SHRIMP DIGESTIVE PROTEINASES HYDROLYZE ENZYME SUPPLEMENTS

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Introduction. The use of proteinases as food and feed supplement to increase assimilation of amino acids from food protein is already in the market, however there is no scientific and experimental evidence supporting the use. Mammalian serine proteinases as bovine and porcine trypsins have been widely used to increase enzyme activity in the digestive tract in farm animals and aquafarming (1). However, in a previous work, it was demonstrated that bovine trypsin was hydrolyzed by digestive serine proteinases from shrimp *Penaeus vannamei* (2), which prevented its contribution to hydrolysis of proteinaceous substrates. In this work we demonstrated that serine proteinases from decapods can hydrolyze mammalian trypsins due to their structural features.

Methods. Proteinolytic activity of mammalian trypsin were mixed with digestive proteinases from crustaceans (shrimp, crab and lobster) and evaluated, in vitro. Electrophoresis and zymograms were used to evaluate proteins and proteinases in the mixtures.

Results. Serine proteinases from decapods hydrolyze mammalian trypsins; the hydrolysis depends on the concentration of shrimp proteinases. When shrimp proteinase concentration was experimentally decreased (Fig. 1 lane 1-7), the mammalian trypsin remained active (Fig.1b lane 5-7).

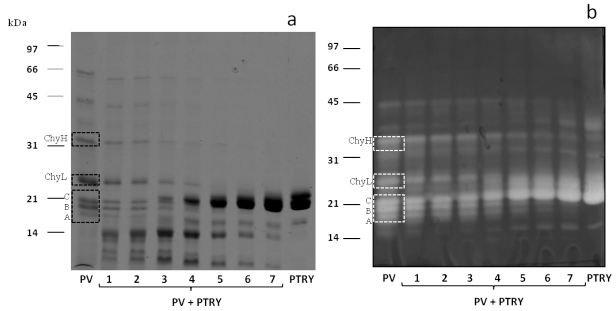

Fig.1 Hydrolysis of mammalian trypsin by shrimp proteinases a) Proteins from mixture of shrimp *Penaeus vannamei* (PV) and porcine trypsin (PTRY) were separated by SDS-PAGE. b) Zymogram from mixture PV plus PTRY, lane 1-7, decreasing concentration of PV. In the figure it shows the major proteinases in PV two chymotrypsins (ChyH and ChyL) and three trypsins (A, B and C).

To know the proteinase responsible for the hydrolysis of the porcine trypsin, ion exchange chromatography was used to fractionate shrimps proteinases. Two chymotrypsins and three trypsins were separated. Fractionated shrimp proteinases were mixed with porcine trypsin; zymograms were used to analyze the activity on the mixtures.

The results demonstrated that shrimp trypsins and chymotrypsins hydrolyzed porcine trypsin (Fig 2).

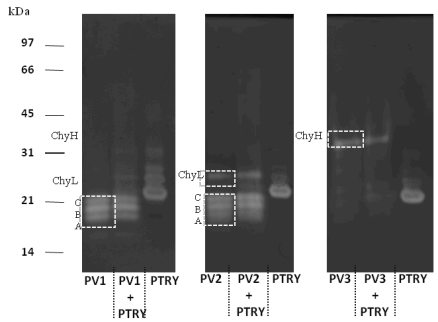

Fig.2 Digestive serine proteinases from shrimp hydrolyzed mammalian trypsin. Fractions obtained by ion exchange chromatography (PV1, PV2 and PV3) were mixed with porcine trypsin (PTRY) and analyzed by zymogram.

Trypsins hydrolyze peptide bonds on the carboxyl side of Arg and Lys. When the primary structure was compared, less number of Lys were found in shrimp than in mammal trypsins, in special Lys^{61} than in combination with Arg^{117} in mammal enzymes are the site of autolysis (3). In this work, when a Lys-methylated porcine trypsin was used, its hydrolysis was reduced significantly when mixed with shrimp trypsins.

Because some decapod digestive proteinases possess collagenase activity (4), and collagenolytic proteinases exhibit a wider specificity toward peptide substrates, we hypothesize that shrimp chymotrypsins hydrolyze porcine trypsin due to their wide range of specificity.

Conclusions. Serine proteinases from decapods hydrolyze mammalian trypsin. Decapods digestive proteinases have proved more resistant to hydrolysis than the mammalian proteinases. Results indicated that it is naive to assume that proteinolytic enzymes from different species will add their catalytic capabilities if mixed; here, we demonstrated that they may antagonize.

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