Crystal structures of BthTX-I chemically modified by BPB reveal important clues on myotoxic inhbition effect.

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Phospholipases A_2 are components of snake venom responsible to disruption of cell membrane integrity via hydrolysis of its phospholipids. A class of this enzymes, the homologues PLA_2s (Lys49-PLA_2s), has a D49K natural substitution and does not show catalytic activity. However, these proteins induce myonecrosis, which is not efficiently neutralized by serum therapy. In an effort to understand the mechanism of action responsible for myotoxicity, we present here the crystallographic structures of the BthTX-I, a Lys49-PLA_2s from *Bothrops pirajai*, chemically modified by *p*-bromophenacyl bromide (BPB) in two different times of incubation between protein and BPB: three and twenty four hours. The BPB is a well-known inhibitor of phospholipases A_2 and in the case of Lys49-PLA_2s reduces their myotoxicity by approximately 50%. Both BthTX-I-BPB-3h and BthTX-I-BPB-24h crystals belong to space group $P2_12_12_1$, and have two molecules in the assymetric unit diffracting at 2.3 Å resolution. These structures support the configuration of the so-called "alternative dimer" as the biological unit. Structural comparisons between apo-BthTX-I and the two structures of BthTX-I-BPB show a small deviation in Ca^{++} binding-loop and C-terminal regions. Furthermore, the alkylation of H48 by BPB provoke similar effects on tertiary and quaternary structures as other complexed structures, including high similarity between the monomers and the formation of the hydrogen bond between them. These data highlight the presence of a ligand in hydrophobic channel to myotoxic inhibition.

Financial Support: FAPESP, CNPq and LNLS.

Key words: Lys49-Phospholipase A_2; myotoxicity inhibition; *p*-bromophenacyl bromide; quaternary structure; X-ray crystallography.