INTRODUCTION: Snake venoms contain a variety of proteolytic enzymes affecting the host coagulation process and one of these enzymes responsible for blood-clotting activity is a serine protease which resembles at least in part thrombin, enzyme that plays a key role in coagulation. MATERIAL AND METHODS: A novel thrombin-like enzyme, designated as BbTL-4, was purified from the venom of Bothrops braziliis after two chromatographic steps (molecular exclusion and RP-HPLC) and some biochemical properties were determined of this principle coagulant. RESULTS AND DISCUSSION: BbTL-4 presented a molecular mass of 25,438.6 Da, as determined by MALDI-TOF mass spectrometry, and exhibited a high specificity for BAρNA, showing a Michaelis-Menten behavior with Km 3.65 mM and Vmax 6.20 nmoles ρ-NNA/lt/min for this substrate. BbTL-4 also showed ability to coagulate bovine fibrinogen and their proteolytic activities were inhibited by PMSF and slightly inhibited by soybean trypsin inhibitor and serum from Didelphis marsupialis (D. mar). This thrombin-like enzyme had the highest activity at 37 ºC and pH 7.5. In addition the enzyme cleaved preferentially the Aα-chain and more slowly the Bβ-chain. This protein didn't degrade the γ-chain of fibrinogen and caused platelet aggregation in platelet rich plasma. CONCLUSIONS: This work reports a procedure for the isolation of a highly purified thrombin-like enzyme from Bothrops braziliis venom, which was named BbTL-4. This protein is of potential interest for the understanding of the pathomechanism of the snake venom action and for the identification of new blood coagulation enzymes of natural sources.

Keywords: Thrombin-like, RP-HPLC, Bothrops brazili

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