PHYSICAL-CHEMICAL CHARACTERIZATION AND STABILITY STUDY OF ALPHA -TRYPSIN AT PH 3.0 BY DIFFERENTIAL SCANNING CALORIMETRY

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α-Trypsin is a serine-protease with a polypeptide chain of 223 amino acid residues and six disulfide bridges. It is a globular protein with predominance of antiparallel β-sheet secondary structure and it has two domains with similar structures. In the present work, a stability study of α-trypsin in the acid pH range was performed and physical-chemical denaturation parameters were measured by using differential scanning calorimetry (DSC). The α-trypsin has a shelf-life ($t_{95\%}$) of about ten months at pH 3.0 and 4 °C and its hydrolysis into the ψ-trypsin isoform is negligible during six months as monitored by mass spectrometry (Micromass Q-ToF). The observed $\Delta H_{cal}/DH_{vH}$ ratio is close to unity for α-trypsin denaturation, which suggests the occurrence of a two-state transition, devoid of molten-globule intermediates. At pH 3.0, α-trypsin unfolded with T_m = 325.9 K and ΔH= 99.10 kcal mol^{-1}, and the change in heat capacity between the native and unfolded forms of the protein was estimated to be 1.96 ± 0.18 kcal mol^{-1} K^{-1}. The stability of α-trypsin calculated at 298 K and at pH 3.0 was DG_U = 6.10 kcal mol^{-1}. These values are in the range expected for a small globular protein. These results show that the thermodynamic parameters for unfolding of β-trypsin do not change substantially after its conversion to α-trypsin.

Key words: stability, thermodynamics, trypsin, calorimetry