EVALUATION OF THE EFFECT OF THE CONCENTRATION OF CALCIUM CHLORIDE ON THE ACTIVITY AND THERMODYNAMIC STABILITY OF ALPHA TRYPSIN

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INTRODUCTION: α-trypsin is a serine-protease with a polypeptide chain of 223 amino acid residues and six disulfide bridges. There are few studies of alpha-trypsin isoform due to difficulty in obtaining and low stability of molecule.

OBJECTIVES: In this work the effect of calcium chloride on the activity and thermodynamics parameters of alpha-trypsin were determined by differential scanning calorimetry.

MATERIAL AND MTETHODS:
Acitivity of alpha trypsin was assayed using the *BAPNA* and reaction product p-nitroaniline was read at 410 nm. Influence of calcium chloride concentration on the activity was tested in range from 0 to 300 mmol.L $^{-1}$.

Alpha-trypsin isoform was applied at 1 mg/mL in the DSC and was then subjected to a Scan rate 60°C/hour maintaining at constant pressure of 20 psi. Were performed tested with calcium concentration range of 0 to 300 mmol.L $^{-1}$.

RESULTS AND DISCUSSION: The results demonstrate that the enzymatic activity is proportional to the ion concentration up to 20 mmol / L but above this value activity decreases abruptly. When a decrease of activity was observed, this could be explained by a reduction of the calcium stabilizing effect, probably because the calcium binding site for enzymes is occupied. The calorimetric results showed that chloride ions and calcium reduce the Tm parameter in the range of 0 to 50 mmol.L of calcium chloride and minimum stability in 50mmol.L. Although of apparent destabilization by ions, when evaluated the relationship ΔH_{cal} / ΔH_{vH} best results are found in the range 20 to 30 mmol/L of clacium chloride.

Conclusion: Thus, our results suggest that there may be different mechanisms of interaction of ions calcium and chloride acting on protein system and that it should be investigated separately.

KEY WORDS: Alpha trypsin, activity, stability
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