Isolation and biochemical characterization of a new serine protease from the venom of *Bothrops pirajai* snake venom


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Snake venoms are complex mixture with varied composition constituted by inorganics and organics elements. Several classes of proteins and peptides have been described, where proteases stand out due them activity on homeostasis processes. *Bothrops pirajai* is a species of venomous snake with distribution restricted to Brazil belonging to the Viperidae family. The aim of this study was the isolation and biochemical characterization of a new serine protease from *B. pirajai* snake venom. Thereby, we made three chromatographic steps (molecular size-exclusion, bioaffinity and reverse phase chromatography). SDS-PAGE and MALDI-TOF mass spectrometry analyses were performed in order to obtain the molecular mass. Coagulant activity, activation of Factor XIII and hydrolysis of on chromogenic substrate N-benzoyl-L-arginine-p-nitroanilide (BApNA) with or without use of phenylmethylsulfonyl fluoride (PMSF) inhibitor assays were performed in order to characterize the enzymatic activity. The protein named BpirSP-39 show 39,408Da, ability to coagulate human plasma (MCD=1.7ug) and it was inhibited by PMSF. The BpirSP-39 also exhibited specificity by BApNA substrate and activate factor XIII of blood coagulation cascade, which it makes this enzyme different of the most others serineproteases from snake venoms. This is the first serineprotease isolated from this specie and it can be used to know better the action mechanism of this toxin. This study was authorized by CGEN/CNPq (010627/2011-1) and IBAMA (27131-2).

Key Words: Serineprotease, *Bothrops pirajai*, purification, snake venom.

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