

Partial Purification of a New Serine Protease from *Bothrops pauloensis* Snake Venom

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Abstract: Serine proteases are a large group of proteins found in plants, microorganisms and animals. They comprise 20% of snake venoms and have many important activities for the pathogenesis of the envenoming. This work shows the partial purification of a new serine protease from *Bothrops pauloensis* snake venom. The fractionation of 150 mg of *B. pauloensis* venom by ion-exchange chromatography on CM-Sepharose column produced six major proteins peaks. Fraction CM1, showing clotting activity, and it was further fractionated on Q-Sepharose and resolved into five protein new fractions, named CMQ1-CMQ5. CMQ1 was applied on Sephacryl S-100 chromatography and resolved into six peaks (CMQS1-CMQS6). The partially isolated serine protease was concentrated in the second peak and was named Bp-Sp. This enzyme showed a major band with molecular weight of 45kDa under reduced conditions by SDS-PAGE. It displayed high clotting activity upon bovine plasma and was able to degrade the bovine fibrinogen (1mg/mL PBS) at different doses, times at 37°C, pH 7.4 by SDS-PAGE analysis. These activities were inhibited by PMSF, benzamidine and leupeptin suggesting the presence of serine residue at active site. The total isolation of this new enzyme is important for its biochemical and structural characterization. In addition, the comprehension of its structural and functional relationship may be important for clinical purposes, which could lead to its use in the treatment and prevention of cardiovascular disorders and strokes.

Keywords: *Bothrops pauloensis*, Serine protease, coagulant activity.

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