the blood. ASNase is a tetrameric protein with identical 34 kDa subunits. The kinetic, thermodynamic, and physico-chemical properties of the ASNase are poorly understood. Here we show the partial characterization of the enzyme using different techniques. A new method to investigate the kinetic properties of the ASNase by using Isothermal Titration Calorimetry was developed. The reaction is exothermic with enthalpy of –11.4 kcal.mol^{-1}. At 25 °C, there is a plateau on the maximum activity between pH 7 and 9. ASNase becomes very unstable after dilution in 50 mM Tris:HCl, pH 8.0 and storage at 4 °C. Similar pH effects was observed in the stability of ASNase as observed by Differential Scanning Calorimetry with a plateau in the transition temperature (Tm) between pH 7 to 9. As expected for oligomeric proteins, the increase in scan rate from 10 to 90 °C.h^{-1} leads to an increase in stability with Tm varying from 58.6 to 63.5 °C. Glucose increased the ASNase stability while glycerol had no effect Similar Tm was found by the changes in intrinsic fluorescence of the ASNase. The center of mass of the fluorescence spectrum of ASNase remained unchanged with the increase on high hydrostatic pressure from 3,000 to 45,000 psi. The urea-induced unfolding of ASNase showed a single transition with a mid-point around 3.0 M urea. The characterization of the ASNase is an important step to the development of new strategies for clinical use and of new formulations that can either decrease the toxicity or increase the stability of the enzyme in the blood stream (or both).