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Journal

Accounts of Chemical Research, 55(23)

ISSN

0001-4842

Authors

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Publication Date

2022-12-06

DOI

10.1021/acs.accounts.2c00497

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Membrane Association Allosterically Regulates Phospholipase A₂ Enzymes and Their Specificity

Published as part of the *Accounts of Chemical Research* special issue “*Chemical Biology of Lipids*”.

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Cite This: *Acc. Chem. Res.* 2022, 55, 3303–3311



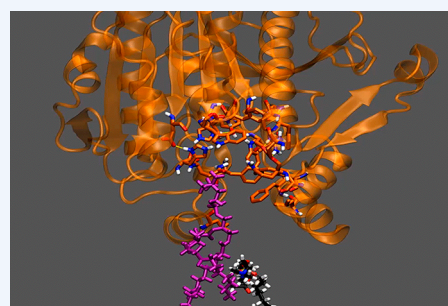
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CONSPECTUS: Water-soluble proteins as well as membrane-bound proteins associate with membrane surfaces and bind specific lipid molecules in specific sites on the protein. Membrane surfaces include the traditional bilayer membranes of cells and subcellular organelles formed by phospholipids. Monolayer membranes include the outer monolayer phospholipid surface of intracellular lipid droplets of triglycerides and various lipoproteins including HDL, LDL, VLDL, and chylomicrons. These lipoproteins circulate in our blood and lymph systems and contain triglycerides, cholesterol, cholesterol esters, and proteins in their interior, and these are sometimes interspersed on their surfaces. Similar lipid–water interfaces also occur in mixed micelles of phospholipids and bile acids in our digestive system, which may also include internalized triglycerides and cholesterol esters. Diacyl phospholipids constitute the defining molecules of biological membranes. Phospholipase A₁ (PLA₁) hydrolyzes phospholipid acyl chains at the *sn*-1 position of membrane phospholipids, phospholipase A₂ (PLA₂) hydrolyzes acyl chains at the *sn*-2 position, phospholipase C (PLC) hydrolyzes the glycerol–phosphodiester bond, and phospholipase D (PLD) hydrolyzes the polar group–phosphodiester bond. Of the phospholipases, the PLA₂s have been the most well studied at the mechanistic level. The PLA₂ superfamily consists of 16 groups and numerous subgroups, and each is generally described as one of 6 types. The most well studied of the PLA₂s include extensive genetic and mutational studies, complete lipidomics specificity characterization, and crystallographic structures. This Account will focus principally on results from deuterium exchange mass spectrometric (DXMS) studies of PLA₂ interactions with membranes and extensive molecular dynamics (MD) simulations of their interactions with membranes and specific phospholipids bound in their catalytic and allosteric sites. These enzymes either are membrane-bound or are water-soluble and associate with membranes before extracting their phospholipid substrate molecule into their active site to carry out their enzymatic hydrolytic reaction. We present evidence that when a PLA₂ associates with a membrane, the membrane association can result in a conformational change in the enzyme whereby the membrane association with an allosteric site on the enzyme stabilizes the enzyme in an active conformation on the membrane. We sometimes refer to this transition from a “closed” conformation in aqueous solution to an “open” conformation when associated with a membrane. The enzyme can then extract a single phospholipid substrate into its active site, and catalysis occurs. We have also employed DXMS and MD simulations to characterize how PLA₂s interact with specific inhibitors that could lead to potential therapeutics. The PLA₂s constitute a paradigm for how membranes interact allosterically with proteins, causing conformational changes and activation of the proteins to enable them to extract and bind a specific phospholipid from a membrane for catalysis, which is probably generalizable to intracellular and extracellular transport and phospholipid exchange processes as well as other specific biological functions. We will focus on the four main types of PLA₂, namely, the secreted (sPLA₂), cytosolic (cPLA₂), calcium-independent (iPLA₂), and lipoprotein-associated PLA₂ (Lp-PLA₂) also known as platelet-activating factor acetyl hydrolase (PAF-AH). Studies on a well-studied specific example of each of the four major types of the PLA₂ superfamily demonstrate clearly that protein subsites can show precise specificity for one of the phospholipid hydrophobic acyl chains, often the one at the *sn*-2 position, including exquisite sensitivity to the number and position of double bonds.

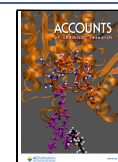


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Received: July 26, 2022

Published: October 31, 2022



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- Mouchlis, V. D.; Hayashi, D.; Vasquez, A. M.; Cao, J.; McCammon, J. A.; Dennis, E. A. Lipoprotein-associated phospholipase A₂: A paradigm for allosteric regulation by membranes. *Proc. Natl. Acad. Sci. U.S.A.* **2022**, *119*, e2102953118.⁴ Demonstration of the conformational change that results from the association of lipoprotein-associated phospholipase A₂ with a membrane and the correlation of lipidomics-determined specificity with sn-2 hydrophobic subsite geometry.

■ INTRODUCTION TO THE PHOSPHOLIPASE A₂ SUPERFAMILY

The most well characterized phospholipase A₂ (PLA₂) enzymes are water-soluble peripheral enzymes that associate with the surface of membranes to extract and bind their phospholipid substrates.^{5,6} However, there are some PLA₂s that are membrane-bound but they have been less well studied, and this Account will focus on the most well studied water-soluble PLA₂s. To access its substrate, a PLA₂ enzyme needs to overcome the lipid/water interface barrier.^{5,6} As a result, PLA₂ enzymes evolved to contain unique interfacial surfaces that contain amphipathic helices facilitating the association process. The association of a PLA₂ with the membrane causes a conformational change in the protein at an allosteric site distinct from its active site.¹ In addition, each enzyme has a unique active site that is either highly specific for a fatty acid at the sn-2 position or more permissive depending on the cellular processes with which the enzyme is involved.² In fact, the PLA₂s have evolved very specific geometries at their specific hydrophobic site, which binds the fatty acyl group at the sn-2 position to result in highly specific substrate specificity for their biological function as determined by lipidomics analysis.^{3,4}

Since PLA₂ enzymes catalyze hydrolysis at the sn-2 position, the type of fatty acid at the sn-1 position is not expected to play a significant role in their activity. However, in some cases it seems that the ester bond at the sn-1 position is preferred versus the ether bond of the platelet activating factor (PAF) or vinyl ethers of plasmalogens.⁷ Hydrogen–deuterium exchange mass spectrometry (HDX-MS) is a powerful biophysical technique that has been used extensively to study the interactions of PLA₂ enzymes with membranes and vesicles.⁸ Molecular dynamics simulations guided by HDX-MS results have proven to be very useful in understanding the association and inhibition

mechanisms of PLA₂ enzymes and in generating enzyme–inhibitor complexes for designing new inhibitors with improved properties.⁹

The PLA₂ superfamily consists of 16 groups and many subgroups of structurally and functionally diverse enzymes.¹⁰ The six types of PLA₂ enzymes include the secreted (sPLA₂), cytosolic (cPLA₂), calcium-independent (iPLA₂), and lipoprotein-associated PLA₂ (Lp-PLA₂) also known as platelet-activating factor acetylhydrolase (PAF-AH), lysosomal PLA₂, and adipose-PLA (AdPLA).^{6,10} Numerous important functions are attributed to the PLA₂ superfamily as summarized in Table 1.⁶ Chief among them is the important role of PLA₂ in

Table 1. Functions of the Human PLA₂ Superfamily^a

type	major groups	major functions
sPLA ₂	groups IB, IIA, IID, IIE, V, X, XIIA	digestion, antibacterial, antiviral, tumorigenesis, pro-inflammatory, anti-inflammatory
cPLA ₂	group IVA (cPLA ₂ α)	pro-inflammatory
iPLA ₂	group VIA (iPLA ₂ β)	remodeling of sn-2 fatty acids, insulin sensitivity, mitochondrial function (Barth syndrome)
LpPLA ₂	group VIIA (PAF-AH)	anti-inflammatory, pro-inflammatory
LPLA ₂		lysosomal phospholipid hydrolysis
AdPLA		phospholipid hydrolysis in adipose tissue

^aAdapted with permission from ref 6. Copyright 2011 American Chemical Society.

generating inflammatory mediators, especially cPLA₂, for which the group IVA cPLA₂ shows dramatic specificity for the release of free eicosatetraenoic acid known as arachidonic acid (AA) from the sn-2 position of membrane phospholipids. Arachidonic acid is an omega-6 fatty acid which is generally considered to be pro-inflammatory.¹¹

The AA is further converted by cyclooxygenases (COX) to various tissue-specific prostaglandins that play potent cell signaling and pro-inflammatory roles by binding to specific G-protein coupled receptors (GPCRs) and activating various peroxisome-proliferator activated receptors (PPARs).¹¹ In certain tissues, the AA is converted enzymatically to various HETEs and leukotrienes by various lipoxygenases, which activate other GPCRs and PPARs. We will discuss the specificity of the various types/groups of PLA₂ at the molecular level herein. Of special current interest is determining which PLA₂ types (and specific groups and subgroups) are responsible for releasing the pro-inflammatory mediator arachidonic acid versus those PLA₂s that favor the omega-3 fish oil-derived fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are often precursors of anti-inflammatory or pro-resolution lipid mediators.¹¹ We recently determined that while GIVA cPLA₂ prefers the omega-6 pro-inflammatory AA as a substrate in the sn-2 position of its phospholipid substrates, omega-3 fatty acid docosahexaenoic acid (DHA), which is generally considered to be anti-inflammatory or pro-resolution, is preferred by group V sPLA₂.³ The functional pro- and anti-inflammatory activities and other functions in specific tissues and cells of each of the sPLA₂s are reviewed elsewhere, including pro-inflammatory properties of GIV cPLA₂ and anti-inflammatory properties of GIID sPLA₂.¹²

In this Account, we will summarize several studies that reveal the association mechanism of group IVA cytosolic (cPLA₂), group VIA calcium-independent (iPLA₂), group V secreted

(sPLA₂), and group VIIA lipoprotein-associated phospholipase A₂ (GVIIA Lp-PLA₂), also known as platelet-activating factor (PAF) acetylhydrolase (PAF-AH) with membranes. Our studies revealed that these enzymes are allosterically regulated by membranes through their interfacial surface. Substrate specificity studies showed that each enzyme contains a distinct binding pocket for the *sn*-2 fatty acid with amino acid side chains that make it specific for a type of fatty acid. Inhibitor studies suggested that these binding sites could be used to achieve inhibition selectivity.

MEMBRANES INDUCE ALLOSTERIC CONFORMATIONAL CHANGES IN ASSOCIATED PROTEINS

PLA₂ enzymes need to associate with an aggregated form of their phospholipid substrate including bilayer membranes and phospholipid vesicles as well as phospholipid monolayers surrounding lipoproteins, lipid droplets, liposomes, and mixed micelles (the subject of a previous Account)¹³ in order to extract and bind a single phospholipid molecule.⁸ “Surface-dilution kinetics” was successfully employed to interpret the kinetics of PLA₂s.^{14,15} It proposed that the enzymes first undergo surface association to the membranes depending on the bulk concentration of enzyme and membranes. In a second distinct step, the enzyme pulls a phospholipid substrate into its catalytic site, which depends on the substrate phospholipid concentration in the two-dimensional interface. Each enzyme contains an interfacial surface, which has unique topology and function, and it facilitates the association with the membrane or any other form of lipid aggregate. A combination of HDX-MS with molecular dynamics simulations has proven to be very successful in understanding the association mechanism of the pure, recombinant human form of specific group/subgroups GIVA cPLA₂, GV sPLA₂, GVIA iPLA₂, and GVIIA Lp-PLA₂. For simplicity, in this Account, we will refer to these enzymes as cPLA₂, sPLA₂, iPLA₂, and Lp-PLA₂. These studies showed that PLA₂ enzymes exist in at least three conformations including the “closed” conformation in water (E), the “open” membrane associated “unbound” conformation (E·M), and the “open” membrane-associated “bound” conformation (ES·M) after the extraction of the phospholipid molecule in the active site (Figure 1).² In this section, we will discuss the mechanism of the association of PLA₂s with the membrane through their interfacial surface and its associated interactions.

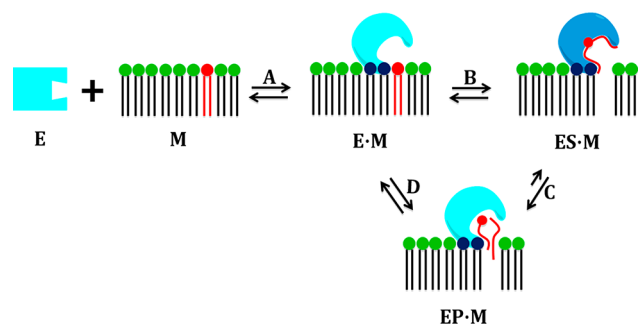


Figure 1. Catalytic cycle of PLA₂ enzymes. The association of each enzyme with the membrane causes an allosteric conformational change that enables it to extract and bind a phospholipid molecule. Reproduced with permission from ref 2. Copyright 2018 American Chemical Society.

HDX-MS studies on the iPLA₂ enzyme in the presence of phospholipid vesicles led to the identification of the peptide regions of the enzyme that interact with the membrane.¹⁶ Among them, peptide region 708–730 showed a significant decrease in deuteration levels in the presence of phospholipid vesicles. Coarse-grained and atomistic molecular dynamics were successful in understanding the association of iPLA₂ with a lipid bilayer.¹⁷ The proposed model suggested that the peptide region 708–730, which is close to the active site, penetrates the lipid/water interface and forms an amphipathic helix with its hydrophilic residues (Arg710 and Lys719) interacting with the headgroups of the fatty acids and its hydrophobic residues (Pro711, Pro714, Trp715, Leu717, Val721, and Phe722) interacting with the acyl chains of the fatty acids (Figure 2A).¹ It is worth mentioning that homology modeling and later the crystal structure revealed an ankyrin repeat region along with the catalytic domain of iPLA₂ that did not show any interactions with the membrane bilayer.^{1,18}

Similar HDX-MS studies were performed on cPLA₂ to identify the peptide regions of the interfacial surface that interact with the membrane. The cPLA₂ structure contains a C2 domain along with the catalytic domain that was thought to assist the association of the enzyme with the membrane through its calcium binding site.^{19,20} The HDX-MS studies showed several peptide regions on the C2 domain and the catalytic domain exhibiting decreases in deuteration levels in the presence of phospholipid vesicles.²¹ According to our model, two regions of the C2 domain (residues 35–39 and 96–98) penetrate the membrane approximately 15 Å, bringing along the catalytic domain to near the membrane surface. Residues 268–279 and 466–470 are two peptide regions of the catalytic domain that are close to the surface of the membrane containing some hydrophobic residues (Trp464 and Met468) interacting with the fatty acid tails of the phospholipids and some hydrophilic residues (Lys273, Lys274, and Arg467) interacting with the headgroups of the phospholipids (Figure 2B).¹

As with the iPLA₂ and cPLA₂ enzymes, sPLA₂ also exhibits an increase in activity due to interfacial activation in the presence of a membrane or any other form of lipid aggregates.^{22,23} HDX-MS studies on group IA sPLA₂ showed that peptide regions 3–8, 18–21, and 56–64 exhibited a significant decrease in the “on-exchange” rates in the presence of dimyristoylphosphatidylcholine phospholipid vesicles. These peptide regions have an amphipathic nature containing hydrophobic residues that penetrate the membrane and hydrophilic residues that interact with the headgroups on the surface of the membrane.²⁴ Along with GIA sPLA₂, we were also interested in the human GV sPLA₂, and this later enzyme was used in our detailed substrate specificity studies. Since there is no crystal structure available for GV sPLA₂, a homology model was generated based on the crystal structure of GIIA sPLA₂ because these two enzymes have 47% identity and 61% homology. In general, there is high homology between sPLA₂ enzymes, and that allowed us to use the HDX-MS peptide regions to generate a structural model for GV sPLA₂ docked on the surface of the membrane (Figure 2C). This model was subjected to extensive molecular dynamics simulations in the presence of various phospholipid substrates. These studies allowed us to determine the structural features that contribute to substrate selectivity, and they will be discussed in detail in the next section of this Account.

Lp-PLA₂ is also membrane-associated and undergoes interfacial activation like c-, i-, and sPLA₂ enzymes. According to our HDX-MS studies, peptide regions 114–120 and 360–

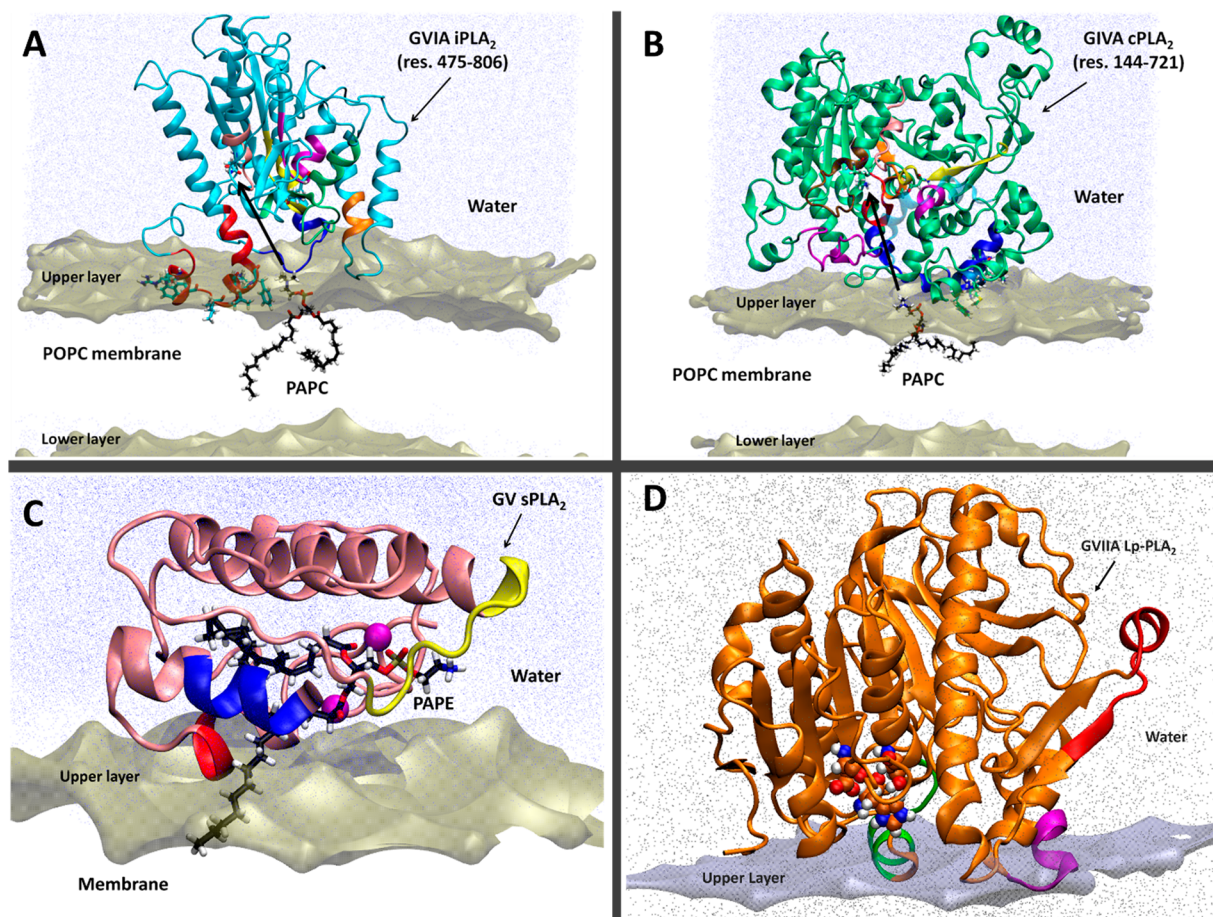


Figure 2. Association of each PLA₂ enzyme with the membrane bilayer through the interfacial surface. (A) iPLA₂, (B) cPLA₂, (C) sPLA₂, and (D) Lp-PLA₂. Adapted with permission from refs 2 and 4. Copyright 2018 American Chemical Society and copyright 2022 National Academy of Sciences, respectively.

368 exhibited decreases in on-exchange rates in the presence of phospholipid vesicles (Figure 2D). These two regions constitute two amphipathic helices that play a significant role in protein–membrane binding because their properties allow them to overcome the water–lipid energy barrier.^{25–27} An enzyme–membrane model was generated based on the HDX-MS data and was subjected to extensive molecular dynamics simulations to study the interfacial activation mechanism of Lp-PLA₂.⁴ The simulations showed that in the presence of the membrane, the volume of the Lp-PLA₂ active site was increased from ~900 to 2000 Å³ due to a conformational change caused by the region 100–130. When the open form of the enzyme was placed in water, a conformational change in the same region caused the enzyme to adopt a closed form.⁴ Similar conformational changes were confirmed for c-, i-, and sPLA₂.²

SPECIFIC PHOSPHOLIPASE A₂ BINDING SITES EXTRACT AND BIND A SPECIFIC PHOSPHOLIPID MOLECULE AND DETERMINE THE PHOSPHOLIPID SUBSTRATE SPECIFICITY

Phospholipids are highly flexible molecules and are often very challenging to cocrystallize with PLA₂ enzymes. Thus, there are no available crystal structures to help understand the binding mode and interactions of phospholipids with the active site of PLA₂ enzymes. Computational methods such as molecular docking and induced-fit docking could not handle the high flexibility of phospholipids.²⁸ Atomistic molecular dynamics at a

microsecond scale have proven successful in developing enzyme–substrate models. These models help understand the binding mode and interactions of phospholipids with each PLA₂ active site and explain each enzyme's specificity for various types of phospholipid molecules. Simulations were carried out for c-, i-, s-, and Lp-PLA₂ enzymes with phospholipids containing various types of fatty acids at the *sn*-2 position.^{1,2,4} Arachidonic (20:4), linoleic (18:2), myristic (14:0), and azeloyl (9:0, COOH) esterified at the *sn*-2 position of membrane phospholipids will be discussed in this Account because they are the optimum substrates for cPLA₂, i and sPLA₂, and Lp-PLA₂, respectively.

The active site of cPLA₂ contains a deep channel containing a large hydrophobic binding pocket that accommodates the two acyl chains of a phospholipid molecule. Enzymatic specificity studies on cPLA₂ showed that this enzyme is highly specific for arachidonic acid at the *sn*-2 position. The simulations showed that cPLA₂ contains a hydrophobic pocket that is rich with aromatic residues including Phe199, Phe291, Phe295, Trp232, Phe397, Phe401, Phe681, Phe683, and Tyr685 that act as a fingerprint recognizing and interacting with the double bonds of arachidonic acid (20:4) through π – π stacking (Figure 3A).² iPLA₂ exhibited optimum activity for phospholipids containing linoleic (18:2) and myristic (14:0) acid at the *sn*-2 position. The simulations showed that iPLA₂ contains two hydrophobic binding pockets for the *sn*-2 fatty acid tail: one that could accommodate myristic (14:0) (not shown) and a second that

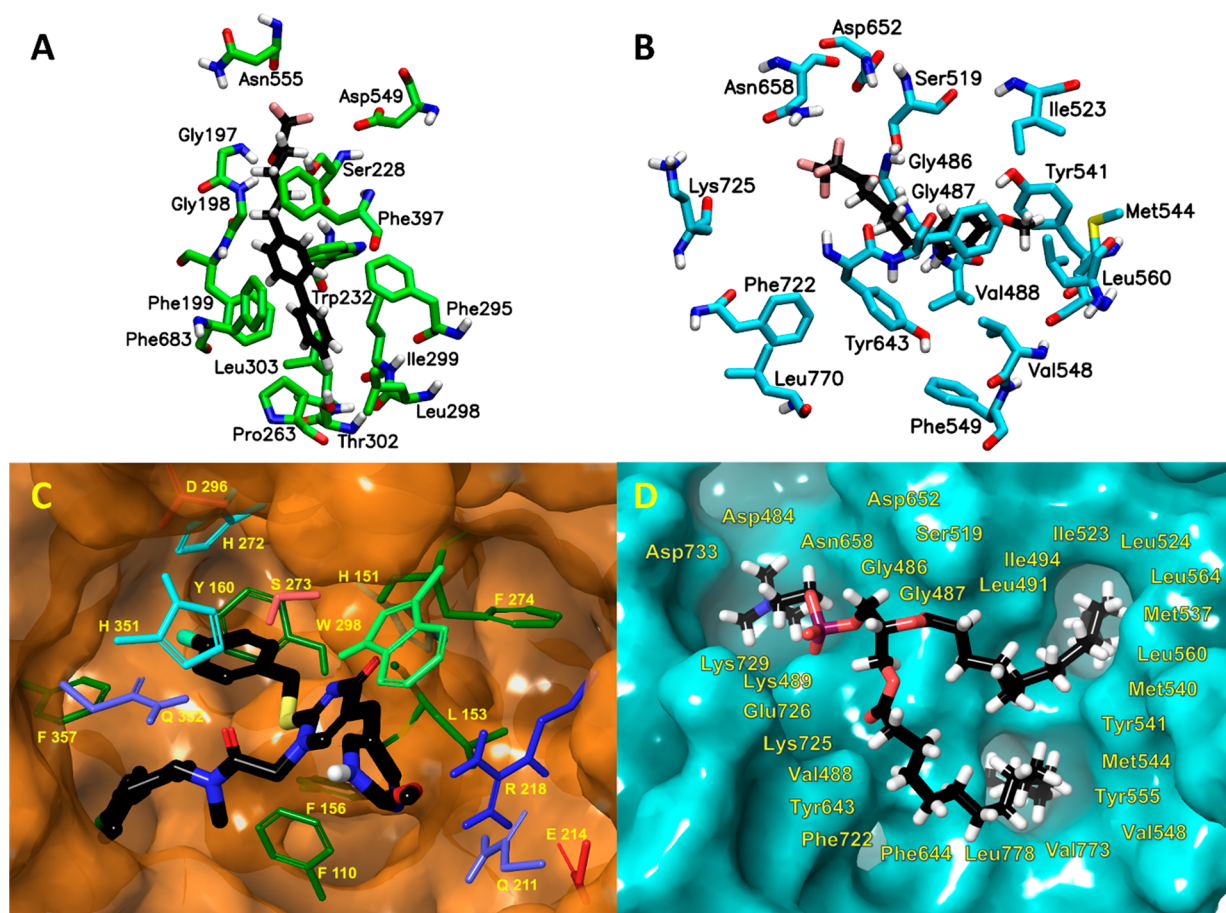


Figure 4. Specificity of each phospholipase A₂ hydrophobic binding site for specific substrates and inhibitors. Binding mode of fluoroketone inhibitors in the active sites of (A) cPLA₂ and (B) iPLA₂. (C) Binding mode of a potent specific inhibitor in the active site of Lp-PLA₂. (D) Binding mode of a phospholipid containing myristic acid at the *sn*-2 position in the active site of iPLA₂. Adapted with permission from refs 2, 4, and 31. Copyright 2018 American Chemical Society, copyright 2022 National Academy of Sciences, and copyright 2016 American Chemical Society, respectively.

accommodates the *sn*-2 arachidonic tail. This pocket contains many aromatic residues that interact with the double bonds of the arachidonic tail. There are two factors that govern tight binding in this hydrophobic pocket: the size of the ligand and its aromaticity. Fluoroketone compounds that contain longer aromatic hydrophobic tails tend to be potent inhibitors for cPLA₂ (Figure 4A). Such compounds contain large enough tails to complement the hydrophobic pocket of cPLA₂ and at the same time form π - π stacking with aromatic residues such as Phe199, Phe291, Phe295, Trp232, Phe397, Phe401, Phe681, Phe683, and Tyr685. Larger compounds such as oxoamides containing long aliphatic or aromatic tails and are also potent and selective inhibitors of cPLA₂.^{34–36}

The hydrophobic part of the iPLA₂ active site consist of two pockets: one that contains aromatic residues and accommodates the *sn*-2 linoleic chain and one that contains aliphatic residues and accommodates the *sn*-2 myristic chain.² Fluoroketone compounds that contain shorter aromatic tails tend to be potent and selective inhibitors for iPLA₂. The aromatic pocket of iPLA₂ has a significantly smaller volume than the one in cPLA₂ because the *sn*-2 site in iPLA₂ is much more specific for linoleic acid at the *sn*-2 position. In addition, the active site of iPLA₂ is more flexible, and the inhibitor locks the enzyme in the closed conformation in which the volume of the hydrophobic pocket is significantly smaller (Figure 4B).³¹ Interestingly, very potent inhibitors of Lp-PLA₂ contain aromatic residues that specifically

associate with aromatic residues in the protein active site (Figure 4C).⁴ It is also worth noting that fluoroketone compounds containing only short aliphatic tails are also potent and selective inhibitors for iPLA₂. Molecular dynamics simulations combined with HDX-MS studies showed that the aliphatic tail binds to the smaller aliphatic pocket of iPLA₂ that accommodates the *sn*-2 myristic chain in myristoyl-containing phospholipids (Figure 4D).³²

Our recently developed lipidomic assay for c-, i-, s-, and Lp-PLA₂ enzymes, using existing potent inhibitors for each enzyme, provided a semihigh throughput setup for performing virtual and experimental screening to identify new chemical matter for PLA₂ inhibition.³⁷ Having in mind the above structural characteristics of the c- and iPLA₂ hydrophobic pockets, a virtual screening workflow was developed, including clustering analysis, docking in multiple enzyme conformations, and interaction criteria to select compounds from libraries to be tested *in vitro*.³⁸ This work led to the identification of novel micromolar hits that are currently under development. sPLA₂ also contains an aromatic and an aliphatic hydrophobic pocket, and it is inhibited by indole compounds that contain short aliphatic and aromatic chains.³⁷ Lp-PLA₂ contains a large hydrophobic pocket that accommodates the *sn*-1 acyl chain and a smaller amphipathic pocket that accommodates short-chain oxidized *sn*-2 chains. Compounds that contain a long chain that occupies the *sn*-1 pocket

and a shorter chain that binds the *sn*-2 chain are potent inhibitors for this enzyme (Figure 4C).⁴

Members of the phospholipase A₂ superfamily are responsible for numerous important biological functions, but they have also been implicated in numerous deleterious physiological effects on lipid metabolism with significant disease implications. This includes primary roles for cPLA₂ in arthritis and a multitude of inflammatory diseases, sPLA₂ and Lp-PLA₂ in various cardiovascular diseases, and iPLA₂ in diabetes and a variety of neurological diseases. Over the years, numerous inhibitors with excellent potency and specificity for a particular PLA₂ were synthesized, and the pharmaceutical industry has pursued the development of several as potential therapeutic moieties. This has resulted in many clinical trials, including several large phase 3 trials. Kokotos and co-workers^{39,40} have recently reviewed the patent and clinical trials literature and report that, surprisingly, to date no approved drug has emerged. Perhaps the insights into the importance of the *sn*-2 site for binding potency and specificity discussed in this Account will provide new avenues of focus for the development of successful therapeutic agents.

CONCLUSIONS

Receptors, phospholipid transfer and exchange proteins, and enzymes often exhibit remarkable specificity for specific phospholipids in each of their *sn*-1 acyl chain, *sn*-2 acyl chain, and polar group subsites. It is likely that membranes induce allosteric conformational changes in proteins associated with bilayer membranes both intra- and extracellularly as well as when associated with monolayers surrounding lipoproteins and lipid droplets. These interactions occur at the lipid–water interface of membranes. The interaction of PLA₂ with bilayer membranes and with its specific phospholipid substrate in its active site and especially the specificity for the *sn*-2 fatty acyl chain serve as a general paradigm for the allosteric regulation of proteins by membranes

A recent review⁴¹ focused on the early development of the detailed kinetic concepts, experimental approaches, and results of molecular dynamics simulations including links to the resulting movies, which led to the concept of allosteric regulation by membranes and the demonstration of the importance of the *sn*-2 site for the specificity of PLA₂ enzymes. In the current Account, we have presented an expanded comprehensive general picture of allosteric regulation by membranes and the specificity of hydrophobic subsites in enzymes that act in or on membranes.

This was possible because of the recent demonstration⁴ of the allosteric regulation and specificity of Lp-PLA₂, which complements the earlier work on the three traditional cellular PLA₂s. Of special note is the recent results³ comparing the differential selectivities of *c*-, *s*-, and iPLA₂ for phospholipids containing in the *sn*-2 position the omega-3 fatty acid EPA or DHA compared to the omega-6 fatty acid AA. In total, these experimental and computational approaches allow us to articulate the structural features of each enzyme that contribute to its substrate potency and specificity. By connecting substrate specificity with inhibitor structure–activity relationships, we can now extend the substrate specificity determinations to determine the precise factors contributing to inhibitor potency and selectivity. This information should aid in the development of a general approach to designing novel chemical moieties for PLA₂-selective inhibition.

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Author Contributions

This manuscript was written and edited by both authors, and they have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

Biographies

Varnavas D. Mouchlis is a senior computational chemist at Bristol Myers Squibb working on developing next-generation therapeutics using novel modalities including targeted protein degradation. He was born in Nicosia, Cyprus, and he received his Ph.D. in organic and computational chemistry from the University of Athens, Greece in 2010 with Prof. George Kokotos. He carried out his postdoctoral studies at the University of California, San Diego with Prof. Edward A. Dennis, during which time he also studied with Prof. J. Andrew McCammon. His postdoctoral research focused on understanding protein–membrane, protein–lipid, and protein–inhibitor interactions using computational and experimental methods including molecular dynamics, hydrogen–deuterium exchange mass spectrometry, homology modeling, lipidomics assays, and virtual screening. He successfully developed structural models to carry out virtual and experimental screenings of compound libraries to identify new chemical matter for phospholipase A₂ inhibition. During his postdoctoral studies, he received several awards including the Wiley Computers in Chemistry Outstanding Postdoc Award by the American Chemical Society, Division of Computers in Chemistry. He then moved to Nova Mechanics Ltd. to work on the development of therapeutics for targets involved in CNS diseases including Alzheimer's and Parkinson's. He applied computational methods including virtual screening and molecular dynamics to develop antiaggregation inhibitors for intrinsically disordered proteins. Subsequently at UCB Biosciences, he worked on small-molecule drug discovery projects and new modalities using computer-aided drug design methods including ternary complex modeling, mixed-solvent molecular dynamics to identify cryptic pockets, molecular dynamics on ternary complexes, scaffold hopping, free-energy perturbation, and virtual screening. His goal is to continue to learn and grow into an experienced drug hunter and to develop new therapeutics that will positively impact the lives of patients.

Edward A. Dennis is the Chancellor I Endowed Chair and Distinguished Professor of Chemistry, Biochemistry, Pharmacology, and the Graduate Division at the University of California, San Diego (UCSD). He was born in Chicago, Illinois, received his B.A. from Yale University (1963) and his Ph.D. from Harvard University (1968), and carried out postdoctoral studies at Harvard Medical School (1967–1969). He received a doctorate in medicine (honorary, 2008) from

Goethe University in Frankfurt, Germany and a doctorate (honorary, 2014) from the University of Lyon INSA in Lyon, France. Dr. Dennis started (1970) as an assistant professor at UCSD and served two terms as Chair of the Department of Chemistry and Biochemistry (1999–2002 and 2017–2019). He received a Guggenheim Fellowship for 1983 to 1984, serving as a visiting professor at Harvard Medical School and a visiting scientist at Brandeis University. Dr. Dennis served as a visiting foreign professor at the Collège de France in Paris (2012), as an adjunct professor at The Scripps Research Institute since 1999, and as a visiting research professor at the Université Pierre et Marie Curie since 2010. He also served as Chair and President of the Keystone Symposia (1996–2004), Editor-in-Chief of the *Journal of Lipid Research* (2003–2018), and Director of the LIPID MAPS Lipidomics Consortium (2003–2014). He is an inaugural fellow of the American Association for the Advancement of Science (AAAS) (1984) and an inaugural fellow of the American Society of Biochemistry and Molecular Biology (ASBMB) (2021). He was the recipient of the ASBMB's Avanti Award in Lipid Enzymology (2000), the European Federation for Lipid Science and Technology's European Lipid Science Award (2008), the Yale Medal (2008), the American Chemical Society, San Diego Section, Distinguished Scientist Award (2016), and the ASBMB Bert Vallee Award in Biomedical Science (2021). Prof. Dennis' career research focus has been on the structure, function, mechanism, and inhibition of the enzyme phospholipase A₂ as well as on protein–membrane and protein–lipid interactions, micelle formation, signal transduction, inflammation, lipid metabolism, eicosanoid action, and especially developing the lipidomics field.

ACKNOWLEDGMENTS

Work on this manuscript and research in our laboratory on phospholipase A₂ have been supported by NIGMS MIRA grant R35 GM139641, which is a renewal of RO1 GM20501-44.

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