Review

Oxidative stress and arachidonic acid mobilization

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Abstract

Reactive oxygen species are known to contribute to tissue damage during injury and inflammation. However, these species can also be sensed by the cells and trigger intracellular signaling cascades. This review examines recent evidence on the involvement of reactive oxygen species in lipid signaling. Attention is focused on activation of phospholipase A2s, enzymes whose action on membrane phospholipids can also render molecules with opposite effects on cells. The participation of Ca2+-dependent and Ca2+-independent phospholipase A2s in arachidonic acid mobilization from phospholipids is discussed, with particular attention to the interplay between cytosolic and secreted Ca2+-dependent forms. The involvement of alternative routes for arachidonic acid mobilization under oxidative stress is also considered.

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1. Introduction

Oxidative damage of tissues and cells has been widely recognized as a key factor for the development of many human diseases [1–4]. Production of reactive oxygen species (ROS) is an inevitable consequence of normal oxidative metabolism. ROS generated at low concentrations by the cells may play roles as signaling molecules within the signal transduction cascades triggered by growth factors, cytokines, and hormones, or may act themselves as direct cellular stimuli, mimicking in this manner the action of these receptor-directed stimuli. However, high ROS levels can be lethal [5]. The cells are therefore endowed with a number of defensive mechanisms against oxidative stress. Occasionally, these antioxidant mechanisms fail and oxidative stress occurs. High ROS levels have a number of direct and indirect consequences on cell signaling pathways that often result in the induction of apoptosis or necrosis [6].

One of the best described effects of ROS on cells is the oxidative modification of fatty acids within membrane phospholipids, i.e., lipoperoxidation. Oxidative modification of membrane phospholipids is a harmful process, altering membrane fluidity, protein structure and cell signaling. The most efficient manner to repair these phospholipids is the selective cleavage of the peroxidized fatty acid residues and their subsequent replacement by native fatty acids [7]. Since most unsaturated and polyunsaturated fatty acids are esterified in the sn-2 position of phospholipids, lipid peroxidation predominantly occurs at this position, which is the one targeted by phospholipase A2 (PLA2). For this reason, PLA2 enzymes may be envisioned to play protective roles against oxidant injury under certain conditions [7]. In contrast with this view, however, oxidative stress and peroxidation of membrane phospholipids have been shown to enhance PLA2 activity in a number of systems, and the associated activation of PLA2 may constitute a critical factor in injury [8]. Increased PLA2 activity in cells responding to oxidants may not only act to help repair damaged membranes but can also give rise to a number of biologically active mediators such as arachidonic acid (AA)-

Abbreviations: AA, arachidonic acid; PLA2, phospholipase A2; iPLA2, calcium-independent phospholipase A2 activity (undefined molecular identity); iPLA2-VIA, Group VIA phospholipase A2; cPLA2α, cytosolic Group IVA phospholipase A2; cPLA2γ, Group IVC phospholipase A2; sPLA2, secreted phospholipase A2; BEL, bromoenoel lactone; MAFP, methyl arachidonoyl fluorophosphonate; lysoPC, lysophosphatidylcholine; ROS, reactive oxygen species

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derived metabolites and lysophosphatidylcholine (lysoPC), with potential protective or deleterious roles on their own.

The PLA2 superfamily of enzymes is comprised of at least 22 distinct mammalian proteins, all of which hydrolyze membrane phospholipids at the sn-2 position of the glycerol backbone, releasing a free fatty acid and a lysophospholipid [9–11]. The PLA2 enzymes have systematically been classified into several group types according to their primary structure [12–14]. From a mechanistic point of view, the PLA2s can be grouped into two major families, namely the low-molecular mass enzymes (<20 kDa), which utilize a catalytic histidine, and the high molecular mass enzymes (>40 kDa), which utilize a catalytic serine [9,10]. For detailed information on the structural features, biochemical characteristics, and cellular functions of the PLA2 enzymes, the reader is kindly referred to recent reviews on the subject [9,10,14–16].

Although the physiological and pathological functions of the PLA2 enzymes appear to overlap in some cases and differ in others, the involvement of particular PLA2 forms in AA mobilization under oxidative stress conditions remains a matter of debate. Nonetheless, a consensus is emerging that PLA2 responsiveness during oxidative stress is a complex process involving multiple enzymes and multiple pathways, and these may vary from one cell to another. To further recognize the role of PLA2 in oxidant-mediated injury and to clarify the different routes for AA mobilization during oxidative stress, we have focused in this review on recent evidence dealing with the role of PLA2-dependent increases in AA release from phospholipids and eicosanoid production during oxidative stress. For the purposes of this review we have treated the Ca2+-dependent PLA2s separately from the Ca2+-independent ones, as the signaling mechanisms involved and general pathophysiological significance appear to clearly differ. While AA mobilization mediated by Ca2+-dependent enzymes generally appears to involve the activation of kinase cascades and intracellular calcium movements, AA mobilization by Ca2+-independent enzymes appears to require neither of these. This may suggest that Ca2+-dependent PLA2s, when involved in the response to oxidants, participate as signalers on their own. On the contrary, involvement of Ca2+-independent phospholipase A2s may potentiate the damage in cells undergoing oxidative stress in the absence of clear regulated signaling.

In addition to PLA2, it is important to note that free AA could also be generated by alternative, PLA2-independent pathways which may be significant in some cases. A particularly relevant one with regard to oxidative stress is the inhibition of fatty acid reacylation. Evidence has been provided that in some systems, oxidants may increase free AA availability by decreasing reacylation of lysoPC with the fatty acid. This will be discussed further below.

2. Ca2+-dependent phospholipase A2s

Two different kinds of Ca2+-dependent PLA2 have been shown to be involved in AA mobilization in response to a variety of receptor-directed and soluble stimuli. These are (i) the Group IVA cytosolic PLA2 (cPLA2), and (ii) certain members of the secreted PLA2 (sPLA2) family, particularly but not exclusively, Groups IIA, V, and X [10,15,17]. Stimulation of the cells by receptor agonists results in the activation of cPLA2α. Under stimulation conditions, the rate of AA release exceeds that of reincorporation into phospholipids, hence net accumulation of AA occurs that is followed by its conversion into different oxygenated compounds, collectively called the eicosanoids. During long-term AA mobilization responses, i.e., those that are typical from immunoinflammatory cells such as macrophages or mast cells, the inducible sPLA2 may also participate in the process, thereby creating an amplification loop that results in a greatly enhanced release of AA for eicosanoid synthesis. There seems to be a cross-talk between these two kinds of PLA2s during cellular activation [18], although the precise molecular details are not yet understood.

To study the interplay between oxidative stress and eicosanoids, Bonventre and colleagues studied the effect of expression of various PLA2s on H2O2-induced AA mobilization in murine mesangial cells [19]. These authors had previously generated a mouse line with the cPLA2α gene mutated [20]. The mouse strains (C57b/6 and SV/129) used to construct the cPLA2α−/− strain have spontaneous null mutations in the gene encoding for Group IIA PLA2 [21], and murine mesangial cells do not express Group V sPLA2 under resting or activation conditions [19]. Bonventre and co-workers employed an adenoviral infection technique to stably express either Group IIA or Group V sPLA2 into the cells [19]. Using this approach, it was possible to study the cross-talk between cPLA2α and either of the sPLA2s during oxidative stress. These are well-defined settings that avoid incomplete PLA2 inhibitions by the use of either pharmacological inhibitors or antisense approaches. cPLA2α was found to be responsible for AA release in response to H2O2 via a mechanism involving activation of protein kinase C and extracellular-signal regulated kinases, and intracellular Ca2+ movements (Fig. 1). Thus, in this system, H2O2 mimics the action of a classical receptor-directed agonist in terms of cPLA2α activation and AA release. Importantly, when present, both Groups IIA and V sPLA2 amplified the cPLA2α-mediated response, this resulting in an increased AA release response [19]. Collectively, these data support the possible existence of cross-talk between cPLA2 and sPLA2 in eliciting a full AA release response. In addition to initiating phospholipid hydrolysis and the subsequent mobilization of AA, cPLA2α might also provide signals that allow sPLA2 to amplify AA release by one of two possible mechanisms: (i) sPLA2 acts itself on AA-containing phospholipids to provide a more or less significant portion of the total AA liberated and/or (ii) sPLA2 provides further stimulatory signals that allow sustained cPLA2α activation, which is responsible for the AA release (Fig. 1).

While the central involvement of cPLA2α in receptor-linked lipid mediator production is demonstrated in a conclusive manner under most pathophysiological conditions [22], the relative contribution of sPLA2 enzymes to lipid mediator production is still controversial. In spite of this, sPLA2s, particularly those belonging to Groups IIA, V, and X, can augment the release of AA and other fatty acids in cell culture studies under different experimental settings [23–27], and pharmacological inhibition of sPLA2s can often attenuate inflammation in experimental animal
models [28–30]. Importantly, a recent gene targeting study of Group V sPLA2 has provided unequivocal evidence for the role of this enzyme in eicosanoid production by macrophages in vivo [31]. Group V sPLA2 might potentially release AA after secretion through two pathways, one involving re-internalization via caveolae [32,33], and the other involving direct interaction with the phosphatidylcholine-rich membranes to directly release AA from various cellular membranes [32,34,35]. Either of these mechanisms would require the sPLA2 to exit the cell as a consequence of the activation process and to subsequently re-associate and/or undergo re-internalization for action in an autocrine or paracrine manner. In contrast, a recent study has uncovered a third mechanism for sPLA2 involvement in AA release in which the de novo synthesized sPLA2s can act intracellularly without the requirement for prior secretion [36].

In the studies of Bonventre and associates [19], a scenario in which sPLA2 enhances the activity of cPLA2α, which then acts to release AA was favored on the basis of experiments demonstrating preferential release of AA over oleic acid in response to H2O2 in cells expressing both kinds of PLA2s. While more direct evidence was not provided, the authors also noted that a correlation exists between the expression level of cPLA2α and the magnitude of AA release. Such a correlation did not appear to occur between the expression level of sPLA2 and the extent of AA release. Moreover, cells expressing sPLA2 but not cPLA2α fail to mobilize AA, whereas cells expressing cPLA2α but not sPLA2 still produce a robust AA release response. These data indicate that cPLA2α alone is a major enzyme for H2O2-induced AA release in mesangial cells even without enhancement from sPLA2.

In agreement with the above scenario, Cho and associates have shown that sPLA2s, acting on the outer membrane of human neutrophils, release fatty acids and lysoPC, which, in turn, can act on the cell to increase cytosolic free calcium levels and phosphorylation of cPLA2α, resulting in cPLA2α-dependent leukotriene biosynthesis [37]. Nonetheless, sPLA2s may also act to release AA in a cPLA2α-independent manner, as demonstrated by studies in cells from mice lacking cPLA2α by genetic disruption [38]. Likewise, products of the 12/15-lipoxygenase pathway generated downstream of cPLA2α mediate the enhanced cellular responses to Groups V and IIA sPLA2s [39,40]. Collectively, these instances, taken from very recent literature, illustrate quite well some of the different possibilities of interplay between cPLA2α and sPLA2 when present within the same cell.

Similar to the results of Han et al. [19] in murine mesangial cells reported above, in rat mesangial cells, H2O2 in combination with platelet-derived growth factor activates the extracellular-signal regulated kinases and p38 mitogen-activated protein kinase, which in turn phosphorylate cPLA2α and lead to enhanced AA release [41]. Although rat mesangial cells are known to express Groups IIA and V sPLA2s in response to stimulation [42], the possible involvement of either of these in H2O2-triggered AA release was not investigated. Importantly however, earlier studies by the same authors in platelets treated with different oxidants had demonstrated an increase in AA release and lysoPC production that was attributed to the enhanced activity of Group IIA sPLA2 [43]. It should be indicated that these studies were performed in rabbit platelets subjected to strong oxidizing conditions, and thus, severe modification of membrane phospholipids by oxidizing reagents occurred, which likely increased accessibility of the membrane to platelet Group IIA sPLA2 [43].

Under most conditions, platelet AA release does not involve participation of Group IIA sPLA2, and this has been correlated with the enzyme’s inability to attack the outer platelet surface if drastic membrane rearrangements do not occur first [44,45].

Expression of sPLA2 proteins is typically low in resting cells. Therefore, before sPLA2s can significantly participate in phospholipid hydrolysis and AA mobilization under activation conditions, their content must be increased by induction of the corresponding gene(s). Induction of various sPLA2s in response to a number of proinflammatory stimuli has been widely documented [42,46,47]. Recently, induction of the AA-releasing sPLA2s, Group IIA and Group V, has also been reported under oxidative stress conditions [48,49]. Ishikawa et al. [48] found that Group V sPLA2 is induced during the acute phase of infarction in human heart. These authors hypothesized that, under these settings, Group V sPLA2 may play a dual role, acting both to remove degraded cell membrane in necrotic areas and to produce bioactive lipid metabolites through cooperation with cyclooxygenase-2. A biphasic increase in the expression of Group IIA sPLA2 was reported by Lin et al. [49] after cerebral ischemia in rats. Initially, there was an increase in the ischemic cortex at 30 min, and this was followed by a secondary increase in the penumbral area at 1 day after ischemia–reperfusion. Interestingly, in this study cPLA2α expression was studied as well, but no changes could be detected under any condition [49].

Studies in Her14 fibroblasts have shown that H2O2 and other oxidants such as cumene hydroperoxide induce the transient activation of cPLA2α in a concentration-dependent manner [50]. cPLA2α is activated in part by a mechanism involving phosphorylation via the extracellular-regulated kinase pathway, although a phosphorylation-independent mechanism was identified as well. The latter was proposed to be due to free radical

![Fig. 1. Role of Ca2+-dependent PLA2s in oxidative stress. H2O2 exposure of the cells results in the activation of intracellular kinase cascades and Ca2+ mobilization. Both of these signals converge at the cPLA2α, which is the dominant enzyme in AA mobilization. When present, sPLA2 may amplify AA release by potentiating cPLA2α activation, perhaps via PKC. Alternatively, cPLA2s may modulate the action of sPLA2, and the latter may also directly affect AA release by directly hydrolyzing membrane phospholipids. PKC, protein kinase C; ERK1/2, extracellular signal-regulated kinases 1/2; MEK, ERK kinase.](image-url)
mediated lipid peroxidation, resulting in increased substrate availability because of the presence of oxidized lipids in the membrane. This is reminiscent of the mechanism described for sPLA₂ in platelets exposed to various oxidants described above.

In cardiomyocytes, oxidative stress is known to cause either cell death or hypertrophy, and the abundance of antioxidant species within the cell appears to constitute a major factor in determining whether the cell commits to one fate or the other. There is discrepancy as to which signaling pathways are activated under hypertrophy-inducing conditions versus cell-death-related conditions, but one of the biochemical events that appears to take place in both cases is the release of AA from its phospholipid storage sites. Utilizing H9c2 cardiomyocyte-like cells, Winstead et al. [51] investigated the pathways responsible for AA release by these cells in response to H₂O₂. AA release was blunted by the dual cPLA₂/iPLA₂ inhibitor methyl arachidonyl fluorophosphonate (MAFP) and the specific cPLA₂ inhibitor pyrrophenone, but not by the iPLA₂ inhibitor bromoenol lactone (BEL). This suggests the involvement of cPLA₂α in the response [51]. Moreover, MAFP and pyrrophenone also prevent alterations in cell morphology induced by H₂O₂, thus highlighting the role of cPLA₂0 in mediating the damage of H9c2 cells undergoing oxidative stress.

3. Ca²⁺-independent phospholipase A₂s

Involvement of Ca²⁺-independent PLA₂ enzymes in the selective release of AA following cellular stimulation with receptor or soluble agonists has remained as a debatable issue because most of the earlier evidence was obtained with the use of BEL, an inhibitor whose selectivity in vivo is unclear. Recently, more selective techniques such as antisense oligonucleotide or RNA interference have been utilized to demonstrate that under certain circumstances, Group VIA calcium-independent PLA₂ (iPLA₂-VIA) may catalyze membrane hydrolysis leading to increased AA mobilization [52].

The first indications for the involvement of iPLA₂ in phospholipid catabolism and fatty acid release during oxidative stress conditions were provided by Gross and colleagues in the 1980s in studies on ischemia–reperfusion injury, although the molecular identity of the iPLA₂ activity involved was not ascertained [53]. More recently, two independent studies in phagocytes have shown that micromolar concentrations of H₂O₂ induce substantial release of AA from H₂O₂-treated cells are used in the assay, the iPLA₂ activity measured is significantly higher than that found in membranes from otherwise unstimulated cells. Therefore, treating the cells with H₂O₂ results in facilitated iPLA₂ attack on membrane phospholipids. Since membranes from H₂O₂-treated cells were found to contain higher amounts of lipid peroxides than membranes from untreated cells [55], these findings suggest that lipid hydrolysis by iPLA₂ occurs more readily in H₂O₂-treated cells because of changes in the physical state of membrane substrates, which may result, at least in part, from lipid peroxide accumulation.

Taken together, the above findings suggest a model for iPLA₂-mediated fatty acid mobilization in H₂O₂-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 iPLA₂ attack, which then results in increased liberation of fatty acids and formation of prostaglandins (Fig. 2). An important aspect of the above model is that this fatty acid release appears to occur in the absence of cPLA₂α activation, which underscores the apparent lack of a regulated signaling component in the process. Still, a mechanism such as this one may be very relevant under oxidative stress, where increased iPLA₂ activity may account for a significant phospholipid hydrolysis before cellular homeostasis is re-established.

![Fig. 2. Role of iPLA₂ in oxidative stress. The continuing action of iPLA₂ membrane phospholipids under resting conditions helps maintaining the steady-state levels of lysoPC and results in the liberation of low levels of free fatty acids such as AA (left panel). When the cells are exposed to H₂O₂, the hydrolytic activity of the iPLA₂ increases as result of lipoxygenation, this leading to increased levels of free AA. Free AA under these conditions may be utilized for prostaglandin synthesis or be used for other cellular functions (right panel).](image)
In the latter study, a possible parallel role for cPLA\(_2\alpha\) was suggested as well on the basis of partial dependence with Ca\(^{2+}\) and the complete abrogation of the response by MAFP but incomplete with BEL [58]. However, the response of astrocytes to H\(_2\)O\(_2\) appears to also proceed independently of protein kinase cascades, which is in agreement with the results in macrophages discussed above [55].

Group IV C PLA\(_2\), also known as cPLA\(_2\gamma\), is a Ca\(^{2+}\)-independent parologue of cPLA\(_2\alpha\). In contrast to cPLA\(_2\alpha\), the information on cPLA\(_2\gamma\) is very limited. It has been shown that cPLA\(_2\gamma\) can be activated in vivo by serum [59]. However, its fatty acid selectivity is controversial. When human embryonic kidney 293 overexpressing cPLA\(_2\gamma\) are exposed to H\(_2\)O\(_2\) and other peroxides, significant release of AA is observed [60]. Inhibitors of tyrosine kinases blunt the effect but immunoprecipitation and immunoblotting assays fail to detect direct tyrosine phosphorylation of cPLA\(_2\gamma\), which suggests the existence of accessory proteins that may help regulate cPLA\(_2\gamma\) during situations of oxidative stress [60]. Thus, the proposal is made that cPLA\(_2\gamma\) is constitutively expressed in the endoplasmic reticulum and Golgi complex, and plays important roles in remodeling and maintaining membrane phospholipids under oxidative stress, both by removing peroxidized fatty acyl chains and enhancing free AA levels [52].

4. PLA\(_2\)-independent mobilization of AA

AA is a participant of a deacylation/reacylation cycle of membrane phospholipids, the so-called Lands cycle, in which a fatty acid is hydrolyzed from phospholipids by PLA\(_2\) and another fatty acid is incorporated by the concerted action of fatty acyl-CoA synthetase and lysophospholipid acyltransferase [61]. It is believed that the Lands pathway constitutes the major route for incorporation of AA and other polysaturated fatty acids in the phospholipids of a variety of cells, most notably inflammatory cells [61]. Since under unstimulated cells, the reacylation pathway dominates over the phospholipolytic step, cellular free AA is kept at a very low level compared to that present in esterified form in phospholipids. However, given this dominance of AA reacylation over AA deacylation in cells, accumulation of free fatty acid may also occur if the reacylation is inhibited, and this is exactly what has been shown to occur in rat alveolar macrophages [62] and vascular smooth muscle cells [63] exposed to H\(_2\)O\(_2\). In both of these studies, a reciprocal correlation was found between inhibition of phospholipid fatty acid acylation and H\(_2\)O\(_2\)-stimulated accumulation of free AA. An effect of H\(_2\)O\(_2\) on the enzymes of the reacylation pathway, arachidonoyl-CoA synthetase and lysophospholipid: arachidonoyl-CoA acyltransferase was discarded by in vitro assay studies. Importantly, a good correlation was observed between decreases in AA acylation and cellular ATP content. Since ATP is required for synthesis of the acylation intermediate arachidonoyl-CoA, the suggestion was made that this was the means by which H\(_2\)O\(_2\) inhibited acylation and hence increased AA release in rat alveolar macrophages [62] and vascular smooth muscle cells [63].

5. Conclusion

Oxidant stress is implicated in numerous proinflammatory responses in mammalian cells. H\(_2\)O\(_2\) is known to trigger AA release and metabolism in various cell types, but the mechanisms involved appear to profoundly vary from one cell to another. Thus, AA mobilization in response to an oxidative stress appears to be a very complex process, potentially involving multiple enzymes and pathways.

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