Thermal stability studies of *Vibrio cholerae* L-asparaginase: Deactivation kinetics and stability enhancement

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Studies on deactivation kinetics as well as quantification of temperature dependent macromolecular properties of protein like activity and stability are ineluctable for predicting economic feasibility for industrial applications. L-asparaginase (L-asparagine amidohydrolase, E.C. 3.5.1.1) is an industrially important enzyme which catalyzes the conversion of L-asparagine to L-aspartate and ammonia. This enzyme has been using as a food processing enzyme as it can effectively reduce the level of acrylamide in a range of starchy foods. L-asparaginase also has application in the treatment of acute lymphoblastic leukemia and its clinical action is attributed to the depletion of L-asparagine, since tumor cells are impotent to synthesize this amino acid and are selectively killed by L-asparagine deprivation. This study reports the temperature dependent deactivation kinetics and stability studies of *Vibrio cholerae* L-asparaginase. Circular dichroism and differential scanning calorimetry studies have been carried out to understand the temperature-dependent conformational changes in the secondary structure of *V. cholerae* L-asparaginase. Thermal stability studies on *V. cholerae* L-asparaginase revealed that the protein is stable up to 40 °C, retaining 70-85% residual activity even after 6 hours of incubation. Moreover it possessed very high melting point of 81 °C and high half-life time of 1100 minutes at 37 °C. Protein was found to be less stable at higher temperatures of 45, 50 and 55 °C and residual activity was declined to 1-6 % in 15 minutes. The effect of various thermal additives like glycerol, glycine and trehalose on thermal stability of *V. cholerae* L-asparaginase was evaluated. Compared to other thermal stabilizers tested, glycine was found to be more effective which resulted in five folds increase in half-life time of enzyme at 50 °C.