Minireview

Phospholipase A_2 regulation of arachidonic acid mobilization

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Abstract Phospholipase A_2 (PLA_2) constitutes a growing superfamily of lipolytic enzymes, and to date, at least 19 distinct enzymes have been found in mammals. This class of enzymes has attracted considerable interest as a pharmacological target in view of its role in lipid signaling and its involvement in a variety of inflammatory conditions. PLA_2s hydrolyze the *sn*-2 ester bond of cellular phospholipids, producing a free fatty acid and a lysophospholipid, both of which are lipid signaling molecules. The free fatty acid produced is frequently arachidonic acid (AA, 5,8,11,14-eicosatetraenoic acid), the precursor of the eicosanoid family of potent inflammatory mediators that includes prostaglandins, thromboxanes, leukotrienes and lipoxins. Multiple PLA_2 enzymes are active within and surrounding the cell and these enzymes have distinct, but interconnected roles in AA release.

Key words: Arachidonic acid; Eicosanoid metabolism; Phospholipase; Inflammation

1. Introduction

Phospholipase A_2 (PLA_2) constitutes a superfamily of enzymes that catalyze the hydrolysis of the phospholipid *sn*-2 ester bond, generating a free fatty acid and a lysophospholipid. The PLA_2 reaction is the primary pathway through which arachidonic acid (AA) is liberated from phospholipids. Free AA is the precursor of the eicosanoids, which include the prostaglandins, generated through the cyclooxygenase reaction, and the leukotrienes, generated through the lipoxygenase reaction [1]. Additionally, the PLA_2 reaction generates a platelet-activating factor (PAF) precursor when the lysophospholipid product possesses a choline headgroup and an alkyl linkage in the *sn*-1 position. In such instances, an acetyltransferase can act upon it to produce PAF (1-*O*-alkyl-2-acetyl-*sn*-3-phosphocholine). Thus, PLA_2 is an important signaling enzyme, through which multiple downstream effectors are generated.

Eicosanoids and PAF are key mediators of inflammation as well as other pathophysiological conditions [1]. Because prostaglandins, leukotrienes and PAF may all be derived from the action of a PLA_2, direct inhibition of such an enzyme would have the potential of blocking all three of the pathways at once, which could be of therapeutic advantage in certain settings. Thus, the pharmaceutical industry has pursued the design of drugs with potential anti-PLA_2 effects.

Targeting and inhibiting the PLA_2 reaction has proved problematic since numerous PLA_2 enzymes have been identified with overlapping properties, including sites of expression and inhibition susceptibility. In mammals, 19 proteins possessing PLA_2 activity have been described (Tables 1 and 2), all of which have been detected in humans, except for Group IIC PLA_2, which is encoded by a pseudogene [2]. Thus a first step for a rational PLA_2 drug design strategy should be to define the different PLA_2 classes present in cells and elucidate the roles of the specific enzymes in eicosanoid and PAF synthesis.

2. PLA_2 classification

PLA_2s have been systematically classified on the basis of their nucleotide and amino acid sequence [2–4]. The latest update to this classification [2], published in October 2000, included 11 groups, most of which also included several subgroups, but since the publication of that latest update, new PLA_2 enzymes have been described, leading to a 12th group [5,6]. Tables 1 and 2 (adapted from Six and Dennis [2]) depict the most current classification of the PLA_2 enzymes. Group XIII PLA_2, identified in parvoviruses [7,8], and Group XIV PLA_2, identified in Symbiotic fungi [9] and *Streptomyces* [10,11], have also now been added. Additionally, a putative sea anemone PLA_2 gene has recently been described [12]. Remarkably, the cysteine residues of the primary structure of this putative sea anemone PLA_2 align perfectly with the mammalian Group V PLA_2s; but, at this time, the sea anemone protein does not meet the criteria for PLA_2 classification as previously delineated, i.e. PLA_2 activity of the expressed protein has not been reported [2]. Very recently, a novel lysosomal Ca^{2+}-independent PLA_2 has also been described. This enzyme also possesses 1-*O*-acylceramide synthase activity; therefore, until the physiological activity of this enzyme is

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Abbreviations: AA, arachidonic acid; PLA_2, phospholipase A_2; $cPLA_2$, cytosolic Ca^{2+}-dependent phospholipase A_2; $iPLA_2$, cytosolic Ca^{2+}-independent phospholipase A_2; $sPLA_2$, secretory phospholipase A_2; PAF, platelet-activating factor; LPS, bacterial lipopolysaccharide

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Table 1
PLA_2 groups utilizing a catalytic histidine[a]

Group		Initial/common sources	Size (kDa)	Disulfides
I	A	Cobra, krait venom	13–15	7
	B	Mammal pancreas	13–15	7
II	A	Human synovial fluid, platelets, rattlesnake, viper venom	13–15	7
	B	Gaboon viper venom	13–15	6
	C	Rat/mouse testis	15	8
	D	Human/mouse pancreas/spleen	14–15	7
	E	Human/mouse brain/heart/uterus	14–15	7
	F[b]	Human/mouse testis/skin	16–17	7
III[c]		Bee/lizard/scorpion/human	15–18	5
V		Mammal heart/lung/macrophage	14	6
X		Human spleen/thymus/leukocyte	14	8
IX		Snail venom (Conodipine-M)	14	6
XI	A	Green rice shoots (PLA_2-I)	12.4	6
	B	Green rice shoots (PLA_2-II)	12.9	6
XII		Mammal heart/kidney/skin, Muscle	18.7	7
XIII		Parvovirus	<10[d]	0
XIV		Symbiotic fungus/*Streptomyces*	13–19	2

[a]These are typically small extracellular PLA_2s requiring Ca^{2+} for activity and possessing an active site histidine and aspartate pair. Table adapted with permission from Six and Dennis [2].
[b]Group IIF has an additional Cys in its C-terminal extension.
[c]Human GIIIPLA$_2$ (55 kDa) has additional novel C-terminal and N-terminal domains.
[d]The parvovirus PLA_2 motif encompasses only approximately 108 residues of the 81–89 kDa VP1up protein.

established or the specific activity is quantified, classification within the PLA_2 system is premature [13].

A broader classification of the PLA_2s that has historically been utilized to describe PLA_2 activities for which sequence data are not available divides the PLA_2 classes into three types: secretory (sPLA$_2$), cytosolic Ca^{2+}-dependent (cPLA$_2$), and cytosolic Ca^{2+}-independent (iPLA$_2$). This classification has numerous caveats, e.g. the Group IVC PLA_2 is generally referred to as cPLA$_2$-γ, despite its being a Ca^{2+}-independent enzyme. However, the system remains useful for making generalizations when describing properties of multiple PLA_2 groups or when the specific identity of a PLA_2 is unknown.

3. PLA_2 properties

With the expansion of the superfamily of PLA_2 enzymes, it has become increasingly difficult to generalize the properties of the PLA_2s. However, in general, the mammalian sPLA$_2$s (Groups IB, IIA,C–F, III, V, X, XII) have low molecular masses (13–19 kDa) and lack specificity for arachidonate-containing phospholipids. The cPLA$_2$s (Group IV, comprising three subgroups) have higher molecular masses (>60 kDa), and preferentially hydrolyze arachidonate-containing phospholipids (although Group IVC PLA_2 exhibits only a marginal preference). Finally, the iPLA$_2$s (Group VI) have high molecular masses (about 85 kDa) but are not selective for arachidonate-containing phospholipids [2]. It is interesting to note that the Ca^{2+} requirements of the PLA_2s do not distribute within this classification system. For example, although most of the sPLA$_2$s require millimolar levels of Ca^{2+} for enzymatic activity, the recently described Group XII sPLA$_2$ appears to be an exception, requiring μM Ca^{2+} levels. Second, Group IVA and Group IVB require Ca^{2+} for translocation to membranes but not for activity. However, Group IVC is an exception, having no Ca^{2+} requirement, indicating some biochemical commonality with the iPLA$_2$s.

4. PLA_2 and AA release

The functions of mammalian PLA$_2$s have largely been studied by examining the cellular regulation and activation of the enzymes in response to specific stimuli. Cellular responses to external stimuli via receptor-dependent or independent pathways elicit a series of signals that ultimately lead to

Table 2
PLA_2 groups utilizing a catalytic serine[a]

Group		Initial/common sources	Alternate names employed	Size (kDa)	Ca^{2+} effects
IV	A	Human U937 cells/platelets, RAW 264.7/rat kidney	cPLA$_2$α	85	$<$mM; membrane translocation
	B	Human pancreas/liver/heart/brain	cPLA$_2$β	114	$<$mM; membrane translocation
	C	Human heart/skeletal muscle	cPLA$_2$γ	61	None
VI	A-1	P388D$_1$ macrophages, CHO	iPLA$_2$ or iPLA$_2$-A	84–85	None
	A-2	Human B-lymphocytes, testis	iPLA$_2$-B	88–90	None
	B	Human heart/skeletal muscle	iPLA$_2$γ or iPLA$_2$-2	88	None
VII	A	Human/mouse/porcine/bovine plasma	PAF-AH	45	None
	B	Human/bovine liver/kidney	PAF-AH (II)	40	None
VIII	A	Human brain	PAF-AH Ib α_1 (subunit of trimer)	26	None
	B	Human brain	PAF-AH Ib α_2 (subunit of trimer)	26	None

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

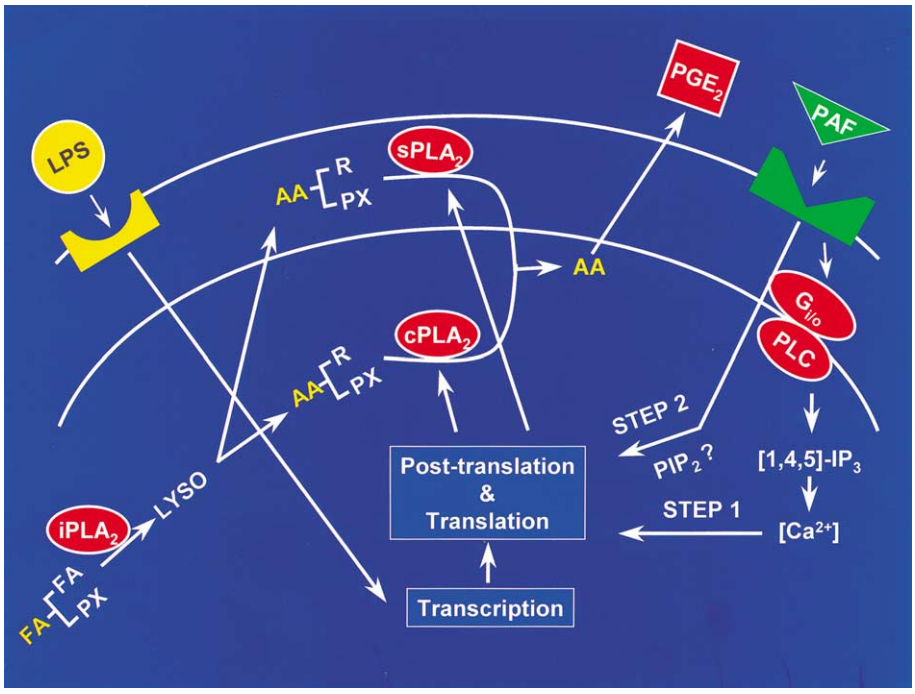

Fig. 1. Signal transduction mechanism in P388D$_1$ macrophages. Group VIA PLA$_2$ (iPLA$_2$) regulates phospholipid reacylation, Group IVA PLA$_2$ (cPLA$_2$) is activated by external stimuli and precedes the activation and/or secretion of Group V PLA$_2$ (sPLA$_2$). Adapted with permission from Balsinde and Dennis [15].

altered PLA$_2$ activity. Elucidation of primary and secondary activating signals has been the subject of much effort for the last 10 years [14]. However, the complexity of the signaling cascade and the identification of numerous mechanisms for PLA$_2$ activation are even further complicated by the overlapping expression of multiple PLA$_2$ enzymes within the cell. By focusing on PLA$_2$ responsiveness to macrophage activation, our laboratory has developed a PLA$_2$ signal transduction model for P388D$_1$ cells [15] (shown in Fig. 1).

The scheme shown in Fig. 1 has been generally confirmed by many other laboratories [16] and thus can be regarded as a currently accepted paradigm for PLA$_2$ signaling in immunoinflammatory cells. The macrophage-like cells generate either an immediate response (induced by the Ca^{2+}-mobilizing agonist, PAF) or a delayed response (induced by bacterial lipopolysaccharide, LPS). PAF elicits the rapid activation of the PLA$_2$ enzymes, whereas LPS primarily acts by inducing the cells to synthesize new protein effectors over the span of hours. In both instances, the foremost event is the translocation and activation of Group IVA PLA$_2$ in an intracellular compartment.

Group IVA (GIVA) PLA$_2$ activation has been the subject of many studies, and generally involves transient elevations of the intracellular Ca^{2+} concentration and the action of the mitogen-activated protein kinase (MAPK) cascade, resulting in the phosphorylation of Ser^{505} within the catalytic domain of GIVA PLA$_2$ [17]. There are a few exceptions, however, in which GIVA PLA$_2$ activation has been described as being activated in a Ca^{2+}-independent manner [18] and/or phosphorylated by kinases other than the MAPK family [19].

Following the activation of GIVA PLA$_2$ is the activation of an sPLA$_2$, which, depending on cellular type, may belong to Groups II, V, or perhaps other groups. Depending on the stimulus, GIVA PLA$_2$ modulation of sPLA$_2$ cellular activity may occur at the level of regulation of enzyme activity itself (immediate responses) [15] or at the gene regulatory level (delayed responses) [20]. In the former case, a variety of cellular mechanisms might account for this activation, including GIVA PLA$_2$-induced rearrangement of membrane phospholipids, which in turn may result in the exposure of preferred substrates to the Group II or V enzymes, or more sophisticated biochemical mechanisms such as inactivation of endogenous sPLA$_2$ inhibitors or Ca^{2+} fluxes. Unlike the Group II and V PLA$_2$s, Group X PLA$_2$ does not require the prior activation of GIVA PLA$_2$; indeed, several groups have shown that Group X PLA$_2$ mediates spontaneous AA release from the outer surface of the plasma membrane [21–23].

While it is clear that GIVA PLA$_2$ acts on perinuclear membranes, the precise site of action of sPLA$_2$ has been the subject of numerous recent studies. The sPLA$_2$ enzyme appears to be released to the extracellular medium, and subsequently reassociates with the outer cellular surface where it hydrolyzes phospholipids. Recent studies have suggested that the enzyme is re-internalized deep into the cell, probably via the caveolin system to the vicinity of nuclear membranes [24]. Whether the enzyme is still active in the cellular interior or whether internalization represents a signal termination mechanism is unclear at present [25]. Data in human neutrophils [26] and mast cells [27] have suggested that sPLA$_2$ internalization leads to protein degradation. In stark contrast, however, in agonist-induced human embryonic kidney (HEK) 293 cells [28,29] internalization resulted in increased AA release and prostaglandin synthesis. The suggestion has been made that this internalization process allows the sPLA$_2$ to localize in the vicinity of COX-2 in the nuclear envelope area, which would ultimately result in a more efficient conversion of AA into prostaglandins [29].

The model depicted in Fig. 1 illustrates a scenario whereby

the concerted action of two distinct PLA_2s leads to a full AA release response. GIVA PLA_2 initiates the response and plays primarily a regulatory role, whereas $sPLA_2$ acts in a second 'wave' to amplify the response by providing a significant portion of the total AA liberated, an intriguing finding considering that $sPLA_2$s are not arachidonyl-selective enzymes [30]. Needless to say, in those cellular systems that do not express an $sPLA_2$ or do so at very low levels [31], GIVA PLA_2 would be the only PLA_2 enzyme responsible for the release.

In addition, $sPLA_2$ may also act in a paracrine manner (i.e. as a stimulus itself) on otherwise unstimulated cells near the site where the $sPLA_2$ enzyme was released. Under these settings, $sPLA_2$ has been shown to induce activation of GIVA PLA_2, thereby propagating the inflammatory response to neighboring cells [25]. Whether $sPLA_2$ activity is actually required for GIVA PLA_2 activation (i.e. whether or not $sPLA_2$ acts on a specific surface receptor) is a matter of debate and probably depends on the type of cell that interacts with the exogenous enzyme [30,32,33].

Studies on the involvement of Group VIA PLA_2 ($iPLA_2$) in AA mobilization have largely been conducted by using bromoenol lactone, a compound that manifests high selectivity for Group VIA PLA_2 in vitro but whose selectivity in vivo is unclear [34–36]. In phagocytic cells, bromoenol lactone has no effect on stimulus-induced AA mobilization, thus ruling out a role for Group VIA PLA_2 in the process [37–40]. In contrast, in other cell types, most notably heart and pancreatic islets [41–43], bromoenol lactone potently suppresses stimulus-induced AA release, which is consistent with the involvement of Group VI PLA_2.

Recently, Group VIA PLA_2 has been shown to be responsive to cellular stimuli including Ca^{2+} ionophore in HEK 293 cells [44] and glucose plus cAMP-elevating agents in INS-1 insulinoma cells [45], suggesting that the enzyme may play a signaling role. These recent studies utilize cells overexpressing Group VIA PLA_2. Although these studies are useful for suggesting enzymatic functions, the cells that overexpress an enzyme may not represent a true physiological responsiveness. Therefore, additional cellular studies will be necessary to confirm if there is a signaling role for this enzyme.

In $P388D_1$ macrophages [46] Group VIA $iPLA_2$ is proposed to mediate phospholipid reacylation reactions by regulating the steady-state level of lysophosphatidylcoline. The lysophospholipid acceptors produced by Group VIA PLA_2 may be used to re-incorporate part of the fatty acids (including AA) that have previously been released by its Ca^{2+}-dependent counterparts. Thus, by regulating AA reacylation reactions, Group VIA PLA_2 may participate in the formation of cellular AA pools. Therefore an emerging model of PLA_2 cellular activity indicates that all three types of PLA_2s ($sPLA_2$, $cPLA_2$, $iPLA_2$) appear to serve important but distinct functions regarding AA mobilization in cells.

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