STUDIES OF ENZYMATIC STABILITY OF A NEW THROMBIN-LIKE ENZYME TL-Bp FROM BOTHROPS PAULOENSIS SNAKE VENOM

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Serineproteinase represents about 20% of the total protein content of Viperidae venoms and they are usually considered to be involved in the blood-clotting disorders. The present work reports studies of enzymatic stability of a thrombin-like enzyme, TL-Bp isolated from BOTHROPS PAULOENSIS snake venom. TL-Bp has been purified using a combination of ion-exchanged, hydrophobic and affinity chromatography. The purity degree was monitored by high-performance liquid chromatography (HPLC). TL-Bp showed to be a glycoprotein with molecular mass of 34kDa under reduced conditions and 30kDa in the absence (by SDS-PAGE). TL-Bp displays esterase activity upon TAME and shows high clotting activity upon bovine plasma. When it was incubated with 75µg of bovine fibrinogen (1mg/mL PBS) and analysed by SDS-PAGE at differents concentrations, pH, temperatures and inhibitors showed high activity, however was inhibited by PMSF. These results confirm one distinctive feature of the serine fibrinogenolytic enzymes on the resistance to inactivation by heat and pH extremes, and show a general agreement that covalently bound carbohydrates avoid the denaturation, therefore, this serineproteinase shares common features with other thrombin-like enzymes of snake venom.

KEY WORDS: thrombin-like, blood-clotting, serineproteinase, snake venom.

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