

THERMODYNAMIC CHARACTERIZATION OF ALFA TRYPSIN AT ACID pH RANGE

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Trypsin was the first discovered and best characterized enzyme, but their structural information just reveals one aspect of the molecule, another is given by energetics associated with this structure. In the present work, was done the determination of the thermodynamic parameters (ΔG_D , ΔC_{pN}^D , ΔS_{Tm} , Tm' , Tm , and ΔH_{cal}) of alpha Trypsin in the acid pH range. The thermal denaturation of enzyme was monitored by the microcalorimeter VP-DSC Microcal with a scanning rate of 60°C / H; cells at constant pressure of 20.0 p.s.i. and Trypsin concentration of 1.0 mg/mL. The alpha trypsin thermodynamic parameters at pH 3.00 showed: Tm (°C)=52.90 ± 0.27; Tm' (cold denaturation, °C)= -24.0; $\Delta Cp=2.02 \pm 0.26$ Kcal.mol⁻¹.K⁻¹; $\Delta H_{(Tm)}=100.35 \pm 0.83$ Kcal.mol⁻¹; $\Delta S_{(Tm)}= 307.0$ cal.mol⁻¹. K⁻¹ and $\Delta G_{D(298K)} = 6.07 \pm 0.80$ Kcal.mol⁻¹. The thermal denaturation of alpha trypsin is highly reversible and thermodynamically driven. Alpha- and beta-Trypsins have similar thermodynamic parameters at acid pH, but the alpha isoform have is less stable and cooperative at thermal denaturation. In the pH range of 2.7-3.4 and with 20.0 mM CaCl₂, alpha trypsin is monomeric, showing a two-states at thermal denaturation.