Review

Modulation of phospholipase A$_2$ activity generated by molecular evolution

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Received 9 March 1999; received after revision 12 May 1999; accepted 18 May 1999

Abstract. Snake venom oligomeric neurotoxins offer several unique examples of modulation of phospholipase A$_2$ (PLA$_2$) activity generated by molecular evolution. This phenomenon was found in evolutionary younger snakes and is probably common for representatives of the genus Vipera. At present, the best-studied example is the heterodimeric neurotoxin vipoxin from the venom of the southeast European snake Vipera ammodytes meridionalis. It is a complex between a basic strongly toxic PLA$_2$ and an acidic and catalytically inactive PLA$_2$-like component (Inh). This is the first reported example of a high degree of structural homology (62%) between an enzyme and its natural protein inhibitor. The inhibitor is a product of the divergent evolution of the unstable PLA$_2$ in order to stabilize it and to preserve the pharmacological activity/toxicity for a long time. Inh reduces both the catalytic activity and toxicity of PLA$_2$. Vipoxin also illustrates evolution of the catalytic into a inhibitory function. Vipoxin analogues have been found in the venom of viperid snakes inhabiting diverse regions of the world. An attempt is made to explain modulation of the toxic function by the three-dimensional structure of vipoxin.

Key words. Phospholipase A$_2$; molecular evolution; inhibitor; pharmacological sites; enzyme activity; enzyme toxicity.

Introduction

Phospholipases A$_2$ (PLA$_2$s) (phosphatide 2-acylhydro-lase, EC 3.1.14) are widespread in living organisms as both intracellular and extracellular enzymes. They are among the smallest enzymes performing various vital physiological functions and have been isolated from a number of sources: snake venom, mammalian pancreas, lung, gastric mucosa, liver, spleen, alveolar macrophages, intestine, membranes, heart, placenta, and brain [1]. The PLA$_2$ enzyme specifically hydrolyzes the 2-acyl ester bond of 1,2-diacyl-3-$sn$-phosphoglycerides releasing fatty acids and lysosphospholipids. The enzyme catalyzes reactions at a lipid-aqueous interface and the phospholipase activity is much higher on aggregated substrates such as monolayers, bilayers, micelles, membranes, and vesicles than with monomolecular dispersed soluble substrates [2]. This phenomenon has been termed ‘interfacial activation’ and includes ‘interfacial binding’ of the enzyme and ‘activation’ steps.

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Structural changes were observed in PLA₂ upon its binding to membranes and were explained as a prerequisite for enzyme activation [3]. Thus it is evident that modulation of membrane-associated PLA₂ activity will influence cellular functions such as chemotaxis, cytotoxicity, and cell differentiation, and it has been established that the interfacial adsorption of PLA₂ on membranes is driven by electrostatic forces. Electrostatic interactions between positive charges from the PLA₂ recognition site and negatively charged anionic headgroups of phospholipids optimize the catalysis and are important for the adsorption and orientation of the enzyme at the lipid-water interface. Thus, two lysyl residues, Lys 7 and Lys 10, mediate the adsorption of the Agkistrodon piscivorus piscivorus PLA₂ to anionic interfaces [4]. Enzyme penetrability into the phospholipid membrane can be improved by positively charged residues flanking hydrophobic segments of the recognition site, which will facilitate hydrolysis of phospholipids [5]. In addition, hydrophobic side chains, such as tryptophyl residues, may penetrate into membranes enhancing PLA₂ binding [6]. Therefore, the hydrophobicity of the enzyme recognition site is important: increased hydrophobicity will improve enzyme binding to the respective target sites on the membrane substrate.

The most important active sites, such as the catalytic site, the Ca²⁺-binding site, and the hydrophobic sub-

![Figure 1](image-url)

Figure 1. (a) Cartoon view of heterodimeric vipoxin complex as representative of the group II PLA₂-s, showing the active PLA₂ in blue and the inhibitor in red. The disulfide bridges for both molecules and the active-site residue side chains are shown for the PLA₂. The intermolecular interaction (PLA₂) Asp 49-Lys 69 (Inh) reducing vipoxin calcium-binding ability is included. The Cz positions of residues of the potential calcium-binding residues inside each calcium-binding loop are indicated as yellow circles. (b) Schematic representation of group I and group II PLA₂-s. α-Helices are indicated as red cylinders, β-strands by blue arrows, disulfide bridges by yellow bars and the Ca²⁺-binding site by a green circle.