Phospholipase A₂ Structure/Function, Mechanism and Signaling

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Abstract

Tremendous advances in understanding the structure and function of the superfamily of phospholipase A₂ (PLA₂) enzymes has occurred in the twenty-first century. The superfamily includes fifteen Groups comprising four main types including the secreted sPLA₂, cytosolic cPLA₂, calcium-independent iPLA₂, and PAF acetyl hydrolase/oxidized lipid LpPLA₂. We review herein our current understanding of the structure and interaction with substrate phospholipid which resides in membranes for a representative of each of these main types of PLA₂. We will also briefly review the development of inhibitors of these enzymes and their roles in lipid signaling.

Introduction

The last twenty-five years has witnessed a virtual explosion in our knowledge about the superfamily of phospholipase A₂ (PLA₂) enzymes. PLA₂ hydrolyzes the fatty acid from the sn-2 position of membrane phospholipids. In vivo, the sn-2 position of phospholipids frequently contains polyunsaturated fatty acids and when released, these can be metabolized to form various eicosanoids and related bioactive lipid mediators (1). The remaining lysophospholipid can also have important roles in biological processes (2).

From the end of the nineteenth and beginning of the twentieth century (3), PLA₂ was known to be a major component of snake venoms and it was later recognized that PLA₂ from old world snakes (Group I) differed in their disulfide bond pattern from new world snakes (Group II). Later it was discovered that the major mammalian digestive enzyme, pancreatic PLA₂ was more similar to that from the old world snakes such as the Indian cobra (Group IA) and hence the pancreatic enzyme was named Group IB. With the isolation, sequencing, and cloning of the PLA₂ from human synovial fluid in 1988 (Group IIA) (4, 5) which had a disulfide bond pattern more similar to the new world rattlesnakes (Group II), the more complicated PLA₂ from bee venom (Group III) (6), and in 1991 the human cytosolic calcium-dependent PLA₂ from macrophages (Group IVA) (7, 8), the need for a more elaborate "Group Numbering System" became obvious (9). As the discovery of additional PLA₂’s continued such as the macrophage secreted Group V PLA₂ (10, 11) and the calcium-independent PLA₂ (Group VI) (12), this system was expanded with fourteen distinct Groups and many subgroups appearing by 2000 (13). The latest review (14) lists fifteen distinct Groups of PLA₂. They cluster in four main categories or types: secreted sPLA₂’s, cytosolic cPLA₂’s, calcium-independent iPLA₂’s, and platelet activating factor (PAF) acetyl hydrolase/oxidized lipid lipoprotein associated (Lp) PLA₂’s. Each of these types has been implicated in diverse kinds of lipid metabolism and disease progression so there has been a tremendous interest in the pharmaceutical and biotechnology industry in developing selective and potent inhibitors of each of these types.

Secreted PLA₂

The secreted PLA₂’s were the first type of PLA₂ enzymes discovered. They are found in sources as diverse as venoms from various snakes, scorpions, etc., components of pancreatic juices, arthritic
synovial fluid, and in many different mammalian tissues (13). They are characterized by a low molecular weight (13-15 kDa), histidine in the catalytic site, Ca\(^{2+}\) bound in the active site, as well as six conserved disulfide bonds, with one or two variable disulfide bonds. These enzymes all catalyze the hydrolysis through the same mechanism of abstraction of a proton from a water molecule followed by a nucleophilic attack on the sn-2 bond. The water molecule is activated by the presence of a histidine/aspartic acid dyad in a Ca\(^{2+}\) dependent manner (15, 16). Most of the secreted PLA\(_2\) enzymes share the property of exhibiting an increase in activity termed interfacial activation when substrate is presented as a large lipid aggregate, rather than in monomeric form. More detailed reviews of interfacial kinetics can be found elsewhere (17, 18).

Understanding the mechanism of interfacial activation as well as the orientation of lipid binding has long been a goal of mechanistic studies of the secreted PLA\(_2\)’s. Experiments using NMR derived nuclear overhauser effect (NOE) results have been used to map the binding sites of a single phospholipid substrate in the cobra venom Group IA PLA\(_2\) as shown in Fig. 1A (19). Recent work using deuterium exchange mass spectrometry with phospholipid surface present has generated a model of how this same enzyme binds to the lipid surface as shown in Fig. 1B (20). The Group IA enzyme appears to bind lipid substrate in the active site through the hydrophobic residues lining the active site channel, and binds neutral membrane substrate through interactions with a group of hydrophobic residues on the lipid binding surface of the molecule. Experiments conducted with the Group III bee venom have used electrostatic potential-modulated spin relaxation magnetic resonance to determine how that enzyme binds the lipid surface (21). The secreted enzymes show similar activity to phospholipids with different fatty acids in the sn-2 position (22). However they have different preferences for the charge on the lipid surface. PLA\(_2\)’s containing a tryptophan in the lipid binding surface display the highest activity toward neutral lipid substrates, and PLA\(_2\)’s with an excess of basic residues on the lipid binding surface display the highest activity toward negatively charged surfaces (22). For a more detailed review of the mechanism of binding to differently charged membranes, see (23).

The primary role of the mammalian secreted PLA\(_2\) enzymes in eicosanoid signaling remains unclear and has been recently reviewed (23). The most well understood function of a mammalian sPLA\(_2\) is Group IIA which has been shown to be a potent anti microbial agent. Many different studies have examined the role the secreted PLA\(_2\)’s play in eicosanoid release and these studies have been inconclusive. They show that the up regulation of Groups IIA, V, and X caused a cytosolic GIVA PLA\(_2\) dependent increase in eicosanoids. However a specific inhibitor of the Group IIA inhibitor has been developed by Lily (24), with clinical trials of its efficacy against arthritis and allergens showing no therapeutic effects (23). The pro-inflammatory role of the secreted PLA\(_2\) has been suggested to possibly be controlled by a protein binding event not dependent on PLA\(_2\) activity. Receptors present in mouse tissues named the M-type receptors have been found to bind different secreted phospholipases, but no M-type receptor in humans has been found that binds PLA\(_2\) (25). Recent work however has shown that Group IIA PLA\(_2\) binds to integrins, and this raises the interesting possibility that integrin-PLA\(_2\) contacts may mediate pro-inflammatory activity (26).

Cytosolic PLA\(_2\)

The first Group IV cytosolic PLA\(_2\), the Group IVA (GIVA), was identified in human platelets in 1986 (27), and was cloned and sequenced in 1991 (7, 8). Many different submembers of the Group IV family have been discovered since then and their properties are reviewed (28). The most well studied cytosolic enzyme is the GIVA PLA\(_2\). It is characterized by an active site serine and aspartic acid dyad, requirement for Ca\(^{2+}\) for activity, and it is the only PLA\(_2\) with a preference for arachidonic acid in the sn-2 position of phospholipids (7, 28). GIVA PLA\(_2\) also possesses lysophospholipase activity, as well as transacylase activity (29). Arachidonic acid is the precursor for the generation of eicosanoids, and this enzyme has been proposed to play a major role in
inflammatory diseases. This was proven through the use of knockout mouse models, where the absence of the GIVA PLA2 gene significantly reduced the effects of many inflammatory diseases (30-32). GIVA PLA2 is now generally considered to be a central enzyme mediating generation of eicosanoids and hence many inflammatory processes.

The structure of this enzyme shows that it is composed of a Ca\(^{2+}\) dependent lipid binding C2 domain and a catalytic \(\alpha/\beta\) hydrolase domain as shown in Fig. 2A (33). Both of these domains are required for full activity (34). The catalytic domain of the enzyme is composed of a core \(\alpha/\beta\) hydrolase region conserved throughout many different lipases, as well as a novel cap region found only in GIVA PLA2. Within the cap region there is a lid region which prevents the modeling of a phospholipid substrate in the active site. It has been proposed that this enzyme must undergo a conformational change in the presence of substrate that opens this lid region. Recent work using lipid substrate, as well as a covalent inhibitor bound in the active site have indeed shown a conformational change of the lid region in the presence of substrate (35).

This enzyme is activated by many different mechanisms. The enzyme is recruited to the membrane by a Ca\(^{2+}\) dependent translocation of the C2 domain. Recent work has localized the lipid binding surface of the enzyme in the presence of Ca\(^{2+}\), as shown in Fig 2B (35). The lipid second messengers ceramide 1-phosphate (36) and phosphatidylinositol (4,5) bisphosphate (37) have been shown to activate the enzyme and working site directed mutagenesis has identified two charged residue patches on the enzyme that bind these lipid second messengers. The enzyme has also been shown to be regulated through phosphorylation on residues 505, 515, and 727, see (38).

Recognition of the importance of the GIVA PLA2 in inflammatory diseases, as well as important structural discoveries has made it a very attractive drug target, and many different laboratories have attempted to develop inhibitors. Two of the most promising drug candidates include the indole derivative inhibitors developed by Wyeth, and the 2-oxoamide inhibitors developed by Kokotos et. al. (39, 40). Both of these inhibitors have been used for \textit{in vivo} animal models of inflammation and have shown potency in reducing inflammatory effects (40, 41). Potential side effects of GIVA PLA2 inhibitors have been suggested by recent work examining a human patient with defects in GIVA PLA2 who showed decreases in PLA2 activity, eicosanoid biosynthesis, and the generation of many small intestinal ulcers (42).

**Ca\(^{2+}\) Independent PLA2**

The Ca\(^{2+}\) independent PLA2’s are members of the GVI family of PLA2 enzymes. The first member of this family the GVI PLA2 was purified from macrophages in 1994 (12). All of the GVI enzymes are characterized by not requiring Ca\(^{2+}\) for catalytic activity. Many new GVI PLA2 members have been identified in the last three years and they are reviewed in (14). The best characterized of the GVI PLA2 enzymes is the GVI PLA2 (43). It is found in cells expressed in multiple different splice variants (44). The active splice forms of the enzyme GIVA-1, and GIVA-2 are composed of 7-8 ankyrin repeats, a linker region and a catalytic domain. This enzyme, similar to GIV PLA2, uses a serine in the active site to catalyze the cleavage of the sn-2 ester bond, however it does not show specificity for an arachidonic acid in the sn-2 position. The GIVA PLA2 also possesses a lysophospholipase activity, as well as transacylase activity (44). The activity of the GIVA PLA2 has been suggested to be regulated through many different mechanisms, including ATP binding, caspase cleavage, calmodulin, and possible ankyrin repeat mediated protein aggregation (38).

The role of the GIVA PLA2 in different signaling pathways has been shown to be very complex. Initial reports of the functions of the GIVA PLA2 were determined using the inhibitor bromoenoallactone (BEL) (44). Recent work has shown that this inhibitor is not specific for GIVA PLA2 and actually functions through activation of the inhibitor by GIVA PLA2 followed by non specific covalent modification of cysteine residues in all proximally located enzymes (45). Therefore it has been hard to evaluate early experiments using this inhibitor to determine the function of the GIVA PLA2. Experiments using the inhibitor BEL are...
reviewed elsewhere (46). Two major factors have allowed the determination of GVI PLA₂’s cellular functions. First the recent generation of GVIA PLA₂ deficient mice has shown the importance of this enzyme in bone formation, apoptosis, insulin secretion, and sperm development (47-50). Secondly the recent development of specific fluoroketone inhibitors of GVIA PLA₂ (51) have shown in mouse models that the GVIA PLA₂ in combination with the GIVA PLA₂ play an important role in Wallerian degeneration and axon regeneration in nerve injury (52). Recent work using antisense oligodeoxyribonucleotide towards GVIA PLA₂ with monocytes has shown decreases in monocyte recruitment and directionality (53).

**PAF Acetyl Hydrolase/Oxidized Lipid Lipoprotein Associated PLA₂**

The PAF acetyl hydrolase/oxidized lipid LpPLA₂ is a member of the GVII family of PLA₂ enzymes. This enzyme was named for its ability to cleave the acetyl group from the sn-2 position of platelet activating factor (PAF), as well as its association with lipoproteins. This name is misleading because this enzyme can cleave oxidized lipids in the sn-2 position up to 9 carbons long, not just PAF (13). This enzyme has also been shown to access substrate in the aqueous phase unlike all other PLA₂’s studied (54). Its active site is composed of a serine, histidine, and aspartic acid hydrolase triad (55) which is unlike all other PLA₂’s which have dyads. Recent work has identified c-terminal regions of the enzyme that are required for binding to HDL and LDL (56). This enzyme was cloned from human plasma in 1995, and was shown to have anti-inflammatory activity in vivo (57). These original studies led to the hypothesis that this enzyme might function in a protective role by stopping the pro inflammatory roles of PAF; however several clinical studies of GVIIA PLA₂ levels in patients have now established this enzyme as a definitive marker of coronary heart disease (58, 59).

With the classification of this enzyme as a positive risk factor in coronary heart disease, it has become a very attractive drug target. A specific inhibitor of this enzyme was developed in 2003 by GlaxoSmithKline (60), and recent clinical trials with this inhibitor have shown a decrease in the complex atherosclerotic lesions that lead to unstable lesions, as well as other cardiovascular disease markers (61-63).

**Conclusion**

Experiments with members of the PLA₂ superfamily of enzymes have been carried out for over 100 years. Early kinetic and structural work established PLA₂ as an important model of lipid enzymology. With the discovery of multiple different family members of PLA₂ and their structural characterization, as well as the discovery of their cellular functions, the PLA₂ family has become a major drug target for many different diseases. The future of this field is very exciting as new knockout mouse models, along with specific inhibitors of these enzymes lead to further elucidation of PLA₂’s roles in cellular processes, along with new potential therapeutics.

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Fig. 1. (A) The Group IA PLA2 with phospholipid substrate modeled in the active site. The active site residues His-48 and Asp-93 and the bound Ca$^{2+}$ is shown in purple. Ca$^{2+}$ is bound by Asp-49 as well as the carbonyl oxygens of Tyr-28, Gly-30, and Gly-32. Aromatic residues are shown in white. Adapted from Dennis (9). (B) Model of the lipid surface binding of the Group IA PLA2 is shown with residues on the interfacial binding surface Tyr-3, Trp-19, Trp 61, and Phe 64 shown in stick form. Adapted from Burke et. al. (20).
Fig. 2. (A) Group IVA PLA2 crystal structure as determined by Dessen et al (33). The C2 domain is shown in orange, with two bound Ca$^{2+}$ ions shown in purple. The catalytic domain is shown on the right with the cap region colored yellow, and the lid region 415-432 colored magenta. The active site residues Ser-228, Asp-549 and Arg-200 are shown in stick form colored red. The PIP2 binding site is shown in dark blue, and the C1P binding site is shown in cyan. (B) Model of the lipid binding surface of the GIVA PLA2 with residues colored based on interaction with lipid membrane. Adapted from Burke et al (35).