Elastase Inhibitor from Hemocytes of Lasiodora sp

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Hemocytes from arthropods contain a variety of bioactive molecules such as antimicrobial peptides, lectins, protease and protease inhibitors with molecular masses between 8 and 120 kDa, revealing it as a rich source of molecules that can be exploited biotechnology. Low molecular weight serine proteinase inhibitors from several families have been identified in arthropod hemocytes or plasma. The aim of this study was to purify and characterize the elastase inhibitor from hemocytes of Lasiodora sp. The hemocyte crude extract of Lasiodora sp inhibited chymotrypsin (22%), trypsin (44%), TPA (52%), urokinase (58%) and neutrophil elastase (99%) and failed to inhibit factor Xa, thrombin, plasmin, kallikrein and subtilisin. Following, hemocyte crude extract was applied on trypsin-Sepharose column and the adsorbed fractions eluted with 0.5M KCI-HCl pH 2.0 were examined for inhibitory activity for trypsin and neutrophil elastase. Purified inhibitor from affinity chromatography was called Lasiodora hemocytes elastase inhibitor (LHEI). Following, the purified LHEI was chromatographed on Sephasil C₁₈ column and the eluted fraction 22 presented neutrophil elastase inhibitory activity. The purified inhibitor from C₁₈ Sephasil column inhibited neutrophil elastase with inhibition constant (Ki) of 0.71 pM, presented molecular mass of 14.2 kDa by SDS-PAGE, as the major polypeptide chain and the determined N-terminal amino acid was L-A-N(T)-P-G-A-D-X(C?)-E-T(E). Few inhibitors have been purified from arachnids, and isolation of neutrophil elastase inhibitor from hemocytes is the first step to elucidating their physiological role in these animals.

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