

Biochemical and Functional Characterization of L-amino Acid Oxidase From
Lachesis muta Venom.

Silva, C. B.¹, Soares, A. M.², Arantes, E.C.¹

¹Departamento de Física e Química, and ²Departamento de Análises Clínicas Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo

Snake venoms are a complex mixture of toxic enzymes such as phospholipases A₂, proteolytic enzymes and other important enzymes such as L-amino acid oxidases. The *Lachesis* genus is regarded as a representative of *Viperidae* family and induces a sort of clinical aspects of ophidian poisoning. The local symptoms include serious tecidual damage, besides grave systemic dysfunction like nausea, vomit, abdominal colic, diarrhea, sweating, low blood pressure and shock. *Lachesis muta* venom contains L-amino acid oxidase (LAAO) which is thought to contribute to the toxicity upon envenomation. For this reasons, the aim of this work was the purification of the L-amino acid oxidase from *Lachesis muta* venom and its biochemical and functional characterization. The purification consisted of three chromatographic steps, including gel filtration in Sephadex G-100[®], followed by high performance ion exchange chromatography of the first fraction in a MonoQ[®] column. All fractions were tested to enzymatic activity performed with a conjugated enzyme's assay which generates hydrogen peroxide by the oxidative deamination of L-leucin. The fraction with major activity was analyzed in a reversed phase chromatography in a C4 column. Afterward, L-amino acid oxidase of *Lachesis muta* was analyzed by SDS-PAGE to confirm the expected high purity of the toxin. The molecular weight estimated for the trimeric enzyme was 200,000 and for the monomer was 73,000. The initial N-terminal amino acid sequence was determined by Edman's degradation using PPSQ 33A Shimadzu Sequencer and showed homology with other LAAOs.

Keywords: *Lachesis muta*, L- amino acid -oxidase, snake venom.

SUPPORT: FAPESP, CAPES and CNPq.