ELSEVIER

J. Lipid Mediators Cell Signalling 12 (1995) 119–130

JOURNAL OF LIPID MEDIATORS AND CELL SIGNALLING

1.1.2

Secretory phospholipases A_2

Makoto Murakami [a], Ichiro Kudo [a,*], Keizo Inoue [b]

[a] *Department of Health Chemistry, School of Pharmaceutical Sciences, Showa University, 1-5-8, Hatanodai, Shinagawa-ku, Tokyo 142, Japan*

[b] *Department of Health Chemistry, Faculty of Pharmaceutical Sciences, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113, Japan*

Accepted 14 February 1995

Keywords: Phospholipase A_2, type I; Phospholipase A_2, type II; Phospholipase A_2, cytosolic; Eicosanoid; Prostaglandin; Inflammation

1. Introduction

Phospholipase A_2 (PLA_2) plays a crucial role in diverse cellular responses including phospholipid digestion and metabolism, host defence, and signal transduction. PLA_2 also provides precursors for eicosanoid generation when the cleaved fatty acid is arachidonic acid, or for platelet-activating factor (PAF) formation when the *sn*-1 position of the phosphatidylcholine contains an alkyl ether linkage. Recent advances in molecular and cellular biology have enabled us to understand the molecular nature, possible function and regulation of each PLA_2 isozyme. Mammalian tissues and cells generally contain more than one enzyme, each of which is regulated independently and performs distinct functions. Cytosolic PLA_2 ($cPLA_2$) and two types of secretory PLA_2s (type I and type II) are most the well characterized PLA_2s amongst a still expanding large family of mammalian PLA_2s. Here we focus on recent studies of secretory isozymes of PLA_2. In particular, we emphasize accumulating information on the possible participation of type II PLA_2 in eicosanoid generation and other cellular functions.

2. Classification and structural features of mammalian secretory PLA_2s

The first mammalian isoform of PLA_2 to be identified was pancreatic PLA_2. Subsequently, mammalian pancreatic PLA_2 as well as a number of secretory PLA_2s

* Corresponding author.

SSDI 0929-7855(95)00013-5

isolated from snake venoms have been well characterized mechanistically and structurally. Traditionally, these secretory PLA_2s have been further subdivided into two main groups (type (group) I and type II) based on their primary structures (Dennis, 1994; Heinrikson et al., 1977). Snake venom PLA_2s from Elapidae and Hydrophidae species are called type I, and the others from Crotalidae and Viperidae species are type II PLA_2. Pancreatic PLA_2 has structural similarities to snake venom type I PLA_2s, and thus, is now termed a mammalian type I PLA_2. As shown in a later section, a wide variety of cells secrete another PLA_2 which has similar structural characteristics to snake venom type II PLA_2. Although the terms '$sPLA_2$' ('s' signifying 'secretory') or 'non-pancreatic PLA_2' have often been used in the last few years to describe this PLA_2 (Clark et al., 1991), we employ the term mammalian type II PLA_2 rather than $sPLA_2$ based on accepted nomenclature and the fact that this enzyme is often detected as a membrane-bound enzyme without detectable secretion.

All secretory PLA_2s possess a similar molecular mass of about 14 kDa, and require the presence of submillimolar to millimolar concentrations of Ca^{2+} for effective hydrolysis of substrate phospholipid at an optimum pH of 8.0–9.0. When purified phospholipid is used as a substrate, type I PLA_2 hydrolyzes both phosphatidylcholine and phosphatidylethanolamine at almost equal rates, whereas type II PLA_2 hydrolyzes phosphatidylethanolamine or phosphatidylserine in preference to phosphatidylcholine and phosphatidylinositol (Kudo et al., 1993).

Structurally, type I and type II PLA_2s contain highly conserved amino acid residues and sequences characteristic of all secretory PLA_2s sequenced to date: (i) an α-helical amino-terminal segment containing the lipophilic residues Leu^2, Phe^5, and Ile^7; (ii) a Ca^{2+}-binding loop with the typical glycine-rich sequence Tyr^{25}-Gly-Cys-X-Cys-Gly-X-Gly-Gly-X-X-X-Pro^{37} and the Asp^{49} residue; (iii) an active site His^{48} residue as well as Tyr^{52}, Tyr^{73}, and Asp^{99} residues; and (iv) common spacing of 12 of 14 cysteine residues. Type I PLA_2s possess characteristic half-cysteines at residues 11 and 77 that are missing in type II PLA_2s, whereas type II PLA_2s have a unique half-cysteine at residue 50 and an extension of several amino acid residues at the carboxy terminus, which ends in a half-cysteine. Further, type I PLA_2s have an extra segment called the 'elapid loop' (residues 54–56), which is absent from type II PLA_2s (Hayakawa et al., 1988; Kramer et al., 1989; Seilhamer et al., 1989). The homology between the amino acid sequences of type I and type II PLA_2s in the same species is about 30% (Fig. 1). The refined three-dimensional crystal structure of human type II PLA_2 indicates that this enzyme is much closer to *Crotalus atrox* type II PLA_2 than to bovine pancreatic

```
rat     type I PLA2   AVWQFRNMIKCTIPGSDPLREYNNYGCYCGLGGSGTPVDDLDRCCQTHDHCYNQAKKLESCKFLID
rat     type II PLA2  SLLEFGQMIL-FKTGKRADVSYGFYGCHCGVGGRGSPKDATDWCCVTHDCCYNRLEKRG-C-----
human type II PLA2    NLVNFHRMIK-LTTGKEAALSYGFYGCHCGVGGRGSPKDATDRCCVTHDCCYKRLEKRG-C-----
human type I PLA2     AVWQFRKMIKCVIPGSDPFLEYNNYGCYCGLGGSGTPVDELDKCCQTHDNCYDQAKKLDSCKFLLD

                      NPYTNTYSYKCSGNVITCSDKNNDCESFICNCDRQAAICFS--KVPYNKEYKDLDTKKHC
                      GTKFLTYKFSYRGGQISCSTNQDSCRKQLCQCDKAAAECFARNKKSYSLKYQFY-PNKFCKGKTPSC
                      GTKFLSYKFSNSGSRITCA-KQDSCRSQLCECDKAAATCFARNKTTYNKKYQYY-SNKHCRGSTPRC
                      NPYTHTYSYSCSGSAITCSSKNKECEAFICNCDRNAAICFS--KAPYNKAHKNLDTKKYCQS
```

Fig. 1. Comparison of the amino acid sequences of type I and type II PLA_2s of human and rat.

type I PLA_2 (Werry et al., 1991). The exon-intron structures of the genes for type I and type II PLA_2s closely resemble each other, indicating that these molecules are members of an evolutionarily conserved family (Kramer et al., 1989; Seilhamer et al., 1986). The 5′-flanking region of the type II PLA_2 gene contains cAMP-responsive and interleukin (IL)-6-responsive elements, suggesting that type II PLA_2 is transcriptionally regulated by transmembrane stimuli including those which elevate cAMP levels and several proinflammatory cytokines (Crowl et al., 1991).

3. Physiological and pathological functions of secretory PLA_2s

3.1. Type I PLA_2

PLA_2 present in pancreatic juice is classified as type I PLA_2 and its main role is in the digestion of phospholipids in food (Heinrikson et al., 1977). Type I PLA_2 is first synthesized as a preproenzyme and processed to an inactive proenzyme, which is further converted into the active form by proteolytic cleavage (De Haas et al., 1968; Peterson et al., 1974). It has become clear that type I PLA_2 is also distributed in non-digestive organs such as the spleen and lung (Tojo et al., 1988), although its function in these tissues is unclear.

Recently, type I PLA_2 has been reported to act as a kind of cytokine/growth factor in several cell lines, such as vascular smooth muscle cells and fibroblasts, where it can regulate proliferation, chemotaxis, and lipid mediator generation by interacting with a specific binding site on the cell surface (Arita et al., 1991; Hanasaki and Arita, 1992). Only the mature form of type I PLA_2 is active on this receptor; the corresponding proenzyme and type II PLA_2 are both ineffective. The type I PLA_2 receptor has recently been cloned. This receptor is a 180-kDa type I transmembrane protein with an NH_2-terminal cysteine-rich domain, a fibronectin type II domain, eight repeats of a carbohydrate recognition domain, and transmembrane and cytoplasmic domains. The structure of this receptor is related to the macrophage mannose receptor (Ishizaki et al., 1994; Lambeau et al., 1994). The NH_2-terminal region including the putative carbohydrate recognition domain is responsible for binding type I PLA_2 (K_d = 1–10 nM), despite the fact that type I PLA_2 is not a glycoprotein. This is the first demonstration of a function for an apparently unrelated to its phospholipid-hydrolyzing activity. The receptor cloned by Lambeau et al. (1994) differs from that cloned by Ishizaki et al. (1994) in that it also binds human type II PLA_2 (K_d = 0.8 nM) tightly.

3.2. Type II PLA_2

Type II PLA_2 is widely distributed in a variety of mammalian tissues and cells, although its levels of expression are rather variable (Kudo et al., 1993). The enzyme is synthesized in a precursor form containing a signal sequence and then processed to a mature enzyme during translocation from the cytosolic to luminal side of the endoplasmic reticulum (Kramer et al., 1989). Unlike type I PLA_2, type II PLA_2 is not synthesized as a zymogen. This indicates that type II PLA_2 is present intracellularly in a

latent form and is maintained in this inactive form by some unknown mechanism. Notably, type II PLA_2 is incapable of eliciting hydrolysis of phospholipids found in the plasma membranes of intact cells (Kudo et al., 1993). This might be due to the preferential substrate specificity of type II PLA_2 toward phosphatidylethanolamine or phosphatidylserine, which are exclusively distributed in the inner leaflet of the bilayer membrane. In some cells, such as neutrophils, type II PLA_2 exists in a 'latent form', whose activity is negligible if purified phospholipid vesicles are used as a substrate but becomes detectable after low pH treatment (Hara et al., 1993; Wright et al., 1990). Another unique feature of type II PLA_2 is its high affinity for heparin (Horigome et al., 1987). This association may be mediated by a cluster of basic amino acid residues present in type II PLA_2. The heparin-binding domain appears to be separate from the active site based upon immunochemical studies with epitope-specific monoclonal antibodies (Murakami et al., 1988).

One of the most abundant sources of type II PLA_2 is platelets. Rat platelets have an extraordinarily high content of type II PLA_2 in α-granules relative to other types of cells in rats or the same cells in other animal species. This platelet enzyme is secreted upon stimulation with thrombin (Horigome et al., 1987). Secretion of type II PLA_2 is also detected in rabbit platelets, but hardly detectable with platelets from mouse, guinea pig, or human (Mizushima et al., 1989). Release of type II PLA_2 from human platelets is detectable when autoclaved *E. coli* is used as a substrate; use of this substrate provided a more sensitive measurement of activity than purified phospholipid suspensions (Kramer et al., 1989). Several lines of evidence have revealed that thromboxane A_2 generation by activated human platelets is exclusively mediated by $cPLA_2$, but not by type II PLA_2 (Riendeau et al., 1994; Bartoli et al., 1994). Therefore, the role of type II PLA_2 in platelets is still unclear. Nevertheless, activation of platelets in vivo results in a transient increase in plasma type II PLA_2, which disappears fairly rapidly from circulation. This rapid clearance of type II PLA_2 from blood is due to a rapid association of type II PLA_2 with liver cells and vascular endothelial cells via heparan sulfate proteoglycan on the cell surface (Murakami et al., 1989). As discussed below, this observation suggests that platelet-derived type II PLA_2 might affect cellular function of liver cells or endothelial cells at foci of thrombosis.

The constitutive expression of type II PLA_2 is detectable in tissues such as spleen, thymus, intestine, tonsil, liver, and bone marrow (Aarsman et al., 1989; Kramer et al., 1989; Ono et al., 1988; Verger et al., 1982). This distribution demonstrates the association of type II PLA_2 with organs related to inflammatory responses. Indeed, constitutive expression of type II PLA_2 is found in inflammatory effector cells, such as neutrophils, macrophages, and mast cells (Wright et al., 1990; Hidi et al., 1993; Murakami et al., 1992a). In intestine, type II PLA_2 is localized in paneth cells, which play a role in anti-microbial defense by the small intestine (Senegas-Balas et al., 1984). In liver, type II PLA_2 is preferentially distributed in Kupffer cells (Inada et al., 1991). These findings strongly suggest that type II PLA_2 may be involved in inflammatory responses and in host defense mechanism.

Indeed, a large amount of type II PLA_2 has been detected at various inflamed sites and in the plasma of patients with rheumatoid arthritis or septic shock, as well as in experimental animal models of inflammation (Chang et al., 1987; Hara et al., 1988;

Kramer et al., 1989; Seilhamer et al., 1989; Vadas and Pruzanski, 1986). It should be emphasized that type II PLA_2 is an 'inducible' isoform of PLA_2 which arises in response to various inflammatory stimuli. When cells such as chondrocytes, vascular smooth muscle cells, hepatocytes, astrocytes, or renal mesangial cells are incubated with proinflammatory cytokines, such as tumor necrosis factor (TNF) α, IL-1 or IL-6, endotoxin, or cAMP-elevating reagents, gene expression of type II PLA_2 is induced, and newly generated PLA_2 protein is released into the extracellular fluid (Crowl et al., 1991; Nakano et al., 1990; Nakazato et al., 1991; Oka and Arita, 1991). Anti-inflammatory glucocorticoids and the anti-inflammatory cytokine, transforming growth factor-β, both suppress the gene expression of type II PLA_2 (Nakano and Arita, 1990; Schalkwijk et al., 1992). Furthermore, induced expression and secretion of type II PLA_2 is associated with generation of prostaglandins (PGs). PG generation occurring in parallel with de novo synthesis of type II PLA_2 progresses slowly extending over hours, and is thereby distinguished kinetically from thromboxane A_2 generation by activated platelets or PGD_2 generation by IgE/antigen-stimulated mast cells, both of which occur within a few minutes and are accompanied by $cPLA_2$ activation in response to an increase in cytosolic Ca^{2+} concentration (Kramer et al., 1993; Nakatani et al., 1994). It is of interest that stimulators or repressors of type II PLA_2 expression overlap with those of prostaglandin endoperoxide synthase (PGHS)-2, an inducible isoform of cyclooxygenase that is definitively required for ligand-initiated slowly progressing PG biosynthesis (Murakami et al., 1994; Reddy and Herschman, 1994).

A relationship between type II PLA_2 expression and cytokine-stimulated PG generation has been demonstrated in human umbilical endothelial cells (HUVEC) and in rat liver BRL-3A cells (Murakami et al., 1993a; Suga et al., 1993). After stimulation with TNFα, these cells produce PGI_2 and PGE_2, respectively, over a period of hours. In these cells, newly synthesized type II PLA_2 arising in response to stimulation with TNFα is not secreted into the medium but rather is retained on cell surface via binding to heparan sulfate proteoglycan. PG generation by these cells is suppressed not only by type II PLA_2 inhibitors, including a neutralizing antibody, but also by 'washing out' of cell surface-associated type II PLA_2 by treatment of cells with heparin or with heparinase/heparitinase. Furthermore, exogenously added type II PLA_2 is captured on heparan sulfate proteoglycan on cell surface and augments TNFα-initiated PG generation. Barbour and Dennis (1993) reported that transfection of mouse macrophage cell line with antisense RNA specific for type II PLA_2 reduced the expression of type II PLA_2 and was accompanied by a diminution of PGE_2 generation elicited by lipopolysaccharide and PAF. Thus, type II PLA_2 associated with the cell surface in a heparin-washable pool appears to be crucial in initiating PG generation by cells stimulated with certain agonists. Because heparan sulfate proteoglycan is ubiquitously expressed on most of mammalian cells, the balance of 'secreted' and 'membrane-associated' forms of type II PLA_2 may depend on the amount of heparan sulfate expressed on the cell surface and the amount of type II PLA_2 generated. Alternatively, heparan sulfate, having a high affinity for type II PLA_2, may be more abundant in endothelial cells or hepatocytes than in cells which secrete the enzyme.

The ability of type II PLA_2 to elicit eicosanoid generation in target cells is dependent on the presence of particular co-stimulators. As mentioned above, exogenously added

type II PLA_2 consistently fails to elicit eicosanoid generation in intact cells. In contrast, cells primed or elicited by certain agonists, such as A23187-treated neutrophilic leukemia cells, IgE/antigen-primed mast cells, and TNFα-stimulated HUVEC and hepatoma cells, respond to type II PLA_2 with generation of more PGs than that elicited by each agonist alone (Hara et al., 1991; Murakami et al., 1991a, 1993a; Suga et al., 1993). Similarly, administration of type II PLA_2 into the hind paw of rats with adjuvant arthritis causes severe exacerbation of edema response which is not observed in normal rats (Murakami et al., 1990). These results indicate that some pathological conditions may be required in order for type II PLA_2 to exhibit a progressive effect on the inflammation. Injection of a monoclonal antibody that neutralizes type II PLA_2 activity into rats with carrageenan-induced pleurisy significantly reduces the pleural exudate volume and the intrapleural leukocyte number (Kakutani et al., 1994). This observation further supports notion that type II PLA_2 is important in the inflammatory reaction.

Taking together these observations, we concluded that type II PLA_2 may attack phospholipids in plasma membranes at certain stages of cell activation. Such 'membrane rearrangement' might be induced by some cell stimulators, such as proinflammatory cytokines. Although the precise mechanisms underlying the rearrangements are not yet fully understood, these stimulators may modify the asymmetric distribution of plasma membrane phospholipids, resulting in a translocation to the outer leaflet of amino phospholipids, which are better substrates for type II PLA_2. Alternatively, the ability of type II PLA_2 to elicit PG synthesis might be coupled to simultaneous induction of PGHS-2, to which type II PLA_2-derived arachidonic acid might be supplied. It is also possible that type II PLA_2 may function as a ligand that stimulates arachidonic acid metabolism through binding to a specific receptor, as in the case of type I PLA_2.

Several investigations have indicated the importance of PLA_2 in degranulation. The classical PLA_2 inhibitors, *p*-bromophenacyl bromide and mepacrine, suppress exocytosis of histamine from activated mast cells (McGivney et al., 1981). They also prevent neurotransmitter release from neuronal cells and chromaffin cells (Moskowitz et al., 1982; Frye and Holtz, 1985). In addition, the PLA_2 activator mellitin, as well as some snake venom PLA_2s, cause dose-dependent degranulation of mast cells (Wang and Teng, 1990). Indeed, histamine release from rat peritoneal mast cells induced by IgE/antigen, A23187, compound 48/80 or substance P is effectively suppressed by type II PLA_2-specific inhibitors (Murakami et al., 1991b, 1992b) This suggests that type II PLA_2 may play an important role in steps common to the process of degranulation induced by various secretagogues. After activation of mast cells, type II PLA_2 is expressed transiently on the cell surface and eventually secreted into the medium (Murakami et al., 1992b). The interaction of type II PLA_2 from heparan sulfate might have an important role in association/dissociation of the enzyme with the plasma membrane, and thus, in the regulation of degranulation. We assume that membrane-associated type II PLA_2 acts on the plasma membrane in an autocrine or paracrine manner to generate some lipid-derived fusogenic molecules (e.g., fatty acids, lysophospholipids or their derivatives) which trigger membrane fusion and lead to progression or enhancement of fusion between the plasma membrane and secretory granules (Karli et al., 1990).

When mast cells are exposed to purified type II PLA_2 at concentrations equivalent to those associated with severe inflammation, a significant release of histamine is induced

(Murakami et al., 1993b). The ability of type II PLA_2 to induce histamine release is inhibited by tyrosine kinase inhibitors, by an antibodies or chemicals that block the activity of type II PLA_2, and by heparin or an antibody recognizing the heparin-binding domain of type II PLA_2. This indicates the importance of both the catalytic domain and the heparin-binding domain of type II PLA_2. Furthermore, these results show that supplementation of quiescent mast cells with exogenous type II PLA_2 allows the cells to bypass the degranulation process.

Type II PLA_2 cannot directly degrade the phospholipids in intact bacterial membranes to manifest anti-microbial activity. In contrast, type II PLA_2 decreases the viability of microorganisms in combination with a neutrophil-derived protein called bactericidal permeability increasing protein (BPI) (Elsbach and Weiss, 1988; Wright et al., 1990) When BPI is included in the reaction mixture, phospholipids of intact bacteria are hydrolyzed by type II PLA_2. BPI does not merely pave the way for PLA_2 since there is a high degree of specificity in the interaction between BPI and the enzyme. Thus, type I PLA_2 is unresponsive to BPI. The clusters of basic residues in the NH_2-terminal region may account in part for the ability of type II PLA_2 but not type I PLA_2 to act on BPI-treated bacterial membranes. The synergistic action of type II PLA_2 with BPI, both of which are secreted from neutrophils, may be important for host defense against bacteria at the initial stages of infection.

Activation of type II PLA_2 is also observed in tissues undergoing ischemia. The breakdown of endogenous phosphatidylethanolamine by type II PLA_2, which is detected during incubation of rat heart homogenates, is accelerated markedly by ischemic treatment of the tissue (Kikuchi-Yanoshita et al., 1993).

4. Novel secretory PLA_2s

Recently, two cDNAs encoding novel secretory PLA_2s have been cloned (Chen et al., 1994a, b). Although these enzymes are secreted when each cDNA is transfected into mammalian cells, and exhibit PLA_2 activity at alkaline pH in the presence of millimolar Ca^{2+}, they have several unique features as follows.

The 'third' isoform of secretory PLA_2 (Chen et al., 1994a), expressed mainly in heart and to a lesser extent in lung, contains neither the elapid loop characteristic of type I PLA_2 nor the six amino acid C-terminal extension characteristic of type II PLA_2. In addition, this newer form of PLA_2 has only 12 of the 14 cysteines found in both type I and type II PLA_2s and, therefore, lacks at least one of the seven characteristic disulfide bonds. The amino acid sequence of the human enzyme shows 30 and 40% identity to human type I and human type II PLA_2, respectively. This third isoform hydrolyzes phosphatidylcholine and phosphatidylethanolamine more efficiently than phosphatidylinositol.

The 'fourth' isozyme is also unique. It contains 16 instead of 14 cysteines which distinguishes it from type I and type II PLA_2s (Chen et al., 1994b). Moreover, Ile^9, which is highly conserved in all of the secretory PLA_2s, is replaced by valine. This fourth PLA_2 is similar to type II PLA_2 in that it lacks the elapid loop and the Cys^{11}-Cys^{77} disulfide bridge, and possesses a C-terminal six amino acid tail. Thus, this

fourth isoform may represent a new subtype of type II PLA_2. The transcript for the fourth isozyme is abundant in testis but not in other tissues. This enzyme hydrolyzes phosphatidylinositol in preference to phosphatidylcholine and phosphatidylethanolamine.

5. Conclusion

Although in comparison to those of $cPLA_2$ the physiological functions of secretory PLA_2s are poorly understood, there is accumulating evidence that mammalian tissues may contain more than one PLA_2, each of which may be regulated independently by a number of stimulants including cytokines and other factors found in the tissue microenvironment (Table 1). Most importantly, secretory PLA_2s can behave as hormones or cytokines by affecting the function of neighboring cells. This is unlike $cPLA_2$ which can only regulate arachidonate metabolism in a single cell. In this regard, the role of secretory PLA_2s could be to amplify or spread the primary responses initiated by intracellular PLA_2s to neighboring cells. Recent studies have revealed that particular cytokines can regulate the expression and function of each step in the biosynthetic pathway of the arachidonic cascade; not only the initial enzyme $cPLA_2$, but the intermediate enzyme PGHS-1 and PGHS-2, and the terminal PG synthase (Murakami et al., 1994, 1995). Hence, more caution should be exercised in interpreting the results of studies of stimulus-initiated modification of eicosanoid production as simply being consequences of changes in expression of several enzymes participating in each step of the post-receptor arachidonic acid cascade. Nonetheless, since PLA_2 regulates the initiation of arachidonic acid metabolism and represents one of the rate-limiting steps of

Table 1
Characteristics of two well characterized secretory PLA_2s (types I and II) and $cPLA_2$

PLA_2	Type I	Type II	$cPLA_2$
Molecular weight	14000	14000	85000
Origins			
Mammalia	pancreatic juice	exudate fluid in inflamed sites; inflammatory cells	cytosol of various cells
Non-mammalia	snake venom (Elapidae, Hydrophiidae)	snake venom (Crotalidae, Viperadae)	not detected
Substrate specificity	PE = PC	PE > PC	PE and PC bearing arachidonoyl residue
Ca^{2+} requirement	mM	mM	μM
Possible function in mammalia	digestion of phospholipids in food; proliferative effect on cells	inflammation; tissue damage; degranulation; destruction of bacterial membrane	receptor-coupled arachidonate release
Unique characteristics	biosynthesized as inactive proenzyme and processed to mature enzyme by proteolytic cleavage	induction of gene expression by inflammatory cytokines; high affinity for heparin	translocation to membrane in a Ca^{2+}-dependent manner; activation by phosphorylation

PE, phosphatidylethanolamine; PC, phosphatidylcholine.

this cascade, an understanding of the molecular nature, regulation of expression and function of each PLA_2 is of particular importance in understanding its universal roles in the maintenance of host homeostasis. Furthermore, as the products generated by the PLA_2 reaction are potentially proinflammatory, the stimulus- and rate-related regulation of PLA_2s may be beneficial or detrimental depending upon the intensity and duration of their activation by cellular perturbations.

References

Aarsman, A.J., De Jong, J.G.N., Arnoldussen, E., Neyes, F.W., Van Wassaner, P.D. and Van den Bosch, H. (1989) Immunoaffinity purification, partial sequence, and subcellular localization of rat liver phospholipase A_2. J. Biol. Chem. 264, 10008–10014

Arita, H., Hanasaki, K., Nakano, T., Oka, S., Teraoka, H. and Matsumoto, K. Novel proliferative effect of phospholipase A_2 in Swiss 3T3 cells via specific binding site. (1991) J. Biol. Chem. 266, 19139–19141

Barbour, S.E. and Dennis, E.A. (1993) Antisense inhibition of type II phospholipase A_2 expression blocks the production of prostaglandin E_2 by P388D1 cells. J. Biol. Chem. 268, 21875–21882

Bartoli, F., Lin, H.-K., Ghomashchi, F., Gelb, M.H., Jain, M.K. and Apitz-Castro, R. (1994) Tight binding inhibitors of 85-kDa phospholipase A_2 but not 14-kDa phospholipase A_2 inhibit release of free arachidonate in thrombin-stimulated human platelets. J. Biol. Chem. 269, 15625–15630

Chang, H.W., Kudo, I., Tomita, M. and Inoue, K. (1987) Purification and characterization of extracellular phospholipase A_2 from peritoneal cavity of caseine-treated rat. J. Biochem. (Tokyo) 102, 147–154

Chen, J., Engle, S.J., Seilhamer, J.J. and Tischfield, J.A. (1994a) Cloning and recombinant expression of a novel human low molecular weight Ca^{2+}-dependent phospholipase A_2. J. Biol. Chem. 269, 2365–2368

Chen, J., Engle, S.J., Seilhamer, J.J. and Tischfield, J.A. (1994b) Cloning and characterization of novel rat and mouse low molecular weight Ca^{2+}-dependent phospholipase A_2s containing 16 cysteines. J. Biol. Chem. 269, 23018–23024

Clark, J.D., Lin, L.-L., Kriz, R.W., Ramesha, C.S., Sultzman, L.A., Lin, A.Y., Milona, N. and Knopf, J.L. (1991) A novel arachidonic acid-selective cytosolic PLA_2 contains a Ca^{2+}-dependent translocation domain with homology to PKC and GAP. Cell 65, 1043–1051

Crowl, R.M., Stoller, T.J., Conroy, R.R. and Stoner, C.R. (1991) Induction of phospholipase A_2 gene expression in human hepatoma cells by mediators of the acute phase response. J. Biol. Chem. 266, 2647–2651

De Haas, G.H., Postema, N.M., Nieuwenhuizen, W. and Van Deenen, L.L.M. (1968) Purification and properties of phospholipase A_2 from porcine pancreas. Biochim. Biophys. Acta 159, 103–117

Dennis, E.A. (1994) Diversity of type types, regulation, and function of phospholipase A_2. J. Biol. Chem. 269, 13057–13060

Elsbach, P. and Weiss, J. (1988) Phagocytosis of bacteria and phospholipid degradation. Biochim. Biophys. Acta 947, 29–52

Frye, R.A. and Holtz, R.W. (1985) Arachidonic acid release and catecholamine secretion from digitonin-treated chromaffin cells: effects of micromolar calcium, phorbol ester, and protein alkylating agents. J. Neurochem. 44, 265–273

Hanasaki, K. and Arita, H. (1992) Characterization of a high affinity binding site for pancreatic-type phospholipase A_2 in the rat. Its cellular and tissue distribution. J. Biol. Chem. 267, 6414–6420

Hara, S., Imai, Y., Murakami, M., Mori, H., Takahashi, K., Kudo, I., Naraba, H., Oh-ishi, S. and Inoue, K. (1993) Dynamics and participation of type II phospholipase A_2 in rat zymosan-induced pleurisy. J. Biochem. (Tokyo) 114, 509–512

Hara, S., Kudo, I., Matsuta, K., Miyamoto, T. and Inoue, K. (1988) Amino acid composition and NH_2-terminal amino acid sequence of human phospholipase A_2 purified from rheumatoid synovial fluid. J. Biochem. (Tokyo) 104, 326–328

Hara, S., Kudo, I. and Inoue, K. (1991) Augmentation of prostaglandin E_2 production by mammalian phospholipase A_2 added exogenously. J. Biochem. (Tokyo) 110, 163–165

Hayakawa, M., Kudo, I., Tomita, S., Nojima, S. and Inoue, K. (1988) The primary structure of rat platelet phospholipase A_2. J. Biochem. (Tokyo) 104, 767–772

Heinrikson, R.L., Kruger, E.T. and Keim, P.S. (1977) Amino acid sequence of phospholipase A_2-alpha from the venom of *Crotalus adamanteus*. A new classification of phospholipase A_2 based upon structural determinants. J. Biol. Chem. 252, 4913–4921

Hidi, R., Vargaftig, B.B. and Touqui, L. (1993) Increased synthesis and secretion of a 14-kDa phospholipase A_2 by guinea pig alveolar macrophages. Dissociation from arachidonic acid liberation and modulation by dexamethasone. J. Immunol. 151, 5613–5623

Horigome, K., Hayakawa, M., Inoue, K. and Nojima, S. (1987) Selective release of phospholipase A_2 and lysophosphatidylserine-specific lysophospholipase from rat platelets. J. Biochem. (Tokyo) 101, 625–631

Inada, M., Tojo, H., Kawata, S., Tarui, S. and Okamoto, M. (1991) Preferential distribution of type II-like phospholipase A_2 in mononuclear phagocytic cells in rat spleen and liver. Eur. J. Biochem. 197, 323–329

Ishizaki, J., Hanasaki, K., Higashino, K., Kishino, J., Kikuchi, N., Ohara, O. and Arita, H. (1994) Molecular cloning of pancreatic type I phospholipase A_2 receptor. J. Biol. Chem. 269, 5897–5904

Kakutani, M., Murakami, M., Okamoto, H., Kudo, I. and Inoue, K. (1994) Role of type II phospholipase A_2 in the inflammatory process of carrageenan-induced pleurisy in rats. FEBS Lett. 339, 76–78

Karli, U.O., Schafer, Y. and Burger, M.M. (1990) Fusion of neurotransmitter vesicles with target membrane is calcium independent in a cell-free system. Proc. Natl. Acad. Sci. USA 87, 5912–5915

Kikuchi-Yanoshita, R., Yanoshita, R., Kudo, I., Arai, H., Takamura, T., Nomoto, K. and Inoue, K. (1993) Preferential hydrolysis of phosphatidylethanolamine in rat ischemic heart homogenates during in vitro incubation. J. Biochem. (Tokyo) 114, 33–38

Kramer, R.M., Hession, C., Johansen, B., Hayes, G., McGray, P., Chow, E.P., Tizard, R. and Pepinski, R.B. (1989) Structure and function of human non-pancreatic phospholipase A_2. J. Biol. Chem. 264, 5768–5775

Kramer, R.M., Roberts, E.F., Manetta, J.V., Hyslop, P.A. and Jakubowski, J.A. (1993) Thrombin-induced phosphorylation and activation of Ca^{2+}-sensitive cytosolic phospholipase A_2 in human platelets. J. Biol. Chem. 268, 26796–26804

Kudo, I., Murakami, M., Hara, S. and Inoue, K. (1993) Mammalian non-pancreatic phospholipases A_2. Biochim. Biophys. Acta 1170, 217–231

Lambeau, G., Ancian, P., Barhanin, J. and Lazdunski, M. (1994) Cloning and expression of a membrane receptor for secretory phospholipase A_2. J. Biol. Chem. 269, 1575–1578

McGivney, A., Morita, V., Crews, F.T., Hirata, F., Axelrod, J. and Siraganian, R.P. (1981) Phospholipase activation in the IgE-mediated and Ca^{2+} ionophore A23187-induced release of histamine from rat basophilic leukemia cells. Arch. Biochem. Biophys. 212, 572–580

Moskowitz, N., Schook, W. and Puszkin, S. (1982) Interaction of brain synaptic vesicles induced by endogenous Ca^{2+}-dependent phospholipase A_2. Science 216, 305–307

Mizushima, H., Kudo, I., Horigome, K., Murakami, M., Hayakawa, M., Kim, D.-K., Kondo, E. and Inoue, K. (1989) Purification of rabbit platelet secretory phospholipase A_2 and its characteristics. J. Biochem. (Tokyo) 105, 520–525

Murakami, M., Kobayashi, T., Umeda, M., Kudo, I. and Inoue, K. (1988) Monoclonal antibodies against rat platelet phospholipase A_2. J. Biochem. (Tokyo) 104, 884–888

Murakami, M., Kudo, I. and Inoue, K. (1989) In vivo release and clearance of rat platelet phospholipase A_2. Biochim. Biophys. Acta 1005, 270–276

Murakami, M., Kudo, I., Nakamura, H., Yokoyama, Y., Mori, H. and Inoue, K. (1990) Exacerbation of rat adjuvant arthritis by intradermal injection of purified mammalian 14-kDa type II phospholipase A_2. FEBS Lett. 268, 113–116

Murakami, M., Kudo, I. and Inoue, K. (1991a) Eicosanoid generation from antigen-primed mast cells by extracellular mammalian type II phospholipase A_2. FEBS Lett. 294, 247–251

Murakami, M., Kudo, I., Fujimori, Y., Suga, H. and Inoue, K. (1991b) Type II phospholipase A_2 inhibitors suppressed lysophosphatidylserine-dependent degranulation of rat peritoneal mast cells. Biochem. Biophys. Res. Commun. 181, 714–721

Murakami, M., Kudo, I., Umeda, M., Matsuzawa, A., Takeda, M., Komada, M., Fujimori, Y., Takahashi, K. and Inoue, K. (1992a) Detection of three distinct phospholipases A_2 in cultured mast cells. J. Biochem. (Tokyo) 111, 175–181

Murakami, M., Kudo, I., Suwa, Y. and Inoue, K. (1992b) Release of 14-kDa group II phospholipase A_2 from

activated mast cells and its possible involvement in the regulation of the degranulation process. Eur. J. Biochem. 209, 257–265

Murakami, M., Kudo, I. and Inoue, K. (1993a) Molecular nature of phospholipase A_2 involved in prostaglandin I_2 synthesis in human umbilical vein endothelial cells. J. Biol. Chem. 268, 839–844

Murakami, M., Hara, N., Kudo, I. and Inoue, K. (1993b) Triggering of degranulation in mast cells by exogenous type II phospholipase A_2. J. Immunol. 151, 5675–5684

Murakami, M., Matsumoto, R., Austen, K.F. and Arm, J.P. (1994) Prostaglandin endoperoxide synthase-1 and-2 couple to different transmembrane stimuli to generate prostaglandin D_2 in mouse bone marrow-derived mast cells. J. Biol. Chem. 269, 22269–22275

Murakami, M., Matsumoto, R., Austen, K.F. and Arm, J.P. (1995) c-Kit ligand mediates increased expression of cytosolic phospholipase A_2, prostaglandin endoperoxide synthase-1, and hematopoietic prostaglandin D_2 synthase and increased IgE-dependent prostaglandin D_2 generation in immature mouse mast cells. J. Biol. Chem. 270, 3239–3246.

Nakano, T. and Arita, H. (1990) Enhanced expression of type II phospholipase A_2 gene in the tissues of endotoxin shock rats and its suppression by glucocorticoid. FEBS Lett. 273, 23–26

Nakano, T., Ohara, O., Teraoka, H. and Arita, H. (1990) Type II phospholipase A_2 mRNA synthesis stimulated by two distinct mechanisms in rat vascular smooth muscle cells. FEBS Lett. 261, 171–174

Nakatani, Murakami, M., Kudo, I. and Inoue, K. (1994) Dual regulation of cytosolic phospholipase A_2 in mast cells after cross-linking of Fcε-receptor. J. Immunol. 153, 796–803

Nakazato, Y., Simonson, M.S., Herman, W.H., Konieczkowski, M. and Sedor, J.R. (1991) Interleukin-1α stimulates prostaglandin biosynthesis in serum-activated mesangial cells by induction of a non-pancreatic (type II) phospholipase A_2. J. Biol. Chem. 266, 14119–14127

Oka, S. and Arita, H. (1991) Inflammatory factors stimulate expression of type II phospholipase A_2 in rat cultured astrocytes. Two distinct pathways of the gene expression. J. Biol. Chem. 266, 9956–9960

Ono, T., Tojo, H., Kuramitsu, S., Kagamiyama, H. and Okamoto, M. (1988) Purification and characterization of a membrane-associated phospholipase A_2 from rat spleen. J. Biol. Chem. 263, 5732–5738.

Peterson, W.A., Volwerk, J.J. and De Haas, G.H. (1974) Interaction of phospholipase A_2 and its zymogen with divalent metal ions. Biochemistry 13, 1439–1445

Reddy, S.T. and Herschman, H.R. (1994) Ligand-induced prostaglandin synthesis requires expression of the TIS10/PGS-2 prostaglandin synthase gene in murine fibroblasts and macrophages. J. Biol. Chem. 269, 15473–15489

Riendeau, D., Guay, J., Weech, P.K., Laliberte, F., Yergey, J., Li, C., Desmarais, S., Perrier, H., Liu, S., Nicoll-Griffith, D. and Street, I.P. (1994) Arachidonyl trifluorometyl ketone, a potent inhibitor of 85-kDa phospholipase A_2, blocks production of arachidonate and 12-hydroxyeicosatetraenoic acid by calcium ionophore-challenged platelets. J. Biol. Chem. 269, 15169–15624

Schalkwijk, C., Pfeilschifter, T., Marki, F. and Van den Bosch, H. (1992) Interleukin 1β and forskolin-induced synthesis and secretion of type II phospholipase A_2 and prostaglandin E_2 in rat mesangial cells is prevented by transforming growth factor-β_2. J. Biol. Chem. 267, 8846–8851

Seilhamer, J.J., Randall, T.L., Yamanaka, M. and Johnson, L.K. (1986) Pancreatic phospholipase A_2: isolation of the human gene and cDNAs from porcine pancreas and human lung. DNA 5, 519–527

Seilhamer, J.J., Pruzanski, W., Vadas, P., Plant, S., Miller, J.A., Kloss, J. and Johnson, L.K. (1989) Cloning and recombinant expression of phospholipase A_2 present in rheumatoid arthritic synovial fluid. J. Biol. Chem. 264, 5335–5338

Senegas-Balas, F., Balas, D., Verger, R., De Caro, A., Figarella, A.C.C., Ferrato, F., Lechene, P., Bertrand, C. and Ribet, A. (1984) Immunohistochemical localization of intestinal phospholipase A_2 in rat paneth cells. Histochemistry 81, 581–584

Suga, H., Murakami, M., Kudo, I. and Inoue, K. (1993) Participation in cellular prostaglandin synthesis of type II phospholipase A_2 secreted and anchored on cell-surface heparan sulfate proteoglycan. Eur. J. Biochem. 218, 807–813

Tojo, H., Ono, T., Inoue, K., Kagamiyama, H., Yamano, T. and Okamoto, M. (1988) A phospholipase A_2 in the supernatant fraction of rat spleen. J. Biol. Chem. 263, 5724–5731

Vadas, P. and Pruzanski, W. (1986) Role of secretory phospholipase A_2 in the pathology of disease. Lab. Invest. 55, 391–404

Verger, R., Ferrato, F., Mansbach, C.M. and Pieroni, G. (1982) Novel intestinal phospholipase A_2: purification and some molecular characteristics. Biochemistry 21, 6883–6889

Wang, J.-P. and Teng, C-M. (1990) Comparison of the enzymatic and edema-producing activities of two venom phospholipase A_2 enzymes. Eur. J. Pharmacol. 190, 347–354

Werry, J.P., Schevitz, R.W., Clawson, D.K., Bobbitt, J.L., Dow, E.R., Gamboa, G., Goodson, Jr., T., Hermann, R.B., Kramer, R.M., McClure, D.B., Mihelish, E.D., Putnum, J.E., Sharp, J.D., Stark, D.H., Teater, C., Warrick, M.W. and Jones, N.D. (1991) Structure of recombinant human rheumatoid arthritic synovial fluid phospholipase A_2 at 2.2 Å resolution. Nature 352, 79–82

Wright, G.W., Ooi, C.E., Weiss, J. and Elsbach, P. (1990) Purification of a cellular (granulocyte) and an extracellular (serum) phospholipase A_2 that participate in the destruction of *Escherichia coli* in a rabbit inflammatory exudate. J. Biol. Chem. 265, 6675–6681