BIOCHEMICAL AND FUNCTIONAL STUDIES OF COLTX-I, A NEW MYOTOXIC PHOSPHOLIPASE A$_2$ FROM CROTALUS OREGANUS LUTOSUS SNAKE VENOM

Almeida, J.R.$^1$, Resende, L.M.$^1$, R.I.M.A., Ribeiro$^2$, Marangoni, S.$^1$, Da Silva, S.L.$^2$

$^1$Department of Biochemistry, Institute of Biology, State University of Campinas (UNICAMP), Campinas, SP, Brazil. $^2$Department of Chemistry, Biochemistry and Bioprocess Engineering, Federal University of São João Del Rei (UFSJ), Ouro Branco, MG, Brazil.

Snake venom phospholipases A$_2$ (PLA$_2$s) play a leading role in the complex pathogenesis of skeletal muscle necrosis, a clinically serious consequence of envenomings, that may lead to permanent loss of tissue and disability. *Crotalus oreganus lutosus* snake venom has not been extensively studied; therefore, the characterization of its components represents a valuable biotechnological tool for studying biological processes destabilized during envenoming. The objectives of this work were to isolate and to characterize a PLA$_2$ from the *Crotalus oreganus lutosus* venom in order to obtain insights into its structure, biological effects, induction of morphological changes of skeletal muscle and its relevance to the pathophysiology of envenomations. The toxin was purified by chromatography steps and characterized using enzymatic, structural and biological assays. In this study, for the first time, a basic PLA$_2$ myotoxin, ColTx-I, was isolated from snake venom by a combination of gel filtration and high performance liquid chromatography. ColTx-I is monomeric with a mass of 14,444 Da, a primary and tertiary structure closely related to basic PLA$_2$s from viperid venoms. The pure enzyme has a specific activity of 15.87 ± 0.65 nmols/min/mg at optimal conditions (pH 8.0 and 37°C). ColTx-I activity was found to be dependent on Ca$^{2+}$, as its substitution by other ionic species as well as the addition of chelating agents significantly reduced its phospholipase activity. *In vivo*, ColTx-I triggered dose-dependent inflammatory responses with an increase in IL-6 levels, systemic and local myotoxicity, characterized by elevated plasma creatine kinase activity. Histological analyzes showed that ColTx-I induced a complex series of degenerative events associated with cellular destruction, edema, inflammatory infiltrate, damage of skeletal fibers and hemorrhage. These biochemical and functional results suggest that ColTx-I, a myotoxic and inflammatory mediator, plays a key role in *Crotalus oreganus lutosus* envenomation.

Keywords: Phospholipase A$_2$; Myotoxin; Snake venom

Acknowledgements: CAPES