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Phospholipase A_2 catalysis and lipid mediator lipidomics

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Abstract

Phospholipase A_2 (PLA_2) enzymes are the upstream regulators of the eicosanoid pathway liberating free arachidonic acid from the *sn*−2 position of membrane phospholipids. Increased levels of intracellular arachidonic acid serve as a substrate for the eicosanoid biosynthetic pathway enzymes including cyclooxygenases, lipoxygenases and cytochrome P450s that lead to inflammation. The Group IVA cytosolic ($cPLA_2$), Group VIA calcium-independent ($iPLA_2$), and Group V secreted ($sPLA_2$) are three well-characterized human enzymes that have been implicated in eicosanoid formation. In this review, we will introduce and summarize the regulation of catalytic activity and cellular localization, structural characteristics, interfacial activation and kinetics, substrate specificity, inhibitor binding and interactions, and the downstream implications for eicosanoid biosynthesis of these three important PLA_2 enzymes.

Keywords

Phospholipase; Catalytic Mechanism; HD-XMS; Molecular Dynamics; LC/MS Assays; Substrate Specificity

Introduction

Phospholipases A_2 (PLA_2) are lipolytic enzymes that play a central role in cellular lipid metabolism and signaling [1]. When PLA_2 enzymes are activated in cells, they catalyze the hydrolysis of the ester bond at the *sn*−2 position of membrane phospholipids which are generally enriched in arachidonic acid (AA) and other polyunsaturated fatty acids (PUFA) [2]. The release of AA and other PUFAs triggers a cascade of cellular processes that involve cyclooxygenases and lipoxygenases that are key in the biosynthesis of eicosanoids including leukotrienes, prostaglandins and thromboxanes [3]. Eicosanoids are lipid mediators that regulate a variety of cellular responses, and they are especially important in immunity and inflammation [4]. Lysophospholipids, which constitutes the other products of PLA_2 catalysis, can lead to a variety of cellular metabolites such as lysophosphatidic acid (LPA)

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which can bind to G protein-coupled receptors [5, 6]. They are also precursors of platelet-activating factor (PAF) which is a potent inflammatory lipid mediator [7, 8].

The PLA_2 superfamily consists of 16 groups of structurally and functionally diverse enzymes [9]. The six main types of PLA_2 enzymes include the secreted ($sPLA_2$), cytosolic ($cPLA_2$), calcium-independent ($iPLA_2$), platelet-activating factor acetylhydrolase (PAF-AH), also known as lipoprotein-associated PLA_2 (Lp-PLA_2), lysosomal PLA_2 ($LPLA_2$), and adipose-PLA_2 (AdPLA) [9]. Our recent studies have focused on three human recombinant enzymes, namely the Group IVA cytosolic ($cPLA_2$), Group VIA calcium-independent ($iPLA_2$), and Group V secreted ($sPLA_2$), which are all water-soluble, membrane-associated enzymes with distinct structures and biological functions [10, 11]. The structure of each enzyme contains a unique active site where the substrate binds and an interfacial surface that mediates association with cellular membranes [12]. Hydrogen/deuterium exchange mass spectrometry (HD-XMS) and molecular dynamics simulations were successfully employed by our laboratory to study the interactions of these enzymes with membranes, substrates, and inhibitors [13–15]. In this review, we discuss what we have now learned about the regulation of activity and cellular localization, structural characteristics, interfacial activation and kinetics, substrate specificity, inhibitor binding and interactions, and implications for the biosynthesis of eicosanoids by these three PLA_2 enzymes under physiological conditions.

Regulation of activity and cellular localization

PLA_2 activity is regulated by complex mechanisms of activation that cause translocation of the PLA_2 enzymes to cellular membranes. $cPLA_2$ activity is regulated by calcium and phosphorylation [16]. The C2 domain contains a calcium binding site that aids in the translocation of $cPLA_2$ to the membrane upon increases in intracellular calcium [17, 18]. Several studies demonstrated a calcium-mediated translocation of $cPLA_2$ to the nuclear envelop and endoplasmic reticulum [19–22]. Phosphorylation of Ser505 has been also found to contribute to $cPLA_2$ activation [23]. In addition to activation by calcium and phosphorylation, phosphatidylinositol 4,5-bisphosphate (PIP2) has been shown to enhance the enzymatic activity of $cPLA_2$ [24, 25]. $iPLA_2$ is a calcium-independent enzyme whose activity is regulated, stabilized, and increased by ATP [9, 26, 27]. It has been shown that $iPLA_2$ is also regulated through other mechanisms of activation including caspase cleavage, calmodulin, and ankyrin repeat mediated oligomerization [9]. Individual $sPLA_2$ enzymes are expressed in specific cell types such as immune cells, epithelial cells, and others [28–30]. It has been shown that $sPLA_2$ hydrolyzes the plasma membrane to release lysophospholipids and free fatty acids which may cause an increase in intracellular calcium concentration that activates $cPLA_2$ [31, 32]. It has been reported that $sPLA_2$ also hydrolyzes oxidized phospholipids in LDL and HDL contributing to atherosclerosis [33, 34]. Figure 1 is a cartoon representation that depicts some of the reported cellular localizations of $cPLA_2$, $iPLA_2$, and $sPLA_2$.

Structural Characteristics

$cPLA_2$ and $iPLA_2$ share a common catalytic Ser/Asp dyad and they have a similar molecular weight of approximately 85 to 90 kDa. In both enzymes, the catalytic Ser lies in a lipase

consensus motif of GXSXG/S [9]. They also contain a very similar sequence motif called the “dual signature nucleotide” (GXGXXG) that contains two important features for catalysis: first an oxyanion hole which stabilizes the tetrahedral intermediate after the attack of the catalytic Ser at the *sn*−2 carbonyl group, and second a positively charged residue of Arg ($cPLA_2$) or Lys ($iPLA_2$) that stabilizes the binding of the phosphate group [14, 35]. The crystal structure of $cPLA_2$ identified the C2 domain and an α/β hydrolase catalytic domain [18]. Human $iPLA_2$ is expressed in two active splice variants that differ by a 54 amino acid insert. Sequence alignment studies revealed an ankyrin repeat region, a linker region, and an α/β hydrolase catalytic domain [36, 37]. The crystal structure of the short splice variant was recently published and showed a dimer [38]. For the long splice variant, a homology model was previously published [13, 14], and the catalytic domain was consistent with the crystal structure of the short form where applicable [38]. $sPLA_2$ is a small 14 kDa protein that contains six disulfide bonds [9]. The crystal structure of the Group V $sPLA_2$ has not been reported, but a homology model was previously published [13]. $sPLA_2$ utilizes a His/Asp catalytic dyad. It has been suggested that $sPLA_2$ activity may be affected by $cPLA_2$ or vice versa [22, 39].

Interfacial activation and kinetics

PLA_2 enzymes can exist in a water-soluble state (**E**), but their water-insoluble phospholipid substrates are part of cellular membranes (**M**). The first step of the PLA_2 catalytic cycle is the association with the surface of the membrane (**EM**) through their interfacial surface (Figure 2). By using HD-XMS and molecular dynamics, we showed that the membrane acts as an allosteric ligand, shifting the conformation of a PLA_2 from the closed form in water to the open form on the surface of the membrane [13, 14]. This process enables the enzyme to extract and bind a phospholipid molecule in the active site (**ES·M**), where it is converted into product (**EP·M**). Figure 3 shows the mechanism for the hydrolysis of a phospholipid molecule by $cPLA_2$ or $iPLA_2$ [14]. The interfacial activation of PLA_2 enzymes can best be explained by the “surface dilution kinetics” model [40, 41]. These studies were conducted by developing interaction models of $cPLA_2$, $iPLA_2$, and $sPLA_2$ with the membrane (Figure 4). Several peptide regions, which are part of the interfacial surface of each enzyme, showed decreased in H/D exchange rates upon association with phospholipid vesicles [13, 14]. These peptide regions were used to place each enzyme on the surface of the membrane. It is worth noting that the membrane interaction models only consider a monomeric association of the enzyme with the membrane, although these enzymes may be aggregated, and $iPLA_2$ was reported to exist as a dimer or a tetramer [42].

Substrate specificity

Determining the activity of PLA_2 enzymes toward a wide variety of phospholipid substrates has always been a very challenging task using traditional radioactive assays. We have recently developed a novel lipidomics-based PLA_2 assay that enabled us to measure the activity of $cPLA_2$, $iPLA_2$, and $sPLA_2$ toward a variety of commercially available synthetic and natural phospholipids [13]. $cPLA_2$ showed distinct specificity for arachidonic acid at the *sn*−2 position, while $iPLA_2$ and GV $sPLA_2$ are more permissive with preference for linoleic and myristic acid (Figure 5). No significant differences in specificity for the fatty acid at the

sn−2 position were observed between palmitic and stearic acid at the *sn*−1 position. $cPLA_2$ and $sPLA_2$ activities are slightly better toward phospholipids containing stearic acid at the *sn* −1 position, whereas $iPLA_2$ activity is somewhat better toward phospholipids containing palmitic acid at the *sn*−1 position [13]. Previously published studies suggested that, although both $cPLA_2$ and $iPLA_2$ contribute to LPC accumulation during stimulation of macrophages, 18:0 LPC appears to be produced primarily by $cPLA_2$ whereas 16:0 LPC appears to be produced primarily by $iPLA_2$ [46, 47]. Molecular dynamics simulations guided by HD-XMS revealed that $cPLA_2$ contains a deep channel-like active site which accommodates a phospholipid molecule in its entirety (see Movie S1 from ref 14: http://movieusa.glencoesoftware.com/video/10.1073/pnas.1424651112/video-1). The active site of $cPLA_2$ is enriched with aromatic residues that interact with the four double bonds of the arachidonic acids through pi-pi stacking (see Movie 1 from ref 13: http://pubs.acs.org/doi/suppl/10.1021/jacs.7b12045/suppl_file/ja7b12045_si_002.avi). In contrast, $iPLA_2$ and $sPLA_2$ contain a more flexible active site that allows them to tightly bind phospholipids containing a larger variety of fatty acids in the *sn*−2 position by recruiting different binding pockets. Linoleic acid containing a single bis-allylic position binds to an aromatic pocket while myristic acid binds to an aliphatic pocket in both $iPLA_2$ and $sPLA_2$ [13, 14].

Inhibitor binding and interactions

The implication of $cPLA_2$, $iPLA_2$, and $sPLA_2$ in chronic inflammatory diseases makes them attractive targets for the development of potent and selective inhibitors [9, 48]. HD-XMS and molecular dynamics simulations were used to understand the binding and interactions of $cPLA_2$ and $iPLA_2$ inhibitors [49–56]. Fluoroketone inhibitors were identified as potent $iPLA_2$ inhibitors in 2010 [57]. In our effort to improve their potency and selectivity, we have developed structureactivity relationships using computer-aided drug design [51, 52]. Using these models, we were able to design, synthesize, and test new fluoroketone compounds. These studies led to the development of new more potent and selective fluoroketone inhibitors. We were also able to identify a novel class of $iPLA_2$ inhibitors that contain a heterocyclic ring instead of the fluoromethyl group [51]. Molecular dynamics simulations showed that the carbonyl group of the inhibitor forms hydrogen bonding with the oxyanion hole (Gly486/Gly487) and the heterocyclic ring with Asn658 (Figure 6). The hydrophobic tail of the inhibitor binds in a pocket where the *sn*2 fatty acid normally binds.

Eicosanoid biosynthesis

$cPLA_2$, $iPLA_2$, and $sPLA_2$ have all been implicated in cellular eicosanoid biosynthesis. $cPLA_2$ is activated by various stimuli that mobilize intracellular calcium and/or phosphorylation, such as Toll-like and purinergic receptors that initiate signaling during an inflammatory response. When activated, $cPLA_2$ translocates to the perinuclear and endoplasmic reticulum membranes, releasing the free arachidonic acid from the phospholipid to which it is esterified. The release of arachidonic acid triggers a cascade of molecular events that involves cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP) enzymes leading to inflammation [3]. $iPLA_2$ is involved in homeostatic cellular functions, primarily membrane homeostasis and remodeling [58]. Since

iPLA$_2$ does not show strong specificity for the esterified fatty acid, it may contribute to the release of arachidonic acid. sPLA$_2$ enzymes are induced in several if not all situations [59].

Eicosanoids are products of the oxidation of arachidonic acid by downstream enzymes including COX [60], LOX [61], and CYP [62]. Oxidation of arachidonic acid can also be caused via non-enzymatic free radical mechanisms [3]. Prostaglandins and thromboxanes are produced by the downstream enzymes of the COX pathway. In particular, functional coupling of thromboxane A synthase 1 (TBXAS1) and PGD synthase (PGDS) with COX1 has been shown to produce the eicosanoids thromboxane A$_2$ (TXA$_2$) and PGD$_2$ during stimulation of macrophages [63]. In addition, PGE synthase 1 (mPGES1; also known as PTGES) and PGI2 synthase (PGIS) are coupled with COX2 to produce PGE$_2$ and PGI$_2$ [63, 64]. Leukotrienes are produced by the downstream enzymes of the LOX pathway. An example is the 5-LOX pathway that involves coupling of cPLA$_2$ to 5-LOX, which are both calcium dependent, and formation of leukotrienes B$_4$ (LTB$_4$) and C$_4$ (LTC$_4$) by LTA4 hydrolase (LTA4H) and LTC4 synthase (LTC4S), respectively [65, 66]. Advances in the lipidomics field now allows routine monitoring of eicosanoid production in stimulated macrophages. This information can be used to develop computational models that predict the outcomes of drug candidates [67].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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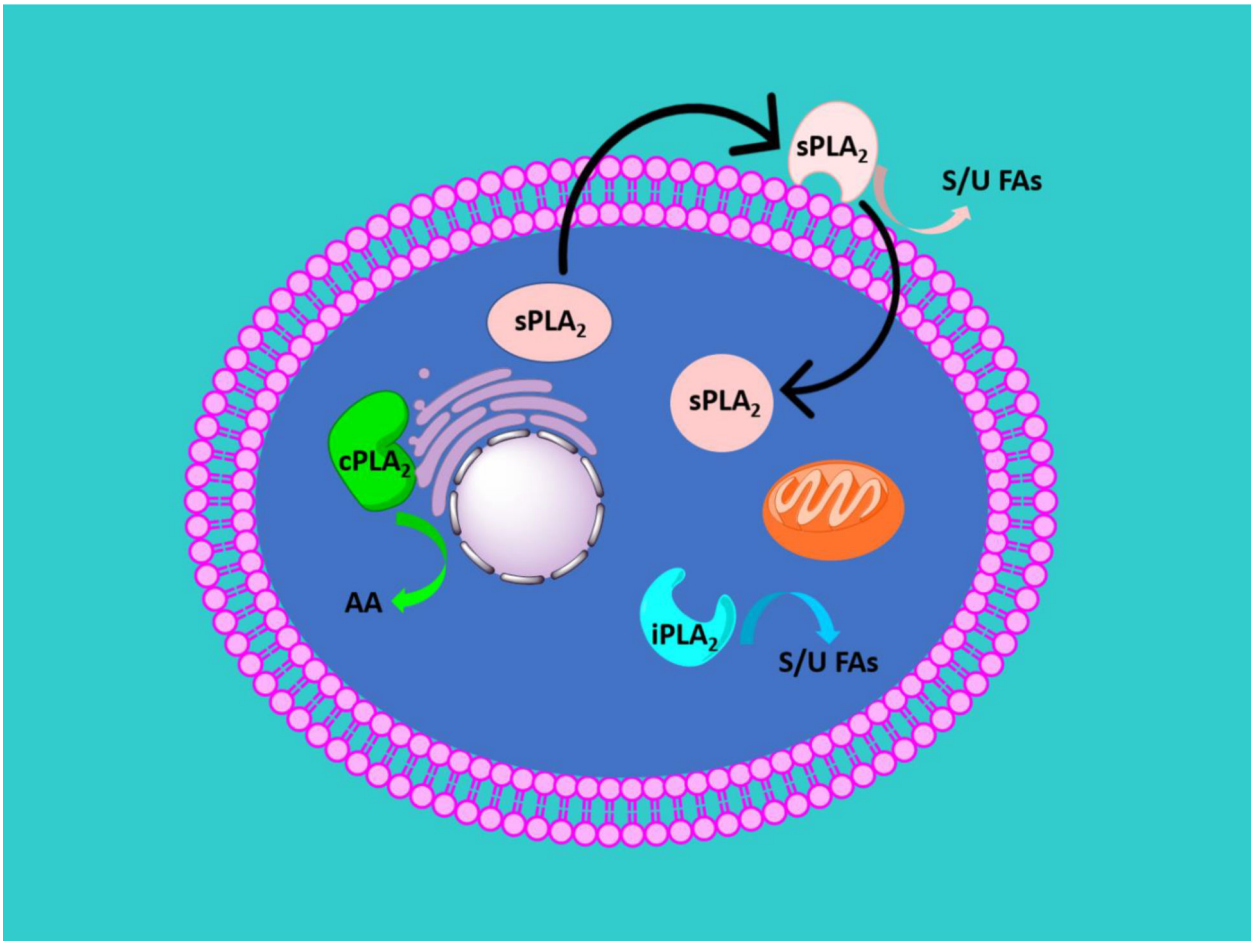

Figure 1.

Cartoon representation that depicts reported cellular localizations of $cPLA_2$, $iPLA_2$, and $sPLA_2$. $cPLA_2$ translocates to the perinuclear membranes including the Golgi. $iPLA_2$ was found in the cytosol and was also found associated with mitochondria and may have different functions/localizations in different cells. $sPLA_2$ was found to be secreted where it may act on cells or may be internalized and act intracellularly. Extracellularly, $sPLA_2$ was found to act on extracellular phospholipids such as microvesicles/exosomes, surfactants, lipoproteins, and bacterial membranes. AA is an abbreviation for arachidonic acid. S/U FAs is an abbreviation for saturated and unsaturated fatty acids.

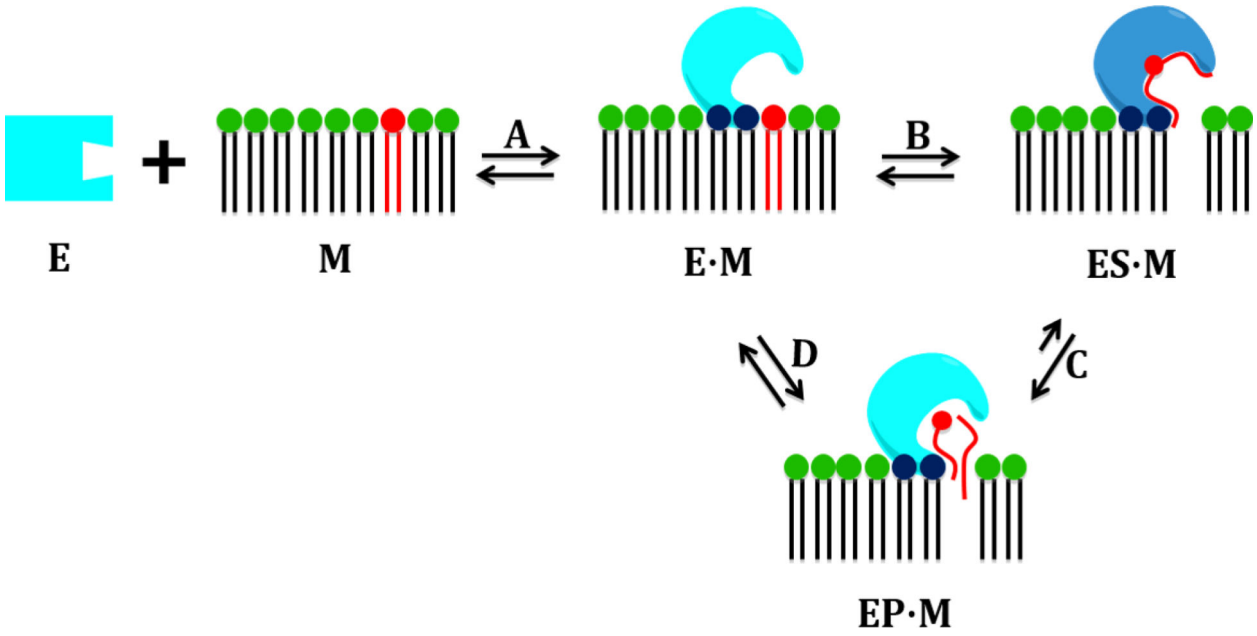

Figure 2.
Cartoon representation of the catalytic cycle of PLA_2 enzymes (reprinted from reference 13).

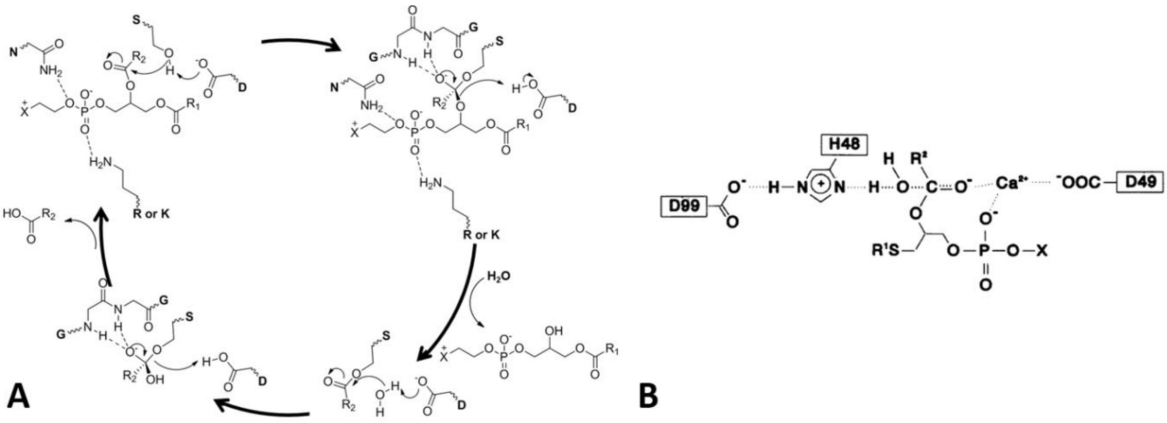

Figure 3.

Mechanism for the hydrolysis of a phospholipid molecule by $cPLA_2$ or $iPLA_2$ (reprinted from reference 14).

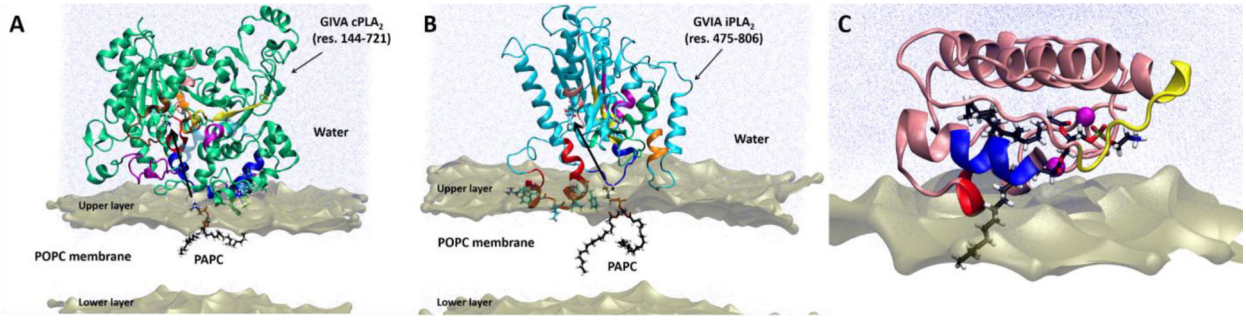

Figure 4.

Interaction models for (A) $cPLA_2$, (B) $iPLA_2$, and (C) $sPLA_2$ with the membrane based on HD-XMS data (adapted form references 13 and 14). The colored peptide regions on each enzyme showed decreased deuteration levels upon association with phospholipid vesicles [43–45].

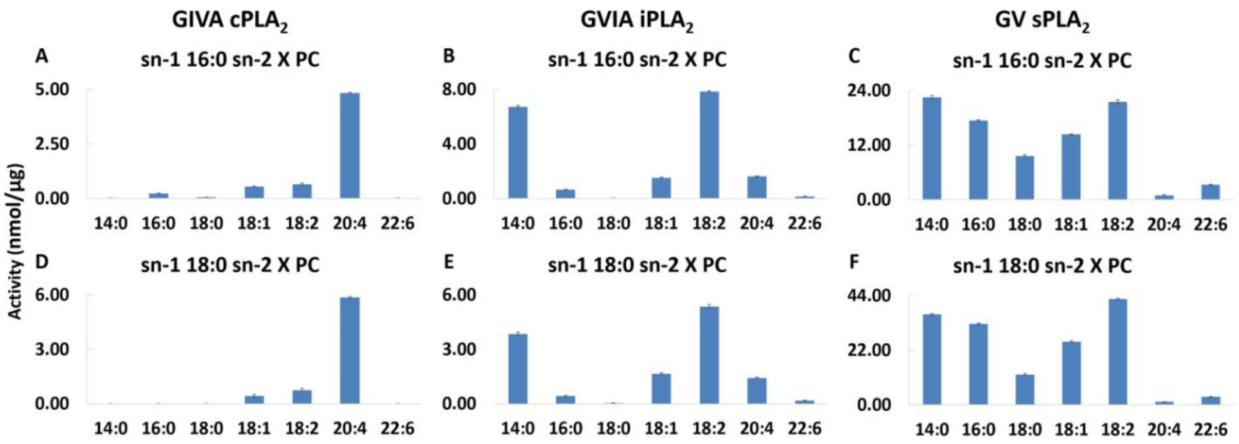

Figure 5.

Enzymatic activity of (A, D) $cPLA_2$, (B, E) $iPLA_2$, and (C, F) $sPLA_2$ toward a variety of phospholipid substrates (reprinted from reference 13).

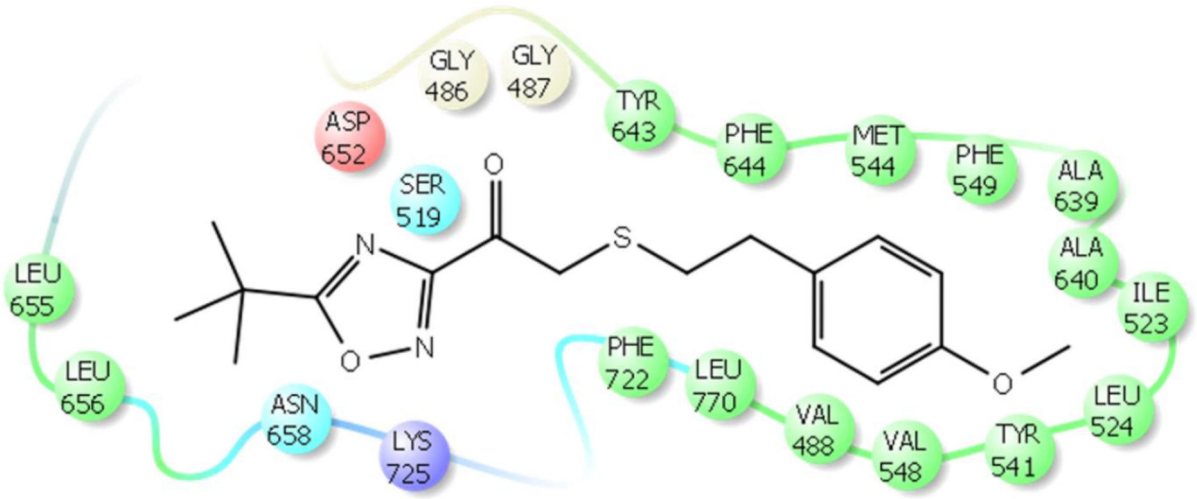

Figure 6.
Binding and interactions of an $iPLA_2$ inhibitor containing a heterocyclic ring.