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Review

# The expanding superfamily of phospholipase $A_2$ enzymes: classification and characterization

David A. Six, Edward A. Dennis *

*Department of Chemistry and Biochemistry, MC 0601, Revelle College and School of Medicine, University of California, San Diego, La Jolla, CA 92093-0601, USA*

**Abstract**

The phospholipase $A_2$ ($PLA_2$) superfamily consists of a broad range of enzymes defined by their ability to catalyze the hydrolysis of the middle (*sn*-2) ester bond of substrate phospholipids. The hydrolysis products of this reaction, free fatty acid and lysophospholipid, have many important downstream roles, and are derived from the activity of a diverse and growing superfamily of $PLA_2$ enzymes. This review updates the classification of the various $PLA_2$'s now described in the literature. Four criteria have been employed to classify these proteins into one of the 11 Groups (I–XI) of $PLA_2$'s. First, the enzyme must catalyze the hydrolysis of the *sn*-2 ester bond of a natural phospholipid substrate, such as long fatty acid chain phospholipids, platelet activating factor, or short fatty acid chain oxidized phospholipids. Second, the complete amino acid sequence of the mature protein must be known. Third, each $PLA_2$ Group should include all of those enzymes that have readily identifiable sequence homology. If more than one homologous $PLA_2$ gene exists within a species, then each paralog should be assigned a Subgroup letter, as in the case of Groups IVA, IVB, and IVC $PLA_2$. Homologs from different species should be classified within the same Subgroup wherever such assignments are possible as is the case with zebra fish and human Group IVA $PLA_2$ orthologs. The current classification scheme does allow for historical exceptions of the highly homologous Groups I, II, V, and X $PLA_2$'s. Fourth, catalytically active splice variants of the same gene are classified as the same Group and Subgroup, but distinguished using Arabic numbers, such as for Group VIA-1 $PLA_2$ and VIA-2 $PLA_2$'s. These four criteria have led to the expansion or realignment of Groups VI, VII and VIII, as well as the addition of Group XI $PLA_2$ from plants. © 2000 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

The phospholipase $A_2$ ($PLA_2$) superfamily consists of a broad range of enzymes defined by their ability to catalyze specifically the hydrolysis of the center (*sn*-2) ester bond of substrate phospholipids (see also previous reviews [1–4]). The hydrolysis products of the $PLA_2$ reaction are free fatty acid and lysophospholipid. The fatty acids released by $PLA_2$, such as arachidonic acid (AA) and oleic acid (OA), can be important as stores of energy, but more importantly AA can also function as a second messenger [5,6] and as the precursor of eicosanoids, which are potent mediators of inflammation and signal transduction [7–9]. The other product of $PLA_2$ action, lysophospholipid, is important in cell signaling, phospholipid remodeling, and membrane perturba-

* Corresponding author. Fax: (858) 5347390;
E-mail: edennis@ucsd.edu

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tion [10,11]. Conversely, the actions of $PLA_2$'s can be important for down-regulating cell signals, as is seen with the $PLA_2$-catalyzed hydrolysis of the bioactive phospholipid, platelet activating factor (PAF), to its inactive, lysophospholipid form [12–14]. While the downstream effects of $PLA_2$'s are diverse, so too are the members of this growing superfamily of enzymes.

The enzymatic activity now characterized as $PLA_2$ activity was first studied in phenomenological detail as early as the 1890's using "poison" or venom from cobras [15–17]. A secreted $PLA_2$ with similar properties was found in large amounts in porcine pancreas (see [16]). Later research defined these small, secreted $PLA_2$'s as being $Ca^{2+}$-dependent, highly disulfide bonded, and possessing catalytic histidine and aspartate residues [1]. Over the years more secreted $PLA_2$'s were discovered in venoms and pancreatic juices of various animals, and these related enzymes were initially divided into two Groups based on disulfide bond positions and unique loops and extensions [1,18,19]. Subsequently, Group II $PLA_2$ was expanded to include mammalian non-pancreatic $PLA_2$, referred to by some as sPLA₂ because of its original isolation as a secreted enzyme from synovial fluid [20,21] and now referred to as Group IIA $PLA_2$. Following that, the unique $PLA_2$ from bee venom was classified as the Group III $PLA_2$ [1]. Many additional forms of secreted $PLA_2$'s utilizing a catalytic histidine have been discovered in recent years, which are clearly related to the Groups I, II, and III $PLA_2$, but do not easily fit into those Groups; this led to the establishment of Groups V, IX, X, and XI.

In 1991 the general classification of $PLA_2$'s as small, secreted enzymes containing a catalytic histidine became outmoded when the cytosolic $PLA_2$ activity initially described in neutrophils [22] and platelets [23] was sequenced and cloned [24,25]. The sequence revealed a completely unrelated 85 kDa $PLA_2$, containing a catalytic serine and no disulfide bonds [24,25]. Since the cloning of this $PLA_2$, often referred to as $cPLA_2$ because of its original isolation from the cytosol of cells, it has been classified as a Group IV $PLA_2$ (now Group IVA $PLA_2$). Subsequently, three more unrelated Groups of $PLA_2$'s have been discovered and classified, including Groups VI, VII, and VIII, bringing the total to 11 Groups as shown in Tables 1 and 2. The advent of genomics has seen an expansion in the number of $PLA_2$ Subgroups, leading to the characterization of exciting new $PLA_2$'s with the anticipation of even more $PLA_2$'s in the future.

## 2. Classification criteria for phospholipase $A_2$ Groups

The diverse $PLA_2$ enzymes have been classified into Groups from I to XI thus far. The criteria for the establishment of a Group or Subgroup has changed as new Groups were identified [1–4,18,19], but with the increases in Group diversity and size, there is a real need for criteria to be codified in a logical classification scheme as follows (see also Table 3). The first essential criterion for an enzyme to be assigned to a $PLA_2$ Group is that it must catalyze the hydrolysis of the *sn*-2 ester bond of a phospholipid substrate. Naturally occurring substrates include platelet activating factor, short fatty acid chain oxidized phospholipids, and long fatty acid chain phospholipids, with *sn*-2 acyl chains ranging from two (acetyl) to 20 carbons (arachidonate) and even longer. While the major activity must be $PLA_2$ activity, members of the $PLA_2$ superfamily may possess other activities, such as $PLA_1$, lysophospholipase $A_1$/$A_2$, acyl transferase, or transacylase activity.

The second essential criterion for an enzyme to be assigned to a $PLA_2$ Group is that the complete amino acid sequence for the mature protein should be known. Moreover, it seems reasonable that future additions to the $PLA_2$ superfamily should be cloned, expressed, and purified to correlate the sequence to specific activity in an unambiguous system, regardless of whether they are discovered by DNA- or activity-based searches.

The third criterion for the classification is that each $PLA_2$ Group should include all of those enzymes which have readily identifiable sequence homology. Specifically, if more than one homologous $PLA_2$ gene exists within a species (paralogs), then each $PLA_2$ gene should be assigned a Subgroup letter, as in the case of Groups IVA, IVB, and IVC $PLA_2$. It is also possible that paralogs will exist only in certain species as is the case with Group IIC $PLA_2$ [26]. Homologs from different species (or-

Table 1
Phospholipase $A_2$ Groups utilizing a catalytic histidine[a]

| Group | | Initial/common sources | Size (kDa) | Disulfides (No.) | Unique disulfides | C-term. extension | Chromosome | | Archetype enzyme | NCBI protein accession No. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Human | Mouse | | |
| I | A | Cobra, krait venom | 13–15 | 7 | 11–77 | none | N/A | N/A | Cobra | P15445 |
| | B[b] | Mammal pancreas | 13–15 | 7 | 11–77 | none | 12q23-24 [58] | 5 [33] | Human | NP_000919 |
| II | A | Human synovial fluid, platelets rattlesnake, viper venom | 13–15 | 7 | 50–137 | 7 res. | 1p34-36 [26] | 4 [59] | Human | NP_000291 |
| | B[c] | Gaboon viper venom | 13–15 | 6 | 50–137 | 6 res. | N/A | N/A | Viper | PSBGA |
| | C | Rat/mouse testis | 15 | 8 | 50–137, 86–92 | 7 res. | 1p34-36 [26][d] | 4 [26] | Mouse | NP_032894 |
| | D | Human/mouse pancreas/spleen | 14–15 | 7 | 50–137 | 7 res. | 1p36.12 [60] | 4 [61] | Human | NP_036532 |
| | E | Human/mouse brain/heart/uterus | 14–15 | 7 | 50–137 | 7 res. | 1p36 [62] | 4 [33] | Human | AAF36541 |
| | F[e] | Mouse testis/embryo | 16–17 | 7 | 50–137 | 30 res. | N.D. | 4 [33] | Mouse | AAF04500 |
| V | | Mammal heart/lung/ macrophage | 14 | 6 | none | none | 1p34-36 [26] | 4 [26] | Human | NP_000920 |
| X | | Human spleen/thymus/ leukocyte | 14 | 8 | 11–77, 50–137 | 8 res. | 16p12-13.1 [63] | 16 [33] | Human | NP_003552 |
| III[f] | | Bee/lizard/scorpion/ human | 15–18 | 5 | N/A | N/A | 22q [64] | N.D. | Honey bee | P00630 |
| IX | | Snail venom (conodipine-M) | 14 | 6 | N/A | N/A | N/A | N/A | Marine snail | AAB33555 |
| XI | A | Green rice shoots ($PLA_2$-I) | 12.4 | 6 | N/A | N/A | N/A | N/A | Rice | CAB40841 |
| | B | Green rice shoots ($PLA_2$-II) | 12.9 | 6 | N/A | N/A | N/A | N/A | Rice | CAB40842 |

[a]These are typically small extracellular $PLA_2$'s requiring millimolar [$Ca^{2+}$] and an active site histidine and aspartate pair. Note that Groups V and X are listed after Groups I and II because of their close homology with many conserved residues including 6+ disulfide bonds, and a histidine and two aspartates, as well as having N-terminal signal peptides that are cleaved to yield the mature $PLA_2$.
[b]Group IB has a five residue insert known as the pancreatic loop.
[c]Group IIB is missing one of the six highly conserved disulfides (approx. 61–94).
[d]Human Group IIC is a pseudogene.
[e]Group IIF has an additional Cys in its C-terminal extension.
[f]Human GIII$PLA_2$ (55 kDa) seems to possess additional novel C-terminal and N-terminal domains.

thologs) should be classified within the same Subgroup wherever such assignments are possible, such as for zebra fish and human Group IVA $PLA_2$. On the other hand, it is sometimes difficult to classify homologous enzymes into established Subgroups. A good example for this situation is for the phospholipase B (PLB) enzymes from various fungi, which are homologous to Group IV $PLA_2$, but not readily classified into any of the current Group IV $PLA_2$ Subgroups. In order to fully classify the PLB's, more sequences of PLB's, as well as further biochemical characterization, should allow the placement of the PLB's into one of the current Subgroups of Group IV $PLA_2$ or justify the creation of a new Subgroup.

The fourth criterion for classification considers active splice variants of the same $PLA_2$ gene to be distinct proteins, but part of the same Subgroup. Each splice variant should be numbered once activity is confirmed, such as for Group VIA $PLA_2$ which has two confirmed, active splice variants, referred to here as Group VIA-1 $PLA_2$ and Group VIA-2 $PLA_2$ [27,28]. In the case of Group VIA $PLA_2$, two confirmed, inactive splice variants have been referred to not as $PLA_2$ enzymes, but still using the Group nomenclature, as Group VIA Ankyrin-1 and Group VIA Ankyrin-2 [28].

These four criteria accommodate historical exceptions, but set out how future additions should be handled. Additionally they do not eliminate ambiguity, but rather point out that some ambiguity exists and will be clarified with further sequence and enzymological data. For simplicity, in the remainder of the text the abbreviation G for Group (i.e., $GIAPLA_2$ for Group IA $PLA_2$) will be employed.

## 3. Phospholipase $A_2$ Groups utilizing a catalytic histidine ($sPLA_2$'s)

### 3.1. General similarities

With the abundant number of closely related $PLA_2$'s from snake venoms and mammalian secretions that fall into Groups I, II, V, and X, the similarities of these enzymes will be described, followed by the specific differences and important characteristics for each Subgroup, and then the Group III, IX, and XI $PLA_2$'s will be considered (see also Table 1). The Group I, II, V, and X $PLA_2$'s are very closely related, and share a common mechanism for cleavage of the *sn*-2 ester bond of phospholipids. Hydrolysis proceeds through the activation and orientation of a water molecule by hydrogen bonding to the active site histidine, which dictates the pH dependence of 7–9 for all $PLA_2$'s with a catalytic histidine [2,29–32]. These $PLA_2$'s that utilize a catalytic histidine, fall into Groups I, II, III, V, IX, X, and XI, and shall be referred to as the Histidine $PLA_2$'s for convenience. Furthermore, located adjacent to the catalytic histidine is a conserved aspartate, forming the so-called His/Asp dyad. This Asp is a ligand of the crucial $Ca^{2+}$, which forms the positively charged oxyanion hole that stabilizes the negatively charged transition state of the $PLA_2$ reaction. This is the origin of the mM $Ca^{2+}$ dependence of the Histidine $PLA_2$'s [2,29–32]. Finally, there are several other conserved residues that participate in the Group I, II, V, and X active sites' hydrogen bonding networks, including tyrosines and glycines in the $Ca^{2+}$-binding loop and a second aspartate residue that activates and orients the catalytic histidine [29–32].

The $PLA_2$ Groups I, II, V, and X are all related evolutionarily as exemplified by their easily identifiable sequence homology and even greater structural homology [1,30,33]. Besides the highly conserved active site residues, there are six absolutely conserved disulfide bonds linking residues 27–131, 29–45, 44–109, 51–102, 61–95, 85–100 (see the sequence alignment in [33] for numbering), and up to two additional unique disulfide bonds in each Group member. In addition all these enzymes have signal sequences which are cleaved in the process of secretion of the mature active protein. The only exception to this is the $GIBPLA_2$, which is secreted with a propeptide that must be cleaved by trypsin to produce the mature active enzyme.

The various $PLA_2$ Group members that employ a catalytic histidine have been compared and contrasted many times in the literature, but there is not space here to mention most of the data. Several key papers in the literature [33–38] as well as several new reviews [39–43] discuss the differences, relationships, and functions of these various secreted $PLA_2$ members in greater detail.

### 3.2. Groups I, II, V, and X phospholipase $A_2$

#### 3.2.1. $GIAPLA_2$

As described above, the cobra venom $PLA_2$ was the first to be characterized, and has the six conserved disulfides plus a seventh one, unique to the Group I enzymes, linking residues 11 and 77 [1]. In addition the $GIPLA_2$'s have a characteristic surface loop termed the elapid loop connecting the second major α-helix to a β-wing. There has been much research about this Group of venom $PLA_2$'s from snakes such as cobras and kraits, including several structures [31,44]. The $GIAPLA_2$, like all histidine $PLA_2$'s, is an interfacial enzyme that requires an aggregated surface to bind to in order to access its phospholipid substrates [45,46]. A particular kinetic property of several of the $GIAPLA_2$'s is termed phosphatidylcholine (PC) activation in which phosphatidylethanolamine (PE) hydrolysis is increased by the presence of PC in the interface [45]. In addition to the well characterized substrate binding site [44], more recently, two distinguishable sites for PC activation and interfacial activation were mapped to the $GIAPLA_2$ from the Indian cobra, greatly increasing the understanding of the $GIAPLA_2$-phospholipid interactions [45].

#### 3.2.2. $GIBPLA_2$

The first non-venom $PLA_2$ was identified in pancreatic juices of cows and subsequently many other animals including humans [1,47,48]. $GIBPLA_2$ has a unique 5 amino acid extension of the elapid loop termed the pancreatic loop as well as the specific seventh disulfide bond between residues 11 and 77, typical of the $GIPLA_2$'s [1]. $GIBPLA_2$ is secreted as a zymogen containing a 7 amino acid leader sequence which impairs interfacial activity until it is cleaved by trypsin [49]. Consistent with its high expression in pancreatic juices, $GIBPLA_2$ has a clear role in the digestion of dietary phospholipids [50,51], as well as less conventional roles in other tissues and cells [33,52]. Many of these other roles are thought to be mediated by the $GIBPLA_2$ activity, but there are also specific receptor-mediated effects that are independent of $PLA_2$ activity [53]. Various aspects of $GIBPLA_2$ will be treated in more detail in the following reviews: [40,41,54].

#### 3.2.3. $GIIAPLA_2$

Initially characterized from venoms of rattlesnakes and vipers as mature proteins of approx. 14 kDa and approx. 120 amino acids, $GIIAPLA_2$ grew to include mammalian non-pancreatic $PLA_2$'s based on the overall sequence homology and specific disulfide bond and loop positioning [18,19,55]. The $GIIAPLA_2$ has the conserved six disulfides plus a seventh between positions 50 and 138 (see [33] for numbering) with Cys-138 present within a C-terminal extension that occurs in all $GIIPLA_2$'s as well as Group X $PLA_2$. The phylogenetic analysis of the various related histidine $PLA_2$'s reveals ambiguities in the current classification system such that both mammalian and venom enzymes are included in $GIIAPLA_2$ despite a larger phylogenetic gap than between all the mammalian Group II and V enzymes [1,33].

$GIIAPLA_2$ has been implicated in eicosanoid production in mammals, but the situation is complex as many of these studies did not distinguish the Group IIC, D, E, F or Group V $PLA_2$ from the $GIIAPLA_2$ [33,56]. Moreover, a naturally occurring frameshift mutation in certain strains of mice that creates a natural "knock-out" for the $GIIAPLA_2$ gene, has no apparent effects on those mice, suggesting compensation by other $PLA_2$'s [57]. The human and mouse $PLA_2$ gene locations, summarized in Tables 1 and 2, show that the $GIIAPLA_2$ is located on chromosome 1p34-36 clustered along with $GVPLA_2$ and three other $GIIPLA_2$ genes [26,27,33,58–70]. A sequence alignment and phylogenetic tree of all the known mouse histidine $PLA_2$ homologs are shown in a recent paper and demonstrate the relationships between the Subgroups of Group II $PLA_2$ [33]. The expression of mouse $GIIAPLA_2$ is quite narrow, being highly expressed in intestine and somewhat in prostate, but undetected elsewhere [33], in contrast to the near ubiquitous expression of human $GIIAPLA_2$ [63]. Given these variable expression patterns between mouse and human homologs of $GIIAPLA_2$, as well as the variations in the core sequence identity (67–89%) for mouse and human species variants, generalization of species-specific data can be problematic. The human $GIIAPLA_2$ is known to bind tightly to anionic phospholipid-containing interfaces such as bacterial membranes and has little or no

Table 2
Phospholipase $A_2$ Groups utilizing a catalytic serine[a]

| Group | | Initial/common sources | Alternate names employed | Size (kDa) | $Ca^{2+}$ effects | Characteristics | Human chromosome | Human NCBI accession No. |
|---|---|---|---|---|---|---|---|---|
| IV | A | Human U937 cells/ platelets RAW 264.7/rat kidney | $cPLA_2$ α | 85 | $< \mu M$; membrane translocation | C2 domain, α/β-hydrolase regulatory phosphorylation | 1q25 [65] | P47712 |
| | B | Human pancreas/liver heart/brain | $cPLA_2$ β | 114 | $< \mu M$; membrane translocation | C2 domain, α/β-hydrolase | 15 [66] | AAD32135 |
| | C | Human heart/skeletal muscle | $cPLA_2$ γ | 61 | None | Prenylated, α/β-hydrolase | 19 [66] | AAC32823 |
| VI | A-1 | $P388D_1$ macrophages, CHO | $iPLA_2$ or $iPLA_2$-A | 84–85 | None | Short splice, 8 ankyrin repeats | 22q13.1 [66,27,67] | AAD41722 |
| | A-2 | Human B-lymphocytes, testis | $iPLA_2$-B | 88–90 | None | Long splice, 7 ankyrin repeats | 22q13.1 [66,27,67] | NP_003551 |
| | B | Human heart/skeletal muscle | $iPLA_2$ γ or $iPLA_2$-2 | 88 | None | Membrane-bound | 7q31 [68,70] | BAA94997 |
| VII | A | Human/mouse/porcine bovine plasma | PAF-AH | 45 | None | Secreted, α/β-hydrolase Ser/His/Asp triad in VIIA and B | N.D. | Q13093 |
| | B | Human/bovine liver/kidney | PAF-AH (II) | 40 | None | Intracellular, myristoylated | N.D. | Q99487 |
| VIII | A | Human brain | PAF-AHIb $\alpha_1$ (subunit of trimer) | 26 | None | Intracellular, G protein fold Ser/His/Asp triad, dimeric | N.D. | Q15102 |
| | B | Human brain | PAF-AHIb $\alpha_2$ (subunit of trimer) | 26 | None | Same as VIIIA; active as heterodimer or homodimer | 11q23 [69] | Q29459 |

[a]Larger, typically intracellular enzymes that utilize a nucleophilic serine for hydrolytic cleavage with no disulfide bonds and no $Ca^{2+}$ requirement for catalysis.

activity against neutral membranes and interfaces [20,71–73]. In recent years the human $GIIAPLA_2$ has been implicated in host-defense responses such as antibacterial activity, consistent with its affinity for anionic bacterial membranes [74]. This topic will be considered in greater detail in a separate review [42].

### 3.2.4. $GIIBPLA_2$

The Group $IIBPLA_2$'s are an anomalous Subgroup of the $GIIPLA_2$'s, not present in mammals, but reported only in two species of vipers, *Bitis gabonica* in 1974 [75] and *Bitis nascicornis* in 1983 [76]. The $GIIBPLA_2$'s are similar to the venom $GIIAPLA_2$'s except that they lack the otherwise universally conserved 61–95 disulfide [1] (see [33] for numbering). Hundreds of venom $PLA_2$'s have been described with no additional homologs to these $GIIBPLA_2$'s, despite a third claim in the literature that is not valid [77]. This apparent discrepancy may be due to peptide sequencing errors of the two known $GIIBPLA_2$'s, as were previously discovered in the initial sequence of $GIIIPLA_2$ [78]. Alternatively, the mutations removing the almost universally conserved 61–95 disulfide bond may represent evolutionary exceptions. Either way, the presence of $GIIBPLA_2$ in the current classification implies more importance to this Subgroup than is probably warranted and creates some confusion.

### 3.2.5. $GIICPLA_2$

The $GIICPLA_2$ was cloned from rodents and the mature protein of approx. 15 kDa was found to be 130 amino acids [79]. $GIICPLA_2$ has eight disulfide bonds including the seven typical $GIIPLA_2$ bonds, as well as an additional disulfide bond between residues 87 and 93 in an extended loop region [33,79]. $GIICPLA_2$ is expressed in rodents, but the absence of a portion of one exon in humans indicates it is a pseudogene [26,79]. The mRNA is expressed highly in rodent testis, specifically those cells undergoing meiosis in the testis [79,80], and has also been seen in brain [79] and pancreas [33]. The recombinant rodent $GIICPLA_2$, secreted from human 293s cells, is active on phosphatidylinositol (PI) more than on PC and PE, but all are hydrolyzed [79].

### 3.2.6. $GIIDPLA_2$

First cloned in 1999, the $GIIDPLA_2$ sequence was identified in a mouse expressed sequence tag (EST), followed by cloning from mouse and human cDNA [60,61]. The $GIIDPLA_2$ sequence encodes a 125 amino acid mature protein of about 14 kDa [60,61]. The mRNA was detected most highly in human and mouse pancreas and spleen with less appearing in thymus and colon [60,61]. Recombinant mouse and human $GIIDPLA_2$'s, secreted from COS and CHO cells, were shown to be active against vesicles of phosphatidylglycerol (PG), PE and PC [60,61] with the substrate affinity for the active site similar for all three [61]. An important distinguishing characteristic is the greater than 1000-fold specificity of inhibition of a particular 1-oxamoylindoline derivative for $GIIAPLA_2$ versus $GIIDPLA_2$ [60].

### 3.2.7. $GIIEPLA_2$

First cloned in 1999, the $GIIEPLA_2$ sequence was identified by sequence searches in a mouse EST [33]. Following cloning, the mature protein was found to contain 127 amino acids (14.5 kDa) containing the histidine $PLA_2$ catalytic residues, and the typical seven disulfide bonds of Group II members [33]. The human $GIIEPLA_2$ was subsequently cloned and shown to match closely the mouse $GIIEPLA_2$ with approx. 89% identity in the mature enzymes [62]. The mRNA (1.5–1.8 kb) was detected in mouse testis, brain, heart and liver by Northern blot [33], in mouse uterus and thyroid by Master blot [33], and in human lung and placenta using RT-PCR [62]. Recombinant mouse $GIIEPLA_2$, secreted from COS cells, was able to hydrolyze PG vesicles much better than PC vesicles [33]. Recombinant, pure human $GIIEPLA_2$ also preferred PG substrate, but was also able to hydrolyze PE and to a lesser extent PC with trends similar to $GIIAPLA_2$ [62]. Finally the expression levels of $GIIEPLA_2$ increased in many tissues of $GIIAPLA_2$-deficient mice upon endotoxin challenge [62]. In situ hybridization of lung from endotoxin-challenged mice showed the appearance of intense staining in the alveolar macrophages, indicating a role for $GIIEPLA_2$ in the inflammatory response in vivo [62].

#### 3.2.8. *GIIFPLA$_2$*

The most recently discovered Histidine PLA$_2$, the GIIFPLA$_2$, was identified by a search of a mouse EST database followed by cloning [33]. The sequence of GIIFPLA$_2$ shows that the mature protein is an unprecedented 148 amino acids due to a 30 amino acid C-terminal extension that contains one free cysteine to which no function has been ascribed [33]. The 4.2 kb mRNA is expressed in the adult testis and embryo [33]. Recombinant GIIFPLA$_2$, secreted by COS cells, is able to hydrolyze PG vesicles much better than PC vesicles [33]. A human homolog has not yet been found, but since GIIFPLA$_2$, like all the Group II and V PLA$_2$'s, is on mouse chromosome 4, the human homolog may be expected to be on the syntenic chromosome 1p34-36 [33].

#### 3.2.9. *GVPLA$_2$ (see especially [39,54])*

The human GVPLA$_2$ discovered in 1994, was cloned and found to be approx. 14 kDa and to contain only the six conserved disulfide bonds of the Group I, II, V, and X PLA$_2$'s, and none of the unique disulfides or loops of Group I or II PLA$_2$. Therefore this unique PLA$_2$ could not be easily assigned to an existing Subgroup, and so became classified separately as Group V PLA$_2$ [81]. GVPLA$_2$ was shown to be highly expressed in rat [82] and human heart [81], but has been shown to be expressed more widely in human and mouse tissues, especially in response to inflammatory stimuli [33,83]. When expressed in vitro, GVPLA$_2$ does have significant activity against PE and PC vesicles in contrast to the GIIAPLA$_2$ [81,82,84–86]. GVPLA$_2$ has also been shown to have a role in inflammation and signal transduction in vivo [34,38,87–89] and has even been identified intracellularly in proximity to its signaling partners, GIVAPLA$_2$ and cyclooxygenase [56]. The recent wealth of structural and functional data for the GVPLA$_2$ will be considered in full detail in a separate review [39].

#### 3.2.10. *GXPLA$_2$*

In 1997 another new PLA$_2$ gene was identified through searches of a human EST database [63]. The sequence encoded a mature protein of 123 amino acids with sequence identity (27–35%) to Group I, II, and V PLA$_2$'s, including the six conserved disulfides and the active site histidine and aspartates [63]. However, this new PLA$_2$ proved difficult to classify with the current system because it contained both the 11–77 disulfide and 50–137 disulfide found typically in the Group I and Group II PLA$_2$'s, respectively, and therefore was named the Group X PLA$_2$ [63]. Human GXPLA$_2$ mRNA expression was detected in spleen, thymus, and blood leukocytes [63], and the protein has been detected in lung alveolar endothelial cells [90]. In contrast to human GXPLA$_2$, mouse GXPLA$_2$ mRNA is expressed in the testis and stomach [33]. The mature protein is approx. 14 kDa, singly *N*-glycosylated, and found both as the 123 residue mature form, and also with an 11 residue propeptide that seems to interfere with full activity [63,90]. Recombinant GXPLA$_2$ secreted from COS cells is active against PE and PC vesicles, but not as effective against anionic phospholipids, and glycosylation appears to have no role in activity or specificity [63,90]. It also has been reported that GXPLA$_2$ can induce the release of AA, OA, and prostaglandin E$_2$ from human monocytic THP-1 cells when added exogenously suggesting a role in inflammation or signal transduction [90].

### 3.3. *Group III phospholipase A$_2$*

The Group III PLA$_2$'s are distantly related to Groups I, II, V, and X [1,78]. In the 1970's the first characterized member of Group III was described from honeybee venom [78,91]. The GIIIPLA$_2$ has many similar biochemical characteristics to the Group I and II PLA$_2$'s, which are easily attributable to their sequence similarities. Having similar sizes and three conserved disulfide bonds between Groups I, II, and III, the structure of the honeybee GIIIPLA$_2$ also shows a homologous His/Asp dyad in the active site which suggests the same hydrolase mechanism as described for the other Histidine PLA$_2$'s [1,29,78]. This is consistent with the pH optimum of 8 for the Group III enzymes [91]. GIIIPLA$_2$ homologs have now been described in lizard, jellyfish, and scorpion venoms [64,92] in addition to bee venoms.

A GIIIPLA$_2$ homolog from scorpion venom, known as imperatoxin I (IpTxi), was identified based on its ability to inhibit ryanodine binding to $Ca^{2+}$ release channels [92]. Reduction of the venom-purified protein yielded two polypeptides of 12 and 3

kDa. The 12 kDa band has significant homology to the other venom $GIIIPLA_2$'s. When the gene was cloned the two polypeptides were found to be encoded by a single gene with a pentapeptide that is evidently excised in the mature protein [92]. IpTxi likely has $PLA_2$ activity based on the $Ca^{2+}$ dependence of its ryanodine binding inhibition, its inhibition by *p*-bromophenacyl bromide, and the similar ryanodine-binding inhibition induced by phospholipid hydrolysis products [92]. A second $GIIIPLA_2$ homolog, named phospholipin, was also identified from scorpion venom and the mature protein is around 15 kDa [93]. Similar to IpTxi, it is made up of two polypeptides (held together by one or more disulfide bonds), and these two polypeptides (108 and 17 residues) are derived from the same gene with a pentapeptide excised in the mature protein [93]. The $PLA_2$ activity was tested and reported to exist although the results were not shown [93]. Therefore, further analysis needs to be carried out to confirm and characterize the $PLA_2$ activity for both of these scorpion $GIIIPLA_2$ homologs.

A $GIIIPLA_2$ human homolog has been cloned and characterized [64]. Screening of human genome sequences led to identification of a fragment with homology to the venom $GIIIPLA_2$ enzymes. The coding region was subsequently cloned from human fetal lung cDNA. The identified protein has a 19 amino acid putative propeptide sequence followed by 490 amino acids encoding a 55 kDa protein with a central $GIIIPLA_2$ domain (16 kDa) that has 31% identity to the honeybee $GIIIPLA_2$. The N- and C-terminal domains of 130 and 219 amino acids, respectively, are not homologous to any other proteins in the databases. The border residues around the central $PLA_2$ domain are suggestive of protease cleavage sites [64], therefore a study of the mature protein from native cells or tissues is required to address the function of these domains. The full length mRNA transcript (4.4 kb) is expressed abundantly in heart, kidney, skeletal muscle, and liver. When human $GIIIPLA_2$ was expressed in COS cells, $PLA_2$ activity accumulated in the culture medium. Partially purified human $GIIIPLA_2$ was found to hydrolyze PG vesicles better than PC vesicles. The pH and $Ca^{2+}$ dependences were consistent with those of the other histidine $PLA_2$'s [64].

The latest PSI-BLAST search [94] of the protein sequence databases using the honeybee $GIIIPLA_2$ sequence revealed all the known $GIIIPLA_2$ members, but most surprisingly five $GIIIPLA_2$ homologs were identified from the fruit fly (data not shown). Recently the *Drosophila melanogaster* genome was sequenced and deposited in the sequence databases [95]. The fruit fly $GIIIPLA_2$-like sequences are also larger than the venom $GIIIPLA_2$, but each of them contains the conserved His/Asp dyad, and four of them contain at least ten cysteines with eight occurring within highly conserved sequence contexts to bee venom and the remaining two in less conserved contexts. The multiple, putative $GIIIPLA_2$ genes in scorpion and fruit flies indicate that $GIIIPLA_2$ will eventually be divided into Subgroups, but the classification criteria (Table 3) need to be met first to make the right assignments.

Table 3
Criteria for phospholipase $A_2$ Group assignments

| | |
|---|---|
| 1. | The identified protein must hydrolyze the fatty acid from the *sn*-2 position of phospholipids with a reasonable specific activity, although the enzyme may also have other activities. |
| 2. | The complete protein sequence of the mature protein must be established to assign a Group or Subgroup number. |
| 3. | Homologous enzymes are distinguished as paralogs (same species) with Subgroup letters (IVA, IVB, etc.), but orthologs (different species) are not assigned separate letters. |
| 4. | Splice variants should be distinguished by Arabic numbers within a Subgroup (VIA-1, VIA-2, etc.). |

### 3.4. Group IX phospholipase $A_2$ (conodipine-M)

$GIXPLA_2$ was purified, sequenced, and characterized from the venom of the marine snail, *Conus magus* [96]. This 14 kDa protein is composed of two polypeptides joined by disulfides, though they may be encoded by the same gene. The only sequence similarity to the other Histidine $PLA_2$ Groups is the putative active site His/Asp dyad and a second Asp that putatively hydrogen bonds to the active site His. In addition, three nearby cysteines are also conserved, though the remaining nine cysteines are unique. The obvious sequence differences and much lower (20 μM) $Ca^{2+}$ requirement for 50% activity

make this a distinct $PLA_2$ Group, and the only Group IX member currently known [96].

### 3.5. *Group XI phospholipase $A_2$ (plant Histidine $PLA_2$'s)*

Recent reports have established the presence of Histidine $PLA_2$'s in plants such as rice, elm, carnation, and *Arabidopsis* [97–99]. Moreover, the rice genome has at least two unique Histidine $PLA_2$ genes that are 31% identical to each other [98]. Comparison to the various Group I, II, III, V, and X $PLA_2$'s indicates that the plant $PLA_2$'s have several conserved residues including two tyrosines and two glycines within the $Ca^{2+}$ binding loop, the active site His and two Asp's, as well as five alignable cysteines [98]. The overall low sequence identity to known Histidine $PLA_2$'s (not including large gaps or insertions, $< 25\%$) and the presence of seven unique cysteines, distinguish the plant $PLA_2$'s from all the other known Histidine $PLA_2$'s. The plant $PLA_2$ activity was conclusively demonstrated using single and double labeled dipalmitoyl-PC and observing the release of radiolabeled palmitic acid only from the *sn*-2 position of PC with a rate of approx. 100 μmoles/min/mg [98]. Having met all the criteria to be classified as $PLA_2$ enzymes, and given the low sequence homology to all other Groups, these two rice Histidine $PLA_2$'s are now defined as the Group XIA and Group XIB $PLA_2$, to correspond to the discoverers' designations of rice $PLA_2$ I and $PLA_2$ II, respectively.

## 4. Phospholipase $A_2$ Groups utilizing a catalytic serine

### 4.1. *Group IV phospholipase $A_2$ ($cPLA_2$)*

Until 1986, the only known $PLA_2$ enzymes fell into the 13–16 kDa $PLA_2$ Groups, which utilize a catalytic histidine as described in Section 3. In 1986 the initial identification and characterization of a cytosolic $PLA_2$ activity was reported from human neutrophils [22] and platelets [23]. In 1991, when the cytosolic $PLA_2$ was sequenced [24,25], this enzyme was referred to as $cPLA_2$ and is now known as the Group IVA $PLA_2$, the first Serine $PLA_2$. $GIVAPLA_2$ is an approx. 85 kDa protein consisting of two domains, a C2 domain and an α/β hydrolase $PLA_2$ domain [24,100,101]. A separate review analyzes the structural details of $GIVAPLA_2$ [102]. Studies over the years have revealed that the mechanism of $GIVAPLA_2$ utilizes a novel catalytic dyad consisting of Ser-228 and Asp-549 [101,103–105]. $GIVAPLA_2$ has marked specificity for AA at the *sn*-2 position of its substrate phospholipids [106–108]. This AA, once liberated, can then be converted into eicosanoids, such as leukotrienes and prostaglandins, potent mediators of inflammatory and signal transduction pathways. $GIVAPLA_2$ knock-out mice have confirmed the central role of $GIVAPLA_2$ in AA release and subsequent inflammatory response in brain trauma and reaffirmed its role in the initiation of parturition [109–112]. Furthermore, mast cells from these mice have confirmed the essential role of $GIVAPLA_2$ in all phases of eicosanoid release [113]. The knock-out studies have been recently reviewed [114].

The molecular basis for the in vivo effects of $GIVAPLA_2$ has been investigated in detail as well. The $GIVAPLA_2$ C2 domain is one of the best studied members of a large family of C2 domains [115] and possesses the specific properties of binding two calcium ions as well as phospholipid membranes [100,116,117]. It is likely that in vivo the C2 domain is responsible for the translocation of $GIVAPLA_2$ from the cytosol to the perinuclear membrane region in response to stimuli that increase intracellular $Ca^{2+}$ concentrations [116–118]. An intriguing mechanism of perinuclear localization involves the specific $Ca^{2+}$-dependent interaction of the C2 domain with vimentin, a cytoskeletal protein that is also localized to the perinuclear region [119].

Beyond the regulatory role of calcium through the C2 domain, $GIVAPLA_2$ is activated up to 3-fold after phosphorylation of serine-505 in vitro [120]. Phosphorylation at Ser-505 by members of the mitogen activated protein kinase family in vivo is implicated in the activation of $GIVAPLA_2$ in response to cell activation [117,120,121]. As yet, it is unclear how phosphorylation affects the activity of $GIVAPLA_2$, since the loop containing Ser-505 is not ordered in the X-ray crystallographic molecular model [101]. Additionally a large percent (50% or larger) of $GIVAPLA_2$ can be found phosphorylated under

basal or non-stimulated conditions, clouding the role of increased phosphorylation towards cellular activation of $GIVAPLA_2$ [5,117,120].

Lastly, it has been shown that $GIVAPLA_2$ has a high affinity and specificity for phosphatidylinositol 4,5-bisphosphate ($PIP_2$) [122]. The $PIP_2$-mediated interaction with the interface also leads to increased activity of $GIVAPLA_2$ [122–124]. As many phosphoinositide-protein interactions are mediated through a pleckstrin homology domain, it is interesting that there is clearly no such domain in $GIVAPLA_2$ [101]. Recently it has been shown that $GIVAPLA_2$ can be activated to release AA without any increase in intracellular calcium concentration [125]. This activation occurs with a concomitant increase in $PIP_2$ levels [125]. Moreover, in the activated cells, inhibiting the synthesis of $PIP_2$ from phosphatidylinositol 4-phosphate correlates to a decrease in AA release [125]. Finally any increase of $PIP_2$ levels in resting cells, by direct addition or stimulation of its production, is sufficient to activate $GIVAPLA_2$, resulting in increased AA release [125].

#### 4.1.1. $GIVBPLA_2$

Searches of the EST databanks revealed two novel $GIVPLA_2$ paralogs in the human genome [66,108,126]. Using a human EST, $GIVBPLA_2$ has been cloned in its entirety, defining a mature protein of 1012 amino acids with approx. 30% identity to $GIVAPLA_2$ [66,126]. The mRNA is expressed ubiquitously, although a much higher level is seen in pancreas, liver, heart and brain [66,126]. The $GIVBPLA_2$ enzyme is about 114 kDa with a unique 242 residue amino-terminal extension and a unique 120 insertion after the C2 domain [66,126]. $GIVBPLA_2$ appears to be calcium dependent in the same manner as $GIVAPLA_2$, has high homology to $GIVAPLA_2$ near the catalytic residues Ser-228 and Asp-549, but does not have any of the four serines that have been shown to be phosphorylated in $GIVAPLA_2$ [66,126]. The homologous active site residues were shown to be critical via site-directed mutagenesis [66]. While Group IVA $PLA_2$ clearly functions as a $PLA_2$ in vivo, it has also been shown to have $lysoPLA_1/A_2$ and transacylase activities in vitro [104,127,128]. Interestingly, lysates from cells overexpressing $GIVBPLA_2$ seem to possess higher $PLA_1$ activity and $lysoPLA_2$ activity than $PLA_2$ activity, but more detailed studies are needed for definitive conclusions [126].

#### 4.1.2. $GIVCPLA_2$

The $GIVCPLA_2$ is a 541 amino acid protein of 61 kDa with slightly less than 30% homology to $GIVAPLA_2$. The mRNA is expressed most highly in heart and skeletal muscle [66,108]. Just as for $GIVBPLA_2$, the active site residues were identified within regions of higher homology and shown to be critical for activity using site-directed mutagenesis [66,108]. Consistent with its smaller size, $GIVCPLA_2$ lacks a C2 domain and has no $Ca^{2+}$-dependence [66,108]. It was also shown that $GIVCPLA_2$ has much lower specificity for AA (3.5-fold) relative to $GIVAPLA_2$ (24.5-fold). $GIVCPLA_2$ was also able to hydrolyze both acyl chains from substrate phosphatidylcholine with clear $PLA_2$ activity [126] and probably $PLA_1$ activity [108], and presumed $lysoPLA_1$ and $A_2$ activities.

#### 4.1.3. Fungal $GIVPLA_2$ homologs

The sequence of $GIVAPLA_2$ contains Ser-228 within a lipase consensus for phospholipase B (PLB) from *Penicillium notatum* (Gly-X-Ser-X-Gly) and has been shown to be the active site serine [101,103,105]. Additional alignments to yeast PLB's show that all three of the critical residues (Ser, Asp, Arg) for $GIVAPLA_2$ align exactly with the PLB's [105]. PLB's by definition can hydrolyze both acyl chains from phospholipid substrates, a phenomenon also reported for the $GIVCPLA_2$, suggesting biochemical similarity along with the sequence similarity. One of the best studied PLB's is from *P. notatum*, and has been studied since the 1960's as a known lecithin acylhydrolase [129]. When the *P. notatum* PLB was sequenced, it was shown to have a large number of asparagine-linked carbohydrates, which is now known to be a common feature of PLB's [130,131].

By far the best studied PLB's include those from *Saccharomyces cerevisiae*, which have four diverse PLB-like genes in their genome [132–134]. Of these four, PLB1 (41% identical to *P. notatum*) was shown to be responsible for much of the PLB and lysoPL activity from yeast, using knock-out and overexpression techniques [133]. Analysis of PLB2 (64% identical to PLB1) shows it also has significant PLB/ly-

soPL activity [132,134], and PLB3 seems to have PLB/lysoPL activity specific for phosphoinositides [134]. Taken together the sequence similarity and biochemical similarities suggest that the PLB's (so far only fungal) be classified as GIVPLA$_2$'s but not yet assigned to any specific Subgroup, pending the biochemical analysis of GIVCPLA$_2$ and cloning of further GIVPLA$_2$ and PLB sequences.

### 4.2. *Group VI phospholipase $A_2$ (iPLA$_2$ – see also [28,135])*

The first cloned and characterized PLA$_2$ with no calcium dependence is often referred to as the iPLA$_2$ for calcium-independent PLA$_2$ and is now classified as GVIA-1 PLA$_2$. GVIA PLA$_2$ was first isolated and characterized from P388D$_1$ macrophages [136]. The GVIA-1 PLA$_2$ was cloned from CHO, P388D$_1$, and human lymphoma cDNAs, and shown to be a novel PLA$_2$ of approx. 750 amino acids and 85 kDa [137,138], containing eight ankyrin repeats [137–139]. Moreover, GVIA-1 PLA$_2$ also contains a lipase consensus sequence (Gly-X-Ser$^{465}$-X-Gly), which does contain the active serine as shown by mutagenesis [137]. Furthermore, inhibitors of the serine-containing Group IVA PLA$_2$ as well as the serine-modifying inhibitor bromoenol lactone (BEL) inhibit the GVIA-1 PLA$_2$, confirming the role of serine as the nucleophile [140]. Moreover, the GVIA-1 PLA$_2$ has some weak transacylase activity, indicating the formation of an acyl-enzyme intermediate on the serine [141]. As yet no further active site residues have been identified, though a truncated protein lacking the N-terminal 405 residues has no activity [137]. GVIA-1 PLA$_2$ was shown to be active as an oligomer through radiation inactivation studies [136]. Additionally, a purified Group VIA PLA$_2$ was initially shown to be activated by ATP [136], although more recently ATP was shown to be acting as a protectant which leads to the appearance of activation [141].

Subsequent to the initial cloning of hamster, human, and mouse GVIA-1 PLA$_2$, it was discovered that there are multiple splice variants of the human GVIAPLA$_2$ gene [27,67,142]. A longer (88 kDa) splice variant, with a 54 amino acid insert within the eighth ankyrin repeat was shown to have activity and accordingly is referred to as GVIA-2 PLA$_2$ [142]. Two other inactive splice variants were identified at the same time, consisting primarily of the first seven ankyrin repeats with variable termini [142]. The first inactive splice variant, GVIA Ankyrin-1, was shown to decrease the activity of GVIA-2 PLA$_2$ when co-transfected, indicating hetero-oligomers may form and be inhibited by the inactive splice variants [142].

The initially described sequence of human GVIA-1 PLA$_2$ has more recently been identified in pancreatic islet cells [27]. Recombinant expression confirmed a very similar activity to the GVIA-1 PLA$_2$ from mouse and hamster, and the GVIA-2 PLA$_2$ from humans [27]. The human GVIA-1 PLA$_2$ and GVIA-2 PLA$_2$ are not identical though, since activity of human GVIA-1 PLA$_2$ seems unaffected by ATP, while the activity of GVIA-2 PLA$_2$ was enhanced by ATP [27]. Further results demonstrated that the GVIA-2 PLA$_2$ was membrane-bound in vivo, perhaps due to the insertion of 54 amino acids [67]. The tissue-dependent expression patterns of each of these splice variants, as well as other splice variants identified but not characterized, are treated in more detail in a separate review [28].

Thus far conclusive evidence based on inhibition of GVIAPLA$_2$ by BEL and specific antisense oligonucleotide-based inhibition has demonstrated in several cell systems that GVIAPLA$_2$ plays a key role in phospholipid remodeling and homeostasis by the production of lysophospholipid [11,28,143–145]. In addition to the clear role GVIAPLA$_2$ plays in phospholipid remodeling and homeostasis, several other reports implicate roles for GVIAPLA$_2$ in signal transduction and other physiological processes, and each of these will also be summarized elsewhere [28]. The existence of multiple splice variants and the oligomeric nature of the active GVIAPLA$_2$ suggest complex roles for GVIAPLA$_2$.

#### 4.2.1. *GVIBPLA$_2$*

Very recently a novel calcium-independent PLA$_2$ was identified based on a preliminary sequence derived from the Human Genome Project, and further analysis of EST's allowed for the cloning of the full sequence from heart and skeletal muscle cDNA [68] and independently from human lymphocyte cDNA [70]. The sequence has approx. 25% identity to GVIA-1 PLA$_2$ over the conserved 437 amino acid

core. The mRNA of around 3.4 kb was detected in heart, placenta, kidney, liver, brain, and skeletal muscle [68,70]. Although lacking a definitive Kozak consensus sequence for an initiator methionine, the predicted maximum size for $GVIBPLA_2$ is 88 kDa [68]. In vitro translation [68] or expression in COS-7 cells [70] yielded $PLA_2$ activity corresponding to an approx. 90 kDa protein as detected by $GVIBPLA_2$ antibodies. Nevertheless, expression of $GVIBPLA_2$ in insect cells gave immunoreactive bands of 77 and 63 kDa, seemingly corresponding to later initiator methionine codons [68]. In all cases thus far $GVIBPLA_2$ protein activity has been identified only in membrane fractions of lysed cells [68,70]. This novel $PLA_2$ was able to hydrolyze PC with a variety of fatty acids at the *sn*-2 position including plasmenyl-PC, indicating $PLA_2$-specific activity [68]. The activity was also inhibited by the irreversible serine hydrolase inhibitor, BEL, with an $IC_{50}$ of around 3 μM with 3 min preincubation [68]. A $GVIBPLA_2$ ortholog has been identified in *Caenorhabditis elegans* as a 546 amino acid protein that has 47% identity to the catalytic region of $GVIBPLA_2$ [70]. Given the sequence similarity and biochemical similarity to $GVIAPLA_2$, this human gene product (or products) and its species homologs are classified as $GVIBPLA_2$'s.

#### 4.2.2. Other $GVIPLA_2$ homologs

Searching the databases reveals proteins from distant species related to the mammalian Group VI $PLA_2$'s. The best studied example is a confirmed $PLA_2$ from potatoes and other plants known as patatin [146]. Potato patatin (approx. 40 kDa) has $PLA_2$ activity that is much higher than its $PLA_1$ activity for PC substrate, although the role of a second lyso$PLA_1/A_2$ reaction was not investigated [146]. While active in the absence of $Ca^{2+}$, patatin has a $Ca^{2+}$-dependent activity increase of up to 3-fold [146], but the significance of this is not clear. One other patatin homolog (48% identity with potato) has been cloned and characterized from cucumber seedlings [147]. It has been shown to have no general lipase activity, and its $PLA_2$ activity is at least 15 times the $PLA_1$ activity on PC substrates [147]. With the recent discovery of $GVIBPLA_2$ it is difficult to determine to which current Subgroup of $GVIPLA_2$ patatin belongs, if any. Both patatins have the same lipase consensus sequence as the $GVIAPLA_2$ (Gly-Thr-Ser-Thr-Gly), but the whole lipase domain is equally conserved between cucumber patatin and both Group VIA and VIB $PLA_2$, so clear Subgroup placement is pending further sequence and enzymological data.

### 4.3. Group VII phospholipase $A_2$ (PAF-acetylhydrolase)

#### 4.3.1. $GVIIAPLA_2$ (plasma PAF-acetylhydrolase)

The $GVIIAPLA_2$ is the well studied plasma platelet activating factor acetylhydrolase (pPAF-AH) (see [12–14,148] for reviews and further references). Platelet activating factor (PAF) is a form of phosphatidylcholine where the *sn*-1 chain is linked by an ether linkage and the *sn*-2 position has an acetyl group. PAF has many physiological effects [13], including potent proinflammatory effects, which can be abrogated by hydrolysis of the *sn*-2 acetyl group to form lyso-PAF [12,13].

First cloned in 1995, $GVIIAPLA_2$ was found to be a 45 kDa protein of 441 amino acids containing a lipase consensus motif of Gly-X-$Ser^{273}$-X-Gly [149]. Furthermore, site-directed mutagenesis has confirmed the identity of a classic hydrolase triad of Ser-273, Asp-296 and His-351 [150]. A molecular model of the $GVIIAPLA_2$ (as well as the $GVIIBPLA_2$) has been created using the distantly related lipase from *Streptomyces exfoliatus*, indicating that both $GVIIPLA_2$ enzymes are α/β hydrolases with catalytic triads [12,151].

$GVIIAPLA_2$, while known best for its PAF-acetylhydrolase activity, can also hydrolyze short chain oxidized fatty acids of up to nine carbons in length from the *sn*-2 position of PC or PE without regard to the nature or length of the *sn*-1 chain [152–154]. $GVIIAPLA_2$ is found circulating in the bloodstream of most animals, and in humans is associated with the apolipoprotein B100 (apoB100) of low density lipoprotein (LDL) and high density lipoprotein [153–156]. The presence of oxidized phospholipids on LDL is associated with pathological conditions such as atherosclerosis, and therefore the localization of $GVIIAPLA_2$, which can cleave the oxidized acyl chain of the oxidized lipid, may function in a protec-

tive role [157–159]. Specifically the human, and not the mouse, GVIIAPLA$_2$ associates with apoB100 and the difference seems to be in the critical residues Trp-115 and Leu-116 [156,160]. While the interactions between GVIIAPLA$_2$ and ApoB100 are clear, the physiological significance of this interaction is not clear. Recently it was shown that the GVIIAPLA$_2$ is not an interfacial enzyme, meaning that it does not access its substrate from a phospholipid aggregate such as a membrane, but rather only accesses PAF or oxidatively-truncated phospholipids from solution as substrate monomers [152]. More details of PAF-acetylhydrolases are presented in separate reviews [14,46].

#### 4.3.2. GVIIBPLA$_2$

The GVIIBPLA$_2$ is an intracellular enzyme first purified and characterized from bovine brain as PAF-AH II [161]. GVIIBPLA$_2$ has significant sequence identity (41%) to the GVIIAPLA$_2$, including the lipase consensus motif Gly-X-Ser-X-Gly, and is also a monomer of around 40 kDa and 392 amino acids [162]. In much the same way as GVIIAPLA$_2$, GVIIBPLA$_2$ is also able to hydrolyze *sn*-2 acyl chains containing not only acetyl (two carbons) but chains as long as glutaroyl (five carbons with terminal carboxylic acid) [161]. It is not yet known whether GVIIBPLA$_2$ also hydrolyzes only monomeric substrate as does GVIIAPLA$_2$ [152].

GVIIBPLA$_2$ is highly expressed in the liver and kidney, and to a lesser extent in other tissues [162]. Further analysis indicates that GVIIBPLA$_2$ is myristoylated at its N-terminus in a consensus sequence of Met-Gly-X-X-X-Ser [163]. Although present in the cytosol, GVIIBPLA$_2$ is partially localized to the endoplasmic reticulum near the nuclear envelope presumably facilitated by its myristoylation [163]. With the addition of oxidative stress agents, GVIIBPLA$_2$ translocated completely to the membrane in a short time, and on a longer timescale translocated to the cytosol in response to antioxidants [163]. Furthermore, cells overexpressing only the active GVIIBPLA$_2$ were resistant to oxidative stress-induced apoptosis [163]. This suggests that while GVIIAPLA$_2$ plays a protective role against oxidation in the plasma, the intracellular GVIIBPLA$_2$ protects cells in the liver and kidneys from oxidative damage.

### 4.4. Group VIII phospholipase A$_2$ (PAF-AHIb)

#### 4.4.1. GVIIIAPLA$_2$ and GVIIIBPLA$_2$

The Group VIIIA and VIIIB PLA$_2$ enzymes are expressed intracellularly in the brain as the active subunits of heterotrimeric PAF-AHIb [164]. The 26 kDa GVIIIAPLA$_2$ and GVIIIBPLA$_2$ have approx. 62% identity and exist as two subunits within the heterotrimeric PAF-AHIb [165–167]. The heterotrimer consists of homodimers or heterodimers of the GVIIIPLA$_2$'s plus one regulatory subunit of 45 kDa [166,168]. Since these are clearly different genes, they have been named here as GVIIIAPLA$_2$ and GVIIIBPLA$_2$. Historically the two catalytic subunits were referred to as the 29 and 30 kDa subunits, then as the β- and γ-subunits, and finally as the $\alpha_1$- and $\alpha_2$-subunits of PAF-AHIb [164,166,168]. The first catalytic subunit to be cloned was GVIIIAPLA$_2$ (the $\alpha_1$-subunit) from bovine brain [165] followed by the GVIIIBPLA$_2$ (the $\alpha_2$-subunit) from bovine brain [166]. The sequences of approx. 230 amino acids for both GVIIIAPLA$_2$ and GVIIIBPLA$_2$ contain a serine in a pseudo-lipase consensus motif of Gly-X-Ser-X-Val [165], which has now been identified as the nucleophilic serine [164,166].

The third, regulatory subunit of the PAF-AHIb was found to be the protein encoded for by the causative gene (LIS-1) for Miller-Deicker lissencephaly, an abnormal neural migration effect resulting in a devastating brain cortex malformation [169,170]. LIS-1, a homolog of the β-subunit of trimeric G proteins, has now been shown to be a microtubule associating phosphoprotein that occurs in a larger complex with several kinases and accessory proteins (see [170] and references therein). PAF itself has also been shown to be important in neural cell migration [171] and as a retrograde messenger in long term potentiation [172]. The exact role of the GVIIIPLA$_2$'s and their activity is not yet clear in the neuronal system, but the LIS-1/PAF pathways are assuredly linked given the genetic defects mapped to these proteins [173].

The GVIIIPLA$_2$ enzymes exhibit exquisite substrate specificity for PAF with $<5\%$ of the PAF activity for priopionyl-PAF in contrast to approx. 50% for GVIIPLA$_2$ [161]. Each GVIIIPLA$_2$ Subgroup is expressed to a different extent in various species, various tissues, and various stages of devel-

opment [167,174,175], and while the significance of this is not clear, a switching mechanism has been postulated for rodents where $GVIIIAPLA_2$ is not expressed in the adult tissues including the brain [176]. All three proteins of PAF-AHIb are extremely well conserved among the known species, perhaps among the best conserved proteins known [175]. In vitro the $GVIIIPLA_2$'s are able to form active homo- or heterodimers that do not seem to exchange [166,173,176]. The three combinations of dimers are all active, but do exhibit different head group preference for the PAF substrate, in that the $GVIIIAPLA_2$ homodimer prefers PE while the $GVIIIBPLA_2$ homodimer and heterodimer prefer phosphatidic acid [173]. The LIS-1 protein was shown to have different effects on the three different dimers, accelerating the $GVIIIAPLA_2$ homodimer activity, not affecting the heterodimer activity, and suppressing the $GVIIIBPLA_2$ homodimer activity [173]. The structure has been determined for $GVIIIAPLA_2$ to 1.7 Å resolution revealing that this novel serine $PLA_2$ has a tertiary fold similar to small GTPases, and remarkably, the heterotrimer is a G protein-like trimer [164].

## 5. Miscellaneous phospholipase $A_2$ activity and homology

Over the years many $PLA_2$ activities have been reported, and most of them are now attributable to a specific Group member of the $PLA_2$ superfamily, either by knowing the sequence or by matching the unique set of enzymatic characteristics. In order to be classified as a novel $PLA_2$ Group or Subgroup member by the criteria given in Table 3, the sequence must be known. Although many of these "orphan" $PLA_2$ activities are mentioned in other detailed reviews, the requirement of a complete sequence leaves only one major and one minor example that will be addressed to reduce significant confusion in the databases and the literature. The minor example is that of the 14-3-3 protein that has some homology to Histidine $PLA_2$'s and was thought to have $PLA_2$ activity [177], but was later thoroughly analyzed and shown to be devoid of $PLA_2$ activity [178].

In 1997 the characterization and sequencing of a protein possessing $Ca^{2+}$-independent $PLA_2$ activity at pH 4.0 (approx. 2 nmol/min/mg), that was inhibited by two serine hydrolase inhibitors, were reported in the literature [179,180]. This enzyme was referred to as lysosomal or acidic calcium-independent $PLA_2$ [179,180]. This same protein was later identified as a 1-cysteine peroxiredoxin, confirmed to possess hydrogen peroxide peroxidase activity in vitro at pH 7.0, localized to the cytosol, and has peroxidase activity in vivo [181]. Moreover, its trace $PLA_2$ activity at pH 4.0 was confirmed, but mutagenesis of the cysteine and a serine had no effect on the $PLA_2$ activity [181]. Further analysis showed this human peroxidase to be identical to a glutathione peroxidase which is important for response to oxidative stress in the eye and skin [182,183]. The resolution of this confusion came when it was conclusively shown that this enzyme is actually a non-selenium glutathione peroxidase that can reduce oxidized phospholipid hydroperoxides with glutathione as an electron donor [184]. Therefore, this non-selenium, 1-cysteine, glutathione peroxidase is not a $PLA_2$.

In addition to those proteins that have been reported to have $PLA_2$ activity, there are many instances of Histidine $PLA_2$-like proteins, whose active sites are modified, thus impairing activity. Several venom or toxin proteins have been shown to have homology to Histidine $PLA_2$'s but have lost $PLA_2$ activity, though they may modulate the $PLA_2$ activity of other homologs [1,185]. Another confusing example that appears in the databases is otoconin-90/95, a 90–100 kDa protein found as $>90\%$ of the protein in the protein-calcium carbonate crystal lattice termed the otoconia [186]. These crystals overlay the inner ear otoconial membranes of the saccule and utricle within the vestibule and are crucial for the detection of linear acceleration and balance control in mammals and amphibians [186,187]. The primary structure of otoconin-90/95 shows that it contains two Histidine $PLA_2$-like domains, related to Groups I, II, V, and X $PLA_2$, that are not thought to be active, since they have several mutations in key active site residues such as an Asp [186–188]. These divergent $PLA_2$ paralogs, found separately from all other known $PLA_2$'s on chromosome 8q24 [189], shall not be classified as $PLA_2$'s solely because they do not have proven $PLA_2$ activity, although their study and comparison to $GIIPLA_2$ structurally may be useful [186,187].

## 6. Conclusions

The pace of discovery of new $PLA_2$ Group members has increased recently, largely beginning with computerized EST and genome searches or low stringency PCR and nucleotide-based searches, leading to the classification of 11 $PLA_2$ Groups with a total of 23 Subgroups described herein. With the completions of the genomes from many model organisms and the impending completion of the human genome project, many more homologous $PLA_2$ genes will certainly be discovered and perhaps more novel Groups as well. This sets the stage for the better classification of various ambiguous orthologs and paralogs within and among the various Groups. Moreover, the growing database of structures for the various $PLA_2$'s may prove just as helpful in determining the relationships between the various enzymes. When the sequencing is completed, it will be much easier to determine the exact relationships between the many Groups and Subgroups that have led to the current classification system and might lead to a more consistent and useful system to understand the phospholipase $A_2$ superfamily relationships.

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