Diversity of Phospholipase A₂ Enzymes

Secretory Phospholipase A₂

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Secretory phospholipase A_2 (sPLA₂) is a growing family of structurally related, disulfide-rich, low molecular weight, lipolytic enzymes with a His-Asp catalytic dyad. sPLA₂s are distributed in a wide variety of vertebrate and invertebrate animals, plants, bacteria, and viruses, and there are 10 catalytically active sPLA₂ isozymes in mammals. Although the structural bases for mammalian sPLA₂s have been well documented, their physiological functions are still subject to debate. Individual mammalian sPLA₂s have distinct enzymatic properties and display distinct tissue expression patterns, suggesting that each enzyme acts on distinct phospholipid membrane moieties *in vivo*. In this article, we briefly review our latest understanding of the possible physiological functions of sPLA₂s, in keeping with their diverse actions on mammalian and nonmammalian cell membranes.

Key words secretory phospholipase A2; arachidonic acid; lipid mediators; lipoprotein; surfactant; bactericidal activity

GENERAL ASPECTS

To date, 10 catalytically active mammalian sPLA₂s (IB, IIA, IIC, IID, IIE, IIF, III, V, X, and XIIA) have been identified in mammals.^{1,2)} Of these, sPLA₂s belonging to the group I/II/V/X collection are closely related, 14-19-kDa secreted enzymes with a highly conserved Ca2+-binding loop (XCGXGG) and a catalytic site (DXCCXXHD). 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. Substrate hydrolysis proceeds through the activation and orientation of a water molecule by hydrogen bonding to the active site histidine. Adjacent to this histidine, there is a conserved aspartate residue, which, together with the Ca²⁺binding loop, acts as a ligand cage for Ca^{2+} . As the genes for sPLA₂-IIA, -IIC, -IID, -IIE, -IIF, and -V are clustered on the same chromosome locus, they are often referred to as the group II subfamily of sPLA2s. sPLA2-III and sPLA2-XIIA share homology with the I/II/V/X collection of sPLA₂s only in the Ca²⁺-binding loop and catalytic site, thereby representing distinct group III and XII collections, respectively.

sPLA₂s hydrolyze the ester bond at the *sn*-2 position of glycerophospholipids in the presence of micromolar to millimolar concentrations of Ca²⁺ without showing strict fatty acid selectivity.^{1,2)} In general, most group II sPLA₂s act on anionic phospholipids in marked preference to charge-neutral phosphatidylcholine (PC), sPLA₂-V and -X hydrolyze both anionic phospholipids and PC efficiently, and sPLA2-IB is intermediate. These head group preferences are due mainly to the differences in their ability to bind to the lipid-vesicle interface. Some of the group II subfamily of sPLA₂s are highly cationic and bind tightly to anionic heparanoids such as heparin and heparan sulfate. The cluster of basic amino acids near the C-termini of sPLA2-IIA, -IID, and -V are essential for their heparanoid binding, with other basic residues diffused throughout the molecules playing a supporting role. The phospholipid head group specificity and heparan sulfate

proteoglycan (HSPG)-binding property affect the cellular functions of each sPLA $_2$.

EXPRESSION AND PROPERTIES OF EACH ${\rm sPLA}_2$ ISOZYME

sPLA₂-IB has a unique five amino acid extension termed the pancreatic loop in the middle part of the molecule and a group I-specific disulfide between Cys¹¹ and Cys^{77,3} It is synthesized in the pancreatic acinar cells, and after secretion into the pancreatic juice, an *N*-terminal heptapeptide of the inactive zymogen is cleaved by trypsin to yield an active enzyme in the duodenum. Although the digestion of dietary phospholipids appears to be a primary function of pancreatic sPLA₂-IB, recent gene targeting of this enzyme has revealed its unexpected role in obesity and diabetes.⁴⁾ sPLA₂-IB is also expressed in some nondigestive organs.⁵⁾ The mature, but not zymogen, form of sPLA₂-IB binds to the M-type sPLA₂ receptor with high affinity,⁶⁾ and mice deficient in the M-type sPLA₂ receptor show resistance to endotoxin shock.⁷⁾

sPLA₂-IIA has a group II-specific disulfide linking Cys⁵⁰ with Cys at the *C*-terminus and a *C*-terminal extension of seven amino acids in length.⁸⁾ The levels of sPLA₂-IIA in sera or exudative fluids are well correlated with the severity of inflammatory disease. The expression of sPLA₂-IIA is markedly induced by proinflammatory stimuli and downregulated by antiinflammatory cytokines and glucocorticoids in a wide variety of cells and tissues in various animal species.^{9–11)} In contrast, its expression is restricted to the intestine or not expressed at all in mice, revealing a notable species difference.¹²⁾ The *sPLA₂-IIA* gene is intrinsically disrupted due to a frameshift mutation in some mouse strains.¹³⁾ Interestingly, these mice are susceptible to colorectal tumorigenesis, suggesting that sPLA₂-IIA plays an antitumor role in this occasion, although the precise molecular mechanism for this is unclear.

 $sPLA_2$ -IIC has an additional disulfide between Cys⁸⁷ and Cys⁹³ in an extended loop region and is expressed in rodent

testis.¹⁴⁾ 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. sPLA₂-IID is structurally most similar to sPLA₂-IIA, and its transcript is constitutively detected in the immune and digestive organs and is upregulated by proinflammatory stimuli in some tissues.¹⁵⁾ Interestingly, its expression is markedly reduced in lymphotoxin α -deficient mice.¹⁶⁾ sPLA₂-IIE, which is another sPLA₂-IIA-related enzyme, is expressed constitutively in several tissues at low levels and is also upregulated by proinflammatory stimuli.¹⁷⁾ The intrinsic enzymatic activity of sPLA₂-IIE is much lower than that of the related sPLA₂s, at least under the standard PLA₂ assay conditions. sPLA₂-IIF possesses a unique 30-amino acid C-terminal extension that contains an additional cysteine residue, which may contribute to formation of a homodimer or a heterodimer with a second protein.¹⁸⁾ The expression of the sPLA₂-IIF transcript is limited to the testis of adult mice, whereas it is detected in various human tissues at low levels. A high level of its expression is found in mouse embryos, implying that its expression is developmentally regulated.

sPLA₂-V does not possess the group I- and group II-specific disulfides and the group II-specific *C*-terminal extension, even though it belongs to the group II subfamily of sPLA₂s.¹⁹⁾ sPLA₂-V is the primary sPLA₂ in the mouse, where its transcript is expressed at higher levels than the other group II subfamily sPLA₂s in various tissues and immune cells.¹²⁾ It is also distributed widely in human tissues, among which the highest expression is found in the heart.¹⁹⁾ As in the case of other group II subfamily sPLA₂s, its expression is also markedly induced by proinflammatory stimuli.¹²⁾

sPLA₂-X has both the group I- and II-specific disulfides, the group II-specific *C*-terminal extension, and the group Ispecific propeptide.²⁰⁾ Like sPLA₂-IB, sPLA₂-X is synthesized as a zymogen, and removal of the *N*-terminal propeptide produces an active mature enzyme.²¹⁾ The mature enzyme interacts with the M-type sPLA₂ receptor with high affinity.²²⁾ sPLA₂-X transcript is expressed in the immune and digestive organs and testis rather constitutively.²⁰⁾

sPLA₂-III is an unusually large protein (55 kDa) among the sPLA₂ family and consists of three domains, in which a central sPLA₂ domain that displays all of the features of group III bee venom sPLA₂s, including 10 cysteines and the key residues of the Ca²⁺ loop and catalytic site, is flanked by large and unique *N*- and *C*-terminal regions.²³⁾ Its transcript is detected in the kidney, heart, liver, and skeletal muscle. It remains unresolved how this enzyme is proteolytically processed in cells.

sPLA₂-XIIA is a 19-kDa enzyme containing a central catalytic domain with a His/Asp catalytic dyad, yet the location of cysteines outside the catalytic domain is distinct from that of other sPLA₂s.²⁴⁾ Furthermore, comparing the consensus segment of the Ca²⁺-binding loop (X₁CG₁X₂G₂), the position of G₂ is replaced by proline in sPLA₂-XIIA. Strong expression of the sPLA₂-XIIA transcript is found in the heart, skeletal muscle, kidney, and pancreas and weaker expression in various tissues in humans. In the mouse, there are two alternative spliced forms with distinct subcellular localization, one of which is expressed in T_{H2} cells.²⁵⁾ ACTIONS OF sPLA₂S ON MAMMALIAN CELL MEM-BRANES

Beyond the essential role of group IVA cytosolic PLA₂ α $(cPLA_2\alpha)$ in lipid mediator production, current cell biology studies have demonstrated that several sPLA2s have the ability to initiate or augment cellular arachidonic acid release. Since the expression of several sPLA₂s are elevated during the inflammatory response, it has been argued that the ameliorating effect of sPLA₂ inhibitors or antibodies on animal inflammation models may be due, at least in part, to suppression of sPLA₂-mediated lipid mediator production.^{26,27)} The modes of cellular action of individual sPLA2s are influenced by their ability to interact with PC, a phospholipid enriched in the outer leaflet of the plasma membrane, and with cell surface HSPG, which presumably plays a role in proper sorting of sPLA₂s into particular membrane microdomains.²⁸⁻³¹⁾ Two pathways for sPLA₂ action on mammalian cells, termed the external plasma membrane pathway and the HSPG-shuttling pathway, are illustrated in Fig. 1.

Although sPLA₂-IIA can act poorly on the surface of quiescent cells because of its weak binding capacity to the PCrich external plasma membrane, it often augments arachidonate release and eicosanoid production in activated cells.^{32,33)} The cationic nature of this enzyme allows its electrostatic interaction with negatively charged heparan sulfate chains of proteoglycans on the cell membrane.²⁸⁾ Thus sPLA₂-IIA, as well as its close relatives sPLA2-IID and -IIE, binds glypican, a glycosylphosphatidylinositol-anchored HSPG, in caveolae or rafts, and upon cell activation they are internalized into vesicular and perinuclear membrane compartments through caveolae/raft-dependent endocytosis.²⁹⁻³¹⁾ It is assumed that this redistribution of HSPG-shuttled sPLA₂s in the intracellular membrane compartments allows their contact with preferred phospholipid substrates and their spatiotemporal colocalization with downstream eicosanoid-biosynthetic enzymes, such as cyclooxygenases and lipoxygenases, leading to efficient eicosanoid biosynthesis. However, the precise intracellular sites on which the HSPG-shuttled sPLA₂s act have not yet been firmly determined. Note that the occurrence of the HSPG-shuttling pathway appears to be cell type specific. The HSPG binding of sPLA₂s results in their inactivation due to lysosomal degradation in certain cases.³⁴⁾

sPLA₂-V shows high affinity for both HSPG and PC, thereby being capable of releasing cellular arachidonic acid in two distinct mechanisms. As in the case of sPLA₂-IIA, the high HSPG affinity of sPLA₂-V allows this enzyme to act on cells through the HSPG-shuttling pathway.^{28–31)} Due to its high interfacial binding to PC, sPLA₂-V can also act on the PC-rich plasma membrane surface to release arachidonic acid and lysophosphatidylcholine (LPC) independently of HSPG.^{34,35)} Since the latter pathway does not require membrane rearrangements of target cells, sPLA₂-V can release arachidonic acid even from quiescent cells far more efficiently than the other group II subfamily of sPLA₂s. In an elegant spatiotemporal analysis, it has been shown that the hydrolysis of the plasma membrane and the perinuclear membrane by sPLA₂-V occurs sequentially and that the plasma membrane hydrolysis by sPLA₂-V is required for the initiation of the HSPG-dependent internalization of this enzyme.36,37)



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Fig. 1. Two Pathways for Exogenous sPLA₂ Action on Mammalian Cells

(A) HSPG-shuttling pathways. sPLA₂-IIA, -IID, and -V bind to the heparan sulfate chains of glycosylphosphatidylinositol-anchored HSPG glypican, are sorted into caveolae or rafts, are internalized to the perinuclear membranous regions through the caveolae/raft-dependent endocytotic machinery, and supply arachidonic acid to adjacent cyclooxygenase (COX) in activated cells. Release of arachidonic acid from the perturbed plasma membrane microdomain by these sPLA₂s is also possible. (B) External plasma membrane pathway. sPLA₂-V and -X are capable of releasing arachidonic acid from the PC-rich external surface of the plasma membrane of quiescent cells. This arachidonic acid is incorporated into the perinuclear COX. sPLA₂-IIF and -III, which exhibit PC-hydrolytic acivity (even though weaker than that of SPLA₂-V and -X), can also utilize the external plasma membrane pathway. Common properties of the sPLA₂s in each situation are shown on the left. For details, see text.

Among mammalian sPLA₂s, sPLA₂-X shows the highest binding affinity for PC and thus exhibits the most potent release of arachidonate and LPC from the plasma membrane of target cells.^{21,30)} The crystal structure reveals that the opening to the active site of sPLA₂-X is considerably larger than that of sPLA₂-IIA and that the electrostatic surface potential of the sPLA₂-X interfacial-binding surface is charge neutral, which allows sPLA₂-X to be as active on zwitterionic as on anionic membranes.³⁸⁾ Since sPLA₂-X shows no affinity for HSPG, it does not act on cells via the HSPG-shuttling pathway.³⁰⁾

sPLA₂-IIF and -III exhibit unique arachidonate-releasing properties, where the regions characteristic of these enzymes play regulatory roles. sPLA₂-IIF, which is acidic and has a long *C*-terminal extension, has the ability to increase cellular arachidonate release, probably through acting on the perturbed microdomain on the plasma membrane with no dependence on HSPG.³⁹⁾ The unique and long *C*-terminal extension, which plays a role in the duration of the interaction between the enzyme and the plasma membrane, is essential for the cellular arachidonate-releasing function of this enzyme. sPLA₂-III acts on cells through the external plasma membrane pathway, which depends on the PC-binding ability of the central sPLA₂ domain, as well as through the HSPGshuttling pathway, which depends on the binding of highly cationic N- and C-terminal domains to HSPG.⁴⁰⁾

Several reports have shown the coordinated actions of sPLA₂s and cPLA₂ α in certain situations, revealing a signal amplification loop. Prior activation of cPLA₂ α is often required for proper induction and membrane-hydrolytic action of the group II subfamily sPLA₂s, as exemplified by sPLA₂-IIA and -V in rat fibroblasts and mouse macrophages, respectively.^{41,42} Certain lipid oxidative product(s) generated *via* the cPLA₂ α -12/15-lipoxygenase pathway promote the transcription of these sPLA₂s.⁴¹ Conversely, sPLA₂s can regulate subsequent activation of cPLA₂ α . For example, sPLA₂-V initially produces LPC at the plasma membrane, which in turn triggers the second wave of membrane hydrolysis by cPLA₂ α at the perinuclear region in human neutrophils.⁴³⁾ In another circumstance, sPLA₂s indirectly activate cPLA₂ α by acting on the sPLA₂ receptors as high-affinity ligands.⁴⁴⁾

In addition to the membranes of live cells, those of apoptotic cells are potential targets for sPLA₂s. sPLA₂s often hydrolyze the membranes of apoptotic cells more efficiently than those of live cells.⁴⁵⁾ Exposure of phosphatidylserine, an anionic phospholipid that is a preferred substrate for the group II subfamily of sPLA₂s, may facilitate the interaction of these enzymes with apoptotic cell surfaces. Microvesicles shed from apoptotic cells (or even from activated cells) also represent a better target for sPLA₂s.⁴⁶⁾ Furthermore, it has



Fig. 2. Diverse Functions of sPLA₂s

(a) $sPLA_2s$ act on mammalian cells to initiate or augment the production of lipid mediators (such as eicosanoids and lysophospholipids), which in turn mediate various biological events leading to cancer, inflammation, and tissue damage or repair. Alternatively, $sPLA_2s$ may be involved in removal of apoptotic cells. (b) $sPLA_2s$ kill bacteria by degrading phospholipids in bacterial membranes, thereby contributing to the innate immunity. (c) $sPLA_2s$ hydrolyze PC or oxidized PC in lipoprotein particles, and progression of this reaction may result in the development of atherosclerosis. (d) In the lung, $sPLA_2s$ hydrolyze surfactant PC. Excessive hydrolysis of surfactant by $sPLA_2s$ leads to serious respiratory disorders, such as ARDS. (e) In the skin, $sPLA_2s$ may contribute to the production of apolar fatty acids, which, together with ceramide, mediate the skin permeability barrier. (f) In male reproductive organs, $sPLA_2s$ may cause infertility.

been recently reported that vimentin, an intermediate filament protein, is exposed on apoptotic T cell surfaces and acts as an adapter for sPLA₂-IIA.⁴⁷⁾

UNCOVERED TARGETS OF sPLA₂S BEYOND MAM-MALIAN CELL MEMBRANES

This section highlights the emerging evidence for potential roles of sPLA₂s in various biological events unrelated to lipid mediator production. The phospholipid moieties listed below may represent more relevant physiologic substrates for sPLA₂s than those in mammalian cell membranes. These events include lipoprotein metabolism, lung surfactant hydrolysis, innate immunity against bacterial infection, the skin permeability barrier, and acrosome reaction of spermatozoa (Fig. 2). Abnormality or dysfunction of sPLA₂s in these processes may be linked to serious human disorders.

Lipoprotein Metabolism Related to Atherosclerosis The sPLA₂ activity present in arterial intima media may be associated with atherosclerosis. Recent clinical studies have indicated that an elevated plasma level of sPLA₂ activity is a risk factor for coronary heart disease.⁴⁸⁾ On the basis of biochemical and immunohistochemical factors, one of the sPLA₂s detected in atherosclerotic lesions has been ascribed to sPLA2IIA.49) In normal human arteries, sPLA2-IIA is associated with vascular smooth muscle cells of the media, whereas in atherosclerotic plaques it is also found in macrophage-rich regions, in the lipid cores of atheromas, and in the extracellular matrices of the diseased intima, in association with collagen fibers.⁴⁹⁾ sPLA₂-IIA is detected during all stages of atherosclerotic development. The association of sPLA₂-IIA with matrix proteoglycans, such as decorin, biglycan and versican, increases the hydrolytic activity of sPLA₂-IIA toward PC in low-density lipoprotein (LDL) several fold.50)

sPLA₂-IIA may exert proatherogenic effects in multiple steps.^{50–53)} sPLA₂-IIA releases free fatty acids and LPC, which may affect the functions and properties of vascular

cells at sites of LDL accumulation. Although (as noted above) the PC-hydrolyzing action of sPLA₂-IIA is very weak, it may act on oxidized LDL more efficiently, since oxidative modification of phospholipids has been shown to increase their sPLA₂-IIA susceptibility. sPLA₂-IIA may also convert lipoproteins to more atherogenic forms since lipoproteins treated with sPLA₂-IIA become more susceptible to further oxidative modification and their affinity for proteoglycans is increased. The close spatial contact between sPLA2-IIA and lipoproteins resulting from the binding of the enzyme to proteoglycan chains may allow their efficient interaction. Furthermore, sPLA₂-IIA may promote aggregation and fusion of the proteoglycan-bound lipoproteins, leading to the progressive deposition of lipids within the extracellular matrices of the arterial intima, a central feature of atherosclerosis. In addition, circulating sPLA₂-IIA can modify LDL and high-density lipoprotein (HDL) in plasma, thereby contributing to the formation of atherogenic lipoprotein particles.

It is interesting to note that transgenic mice overexpressing human sPLA₂-IIA show increased susceptibility to atherosclerosis.^{54,55)} In these mice, sPLA₂-IIA is present in atherosclerotic lesions in the aorta, as is the case in human atherosclerosis. Plasma levels of HDL are lower, while those of LDL and biologically active oxidized phospholipids are higher, in transgenic mice than in their control littermates. Furthermore, engraftment of bone marrow cells from sPLA₂-IIA transgenic mice into LDL receptor-knockout mice results in the development of atherosclerosis without altering serum lipoprotein levels, suggesting that sPLA₂-IIA expression in macrophages is sufficient to induce athersclerotic lesion formation.⁵⁵⁾

However, one may doubt whether the hydrolysis of PC in lipoproteins is explained by sPLA₂-IIA alone, since this enzyme exhibits only poor activity toward PC. In this regard, it has recently been shown that sPLA₂-V and -X are much more efficient than sPLA₂-IIA in the hydrolysis of PC in lipoprotein particles.^{56,57} Moreover, sPLA₂-X-modified LDL is efficiently incorporated into macrophages, resulting in ac-

cumulation of cytoplasmic lipid droplets. sPLA₂-X is present in foam cell lesions in the arterial intima as well as in smooth muscle cells in the medial layer of the artery wall of high fatfed apolipoprotein E-deficient mice.⁵⁷⁾ Given these observations, it is plausible that the modification of LDL by these PC-hydrolytic sPLA₂s in the arterial vessels is responsible for the generation of atherogenic lipoprotein particles. A remaining question that needs to be clarified is whether sPLA₂-V or -X is present in atherosclerotic lesions in humans.

Lung Surfactant Hydrolysis Linked to Acute Respiratory Distress Syndrome Pulmonary surfactant is a lipidprotein complex, synthesized by the alveolar type II epithelial cells, which lowers surface tension along the alveolar epithelium, thereby promoting alveolar stability. This complex is composed of 10% protein and 90% lipid, with a high proportion of dipalmitoyl-PC. The destruction of surfactant results in loss of alveolar stability and severe impairment of gas exchange, leading to the alveolar collapse known as acute respiratory distress syndrome (ARDS). Bronchioalveolar lavage fluids (BALF) of patients with ARDS or with severe bronchial asthma contain sPLA₂ activity as well as LPC as a degradation product of surfactant, the levels of which often positively correlate with the severity of disease.58,59) Moreover, accumulation of LPC damages type I alveolar cells, increases capillary permeability, reduces the surfactant tensioactivity, and recruits inflammatory cells into the lung.

In several experimental animal models of ARDS, the main sPLA₂ isozyme that participates in the destruction of surfactant phospholipids has been assigned to sPLA₂-IIA.⁶⁰⁻⁶²⁾ Increased sPLA₂ activity in BALF occurs in parallel with that in sPLA2-IIA expression, and this activity can be neutralized with anti-sPLA2-IIA antibody. The surfactant lipoprotein SP-A, which shows homology with sPLA₂-inhibitory protein in snakes, inhibits sPLA₂-IIA activity by direct protein-protein interaction, and the levels of SP-A and surfactant hydrolysis show an inverse relationship. SP-A also suppresses sPLA₂-IIA expression in alveolar macrophages. The synthetic surfactant containing an sPLA2-resistant PC analogue protects the lungs from ARDS-associated injury.63) Moreover, the symptoms of experimental ARDS are markedly attenuated by treatment of animals with an agent that potently inhibits sPLA₂-IIA.⁶⁴⁾

Although these observations provide strong support for the involvement of sPLA2 in lung surfactant hydrolysis, the possibility that sPLA₂ isozyme(s) other than sPLA₂-IIA contributes to this event cannot be ruled out. The sPLA₂ inhibitor used in the pharmacologic study is not strictly specific for sPLA₂-IIA but can also inhibit other sPLA₂ isozymes. Intratracheal instillation of sPLA2-IIA results in the reduction of phosphatidylglycerol, a minor surfactant component, but not that of PC,⁶⁵⁾ in agreement with the substrate specificity of this enzyme. Diagnostically, however, it is LPC that accumulates in the BALF of ARDS patients. It should be noted that surfactant hydrolysis in vivo is facilitated in SP-A-deficient mice and, conversely, reduced in SP-A-overexpressing mice.⁶⁵⁾ Since the mouse strain used in this study is intrinsically deficient in sPLA2-IIA, this observation strongly indicates that an sPLA₂ isozyme(s) other than sPLA₂-IIA contributes to the surfactant hydrolysis in this situation.

An in vitro study demonstrated that the two PC-hydrolyz-

ing sPLA₂s, sPLA₂-V and -X, are capable of degrading surfactant.⁶⁵⁾ More importantly, *in situ* hybridization of human lungs reveals that these two enzymes, but not sPLA₂-IIA, are expressed in airway epithelial cells of human lungs.⁶⁶⁾ Our recent immunohistochemical analysis of human lungs with pneumonia supports this observation (Murakami M., unpublished data). In this context, it is conceivable that the inhibitors that block sPLA₂-V or -X would be beneficial for the treatment of patients with ARDS, the mortality rate of which is still in excess of 50% despite recent advances in intensive care.

Bacterial Membrane Hydrolysis as an Arsenal of Innate Immunity Perhaps the best-recognized physiologic function of sPLA2-IIA is the degradation of bacterial membranes, thereby providing the first line of antimicrobial defense of the host. sPLA₂-IIA is abundantly present in tears, in which the majority of bactericidal action is due to sPLA2-IIA in combination with lysozyme.⁶⁷⁾ sPLA₂-IIA effectively kills Gram-positive bacteria, such as staphylococci, enterococci, and streptococci, *in vitro*,^{68,69)} and sPLA₂-IIA transgenic mice show decreased mortality following Staphylococcus aureus infection, associated with improved clearance of bacteria from organs.⁷⁰⁾ The bacterial envelope sites engaged in cell growth may represent preferential sites for the bactericidal action of sPLA₂-IIA, since bacteria in the growth phase are more susceptible to those in the stationary phase.⁶⁹ Although bacterial cell wall components outside of the phospholipid membrane seem to provide a barrier for the access of sPLA₂-IIA, this enzyme is also bactericidal against Gramnegative Escherichia coli by acting synergistically with the bacterial permeability-increasing protein (BPI), which is produced by neutrophils.71) Through its interaction with lipopolysaccharide, BPI perturbs the capsules of Gram-negative bacteria and enables sPLA₂-IIA to hydrolyze bacterial membrane. Furthermore, sPLA2-IIA enhances the lytic action of complement components against Escherichia coli.72)

Initial binding of $sPLA_2$ -IIA to the surface of Gram-positive bacteria involves electrostatic interactions between the enzyme and the bacterial cell surface. A highly cationic nature of $sPLA_2$ -IIA is required for this enzyme to promote the initial interaction and penetration of the cell wall. Polyanionic properties of lipoteichonic acids in the Gram-positive cell wall facilitate the subsequent hydrolysis of membrane phospholipids by bound $sPLA_2$ -IIA.⁷³

It has become apparent that several other sPLA₂s also exhibit bactericidal activity *in vitro*.^{74,75} The rank order potency among human sPLA₂s against Gram-positive bacteria is IIA>X>V>XII>IIE>IB, IIF. Thus, with the exception of acidic sPLA₂-X, the bactericidal activity of sPLA₂s depends on positive charges on their molecular surfaces. It is also notable that sPLA₂-XIIA kills Gram-negative bacteria even in the absence of BPI, even though the enzymatic activity of this enzyme toward pure phospholipid vesicles is very low.

Regulation of the Permeability Barrier in the Skin sPLA₂ activity is needed for the degradation of polar lipids in the upper epidermal layers for the generation of fatty acids, which, together with ceramide, are major components of the stratum corneum. This layer of apolar lipids is called the permeability barrier, which prevents transepidermal water loss. The physiologic role of sPLA₂s in barrier integrity has been proposed based on the observation that inhibition of sPLA₂

activity in mouse skin results in the destruction of epidermal integrity and in transepidermal water loss.⁷⁶⁾ sPLA₂s may be also involved in inflammatory processes and keratinocyte growth in the skin, a concept that is supported by the finding that sPLA₂-IIA transgenic mice develop hyperkeratosis and hyperplasia.⁷⁷⁾ It has recently been shown that sPLA₂-IB, -IIE, -IIF, -V, and -XIIA are expressed in the upper differentiated layers, sPLA₂-IIA, -IID, and -X are present in the basal and suprabasal layers, and sPLA₂-IIC is found in all layers of mouse skin.⁷⁸⁾ Furthermore, the expression of these sPLA₂s is differently regulated during *in vitro* differentiation of kerationocytes.

Acrosome Reaction of Spermatozoa Human seminal plasma contains high $sPLA_2$ activity, and purification of this enzyme revealed its identity to be $sPLA_2$ -IIA.⁷⁹⁾ Immunohistochemical analyses using anti- $sPLA_2$ -IIA antibody shows its predominant location in epithelial cells in human and bovine prostates, particularly in the posterior lobe and paraurethral glands,⁸⁰⁾ where this enzyme may be a part of the antimicrobial arsenal to protect this organ and spermatozoa against microbial invasion. It has become apparent that, in addition to $sPLA_2$ -IIA, transcripts for other $sPLA_2$ s are detected in male genital organs more abundantly than in many other tissues,¹⁸⁾ suggesting their specific roles in the male reproductive system.

The PLA₂ reaction has been implicated in the acrosome reaction of spermatozoa and the plasma membrane fusion between sperm and oocytes.^{81,82)} It has been proposed that a certain sPLA₂(s) exists on the acrosomal surface and in the middlepiece mitochondria of hamster and human spermatozoa.^{83,84)} The acrosome reaction of hamster spermatozoa and subsequent fertilization *in vitro* is suppressed by anti-sPLA₂ antibody, and this effect was reversed by LPC, a major PLA₂ reaction product.⁸⁵⁾ Accumulation of LPC occurs following *in vitro* incubation of spermatozoa and induces the changes in the zona pellucida and the oolemma which allow sperm-egg fusion.^{86–88)}

Our recent immunohistochemical study has revealed that sPLA₂-IID, -IIE, -IIF, -V, and -X are expressed in spermatogenic cells (Murakami M., unpublished data). Expression of sPLA₂-IIC, a rodent-specific isozyme, in mouse spermatogenic cells was also demonstrated by in situ hybridization.89) Thus, it is tempting to speculate that these germ cell-associated sPLA₂s may play a role in the sperm acrosome reaction by producing LPC. The possible role of $sPLA_2s$ in the acrosome reaction, an exocytosis event in which the acrosomal content is released prior to fertilization, is reminiscent of the ability of sPLA2s to promote exocytosis in mast cells and neuroendocrine cells.^{90,91} Abnormal expression or function of sPLA₂s in male genital organs may cause developmental and functional defects in spermatozoa, as evidenced by the observation that transgenic overexpression of sPLA2-IIA in mouse testis leads to infertility due to the impairment of spermatogenesis.⁹²⁾

CONCLUDING REMARKS

In this review, we describe the up-to-date knowledge of mammalian sPLA₂s, focusing on their potential target membranes in various biological events. The control of particular

sPLA₂s should have advantages over the inhibition of selective lipid mediator pathways and of some other biological events in the treatment of pathologic states. Since more than one PLA₂ can be involved in the pathology of various diseases, the understanding of the expression, function, and regulation of each sPLA₂ in specific tissues and disease states is of particular importance. In certain situations, it would be favorable to control the activity of the different PLA₂s for the treatment of disorders. Accumulating information, combined with the use of proteomics and genetically manipulated animal models of various diseases, will increase our knowledge of the specific or overlapping pathophysiologic functions of individual sPLA₂s. Hopefully, such improved understanding will enable the development of specific agents aimed at decreasing the potential contribution of each sPLA₂ to human pathology.

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