Characterization of a New Thrombin "Like" Factor from Crotoxin *Crotalus durissus cumanensis* Venom

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We have investigated the thrombin "like" and platelet aggregating activity of whole crotoxin and its subunits isolated from *Crotalus durissus cumanensis* venom. Using HPLC molecular exclusion, we detected the presence of two different serine protease activities in the fraction I, and another in the (crotoxin) fraction III, which induced strong thrombin "like" activity, From crotoxin, we isolated PLA_2, crotapotin and another minor fraction (III-3) that exhibited serine protease activity. After a new fractionation on RP-HPLC chromatography, we obtained two other fractions: TL101 and TL102. TL101 was obtained with high degree of molecular homogeneity with molecular mass of approximately 28 kDa and a high content of acidic amino residues, such as aspartic acid and glutamic acid. Other important amino acids were histidine, cysteine and lysine. This protein exhibited high specificity for BApNA, a Michaelis-Menten behavior with Vmax estimated in 5.37 lM/min and Km value of 0.48 mM for this substrate. TL101 also degraded fibrinogen and a and b chain cleavage. Enzymatic as well as the platelet aggregation activities were strongly inhibited when incubated with TLCK and PMSF, specific inhibitors of serine protease. Also, TL101 induced platelet aggregation in washed and platelet-rich plasma, and in both cases, TLCK inhibited its activity.

The N-terminal amino acid sequence of TL101 presented a high amino acid sequence homology with other thrombin-like proteins, but it was significantly different from gyroxin. These results showed that crotoxin is a highly heterogeneous protein composed of PLA_2, thrombin-like and other fractions that might explain the diversity of pharmacological activities.

Keywords: Thrombin-Like, TL101, serine protease activity, *Crotalus durissus cumanensis.*

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