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 15 groups comprising four main types including the secreted sPLA₂, cytosolic cPLA₂, calcium-independent iPLA₂, and platelet activating factor (PAF) acetyl hydrolase/oxidized lipid lipoprotein associated (Lp)PLA₂. We review herein our current understanding of the structure and interaction with substrate phospholipids, 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.—Burke, J. E., and E. A. Dennis. Phospholipase A₂ structure/function, mechanism, and signaling. J. Lipid Res. 2009. 50: S237–S242.

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The last 25 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 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 PLA₂s continued such as the macrophage secreted group V PLA₂ (10, 11) and the calcium-independent PLA₂ (group VI) (12), this system was expanded with 14 distinct groups and many subgroups appearing by 2000 (13). The latest review (14) lists 15 distinct groups of PLA₂. They cluster in four main categories or types: secreted sPLA₂s, cytosolic cPLA₂s, calcium-independent iPLA₂s, and platelet activating factor (PAF) acetyl hydrolase/oxidized lipid lipoprotein associated (Lp)PLA₂s. 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 PLA₂s 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, Ca²⁺ 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 nuclear magnetic resonance derived nuclear overhauser effect 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 $s$n-$2$ 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 Ref. 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 antimicrobial 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 group IVA (GIVA) PLA$_2$ dependent increase in eicosanoids. However a specific inhibitor of the group IIA inhibitor has been developed by Schevitz et al. (24), with clinical trials of its efficacy against arthritis and allergens showing no therapeutic effects (23).

The proinflammatory 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 shown 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 proinflammatory activity (26).

**CYTOSOLIC PLA$_2$**

The first group IV cytosolic PLA$_2$, 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 PLA2. It is characterized by an active site serine and aspartic acid dyad, requirement for Ca$^{2+}$ for activity, and it is the only PLA2 with a preference for arachidonic acid in the sn-2 position of phospholipids (7, 28). GIVA PLA2 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 that 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, has 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 work using 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 Ref. 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 Six et al. (39) and McKew et al. (40). Both of these inhibitors have been used for 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 PLA2s are members of the GVI family of PLA2 enzymes. The first member of this family the GVIA 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.
(as reviewed in Ref. 14). The best characterized of the GVI PLÀ enzymes is the GVI PLÀ (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 PLÀ, 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 PLÀ also possesses a lysophospholipase activity, as well as transacylase activity (44). The activity of the Givia PLÀ 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 PLÀ in different signaling pathways has been shown to be very complex. Initial reports of the functions of the GIVA PLÀ were determined using the inhibitor bromoenoallactone (44). Recent work has shown that this inhibitor is not specific for GIVA PLÀ and actually functions through activation of the inhibitor by GIVA PLÀ followed by nonspecific 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 PLÀ. Experiments using the inhibitor bromoenoallactone are reviewed elsewhere (46). Two major factors have allowed the determination of GIVA PLÀ’s cellular functions. First the recent generation of GIVA PLÀ deficient mice has shown the importance of this enzyme in bone formation, apoptosis, insulin secretion, and sperm development (47–50). Second the recent development of specific fluoroketone inhibitors of GIVA PLÀ (51) have shown in mouse models that the GIVA PLÀ in combination with the GIVA PLÀ play an important role in Wallerian degeneration and axon regeneration in nerve injury (52). Recent work using antiserum oligodeoxyribonucleotide toward GIVA PLÀ with monocytes has shown decreases in monocyte recruitment and directionality (53).

PAF ACETYL HYDROLASE/OXIDIZED LIPID LPLÀ

The PAF acetyl hydrolase/oxidized lipid LPLÀ is a member of the GIVII family of PLÀ enzymes. This enzyme was named for its ability to cleave the acetyl group from the sn-2 position of 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 PLÀs studied (54). Its active site is composed of a serine, histidine, and aspartic acid hydrolase triad (55), which is unlike all other PLÀs, 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 proinflammatory roles of PAF; however several clinical studies of GIVIIA PLÀ 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 PLÀ superfamily of enzymes have been carried out for over 100 years. Early kinetic and structural work established PLÀ as an important model of lipid enzymology. With the discovery of multiple different family members of PLÀ and their structural characterization, as well as the discovery of their cellular functions, the PLÀ 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 PLÀ’s roles in cellular processes, along with new potential therapeutics.

REFERENCES


Structure/function of PLA2 S241


