Trypsin-inhibitor in the digestive juice from the spider *Nephilengys cruentata*

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Spiders are efficient predators and insects constitute the major source of prey for these animals. Prey digestion starts extraorally by the digestive juice produced in the hepatopancreas. Some data in literature indicate that spiders´digestive juice (SDJ) should have some peptidase inhibitors in order to control preys proteases ingested. In order to investigate the presence of protease inhibitors in both, SDJ and hepatopancreas homogenate samples chromatographic separations in anion exchange and gel filtration chromatographies were used. Chromatographic fractions were then tested against different insect and mammalian peptidases. *Periplaneta americana* midgut homogenate, *Spodoptera frugiperda* midgut homogenate and *Gryllus sp* midgut homogenate were used as sources of insect trypsins. As mammalian trypsin source comercial bovine trypsin was tested. SDJ samples were submitted to a gel filtration chromatography in 20 mM Tris-HCl pH 7.0 buffer containing 0.5M NaCl. Identification of inhibitory fractions was done with the mixture of 25 μL of chromatographic fractions and 25 μL of enzyme source. These mixtures were pre-incubated for 20 minutes at 30°C. After that Benzoyl-Arg-p-nitroanilide (BApNa) was added to mixtures as trypsin substrate. Fractions with diminished trypsin activity were pooled and applied to an anion-exchange after a desalting process. The anion-exchange step was done in a Hitrap Q column equilibrated with 0.02 M Tris-HCl buffer pH 7.0. and eluted with a linear NaCl gradient. Fractions with trypsin-inhibitory activity were individually submitted to a 15% SDS-PAGE electrophoresys. The gel evidenced the isolation and enrichment of a protein of approximately 13 kDa correspondent to the inhibitory activity. Hepatopancreas samples were applied to an anion exchange column in the same conditions described before. Active fractions were then applied to a gel filtration column. Electrophoresis of these fraction indicated a partial purification of the inhibitory activity. Supported by: FAPESP.