Interfacial refolding and tuning of lipolytic enzymes at membrane like structures

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Introduction

➢ Lipolytic enzymes hydrolyze their water insoluble substrates selfassembled in supramolecular aggregates on the lipid – water interface (necessary conditions, NC) [1].

➢ Activated state of these enzymes is achieved at corresponding pH, T, [Me^{2+}], ionic strength, degree of dispersion, surface curvature of vesicle or micelles, by some of reaction products and non intrinsic additives (organic solvents, detergents, adsorbents (SC) [1,2] and reaction courses are characterized by an induction period [4], (Fig.1,2).

➢ Formation of anisotropic clusters, rafts or microdomains from molecules products by Ca^{2+} helation into initial isotropic media of substrate is the reason of activation and control for example of PLD, modifying their charge and curvature on the surface of mixed micelles [2,3], (Fig.2,3)

➢ This enzymatic process obtains planar directivity to the side of cluster formation and thus lipolytic enzymes which produce anisotropic clusters may be relevant to the lateral vector enzymes [2-4], Fig.3,4 as opposed to transmembrane enzymes-vectors.

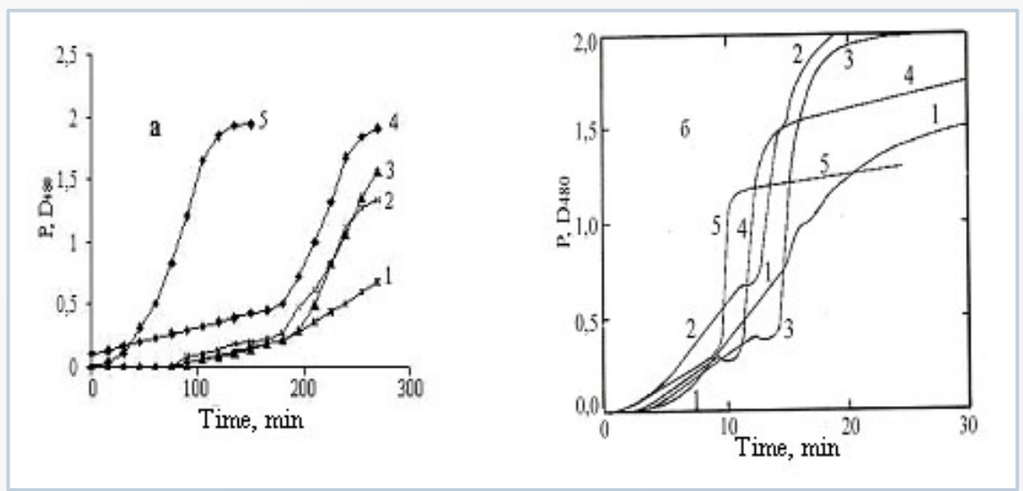

Fig. 1. Effect of anionic detergents on PLD activation and regulation. **a**) Hydrolysis of LPC by purified PLD from daikon *R. sativus longipinatus*. Reaction conditions: without detergent (*1*) or in the presence of DOCA (*2*), TCA (*3*), LPA (*4*), SDS (*5*), each at 0.2 mM concentration; [LPC] = 2 mM, [Ca^{2+}] = 20 mM. **b**) Dependence of kinetics of LPC hydrolysis by PLD from *Str. chromofuscus* on SDS concentrations (mM): *1*) 0; *2*) 0.2; *3*) 0.5; *4*) 0.7; *5*) 1.0. Reaction conditions: PLD, 2.25 μg; [Ca^{2+}] = 20 mM, pH 5.6, 30°C. P is LPA, the product of the reaction.

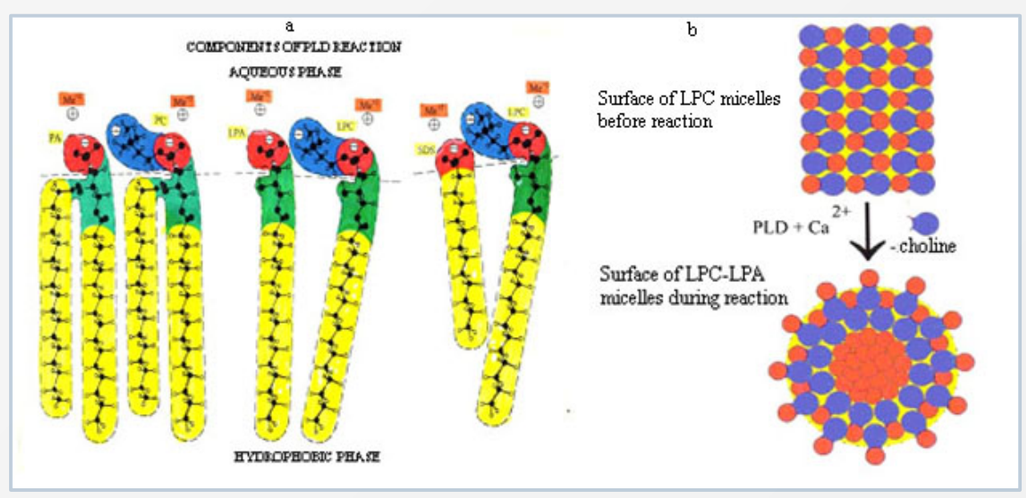

Fig. 2. Scheme of an components in reaction with phospholipase D (**a**) and cluster formation of LPA (product of hydrolysis) on the surface of LPC micelles (**b**).

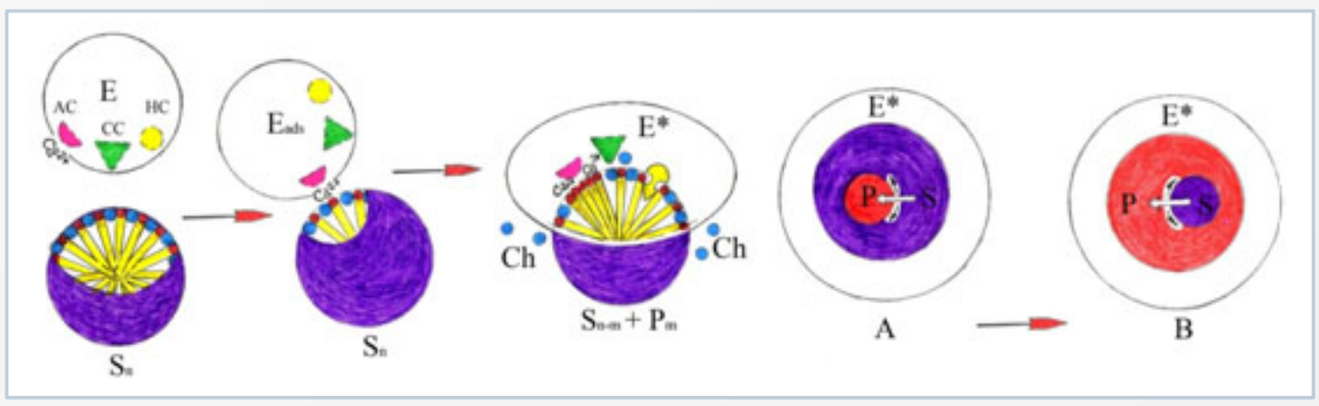

Fig. 3. Scheme of hydrolysis of micellar LPC by phospholipase D. Designations: E, water-soluble PLD; Eads, PLD adsorbed on micelle; E*,activated PLD in enzyme-substrate complex; AC, anionic PLD center; CC, catalytic PLD center; HC, hydrophobic PLD center; S, LPC (substrate); Sn, substrate micelle; Sn-m + Pm, mixed LPC–LPA micelle; P, Pm (one of the reaction products, LPA, in mixed micelle); Ch, choline (water-soluble product); **A** - S/P at the beginning of reaction; **B** - S/P at the end of reaction. Directions of biotransformation and PLD oscillation in the course of hydrolysis of substrate to product are shown by arrows.

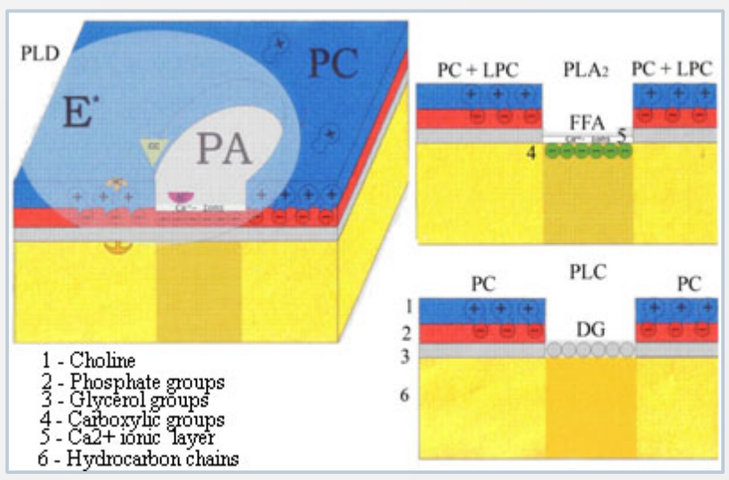

Fig. 4. Scheme of cluster formation of products of lecithin hydrolysis by phospholipases.

Conclusions

➢ The suggested hypothesis for PLD activation and functioning differs from the previously existing ones for lipolytic enzymes in domain formation of the reaction product, depositing important mediators and second messenger (PA or LPA and Ca^{2+}), by vectority of process of biotransformation of lipids on the surface of their supramolecular structures, and rationalization of regulatory properties of the formed microdomain.

➢ Clusters (rafts) produced by lipolytic enzymes can play a role in membrane depots and transporters of biologically active compounds, lipid chaperons, tuners and modulators of biomembrane components and can clear up the polyfunctional effects of lipolytic enzymes.

➢ The interdomain interface with the neighboring lipid phases is especially important for refolding, tuning and functioning of lipolytic enzymes.

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