Chapter 4

Phospholipases: An Overview

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Abstract

Phospholipids are present in all living organisms. They are a major component of all biological membranes, along with glycolipids and cholesterol. Enzymes aimed at cleaving the various bonds in phospholipids, namely phospholipases, are consequently widespread in nature, playing very diverse roles from aggression in snake venom to signal transduction, lipid mediators production, and digestion in humans. Although all phospholipases target phospholipids as substrates, they vary in the site of action on the phospholipids molecules, physiological function, mode of action, and their regulation. Significant studies on phospholipases characterization, physiological role, and industrial potential have been conducted worldwide. Some of them have been directed for biotechnological advances, such as gene discovery and functional enhancement by protein engineering. Others reported phospholipases as virulence factors and major causes of pathophysiological effects. In this introductory chapter, we provide brief details of different phospholipases.

Key words: Phospholipases, Classification, Physiological function, Industrial application

1. Introduction

Phospholipases are classified according to the site of bond cleavage in their phospholipid substrates (Fig. 1). The phospholipases A1 (PLA1) and A2 (PLA2) produce free fatty acids and 2-acyl lysophospholipid or 1-acyl lysophospholipid, respectively. The fatty acid linked to the lysophospholipid is cleaved by lysophospholipase activity of phospholipase B (PLB). Phospholipases C (PLC) are defined as phosphodiesterases that cleave the glycerophosphate bond. Finally, the base group of the phospholipid is removed by phospholipase D (PLD) (1, 2). Catalytically active phospholipases

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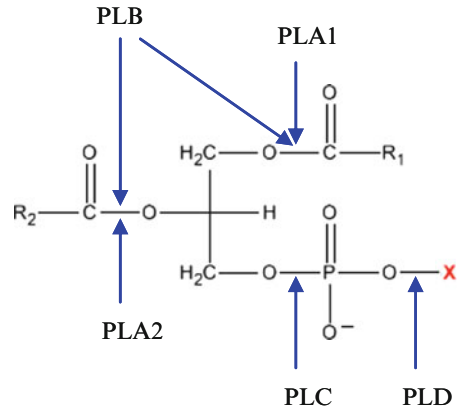

Fig. 1. The site of action of various phospholipases on phospholipid. A generalized depiction of a phospholipid, where X = phospholipid common base (e.g., choline, ethanolamine, inositol). The various sites of attack for hydrolytic cleavage of the various phospholipase types A1, A2, B, C, and D are shown with *arrows*.

share a common substrate, phospholipids, and phosphatidylcholine (PC) as major substrate. However, they can also act on phosphatidylethanolamine, phosphatidylinositol, sphingomyelin, lysophosphatidylcholine (LPC), and lysophosphatidylinositol in some organisms and tissues (3).

The International Union of Biochemistry and Molecular Biology (IUBMB) enzyme nomenclatures for phospholipases A1 and A2 are 3.1.1.32 and 3.1.1.4, respectively. The lipases, which act mainly on triglycerides, are designated 3.1.1.3. The IUBMB enzyme nomenclatures for phospholipases C and D are 3.1.4.3 and 3.1.4.4, respectively. One must, however, realize that many enzymes have broad substrate specificity and that a phospholipase being classified as a 3.1.1.32 enzyme might also have activity, for example, as a triacylglycerol lipase (3.1.1.3) (4–6).

2. Phospholipases A1

PLA1 activities have been detected in many cells and tissues from various organisms (5, 7). However, a limited number of PLA1s were purified and cloned so far. These include phosphatidylserine-specific PLA1s (PS-PLA1s) from rat platelets (8), PLA1 from vespid venom (9), and a phosphatidic acid-preferential PLA1 (PA-PLA1) from bovine and human testis (10). These enzymes are also members of the triacylglycerol lipase family and show considerable sequence similarity to human hepatic and pancreatic lipases and the guinea pig pancreatic lipase-related protein 2 (GPLRP2) (11).

Two molecular characteristics of these PLA1s emerge from these sequence/structure comparisons: the presence of a very short lid and the deletion of a loop (the β9 loop) compared to the pancreatic lipases. These differences were suggested to be the key to the PLA1 activity (11). All these enzymes have the typical Ser-His-Asp catalytic triad.

Among the bacterial lipases, the 46 kDa lipase from the animal skin pathogen *Staphylococcus hyicus* (SHL) is unique in having high PLA1 activity, besides having considerable lipase activity (12). The crystal structure of SHL in an open conformation showed the importance of Lys295 and Ser356 for phospholipid binding and PLA1 activity (13). This enzyme adopts the α/β hydrolase fold common to many lipases/esterases (14) and has a Ser-Asp (Glu)-His catalytic triad. As well as in its phospholipase activity, SHL differs from its staphylococcal homologues and most other bacterial lipases in its broad substrate specificity (15).

Bacterial PLA1 enzymes are present in both the outer membrane and cytoplasmic compartment. The outer membrane phospholipase A (OMPLA) is one of the few enzymes present in the outer membrane of gram-negative bacteria. It is known to have a broad range of activities, i.e., PLA2, lyso-PLA1, and lyso-PLA2 (16). OMPLA is a dimer and has an atypical Ser-His-Asn catalytic triad (17). This enzyme is unusually resistant to inactivation by heat and ionic detergents and requires calcium for maximal activity (18). Cytoplasmic PLA1 can be inactivated by heat and detergent and can also act as a transacylase.

Recently, it has been reported that PLA1 plays important biological role in both phospholipidosis, a pathological condition in which phospholipids accumulate in lysosomes, and virulence factors for bacterial and fungal pathogenesis (19).

3. Phospholipases A2

PLA2s are the most widely studied phospholipases. The 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, free fatty acids and lysophospholipids, have many different important physiological roles. The PLA2 superfamily is subdivided into several classes, including small molecular mass-secreted PLA2s (sPLA2), cytosolic PLA2s (cPLA2), Ca^{2+}-independent PLA2s (iPLA2), platelet-activating factor acetylhydrolases (PAF-AH), lysosomal PLA2s, and a recently identified adipose-specific PLA2 (20). Assignment of the enzymes to a certain group is based on their catalytic mechanism (His/Asp, Ser/Asp, or Ser/His/Asp hydrolase), localization, evolutionary relationships, as well as functional and structural features.

Over the last 10–15 years, new members of the PLA2 superfamily have been discovered, and this has led to several updates of the group numbering system for these enzymes (20, 21). During the past decade, data concerning the unexplored roles of various PLA2 enzymes in pathophysiology have emerged on the basis of studies using knockout and transgenic mice, specific inhibitors, and information obtained from analysis of human diseases caused by mutations in PLA2 genes.

3.1. The sPLA2 Family

sPLA2s form a broad family of structurally conserved proteins that have a small molecular mass (14–19 kDa) and utilize a His/Asp dyad and a calcium ion for catalysis. sPLA2s belonging to the group I/II/V/X are closely related by a highly conserved Ca^{2+}-binding loop. In addition to these elements, there are six absolutely conserved disulfide bonds and up to two additional unique disulfide bonds, which contribute to the high degree of stability of these enzymes (Table 1) (21). Among the I/II/V/X group of sPLA2s, the genes for sPLA2-IIA, -IIC, -IID, -IIE, -IIF, and -V are clustered on the same chromosome locus in both human and mouse (22). sPLA2-III and sPLA2-XIIA/XIIB share homology with the I/II/V/X collection of sPLA2s only in the Ca^{2+}-binding loop and catalytic site, thereby representing distinct group III and XII collections, respectively.

The sPLA2s are found in plants (23), insects (24), molluscs (25), reptiles (26), and mammals (27). Two forms, containing 0 or 2 disulfide bonds, have been identified in parvovirus (28) and in the symbiotic fungus *Tuber borchii* (29), respectively. A bacterial sPLA2 from *Streptomyces violaceoruber* also contains only two disulfide bonds (30, 31). Mammalian Group III PLA2 enzymes possess a larger molecular mass of around 55 kDa due to N-terminal and C-terminal extensions (32).

sPLA2s do not exhibit acyl-chain specificity, whereas there is some specificity for certain head groups of the phospholipid substrate: generally, sPLA2s show a high activity with anionic phospholipids, but only the GV and GX PLA2s also hydrolyze PC vesicles (33).

Although they are secreted enzymes, recent work indicated that certain sPLA2s can also act intracellularly to release *sn*-2 acyl chains, including arachidonic acid (AA), before being secreted (34, 35). Most mammalian cells contain several sPLA2s suggesting that they have distinct functions. Some sPLA2s, such as Groups V and X sPLA2, effectively hydrolyze cell surface phospholipids upon secretion (33, 36). Others, such as Group IIA sPLA2, are unable to hydrolyze cell surface phospholipids but play a role in host defense by degrading bacterial phospholipids (37). Certain sPLA2s have also been shown to use phospholipids in serum lipoproteins and may function to promote atherogenesis (38). Little is known about the *in vivo* role of sPLA2s in host defense against viruses or parasites.

Table 1
Secreted phospholipases A2

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	14	6
X	Human spleen/thymus/leukocyte	14	8
XIA	Green rice shoots (PLA2-I)	12.4	6
XIB	Green rice shoots (PLA2-II)	12.9	6
XII	Human/murine	19	7
XII	Parvovirus	<10	0
XIV	Symbiotic fungus/bacteria	13–19	2

Animal and early-phase clinical studies suggest that sPLA2s could be exciting therapeutic targets for atherosclerosis.

Since each sPLA2 displays a unique expression pattern in different cell types within restricted tissues, one should consider when and where the different sPLA2 isoforms are expressed, which isoforms are involved in what types of pathophysiology, and how they exhibit their specific functions in order to comprehensively understand the specific functions of individual sPLA2s.

The action of sPLA2s on cellular membranes should not be limited to the release of AA, since other unsaturated fatty acids could also be released, such as oleic acid, linoleic acid, and ω-3 fatty acids (e.g., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are precursors of anti-inflammatory lipid mediators), as well as lysophospholipids, such as LPC and lysophosphatidic acid.

sPLA2-IB is synthesized in the pancreatic acinar cells. After secretion into pancreatic juice, an N-terminal heptapeptide of the

inactive zymogen is cleaved by trypsin to yield an active enzyme in the duodenum. sPLA2-IB binds more tightly to anionic phospholipids than to PC, yet the hydrolysis of PC is greatly accelerated in the presence of a low concentration of detergent, such as deoxycholate (39). This property seems important since the digestion of phospholipids in dietary foods by pancreatic sPLA2-IB occurs in the presence of bile acids in the intestinal tract.

The levels of sPLA2-IIA in serum or exudative fluids are well correlated with the severity of inflammatory diseases, such as rheumatoid arthritis and sepsis (40). sPLA2-IIA is often referred to as an "inflammatory sPLA2". Because of its weak binding to PC, sPLA2-IIA hardly releases AA from resting mammalian cells by acting directly on the outer leaflet of the plasma membrane, which is PC rich. As such, the intracellular redistribution of sPLA2-IIA may allow targeting of the enzyme to its preferred anionic phospholipid substrates (41). In addition, sPLA2-IIA can release AA during its own secretion, probably at the luminal surface of the endoplasmic reticulum (ER), Golgi, or secretory vesicle membranes (35). The enzyme can also act on apoptotic cells or microvesicles (exosomes) shed from activated cells, on which anionic substrates, such as phosphatidylserine or phosphatidic acid, are exposed on the surface (42–44). The best substrates for sPLA2-IIA are phosphatidylethanolamine and phosphatidylglycerol, which are major components of bacterial membranes. The best-recognized physiologic function of sPLA2-IIA is the degradation of bacterial membranes, thereby providing the first line of host antimicrobial defense (45, 46). Since bacterial infection can occur anywhere in the body, marked induction of sPLA2-IIA in the circulation as well as in local areas of inflammation or injury can be regarded as an innate defense response for clearance of growing bacteria.

The expression and functions of other Group II sPLA2s have not yet been firmly established. sPLA2-IIC is expressed in meiotic cells within rodent testis (47). In contrast, the absence of a portion of one exon in the human genome indicates that it is a pseudogene and not expressed as a functional protein in humans (48). sPLA2-IID is structurally most similar to sPLA2-IIA, and its transcript is constitutively detected in lymphoid organs, such as the spleen and lymph nodes (49, 50). sPLA2-IIE, which is expressed constitutively in several tissues at low levels, has much lower catalytic activity than other Group II sPLA2s, at least under the standard PLA2 assay conditions (33).

sPLA2-V is often classified into the Group II subfamily of sPLA2s since its gene is located in the Group II sPLA2 cluster locus (51). At the cellular level, sPLA2-V is capable of releasing AA more potently than sPLA2-IIA (52–54). The transcellular (paracrine) action of sPLA2-V in inducing eicosanoid synthesis by neighboring cells is superior to that of sPLA2-IIA (55, 56).

sPLA2-V can also hydrolyze PC in lung surfactant (57) and plasma lipoproteins (58–60) much more potently than sPLA2-IIA *in vitro*. Recent lipidomics approaches have shown that sPLA2-V releases oleic acid in preference to AA from cellular membranes, lipoproteins, and even pure phospholipid vesicles (61). Since oleic acid is generally the most abundant fatty acid in cellular membrane PC, a primary role of sPLA2-V might be to release LPC from abundant oleate-containing PC. sPLA2-V has both proinflammatory and anti-inflammatory potential in the context of disease models (62, 63). Hence, the two Group II subfamily sPLA2s, sPLA2-IIA and sPLA2-V, exert opposite (offensive and defensive, respectively) impacts on inflammatory arthritis. This may explain why clinical trials of pan-sPLA2 inhibitors, which block both enzymes, for treatment of rheumatoid arthritis have been unsuccessful (64). Hydrolysis of PC in lipoproteins by sPLA2 produces nonesterified fatty acids and LPC, which can trigger vasoactive, chemotactic, and proinflammatory actions leading to the acceleration of atherosclerosis. Recent findings have indicated that sPLA2-V is more important than sPLA2-IIA for the promotion of atherosclerosis (58, 60, 65–67). Since sPLA2-V is expressed in alveolar macrophages and bronchial epithelial cells in the airways and urinary ducts of the kidney (68, 69), where fungal infection frequently occurs in immunocompromised patients, the enzyme may contribute to the first innate immune response against fungi by facilitating phagocytosis and killing through a mechanism that is likely dependent on phagolysosome fusion (63, 70).

Like sPLA2-IB, sPLA2-X is synthesized as a zymogen, and removal of the N-terminal propeptide produces an active mature enzyme (71). In the gastrointestinal tract, where sPLA2-X is most highly expressed among tissues (72, 73), the enzyme is located in columnar epithelial cells, Paneth cells, and interstitial ganglion cells (74). Because of this location, the roles of sPLA2-X in gut homeostasis, such as dietary lipid digestion, food intake and obesity, intestinal bacterial flora regulation, peristaltic reflex, and nociception, controlled by the enteric nervous system should be taken into consideration. sPLA2-X is highly expressed in the gastrointestinal tract (74). Among the mammalian sPLA2s, sPLA2-X shows the highest binding affinity for PC and thus elicits the most potent release of AA and LPC from the PC-rich outer leaflet of the plasma membrane of even intact cells (36). sPLA2-X can also hydrolyze PC in lung surfactant (75) and plasma lipoproteins (76, 77) very efficiently *in vitro*. sPLA2-X is one of the major sPLA2 isoforms detected in airway cells of humans with asthma, where the enzyme is localized to airway epithelial cells and bronchial macrophages (68, 78). Recent studies using sPLA2-deficient mice have identified sPLA2-X as new key player in asthma (79). However, it remains uncertain how distinct types of PLA2 enzymes interplay in the pathogenesis of allergic airway inflammation (80, 81). It has also

been proposed that sPLA2-X participates in the development of atherosclerosis (77, 82). Other sPLA2s, including sPLA2-IID, -IIE, and -IIF, are also detectable in human atherosclerotic plaques (83), and sPLA2-IIF is capable of hydrolyzing lipoprotein-associated PC *in vitro* (82). However, the impact of overexpression or deletion of these sPLA2s on atherosclerosis *in vivo* is currently unknown.

sPLA2-III, which is the only enzyme belonging to the Group III collection, is an unusually large protein (55 kDa) among the sPLA2 family members (32). sPLA2-III can hydrolyze PC and phosphatidylethanolamine equally and augment AA release from cell membranes more efficiently than sPLA2-IIA and less efficiently than sPLA2-X and sPLA2-V. Examination of the expression profiles of a full set of sPLA2s in the human colon suggests that sPLA2-III might be a good candidate biomarker for colon cancers (72). sPLA2-III and sPLA2-X, that are expressed in different locations within the male reproductive organs, exert nonredundant functions in two major steps of male fertility, one during sperm maturation in the epididymis and the other during capacitation and acrosome reaction likely after ejaculation into the uterus and oviduct. This concept has provided new insights into the physiological role of the sPLA2 family in reproduction biology. It should be noted that male reproductive organs also express other sPLA2s, which show distinct cellular localizations: for instance, sPLA2-IIA is abundantly expressed in the prostate epithelium, whereas sPLA2-IID is expressed in the tail of elongated spermatids, sPLA2-V in spermatogonia and Leydig cells, and sPLA2-IIF in Leydig cells within the testicular niche (84, 85). The functions of these sPLA2s in male reproduction as well as expression and functions of sPLA2s in female reproductive organs require further investigation.

The Group XII family includes two isoforms, XIIA and XIIB. High expression of sPLA2-XIIA is found in various tissues, suggesting a housekeeping function. Even though the catalytic activity of sPLA2-XIIA is very weak under the standard enzyme assay conditions (86), it can kill gram-negative bacteria, such as *Escherichia coli* and *Helicobacter pylori*, more efficiently than sPLA2-IIA (37, 87). Since sPLA2-XIIA is abundantly and ubiquitously expressed in various tissues, its primary role might be protecting the host from infection of gram-negative bacteria. The exact roles of sPLA2-XIIA and its catalytically inactive homolog sPLA2-XIIB, in which the catalytic center His is replaced with Leu (88), are still unknown.

Finally, the presence of catalytically inactive or very weak sPLA2s lends support to the idea that some functions of sPLA2s may depend on their ligand-like action. A number of membrane receptors and soluble binding proteins interacting with sPLA2s have been described (27, 89–92).

Although the fact that pan-sPLA2 inhibitors prevent several disease models in experimental animals (93–95) suggesting their potential therapeutic efficacies, inhibition of multiple sPLA2s altogether could inhibit both offensive and defensive sPLA2 isozymes and thereby cancel the therapeutic effect resulting from the inhibition of the proinflammatory one(s). This might account for the reason why some pan-sPLA2 inhibitors have no beneficial impact on allergen-induced bronchoconstriction, rheumatoid arthritis, and severe sepsis in human subjects (64, 96, 97). From this viewpoint, an inhibitor that specifically blocks offensive sPLA2(s) but not defensive isozyme(s) may be more desirable than the currently tested pan-sPLA2 inhibitors, which block group I/II/V/X sPLA2s altogether. It should also be noted that sPLA2s have a beneficial action as antimicrobial proteins. For instance, sPLA2-IIA has the ability to effectively kill bacteria by directly degrading bacterial membranes while sPLA2-V exhibits an antifungal function by facilitating macrophage uptake of fungal particles (see above). Thus, application of pan-sPLA2 inhibitors to patients with infectious airway diseases might result in expansion of microbial infection, thereby having an unwanted potential to worsen the disease. Nonetheless, we would like to draw attention to the fact that a strong side effect should warrant a strong beneficial effect also. The fact that pan-PLA2 inhibitors are being tested in an atherosclerosis trial means that an untoward effect may not be the limiting factor for this disease. All of the above knowledge should help lead to the proper identification of certain PLA2s as therapeutic targets or novel biotherapeutic molecules in various diseases, such as inflammation, tissue injury, metabolic diseases, and cancer.

3.2. The cPLA2 Family

The Group IV PLA2 family contains six members, Group IVA, Group IVB, Group IVC, Group IVD, Group IVE, and Group IVF (98). These enzymes are also commonly referred to as cPLA2 α, β, γ, δ, ε, and ζ, respectively. cPLA2α, the most extensively studied Group IV PLA2, is widely expressed in mammalian cells and mediates the production of functionally diverse lipid products in response to extracellular stimuli (99, 100). Structural analysis of the Group IV PLA2 revealed the presence of an N-terminal β-sandwich C2 domain attached to a C-terminal catalytic domain by a flexible linker, with the exception of cPLA2γ in which the C2 domain is absent (98). A large number of mammalian proteins contain a C2 domain, which functions primarily to promote interaction of proteins with membranes (101). Full activation of the cPLA2α requires Ca^{2+} binding to the N-terminal C2 domain and phosphorylation on serine residues (102). The signature of the Group IV family is a conserved active site dyad composed of a nucleophilic serine (Ser228 in cPLA2α) in the modified lipase consensus sequence GXSXS/A and an aspartic acid (Asp549 in cPLA2α) (103, 104). A conserved arginine (Arg200 in cPLA2α) is

also required for activity (103). The mammalian Group IV paralogs cPLA2s β, γ, δ, ε, and ζ share approximately 30–37% amino acid identity with cPLA2α (105). It has been previously reported that cPLA2α displays PLA1, PLA2, and lysophospholipase activities (106). This is in marked contrast to secreted PLA2s, which are strict PLA2s. There are no X-ray structures on phospholipid analogs bound to the active site of cPLA2s, so it is not possible to understand why these enzymes have multiple phospholipase activities; this degree of multiple substrate tolerance is unusual for enzymes. A recent study of the full set of mammalian isoforms shows that they all display PLA1 and PLA2 activities, but the relative amount of each is dramatically different among the isoforms (107). Current information available about the biochemical properties and tissue distribution of the other Group IV PLA2s suggests that they may have distinct mechanisms of regulation and functional roles (98, 107).

The cPLA2α knockout mouse model has provided important information about its role in physiological processes and disease. Deleting cPLA2α has profound phenotypic effects in models of disease. cPLA2α can act to promote or suppress disease pathology, which is not unexpected since its activation results in the production of numerous mediators with diverse functions. cPLA2α contributes to the pathogenesis of a variety of diseases particularly those in which inflammation plays a primary role, such as allergic reactions, acute lung injury, pulmonary fibrosis, brain injury, arthritis, bone resorption, Alzheimer, and autoimmune encephalomyelitis (a model of human multiple sclerosis), implicating a role for proinflammatory lipid mediators in disease pathogenesis (108–113). cPLA2α activation also results in the production of mediators, particularly prostaglandin E2 (PGE_2) and prostacyclin, which can exert anti-inflammatory or protective effects. PGE_2 plays a bronchoprotective role by attenuating airway responsiveness, and prostacyclin protects against hypertension, cardiac hypertrophy, and cardiac fibrosis (114, 115). cPLA2α-derived mediators can either promote or suppress tumorigenesis depending on the specific target tissue (116, 117). The physiological function of cPLA2α is difficult to predict because of the number of mediators that are produced as a result of its activation and the specific impact of these mediators in different tissues.

A role for cPLA2α in regulating normal processes and disease pathogenesis has been established. However, the knockout mouse model has also revealed that there are alternative pathways for production of lipid mediators. For example, there is significant residual production of urinary PGE_2 in cPLA2α-deficient mice (118). Also, there are cPLA2α-dependent and -independent pathways for thromboxane A_2 production in platelets (119). These results point to a possible role for Group VI PLA2s, secreted PLA2s, or compensation by other Group IV PLA2s. The cPLA2α knockout

mouse has provided important insight into the physiological function of cPLA2α in the mouse. cPLA2α has been highly conserved throughout evolution and is widely expressed in all tissues of rodents and humans suggesting that results in the murine model can be extrapolated to humans. However, there is less conservation of other Group IV PLA2s and their tissue distribution differs between mice and humans. This suggests that the function of other Group IV PLA2s may differ among species, although this remains to be determined.

Considerable understanding of cPLA2α function has been derived from investigations of the enzyme and from cPLA2α null mice, but knowledge of discrete roles for this enzyme in humans is limited. The identification of inherited mutations in human cPLA2α that are associated with a profound defect in cPLA2α catalytic activity, impaired eicosanoid biosynthesis, small intestinal ulceration, and platelet dysfunction suggested the physiological importance of cPLA2α in human disease and physiological processes (120, 121).

These findings have significant implications for the safety and effectiveness of pharmacologic inhibition of the cPLA2α enzyme.

3.3. The iPLA2 Family

The intracellular Group VI calcium-iPLA2s and Group IV cPLA2s have no homology to sPLA2s. Unlike the sPLA2s, they do not strictly hydrolyze *sn*-2 acyl chains, but also exhibit PLA1, lysophospholipase, and transacylase activities. Several enzymes have been classified in the Group VI iPLA2 family based on the presence of conserved nucleotide binding ((G/A)XGXXG) and lipase (GXSXG) consensus sequences (122).

Group VIA iPLA2, also called iPLA2β, has been studied most extensively (122, 123). It may function in phospholipid acyl remodeling, but clearly is a regulated enzyme that plays a signaling role in mediating AA release and regulating calcium homeostasis (99, 123). Group VIA iPLA2 has PLA2 activity with no acyl chain selectivity and weaker lysophospholipase, PLA1, and transacylase activities (124). It has recently been demonstrated that Group VIA iPLA2 also hydrolyzes fatty acyl CoAs utilizing the same active-site nucleophile that catalyzes phospholipid hydrolysis (125). A knockout mouse model has revealed that endothelial PAF production is entirely dependent on iPLA2β activity (126). Because inflammatory cell recruitment involves the interaction of endothelial cell PAF with PAF receptors on circulating cells, these data suggest that iPLA2β may be a suitable therapeutic target for the treatment of inflammatory lung diseases.

Another iPLA2 member, Group VIB iPLA2 (also called iPLA2γ), exhibits PLA2 and lysophospholipase activities, but also has PLA1 activity specific for 1-palmitoyl-2-arachidonoyl-PC resulting in production of 1-lyso-2-arachidonoyl-PC (127). This product may serve as a precursor for a variety of lipid mediators,

including free AA and endocannabinoid 2-arachidonoylglycerol and its glycerol-linked prostaglandin derivatives.

Three Group VI iPLA2s (iPLA2 ε, ζ, and η) have recently been identified that possess robust triacylglycerol lipase and acylglycerol transacylase activities (128). The enzymatic profile of iPLA2ε and iPLA2ζ, and the observation that their expression increases during adipogenesis, suggests a role in triglyceride homeostasis in adipocytes (128).

There is evidence that iPLA2s in the Group VI family exhibit multiple enzymatic activities, illustrating their enzymatic and functional diversity. The potential for expression of different splice variants of the Groups VI and IV PLA2s underscores the importance of identifying the endogenous proteins, produced in different cell types and tissues, and characterizing their enzymatic properties (129).

3.4. The PAF-AH Family

PAF-AH enzymes represent a unique group of acyl hydrolases with a catalytic serine that are capable of releasing acetate from the *sn*-2 position of PAF. To date, four enzymes that specifically catalyze this reaction have been identified. One enzyme is a secreted protein known as plasma-type PAF-AH or "lipoprotein-associated PLA2", which has recently attracted attention as a new therapeutic target for atherosclerosis (130, 131). The other three enzymes are intracellular referred to as PAF-AH type II (Group VII PLA2), which is homologous to plasma-type PAF-AH, and PAF-AH type I (Group VIII PLA2), which is composed of a homo- or heterodimer of the two closely related catalytic subunits, α1 and α2, complexed with another regulatory β subunit (132).

The plasma-type PAF-AH, or Group VIIA PLA2, is a 45-kDa secreted protein containing a lipase consensus motif GXS273XG (133). The crystal structure of this enzyme shows that it has a classic lipase α/β hydrolase fold and contains the Ser273, His351, and Asp296 catalytic triad (134). Ca^{2+} is not required for catalysis, as is the case for other PAF-AH enzymes. A major fraction of the human enzyme is associated with the apo-B100 of LDL and HDL in plasma (135). Although earlier workers have hypothesized that plasma-type PAF-AH has a protective role against the development of atherosclerotic lesions by removing oxidized LDL (136), most of the data obtained recently suggest that it has an active role in atherosclerotic development and progression (137, 138). Selective inhibition of PAF-AH inhibits progression to advanced coronary atherosclerotic lesions and confirms the crucial role of vascular inflammation independently of hypercholesterolemia in the development of lesions implicated in the pathogenesis of myocardial infarction and stroke (139). Moreover, PAF-AH selective inhibition prevents necrotic core expansion, a key determinant of plaque vulnerability in humans (140). These findings suggest that PAF-AH inhibition may represent a novel therapeutic approach for atherosclerosis.

Accumulated evidence suggests that PAF-AH-II plays a role in defense against oxidative stress, most likely by degrading oxidized phospholipids in the membranes (141). However, the PAF-AH-I pathway may be linked to normal neuronal migration (142).

3.5. The Lysosomal PLA2 Family

Currently, two distinct types of PLA2s, that show lysosomal localization, have been identified: acidic Ca^{2+}-iPLA2 (aiPLA2) and lysosomal PLA2 (LPLA2) (143). Although these two proteins are structurally quite distinct, they are localized in lysosomes, show acidic pH preference for catalysis, and play a common role in homeostatic surfactant metabolism. aiPLA2 is identical to peroxiredoxin 6 (Prdx6), a nonselenoprotein with glutathione peroxidase activity (144). The peroxidase and PLA2 enzymatic functions of this protein have distinctly different active sites; the PLA2 activity is Ser32 dependent, whereas the peroxidase activity requires Cys47 (145). Gene targeting of this enzyme hampers surfactant synthesis and causes neonatal death due to respiratory distress syndrome (146).

LPLA2, or Group XV PLA2, is highly homologous with lecithin cholesterol acyltransferase and is phylogenetically related to a large group of plant phospholipases. Deficiency of LPLA2 results in foam cell formation, surfactant lipid accumulation, splenomegaly, and phospholipidosis in mice (147).

3.6. AdPLA: An Adipose-Specific PLA

A new PLA2 that is expressed abundantly in white adipose tissue has recently been identified (148). The enzyme, termed AdPLA or Group XIV PLA2, belongs to the lecithin retinol acyltransferase family and is identical to H-Rev107, which was originally cloned as a negative regulator of proto-oncogene Ras and classified as a class II tumor suppressor (149). AdPLA is induced during preadipocyte differentiation into adipocytes (148). Mutational studies show that His23 and Cys113 are critical for AdPLA activity. The enzyme is able to liberate both *sn*-1 and *sn*-2 fatty acids. Depending on the assay conditions, its PLA1 activity can exceed the PLA2 activity (150). Evidence from AdPLA knockout mice suggests that AdPLA, which is a PLA1/2 rather than a PLA2, may promote obesity through a mechanism distinct from PGE_2 signaling (151).

4. Phospholipases B

PLB enzymes catalyze three distinct activities: an *sn*-1 and *sn*-2 fatty acid ester hydrolase, a lysophospholipase and a transacylase activity. Thus, there is some confusion in nomenclature for these enzymes (152). Synonyms, including lysophospholipase and lysophospholipase–transacylase, are used for PLB. The hydrolase activity allows the enzyme to cleave fatty acids from both phospholipids

(PLB activity) and lysophospholipids (lysophospholipase activity) while the transacylase activity allows the enzyme to produce phospholipids by transferring a fatty acid to a lysophospholipid. Many fungal species appear to produce PLB with both hydrolyzing and acyltransferase activity (153). These activities have also been described in bacteria (2), *Dictyostelium discoideum* (protozoa) (152), and in mammalian cells (154). The fungal PLBs range 600–700 amino acids in length and have a potential role in virulence and fungal pathogenesis (155). However, there is no evidence that PLBs produced by pathogenic bacteria, such as *Moraxella bovis*, are toxic or play a role in virulence.

5. Phospholipases C

According to their substrate specificities, PLCs can be classified as (a) phosphatidylinositol-specific PLC (PI-PLC), (b) PC-preferring PLC (PC-PLC), and (c) nonspecific PLC.

PI-PLCs were found in a broad spectrum of organisms, including bacteria, plants, and animals. PC-PLCs have been detected in a variety of bacteria. *Listeria monocytogenes* showed PC-PLC as virulence factor (156, 157). PI-PLCs in eukaryotes play a key role in signal transduction cascades generating membrane-associated second messengers (e.g., inositol-1,4,5-triphosphate) in response to cell surface receptor activation (157, 158). 1,2-Diacylglycerol, one of the reaction products, acts as second messenger in cellular signaling through activation of protein kinase C. In prokaryotes, PI-PLCs act as virulence factor in some pathogenic bacteria (159). Bacterial PI-PLCs consist of a single domain of 30–35 kDa while the much larger eukaryotic enzymes (80–150 kDa) are organized in several distinct domains. Both the eukaryotic and prokaryotic enzymes need cofactors, calcium and zinc, respectively, for optimum catalytic activity (158).

6. Phospholipases D

PLD is a ubiquitous enzyme found in bacteria, fungi, plants, and mammals. The PLD family includes enzymes that are involved in phospholipid metabolism, nucleases, toxins, and virus envelope proteins of unknown function (160). Phosphatidic acid, one of the reaction products, is involved in signal transduction. The PLD enzymes have a characteristic modular structure in which a common catalytic domain is flanked by regulatory sequences. These include lipid-binding Phox (PX) and Pleckstrin Homology (PH) domains and motifs that are unique to the PLD enzymes.

The active-site region of the PLD family members consists of a conserved sequence motif, the $HXK(X)_4D$ motif (or the HKD motif), with a few exceptions (161). PLDs also exhibit a unique transphosphatidylation activity that transfers the phosphatidyl moiety of the substrate to certain nucleophiles, such as ethanol, thereby forming phosphatidylethanol (162).

The role of PLD as a virulence factor for *Corynebacterium pseudotuberculosis*, *Corynebacterium ulcerans*, and *Arcanobacterium haemolyticum* has been reported (163).

Mammalian PLDs are key enzymes in intracellular signaling, where phosphatidic acid is involved in signal transduction (2). PLD was thought to be an integral part of the signaling network involving various phospholipases (162). Many factors (e.g., fatty acids, phosphoinositol, small GTP-binding proteins, protein kinase C, Ca^{2+}, phosphorylation) regulate PLD activation in a synergistic or antagonistic manner. PLD generates phosphatidic acid, which subsequently can be either metabolized by PLA2-generating lysophosphatidic acid, a potent cellular mitogen, or by phosphatidate phosphohydrolase yielding diacylglycerol. PLD is a membrane-associated enzyme, and its activities have been reported for diverse subcellular organelles, including plasma membrane, Golgi vesicles, endoplasmic reticulum, secretary vesicles, and nuclear membranes.

More physiological functions of PLD need to be clarified. Genetic and pharmacological approaches implicated PLD for control of intracellular membrane transport and recognition of the actin cytoskeleton (160).

In summary, the phospholipases generate numerous lipid products which control much of cellular signaling. Although the importance of these enzymes in eicosanoid metabolism signal translation is not questioned, much remains to be studied regarding the regulation of these enzymes and their pathophysiological roles.

7. Industrial Applications of Phospholipases

There is a lot of interest in phospholipases from a physiological and pharmaceutical perspective (164). Their use in industrial processes has grown hand in hand with our ability to clone and express the genes in microbial hosts and in commercially attractive amounts (165). Further, the use in industrial processes is increasing through the ability of optimizing the enzymes by protein engineering (5).

Phospholipases are commonly used to produce emulsifier-like molecules (e.g., lysolecithin and monoglycerides) by acting on the phospholipids already present in the ingredients.

A phospholipase from *F. oxysporum* with both lipase and phospholipase activity was introduced to the market by Novozymes A/S for baking application under the trade name Lipopan F™. The lipids and the hydrolyzed products are important for gas bubble stability in the dough (166). The value of lipases in bread making lies in their ability to increase loaf volume and improve softness. Phospholipase and galactolipase activities were shown to provide even better emulsification in the dough (5).

PLA2 was also used for enzymatic hydrolysis of egg yolk to give better emulsion in mayonnaise preparations (167). Enhancement of interaction between proteins and phospholipids may be the reason why PLA2 increases emulsion stability. Structural and functional changes of low-density lipoprotein were observed in PLA2-treated hen egg yolk (168).

Removal of impurities in newly produced vegetable oil with the help of enzymes constitutes another large application of phospholipases, such as vegetable oil degumming. Phospholipase A-mediated oil degumming is a well-established process step in the physical refining of vegetable oils (rape seed, soybean, sunflower seed) (165). Vegetable oil contains phospholipids (commonly known as gums), which have a negative impact on the storage stability and the downstream processing of the oil. A large part of the gums can be removed with water. The remaining "nonhydratable" gums can be removed by either a caustic process or an enzymatic process based on phospholipase A.

Lysolecithases from different fungal origins were reported and patented, but there is only one industrial application within the starch industry. In the starch industry, during glucose syrup production, lysolecithin contamination blocks the filters. Lysolecithase addition improves filtration (169).

The hydrolysis of milk phospholipids with PLA1 from *Fusarium venenatum* significantly increased the cheese yield through better moisture and fat retention during whey drainage and stretching (170). The fungal PLA1 from *F. venenatum* hydrolyzed the major milk phospholipids, phosphatidylethanolamine and phosphatidylcholine. The PLA1 was used to increase cheese yield and decrease the oiling-off effect in cheese as well. The oiling-off effect is the tendency of the cheese to form free oil upon storage and/or melting.

It is worth noting that the use of a phospholipase for industrial applications leads to substantial environmental benefits. The use of enzymes as an alternative to chemical processes to make products often provides a cleaner solution for the industrial processes. In a world with great demands on nonpolluting and energy-saving technical solutions, white biotechnology is a strong alternative.

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