

Single Molecule and X-ray Crystallographic Studies on the Interfacial Action Mechanism of Phospholipase A2 on Phospholipid Membranes

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Secretory phospholipase A2 (PLA2) is known to be activated by membrane binding to facilitate the entry of the substrate and promote calcium dependent hydrolysis of glycerol phospholipid such as phosphatidylcholine (PC) for its physiological activities of inflammation, allergy and tumorigenesis. Despite the availability of over hundreds of 3D structures of PLA2 in the presence and absence of its cofactor, inhibitors and transition state analogues for the depiction of the catalytic mechanism of the enzyme, it is still not clear how the lipid structure and its packing properties could regulate enzymatic activities.

We report here the crystal structures of native PLA2 and in complexed with phospholipid micelles in the absence of calcium ions from Taiwan cobra and show that phospholipids binding occur not only at the PC activation site of Asp23, but also at the half way of the hydrophobic channel to form several unexpected calcium independent substrate binding intermediates. This result, together with fluorescence images of RhB-PLA2 on phospholipid supported bilayer, allow us to propose a multiple energy landscape for PC activation, in addition to the interfacial activation mechanism proposed for the anionic lipid binding at the interfacial binding surface.

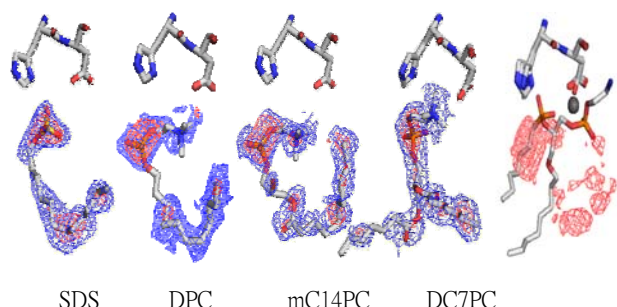


Fig. 1: The 2Fo – Fc electron density difference maps, contoured at 0.7 σ , of three dC7PC substrate molecules with fitted stick model. Amino acid residues from the respective Mol A, B, and C involved in hydrophobic interaction with phospholipid molecules are also shown. PyMol was used to generate the related figures (pymol.sourceforge.net).

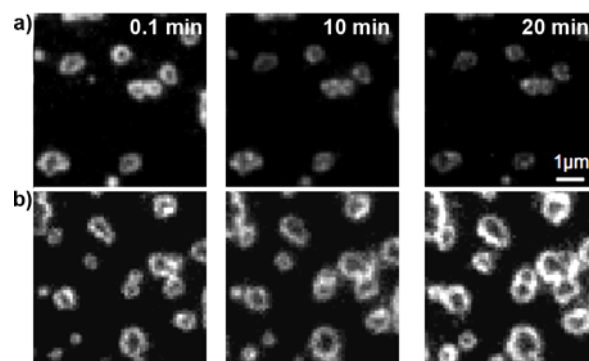


Fig. 2: Fluorescence images of RhB-PLA2 on DPPC supported bilayer with multiple circular defects after injection of 20 nM. RhB-PLA2 in 10 μ M EDTA (a) and 5mM CaCl₂ (b), respectively at room temperature. Solutions of supported bilayer all contain 100 mM NaCl, 10 mM Tris and pH 8.0.

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