Dissecting the Pathways and Enzymes Involved in the Maintenance of Cellular Lysophospholipid levels: Studies on Calcium-independent Phospholipase A_2

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Arachidonic acid is an intermediate of a reacylation/deacylation cycle of membrane phospholipids in which the fatty acid is hydrolyzed from phospholipid by phospholipase A_2 and incorporated back by CoA-dependent acyltransferases. In resting cells, reacylation dominates; in stimulated cells the dominant reaction is deacylation. Enhanced arachidonate reacylation during cellular activation is still very significant, as reflected by the finding that the major part of the free fatty acid released by phospholipase A_2 is effectively re-incorporated into phospholipids.

Arachidonic acid incorporation into phospholipids depends on the availability of lysophospholipid acceptors, particularly lysophosphatidylcholine (lysoPC). In many cells, the levels of lysoPC appear to be maintained by the continuing action of the Ca^{2+}-independent Group VIA phospholipase A_2 (iPLA$_2$-VIA) on cellular phospholipids. Thus a decrease in the activity of iPLA$_2$-VIA frequently results in the diminished production of lysoPC and hence in the inhibition of AA incorporation into phospholipids.

In resting conditions, iPLA$_2$-VIA is thought to constitute the primary phospholipase involved in the liberation of fatty acids, including arachidonate, during the continuous recycling of membrane phospholipids that takes place under these conditions. Since, as indicated above, the rate of arachidonate release by iPLA$_2$-VIA is lesser than the rate of its reacylation back into phospholipids, no net accumulation of free fatty acid occurs. Once arachidonic acid is initially incorporated into lysoPC by the action of CoA-dependent acyltransferases, it is transferred to certain lysophospholipids, particularly the ethanolamine lysophospholipids (lysoPE). Such a transfer is catalysed by the enzyme CoA-independent transacylase.

Within the framework of the model depicted in Fig. 1, recent studies have further explored the involvement of other phospholipases in addition to iPLA-VIA in *housekeeping* phospholipid and fatty acid remodeling. Possible iPLA$_2$ roles in inflammatory signaling distinct from receptor-regulated arachidonate metabolism have also been investigated. These will be discussed later in this talk.

Our first objective [1] is to clarify the biochemical features of arachidonate incorporation into, and remodeling within, phospholipid species. Arachidonic acid incorporation into phospholipids was compared with the incorporation of eicosa-5,8,11,14,17-pentaenoic acid, an *n-3* fatty acid.

In the second part [2], we discuss a model for fatty acid mobilization in H_2O_2-treated cells whereby the oxidant induces lipid oxidation, which results in accumulation of lipid peroxides at the membrane. These lipid peroxides destabilize the membrane and render it more susceptible to iPLA$_2$-VIA attack, which

results in increased liberation of fatty acids. A mechanism like this may be relevant under pathophysiological conditions such as oxidative stress, where increased iPLA$_2$-VIA activity may account for a significant phospholipid hydrolysis before cellular homeostasis is re-established.

The third part of the talk concerns with the inhibitor bromoenol lactone [3]. Originally described as a serine protease inhibitor, bromoenol lactone has been found to potently inhibit iPLA$_2$-VIA. Thus, it has been frequently used to define biological roles of iPLA$_2$-VIA in cells. However, bromoenol lactone is also known to inhibit another key enzyme of phospholipid metabolism, namely the magnesium-dependent phosphatidate phosphohydrolase-1. I will discuss the role of the latter enzyme in cell integrity and survival. Caution should be exercised when using bromoenol lactone in studies involving long incubation times, due to the capacity of this drug to induce iPLA$_2$-VIA-independent apoptosis in a variety of cells.

To further expand our investigations on cellular functions of iPLA2-VIA, we prepared stably transfected cells overexpressing this enzyme [4]. Utilizing this strategy, we reassessed the role of iPLA$_2$-VIA in oxidant-induced AA release and incorporation into phospholipids and extended our studies to the role of iPLA$_2$ in oxidant-induced apoptosis. These results indicate that, although iPLA$_2$-VIA-mediated phospholipid hydrolysis occurs during apoptosis, iPLA$_2$-VIA may actually be dispensable for the apoptotic process to occur. Thus, beyond a mere destructive role, the enzyme may play other roles during apoptosis.

In this regard [5], we have obtained data to suggest that formation of lysophosphatidylcholine by iPLA$_2$-VIA in hydrogen peroxide-treated U937 cells to induce apoptosis directly contributes to their efficient clearance by macrophages. Thus, iPLA$_2$-VIA-mediated membrane phospholipid hydrolysis in apoptotic cells plays a role in enabling macrophages to engulf the dying cells. iPLA$_2$-VIA-induced lysoPC within the dying cell may function as a direct “eat me” signal that helps macrophages recognize an engulf the apoptotic cell.

An aspect that remains to be clarified is whether iPLA$_2$-VIA, in addition to its housekeeping role in phagocytic cells in general, also plays some role in regulated phospholipid hydrolysis in phagocytic cells. The fact that multiple splice variants of iPLA$_2$ exist in some cells suggest the possibility that the enzyme may be subject to different regulatory mechanisms that differ among cell types. In this regard, we have obtained data that suggest that iPLA$_2$-VIA may be capable of playing some signaling roles in cells aside from arachidonate metabolism [6]. Our data have identified iPLA$_2$-VIA-mediated lysoPC production as a necessary component of the molecular machinery leading to lysozyme secretion in U937 cells and rule out a role for cytosolic group IVA phospholipase A$_2$ in the response, which in turn is responsible for all of the arachidonic acid mobilization. Collectively, the results demonstrate distinct roles in inflammatory cell signaling for two intracellular phospholipases.

In more recent results [7], we have noted that free arachidonic acid levels within the cells may provide an important cellular signal for the onset of apoptosis and that perturbations of the mechanisms controlling arachidonate reacylation, and hence free arachidonic acid availability, may decisively affect cell survival. The role of iPLA$_2$-IVA in these events was studied in cells overxpressing the enzyme. These cells exhibit an increased basal hydrolysis of arachidonate-containing phospholipids, and hence of free arachidonic acid and, interestingly, also show augmented apoptosis rates when treated with hydrogen peroxide for extended periods of time.

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