# Phylogenetic analyses support different oligomeric assemblies for myotoxic phospholipases $A_2$ indicated by structural studies

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Snakes from *Bothrops* genus are responsible for more than 90% of all ophidic accidents that happen in Latin America. Phospholipases $A_2$ ($PLA_2$s) and their homologues are among their major compounds. $PLA_2$s hydrolyze the *sn*-2 acyl groups of phospholipids in contrast to $PLA_2$s homologues (or Lys49-$PLA_2$s) which are enzimatically inactive toxins that induce a large spectrum of pharmacological activities, such as myotoxicity, neurotoxicity and hypotension. Although many studies show these snake venom Lys49-$PLA_2$s present similar tertiary structures, biophysical and biochemical studies show they can be found as monomers, dimers, and tetramers in solution. Therefore, the key to understand this molecular puzzle may be related to the different oligomeric assemblies they assume. The myotoxic effect caused by these proteins may lead to pemanent tissue loss, disability and amputation. Considering this effect is not efficiently neutralized by serum therapy, the knowledge of the pharmacological site and the mechanism of action of these toxins have been the aim of many studies. Recently, our group solved three crystallographic structures of these homologue proteins under the same crystallization conditions, two of them grown in the presence of alpha-tocopherol. Comparative structural studies between them and other crystallographic structures available in the Protein Data Bank were performed. Based on this analysis, site-directed-mutagenesis, synthetic-peptide and anion-assisted interface studies, we suggest the probable residues that constitute the myotoxic site of these dimeric toxins; additionally, we propose these residues are specific for *Bothrops* genus. To evaluate this hypothesis, phylogenetic analyses studies with amino acid sequences were performed using Bayesian/MCMC method. All bothropic homologue $PLA_2$s amino acid sequences formed a monophyletic group, supporting our hypothesis. Furthermore, myotoxic proteins were grouped into eleven different branches, being this classification probably related to the different patterns of oligomeric assemblies they exhibit.

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