Crystal Structure of Phospholipase A2 from *Bothrops neuwiedi* Complexed to Fatty Acid.

Rocha, B.A.[1], Delatorre, P. [2], Toyama, M.H.[3], Cavada, B.S.[4]

[1]Instituto de química e Biotecnologia, Universidade Federal de Alagoas, Maceió, Brasil; [2]Departamento de Biologia Molecular, Universidade Federal da Paraíba, João Pessoa, Brazil; [3]Campus do Litoral Paulista - Unidade São Vicente, UNESP, São Paulo, Brazil; [4]Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, Fortaleza, Brasil.

The enzyme phospholipase A_2 (PLA2) catalyzes the hydrolysis of the *sn*-2 acyl chain of phospholipids, forming fatty acids and lysophospholipids. Many basic snake phospholipases with molecular mass between 13KDa and 16 KDa were purified from Bothrops genus. These proteins present an amino acid substitution at position 49 (Aspartic to Lysine). Due to this modification they display a weaker catalytic activity, which occurs by the reduction of Ca^{2+} binding properties. Although, PLA2 (Lys-49) is still capable to disrupt biologic membranes by phospholipids hydrolysis Ca^{2+}-independent. This work objective obtaining phospholipase (K49) tridimentional structure from *Bothrops neuwiedi*, and intend to understand the catalytic mechanism calcium-independent by the interaction with a fatty acid. The purified protein was crystallized with 2.0 *M* ammonium sulfate as a precipitating agent using the sitting-drop vapour-diffusion method. The crystals belonged to the monoclinic space group $P2_1$, with unit-cell parameters a = 38.8 Å, b = 70.4 Å, c = 44.0 Å, α = 90.0°, β = 109.3° and γ= 90.0°. The structure was refined at 2.2 Å of resolution to a final R factor of 0.211 and R free of 0.258, using 11,130 unique reflections. This is a dimmeric PLA_2 structure in which the catalytic site is comprised by HIS48, LYS49 and TYR49. The active sites of all molecules are located on the surface and are fully exposed to the solvent, resulting in a highly potent enzymatic unit. In this site can be observed a fatty acid bound, presenting 12 carbon atoms and a cis-bound in carbon 9. The final protein model consists of 121 amino-acid residues and 100 water molecules.

Keywords: Phospholipase A2*, Bothrops neweidje*, Fatty acid
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