Functional Characterization of BthSP, a New Serine Protease from *Bothrops moojeni* Snake Venom

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INTRODUCTION: Snake venom comprises a complex mixture of proteins and peptides, which interact with components of the human hemostatic system. Some of these components are enzymes, such as serine proteases, that may act on the coagulation cascade, making the blood incoagulable. **OBJECTIVES:** In this work, we describe the purification and functional characterization of the BthSP, a new serine protease from *B. moojeni* snake venom. **MATERIAL AND METHODS:** The fractionation of the venom was carried out through two chromatographic steps (DEAE Sephacel followed by Sephadex G-75) and the purification of the BthSP was monitored by SDS-PAGE. Coagulant activity was determined by incubating 5µg of BthSP with bovine plasma or bovine fibrinogen at 37°C. The activity was characterized by the immediate appearance of the fibrin clot. Stability of the BthSP on coagulant activity was assayed with the enzyme previously incubated at different temperatures (30-100°C), pH values (3.0-11.0) and inhibitors (aprotinin, EDTA, PMSF, β-mercaptoethanol, 1,10-phenanthroline and leupeptin). Defibrinating activity was tested by intraperitoneal injection of 10µg of crude venom or 5µg of BthSP into mice. After 1 h, the animals were euthanized and bled by cardiac puncture. Whole blood was placed in tubes and kept at 25-30°C until clotting occurred. **RESULTS AND DISCUSSION:** BthSP was purified by an anion-exchange and molecular exclusion chromatography and was found to be a single-chain protein with an apparent molecular mass of 36,000 and 32,000 under reduced and non-reduced conditions, respectively. BthSP induced blood-clotting *in vitro* and defibrination *in vivo*. The enzyme was stable at high temperatures (up to 100°C), showed maximum coagulant activity at pH around 7.0 and it was inhibited by PMSF (serine protease inhibitor). **CONCLUSIONS:** In this work, we purified a new serine protease from *B. moojeni* snake venom. This enzyme presents medical interest because it may making the animal's blood incoagulable.

Key Words: *Bothrops moojeni*, serine proteases, hemostasis
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