Fractionating and Identifying Coagulant Proteins from *Bothrops alternatus* Snake Venom

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Introduction: Serine proteases, metalloproteases, phospholipases A2, and others components of the snake venom can affect hemostasis in different points, like on coagulation cascade factors, vascular integrity or platelet aggregation. **Objectives:** This work describes the fractionating of the *B. alternatus* venom and the identification of proteins with clotting effect. **Materials and methods:** Crude venom of *B. alternatus* (50mg) was chromatographed on a Sephadex-G-75 column, which separates molecules by size. The coagulant fraction was applied on a heparin-agarose column. Molecular mass of the fractions were determined using polyacrylamide gel electrophoresis (14%) in the presence of sodium dodecyl sulfate (SDS-PAGE). Coagulant activity was performed by mixing 20 µg of crude venom or 20 µg of the fractions with 200 µl of citrated bovine plasma solution at 37°C in triplicate. Clotting formation was monitored by a coagulometer (CLO Timer). The effect of inhibitors on the clotting activity was assayed after preincubation of samples with specific inhibitors of the serine proteases (PMSF), cysteine proteases (leupeptin) and metaloproteases (1, 10-phenantroline). **Results and Discussions:** Fractionation of *B. alternatus* crude venom on a Sephadex-G75 column produced three major protein peaks (G1, G2 and G3). The main coagulant activity was detected in peak G1, which was further fractioned using affinity chromatography on a heparin-agarose column. This chromatography resulted in two fractions (H1 and H2) and the H2 fraction was used because its clotting activity was conserved. The H2 fraction presented two protein bands with estimated masses of 67kDa and 35kDa under non-reduced conditions. The crude venom induced blood-clotting in 20s while the H2 fraction induced blood-clotting in 51s. The clotting activity of the H2 fraction when incubated with PMSF, leupeptin and 1-10 phenantroline was inhibited by 75%, 83% and 100%, respectively. **Conclusions**: These results suggest that H2 fraction contains coagulant proteins and the metalloproteinases are the most important to your clotting activity.

Key words: snake venom, Bothrops alternatus, blood coagulation.
Acknowledgements: FAPEMIG, CNPq, CAPES, MCTI and UFU.