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COMMENTARY

PHOSPHOLIPASE A_2 ENZYMES: REGULATION AND PHYSIOLOGICAL ROLE

ANIL B. MUKHERJEE,*† LUCIO MIELE* and N. PATTABIRAMAN‡§

*Section on Developmental Genetics, Human Genetics Branch, NICHD, National Institutes of Health, Bethesda, MD 20892; and ‡Code 6030, Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, DC 20375, U.S.A.

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In recent years, interest in phospholipid metabolism has increased dramatically. One of the primary reasons for this increased scientific interest is the recognition that enzymatic reactions involving phospholipids participate in many vital cellular functions, including signal transduction. Moreover, compelling evidence has been accumulated indicating that phospholipid-metabolizing enzymes are involved in the pathogenesis of many devastating disease processes and/or contribute to aggravating existing pathological conditions. As a result, these enzymes have become prime targets for biochemical, genetic and pharmacological studies. Among phospholipid hydrolases, PLA_2s‖ have received considerable attention, due to their involvement in inflammation, cell proliferation and signal transduction. Recent discoveries of several structurally unrelated mammalian PLA_2s have raised new questions on the physiological roles of this family of enzymes.

In this article, we will discuss several mechanisms of regulation and the possible biological roles of PLA_2s. We will also discuss current efforts to develop pharmacological agents to specifically regulate the activity of these enzymes. A comprehensive review of the literature on PLA_2s is beyond the scope of this commentary, and the reader is referred to appropriate review articles whenever indicated.

General features of PLA_2s

PLA_2s (EC 3.1.1.4, phosphatide *sn*-2 acylhydrolases) are esterases that hydrolyze the *sn*-2 ester bonds in phosphoglyceride molecules releasing a free fatty acid and a lysophospholipid [for review see Refs. 1–8]. This is the predominant (but not the only) pathway for the production of arachidonic acid from 2-arachidonyl glycerophospholipids. Arachidonic acid is the substrate for cyclo- and lipoxygenases in the arachidonic acid cascade generating various eicosanoids (e.g. prostaglandins, leukotrienes and thromboxanes). By hydrolyzing 1-*O*-alkyl, 2-arachidonyl glycerophosphocholine, some PLA_2s generate lyso-PAF, the precursor of PAF. Recent investigations have revealed that in mammals this reaction is catalyzed by several PLA_2 isoforms that differ in structure, substrate specificity, ion requirements and catalytic mechanism. Table 1 describes some of the best characterized types of mammalian PLA_2s described thus far. An extensive review of the literature on PLA_2 activities in mammalian tissues can be found elsewhere [2]. Unlike other enzymes involved in signal transduction (e.g. adenylate and guanylate cyclases, phospholipases C), PLA_2s are found both intra- and extracellularly. Historically, the first PLA_2s to be characterized in detail were those from the mammalian pancreas and the venoms of various snakes. These PLA_2s show considerable sequence similarity [1–4] and are evolutionarily related, forming the superfamily of group I/II "low molecular weight" PLA_2s. Structurally similar low molecular weight PLA_2s, which are suggested to be distantly related to the group I and II enzymes, are found in the venoms of bees and wasps [2]. The low molecular weight family includes some of the best characterized PLA_2s from structural and mechanistic points of view. Studies of over 50 of these enzymes from different phyla reveal conservation of several important features, namely, a relatively low molecular mass (≈14 kDa), an amphipathic amino terminal α-helix, the Ca^{2+}-binding loop and the active site, and a large number of intramolecular disulfide bonds (generally seven, but variations exist). These PLA_2s are relatively thermostable and require calcium ions for activity. Despite these similarities, there are certain structural differences that permit classification of these enzymes into two different groups [2]. Group I enzymes are found mainly in the mammalian pancreas and in the venoms of *Elapidae* (e.g. *Naja naja*) and *Hydrophidae* snakes. Group II enzymes are found mostly in *Crotalidae* and *Viperidae* snakes [2]. Molecular models of group I and group II enzymes are shown

† Corresponding author: Dr. Anil B. Mukherjee, National Institutes of Health, Bldg. 10, Room 9S242, Bethesda, MD 20892. Tel. (301) 496-7213; FAX (301) 402-0234.

§ Present address: Bldg. 430/Room 206, NCI/PRI/FCRDC, P.O. Box B, Frederick, MD 21702-1201.

‖ Abbreviations: PLA_2, phospholipase A_2; $cPLA_2$, cytosolic PLA_2; PAF, platelet-activating factor; PKC, protein kinase C; GAP, GTPase-activating protein; DTNB, 5,5′-dithiobis-2-nitrobenzoate; IL, interleukin; TNF, tumor necrosis factor; TG, transglutaminase; MAPK, microtubule-associated protein kinase; RDS, respiratory distress syndrome; EGF, epidermal growth factor; and TGF, transforming growth factor.

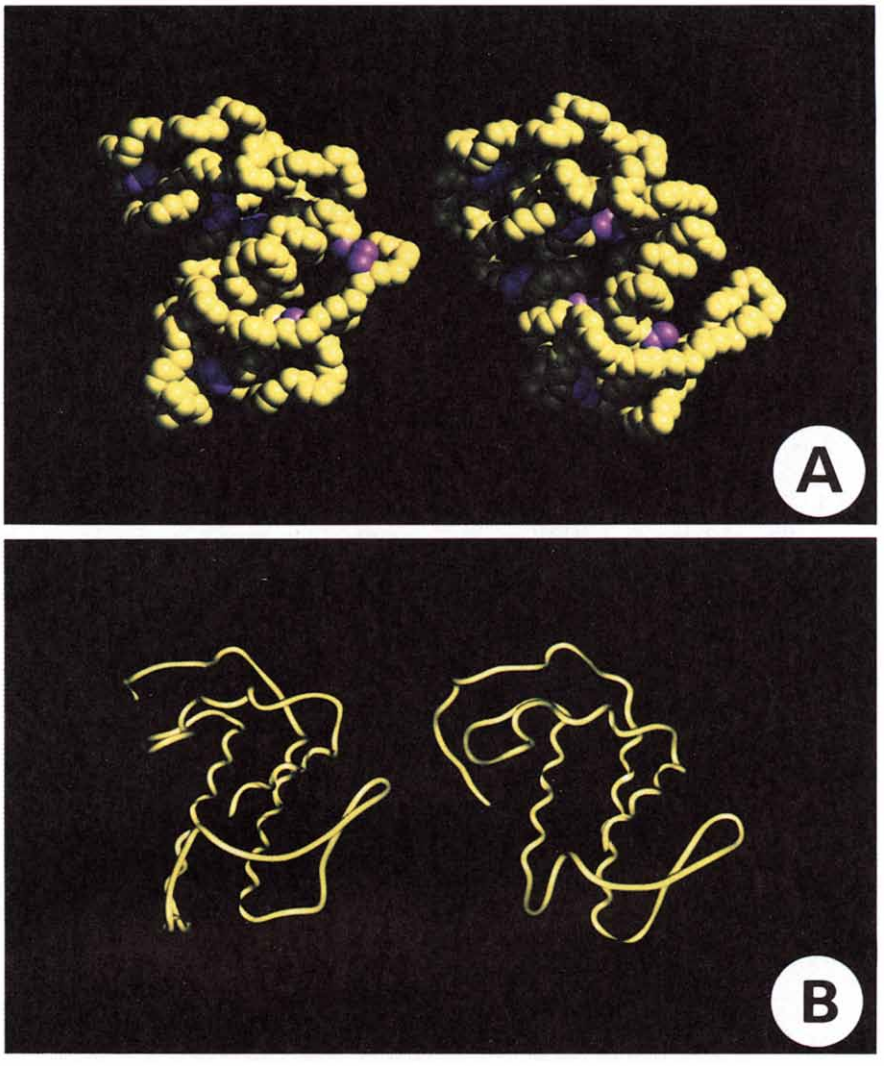

Fig. 1. Molecular model of groups I and II phospholipases A_2. (A) van der Waals' surface representation of the backbone atoms of group I (left) and group II (right) PLA_2 structures. The purple spheres represent disulfide bonds. (B) Ribbon representation of group I (left) and group II (right) PLA_2 structures.

in Fig. 1. The critical structural difference between these two groups is the invariable disulfide bond between cysteine residues 11 and 77 in group I, which is absent in group II enzymes [9]. In group II PLA_2s, an additional disulfide bond is present "connecting the middle of the C-helix to the C-terminus of an extra long (by 7-residues) tail" [9]. Another difference is the presence of the short D-helix or "Elapid loop" in group I enzymes. Some low molecular weight PLA_2s (e.g. pancreatic enzymes) are monomeric in solution, while others form dimers (e.g. *Crotalus atrox*). Other PLA_2s (e.g. *N. naja*) dimerize only at relatively high concentrations. Trimeric forms, and forms containing chaperone subunits are described in animal venoms [2, 9]. In mammals, both group I and II low molecular weight enzymes are present in many tissues and extracellular fluids [7, 8] (Table 1). Group I PLA_2s are secreted as zymogens by the pancreas, but are also found in other organs such as the lung, spleen and gastric mucosa. In the latter two organs, the enzyme is intracellular. A non-pancreatic, group I PLA_2 is also present in human spermatozoa, which is distinct from the seminal fluid group II enzyme [8]. Group II PLA_2 is found in several tissues as secretory and cell surface associated enzymes (e.g. placenta, platelets, neutrophils and peritoneum). At least in humans, there seems to be a single gene for group II PLA_2 [7]. In addition to the low molecular weight enzymes, intracellular PLA_2s of diverse structure and characteristics have been described in various cells and tissues including platelets, macrophages, monoblast and macrophage cell lines, kidney and myocardium [7, 8]. An ischemia-induced myocardial PLA_2 that preferentially hydrolyzes *sn*-2 arachidonyl plasmalogen substrates has been

Table 1. Mammalian phospholipases A_2

I	Low molecular weight (≈14 kDa) PLA_2s
	(a) Group I (secretory and cytosolic): pancreas, lung, spleen, gastric mucosa, sperm, serum
	(b) Group II (secretory and membrane-associated): synovial fluid, placenta, platelets, neutrophils, mesangium, astrocytes, hepatocytes, seminal fluid, peritoneal fluid, serum
II	Ca^{2+}-independent PLA_2s
	40 kDa (microsomal): plasmalogen-selective; myocardium
	58 kDa (cytosolic): dimeric, phosphatidyl-ethanolamine-selective; platelets
III	$cPLA_2$
	85 kDa (cytosolic) regulated by Ca^{2+}, G-proteins and phosphorylation: neutrophils, platelets, macrophage-like cells, liver, lung, brain, small amounts in kidney and spleen
IV	Lysosomal PLA_2s
	Acidic pH optimum; ubiquitous

described [10–12]. This is a microsomal, 40 kDa, Ca^{2+}-independent enzyme. Isolation of a dimeric 58 kDa Ca^{2+}-independent PLA_2 from sheep platelets and cloning of its cDNA have been reported [13]. This enzyme is cytosolic and hydrolyzes selectively 2-arachidonyl phosphatidylethanolamine [13]. A distinct $cPLA_2$ has received considerable attention recently because of its possible involvement in several signal transduction pathways [7, 8]. This PLA_2 is an 85 kDa protein that has an apparent molecular mass of 100–110 kDa in SDS–PAGE. It does not show significant sequence similarity with any other PLA_2, and does not contain disulfide bonds. $cPLA_2$ prefers *sn*-2 arachidonyl phospholipids [7, 8]. Clark *et al.* [14] and Sharp *et al.* [15] have cloned and expressed cDNAs coding for human Ca^{2+}-dependent $cPLA_2$. There are significant local amino acid sequence similarities between $cPLA_2$, Ca^{2+}-dependent PKC isoforms, and GAP [14]. Like these proteins, $cPLA_2$ can translocate from cytosol to cell membrane in the presence of micromolar concentrations of Ca^{2+} [14]. Moreover, this enzyme can be phosphorylated and activated by several protein kinases (see below). Other PLA_2 activities have been described in lysosomes, which have low pH optima and are Ca^{2+} independent [2, 8].

Catalytic mechanism and activation of PLA_2s

A detailed characterization of the catalytic mechanism is available only for low molecular weight PLA_2s [16–20]. From a mechanistic point of view, these enzymes resemble serine proteases, but instead of a Ser hydroxyl, the nucleophile in PLA_2s is a water molecule. The side chain of invariant His 48 in PLA_2s acts as a general acid-base catalyst abstracting a proton from the water molecule, which leads to the formation of a tetrahedral, negatively charged transition state. The protonated His 48 is stabilized by a hydrogen bond network including the β-carboxylate of Asp 99 [17]. The negative charge on the transition state intermediate is stabilized by interactions with the calcium ion and the amido nitrogen of Gly 30. The calcium ion is also coordinated in a complex with the phosphate oxyanion of the phospholipid substrate and with the β-carboxylate of Asp 49. The transition state collapses into products as the His 48 imidazolium donates its proton to the *sn*-2 ester oxygen, leading to the formation of a lysophospholipid and a fatty acid. This mechanism is supported by structural data on *N. naja*, bee venom, pancreatic and human group II PLA_2s [16–20]. A slightly different mechanism has been suggested for "Lys 49" snake venom enzymes, which lack Asp 49 [2]. The catalytic mechanisms of other PLA_2s have not been clarified in detail, but appear to be different from that of low molecular weight PLA_2s [7]. For example, the fact that DTNB inactivates both $cPLA_2$ and Ca^{2+}-independent PLA_2s suggests that one or more Cys sulfydryls are essential for catalysis, perhaps through the formation of a covalent acyl-enzyme thioester. In fact, a covalent acyl-enzyme intermediate has been demonstrated for the 58 kDa dimeric Ca^{2+}-independent PLA_2 [13]. The study of PLA_2 enzymology is complicated by the fact that the activity of all these enzymes depends upon binding to a lipid-water interface [1, 2]. This binding equilibrium, as well as the characteristics of the interface itself, affect the observed enzyme kinetics in ways that are different with different enzymes [1, 2]. Interfacial catalysis has been studied in detail only in low molecular weight PLA_2s. All these enzymes are more active on aggregated phospholipid substrates (e.g. pure or mixed micelles, vesicles, monolayers or bilayers) compared with monomeric soluble substrates. This phenomenon has been called "interfacial recognition" [21]. In addition, some PLA_2s also show a time lag after addition of the enzyme to a solution in contact with a phospholipid surface before full activity is observed [21]. This process, known as "interfacial activation," appears to be limited to those PLA_2s that tend to be monomeric in solution [21]. Some confusion exists in the literature as the term "interfacial activation" has sometimes been used to describe both these phenomena. A possible structural explanation of interfacial recognition has been suggested [17]. The active site of low molecular weight PLA_2s is accessed through a hydrophobic channel, which facilitates the diffusion of a phospholipid molecule from a monolayer into the catalytic site. The opening of the channel is surrounded by residues that can provide a hydrophobic "seal" by binding to the interface, thereby allowing diffusion of phospholipid molecules into the active site without need for solvation of the hydrophobic acyl groups. A universally acceptable

explanation for the interfacial activation lag displayed by monomeric PLA_2s remains to be proposed. One important caveat in the interpretation of these studies is that the details of interfacial binding and activation may vary, depending on the type of enzyme and substrate used. Thus, results obtained with one PLA_2 in one assay system cannot be easily extrapolated to another enzyme and/or another assay system [2, 21]. The equilibrium between enzyme in solution and surface-bound enzyme is suggested to contribute to the anomalous kinetic behavior of some PLA_2s [22]. Dimerization of interface-bound enzymes, accompanied by an increase in catalytic activity, is supported by strong kinetic evidence [21, 23, 24]. Direct chemical evidence for the formation of PLA_2 dimers has been obtained for Asp 49 *Agkistrodon piscivorus* and porcine pancreatic PLA_2 [21]. In these cases, dimerization is triggered by autoacylation of Lys residues (Lys 7 and 10 for *A. piscivorus* and Lys 56 for porcine pancreatic PLA_2). Autoacylation is suggested to occur as a slow side reaction in which the ε amino group of Lys residues accepts an acyl group from a phospholipid substrate during the initial catalytic cycles (which would explain the lag before full activity is observed).

Regulation of PLA_2s

The mechanisms of regulation of extracellular group II PLA_2 identified thus far operate mainly through modulation of its gene expression. Several cytokines (IL-1, IL-6 and TNF) appear to up-regulate PLA_2 expression [25, 26], whereas glucocorticoids are suggested to down-regulate it [27]. In addition, group II PLA_2 binds heparin and heparan sulfate [28, 29]. This could provide possible mechanisms for scavenging circulating enzyme and for enzyme binding to extracellular matrix or cell membranes. Cell-bound enzyme could contribute to arachidonate mobilization in several cell types (see below).

Our laboratory has described a novel mechanism of activation of group I PLA_2s through post-translational modifications of these enzymes catalyzed by TGs (EC 2.3.2.13). TGs, which include coagulation factor XIIIa, catalyze at least two different modifications of group I PLA_2s: (i) the formation of an intramolecular ε-(γ-glutamyl)lysine isopeptide bond between Gln 4 and an as yet unidentified Lys residue [30], and (ii) the covalent conjugation of polyamines and other mono and diamines to Gln 4 [31]. Both modifications cause a considerable increase in PLA_2 catalytic activity, with two slightly different mechanisms. The intramolecular isopeptide bond formation promotes a non-covalent dimerization of porcine pancreatic PLA_2 in the absence of substrate, while the same phenomenon could not be demonstrated for polyaminated PLA_2 [31]. It is unclear whether polyaminated PLA_2 has a higher tendency to dimerize when bound to lipid–water interfaces or if its affinity for such interfaces is increased by the polyamine moiety, which is known to bind phospholipids. It is also possible that the TG-catalyzed transamidation of the Gln 4 side chain by itself may have an effect on the structure of the active site of PLA_2 [31].

Numerous agonists induce receptor-mediated PLA_2 activation in a variety of cells [32]. The catalytic characteristics of $cPLA_2$, and its complex regulation (see below), indicate that this enzyme is involved in agonist-induced arachidonate release. However, we cannot exclude that other PLA_2s (low molecular weight Ca^{2+}-independent, or as yet undiscovered PLA_2s) are also involved in signal transduction [33]. G-proteins have been shown to activate PLA_2s in several systems [32, 34]. At least in some cases, it has been suggested that the $\beta\gamma$ dimer, rather than the α subunit, causes PLA_2 stimulation [34]. In this respect, it is interesting that a PLA_2-activating protein originally identified because of its structural similarity to melittin [35] has been recognized recently to be a member of the G-protein β subunit superfamily [36]. A scheme for phosphorylation-dependent activation of $cPLA_2$ by G-protein and Ca^{2+} in HL-60 granulocytes has been proposed recently [37]. It appears that $cPLA_2$ activation by G-protein coupled receptors in intact cells requires phosphorylation of this enzyme by PKC or PKC-stimulated kinases, as well as an increase in intracellular Ca^{2+}. Phosphorylation is suggested to eliminate the influence of an unidentified inhibitory constraint(s), which reduces the activity of unphosphorylated $cPLA_2$ and prevent its activation by G-protein(s). In addition, $cPLA_2$ is activated by ATP, thrombin, Ca^{2+}-ionophores, and PKC-catalyzed phosphorylation in intact cells [38]. This enzyme is also phosphorylated and activated *in vitro* by p42 MAPK [39]. Moreover, $cPLA_2$ or $cPLA_2$-associated proteins are phosphorylated on tyrosine in HEL-30 keratinocytes stimulated by TGF-α [40]. The complex pattern of phosphorylation of $cPLA_2$ by serine/threonine and tyrosine kinases suggests that this enzyme may be part of the signal transduction pathway of growth factor receptors and possibly that *ras* activation could result in $cPLA_2$ stimulation (e.g. through MAPK). The importance of the arachidonate cascade in mitogenesis has been recognized for a long time [41]. The most important regulatory pathways that can lead to activation of $cPLA_2$ are schematically illustrated in Fig. 2.

Physiological and pathological roles of PLA_2s

Assimilation and remodeling. Prototypic PLA_2s with digestive functions are present in pancreatic juice. Lysosomal PLA_2s can also play a digestive role in hydrolyzing cell membrane phospholipids after phago-lysosomal fusion. Intracellular low molecular weight PLA_2(s) may participate in membrane turnover. It is still unclear whether the group I PLA_2 expressed in the mammalian lung [43] participates in the turnover of surfactant phospholipids and/or in surfactant degradation under pathological conditions such as RDS [44]. Interestingly, the respiratory epithelia of several mammals secrete large amounts of uteroglobin/Clara cell 10 kDa protein, a family of proteins that inhibit low molecular weight PLA_2s [45].

Roles of extracellular PLA_2s in immunologic responses, inflammation, cellular proliferation, vasoconstriction and bronchoconstriction. Several lines

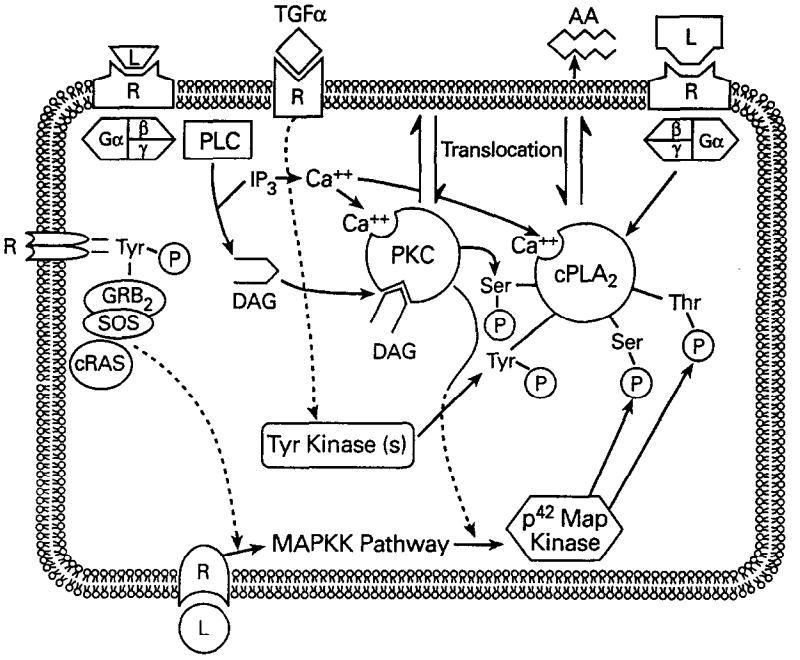

Fig. 2. Diagrammatic representation of some possible mechanisms of regulation of $cPLA_2$. Abbreviations: AA, arachidonic acid; L, ligand; R, receptor; $G\alpha/\beta/\gamma$, G-protein trimer; PLC, phospholipase C; DAG, diacylglycerol; IP_3, inositol 1,4,5-triphosphate; PKC, protein kinase C; MAPKK, microtubule-associated protein kinase kinase; cRAS, cellular *ras*; P, phosphoryl; TGF-α, transforming growth factor α; and GRB_2/SOS, one of the possible pathways of activation of p21 *ras* by Tyr-phosphorylated receptors [42]. Solid arrows = direct pathways; dashed arrows = indirect pathways.

of evidence suggest that group II PLA_2 may participate in the defense against microorganisms, as well as in local and systemic inflammatory responses. Group II PLA_2 secreted by neutrophils upon activation may participate in the lysis of bacteria, in conjunction with a "permeability factor" protein [2, 46]. Group II PLA_2 activity is increased in the serum and synovial fluids of patients with adult and juvenile rheumatoid arthritis and in the serum of patients with septic shock [7, 44]. Its regulation by IL-6 in HepG2 cells and the presence of an IL-6 responsive element in its gene indicate that this enzyme may be an acute phase protein [25]. Extracellular PLA_2s have been suggested to be the causative agent of inflammatory hyperemia and can cause an arthritis-like inflammation when injected in rabbit joints [7, 44]. The mechanism of these proinflammatory effects is not understood completely. Direct cytotoxicity due to hydrolysis of cell membrane phospholipids, and the generation of proinflammatory lipid mediators from membranes of intact and necrotic cells in inflamed tissues are putative mechanisms. Group II PLA_2 enhances eicosanoid generation from activated neutrophils and mast cells [7], and binds specifically to cell membranes of platelets, neutrophils, chondrocytes and macrophage-like cells [7]. Recent work by Inoue and colleagues [47] has shown that the addition of group II PLA_2 to resting T-lymphocytes causes a considerable increase in lymphocyte response to diacylglycerol plus ionomycin-induced activation, as measured by [^{3}H]thymidine incorporation and expression of IL-2 receptor. In the absence of diacylglycerol and ionophore, PLA_2 alone was ineffective. These novel observations may indicate that secretory group II PLA_2 present in inflammatory exudates can enhance T-lymphocyte activation. This, in turn, could lead to a propagation of the inflammatory response (e.g. through cytokine secretion). It is tempting to speculate that a similar mechanism may create a positive feedback loop sustaining chronic inflammation, e.g. in the synovium of patients with rheumatoid arthritis. The ability of group II PLA_2 to bind sulfated polysaccharides and the remarkable stability of this enzyme suggest that this PLA_2 could remain active for prolonged periods of time in the extracellular matrix of cartilage and other connective tissues. In addition, these data may indicate a cross-talk between extra- and intracellular PLA_2s, since in many cells treatment with diacylglycerols and Ca^{2+} ionophores causes activation of intracellular $cPLA_2$. All in all, these observations suggest that group II extracellular PLA_2s have proinflammatory effects *in vivo* and can enhance the activation of inflammatory cells by other agents *in vitro*.

Arita *et al.* [48, 49] have demonstrated that group I pancreatic type PLA_2s have several biological activities that are receptor-mediated. These effects are apparently unrelated to the catalytic activity of these enzymes, and are specific for group I PLA_2s. Pancreatic PLA_2s are suggested to have a receptor-

mediated proliferative effect on Swiss 3T3, rat synovial, smooth muscle cells, and chondrocytes [48–50]. Additional receptor-mediated effects of group I PLA_2 include: (i) contraction of guinea pig lung parenchyma [51]; (ii) contraction of isolated porcine cerebral arteries [52]; and (iii) chemokinetic migration of embryonal rat aortic smooth muscle cells [53]. The contractile activities on lung parenchyma and porcine cerebral arteries are blocked by cyclooxygenase inhibitors, suggesting a previously unrecognized effect of extracellular group I PLA_2s on the arachidonate cascade, and possibly a receptor-mediated cross-talk between group I PLA_2 and $cPLA_2$. The putative PLA_2 receptor has been purified [54*] from bovine corpus luteum membranes, and shown to be a 190 kDa glycoprotein that binds PLA_2 with high affinity ($K_D \approx 1$ nM). The carbohydrate moiety of the receptor is necessary for optimal PLA_2 binding. These highly interesting results, when firmly established *in vivo*, may indicate novel physiological roles of group I PLA_2s. The fibroblast proliferative effect may well be related to the development of fibrosis, which often follows the resolution of an inflammatory process. For example, one might speculate that the release of pancreatic PLA_2 from disrupted acinar cells may contribute to the pancreatic fibrosis which is observed frequently in chronic pancreatitis and cystic fibrosis. The contractile effects may be involved in the pathogenesis of vasoconstriction and perhaps bronchoconstriction characteristic of diseases such as asthma. In this connection, it should be noted that the lung produces a group I PLA_2, and a similar enzyme has been identified in the secretory granules of mast cells [55]. The possible systemic effects of pancreatic PLA_2 released into the bloodstream during pancreatitis [44] remain to be established. The presence of the PLA_2 receptor in the corpus luteum may indicate the function(s) of this receptor in reproductive physiology. Important questions worth investigating are whether or not additional physiological agonists and possibly antagonists of this receptor can be identified, and whether synthetic antagonists with pharmacological activity can be developed. Such studies may provide new opportunities for the prevention of post-inflammatory fibrosis, the inhibition of endothelial contraction, and possibly the inhibition of bronchospasm in asthma.

Roles of intracellular PLA_2s in signal transduction. Ligand-activated arachidonate release with subsequent eicosanoid production is a major signal transduction pathway that is part of the cellular response to a very large number of agonists, including hormones, neurotransmitters and local mediators. Some of the agonists that induce arachidonate release in their target cells include: bradykinin [56], cerulein [57], estradiol followed by progesterone pretreatment [58], 1,25-$(OH)_2$ vitamin D [59], vasopressin, EGF [60], serotonin, [61], acetylcholine through muscarinic receptors [62], histamine [63], norepinephrine via α-adrenergic receptors [64], glutamate [65], IgE antibodies through their Fc receptors [66], dopamine through D2 receptors [67] and TGFα [40]. In most of these cases, arachidonate release has been directly or indirectly shown to be PLA_2 mediated. At least in some systems, such as neutrophil-like HL-60 cells [37], HEL-30 keratinocytes [40] and CHO cells transfected with $cPLA_2$ cDNA [38], $cPLA_2$ has been directly linked to agonist-induced arachidonate release. The presence of this enzyme in macrophage-like cell lines, platelets and mesangial cells suggests that it may play a role in arachidonate release during inflammation, platelet activation and renal prostaglandin production [7]. However, direct *in vivo* evidence for the participation of $cPLA_2$ in such phenomena has not been reported yet. PLA_2-catalyzed arachidonic acid release is suggested to play vital roles in the CNS [68]. A bidirectional regulation of neuronal PLA_2 by Ca^{2+}/calmodulin-dependent protein-kinase II, cAMP-dependent protein kinase and casein kinase II has been proposed as a possible mechanism for the modulation of neurotransmitter release [68]. Glutamate-induced arachidonate release has been suggested to participate in long-term potentiation and thus, in long-term memory [65]. Additionally, PLA_2 activation has been shown to be one of the functions of transducin, the G-protein mediating signal transduction from light stimuli in retinal rods [69].

PLA_2 and cell damage. Recent reports indicate that PLA_2 activation can contribute to anoxic injuries in different organs. Ca^{2+}-independent, intracellular PLA_2s have been suggested to participate in cardiac ischemic damage. Hazen *et al.* [10, 11] and Miyazaki *et al.* [12] have demonstrated that a 10-fold increase in activity of a membrane-associated, Ca^{2+}-independent and plasmalogen-selective PLA_2 occurs during brief myocardial ischemia. Using an animal model Chiarello *et al.* [70] have demonstrated that ischemic myocardial damage can be reduced substantially by inhibition of PLA_2 activity by quinacrine. Quinacrine is not a specific inhibitor of PLA_2, and these results await confirmation with more specific inhibitors of this enzyme. Recently, Nakamura and colleagues [71] reported increased PLA_2 activity in anoxic rat kidneys, and Portilla *et al.* [72] demonstrated that anoxia dramatically induces an intracellular PLA_2 activity in rabbit kidney cells. These authors also suggested that this activated enzyme of renal origin may be a group I PLA_2. Further studies are necessary to confirm these findings. Brain ischemia also causes activation of various PLA_2 activities [73]. Otamiri and his colleagues [74] have demonstrated that ischemia-induced mucosal destruction in the gastrointestinal tract is caused by the activation of phospholipase A_2. They also found that inhibition of PLA_2 activity prevents mucosal cell damage associated with small intestinal ischemia. Additionally, PLA_2s are suggested to be involved in the mechanism of action of several cytotoxic agents. Cell death caused by the toxic peptide *Pyrularia* thionin is mediated by Ca^{2+} influx and the activation of an intracellular PLA_2 [75]. It has been suggested that the cytotoxicity of the cancer chemotherapeutic agent Adriamycin®

* Since the manuscript was submitted, two groups have reported the cloning and characterization of cDNAs coding for the PLA_2 receptor. For details the reader is referred to Refs. 84 and 85.

may be partially related to its ability to activate PLA_2 *in vivo* [76] and *in vitro* [77].

Therapeutic potential of PLA_2 inhibition

The evidence presented above suggests that inhibition of various PLA_2s is potentially a novel and effective therapy for several important disorders including rheumatoid arthritis, autoimmune uveitis, RDS, myocardial infarction, and septic and endotoxic shock [3–8]. Thus, specific inhibitors of PLA_2 activity may have numerous therapeutic applications. However, no specific PLA_2 inhibitory drugs are presently available for clinical use. Therefore, such molecules are actively sought by many investigators around the world. Already a large number of substances are available, which inhibit PLA_2 activity *in vitro* [5, 7, 78, 79], and the list is growing rapidly. The most important problem encountered with "classic" PLA_2 inhibitors, which include antimalarial drugs like quinacrine, local anesthetics, alcohols, organic solvents, polyamines and aminoglycoside antibiotics, is their lack of specificity. These molecules act mostly through interactions with the lipid–water interface, rather than with the enzyme [5]. Other substances, such as *p*-bromophenacylbromide or monoalide, are non-specific covalent modifying reagents [5, 7]. More specific, mechanism-based competitive inhibitors of low molecular weight PLA_2s have been described [7, 78, 79]. These molecules are only weakly active against $cPLA_2$ [7]. However, several phospholipid and transition state analogs designed on the basis of PLA_2 structure are pharmacologically inactive *in vivo* [78]. Competitive inhibitors derived from dehydroabietylamine are anti-inflammatory *in vivo*, but are also cytotoxic and inhibit 5-lipoxygenase in addition to PLA_2 [79]. Secretory proteins that inhibit low molecular weight PLA_2s have been described in rodents and humans [44], and synthetic peptides derived from these proteins are potent anti-inflammatory agents *in vivo* [6, 80]. However, it is unclear whether the biological activities of these compounds result from direct inhibition of PLA_2 activity or by as yet unexplained mechanisms [6, 80]. It should be mentioned that at present we have no clear notion as to the long-term effects of PLA_2 inhibition. Because PLA_2s play physiologically important roles in numerous cellular processes, it is very difficult to predict the potential side-effects of chronic use of cell-permeable, non-specific inhibitors of these enzymes. In particular, the proposed roles of intracellular PLA_2s in the CNS (e.g. long-term potentiation) raise concerns over possible neurological side-effects of PLA_2 inhibitory drugs that cross the blood–brain barrier. Future progress in this field will require the design of pharmacologically active inhibitors with low toxicity, that are specific for a particular type of PLA_2. Antisense oligonucleotides are promising candidates, and the use of such substances has already been described for the inhibition of group II PLA_2 expression [81]. However, even these molecules are not devoid of non-specific effects, and their usefulness should be established on a case-by-case basis [82]. Another potentially important strategy to inhibit extracellular PLA_2s or to neutralize their receptor-binding activity is the use of monoclonal antibodies or derivatives thereof (e.g. peptide or peptidomimetics derived from the complementarity-determining region of monoclonal immunoglobulins). We have identified recently a region of porcine pancreatic PLA_2, which includes part of the Ca^{2+}-binding loop as a potential target for the structure-based development of non-competitive inhibitors [83]. Binding of neutralizing IgG or Fab fragments to this region leads to complete inhibition of PLA_2 catalytic activity. The inhibition is non-competitive with respect to both phospholipid substrate and Ca^{2+}, and does not involve immunoprecipitation. Since the Ca^{2+}-binding loop is a conserved region in all low molecular weight PLA_2s, it may be a target for the design of potent and specific inhibitors of these enzymes (e.g. from monoclonal antibodies). One possible application of such inhibitors is the neutralization of circulating PLA_2s in septic shock. Since the three-dimensional structures of $cPLA_2$ and Ca^{2+}-independent intracellular PLA_2s remain unresolved, rational inhibitor design is still difficult for these enzymes. Future research along these lines may allow the development of novel, therapeutically useful inhibitors of this class of enzymes.

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