Phospholipase A_2 structure/function, mechanism, and signaling[1]

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Abstract Tremendous advances in understanding the structure and function of the superfamily of phospholipase A_2 (PLA_2) enzymes has occurred in the twenty-first century. The superfamily includes 15 groups comprising four main types including the secreted $sPLA_2$, cytosolic $cPLA_2$, calcium-independent $iPLA_2$, and platelet activating factor (PAF) acetyl hydrolase/oxidized lipid lipoprotein associated (Lp)PLA_2. 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 PLA_2. 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 A_2 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 A_2 (PLA_2) enzymes. PLA_2 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), PLA_2 was known to be a major component of snake venoms, and it was later recognized that PLA_2 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 PLA_2, 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 PLA_2 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 PLA_2 from bee venom (group III) (6), and in 1991 the human cytosolic calcium-dependent PLA_2 from macrophages (group IVA) (7, 8), the need for a more elaborate "group numbering system" became obvious (9). As the discovery of additional PLA_2s continued such as the macrophage secreted group V PLA_2 (10, 11) and the calcium-independent PLA_2 (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 PLA_2. They cluster in four main categories or types: secreted $sPLA_2$s, cytosolic $cPLA_2$s, calcium-independent $iPLA_2$s, and platelet activating factor (PAF) acetyl hydrolase/oxidized lipid lipoprotein associated (Lp)PLA_2s. 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 PLA_2

The secreted PLA_2s were the first type of PLA_2 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

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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_2s. 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 *sn*-2 position (22). However they have different preferences for the charge on the lipid surface. PLA_2s containing a tryptophan in the lipid binding surface display the highest activity toward neutral lipid substrates, and PLA_2s 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_2s 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

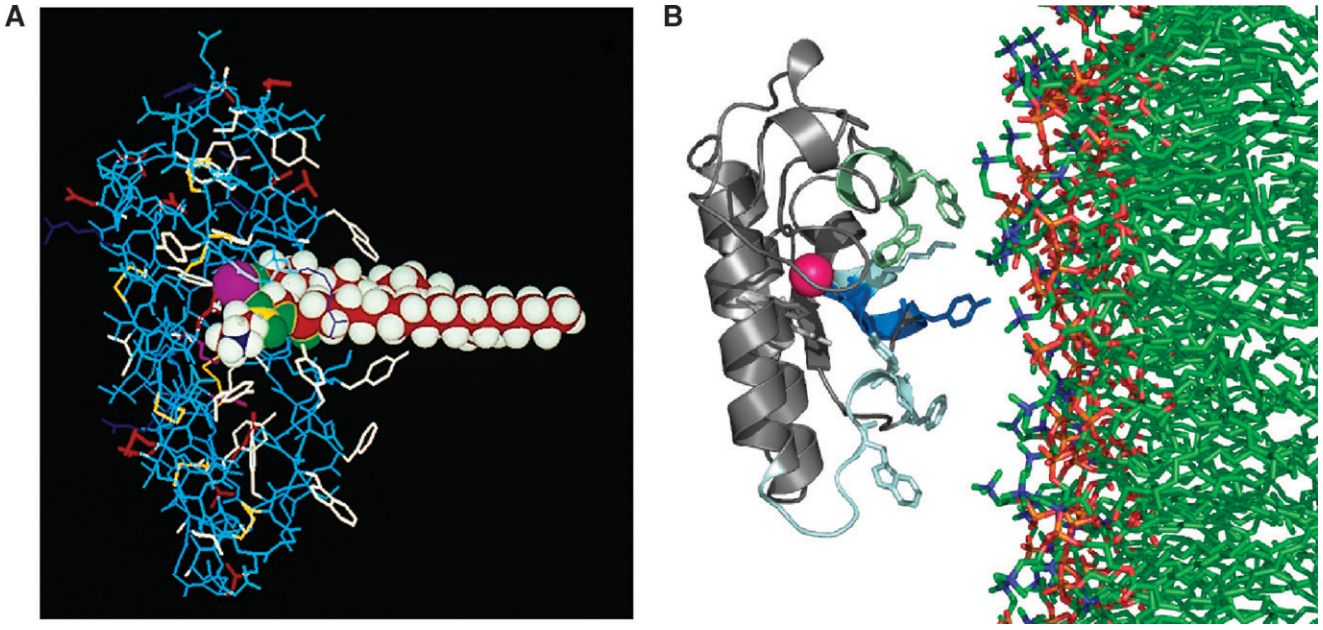

Fig. 1. A: The group IA phospholipase A_2 (PLA_2) with phospholipid substrate modeled in the active site. The active site residues His-48 and Asp-93 and the bound Ca^{2+} is shown in purple. Ca^{2+} is bound by Asp-49 as well as the carbonyl oxygens of Tyr-28, Gly-30, and Gly-32. Aromatic residues are shown in white. Adapted from Dennis (9). B: Model of the lipid surface binding of the group IA PLA_2 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).

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 PLA_2 gene significantly reduced the effects of many inflammatory diseases (30–32). GIVA PLA_2 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 α/β 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 α/β hydrolase region conserved throughout many different lipases, as well as a novel cap region found only in GIVA PLA_2. 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 PLA_2 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 PLA_2 inhibitors have been suggested by recent work examining a human patient with defects in GIVA PLA_2 who showed decreases in PLA_2 activity, eicosanoid biosynthesis, and the generation of many small intestinal ulcers (42).

CA^{2+} INDEPENDENT PLA_2

The Ca^{2+} independent PLA_2s are members of the GVI family of PLA_2 enzymes. The first member of this family the GVIA PLA_2 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 PLA_2 members have been identified in the last three years

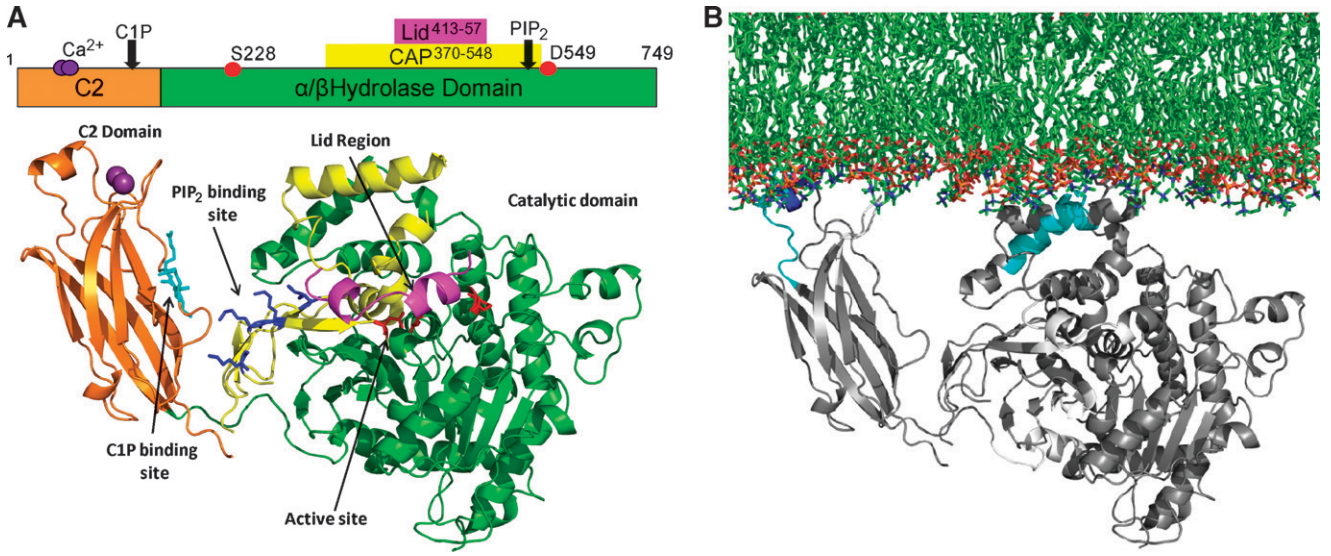

Fig. 2. A: Group IVA PLA_2 crystal structure as determined by Dessen et al. (33). The C2 domain is shown in orange, with two bound Ca^{2+} ions shown in purple. The catalytic domain is shown on the right with the cap region colored yellow, and the lid region 415–432 colored magenta. The active site residues Ser-228, Asp-549, and Arg-200 are shown in stick form colored red. The PIP_2 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 group IVA (GIVA) PLA_2 with residues colored based on interaction with lipid membrane. Adapted from Burke et al (35).

(as reviewed in Ref. 14). The best characterized of the GVI PLA_2 enzymes is the GVIA PLA_2 (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 PLA_2, 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 GVIA PLA_2 also posseses a lysophospholipase activity, as well as transacylase activity (44). The activity of the GVIA PLA_2 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 GVIA PLA_2 in different signaling pathways has been shown to be very complex. Initial reports of the functions of the GVIA PLA_2 were determined using the inhibitor bromoenollactone (44). Recent work has shown that this inhibitor is not specific for GVIA PLA_2 and actually functions through activation of the inhibitor by GVIA PLA_2 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 GVIA PLA_2. Experiments using the inhibitor bromoenollactone are reviewed elsewhere (46). Two major factors have allowed the determination of GVI PLA_2's cellular functions. First the recent generation of GVIA PLA_2 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 GVIA PLA_2 (51) have shown in mouse models that the GVIA PLA_2 in combination with the GIVA PLA_2 play an important role in Wallerian degeneration and axon regeneration in nerve injury (52). Recent work using antisense oligodeoxyribonucleotide toward GVIA PLA_2 with monocytes has shown decreases in monocyte recruitment and directionality (53).

PAF ACETYL HYDROLASE/OXIDIZED LIPID LPPLA_2

The PAF acetyl hydrolase/oxidized lipid LpPLA_2 is a member of the GVII family of PLA_2 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 PLA_2s studied (54). Its active site is composed of a serine, histidine, and aspartic acid hydrolase triad (55), which is unlike all other PLA_2s, 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 GVIIA PLA_2 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 PLA_2 superfamily of enzymes have been carried out for over 100 years. Early kinetic and structural work established PLA_2 as an important model of lipid enzymology. With the discovery of multiple different family members of PLA_2 and their structural characterization, as well as the discovery of their cellular functions, the PLA_2 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 PLA_2s' roles in cellular processes, along with new potential therapeutics.

REFERENCES

1. Funk, C. D. 2001. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science.* **294:** 1871–1875.
2. Rivera, R., and J. Chun. 2008. Biological effects of lysophospholipids. *Rev. Physiol. Biochem. Pharmacol.* **160:** 25–46.
3. Stephens, W. W., J. L. Walker, and W. Myers. 1898. The action of cobra poison on the blood: a contribution to the study of passive immunity. *J. Pathol. Bacteriol.* **5:** 279–301.
4. Seilhamer, J. J., W. Pruzanski, P. Vadas, S. Plant, J. A. Miller, J. Kloss, and L. K. Johnson. 1989. Cloning and recombinant expression of phospholipase A2 present in rheumatoid arthritic synovial fluid. *J. Biol. Chem.* **264:** 5335–5338.
5. Kramer, R. M., C. Hession, B. Johansen, G. Hayes, P. McGray, E. P. Chow, R. Tizard, and R. B. Pepinsky. 1989. Structure and properties of a human non-pancreatic phospholipase A2. *J. Biol. Chem.* **264:** 5768–5775.
6. Kuchler, K., M. Gmachl, M. J. Sippl, and G. Kreil. 1989. Analysis of the Cdna for phospholipase-A2 from honeybee venom glands - the deduced amino-acid sequence reveals homology to the corresponding vertebrate enzymes. *Eur. J. Biochem.* **184:** 249–254.
7. Clark, J. D., L. L. Lin, R. W. Kriz, C. S. Ramesha, L. A. Sultzman, A. Y. Lin, N. Milona, and J. L. Knopf. 1991. A novel arachidonic acid-selective cytosolic PLA2 contains a Ca(2+)-dependent translocation domain with homology to PKC and GAP. *Cell.* **65:** 1043–1051.
8. Kramer, R. M., E. F. Roberts, J. Manetta, and J. E. Putnam. 1991. The Ca2(+)-sensitive cytosolic phospholipase A2 is a 100-kDa protein in human monoblast U937 cells. *J. Biol. Chem.* **266:** 5268–5272.
9. Dennis, E. A. 1994. Diversity of group types, regulation, and function of phospholipase A2. *J. Biol. Chem.* **269:** 13057–13060.
10. Chen, J., S. J. Engle, J. J. Seilhamer, and J. A. Tischfield. 1994. Cloning and recombinant expression of a novel human low-molecular-weight Ca2+-dependent phospholipase-a(2). *J. Biol. Chem.* **269:** 2365–2368.
11. Balboa, M. A., J. Balsinde, M. V. Winstead, J. A. Tischfield, and E. A. Dennis. 1996. Novel group V phospholipase A2 involved in arachidonic acid mobilization in murine P388D1 macrophages. *J. Biol. Chem.* **271:** 32381–32384.

12. Ackermann, E. J., E. S. Kempner, and E. A. Dennis. 1994. Ca(2+)-independent cytosolic phospholipase A2 from macrophage-like P388D1 cells. Isolation and characterization. *J. Biol. Chem.* **269:** 9227–9233.
13. Six, D. A., and E. A. Dennis. 2000. The expanding superfamily of phospholipase A(2) enzymes: classification and characterization. *Biochim. Biophys. Acta.* **1488:** 1–19.
14. Schaloske, R. H., and E. A. Dennis. 2006. The phospholipase A2 superfamily and its group numbering system. *Biochim. Biophys. Acta.* **1761:** 1246–1259.
15. Scott, D. L., S. P. White, Z. Otwinowski, W. Yuan, M. H. Gelb, and P. B. Sigler. 1990. Interfacial catalysis: the mechanism of phospholipase A2. *Science.* **250:** 1541–1546.
16. Yu, L., and E. A. Dennis. 1991. Critical role of a hydrogen bond in the interaction of phospholipase A2 with transition-state and substrate analogues. *Proc. Natl. Acad. Sci. USA.* **88:** 9325–9329.
17. Gelb, M. H., M. K. Jain, A. M. Hanel, and O. G. Berg. 1995. Interfacial enzymology of glycerolipid hydrolases: lessons from secreted phospholipases A2. *Annu. Rev. Biochem.* **64:** 653–688.
18. Carman, G. M., R. A. Deems, and E. A. Dennis. 1995. Lipid signaling enzymes and surface dilution kinetics. *J. Biol. Chem.* **270:** 18711–18714.
19. Plesniak, L. A., L. Yu, and E. A. Dennis. 1995. Conformation of micellar phospholipid bound to the active site of phospholipase A2. *Biochemistry.* **34:** 4943–4951.
20. Burke, J. E., M. J. Karbarz, R. A. Deems, S. Li, V. L. Woods, Jr., and E. A. Dennis. 2008. Interaction of group IA phospholipase A2 with metal ions and phospholipid vesicles probed with deuterium exchange mass spectrometry. *Biochemistry.* **47:** 6451–6459.
21. Lin, Y., R. Nielsen, D. Murray, W. L. Hubbell, C. Mailer, B. H. Robinson, and M. H. Gelb. 1998. Docking phospholipase A2 on membranes using electrostatic potential-modulated spin relaxation magnetic resonance. *Science.* **279:** 1925–1929.
22. Singer, A. G., F. Ghomashchi, C. Le Calvez, J. Bollinger, S. Bezzine, M. Rouault, M. Sadilek, E. Nguyen, M. Lazdunski, G. Lambeau, et al. 2002. Interfacial kinetic and binding properties of the complete set of human and mouse groups I, II, V, X, and XII secreted phospholipases A2. *J. Biol. Chem.* **277:** 48535–48549.
23. Lambeau, G., and M. H. Gelb. 2008. Biochemistry and physiology of mammalian secreted phospholipases A2. *Annu. Rev. Biochem.* **77:** 495–520.
24. Schevitz, R. W., N. J. Bach, D. G. Carlson, N. Y. Chirgadze, D. K. Clawson, R. D. Dillard, S. E. Draheim, L. W. Hartley, N. D. Jones, E. D. Mihelich, et al. 1995. Structure-based design of the first potent and selective inhibitor of human non-pancreatic secretory phospholipase A2. *Nat. Struct. Biol.* **2:** 458–465.
25. Lambeau, G., and M. Lazdunski. 1999. Receptors for a growing family of secreted phospholipases A2. *Trends Pharmacol. Sci.* **20:** 162–170.
26. Saegusa, J., N. Akakura, C. Y. Wu, C. Hoogland, Z. Ma, K. S. Lam, F. T. Liu, Y. K. Takada, and Y. Takada. 2008. Pro-inflammatory secretory phospholipase A2 type IIA binds to integrins {alpha}v{beta}3 and {alpha}4{beta}1 and induces proliferation of monocytic cells in an integrin-dependent manner. *J. Biol. Chem.* **283:** 26107–26115.
27. Kramer, R. M., G. C. Checani, A. Deykin, C. R. Pritzker, and D. Deykin. 1986. Solubilization and properties of Ca2+-dependent human platelet phospholipase A2. *Biochim. Biophys. Acta.* **878:** 394–403.
28. Ghosh, M., D. E. Tucker, S. A. Burchett, and C. C. Leslie. 2006. Properties of the group IV phospholipase A2 family. *Prog. Lipid Res.* **45:** 487–510.
29. Reynolds, L., L. Hughes, A. I. Louis, R. A. Kramer, and E. A. Dennis. 1993. Metal ion and salt effects on the phospholipase A_2, lysophospholipase, and transacylase activities of human cytosolic phospholipase A_2. *Biochim. Biophys. Acta.* **1167:** 272–280.
30. Bonventre, J. V., Z. Huang, M. R. Taheri, E. O'Leary, E. Li, M. A. Moskowitz, and A. Sapirstein. 1997. Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A2. *Nature.* **390:** 622–625.
31. Uozumi, N., K. Kume, T. Nagase, N. Nakatani, S. Ishii, F. Tashiro, Y. Komagata, K. Maki, K. Ikuta, Y. Ouchi, et al. 1997. Role of cytosolic phospholipase A2 in allergic response and parturition. *Nature.* **390:** 618–622.
32. Uozumi, N., and T. Shimizu. 2002. Roles for cytosolic phospholipase A2alpha as revealed by gene-targeted mice. *Prostaglandins Other Lipid Mediat.* **68–69:** 59–69.
33. Dessen, A., J. Tang, H. Schmidt, M. Stahl, J. D. Clark, J. Seehra, and W. S. Somers. 1999. Crystal structure of human cytosolic phospholipase A2 reveals a novel topology and catalytic mechanism. *Cell.* **97:** 349–360.
34. Hsu, Y. H., J. E. Burke, D. L. Stephens, R. A. Deems, S. Li, K. M. Asmus, V. L. Woods, Jr., and E. A. Dennis. 2008. Calcium binding rigidifies the C2 domain and the intra-domain interaction of GIVA phospholipase A2 as revealed by hydrogen/deuterium exchange mass spectrometry. *J. Biol. Chem.* **283:** 9820–9827.
35. Burke, J. E., Y. H. Hsu, R. A. Deems, S. Li, V. L. Woods, Jr., and E. A. Dennis. 2008. A phospholipid substrate molecule residing in the membrane surface mediates opening of the lid region in group IVA cytosolic phospholipase A2. *J. Biol Chem* **283:** 31227–31236.
36. Stahelin, R. V., P. Subramanian, M. Vora, W. Cho, and C. E. Chalfant. 2007. Ceramide-1-phosphate binds group IVA cytosolic phospholipase a2 via a novel site in the C2 domain. *J. Biol. Chem.* **282:** 20467–20474.
37. Six, D. A., and E. A. Dennis. 2003. Essential Ca(2+)-independent role of the group IVA cytosolic phospholipase A(2) C2 domain for interfacial activity. *J. Biol. Chem.* **278:** 23842–23850.
38. Burke, J. E., and E. A. Dennis. 2008. Phospholipase A2 biochemistry. *Cardiovasc. Drugs Ther.* In press.
39. Six, D. A., E. Barbayianni, V. Loukas, V. Constantinou-Kokotou, D. Hadjipavlou-Litina, D. Stephens, A. C. Wong, V. Magrioti, P. Moutevelis-Minakakis, S. F. Baker, et al. 2007. Structure-activity relationship of 2-oxoamide inhibition of group IVA cytosolic phospholipase A(2) and group v secreted phospholipase A(2). *J. Med. Chem.* **50:** 4222–4235.
40. McKew, J. C., K. L. Lee, M. W. Shen, P. Thakker, M. A. Foley, M. L. Behnke, B. Hu, F. W. Sum, S. Tam, Y. Hu, et al. 2008. Indole cytosolic phospholipase A2 alpha inhibitors: discovery and in vitro and in vivo characterization of 4-{3-[5-chloro-2-(2-{[(3,4-dichlorobenzyl) sulfonyl]amino}ethyl)-1-(dipheny lmethyl)-1H-indol-3-yl]propyl} benzoic acid, efipladib. *J. Med. Chem.* **51:** 3388–3413.
41. Yaksh, T. L., G. Kokotos, C. I. Svensson, D. Stephens, C. G. Kokotos, B. Fitzsimmons, D. Hadjipavlou-Litina, X. Y. Hua, and E. A. Dennis. 2006. Systemic and intrathecal effects of a novel series of phospholipase A(2) inhibitors on hyperalgesia and spinal prostaglandin E-2 release. *J. Pharmacol. Exp. Ther.* **316:** 466–475.
42. Adler, D. H., J. D. Cogan, J. A. Phillips 3rd, N. Schnetz-Boutaud, G. L. Milne, T. Iverson, J. A. Stein, D. A. Brenner, J. D. Morrow, O. Boutaud, et al. 2008. Inherited human cPLA(2alpha) deficiency is associated with impaired eicosanoid biosynthesis, small intestinal ulceration, and platelet dysfunction. *J. Clin. Invest.* **118:** 2121–2131.
43. Balboa, M. A., J. Balsinde, S. S. Jones, and E. A. Dennis. 1997. Identity between the Ca2+-independent phospholipase A2 enzymes from P388D1 macrophages and Chinese hamster ovary cells. *J. Biol. Chem.* **272:** 8576–8580.
44. Winstead, M. V., J. Balsinde, and E. A. Dennis. 2000. Calcium-independent phospholipase A(2): structure and function. *Biochim. Biophys. Acta.* **1488:** 28–39.
45. Song, H., S. Ramanadham, S. Bao, F. F. Hsu, and J. Turk. 2006. A bromoenol lactone suicide substrate inactivates group VIA phospholipase A2 by generating a diffusible bromomethyl keto acid that alkylates cysteine thiols. *Biochemistry.* **45:** 1061–1073.
46. Balsinde, J., and M. A. Balboa. 2005. Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A(2) in activated cells. *Cell. Signal.* **17:** 1052–1062.
47. Bao, S., D. J. Miller, Z. Ma, M. Wohltmann, G. Eng, S. Ramanadham, K. Moley, and J. Turk. 2004. Male mice that do not express group VIA phospholipase A2 produce spermatozoa with impaired motility and have greatly reduced fertility. *J. Biol. Chem.* **279:** 38194–38200.
48. Bao, S., H. Song, M. Wohltmann, S. Ramanadham, W. Jin, A. Bohrer, and J. Turk. 2006. Insulin secretory responses and phospholipid composition of pancreatic islets from mice that do not express Group VIA phospholipase A2 and effects of metabolic stress on glucose homeostasis. *J. Biol. Chem.* **281:** 20958–20973.
49. Bao, S., Y. Li, X. Lei, M. Wohltmann, W. Jin, A. Bohrer, C. F. Semenkovich, S. Ramanadham, I. Tabas, and J. Turk. 2007. Attenuated free cholesterol loading-induced apoptosis but preserved phospholipid composition of peritoneal macrophages from mice that do not express group VIA phospholipase A2. *J. Biol. Chem.* **282:** 27100–27114.
50. Ramanadham, S., K. E. Yarasheski, M. J. Silva, M. Wohltmann, D. V. Novack, B. Christiansen, X. Tu, S. Zhang, X. Lei, and J. Turk. 2008. Age-related changes in bone morphology are accelerated in group VIA phospholipase A2 (iPLA2beta)-null mice. *Am. J. Pathol.* **172:** 868–881.
51. Baskakis, C., V. Magrioti, N. Cotton, D. Stephens, V. Constantinou-Kokotou, E. A. Dennis, and G. Kokotos. 2008. Synthesis of poly-

fluoro ketones for selective inhibition of human phospholipase A2 enzymes. *J. Med. Chem.* **51(24):** 8027–8037.
52. Lopez-Vales, R., X. Navarro, T. Shimizu, C. Baskakis, G. Kokotos, V. Constantinou-Kokotou, D. Stephens, E. A. Dennis, and S. David. 2008. Intracellular phospholipase A2 group IVA and group VIA play important roles in Wallerian degeneration and axon regeneration after peripheral nerve injury. *Brain.*
53. Mishra, R. S., K. A. Carnevale, and M. K. Cathcart. 2008. iPLA2beta: front and center in human monocyte chemotaxis to MCP-1. *J. Exp. Med.* **205:** 347–359.
54. Gelb, M. H., J. H. Min, and M. K. Jain. 2000. Do membrane-bound enzymes access their substrates from the membrane or aqueous phase: interfacial versus non-interfacial enzymes. *Biochim. Biophys. Acta.* **1488:** 20–27.
55. Tjoelker, L. W., C. Eberhardt, J. Unger, H. L. Trong, G. A. Zimmerman, T. M. McIntyre, D. M. Stafforini, S. M. Prescott, and P. W. Gray. 1995. Plasma platelet-activating factor acetylhydrolase is a secreted phospholipase A2 with a catalytic triad. *J. Biol. Chem.* **270:** 25481–25487.
56. Gardner, A. A., E. C. Reichert, M. K. Topham, and D. M. Stafforini. 2008. Identification of a domain that mediates association of platelet-activating factor acetylhydrolase with high density lipoprotein. *J. Biol. Chem.* **283:** 17099–17106.
57. Tjoelker, L. W., C. Wilder, C. Eberhardt, D. M. Stafforini, G. Dietsch, B. Schimpf, S. Hooper, H. Le Trong, L. S. Cousens, G. A. Zimmerman, et al. 1995. Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. *Nature.* **374:** 549–553.
58. Lavi, S., J. Herrmann, R. Lavi, J. P. McConnell, L. O. Lerman, and A. Lerman. 2008. Role of lipoprotein-associated phospholipase A2 in atherosclerosis. *Curr. Atheroscler. Rep.* **10:** 230–235.
59. Lerman, A., and J. P. McConnell. 2008. Lipoprotein-associated phospholipase A2: a risk marker or a risk factor? *Am. J. Cardiol.* **101:** 11F–22F.
60. Blackie, J. A., J. C. Bloomer, M. J. Brown, H. Y. Cheng, B. Hammond, D. M. Hickey, R. J. Ife, C. A. Leach, V. A. Lewis, C. H. Macphee, et al. 2003. The identification of clinical candidate SB-480848: a potent inhibitor of lipoprotein-associated phospholipase A2. *Bioorg. Med. Chem. Lett.* **13:** 1067–1070.
61. Serruys, P. W., H. M. Garcia-Garcia, P. Buszman, P. Erne, S. Verheye, M. Aschermann, H. Duckers, O. Bleie, D. Dudek, H. E. Botker, et al. 2008. Effects of the direct lipoprotein-associated phospholipase A(2) inhibitor darapladib on human coronary atherosclerotic plaque. *Circulation.* **118:** 1172–1182.
62. Mohler 3rd, E. R., C. M. Ballantyne, M. H. Davidson, M. Hanefeld, L. M. Ruilope, J. L. Johnson, and A. Zalewski. 2008. The effect of darapladib on plasma lipoprotein-associated phospholipase A2 activity and cardiovascular biomarkers in patients with stable coronary heart disease or coronary heart disease risk equivalent: the results of a multicenter, randomized, double-blind, placebo-controlled study. *J. Am. Coll. Cardiol.* **51:** 1632–1641.
63. Wilensky, R. L., Y. Shi, E. R. Mohler 3rd, D. Hamamdzic, M. E. Burgert, J. Li, A. Postle, R. S. Fenning, J. G. Bollinger, B. E. Hoffman, et al. 2008. Inhibition of lipoprotein-associated phospholipase A(2) reduces complex coronary atherosclerotic plaque development. *Nat. Med.* In press.