Poster I-4

Crystal structure of PrTX-I complexed with alpha-tocopherol and comparison studies with native Lys49-PLA2s

Authors:
Juliana Izabel dos Santos (*Depto. de Física e Biofísica - Iinstituto de Biociências, UNESP / Botucatu*)
Andreimar Martins Soares (*Depto. de Análises Clínicas, Bromatológicas e Toxicológicas, FCFRP, USP / Ribeirão Preto*)
Marcos Roberto de Mattos Fontes (*Depto. de Física e Biofísica - Iinstituto de Biociências, UNESP / Botucatu*)

Short Abstract: This work reports the crystal structure of PrTX-I complexed with alpha-tocopherol and the comparison with native PrTX-I. Additionally, other native Lys49-PLA2s are used in the comparison. This study may bring insights into the myotoxic / cytotoxic mechanisms of Lys49-PLA2s and the role of C-terminal region.

Long Abstract:

Phospholipases A2 (PLA2s) are small calcium-dependent proteins that cause the liberation of fatty acids and lysophospholipids by hydrolyzis of membrane phospholipids. These enzymes (PLA2s) are the main components of snake venoms from Bothrops genus. They also present many farmacological effects like hypotensive and myotoxic activities that are related to its different regions. Piratoxin-I (PrTX-I) is a myotoxic PLA2 homologue (Asp49 is substituted for Lys 49) from the venom of Bothrops pirajai. It was believed that the substitution of Asp for Lys in the position 49 of the protein was responsible for the loss or reduction of its membrane disruption activity. It has been observed by site-direct mutagenesis of C-terminal region in Lys49-PLA2 BthTX-I that there are two distinct regions (in the C-terminal) related to myotoxic and Ca+ independent membrane damage activities (Chioato et al., 2002). The substitution of Arg and Lys residues with Ala in the region 117-122 resulted in a significant reduction of myotoxic activity, and the substitutions of residues 115, 116 and 122 with Ala resulted in a reduced Ca2+ independent membrane damage activity. However, the substitution of Lys122 with Ala alters both activities (Chioato et al., 2002). Therefore, detailed studies with other Lys49-PLA2s and different ligands seems to be fundamental for understanding the role of C-terminal region, particularly involving the Lys122 and the residue of catalytic site of PLA2s. Other studies are also correlating the importance of the C-terminal region for myotoxic and cytotoxic activities for Lys49-PLA2s. The co-crystallization of these proteins with inhibitors and site-directed mutagenesis are possible methodologies that can help to confirm these data. Alpha-tocopherol (vitamin E) is involved in the regulation of the production of eicosanoids by inhibition of both PLA2 and cyclooxygenase activities. It is also a promising drug for the treatment of some neurodegenerative diseases like schizofrenya and Parkinson, where the levels of PLA2s are higher than the normal levels, and to avoid the infection of cells by some viruses that use this enzyme to get into them. This work reports the high resolution crystal structure of the PrTX-I and alpha-tocopherol complex (1.8 Å resolution) and the comparison with native PrTX-I solved at 2.2 Å resolution. Additionally, other native Lys49-PLA2s are used in the

comparison. PrTX-I and PrTX-I-alpha-tocopherol crystals were obtained under the same condition of crystallization; however, the crystals are not isomorphous (P21– PrTX-I-alpha-tocopherol and P3121 - PrTX-I) probably due to the changes in the quaternary structures. Based on the electron density alpha-tocopherol molecule could be positioned in the active site of each PrTX-I monomer. The ligand is stabilized mainly by hydrophobic interactions with apolar amino acid residues of the substrate binding cleft. This study may bring insights into the myotoxic and cytotoxic mechanisms of Lys49-PLA2s and the role of C-terminal region.

Supported by FAPESP, FUNDUNESP, LNLS and CNPq.

Chioato et al., 2002. Distinct sites for myotoxic and membrane-damaging activities in the C-terminal region of a Lys49-phospholipase A2. Biochem J.