STRUCTURAL STUDIES WITH THE MYOTOXIN BnSP-7 FROM SNAKE VENOM IN NATIVE STATE AND COMPLEXED WITH CAFFEIC ACID.

De Lima, L. F. G.¹; Salvador, G. H. M.¹; Fernandes, C. A. H.¹; Fontes, M. R. M.¹

¹Laboratório de Biologia Molecular Estrutural, Departamento de Física e Biofísica, Instituto de Biociências de Botucatu, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Botucatu, Brasil.

Introduction: Phospholipases A₂ (PLA₂)s are the most abundant proteins found in Viperidae snake venoms. Lys49-PLA₂ is a subclass of these proteins which does not present catalytic activity but it can display myotoxic effects by a muscle membrane−destabilizing activity. Studies involving the interaction of Lys49-PLA₂ with potential neutralizing molecules have been employed to elucidate their mechanism of action and structural determinants of their biological activities. Furthermore, PLA₂ inhibitors may provide therapeutic molecular models with antiophidian properties and may be applicable as a supplement to the conventional serum therapy. Objectives: The aim of this work was structural studies with the BnSP-7, a Lys49-PLA₂ from B. pauloensis venom, in the native form and complexed to caffeic acid. Materials and Methods: BnSP-7 was isolated from B. pauloensis crude venom by ionic exchange and reverse phase chromatography techniques. The secondary structure of BnSP-7 was analyzed by circular dichroism (CD) showing a typical CD spectrum from PLA₂. Crystals of the native BnSP-7 and BnSP-7 complexed to caffeic acids were obtained using with a concentration protein of 12 mg/mL using vapor-diffusion sitting drop method. X-ray diffraction data were collected using a synchrotron radiation source (MX2 station - LNLS, Campinas, Brazil). The crystal structures were solved by molecular replacement using the Phenix program package. Discussion and Results: Crystals of the BnSP-7 and BnSP-7/caffeic acid complex diffracted X-rays to 1.59 Å and 2.35 Å resolution, respectively. After molecular replacement and cycles of manual and automated refinement, an electron density that corresponds to a CA molecule was observed at the Membrane Disruption-Site (MDiS) which is part of the C-terminal region. A polyethylene glycol molecule was found at the hydrophobic channel. Conclusions: These results highlight the relevance of the C-terminal region and MDiS site on myotoxic mechanism of Lys49-PLA₂ as proposed recently.

Key words: X-ray crystallography, PLA₂-like, PLA₂ inhibitors

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