DETERMINATION OF ACTIVITY AND THERMODYNAMIC STABILITY OF THE BOVINE ALPHA-TRYPSIN ISOFORM IN ORGANIC–AQUEOUS SOLVENT.

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INTRODUCTION. The use of enzymes in organic solvents has risen the scale of their practical applications and allowed the syntheses of polymeric, biologically active enantiomers that are difficult to obtain with conventional chemical catalysts. Trypsin belongs to the group of serine proteases; it specifically hydrolyzes peptide bonds at the carboxyl side of lysine and arginine residues. This work studied the effect of monoalcohols concentration on kinetic and thermodynamic parameters of bovine α- trypsin.

MATERIAL AND METHODS. The amidasic activity of α-trypsin using BApNA as substrate was tested in aqueous solutions of monoalcohols, methanol, ethanol and propanol monitored by UV-VIS spectroscopy. The study of thermal unfolding of the α- trypsin in buffer and monoalcohols was performed by monitoring the aromatic residue followed by UV spectroscopy. The enzyme kinetics test was performed in ethanol-buffer 60% v/v.

RESULTS AND DISCUSSION. The results demonstrate that the abrupt fall in α-trypsin activity is dependent on the type and concentration of organic solvent. However there was not linear correlation between the decrease in catalytic activity and the dielectric constant of monoalcohols. The TM values for the α-trypsin in buffer system and monoalcohols-buffer systems (Cm) not was significantly different, showing that this concentration of organic solvents (Cm) monoalcohols practically does not change the thermal stability of the protein. However, the values of ΔH decreased by half, suggesting that irreversible process occurred. The kinetic parameters for α-trypsin in a buffer system and ethanol-buffer (Cm) showed that the KM values remained constant for both systems, but decreased of Vmax in ethanol-buffer system showed that ethanol acted as non-competitive inhibitor.

CONCLUSIONS. Thus we conclude that the monoalcohols decreases the enzymatic activity of trypsin in two ways: by acting as non-competitive inhibitor and causing irreversible changes, therefore decreasing number of protein molecules available to perform catalysis.

Key words: physical-chemical, enzyme kinetics, α-trypsin.

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