Published in final edited form as:

Adv Biol Regul. 2018 January ; 67: 212–218. doi:10.1016/j.jbior.2017.10.009.

Review of four major distinct types of human phospholipase A₂

Alexis M. Vasquez, Varnavas D. Mouchlis, and Edward A. Dennis*

Department of Chemistry and Biochemistry and Department of Pharmacology, School of Medicine, University of California, San Diego, La Jolla, California 92093-0601

Abstract

The phospholipase A₂ superfamily of enzymes plays a significant role in the development and progression of numerous inflammatory diseases. Through their catalytic action on membrane phospholipids, phospholipases are the upstream regulators of the eicosanoid pathway releasing free fatty acids for cyclooxygenases, lipoxygenases, and cytochrome P450 enzymes which produce various well-known inflammatory mediators including leukotrienes, thromboxanes and prostaglandins. Elucidating the association of phospholipases A₂ with the membrane, the extraction and binding of phospholipid substrates, and their interaction with small-molecule inhibitors is crucial for the development of new anti-inflammatory therapeutics. Studying phospholipases has been challenging because they act on the surface of cellular membranes and micelles. Multidisciplinary approaches including hydrogen/deuterium exchange mass spectrometry, molecular dynamics simulations, and other computer-aided drug design techniques have been successfully employed by our laboratory to study interactions of phospholipases with membranes, phospholipid substrates and inhibitors. This review summarizes the application of these techniques to study four human recombinant phospholipases A₂ *in vitro*.

1. Introduction to phospholipase A₂ superfamily

Phospholipases A₂ were initially purified from the venom of poisonous snakes. As the ability to sequence and purify new proteins became more technologically advanced in the 1970s, it became apparent that these enzymes were abundant with cysteines that formed disulfide bonds (Davidson and Dennis, 1990a, b). At the same time, a separate novel human mammalian enzyme that also maintained this pattern of disulfide bonds and hydrolyzed phospholipid substrates in the body was discovered (Kramer et al., 1989; Seilhamer et al., 1989). Later as intracellular non-disulfide containing PLA₂s were discovered, it was necessary to create a group numbering system that could categorize the rapidly expanding number of PLA₂ enzymes together in a logical manner and make it easy to accurately identify which phospholipase A₂ was being studied (Dennis, 1994). Over the last 50 years, six major types of phospholipase A₂ enzymes have been identified: sPLA₂, cPLA₂, iPLA₂, Lp-PLA₂ (also known as PAF-AH), LPLA₂, and AdPLA₂ (Table 1.1).

*To whom correspondence should be addressed: edennis@ucsd.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

PLA₂ enzymes utilize either a catalytic dyad or triad to catalyze the hydrolysis of the ester bond at the *sn*-2 position of phospholipid substrates. Hydrolysis of substrates releases free fatty acids and lysophospholipids that can be acted upon in a variety of manners including, when it is arachidonic acid, being converted further to lipid signaling mediators (Figure 1). While features such as structure and the specific activity toward various substrates reveal commonality among the several types of PLA₂s, localization of them varies. Lp-PLA₂ and sPLA₂ are both secreted enzymes and act on membrane lipids extracellularly, while cPLA₂ and iPLA₂ hydrolyze intracellular lipids of bilayer membranes including various sub-cellular organelles, less is understood about the localization of LPLA₂ and AdPLA₂.

It is a particular challenge to study these water-soluble enzymes because they act on phospholipids which typically aggregate in an aqueous environment forming micelles, vesicles, liposomes, and other large structural aggregates (Dennis, 2016). Carrying out kinetics in a mixed micelle system with the non-ionic detergent Triton X-100 using “surface-dilution kinetics” has been a particularly informative model system to study PLA₂ activity since the hydrolysis of the phospholipid substrates occurs at the lipid-water interface (Deems et al., 1975). Studies on phospholipase A₂ enzymes must consider how experimental techniques will operate in this type of cellular environment (substrate aggregation, membrane association, etc.); and since no one technique offers full characterization of PLA₂s, we have had to utilize a wide array of approaches.

Whether it be enzymatic assays or molecular dynamics, each unique method offers advantages and disadvantages to studying PLA₂ enzymes. It has been necessary to utilize a multidisciplinary approach to understand the mechanism of action of these enzymes. Learning about their interactions with membranes (Bucher et al., 2013), phospholipid substrates (Mouchlis et al., 2015) and inhibitors (Mouchlis et al., 2011a) was achieved using a combination of enzyme assays, deuterium exchange mass spectrometry and molecular dynamics (Mouchlis and Dennis, 2016). In this review, results of studies on four human PLA₂s including their structure, function and activity are discussed in detail.

2. Lipoprotein-associated Phospholipase A₂

Platelet activating factor (PAF) is a potent phospholipid mediator that plays a major role in clotting and inflammatory pathways (Prescott et al., 2000). Two groups of phospholipases, Group VII and Group VIII, catalyze the hydrolysis of the *sn*-2 fatty acyl bond in PAF and other lipid substrates (Dennis et al., 2011). Therefore, these enzymes were initially called PAF acetylhydrolases (PAF-AH) (Schaloske and Dennis, 2006). Further studies of the Group VIA enzyme revealed that it associates with both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) leading to the name lipoprotein-associated phospholipase A₂ (Lp-PLA₂).

Lp-PLA₂ is calcium independent, secreted extracellularly and has been proposed to play a significant role in the development of atherosclerosis (Dennis et al., 2011; Tjoelker et al., 1995b). Group VIIA Lp-PLA₂ is a 45 kDa, secreted protein that contains an alpha/beta hydrolase fold and a GX SXG motif that is characteristic of esterases and lipases (Samanta and Bahnson, 2008). The active site contains a catalytic triad composed of Ser/Asp/His

(Tjoelker et al., 1995a) (Figure 2). Group VIIB Lp-PLA₂ is about 40 kDa long and contains the same catalytic triad as GVIIA Lp-PLA₂. Group VIII Lp-PLA₂ consists of two 26 kDa catalytic subunits and one 45 kDa non-catalytic regulatory subunit. GVII and GVIII Lp-PLA₂ do not show much amino acid sequence identity but both are related to each other since these enzymes are in principle capable of retrieving substrates from the aqueous phase or through direct extraction from membranes (Cao et al., 2011; Min et al., 1999).

Some of the most common substrates for Lp-PLA₂ include PAF, short fatty acyl chain phosphatidylcholines, oxidized phospholipids, and F₂-isoprostanes (Stafforini et al., 1997; Stafforini et al., 2006; Stremmer et al., 1991; Stremmer et al., 1989). GVIII Lp-PLA₂ utilizes similar substrates but are more restricted in their activity towards the *sn*-2 position by having specificity towards acetyl groups (Manya et al., 1999). Catalytic hydrolysis of these substrates is believed to be stabilized by an oxyanion hole formed by the backbone amides of residues Leu153 and Phe274 (Samanta et al., 2009). H/D exchange studies suggested that residues 360–368 and 113–120 might serve as anchors for the association of the enzyme to the surface of the membrane (Cao, Jian et al., 2013; Cao, J. et al., 2013).

Since Lp-PLA₂ has been found to associate with both HDL and LDL from which it presumably retrieves its oxidized phospholipid substrate, abnormal distribution of this enzyme has been suggested to be linked to cardiovascular diseases (Tellis and Tselepis, 2009). Because the hydrolysis of substrates by Lp-PLA₂ has major upstream implications for inflammatory diseases (such as atherosclerosis and neonatal necrotizing enterocolitis), development of inhibitors and therapeutic interventions has been of great interest for many years. Azetidinones and pyrimidones have demonstrated inhibitory activity for Lp-PLA₂. GlaxoSmithKline has also developed the potent and selective inhibitor Darapladib that reached clinical trials (phase III) (Blackie et al., 2002; Blackie et al., 2003). Since then further investigation has been underway to learn more about the biological relevance of Lp-PLA₂ and its inhibitors.

3. Calcium-independent Phospholipase A₂

Group VIA calcium-independent phospholipase A₂ (iPLA₂), also known as PNPLA9 or iPLA₂β, is a calcium independent phospholipase A₂ and plays a key role in membrane remodeling (Ackermann et al., 1994; Balsinde et al., 1997). The many isoforms of iPLA₂ differentiate themselves from the many other phospholipase A₂ enzymes that are calcium dependent and they have been thought to be less specific as to the fatty acid at the *sn*-2 position (Lio and Dennis, 1998). These enzymes are the most widespread PLA₂s throughout human tissues. Although the crystal structure of iPLA₂ has not been solved, homology models and sequence alignments revealed that the longest splice variant (806 amino acid residues and 89 kDa) utilizes a catalytic dyad of Ser/Asp and is comprised of seven ankyrin repeats, a linker region, and a patatin-like α/β hydrolase catalytic domain (Larsson Forsell et al., 1999; Ma et al., 1999) (Figure 3). Despite studies demonstrating that iPLA₂ activity is regulated by many different mechanisms, it is still unclear how this enzyme is activated to bind and hydrolyze a specific substrate.

Inference of the structure of iPLA₂ from homology models and sequence alignments to previously crystallized structures of patatin lipase and human ankyrin-R d34 repeats has been fundamental to establishing the mechanism of action of iPLA₂ (Kienesberger et al., 2009; Michaely et al., 2002; Wilson et al., 2006). Regions of iPLA₂ that show the most similarity to patatin are proposed to be the catalytic domain (α/β hydrolase domain) while the rest of the enzyme is considered a regulatory unit (Mouchlis et al., 2015). The ankyrin repeats have been shown by hydrogen/deuterium exchange data to be highly flexible and may be key in regulation of iPLA₂ activity.

It has been demonstrated that iPLA₂ activity is regulated by ATP binding, caspase cleavage of the ankyrin repeats that results in a hyperactive form of the enzyme, calmodulin inhibition, oligomerization of the enzyme due to aggregation of ankyrin repeats, and membrane allosteric regulation (Mouchlis et al., 2015). ATP activation has been repeatedly demonstrated to be a major regulator of iPLA₂ activity. This enzyme is often found to localize in mitochondria, where ATP levels are critical for cellular regulation. iPLA₂ is the only PLA₂ believed to be regulated by ATP. ATP does not act as a substrate or cofactor, but rather activates the enzyme, presumably through allosteric regulation (Lio and Dennis, 1998; Ma et al., 1999). It is important to develop iPLA₂ inhibitors because the enzyme plays a significant role in many diseases including Barth syndrome and diabetes. Current known inhibitors include trifluoromethyl ketones, tricarbonyls, lactones and polyfluoroketones (Mouchlis, V. D. et al., 2016; Mouchlis, Varnavas D. et al., 2016).

4. Cytosolic Phospholipase A₂

Multiple subgroups of cytosolic phospholipase A₂ (cPLA₂) have been discovered in different cell types (Dennis et al., 2011). The first cPLA₂ purified and sequenced was Group IVA cPLA₂, an 85kDa protein that is regulated by intracellular calcium levels (Clark et al., 1991; Dessen et al., 1999; Kramer et al., 1991). This enzyme is widely distributed in cells throughout most types of human tissue and consists of a C2 domain, linker, and α/β hydrolase domain as determined by x-ray crystallography (Dessen et al., 1999). Calcium binding to the C2 domain in the N-terminal causes localization of the protein to a phospholipid membrane (Channon and Leslie, 1990). cPLA₂ also utilizes a catalytic dyad of Ser/Asp within the C-terminal α/β hydrolase domain (Pickard et al., 1996) as illustrated in Figure 4).

cPLA₂ acts on many lipid substrates including but not limited to phosphatidylcholines, phosphatidylethanolamines, and phosphatidylinositols (Leslie et al., 1988). Hydrolysis of phospholipid substrates has shown high specificity towards arachidonic acid at the *sn*-2 fatty acyl bond (Clark et al., 1991). In addition to this activity, cPLA₂ also displays lysophospholipase and trans-acylase activity (Reynolds et al., 1991). It has been demonstrated that the lipid mediator phosphatidylinositol 4,5-bisphosphate (PIP₂) further increases cPLA₂ activity (Mosior et al., 1998; Tamiya-Koizumi et al., 1989) as well as ceramide-1-phosphate (C1P) (Nakamura et al., 2006). Besides calcium, PIP₂, and C1P activation, cPLA₂ is regulated by phosphorylation and membrane interactions (Mouchlis et al., 2015). Although no specific disease is associated with regulation of this enzyme, it is known to play a role in inflammation through downstream regulation of COX genes.

Multiple types of inhibitors have been developed including 2-oxoamides and 2-oxoesters (Kokotou et al., 2017; Mouchlis et al., 2012).

5. Secreted Phospholipase A₂

The secreted phospholipase A₂ (sPLA₂) was the first phospholipase A₂ enzyme identified and studied in detail from snake venom, mammalian pancreas and mammalian cells (Dennis et al., 2011). sPLA₂ is a group of small enzymes (14–18kDa) that use a His/Asp dyad and also required calcium for catalytic activity (Schaloske and Dennis, 2006) (Figure 4). Mechanistic insights were developed from the crystal structure of the cobra venom (*Naja naja naja*) which was reported (Fremont et al., 1993) by our laboratory as well as crystal structures of other venom and pancreatic PLA₂s by many groups over the years (Seilhamer et al., 1989; Kramer et al., 1989; Guy et al., 2009; Matoba et al., 2002). For mammalian sPLA₂s, sequence alignments have been used to interpret the structure of some of these enzymes, such as the Group V sPLA₂ based on the crystal structure of the Group IIA sPLA₂ (Edwards et al., 2002). Multiple calcium binding sites have been reported according to DXMS studies (Burke et al., 2008). Mechanisms of activation and inhibition of this enzyme have been studied, and it has been shown that the sPLA₂ s enzyme do not hydrolyze the sn-1 position of the lysophospholipid products while some of the intracellular enzymes do. Surprisingly, sPLA₂ does display an increase in activity when substrate forms aggregates rather than monomers, known as “interfacial activation” (Carman et al., 1995).

Major functions of sPLA₂ include antibacterial and antiviral properties, regulating HDL and LDL levels and playing a role in inflammatory diseases such as atherosclerosis. Numerous inhibitors have been developed for sPLA₂ including dicarboxylic acids, sulfonamides, amides, indoles, and oxadiazolones, many of which were helped and better understood by *in silico* studies (Mouchlis et al., 2011b; Mouchlis et al., 2010a, b).

6. Conclusion

Understanding the cellular function of phospholipases is a very challenging task. PLA₂ (Dennis and Norris, 2015) and other related enzymes (Grkovich and Dennis, 2009) play a significant role in the development of inflammatory diseases and thus understanding how to regulate their activity is necessary for the advancement of interventional therapeutics and inhibitors. Computational techniques combined with hydrogen-deuterium exchange mass spectrometry experiments have been successfully employed to understand the mechanism of action of phospholipases as well as the structural and physicochemical properties of their active sites (Mouchlis and Dennis, 2016). In conjunction with these approaches, enzymatic assays have also been used to further investigate PLA₂ enzymatic activity. This review summarizes the application of these techniques to study four human phospholipases A₂

Acknowledgments

This work was generously supported by NIH grant GM20501-41.

References

- Ackermann EJ, Kempner ES, Dennis EA. Ca^{2+} -independent cytosolic phospholipase A_2 from macrophage-like P388D1 cells. Isolation and characterization J Biol Chem. 1994; 269:9227–9233. [PubMed: 8132660]
- Balsinde J, Balboa MA, Dennis EA. Antisense inhibition of group VI Ca^{2+} -independent phospholipase A_2 blocks phospholipid fatty acid remodeling in murine P388D1 macrophages. J Biol Chem. 1997; 272:29317–29321. [PubMed: 9361012]
- Blackie JA, Bloomer JC, Brown MJ, Cheng HY, Elliott RL, Hammond B, Hickey DM, Ife RJ, Leach CA, Lewis VA, Macphee CH, Milliner KJ, Moores KE, Pinto IL, Smith SA, Stansfield IG, Stanway SJ, Taylor MA, Theobald CJ, Whittaker CM. The discovery of SB-435495. A potent, orally active inhibitor of lipoprotein-associated phospholipase A_2 for evaluation in man. Bioorg Med Chem Lett. 2002; 12:2603–2606. [PubMed: 12182870]
- Blackie JA, Bloomer JC, Brown MJ, Cheng HY, Hammond B, Hickey DM, Ife RJ, Leach CA, Lewis VA, Macphee CH, Milliner KJ, Moores KE, Pinto IL, Smith SA, Stansfield IG, Stanway SJ, Taylor MA, Theobald CJ. The identification of clinical candidate SB-480848: a potent inhibitor of lipoprotein-associated phospholipase A_2 . Bioorg Med Chem Lett. 2003; 13:1067–1070. [PubMed: 12643913]
- Bucher D, Hsu YH, Mouchlis VD, Dennis EA, McCammon JA. Insertion of the Ca^{2+} -independent phospholipase A_2 into a phospholipid bilayer via coarse-grained and atomistic molecular dynamics simulations. PLoS Comput Biol. 2013; 9:e1003156. [PubMed: 23935474]
- Burke JE, Karbarz MJ, Deems RA, Li S, Woods VL Jr, Dennis EA. Interaction of group IA phospholipase A_2 with metal ions and phospholipid vesicles probed with deuterium exchange mass spectrometry. Biochemistry. 2008; 47:6451–6459. [PubMed: 18500818]
- Cao J, Burke J, Dennis E. Using hydrogen/deuterium exchange mass spectrometry to define the specific interactions of the phospholipase A_2 superfamily with lipid substrates, inhibitors, and membranes. J Biol Chem. 2013; 288:1806–1813. [PubMed: 23209293]
- Cao J, Hsu YH, Li S, Woods VL, Dennis EA. Lipoprotein-associated phospholipase A_2 interacts with phospholipid vesicles via a surface-disposed hydrophobic alpha-helix. Biochemistry. 2011; 50:5314–5321. [PubMed: 21553808]
- Cao J, Hsu YH, Li S, Woods VL Jr, Dennis EA. Structural basis of specific interactions of Lp-PLA $_2$ with HDL revealed by hydrogen deuterium exchange mass spectrometry. J Lipid Res. 2013; 54:127–133. [PubMed: 23089916]
- Carman G, Deems R, Dennis E. Lipid signaling enzymes and surface dilution kinetics. J Biol Chem. 1995; 270:18711–18714. [PubMed: 7642515]
- Channon JY, Leslie CC. A calcium-dependent mechanism for associating a soluble arachidonoyl-hydrolyzing phospholipase A_2 with membrane in the macrophage cell line RAW 264.7. J Biol Chem. 1990; 265:5409–5413. [PubMed: 2108137]
- Clark JD, Lin LL, Kriz RW, Ramesha CS, Sultzman LA, Lin AY, Milona N, Knopf JL. A novel arachidonic acid-selective cytosolic PLA $_2$ contains a Ca^{2+} -dependent translocation domain with homology to PKC and GAP. Cell. 1991; 65:1043–1051. [PubMed: 1904318]
- Davidson FF, Dennis EA. Amino acid sequence and circular dichroism of Indian cobra (*Naja naja*) venom acidic phospholipase A_2 . Biochim Biophys Acta. 1990a; 1037:7–15. [PubMed: 2294972]
- Davidson FF, Dennis EA. Evolutionary relationships and implications for the regulation of phospholipase A_2 from snake venom to human secreted forms. J Mol Evol. 1990b; 31:228–238. [PubMed: 2120459]
- Deems RA, Eaton BR, Dennis EA. Kinetic analysis of phospholipase A_2 activity toward mixed micelles and its implications for the study of lipolytic enzymes. J Biol Chem. 1975; 250:9013–9020. [PubMed: 1194274]
- Dennis E, Cao J, Hsu YH, Magrioti V, Kokotos G. Phospholipase A_2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. Chem Rev. 2011; 111:6130–6185. [PubMed: 21910409]

- Dennis EA. Diversity of group types, regulation, and function of phospholipase A₂. *J Biol Chem*. 1994; 269:13057–13060. [PubMed: 8175726]
- Dennis EA. Liberating Chiral Lipid Mediators, Inflammatory Enzymes, and LIPID MAPS from Biological Grease. *J Biol Chem*. 2016; 291:24431–24448. [PubMed: 27555328]
- Dennis EA, Norris PC. Eicosanoid storm in infection and inflammation. *Nat Rev Immunol*. 2015; 15:511–523. [PubMed: 26139350]
- Dessen A, Tang J, Schmidt H, Stahl M, Clark J, Seehra J, Somers W. Crystal structure of human cytosolic phospholipase A₂ reveals a novel topology and catalytic mechanism. *Cell*. 1999; 97:349–360. [PubMed: 10319815]
- Edwards SH, Thompson D, Baker SF, Wood SP, Wilton DC. The crystal structure of the H48Q active site mutant of human group IIA secreted phospholipase A₂ at 1.5 Å resolution provides an insight into the catalytic mechanism. *Biochemistry*. 2002; 41:15468–15476. [PubMed: 12501175]
- Fremont DH, Anderson DH, Wilson IA, Dennis EA, Xuong NH. Crystal structure of phospholipase A₂ from indian cobra reveals a trimeric association. *Proc Natl Acad Sci U S A*. 1993; 90:342–346. [PubMed: 8419939]
- Grkovich A, Dennis EA. Phosphatidic acid phosphohydrolase in the regulation of inflammatory signaling. *Adv Enzyme Regul*. 2009; 49:114–120. [PubMed: 19534028]
- Kienesberger PC, Oberer M, Lass A, Zechner R. Mammalian patatin domain containing proteins: a family with diverse lipolytic activities involved in multiple biological functions. *J Lipid Res*. 2009; 50(Suppl):S63–68. [PubMed: 19029121]
- Kokotou MG, Galiatsatou G, Magrioti V, Koutoulougenis G, Barbayianni E, Limnios D, Mouchlis VD, Satpathy B, Navratil A, Dennis EA, Kokotos G. 2-Oxoesters: A Novel Class of Potent and Selective Inhibitors of Cytosolic Group IVA Phospholipase A₂. *Sci Rep*. 2017; 7:7025. [PubMed: 28765606]
- Kramer RM, Hession C, Johansen B, Hayes G, McGray P, Chow EP, Tizard R, Pepinsky RB. Structure and properties of a human non-pancreatic phospholipase A₂. *J Biol Chem*. 1989; 264:5768–5775. [PubMed: 2925633]
- Kramer RM, Roberts EF, Manetta J, Putnam JE. The Ca²⁺-sensitive cytosolic phospholipase A₂ is a 100-kDa protein in human monoblast U937 cells. *J Biol Chem*. 1991; 266:5268–5272. [PubMed: 2002059]
- Larsson Forsell PK, Kennedy BP, Claesson HE. The human calcium-independent phospholipase A₂ gene multiple enzymes with distinct properties from a single gene. *Eur J Biochem*. 1999; 262:575–585. [PubMed: 10336645]
- Leslie CC, Voelker DR, Channon JY, Wall MM, Zelarney PT. Properties and purification of an arachidonoyl-hydrolyzing phospholipase A₂ from a macrophage cell line, RAW 264.7. *Biochim Biophys Acta*. 1988; 963:476–492. [PubMed: 3143418]
- Lio YC, Dennis EA. Interfacial activation, lysophospholipase and transacylase activity of group VI Ca²⁺-independent phospholipase A₂. *Biochim Biophys Acta*. 1998; 1392:320–332. [PubMed: 9630702]
- Ma Z, Wang X, Nowatzke W, Ramanadham S, Turk JC. Human pancreatic islets express mRNA species encoding two distinct catalytically active isoforms of group VI phospholipase A₂ (iPLA₂) that arise from an exon-skipping mechanism of alternative splicing of the transcript from the iPLA₂ gene on chromosome 22q13.1. *J Biol Chem*. 1999; 274:9607–9616. [PubMed: 10092647]
- Manya H, Aoki J, Kato H, Ishii J, Hino S, Arai H, Inoue K. Biochemical characterization of various catalytic complexes of the brain platelet-activating factor acetylhydrolase. *J Biol Chem*. 1999; 274:31827–31832. [PubMed: 10542206]
- Michael P, Tomchick D, Machius M, Anderson R. Crystal structure of a 12 ANK repeat stack from human ankyrinR. *EMBO J*. 2002; 21:6387–6483. [PubMed: 12456646]
- Min JH, Jain MK, Wilder C, Paul L, Apitz-Castro R, Aspleaf DC, Gelb MH. Membrane-bound plasma platelet activating factor acetylhydrolase acts on substrate in the aqueous phase. *Biochemistry*. 1999; 38:12935–12942. [PubMed: 10504265]
- Mosior M, Six DA, Dennis EA. Group IV cytosolic phospholipase A₂ binds with high affinity and specificity to phosphatidylinositol 4,5-bisphosphate resulting in dramatic increases in activity. *J Biol Chem*. 1998; 273:2184–2191. [PubMed: 9442060]

- Mouchlis VD, Barbayianni E, Mavromoustakos TM, Kokotos G. The application of rational design on phospholipase A₂ inhibitors. *Curr Med Chem*. 2011a; 18:2566–2582. [PubMed: 21568891]
- Mouchlis VD, Bucher D, McCammon JA, Dennis EA. Membranes serve as allosteric activators of phospholipase A₂, enabling it to extract, bind, and hydrolyze phospholipid substrates. *PNAS*. 2015; 112:E516–E525. [PubMed: 25624474]
- Mouchlis VD, Dennis EA. Membrane and inhibitor interactions of intracellular phospholipases A₂. *Adv Biol Regul*. 2016; 61:17–24. [PubMed: 26774606]
- Mouchlis VD, Limnios D, Kokotou MG, Barbayianni E, Kokotos G, McCammon JA, Dennis EA. Development of potent and selective inhibitors for group VIA calcium-independent phospholipase A₂ guided by molecular dynamics and structure-activity relationships. *J Med Chem*. 2016; 59:4403–4414. [PubMed: 27087127]
- Mouchlis VD, Magrioti V, Barbayianni E, Cermak N, Oslund RC, Mavromoustakos TM, Gelb MH, Kokotos G. Inhibition of secreted phospholipases A₂ by 2-oxoamides based on alpha-amino acids: Synthesis, in vitro evaluation and molecular docking calculations. *Bioorg Med Chem*. 2011b; 19:735–743. [PubMed: 21216150]
- Mouchlis VD, Mavromoustakos TM, Kokotos G. Design of new secreted phospholipase A₂ inhibitors based on docking calculations by modifying the pharmacophore segments of the FPL67047XX inhibitor. *J Comput-Aided Mol Des*. 2010a; 24:107–115. [PubMed: 20130961]
- Mouchlis VD, Mavromoustakos TM, Kokotos G. Molecular docking and 3D-QSAR CoMFA studies on indole inhibitors of GIIA secreted phospholipase A₂. *J Chem Inf Model*. 2010b; 50:1589–1601. [PubMed: 20795712]
- Mouchlis VD, Michopoulou V, Constantinou-Kokotou V, Mavromoustakos T, Dennis EA, Kokotos G. Binding conformation of 2-oxoamide inhibitors to group IVA cytosolic phospholipase A₂ determined by molecular docking combined with molecular dynamics. *J Chem Inf Model*. 2012; 52:243–254. [PubMed: 22196172]
- Mouchlis VD, Morisseau C, Hammock BD, Li S, McCammon JA, Dennis EA. Computer-aided drug design guided by hydrogen/deuterium exchange mass spectrometry: A powerful combination for the development of potent and selective inhibitors of Group VIA calcium-independent phospholipase A₂. *Bioorg Med Chem*. 2016; 24:4801–4811. [PubMed: 27320659]
- Nakamura H, Hirabayashi T, Shimizu M, Murayama T. Ceramide-1-phosphate activates cytosolic phospholipase A₂α directly and by PKC pathway. *Biochem Pharmacol*. 2006; 71:850–857. [PubMed: 16443193]
- Pickard RT, Chiou XG, Striffler BA, DeFelippis MR, Hyslop PA, Tebbe AL, Yee YK, Reynolds LJ, Dennis EA, Kramer RM, Sharp JD. Identification of essential residues for the catalytic function of 85-kDa cytosolic phospholipase A₂. Probing the role of histidine, aspartic acid, cysteine, and arginine. *J Biol Chem*. 1996; 271:19225–19231. [PubMed: 8702602]
- Prescott SM, Zimmerman GA, Stafforini DM, McIntyre TM. Platelet-activating factor and related lipid mediators. *Annu Rev Biochem*. 2000; 69:419–445. [PubMed: 10966465]
- Reynolds LJ, Washburn WN, Deems RA, Dennis EA. Assay strategies and methods for phospholipases. *Methods Enzymol*. 1991; 197:3–23.
- Samanta U, Bahnson BJ. Crystal structure of human plasma platelet-activating factor acetylhydrolase: structural implication to lipoprotein binding and catalysis. *J Biol Chem*. 2008; 283:31617–31624. [PubMed: 18784071]
- Samanta U, Kirby SD, Srinivasan P, Cerasoli DM, Bahnson BJ. Crystal structures of human group-VIIA phospholipase A₂ inhibited by organophosphorus nerve agents exhibit non-aged complexes. *Biochem Pharmacol*. 2009; 78:420–429. [PubMed: 19394314]
- Schaloske RH, Dennis EA. The phospholipase A₂ superfamily and its group numbering system. *Biochim Biophys Acta*. 2006; 1761:1246–1259. [PubMed: 16973413]
- Seilhamer JJ, Pruzanski W, Vadas P, Plant S, Miller JA, Kloss J, Johnson LK. Cloning and recombinant expression of phospholipase A₂ present in rheumatoid arthritic synovial fluid. *J Biol Chem*. 1989; 264:5335–5338. [PubMed: 2925608]
- Stafforini DM, McIntyre TM, Zimmerman GA, Prescott SM. Platelet-activating factor acetylhydrolases. *J Biol Chem*. 1997; 272:17895–17898. [PubMed: 9218411]

- Stafforini DM, Sheller JR, Blackwell TS, Sapirstein A, Yull FE, McIntyre TM, Bonventre JV, Prescott SM, Roberts LJ 2nd. Release of free F₂-isoprostanes from esterified phospholipids is catalyzed by intracellular and plasma platelet-activating factor acetylhydrolases. *J Biol Chem.* 2006; 281:4616–4623. [PubMed: 16371369]
- Stremmler KE, Stafforini DM, Prescott SM, McIntyre TM. Human plasma platelet-activating factor acetylhydrolase. Oxidatively fragmented phospholipids as substrates. *J Biol Chem.* 1991; 266:11095–11103. [PubMed: 2040620]
- Stremmler KE, Stafforini DM, Prescott SM, Zimmerman GA, McIntyre TM. An oxidized derivative of phosphatidylcholine is a substrate for the platelet-activating factor acetylhydrolase from human plasma. *J Biol Chem.* 1989; 264:5331–5334. [PubMed: 2494162]
- Tamiya-Koizumi K, Umekawa H, Yoshida S, Ishihara H, Kojima K. A novel phospholipase A₂ associated with nuclear matrix: stimulation of the activity and modulation of the Ca²⁺ dependency by polyphosphoinositides. *Biochim Biophys Acta.* 1989; 1002:182–188. [PubMed: 2539188]
- Tellis CC, Tselepis AD. The role of lipoprotein-associated phospholipase A₂ in atherosclerosis may depend on its lipoprotein carrier in plasma. *Biochim Biophys Acta.* 2009; 1791:327–338. [PubMed: 19272461]
- Tjoelker LW, Eberhardt C, Unger J, Trong HL, Zimmerman GA, McIntyre TM, Stafforini DM, Prescott SM, Gray PW. Plasma platelet-activating factor acetylhydrolase is a secreted phospholipase A₂ with a catalytic triad. *J Biol Chem.* 1995a; 270:25481–25487. [PubMed: 7592717]
- Tjoelker LW, Wilder C, Eberhardt C, Stafforini DM, Dietsch G, Schimpf B, Hooper S, Le Trong H, Cousens LS, Zimmerman GA, Yamada Y, McIntyre TM, Prescott SM, Gray PW. Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. *Nature.* 1995b; 374:549–553. [PubMed: 7700381]
- Wilson PA, Gardner SD, Lambie NM, Commans SA, Crowther DJ. Characterization of the human patatin-like phospholipase family. *J Lipid Res.* 2006; 47:1940–1949. [PubMed: 16799181]

Figure 1. Phospholipases A₂ function as degradative enzymes when they produce lysophospholipids and free fatty acids as products, as biosynthetic enzymes when the lysophospholipid product is reacylated with a PUFA to produce important phospholipids, and as signaling enzymes when the products are converted to agonists of GPCRs (adapted from (Dennis, 2016)).

Figure 2. A model of GVIIA Lp-PLA₂ binding to the surface of a DMPC membrane
The proposed membrane binding region of Lp-PLA₂ is shown in blue (amides 113–120).
The proposed region for liposome association (amides 115 and 116) as well as the catalytic triad (Ser273, Asp296, and His351) are shown in red (adapted from (Cao et al., 2011)).

Figure 3. Three-dimensional structure of calcium-independent phospholipase A₂
 A homology model of GVIA iPLA₂ is shown (adapted from (Mouchlis et al., 2015)).

Figure 4. Three-dimensional structure of cytosolic phospholipase A₂

The X-ray crystal structure of GIVA cPLA₂ (PDB ID 1CJY) (adapted from (Mouchlis et al., 2015)).

Figure 5. Hypothetical model of GIA sPLA2 before (left) binding to the surface of a DMPC membrane and after (right)

Areas with decreases in deuterium exchange are colored in blue and turquoise (adapted from (Burke et al., 2008)).

Table 1
Phospholipase A₂ superfamily

(adapted from Dennis et al, 2011).

type	group	subgroup	molecular mass (kPa)	catalytic residues
sPLA ₂	GI	A, B	13–15	His/Asp
	GII	A, B, C, D, E, F	13–17	
	GIII		15–18	
	GV		14	
	GIX		14	
	GX		14	
	GXI		13	
	GXII	A, B	19	
	GXIII	A, B	<10	
cPLA ₂	GXIV		13–19	Ser/Asp
	GIV	A(α), B(β), C(γ), D(δ), E(ϵ), F(ζ)	60–114	
iPLA ₂	GVI	A(β), B(γ), C(δ), D(ϵ), E(ζ), F(η)	84–90	Ser/Asp
PAF-AH	GVII	A (Lp-PLA ₂), B(PAF-AH II)	40–45	Ser/Asp/His
	GVIII	A(α_1), B(α_2), β	26–40	
LPLA ₂	GXV		45	Ser/Asp/His
AdPLA ₂	GXVI		18	His/Cys