

Structural Studies with Phospholipase A₂ homologue Bothropstoxin-I complexed to Ca⁺² Ions: Insights into the myotoxic activity partial inhibition

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Phospholipases A₂ (PLA₂) are among the main components of *Bothrops* venoms and consists of a broad range of enzymes defined by their ability to catalyze specifically the hydrolysis of the center (sn-2) ester bond of substrate phospholipids. Bothropstoxin-I (BthTX-I) is a PLA₂ myotoxic, catalytically inactive, purified from *Bothrops jararacussu* venom, with a single chain of 121 amino acid residues, containing a single Met and 7 disulfide bonds. In increase of Ca⁺², reduce the PLA₂ activity by 48%, as determined by fatty acids, and by 42%, as determined by lysophosphatidylcholine. Crystals of BthTX-I complexed to Ca⁺² ions were obtained by hanging-drop vapour-diffusion, in similar conditions to the native protein (temperature and crystallization solution). X-ray diffraction data of a single crystal of BthTX-I+Ca⁺² was collected using a Synchrotron Radiation Source (LNLS-MX1, Campinas, Brazil). Data were processed using the HKL2000 package program at 1.93Å resolution. After the refinement, strong electron densities were found and attributed to the Ca²⁺ ions, despite of the quaternary structure of protein has been highly conserved in comparison to the native structure. This study can add insights to understanding the influence of bivalent ions in the myotoxic and enzymatic activities of phospholipase A₂ homologues.

Keywords: Bothropstoxin-I, BthTX-I, Lys49-PLA₂, Myotoxic and Phospholipase A₂.
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