Phospholipase A_2 in Eicosanoid Generation

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Phospholipase A_2 (PLA_2) catalyzes the hydrolysis of the *sn*-2 fatty acyl bond of phospholipids, liberating free fatty acids and lysophospholipids (for a review *see* Reference 1). Its presence in pancreatic juices and in cobra venom was discovered around the beginning of the twentieth century (for a review *see* Reference 2). The role of these secreted PLA_2s in digestion and envenomization has since been well documented. Evidence began to appear in the last several decades that PLA_2s are also present in most types of cells and that they are involved in many different cellular functions including general lipid metabolism and membrane homeostasis. During this same period the view of phospholipids has also changed. What were once considered the inert building blocks of cellular membranes are now known to be important metabolic entities. Phospholipids and their metabolites are involved in a large number of important cellular control systems. This discovery has elevated PLA_2s from being simple maintenance enzymes to being important players in such crucial systems as signal transduction and eicosanoid production. Through these systems, the actions of PLA_2s affect a wide range of human physiological functions and diseases including asthma and allergy, the initiation and maintenance of parturition, blood clotting, atherosclerosis, sepsis, asthma, inflammatory bowel disease, and arthritis and other inflammatory diseases (for a review *see* Reference 3). The development of PLA_2 inhibitors is being vigorously pursued. A drug that modulates PLA_2 activity would have a large number of potential uses, especially as a nonsteroidal antiinflammatory agent (for a review *see* Reference 4). Such an inhibitor could supplement the current armament of cyclooxygenase inhibitors and new cyclooxygenase 2 inhibitors being developed to block prostaglandin biosynthesis and the multitude of approaches (for a review *see* Reference 5) currently being explored for the inhibition of enzymes responsible for leukotriene biosynthesis and leukotriene receptor antagonists as illustrated in Figure 1.

PHOSPHOLIPASE A_2 GROUPS AND CLASSIFICATION

For many years, the only known PLA_2s were the secreted pancreatic and venom enzymes (for reviews *see* References 1 and 6–9). These are soluble, extracellular enzymes that have high disulfide bond content, low molecular mass ($\sim$ 14 kD), and require millimolar levels of Ca^{2+} for catalysis. These enzymes have been the subject of numerous diverse studies, including crystallographic (10), and a large body of knowledge has accumulated about how the secreted enzymes act and how they interact with lipid interfaces (for a review *see* Reference 1).

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As it became apparent that PLA_2 was far more than just a digestive enzyme, researchers began searching for intracellular PLA_2s that could account for these new roles. While versions of the secreted enzymes have been found in most cells, several new PLA_2s have also been found that differ dramatically from the secreted enzymes and from one another. The appearance of these new enzymes forced a reevaluation of the PLA_2 classification scheme that had been used for many years. By tradition these enzymes had been divided into three groups based on sequence homology and disulfide bond formation (11). The Group I enzymes are obtained from the venom of Elapidae snakes and from mammalian pancreas. They have a disulfide bridge between residues 11 and 77. The Group II enzymes were first isolated from Viperidae snakes. They do not contain the 11–77 disulfide bridge, but they do have an additional six residues at the carboxy terminus ending with a disulfide bridge between the last amino acid and residue 50. The Group III enzyme was obtained from bee venom and its structure differs significantly from those found in Groups I and II. Several human counterparts to these enzymes have now been identified. The best characterized are the Group IB PLA_2 from pancreas and the Group IIA PLA_2 originally isolated from the synovial fluid of patients with rheumatoid arthritis (12, 13). Group II enzymes have also been found in many different cell types including rat liver mitochondria (for a review *see* Reference 14).

Several other PLA_2s have been discovered that have quite different characteristics and clearly do not fit into these categories. One of the first such enzymes was the human "cytosolic" PLA_2 (Group IV PLA_2) (15, 16). This enzyme has been identified in a variety of cells; it has a molecular mass of 85 kD, an apparent preference for arachidonate-containing phospholipids, and translocates from the cytosol to membranes in the presence of submicromolar levels of Ca^{2+}. Specificity studies including its lysophospholipase activity (17) and a novel activation by phosphatidylinositol biphosphate (PIP_2) (18) have been reported. This has been designated as a Group IV enzyme (1).

Two novel low molecular weight PLA_2s have been cloned and characterized by Tischfield, Seilhamer, and colleagues (19, 20). These new proteins are unique in that they contain 12 and 16 cysteine residues instead of the usual 14, suggesting 6 and 8 disulfide bonds, respectively. These enzymes were originally identified when screening genomic libraries with cDNA probes to the Group II and Group IB pancreatic PLA_2s (21, 22). Analysis of the deduced amino acid sequences of these proteins reveals a high degree of homology with the known PLA_2s and retention of many of the conserved amino acids located in the active site and in the Ca^{2+}-binding site. The 12-cysteine protein lacks the disulfide bond characteristic of the Group I enzymes as well as the disulfide bond characteristic of the Group II enzymes. This enzyme is classified as a Group V PLA_2 and is released by activated macrophages (23). We have cloned and expressed this novel human enzyme (24). The 16-cysteine protein contains the disulfide bond characteristic of the Group II enzymes, lacks the Group I disulfide bond, and contains one novel additional disulfide bond. Because it contains the Group II disulfide, it is classified as Group IIC, as op-

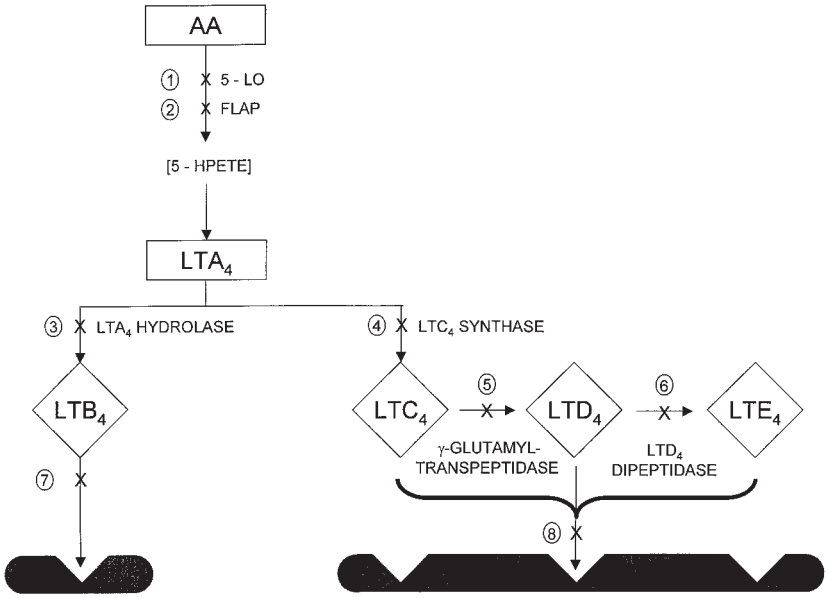

Figure 1. Potential inhibitor and antagonist sites specific for leukotriene biosynthesis and receptor action. 5-LO = 5-lipoxygenase; FLAP = 5-lipoxygenase-activating protein; 5-HPETE = 5-hydroperoxyeicosatetraenoic acid. (Reprinted with permission from Reference 5.)

posed to the "synovial fluid" enzyme, which is Group IIA (1, 25). We have identified and purified another, apparently different, PLA_2 from the $P388D_1$ cell line (26). It is an intracellular, cytosolic PLA_2 that does not require Ca^{2+}. We have sequenced and cloned this 80-kD enzyme (27), which seems to be stabilized by ATP. We have also characterized the inhibition of the enzyme (28). This enzyme has been designated as a Group VI enzyme and currently constitutes the best characterized Ca^{2+}-independent intracellular PLA_2.

We updated the original classification scheme (1) that now includes all the preceding enzymes as well as the platelet-activating factor (PAF) acetylhydrolases and additional secreted PLA_2s (25) (*see* Table 1). An even more up-to-date listing of group numbers will appear shortly (4). There are numerous other Ca^{2+}-dependent and Ca^{2+}-independent intracellular PLA_2 activities described in the literature (for reviews *see* References 14 and 29). However, most of these enzymes have not been characterized sufficiently to allow their assignment to a partic-

TABLE 1

CHARACTERISTICS OF THE MAJOR GROUPS OF PHOSPHOLIPASE A_2*

Group	Source	Location	Size (*kD*)	Ca^{2+} Requirement	No. of Disulfide Bonds	Molecular Characteristics
I						
A	Cobras, kraits	Secreted	13–15	mM	7	His–Asp pair
B	Porcine/human pancreas	Secreted	13–15	mM	7	His–Asp pair, elapid loop
II						
A	Rattlesnakes, vipers, human synovial fluid/platelets	Secreted	13–15	mM	7	His–Asp pair, carboxyl extension
B	Gaboon viper	Secreted	13–15	mM	6	His–Asp pair, carboxyl extension
C	Rat/mouse testes	Secreted	15	mM	8	His–Asp pair, carboxyl extension
III	Bees, lizards	Secreted	16–18	mM	5	His–Asp pair
IV	Raw 264.7, rat kidney, human U937/platelets	Cytosolic	85	$< \mu$M		Ser-228 in GLSGS consensus sequence, Arg-200, Asp-549 required; Ser-505 phosphorylation site; CaL B domain
V	Human/rat/ mouse heart/ lung, $P388D_1$ macrophages	Secreted	14	mM	6	His–Asp pair, no elapid loop, no carboxyl extension
VI	P338D1 macrophages, CHO cells	Cytosolic	80–85	None		GXSXG consensus sequence, ankyrin repeats, 340-kD complex
VII	Human plasma	Secreted	45	None		GXSXG consensus sequence, Ser-273, Asp-296, His-351
VIII	Bovine brain	Cytosolic	29	None		Ser-47
IX	Marine snail	Secreted	14	$<$ mM	6	His–Asp pair

Definition of abbreviation: CaL B = Ca^{2+}-lipid or C-2.
* Reprinted with permission from Reference 25.

ular group. PLA_2 may indeed be a much more diverse family of enzymes than had been previously believed (1, 4, 25).

INTRACELLULAR PLA_2s IN P388D_1 CELLS

As already noted, intracellular PLA_2s are involved in numerous and diverse cell functions. Most cells contain more than one type of PLA_2 and many contain several. All of these enzymes carry out the same reaction, the hydrolysis of the *sn*-2 fatty acid ester of phospholipids. These facts pose an intriguing series of questions. With multiple enzymes and multiple functions, which enzymes are involved with which functions? Does a single type of PLA_2 participate in one and only one function, or does each enzyme participate in several functions? If an enzyme participates in only a few functions, is this controlled via enzyme specificity or via subcellular localization?

We have decided to tackle these questions by studying the PLA_2s found in a single type of cell, using a well-established cell line. In such a system, the environment of the cells is completely and uniformly controlled and large numbers of identical cells can be obtained, experiment after experiment. The use of a cell line also offers an enzyme source that can be grown in large enough quantities that the various enzymes can be isolated and studied in detail.

We have chosen to study the P388D_1 macrophage-like cell line. P388D_1 cells have been characterized both morphologically and functionally as being a macrophage-like cell line (30, 31). As such, these cells can be activated to produce various eicosanoids and exhibit many of the other functions in which PLA_2s have been implicated. We have extensively studied general lipid metabolism and prostaglandin production in these cells. We have shown that these cells are primed by LPS and activated by PAF (32) to produce both eicosanoids and free arachidonic acid. P388D_1 cells contain a number of different phospholipase activities (23, 27). Our studies have allowed us to develop a model of PLA_2 action in these cells (33–35). We have found that there are at least four PLA_2s that have potentially significant roles in these and other cells, i.e., the Group IIA, Group IV, Group V, and Group VI PLA_2s.

The Group VI PLA_2 is involved with the phospholipid remodeling pathway. There exists in cells an ongoing deacylation/reacylation cycle of membrane phospholipids, the so-called Lands cycle, whereby a preexisting phospholipid is cleaved by an intracellular PLA_2 to generate a 2-lysophospholipid, which in turn is reacylated with a different fatty acid to generate a new phospholipid (for a review *see* Reference 35). Macrophages and macrophage cell lines possess a high capacity to incorporate arachidonic acid into their membrane phospholipids. We have shown that for the P388D_1 cells the arachidonic acid incorporation proceeds via the remodeling pathway and that the PLA_2 responsible for the lysophospholipid production is the Group VI PLA_2. This enzyme would affect eicosanoid production only indirectly as a remodeling enzyme by modulating the levels of arachidonic acid in phospholipids by regulating the supply of lysophospholipid.

The Group V and Group IV enzymes are more directly involved in arachidonic acid release and thus prostaglandin production. The Group V enzyme is secreted and acts on the plasma membrane of cells to liberate arachidonic acid and enable prostaglandin production. The Group IV PLA_2 carries out the same function except that it acts internally. While they can act separately, there is evidence that there is cross-talk between these activities and that the activity of one affects the other (34). In addition, there appears to be coupling to one of the two cyclooxygenase (33).

In other cells, a different combination of enzymes can be involved. In WISH cells the Group IIA PLA_2 has replaced the Group V enzyme, which is not detected in these cells (36). In platelets, the secreted PLA_2s (sPLA_2s) do not seem to have an effect and the Group IV PLA_2 appears to be totally responsible for prostaglandin production. Clearly, each type of cell must be examined to determine which enzymes are involved.

Thus, for P388D_1 cells arachidonic acid is taken up by the cells and incorporated into phospholipids via the remodeling pathway and the Group VI PLA_2. The binding of PAF to specific receptors at the plasma membrane after lipopolysaccharide (LPS) priming initiates the activation process by increasing the intracellular Ca^{2+} levels. PAF also triggers a second as yet not completely identified signal. These signals act in concert to initiate translational/posttranslational events that result in the activation of the Group V PLA_2 and Group IV PLA_2. These enzymes are responsible for mobilizing arachidonic acid for prostaglandin production on PAF receptor stimulation in these cells.

THE KINETICS OF PHOSPHOLIPASE ENZYMES

As the list of PLA_2s increases, so too does the diversity of their characteristics (for a review *see* Reference 4). These enzymes, however, do share one important characteristic: they all must interact with large aggregated lipid structures (for a review *see* Reference 37). The nature of this interaction has been defined, in large part, by the studies carried out on the Group I and II PLA_2s. In this sense, the cobra venom and pancreatic PLA_2s have stood as paradigms not only of phospholipid metabolism but for all of lipid enzymology as well. These enzymes continue to play an important role in understanding how soluble enzymes interact with phospholipid interfaces and in defining the parameters of this interaction. This knowledge is crucial if we are to understand how the new, more diverse PLA_2s function.

The studies of the Group I, II, and III enzymes have defined three areas of enzyme activity that seem to be characteristic of PLA_2s and their interactions with interfaces. These are surface dilution kinetics, interfacial activation, and lipid activation. Whether all PLA_2s exhibit these three phenomena has yet to be determined. One of the needs in the phospholipase field is to survey the new intracellular PLA_2s to see if and how these phenomena affect their activity. By comparing the protein structures with the expression of these phenomena, valuable information will be gained relating structure to function. This will be important in the design of inhibitors for these important enzymes which can have important implications in the development of new antiinflammatory agents to prevent the generation of leukotrienes and prostaglandins.

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