Digestive astacin-like enzyme from *Nephilengys cruentata*.

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Astacins are metalloendopeptidases (family of zinc-endopeptidases) which present a consensus motif HEXXHXXGXXH and was firstly identified in the crayfish *Astacus astacus*. Our group isolated the major astacin-like enzyme present in the hepatopancreas from the giant spider *Nephilengys cruentata*. Adult females were not fed for at least two weeks. After that, cannibalism among spiders was favored. Spiders were dissected, the hemolymph was collected in the presence of sodium cacodylate and the hepatopancreas was isolated and then homogeneized in cold Milli Q water. Homogenate samples were applied into a Hitrap Q column equilibrated in 0.02 M Tris HCl buffer pH 9.0 and eluted with a linear NaCl gradient. Fractions active on casein-FITC were pooled and submitted to gel filtration in a Superdex G-75 column. Active fractions were individually applied on a 15% polyacrylamide gel and submitted to electrophoresis showing the isolation of a peptidase of 14 kDa. These fractions were also pooled and used in a zymography with gelatin as substrate. The low molecular mass of this enzyme and data on inhibition with 1,10-Phenanthroline and EDTA indicated that this enzyme is an astacin-like enzyme. This peptidase is inactivated at 55°C with a half-life of 46 minutes, present a Km of 0.48% using casein-FITC as substrate and a pH optimum of 8.0. Tests of inhibition of astacin with hemolymph samples from *Nephilengys cruentata* indicated an inhibition of 70% of astacin activity evidencing a specific inhibitor in the hemolymph. Isolation of this inhibitor and the hydrolysis of spider silk by astacin are being tested. **Supported by: FAPESP**.