

Serineproteases and immunological cross reaction in activated and *in natura* *Brotheas amazonicus* venom

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Introduction: *Brotheas amazonicus* Lourenço, 1988 is one of the largest scorpion species from Brazil, living in Amazon rainforest and its venom has low toxicity in mammals (2,3). In early studies, scorpion venoms toxic to humans showed proteolytic activity in different substrates (1,4). There are scarce literature about proteolytic activity in non-toxic scorpions, as well as venom transformation. Here we describe results from a venom activation process, which modifies proteins from *B. amazonicus* venom, and once activated, it shows affinity to fibrinogen and collagen substrates. It also reacts differently to cross reaction from specific antivenoms. **Objectives:** Detection of two or more different responses in biological tests resulted from activation process of *B. amazonicus* venom. **Material and Methods:** For enzymatic studies, we used SDS-PAGE zymograms, with bovine collagen and fibrinogen as substrates. In immunochemical response, we used Western Blotting technique and Brazilian scorpionic and arachnidic antivenoms with polyclonal antibodies. In these two tests, activated and non-activated (*in natura*) venom were compared. **Results and Discussion:** Only activated *B. amazonicus* venom showed enzymatic activity in bovine fibrinogen and collagen substrates (fig.1,2). This activity was inhibited by specific serineprotease inhibitor (fig.3), and comparison between activated and non-activated venom, when submitted to zymogram test, suggest non-activated enzymes inside *B. amazonicus* venom, where only the activated venom was able to perform substrate degradation. Western Blotting technique in *B. amazonicus* venom shows toxins with ≥ 30 kDa interacting with antibodies from Brazilian arachnidic and scorpionic antivenoms. Activated *B. amazonicus* venom showed an increased sensibility to arachnidic antivenom, besides non-activated (*in natura*) *B. amazonicus* venom showed an increased sensibility to scorpionic antivenom (fig.4). **Conclusions:** Such results suggest common epitopes between hazardous Brazilian scorpion and spider species, and such activation process is able to modify protein structure, giving catalytic properties and also showing or hiding epitopes. Venom activation pathway remains unclear but this process study is in progress.

Keywords: *Brotheas amazonicus*, venom activation, toxinology.

Sponsors: CAPES, CNPq and FINEP.

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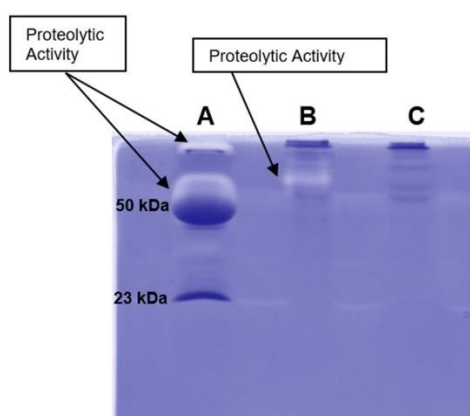


Figure 1: Zymogram from proteolytic activity on bovine collagen. A – *Bothrops atrox* venom; B – Activated *B. amazonicus* venom; C – Non-activated *B. amazonicus* venom.

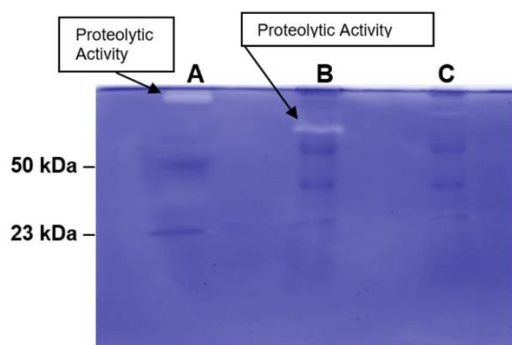


Figure 2: Zymogram from proteolytic activity on bovine fibrinogen. A – *Bothrops atrox* venom; B – Activated *B. amazonicus* venom; C – Non-activated *B. amazonicus* venom.

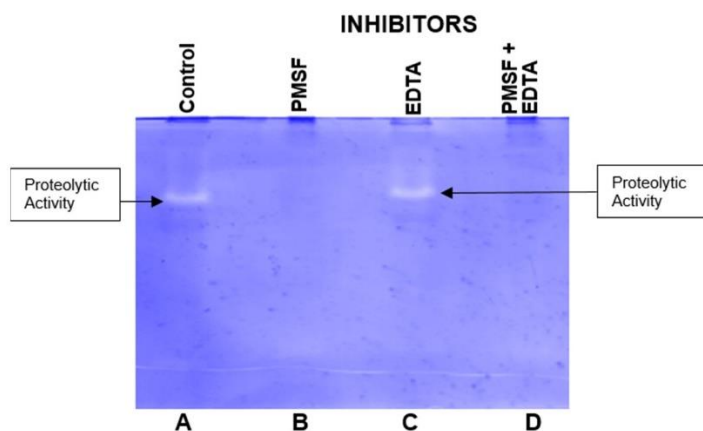


Figure 3: Zymogram from proteolytic activity evaluation on bovine fibrinogen from activated *B. amazonicus* venom. **A** – Activated *B. amazonicus* venom; **B** – Activated *B. amazonicus* venom + PMSF (20 mMol); **C** – Activated *B. amazonicus* venom + EDTA (20 mMol); **D** – Activated *B. amazonicus* venom + PMSF (20 mMol) + EDTA (20 mMol).

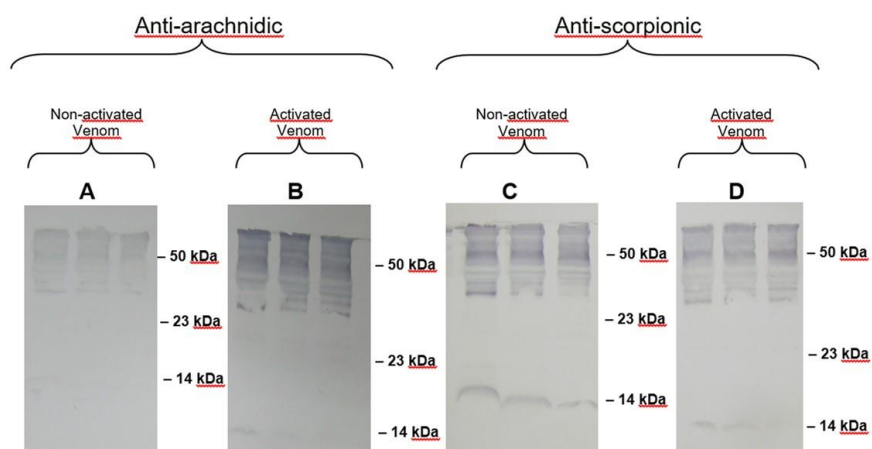


Figure 4: Immunoprofile by western Blotting from *B. amazonicus* venom in non-reduced conditions. **A** – Non-activated venom + anti-arachnidic antivenom; **B** – Activated venom + anti-arachnidic antivenom; **C** – Non-activated venom + anti-scorpionic antivenom; **D** – Activated venom + anti-scorpionic antivenom.