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Phospholipase A₂ Biochemistry

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Abstract

The phospholipase A₂ (PLA₂) superfamily consists of many different groups of enzymes that catalyze the hydrolysis of the sn-2 ester bond in a variety of different phospholipids. The products of this reaction, a free fatty acid, and lysophospholipid have many different important physiological roles. There are five main types of PLA₂: the secreted sPLA₂'s, the cytosolic cPLA₂'s, the Ca²⁺-independent iPLA₂'s, the PAF acetylhydrolases, and the lysosomal PLA₂'s. This review focuses on the superfamily of PLA₂ enzymes, and then uses three specific examples of these enzymes to examine the differing biochemistry of the three main types of these enzymes. These three examples are the GIA cobra venom PLA₂, the GIVA cytosolic cPLA₂, and the GVIA Ca²⁺-independent iPLA₂.

1. Introduction

PLA₂s form a superfamily that currently contains fifteen separate, identifiable groups and numerous subgroups of PLA₂ [1–3]. These enzymes are characterized by their ability to specifically hydrolyze the sn-2 ester bond of phospholipid substrate as shown in Fig. 1. Enzymes are assigned to groups based on sequence, molecular weight, disulfide bonding patterns, the requirement for Ca²⁺, etc. There are five main categories of PLA₂: the secreted small molecular weight sPLA₂s, the larger cytosolic Ca²⁺-dependent cPLA₂s, the Ca²⁺-independent iPLA₂s, the PAF acetylhydrolases, and the lysosomal PLA₂'s.

The products of the hydrolysis of the sn-2 ester bond of phospholipid are a free fatty acid and lysophospholipid. Both of these products represent the first step in generating important second messengers that play important physiological roles. Arachidonic acid (AA) when released from the sn-2 position of phospholipids can be converted into eicosanoids through the action of a variety of different downstream enzymes [4]. These eicosanoid molecules can exert a wide range of physiological and pathological effects. The lysophospholipid can be converted into lyso phosphatidic acid or be acetylated into platelet activating factor which also plays a variety of physiological roles [5,6].

This review aims to introduce the superfamily of PLA₂ enzymes, their mechanism of action, as well as focusing on three well defined enzymes of the sPLA₂, cPLA₂, and iPLA₂ types.

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2. The PLA₂ Superfamily of Enzymes

2.1. Secreted PLA₂ Enzymes

PLA₂ activity was first studied in phenomenological detail as early as the 1890's using "poison" or venom from cobras [7,8]. The group numbering system was originally used to distinguish between different snake venoms, with the first use of the group numbering system seen in 1977 with Group I/II to distinguish between venoms from rattlesnakes and vipers from cobra and kraits [9], based on differences in disulfide bonding patterns. The secreted PLA₂s are characterized by their requirement for histidine in the active site, low molecular weight, Ca²⁺ requirement for catalysis, and the presence of six conserved disulfide bonds, with one or two variable additional disulfide bonds [1,2]. The mechanism of action/structure of the GIA PLA₂ will follow in the following section, as well as mechanistic differences between the GIA sPLA₂ and other group members. The first non-venom PLA₂ named GIB was isolated from the pancreatic juices of cows, and was also found in many other animals [10]. There is strong evidence that this enzyme plays a major role in the digestion of phospholipids in the stomach [11,12]. This was followed by the isolation of many other mammalian and other forms of secreted PLA₂'s shown in Table 1. The name sPLA₂ was coined from the high content of GIIA PLA₂ in the synovial fluid of patients with rheumatoid arthritis [13], but has come to stand for secreted.

All of the sPLA₂ enzymes (except group III) [14] display a characteristic increase in activity when substrate is switched from monomeric to higher ordered lipid aggregates, and this is known as interfacial activation [15]. The mechanism of interfacial activation will be considered in the next section on the GIA PLA₂.

The sPLA₂ group of enzymes has a vast variety of physiological functions. One of the most defined biological functions is for Group IIA PLA₂, which has been shown to be important as an antimicrobial agent by hydrolyzing the negatively charged membranes of gram negative bacteria [16,17]. The exact physiological role of sPLA₂ enzymes in production of eicosanoids has remained undefined. For more in depth analysis of the biological actions of the mammalian sPLA₂ enzymes please see [18,19].

2.2 Cytosolic PLA₂'s

The cytosolic PLA₂'s are larger than the sPLA₂ enzymes (61–114 kDa) and do not have the same disulfide bonding network as the sPLA₂ enzymes. The first cytosolic PLA₂ was isolated from neutrophils and platelets in 1986. The complete list of these enzymes is shown in Table 2. The detailed mechanism and biology of the GIVA PLA₂ will be explained in detail in Section 4. These enzymes all function through the action of a serine/aspartic acid dyad. All of the cytosolic PLA₂'s (except GIVC [20]) require Ca²⁺ for activity, due to the presence of C2 domains [21–24]. The different GIV enzymes have different specificity for fatty acids in the sn-2 position. GIVA is specific for AA containing phospholipids [25], GIVB and GIVC have very little specificity [22,23], GIVD appears to be specific for linoleic acid (LA) containing fatty acids [24], while GIVE and GIVF hydrolyze both AA and LA [21]. For a more in-depth analysis of the biology of the other cytosolic enzymes see reviews [1,26].

2.3 Ca²⁺ Independent PLA₂'s

The Ca²⁺ independent PLA₂ (iPLA₂s) includes six different types GVIA, GVIB, GVIC, GVID, GVIE, and GVIF PLA₂ as shown in Table 3. The term Ca²⁺ independent PLA₂ is misleading for the Group VIA enzymes. All of the enzymes in this group do not require Ca²⁺ for activity, but the GIVC enzyme also does not require Ca²⁺ for activity, but was placed in the Group IV of PLA₂ enzymes due to homology to other GIV enzymes [20]. All of these enzymes function through a catalytic serine at the active site. There is no fatty acid chain specificity seen in any

of the GVI enzymes. The inhibitor BEL inhibits specifically GVIA, and GVIB, however the inhibition is mediated through different enantiomers [27]. This property has allowed for studies focusing on specific GVI enzymes [28]. For reviews of the other GVI enzymes see [1].

2.4 PAF acetylhydrolases

The PAF acetylhydrolases are composed of two PLA₂ groups that both hydrolyze the acetyl group from the sn-2 position of platelet activating factor (PAF) as shown in Table 4. The function of this class of enzymes is of high interest due to the important roles played by PAF in the body. All of these enzymes function through the action of a catalytic serine. The PAF acetylhydrolases all have a Ser/His/Asp catalytic triad mediating hydrolysis [29,30]. These enzymes do not require Ca²⁺ for activity. The GVIIA PLA₂ is a secreted enzyme that can also hydrolyze short chain fatty acids from the sn-2 position [31]. This enzyme is not interfacially activated [31]. This enzyme is also known as the lipoprotein associated PLA₂. Recent work has identified regions in the catalytic domain important for binding to both HDL and LDL cholesterol molecules [32]. The mechanisms that impart preferences for HDL and LDL are currently poorly understood. The GVIII PAF acetylhydrolases are also regulated through aggregation of the catalytic and regulatory subunits [33]. For excellent reviews on the biology of lipoprotein associated PLA₂ and relation to cardiovascular disease see [34,35].

2.5 Lysosomal PLA₂

The lysosomal PLA₂ is the newest type; it was purified from bovine brain and acylates ceramide using the acyl group from the sn-2 position of phospholipid as substrate [36,37]. This enzyme contains a conserved Ser-His-Asp triad and has four cysteine residues that are required for catalytic activity [38].

3. Group IA PLA₂

One of the best studied PLA₂ enzymes is the cobra venom Group IA (GIA) PLA₂. This enzyme has acted as not only an important model of phospholipid metabolizing enzymes, but of all lipid enzymology. Many different crystal structures of this enzyme exist from different venom sources [39–42]. These crystal structures all show some important traits as shown in Fig. 2. The enzyme contains the six conserved disulfide bonds from 28–44, 26–118, 43–99, 50–92, 60–85, and 78–80, as well as the additional disulfide bridge from 11–71 [39]. The active site dyad is composed of the conserved His-48, and Asp-99. The active site histidine is found to be conserved in all sPLA₂ enzymes [15,43–45]. The enzyme catalyzes hydrolysis through the activation of a water molecule by extraction of a proton, and attack at the sn-2 ester bond [15,46,47]. This mechanism explains the pH dependence of these enzymes at around 7–9. Recent work using unnatural phospholipid substrate with PC headgroups in the sn-2 position have shown that phospholipid hydrolysis is proportional to the ease of water accessibility to the active site [48,49]. The enzyme binds Ca²⁺ through the conserved Asp-49 [50,51], as well as the carbonyl oxygens of Tyr-28, Gly-30, and Gly-32 [39]. The Ca²⁺ ion is required for hydrolysis through orientation of the lipid substrate by coordination of the negative charge from the phosphate oxygen [40]. Some structures have shown the presence of a secondary Ca²⁺ ion that may act as a supplementary electrophile [40].

NMR studies of the GIA PLA₂ with inhibitor bound in the active site have allowed for the creation of a model of substrate bound in the active site as shown in Fig. 2. [52,53]. The fatty acid tails of the substrate are surrounded by the hydrophobic residues Leu-2, Phe-5, Trp-19, Tyr-52, and Tyr-69. Crystal structures of the GIA PLA₂ with bound inhibitors closely matches this result [42]. The enzyme has very little selectivity for the fatty acid in the sn-2 position [54].

This enzyme is able to hydrolyze monomeric phospholipid substrates, but there is a substantial increase in activity when the enzyme acts on large lipid aggregates [55]. This enzyme has also been shown to be activated by phospholipids containing phosphatidylcholine (PC) head groups [56], and two possible sites for this interaction have been suggested [41,57]. A combination of site-directed mutagenesis and equilibrium dialysis has identified and confirmed there is an activator site distinct from the catalytic site [58,59].

The different sPLA₂'s all share different preferences for the charge state of the lipid membrane. For an excellent analysis of the different mouse and human sPLA₂ membrane preferences see [60]. The majority of sPLA₂ enzymes preferentially hydrolyze anionic substrates [18]. The GIA enzyme however is able to hydrolyze zwitterionic substrate equally as well as negatively charged lipid surfaces [56,61]. This is most likely due to the aromatic residues present on the interfacial binding surface of the GIA PLA₂ as shown in Fig. 2. Mutation of these residues significantly decreases the membrane binding of this enzyme [59,61].

In comparison, the crystal structures of the GIB and GIIA enzymes demonstrate these enzymes have a cationic interfacial binding surface, and this may play a large role in their preference for anionic lipids [44,62]. It has been shown that a mutant form of the GIB enzyme with the pancreatic loop from 62–66 removed has increases in activity against zwitterionic substrate, and a decrease in activity against negatively charged substrate [63]. Recent studies using a GIIA enzyme with a Trp residue mutated into the interfacial binding region dramatically increased zwitterionic phospholipid hydrolysis [64], as well as penetration [65,66]. The only other sPLA₂ enzymes to have high affinity for zwitterionic vesicles are the GV, and GX enzymes [67–69] which also share the characteristic of having Trp residues in the interfacial binding region.

4. Group IVA PLA₂

GIVA PLA₂ is an 85 kDa enzyme that utilizes a catalytic serine for hydrolysis rather than histidine, as in the sPLA₂ enzymes. This enzyme was initially isolated from human neutrophils [70], and platelets [71]. This enzyme was sequenced in 1991, and was shown to be specific for phospholipids containing arachidonic acid in the *sn*-2 position [72]. The crystal structure of the C2 domain was solved in 1998 [73] followed by the whole enzyme in 1999 and showed a Ca²⁺ binding C2 domain important for Ca²⁺ mediated membrane translocation, and a α/β hydrolase domain that contains the catalytic site [74]. The crystal structure is shown in Fig. 3. Of special note in this structure is the presence of a lid region that spans regions 415–432 that prevents the modeling of a phospholipid substrate in the active site. This structure confirmed previous work using mutant constructs showing the two independent functions of the C2, and catalytic domain [75]. This structure showed the presence of a novel Ser/Asp dyad that mediated the hydrolysis of phospholipid substrate. The enzyme hydrolyzes substrate through the formation of a serine-acyl intermediate [76,77]. Previous work had suggested that the active site residues would consist of Ser-228, Asp-549, and Arg-200 due to inactivity of mutants containing mutations at any of these locations [78]. This crystal structure shows that Ser-228, and Asp-549 are in the correct orientation to act as an active site dyad, but Arg-200 is too far away to form any contacts with either Ser-228, or Asp-549. This led to the proposal that Arg-200 may be important in binding the charged headgroups of phospholipid substrate [74].

This enzyme also has both lysophospholipase, and transacylase activity [79,80], however the lysophospholipase activity of this enzyme is insensitive to Ca²⁺ concentration. The PLA₂ activity of the enzyme is active against monomeric substrate, but there is a substantial activation upon binding a membrane surface [75]. For the enzyme to be active, it must be sequestered to a phospholipid interface. The binding of the GIVA PLA₂ to the membrane is mediated through

three mechanisms: Ca^{2+} mediated translocation, binding of secondary lipid messengers, and phosphorylation.

Ca^{2+} binding in the GIVA PLA_2 is not required for catalysis as in the s PLA_2 enzymes, but is required for translocation to the membrane surface [81–86]. Ca^{2+} binding is mediated through the C2 domain of the enzyme. C2 domains are conserved domains present on many different lipid binding proteins (For an excellent review on membrane binding domains see [87]). The mechanism of Ca^{2+} binding to the C2 domain, and how this mediates phospholipid binding, has been studied through a variety of techniques, including x-ray reflectivity, site directed mutagenesis, NMR, EPR, and computational methods [88–94]. These studies have shown that Ca^{2+} binding to this domain sequesters the protein to the lipid surface through penetration of Ca^{2+} binding loops one and three, composed of amino acids 35–39, and 96–98, into the interface. The C2 domain of the GIVA PLA_2 is specific for membranes with PC headgroups [92,95].

The GIVA PLA_2 is also activated by binding many different lipid second messengers. It has been shown that phosphatidylinositol (4,5) bis phosphate (PIP_2) significantly activates the enzyme in a Ca^{2+} independent manner [96,97]. The location of the PIP_2 binding site was identified through the use of site directed mutagenesis and is located at four lysines at position 485, 541, 543, and 544 [97,98] as shown in Fig. 3. We have also shown that this PIP_2 activation requires the presence of the C2 domain, even though the PIP_2 binding site is completely contained on the catalytic domain [97]. Recently the lipid ceramide 1 kinase was discovered to also be an activator of GIVA PLA_2 [99–101]. Ceramide 1-phosphate (C1P) binds to the enzyme at a specific site in the C2 domain consisting of Arg-57, Lys-58, and Arg-59 shown in Fig. 3. [102]. Studies have also shown that the mechanism of C1P activation is Ca^{2+} dependent, and decreases the kinetic dissociation constant from the membrane surface [103] while PIP_2 activation is caused by an increase in the catalytic efficiency, potentially through a conformational change [103].

The phosphorylation state of the GIVA PLA_2 also plays an important role in mediating lipid-enzyme interactions. Many different residues on the GIVA enzyme can be phosphorylated by a myriad of different kinases. The main residues found phosphorylated are Ser-505, Ser-515, and Ser-727. These residues are phosphorylated by mitogen activated protein kinases (MAPKs), mitogen activated protein kinase interacting kinase (MNK1), calmodulin kinase II (CamKII), and mitogen activated protein kinase interacting kinase (MNK1) respectively [104–107]. Other phosphorylation sites have been reported at Ser-437, and Ser-454 in Sf9 cells [108], but there is currently no information on the effects of phosphorylation at these residues. Interestingly all of the residues that have been found to be phosphorylated are located in areas of the crystal structure with no traceable electron density [74]. It has been shown that Ser-505, and Ser-727 are common phosphorylation sites in agonist-stimulated human platelets and HeLa cells [104], while Ser-505, and Ser-515 phosphorylation are found in vascular smooth muscle cells [109]. Ser-505 phosphorylation has been shown to cause a very small increase in activity [110,111], however recent work studying membrane binding found a 60 fold increase in membrane affinity at $2.5 \mu\text{M}$ Ca^{2+} , and it is suggested that it induces a conformational change that causes tighter binding to the lipid surface [112]. It has recently been shown that Ser-727 phosphorylation mediates GIVA PLA_2 activity through disruption of the complex formed between annexin A2, p11, and GIVA PLA_2 [113]. Ser-515 phosphorylation has been shown to increase in-vitro activity of the enzyme 3 fold [106], and may also activate the enzyme through a conformational change.

The importance of GIVA PLA_2 in many different inflammatory processes has been proven through the use of knockout mice deficient in GIVA PLA_2 . These mice showed significant

decreases in allergic response, damage from acute lung injury, and postischaemic brain injury [114–116]. For review see [117].

5. GVIA PLA₂

The human Group VIA PLA₂ gene yields multiple splice variants, including GVIA-1, GVIA-2, GVIA-3 PLA₂, GVIA Ankyrin-1 and GVIA Ankyrin-2 [118,119]. At least two of these isoforms, GVIA-1 and GVIA-2 iPLA₂ are active. The human GVIA iPLA₂ contains 7–8 ankyrin repeats, a linker region, and a catalytic domain. The 85-kDa GVIA-2 iPLA₂ was first purified and isolated from the p388D1 cell line [120,121], which possesses PLA₂ activity as well as lysophospholipase and transacylase activity [122]. The active site serine of the GVIA iPLA₂ lies within a lipase consensus sequence (Gly-X-Ser⁵¹⁹-X-Gly) [123]. The enzyme is not specific in what fatty acid is being released [120,123]. The activity of GVIA iPLA₂ has been reported to be regulated through many mechanisms. The enzyme possesses a caspase-3 cleavage site that is clipped in vitro [124–126]. The caspase truncated enzyme was hyperactive and reduced cell viability when overexpressed in HEK293 cells [124]. Caspase mediated activation has also been recently shown to be important in mediating cell migration in ovarian cancer cells [127]. The enzyme is also regulated through ATP binding. ATP binding seems to protect the GVIA PLA₂ from losing activity [122]. Fatty acyl-CoA was also shown to be a substrate for GVIA iPLA₂, showing a potential role for nucleotide binding [128].

The GVIA PLA₂ contains multiple ankyrin repeats which may be important in mediating protein-protein interactions. The enzyme when originally isolated was shown to be active as a tetramer [120]. The splice variant GVI Ankyrin-1 was also suspected to be a negative regulator of GVIA PLA₂ through blocking potential formation of the active aggregate.[119]. The importance of the ankyrin repeats is shown by studies done on the catalytic domain alone of GVIA PLA₂ showing no activity [119]. The enzyme has also been shown to be regulated by calmodulin which negatively regulates GVIA PLA₂ through direct binding on the residues 694–705 of GVIA-1 PLA₂ [129,130].

GVIA-2 iPLA₂ is membrane associated when overexpressed in COS-7 cells as well as rat vascular smooth muscle cells [118,131]. The other active splice variant, GVIA-1, is cytosolic and not specific in targeting membrane surfaces [118,131]. This enzyme has been shown to be important in membrane homeostasis and remodeling [132], and it appears that this enzyme is the primary PLA₂ for day to day metabolic functions within the cell. More recently, others have found that GVIA iPLA₂ is involved in cell proliferation [133–136], mediating cell growth [28], apoptosis [137] and glucose-induced insulin secretion [138].

Conclusion

The PLA₂ superfamily of enzymes mediates a variety of important cellular functions. Research in this field has continued to expand with the discovery of important new functions for many of these enzymes. With the continued work of dedicated researchers this field of study will most certainly continue to generate important discoveries of novel PLA₂ activities, as well as understanding the mechanism and function of these enzymes. Further knowledge of this superfamily of enzymes should raise the potential for many possible new and exciting drug targets in the years to come.

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Figure 1. Reaction catalyzed by the PLA₂ superfamily of enzymes. Phospholipid on the left is hydrolyzed at the sn-2 position to yield lysophospholipid and free fatty acid on the right.

Figure 2.

Group IA PLA₂ with phospholipid substrate modeled in the active site based on crystal structure by Fremont et al. [39], along with NOE measurements [52] using amide pseudo-substrate inhibitor [53]. The active site residues His-48 and Asp-93 and the bound Ca²⁺ is shown in purple. Ca²⁺ is bound by Asp-49 as well as the carbonyl oxygens of Tyr-28, Gly-30, and Gly-32. Aromatic residues are shown in white; of special interest are the aromatic residues on the interfacial binding surface Tyr-3, Trp-19, Trp 61, and Phe 64. Adapted from Dennis [3]

Figure 3. Group IVA PLA₂ crystal structure as determined by Dessen et. Al. [74]. The C2 domain is shown in orange, with two bound Ca²⁺ ions shown in light yellow. The catalytic domain is shown on the right with the cap region colored yellow, and the lid region 415–432 colored magenta. The active site residues Ser-228, Asp-549 and Arg-200 are shown in stick form colored red. The PIP₂ binding site is shown in dark blue, and the C1P binding site is shown in cyan.

Table 1Secreted phospholipases A₂ (sPLA₂)

Group	Source	Molecular mass (kDa)	Disulfide bonds
IA	Cobras and Kraits	13–15	7
IB	Human/porcine pancreas	13–15	7
IIA	Rattlesnakes: human synovial	13–15	7
IIB	Gaboon viper	13–15	6
IIC	Rat/murine testis	15	8
IID	Human/murine pancreas/spleen	14–15	7
IIE	Human/murine brain/heart/uterus	14–15	7
IIF	Human/murine testis/embryo	16–17	6
III	Human/murine/lizard/bee	15–18 55 (human/murine)	8
V	Human/murine heart/lung/macrophage	14	6
IX	Snail venom (conodipine-M)	14	6
X	Human spleen/thymus/leukocyte	14	8
XIA	Green rice shoots (PLA ₂ -I)	12.4	6
XIB	Green rice shoots (PLA ₂ -II)	12.9	6
XII	Human/murine	19	7
XIII	Parvovirus	<10	0
XIV	Symbiotic fungus/ bacteria	13–19	2

Adapted from [1,2]

Table 2Cytosolic Group IV phospholipases A₂ (cPLA₂)

Groups	Source	Molecular mass (kDa)	Features	Alternate names
IVA	Human/murine	85	C2 domain	cPLA ₂ α
IVB	Human	114	C2 domain	cPLA ₂ β
IVC	Human	61	acylated	cPLA ₂ γ
IVD	Human/murine	92–93	C2 domain	cPLA ₂ δ
IVE	Murine	100	C2 domain	cPLA ₂ ε
IVF	Murine	96	C2 domain	cPLA ₂ ξ

Adapted from [1,2]

Table 3Ca²⁺ independent Group VI phospholipases A₂ (iPLA₂)

Group	Source	Molecular mass (kDa)	Features	Alternate names
VIA-1	Human/murine	84–85	8 ankyrin repeats	iPLA ₂
VIA-2	Human/murine	88–90	7 ankyrin repeats	iPLA ₂ β
VIB	Human/murine	88–91	Membrane-bound	iPLA ₂ γ
VIC	Human/murine	146	Integral membrane protein	iPLA ₂ δ, neuropathy target esterase (NTE)
VID	Human	53	Acylglycerol transacylase, triacylglycerol lipase	iPLA ₂ ε, adiponutrin
VIE	Human	57	Acylglycerol transacylase, triacylglycerol lipase	iPLA ₂ ζ, TTS-2.2
VIF	Human	28	Acylglycerol transacylase, triacylglycerol lipase	iPLA ₂ η, GS2

Adapted from [1,2]

Table 4Group VII and Group VIII phospholipases A₂ displaying PAF acetylhydrolase (PAF-AH) activity

Group	Source	Molecular mass(kDa)	Features	Alternate names
VIIA	Human, murine, porcine, bovine	45	Secreted, α/β hydrolase	Lipoprotein-associated PLA ₂ (Lp-PLA ₂), Plasma PAF-AH
VIIIB	Human, bovine	40	Intracellular, myristoylated, α/β hydrolase	PAF-AH II
VIIIA	Human	26	Intracellular, Ser/His/Asp triad, homodimer or heterodimer with GVIIIB associates with regulatory β -subunit	PAF-AH Ib (α 1 subunit)
VIIIB	Human	26	Intracellular Ser/His/Asp triad, homodimer or heterodimer with GVIIIA associates with regulatory β -subunit	PAF-AH Ib (α 2 subunit)

Adapted from [1,2]

Table 5Lysosomal Phospholipase A₂

Group	Source	Molecular mass (kDa)	Features	Alternate names
XV	Human, murine, bovine	45 (deglycosylated)	Ser/His/Asp triad, glycosylated, N-terminal signal sequence	ACS, lysosomal PLA ₂ (LPLA ₂), LLPL

Adapted from [1,2]