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High resolution crystal structure of BthTX-I-BPB and comparison analysis with native BthTX-I - insights into the lack of the toxic activities

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Short Abstract: Structure of a non-catalytic and myotoxic Lys49-PLA2 was solved with BPB inhibitor in two different oligomeric states. BthTX-I with the His48 chemically modified by BPB shows strong myotoxic and citotoxic activities reduction. The comparison between monomeric and dimeric BthTX-I-BPB and three different native conformations is analyzed.

Long Abstract:

The PLA2s are probably among the most widely studied of all enzyme families both in terms of their structural characteristics and catalytic properties however their toxic activities, catalytic mechanism and pharmacological effects it is not completely understood. From a structural perspective, enzymes isolated from the venoms of serpents and insects as well as from biological fluids and cells, such as saliva, venom, synovial fluid, macrophages, platelets, pancreas, spleen smooth muscle and placenta have been the subject of intense investigation by experimental data (X-ray diffraction and NMR) and theoretical techniques such as molecular dynamics simulations that also been used to complement the experimental data[1]. PLA2s are 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 bonds of substrate phospholipids liberating free fatty acids and lysophospholipids, and products of phospholipids hydrolysis may subsequently be utilized for the synthesis of eicosanoids, which serve as secondary messengers or as metabolic precursors in a variety of inflammatory reactions[2].

Crystallographic studies have found application in the rational design of potent inhibitors of human non-pancreatic secretory PLA2[3] and the use of transition state and substrate analogues has also contributed to our current understanding of the catalytic mechanism. In PLA2 catalytically active (Asp49-PLA2), the alkylation of His48 residue of active site by the p-bromophenacyl bromide (BPB) not only abolisher the catalytic activity, but also decreases other toxic and pharmacological effects. But, in Lys49-PLA2 (catalytically inactive) the alkylation of His48 residue with BPB reduced 45%, 85% and 15% of its myotoxic, cytotoxic and edema inducing activity, respectively, with no significant changes in its liposome-disrupting activity[4]. The reduction of these toxic activities and the relationship with conserved residues related to the catalytic domain are not understood yet. BthTX-I or Bothropstoxin is a non-catalytic and basic myotoxic purified from Bothrops jararacussu venom. BthTX-I was solved by X-ray crystallography in two conformations (“open” and

“closed”); this was also demonstrated in solution using fluorescence emission experiments[5]. These different conformations are mainly due to the beta-wing region functioning as a molecular hinge. However, Magro et al (2003) observed there are not just two conformations (“open” and “closed”) but at least six different ones, and Lys49-PLA2 monomers are probably very flexible or adopt many states in solution, leading to different conformations in the crystals according to the best crystal packing of each protein[6,7]. Open-BthTX-I crystals belong to P3221 space group while closed-BthTX-I crystals belong to P3121 leading to the hypothesis that the different conformation may be due to the different crystals packing. Recently, we also solved BthTX-I in an intermediate state with the crystals belonging to the P21 space group.

Here, we report the structure determination and preliminary analysis of the first Lys49-PLA2 chemically modified by p-bromophenacyl bromide. Additionally, the structural comparison between the native BthTX-I in three different conformations ("open", "closed" and "intermediated" states) and, monomeric and dimeric BthTX-I-BPB is analyzed.

Crystals of BthTX-I-BPB were obtained in the same crystallization condition, but in different temperatures: 10ºC and 18°C. The structures were solved at 1.48Å and 2.28Å resolution for monomeric and dimeric BthTX-I-BPB, respectively. These crystals belong to different spaces groups: C2221 and P212121. The crystal structures were determined using molecular replacement techniques implemented in the program AMoRe using the coordinates of a monomer of BthTX-I native.

Packing parameter calculations based on a protein molecular weight indicate the presence of two molecules in the asymmetric unit for the crystals of P212121 space group. This corresponds to a Matthews coefficient VM (volume per Dalton) (Vm) of 2.5 Å3/Da, with a calculated solvent content in the crystals of 51.8% solvent content. In contrast, the volume of the unit cell for the C2221 space group is compatible with a monomer in the asymmetric unit (Vm=2.7 Å3/Da, 55.2% solvent content) There is a BPB bound to the His48 located in the region corresponding to the catalytic domain of Asp49-PLA2s for both conformations (monomeric and dimeric). The structure was refined using the CNS program and the manual correction of atomic positions was made by the “O” program.

The BthTX-I-BPB crystals are not isomorphous with those of the native protein for both their oligomeric conformations (open, closed and intermediated). This suggests the inhibitor binding has lead to changes in the quaternary structure and an alternative conformation for the protein may have been obtained. The BPB ligand binds covalently to the His48 residue of catalytic sites of PLA2s however, the myotoxic and cytotoxic activities of non-catalytic BThTX-I decrease dramatically after the ligand binding. Possible explanations for this fact are that the BPB binding may result in conformational changes and/or C-terminal residues (e.g. Lys122) may be indirectly interacting with the active site affecting the toxic mechanisms. Then, detailed studies with this complex might add insights into the myotoxic and cytotoxic mechanisms of Lys49-PLA2s and, eventually, the role of C-terminal region.

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