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# Involvement of Calcium-independent Phospholipase $A_2$ in Hydrogen Peroxide-induced Accumulation of Free Fatty Acids in Human U937 Cells*

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**Previous studies have demonstrated that U937 cells are able to mobilize arachidonic acid (AA) and synthesize prostaglandins in response to receptor-directed and soluble stimuli by a mechanism that involves the activation of Group IV cytosolic phospholipase $A_2\alpha$. In this paper we show that these cells also mobilize AA in response to an oxidative stress induced by $H_2O_2$ through a mechanism that appears not to be mediated by cytosolic phospholipase $A_2\alpha$ but by the calcium-independent Group VI phospholipase $A_2$ ($iPLA_2$). This is supported by the following lines of evidence: (i) the response is essentially calcium-independent, (ii) it is inhibited by bromoenol lactone, and (iii) it is inhibited by an $iPLA_2$ antisense oligonucleotide. Enzyme assays conducted under a variety of conditions reveal that the specific activity of the $iPLA_2$ does not change as a result of $H_2O_2$ exposure, which argues against the activation of a specific signaling cascade ending in the $iPLA_2$. Rather, the oxidant acts to perturb membrane homeostasis in a way that the enzyme susceptibility/accessibility to its substrate increases, and this results in altered fatty acid release. In support of this view, not only AA, but also other fatty acids, were found to be liberated in an $iPLA_2$-dependent manner in the $H_2O_2$-treated cells. Collectively, these studies underscore the importance of the $iPLA_2$ in modulating homeostatic fatty acid deacylation reactions and document a potentially important route under pathophysiological conditions for increasing free fatty acid levels during oxidative stress.**

Phospholipase $A_2$ ($PLA_2$)[1] constitutes a key regulatory step in the production of prostaglandins, because it catalyzes the release of arachidonic acid (AA) from the sn-2 position of phospholipids, making the free fatty acid accessible to prostaglandin synthases. At present, 14 different $PLA_2$ groups have been identified (1, 2). These include ten groups of enzymes utilizing a catalytic histidine, which show millimolar requirements for $Ca^{2+}$ and are collectively referred to as the secreted $PLA_2$s (Groups I, II, III, V, IX, X, XI, XII, XIII, and XIV) (1, 2), and two groups of intracellular, high molecular mass enzymes, which utilize a catalytic serine (Groups IV and VI). Group IVA $PLA_2$, also known as cytosolic $PLA_2\alpha$ ($cPLA_2\alpha$), is a highly regulated, $Ca^{2+}$-dependent enzyme (1, 2), whereas Group VI $PLA_2$, or $iPLA_2$, is $Ca^{2+}$-independent (1, 2).

Among these $PLA_2$s, Groups IIA, V, and IVA have repeatedly been shown to be responsible for AA release and prostaglandin generation in different systems (3–5). In phagocytic cells, Group VI $PLA_2$ has been primarily implicated in basal fatty acid reacylation reactions by controlling the cellular level of lysophosphatidylcholine acceptors (6). In other cell types, notably heart and pancreatic islets, the enzyme has also been implicated in receptor-mediated AA release, based on the effects of a bromoenol lactone suicide inhibitor (BEL) (6).

Recent work has shown that reactive oxygen intermediates enhance AA release and prostaglandin production in different cell systems, but the molecular mechanism responsible for these effects has not been clarified. Activation of an intracellular $PLA_2$ has been pointed out as the most likely mechanism for AA mobilization in vascular smooth muscle cells, stromal cells, and striatal neurons exposed to $H_2O_2$ (7–11). In other systems however, diminished AA incorporation into phospholipids, not $PLA_2$ activation, has been suggested to be the event responsible for free AA accumulation (12, 13). In an attempt to reconcile these conflicting results, we sought to investigate the ability of $H_2O_2$ to induce AA mobilization from human monocytic U937 cells and the molecular mechanism involved in this process. U937 cells contain both $cPLA_2\alpha$ and $iPLA_2$ and have been shown to release AA and produce prostaglandins in response to a variety of receptor-mediated and soluble agonists in a $cPLA_2\alpha$-regulated manner (14, 15). Utilizing a variety of approaches, we show here that $H_2O_2$-induces AA mobilization in U937 cells by a $Ca^{2+}$-independent mechanism that involves not $cPLA_2\alpha$, but rather $iPLA_2$. Importantly, however, the results indicate that the $iPLA_2$-mediated AA release does not reflect a true activation of the enzyme (*i.e.* a stable increase in the specific activity of the enzyme) but rather an increased accessibility of the $iPLA_2$ toward its substrate. These results underscore the key role of $iPLA_2$ in modulating basal fatty acid deacylation reactions.

## EXPERIMENTAL PROCEDURES

*Materials*—[5,6,8,9,11,12,14,15-$^3$H]AA (100 Ci/mmol) was from Amersham Biosciences. BEL and methyl arachidonyl fluorophosphonate (MAFP) were from Cayman (Ann Arbor, MI). The specific $cPLA_2\alpha$ inhibitor pyrrophenone was generously provided by Dr. K. Seno

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[1] The abbreviations used are: $PLA_2$, phospholipase $A_2$; AA, arachidonic acid; $cPLA_2\alpha$, cytosolic phospholipase $A_2\alpha$; $iPLA_2$, $Ca^{2+}$-independent phospholipase $A_2$; BEL, bromoenol lactone; MAFP, methyl arachidonyl fluorophosphonate; ConA, concanavalin A.


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(Shionogi Co., Osaka, Japan) (16–18). All other reagents were from Sigma.

*Cell Culture*—U937 cells were maintained in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum, 2 mM glutamine, penicillin (100 units/ml), and gentamycin (24 μg/ml). The cells were incubated at 37 °C in a humidified atmosphere of $CO_2/O_2$ (1:19) at a cell density of 0.5–1 × $10^6$ cells/ml in 12-well plastic culture dishes (Costar). Cell differentiation was induced by treating the cells with 35 ng/ml PMA for 24 h (19, 20).

*AA Release Experiments*—The cells were labeled with 0.5 μCi/ml [$^3$H]AA for 18 h. After this period, the cells were washed and placed in serum-free medium for 1 h before the addition of the appropriate stimulus in the presence of 0.5 mg/ml bovine serum albumin. The supernatants were removed, cleared of cells by centrifugation, and assayed for radioactivity by liquid scintillation counting.

For analysis of [$^3$H]AA metabolites released into the supernatant, the stimulations were conducted in the absence of albumin. The supernatant was acidified to pH 3.5 with 5 M formic acid and extracted twice with 3 ml of ethyl acetate. The ethyl acetate was dried under a stream of nitrogen, and the residue was dissolved in a few drops of chloroform/methanol (2:1, v/v) and chromatographed on Silicagel G-60 plates. Unlabeled prostaglandin standards were used as carriers. The solvent system used was chloroform/methanol/acetic acid/water (90:8:1:0.8, by volume) (21).

*Treatment of the Cells with Antisense Oligonucleotides*—The antisense oligonucleotides utilized in these studies were derived from prior publications reporting their effects (22–24). The $iPLA_2$ antisense sequence corresponded to nucleotides 59–78 in the murine group VI $iPLA_2$ sequence, which is conserved in human group VI $iPLA_2$ (25, 26). The antisense or sense oligonucleotides were mixed with LipofectAMINE, and complexes were allowed to form at room temperature for 10–15 min. The complexes were then added to the cells, and the incubations were allowed to proceed under standard cell culture conditions. The final concentrations of oligonucleotide and LipofectAMINE were 1 μM and 10 μg/ml, respectively. Oligonucleotide treatment and culture conditions were not toxic for the cells as assessed by the trypan blue dye exclusion assay and by quantitating adherent cell protein.

*$iPLA_2$ Assay*—Briefly, U937 cell aliquots were incubated for 30 min at 37 °C in 100 mM Hepes (pH 7.5) containing 5 mM EDTA and 100 μM phospholipid substrate (pH 7.5) in a final volume of 250 μl. The substrates utilized in the assay were [$^3$H]AA-labeled choline glycerophospholipids and [$^3$H]AA-labeled ethanolamine glycerophospholipids, and they were used in the form of sonicated vesicles in buffer (15). In some experiments, the EDTA was replaced by 1 mM $CaCl_2$. $iPLA_2$ activity was also measured utilizing the mixed-micelle assay described by Dennis and co-workers (27) and the mammalian membrane substrate assay described by Diez *et al.* (28).

*Preparation of Substrates for the $iPLA_2$ Assay*—[$^3$H]AA-labeled choline glycerophospholipids and ethanolamine glycerophospholipids were isolated from cellular lipids of U937 cells incubated for 24 h with the exogenous $^3$H-labeled fatty acid (0.5 μCi/ml). Labeled phospholipids were purified by thin-layer chromatography and tested for purity as described previously (15). Labeled U937 cell membranes were prepared by adding 0.5 μCi/ml [$^3$H]AA to the U937 cell cultures for 18 h. Total cellular membranes were prepared by sucrose centrifugation exactly as described by Diez *et al.* (28).

*Lipid Peroxide Determination*—The amount of lipid peroxides in membranes was quantified by the thiobarbituric acid-reactive substance assay (29). The samples were mixed with 1 ml of 0.67% thiobarbituric acid and 0.5 ml of 20% tricholoroacetic acid, and the mixtures were incubated in a boiling water bath for 20 min. After cooling the tubes on ice, the reaction mixture was centrifuged at 3000 × *g* for 10 min, and absorbance of the supernatant was read at 532 nm. The concentration of thiobarbituric acid-reactive substances, which is directly proportional to the amount of lipid peroxides in the samples, was calculated using tetraethoxy propane as a reference standard.

*Data Presentation*—Assays were carried out in duplicate or triplicate. Each set of experiments was repeated at least three times with similar results. Unless otherwise indicated, the data presented are from representative experiments.

## RESULTS

*AA Mobilization in $H_2O_2$-treated U937 Cells*—We began the current study by determining whether $H_2O_2$ was capable of causing the extracellular release of AA from U937 cells. To this end, the cells, labeled with 0.5 μCi of [$^3$H]AA, were exposed to different concentrations of $H_2O_2$ for various periods of time. As shown in Fig. 1, $H_2O_2$ did induce a concentration- and time-dependent release of [$^3$H]AA from the cells (Fig. 1). Maximal effects of $H_2O_2$ on AA release were observed at a concentration of 500 μM (Fig. 1*A*). Such a concentration was therefore used in all subsequent experiments. Fig. 1*B* shows that, after a lag of about 5–15 min, $H_2O_2$-induced AA release proceeded linearly for the following hour, proceeding at a slower rate thereafter. That the kinetics of AA release in response to $H_2O_2$ does not show saturation within 1 h of treatment is in stark contrast with the kinetics of AA release in response to the receptor-directed agonist ConA (20), which is also shown in Fig. 1*B* for comparison.

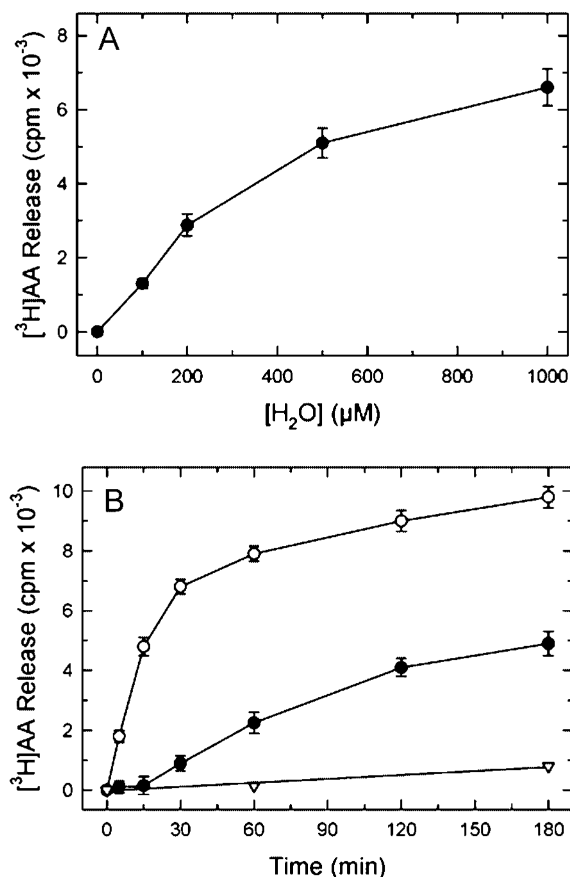


FIG. 1. **$H_2O_2$-induced [$^3$H]AA release from U937 cells.** *A*, dose response of the $H_2O_2$ effect (60-min incubation). *B*, time course of [$^3$H]AA release in response to 500 μM $H_2O_2$ (*closed circles*) and 100 μg/ml ConA (*open circles*) and in the absence of stimulation (*open triangles*).

The composition of the $^3$H-released material was analyzed by thin-layer chromatography, and the results are shown in Table I. Treatment of the cells with $H_2O_2$ significantly increased prostaglandin production, most notably of prostaglandin $E_2$ and $D_2$, but unmetabolized free AA was the most abundant labeled compound released into the medium.

*$PLA_2$ Inhibition Studies*—To address the involvement of the different $PLA_2$ forms in $H_2O_2$-induced AA release, we first utilized MAFP, a dual $cPLA_2/iPLA_2$ inhibitor (30). As shown in Fig. 2*A*, MAFP significantly blocked the response to $H_2O_2$. To distinguish whether the inhibition of MAFP on AA release was because of either $cPLA_2\alpha$ or $iPLA_2$, we conducted studies with BEL, a compound that manifests a marked selectivity for inhibition of $iPLA_2$ *versus* $cPLA_2\alpha$ *in vitro* (6). Fig. 2*A* also shows that BEL, at concentrations that are known to block cellular $iPLA_2$, exerted a significant inhibitory effect on the $H_2O_2$-induced AA mobilization. As a control for these experiments,

TABLE I
*Prostaglandin production by U937 cells exposed to $H_2O_2$*

U937 cells, prelabeled with [$^3$H]AA, were untreated or treated with 500 1/4M $H_2O_2$ for 60 min. Supernatants were taken off, and the different metabolites were separated by thin-layer chromatography as described under "Experimental Procedures." The data are expressed in dpm × $10^{-3}$. $PGE_2$, prostaglandin $E_2$, $PGD_2$, prostaglandin $D_2$, 6-keto-$PGF_1\alpha$, 6-ketoprostaglandin $F_1\alpha$, $TXB_2$, thromboxane $B_2$.

| Eicosanoid | Control | $H_2O_2$ |
|---|---|---|
| $PGE_2$ | 2.7 ± 0.4 | 7.1 ± 0.2 |
| $PGD_2$ | 1.9 ± 0.3 | 6.7 ± 0.2 |
| 6-Keto-$PGF_1\alpha$ | 0.9 ± 0.1 | 1.9 ± 0.1 |
| $TXB_2$ | 0.5 ± 0.1 | 1.5 ± 0.1 |
| AA | 8.7 ± 0.4 | 20.7 ± 0.4 |

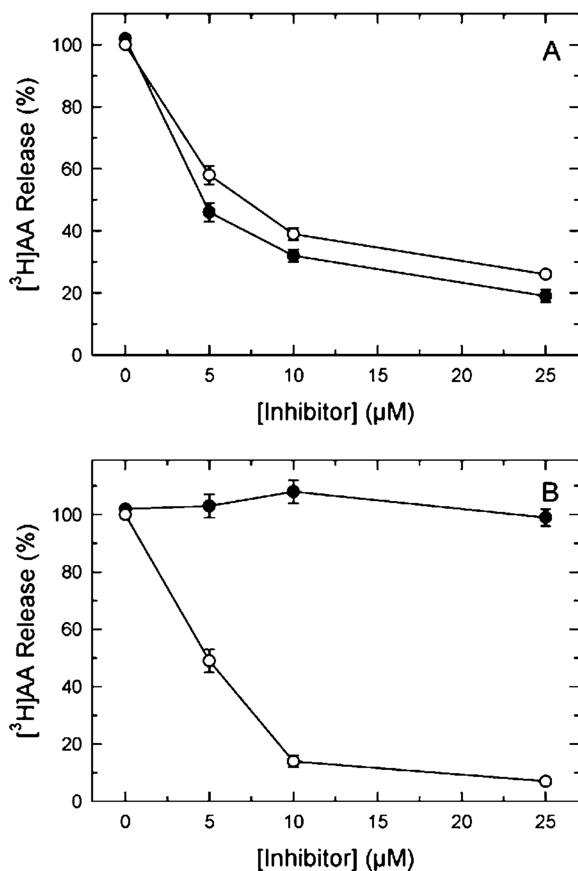


FIG. 2. **Effect of MAFP and BEL on [$^3$H]AA release from U937 cells.** The cells were treated with the indicated concentrations of MAFP (*open circles*) or BEL (*closed circles*) for 30 min before the addition of 500 $\mu$M $H_2O_2$ (*A*) or 100 $\mu$g/ml ConA (*B*), and the incubations proceeded for 60 min. Results are given as a percentage of the response obtained in the absence of inhibitors.

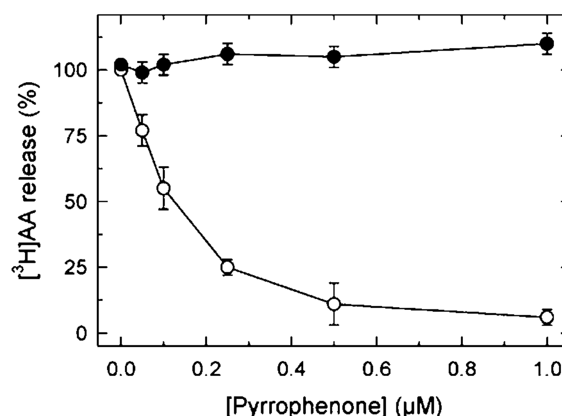


FIG. 3. **Effect of pyrrophenone on [$^3$H]AA release from U937 cells.** The cells were treated with the indicated amounts of pyrrophenone for 30 min before the addition of 500 $\mu$M $H_2O_2$ (*closed symbols*) or 100 $\mu$g/ml ConA (*open symbols*), and the incubations proceeded for 60 min. Results are given as a percentage of the response obtained in the absence of inhibitors.

the effects of these inhibitors on ConA-induced AA mobilization were also studied (Fig. 2*B*). MAFP, but not BEL, inhibited the response, thus suggesting the involvement of $cPLA_2\alpha$ but not of $iPLA_2$ under these conditions.

Fig. 3 shows the effect of pyrrophenone, a compound that exhibits more than 1000-fold selectivity for inhibition of $cPLA_2\alpha$ *versus* $iPLA_2$ (18, 31). Whereas pyrrophenone exerted no significant effect on the $H_2O_2$-induced AA release, it completely inhibited the response to ConA ($IC_{50}$ ~0.2 $\mu$M). Collectively, these results suggest that $cPLA_2\alpha$ mediates the ConA-induced release of AA but has no effect on the $H_2O_2$ response. The latter appears to involve the $iPLA_2$.

To further define the role of $iPLA_2$ in U937 cell release, we also examined the effects of an antisense oligonucleotide to $iPLA_2$. The $iPLA_2$ antisense oligonucleotide used was the human counterpart of the murine one that we and others have successfully employed elsewhere (22–24). Using this antisense, an ~70% decrease of both the immunoreactive $iPLA_2$ protein (Fig. 4*A*) and of cellular $iPLA_2$ activity (Fig. 4*B*) was achieved, in agreement with previous estimates (22, 23). The antisense to $iPLA_2$ had no effect on the expression of $cPLA_2\alpha$ (Fig. 4*A*). Under these conditions, a significant decrease in the AA release response of $H_2O_2$-treated cells was observed (Fig. 4*C*), which provides additional evidence for the involvement of the $iPLA_2$ in this process. Control antisense experiments utilizing ConA as a trigger for AA release revealed the expected lack of effect of the $iPLA_2$ antisense (Fig. 4*C*).

*Characterization of the $H_2O_2$ Effect on $iPLA_2$*—Collectively, the above data suggest the involvement of $iPLA_2$ in the AA mobilization response induced by $H_2O_2$ in U937 cells. Because $iPLA_2$ is a $Ca^{2+}$-independent enzyme, one might expect the $H_2O_2$-induced AA mobilization process to be $Ca^{2+}$-independent, as well. To evaluate this possibility, the following approaches were undertaken. In the first place, the cells were exposed to $H_2O_2$ in the absence of $Ca^{2+}$ in the incubation medium, and the effect on AA mobilization was studied. Fig. 5 shows that this strategy did not modify the $H_2O_2$ response. As a control, the effect of $Ca^{2+}$ deprivation on the ConA response was also studied, and the response was strongly blunted (Fig. 5). In the next series of experiments, the cells were depleted of their intracellular $Ca^{2+}$ by treating them with 40 $\mu$M quin2/AM plus 1 mM EGTA in a $Ca^{2+}$-free medium. This procedure buffers and clamps the intracellular calcium concentration at very low levels (about $10^{-8}$ M) (32). Under these conditions, the AA response to $H_2O_2$ remained unchanged, whereas the ConA response was abolished (Fig. 5). Collectively, these results indicate that AA mobilization in response to $H_2O_2$ does not require $Ca^{2+}$, which is consistent with the participation of an $iPLA_2$.

Unlike $cPLA_2\alpha$, $iPLA_2$ does not show any apparent substrate specificity (6). Thus, if $iPLA_2$ is involved in fatty acid release in the $H_2O_2$-treated cells, one might expect to observe the release of not only AA but also of other fatty acids. To address this possibility, experiments were conducted where the cells were labeled with [$^3$H]oleic acid prior to exposure to $H_2O_2$. $H_2O_2$ induced a low but measurable release of oleic acid. When the cells were exposed to ConA instead, release of oleic acid was not observed (Fig. 6). Altogether, the results are consistent with the finding that ConA signals through the AA-specific $cPLA_2\alpha$ but not through the $iPLA_2$. $H_2O_2$, in contrast, appears to cata-

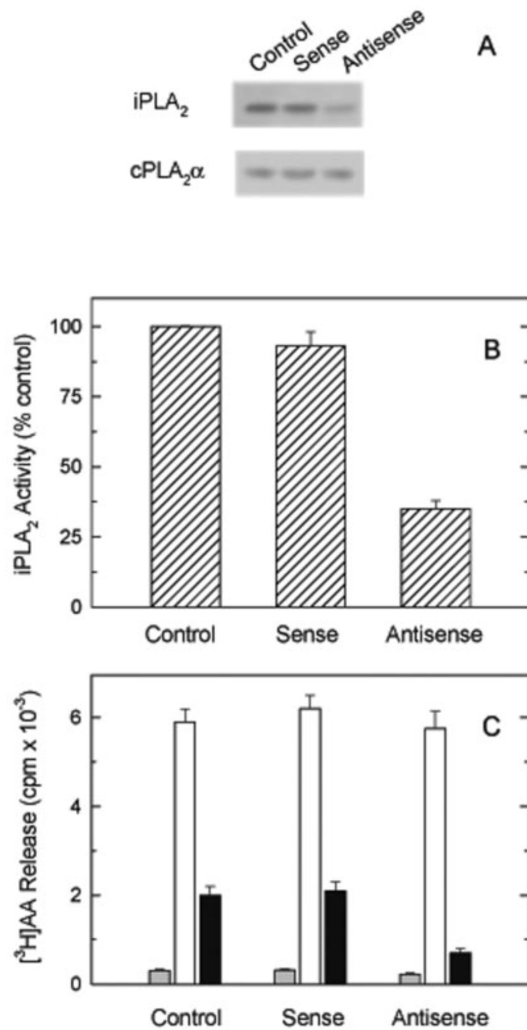


FIG. 4. **$iPLA_2$ antisense oligonucleotide inhibits $iPLA_2$ protein expression and activity and AA release in $H_2O_2$-treated U937 cells.** The cells were either untreated (*Control*) or treated with sense or antisense oligonucleotides. *A*, total cytoplasmic protein was evaluated by immunoblot for $iPLA_2$ (*top*) or $cPLA_2\alpha$ (*bottom*). *B*, effect on cellular $iPLA_2$ activity. *C*, effect on the AA mobilization response triggered by 500 $\mu$M $H_2O_2$ (*closed bars*), 100 $\mu$g/ml ConA (*open bars*), or neither (*gray bars*).

lyze fatty acid mobilization through the fatty acid-nonspecific $iPLA_2$.

*Studies on the Regulation of $iPLA_2$ Activity*—If the $H_2O_2$ effect on the $iPLA_2$ is truly an activating one, an increase in the specific activity of the enzyme is to be expected. Homogenates of U937 cells, either untreated or treated with $H_2O_2$, were prepared, and assays were conducted to assess $iPLA_2$ activity utilizing a vesicle substrate assay. Under these conditions we failed to detect any change in the $iPLA_2$-specific activity of homogenates from $H_2O_2$-treated cells *versus* untreated cells. Conversely, definite increases in the $Ca^{2+}$-dependent activity of the homogenates could be detected if the cells were previously treated with ConA (Fig. 7). These changes, which most likely correspond to increases in $cPLA_2$ activity (14, 15), suggest that our inability to detect changes in the $iPLA_2$ specific activity may not be because of technical issues. Experiments in which $iPLA_2$ activity was measured utilizing the mixed micelle assay described by Dennis and co-workers (27) also failed to reveal any change in the $iPLA_2$ activity of the homogenates (not shown).

As a third approach, we utilized the mammalian membrane assay system described by Diez and co-workers (28). In this system, purified [$^3$H]AA-labeled mammalian membranes are used as a substrate. Utilizing this assay, again no differences in the $iPLA_2$ activity of untreated cells *versus* $H_2O_2$-treated cells could be demonstrated. Importantly however, when $iPLA_2$ activity of homogenates from either untreated cells or $H_2O_2$-treated cells was assayed toward $H_2O_2$-treated membranes, a low but significant increase in the $iPLA_2$ activity could be

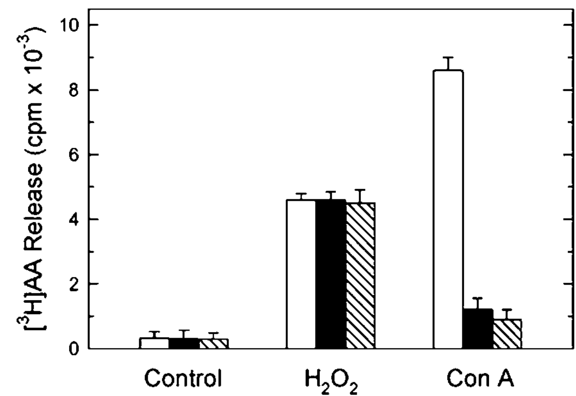


FIG. 5. **Effect of $Ca^{2+}$ on [$^3$H]AA release from U937 cells.** The cells were treated with 500 $\mu$M $H_2O_2$, 100 $\mu$g/ml ConA, or neither (*Control*) as indicated for 60 min in medium with 1.3 mM $CaCl_2$ (*open bars*), $Ca^{2+}$-free medium with 1 mM EGTA (*closed bars*), or $Ca^{2+}$-free medium with 1 mM EGTA plus 40 mM quin2/AM (*hatched bars*). Afterward, supernatants were assayed for [$^3$H]AA release.

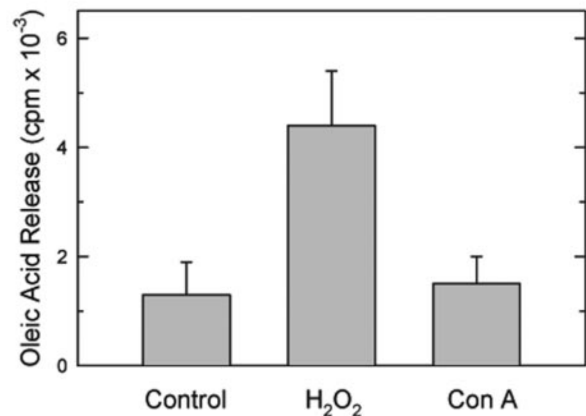


FIG. 6. **Oleic acid release from U937 cells.** The cells, labeled with [$^3$H]oleic acid, were treated with 500 $\mu$M $H_2O_2$, 100 $\mu$g/ml ConA, or neither (*Control*) as indicated, for 60 min. Afterward, supernatants were assayed for [$^3$H]oleic acid release.

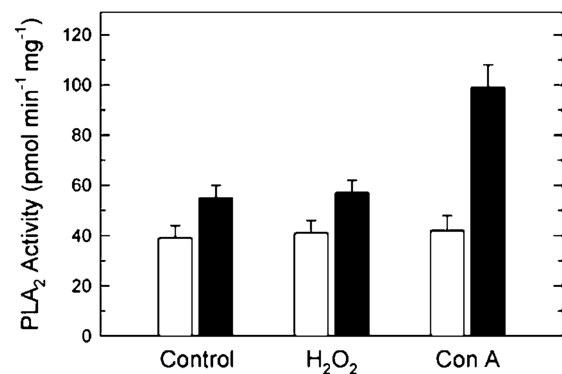


FIG. 7. **$PLA_2$ activity of homogenates from U937 cells.** Homogenates from untreated cells (*Control*) or from cells treated with either 500 $\mu$M $H_2O_2$ or 100 $\mu$g/ml ConA were prepared, and $PLA_2$ activity was measured in the absence (*open bars*) or presence (*closed bars*) of 1 mM $CaCl_2$ in the assay mix.

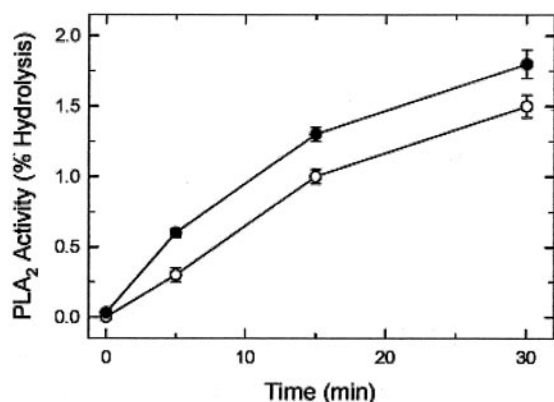


FIG. 8. **Time course of $PLA_2$ activity using a natural membrane as substrate.** Untreated (*open circles*) and $H_2O_2$-treated (*closed circles*) [$^3$H]AA-labeled membranes were incubated with U937 cell homogenates (as a source of enzyme). Reactions were stopped at different time points, and free [$^3$H]AA was isolated by thin-layer chromatography. $PLA_2$ activity was expressed as the percentage of hydrolysis of the labeled membrane substrate.

measured (Fig. 8). Thus, it must be the physical state of the substrate and not the intrinsic activity of the enzyme that changes after $H_2O_2$ exposure. Moreover, the membranes from $H_2O_2$-treated cells showed significantly elevated levels of lipid peroxides, as quantified by measuring thiobarbituric acid-reactive substances (73 ± 12 pmol/mg protein in $H_2O_2$-treated membranes *versus* 31 ± 9 pmol/mg protein in membranes from untreated cells; mean ± S.E., $n = 4$).

## DISCUSSION

Phagocytic cells produce reactive oxygen intermediates such as superoxide anion and hydrogen peroxide in response to a variety of agonists (33). Although the production of these oxygen metabolites plays an important role in cellular signaling and host defense, their uncontrolled production constitutes a serious pathophysiological factor for a wide variety of vascular-based disorders (33). Oxidative damage is often associated with AA mobilization from cells from the vascular system, such as endothelial cells, smooth muscle cells, platelets, and phagocytes. Thus, interactions between reactive oxygen intermediates and AA metabolites are of particular importance.

In this study, $H_2O_2$ was used to investigate mechanisms of AA mobilization in phagocytic cells under an oxidative stress, and the data suggest that oxidant-induced fatty acid mobilization from U937 phagocytes does not depend on $cPLA_2\alpha$ but rather on an $iPLA_2$-like activity. This is based on several lines of evidence, such as the use of chemical inhibitors and of antisense oligonucleotide techniques. Examination of the time course of AA mobilization in response to $H_2O_2$ revealed that, after a short lag, the response proceeded linearly with time, showing signs of saturation only after 2 h of exposure to the oxidant. Such a kinetics strongly contrasts with the response of the cells to ConA, a well known receptor agonist of U937 cells, which shows the typical saturation kinetics that is expected from a highly regulated cellular response such as AA release. In keeping with the above, when assayed in a cell-free system, cellular $iPLA_2$ activity did not change. Of note, assays were conducted under three different experimental conditions, namely a vesicle assay, a mixed micelle assay, and a natural membrane assay. Because the results were the same regardless of the assay system utilized, it appears likely that the intrinsic activity of the $iPLA_2$ does not change after exposure of the cells to $H_2O_2$. This conclusion argues against the possibility of a stable activation of the $iPLA_2$ as the mechanism for $H_2O_2$-mediated AA release in U937 cells.

Interestingly, when membranes from $H_2O_2$-treated cells were used in the assay, the $iPLA_2$ activity measured was found to be significantly higher than that found in membranes from otherwise unstimulated cells. Therefore, treating the cells with $H_2O_2$ results in facilitated $iPLA_2$ attack on membrane phospholipids. We have found that membranes from $H_2O_2$-treated cells contain significantly higher amounts of lipid peroxides than membranes from untreated cells. Thus the data suggest that lipid hydrolysis by $iPLA_2$ occurs more readily in $H_2O_2$-treated cells because of changes in the physical state of membrane substrates, which may result, at least in part, from lipid peroxide accumulation. How this facilitated catalysis occurs is presently unknown, but a number of factors that alter membrane lipid packing are well documented to increase fatty acid release both *in vitro* and *in vivo* (34).

Taken together, these results suggest 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 susceptible to attack by the $iPLA_2$, which then starts releasing increased amounts of fatty acids. An important aspect of the above model is that this fatty acid release occurs in the absence of $cPLA_2\alpha$ activation, which underscores the apparent lack of a regulated signaling component in the process. Still, a mechanism such as the one proposed here may be of importance under certain pathophysiological settings (*i.e.* oxidative stress), where increased $iPLA_2$ activity may account for a significant phospholipid hydrolysis before cellular homeostasis is re-established. In turn, these results highlight the key role of $iPLA_2$ in modulating basal fatty acid deacylation reactions.

Whether $iPLA_2$ is also involved in regulated phospholipid hydrolysis in phagocytic cells is unknown at present. However, the fact that multiple splice variants of $iPLA_2$ exist in some cells and that other $iPLA_2$s distinct from the classical group VI enzyme have recently been described (6) suggest the possibility that $iPLA_2$ may be subject to complex regulatory mechanisms that differ among cell types. Two recent reports utilizing cells overexpressing group VI $iPLA_2$ have shown the enzyme to be responsive to $Ca^{2+}$ ionophore in HEK293 cells (35) and to glucose plus cAMP-elevating agents in INS-1 insulinoma cells (36), thus suggesting that the enzyme is capable of playing some signaling roles in cells. Whether, in addition to its housekeeping role in U937 cells and phagocytic cells in general, the group VI $iPLA_2$ also plays a signaling role is currently under study.

Analysis of the AA metabolites produced after exposure to the cells to $H_2O_2$ revealed a significant production of prostaglandins, particularly the pro-inflammatory prostaglandins $E_2$ and $D_2$. This suggests that an immediate biological consequence of $H_2O_2$-induced AA release is to generate mediators that propagate and/or amplify the oxidative injury. Interestingly, a major portion of the material released after $H_2O_2$ exposure remained as free unmetabolized AA, which raises the possibility that its metabolism to eicosanoid mediators might not be its only biological fate. $H_2O_2$ is known to induce apoptosis in a number of cells including phagocytes (37, 38), and there is evidence that unesterified AA within cells can signal apoptosis (39, 40). Moreover, treating U937 cells with BEL has been show to retard Fas- and tumor necrosis $\alpha$/cycloheximide-mediated apoptosis (41, 42). Taking all these findings together, it is tempting to speculate that the AA liberated by $iPLA_2$ in $H_2O_2$-treated cells may play a role in oxidant-induced apoptosis in these cells. Studies are currently in progress to investigate this attractive possibility.

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