Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9741–9745, December 1990
Medical Sciences

Phospholipase A_2 activity of low density lipoprotein: Evidence for an intrinsic phospholipase A_2 activity of apoprotein B-100

(oxidized low density lipoprotein/lipid peroxidation/platelet-activating factor/*p*-bromophenacyl bromide)

SAMPATH PARTHASARATHY* AND JOELLEN BARNETT

Department of Medicine, University of California, San Diego, La Jolla, CA 92093-0613

Communicated by Daniel Steinberg, September 24, 1990

ABSTRACT **During oxidative modification of low density lipoprotein (LDL) there is extensive degradation of phosphatidylcholine (PtdCho) to lysophosphatidylcholine (lyso-PtdCho), with the removal of fatty acids from the 2 position. The phospholipase A_2 (PLA2) activity responsible for hydrolysis is closely associated with LDL. By use of lipoxygenase-oxidized 2-[1-^{14}C]linoleoyl PtdCho as the substrate and delipidated apoprotein B (apo-B), evidence is presented to show that (*i*) the activity is destroyed progressively during the oxidative modification of LDL; (*ii*) *p*-bromophenacyl bromide (pBPB), a histidine modifier that inhibits the oxidative modification of LDL, also substantially inhibits the PLA2 activity; and (*iii*) photooxidation of LDL in the presence of Rose Bengal completely inactivates the enzyme with concomitant loss of apo-B histidine residues. High molecular weight proteins from delipidated LDL, separated by polyacrylamide gel electrophoresis, showed PLA2 activity. It is suggested that apo-B itself may possess PLA2 activity.**

We have previously shown that when low density lipoprotein (LDL) undergoes oxidative modification in the presence of cultured endothelial cells, there is extensive degradation of phosphatidylcholine (PtdCho) to lysophosphatidylcholine (lyso-PtdCho) with selective release of the fatty acid at the 2 position (1). Membrane-associated phospholipases may be involved in this cell-induced modification of LDL. However, degradation of PtdCho is also seen when the oxidation of LDL occurs in the absence of cells, indicating that there is a phospholipase A_2 (PLA2) activity closely associated with LDL itself (1). We suggested that the propagation of lipid peroxidation is more effective when the oxidized fatty acid and/or its degradation products can be released from the glycerol backbone and therefore diffuse more freely within the lipid environment of the LDL (2). In the present studies, using oxidized 2-[1-^{14}C]linoleoyl PtdCho as substrate, we demonstrate that the PLA2 activity of LDL is inhibited by *p*-bromophenacyl bromide (pBPB). We also show that photochemical oxidation of histidine residues of LDL apoprotein completely inactivates the PLA2 activity. It has been suggested that plasma platelet-activating factor (PAF) hydrolase, which is known to be associated with LDL, may account for all of the hydrolysis of oxidized PtdCho (3, 4). In this study, we show that extraction of apoprotein B-100 (apo-B) with buffers that remove soluble proteins (5) failed to remove the PLA2 activity from apo-B. On the other hand, apo-B and lower molecular weight fragments of apo-B isolated by PAGE catalyzed the hydrolysis of oxidized PtdCho.

MATERIALS AND METHODS

Carrier-free Na^{125}I and [1-^{14}C]linoleic acid were purchased from New England Nuclear. 2-[1-^{14}C]Linoleoyl PtdCho was synthesized as described (2). pBPB, Rose Bengal, and soybean lipoxygenase were purchased from Sigma.

Human LDL (d = 1.019–1.057), very low density lipoprotein (VLDL; d < 1.006), and high density lipoprotein (HDL; d = 1.063–1.21) were isolated from pooled human plasma as described (6). In some instances, light (d = 1.019–1.056) and heavy (d = 1.056–1.063) LDL subfractions were separately isolated. All lipoproteins were dialyzed at 4°C against phosphate-buffered saline (PBS) containing 0.01% EDTA before use. Lipoprotein concentrations are expressed in protein equivalents.

pBPB was dissolved in warm ethanol and kept warm prior to addition (usually 5 μl) to the incubation mixture. For phospholipase assay, 200 μg of LDL in 1 ml of PBS was preincubated with pBPB at the specified concentrations for 120 min at 37°C. The LDL was delipidated with acetone before enzyme activity was measured.

[^{14}C]pBPB was synthesized from [U-^{14}C]benzene as follows. Benzene was converted to bromobenzene by direct addition of Br_2 in carbon tetrachloride. The product was treated with equimolar amounts of bromoacetylbromide in the presence of anhydrous aluminum chloride. Labeled pBPB was purified by preparative TLC using hexane/diethyl ether (50:50; vol/vol). The product comigrated with authentic pBPB standard and was effective in inhibiting snake venom PLA2 activity.

Photooxidation of LDL was performed in an ice bath by irradiating LDL (500 μg/ml) in 0.154 M NaCl/0.05 M Tris·HCl, pH 8.0, as follows. Rose Bengal (final concentration, 25–50 μg/ml) was added to LDL samples and the tubes were exposed to two tungsten lamps (100 W each) at a distance of ≈22 cm for 15–180 min as specified. LDL exposed to light without Rose Bengal and a LDL/Rose Bengal mixture not exposed to light were used as controls. Two hundred micrograms of the delipidated protein was used for the PLA2 assay.

SDS Agarose/PAGE. The method of Gabelli *et al.* (7) was used to isolate apo-B without small molecular weight protein contamination. By use of a 3-mm-thick agarose/polyacrylamide gel, 1.2 mg of LDL protein was delipidated with acetone/butanol and was dissolved without boiling in 0.89 M Tris borate buffer (pH 8.3) containing 0.1% sodium deoxycholate, 15% (vol/vol) glycerol, and 0.2% Tween 20. Delipidated boiled LDL was used as a control. In some experiments, albumin was used as an internal standard. Electrophoresis was performed at ≈200 V for 2 hr at 4°C. A portion of the gel was stained with Coomassie blue. Bands were cut horizontally across the gel, minced, and directly used for

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; TBARS, thiobarbituric acid-reactive substance(s); PtdCho, phosphatidylcholine; lyso-PtdCho, lysophosphatidylcholine; PAF, platelet-activating factor; pBPB, *p*-bromophenacyl bromide; apo-B, apoprotein B-100; BHT, butylated hydroxytoluene; PLA2, phospholipase A_2.

*To whom reprint requests should be addressed.

phospholipase assay. Gels without any proteins applied served as controls.

PLA_2 Assay. Oxidized 2-[1-^{14}C]linoleoyl PtdCho (2500 dpm/nmol) was prepared as described earlier using soybean lipoxygenase (2). One milligram of sodium deoxycholate was added to 200 μg of LDL in 300 μl of PBS and the lipids were extracted by stepwise extraction with ice-cold acetone and butanol (3 ml) followed by a final wash with acetone (3 ml). The protein was gently dried in nitrogen and was immediately resuspended in 200 μl of 0.2 M Tris buffer (pH 7.2) containing 1 mg of sodium deoxycholate. Final volume was adjusted to 800 μl and incubation was started by the addition of 35–50 nmol of labeled oxidized PtdCho. After 2 hr of incubation at 37°C, the samples were extracted by the method of Bligh and Dyer (8) using 1 ml of 0.2 M HCl for acidification. The chloroform phase was dried under nitrogen and the fatty acid products were separated by TLC on silicic acid plates using chloroform/methanol/acetic acid/water (90:10:0.5:0.5; vol/vol) as the solvent. Linoleic acid and oxidized linoleic acid were run as standard carriers. Fatty acid products were identified after exposure to iodine vapors and the radioactivity was determined as described (2).

Labeled PAF was synthesized by the acetylation of lyso-PAF with [^{14}C]acetic anhydride in the presence of pyridine. The product was purified by TLC. The hydrolysis of PAF was measured by determining the radioactivity in the aqueous phase after extraction of the incubation system by the method of Bligh and Dyer (8). The chloroform phase was also dried, lipids were separated by TLC, and the radioactivity remaining in PAF was determined.

Modification of LDL was carried out as described elsewhere using cultured endothelial cells or 5 μM copper in Ham's F-10 medium (1, 2). Degradation of the modified LDL by mouse peritoneal macrophages was measured as described (1).

Thiobarbituric acid-reactive substances (TBARS) and protein were measured by the methods of Patton and Kurtz (9) and Lowry *et al.* (10), respectively. Amino acid analysis was performed after extraction of LDL lipids (8) as described (11).

RESULTS

It has been previously reported that LDL, as conventionally isolated, contains PLA2 activity (2). This activity preferentially utilized oxidized PtdCho as a substrate. In fact, even when a trace of activity was observed with "unoxidized PtdCho" as substrate, the released fatty acid was actually an oxidized fatty acid, as shown by its polarity on TLC. PLA2 activity against oxidized PtdCho was mostly present in the apo-B-containing particles—VLDL and LDL (Table 1). Heavy LDL was usually 1.5–3 times more active than the light LDL fraction. This suggested the possibility that the lipoprotein(a) [Lp(a)] fraction might contain PLA2 activity.

Table 1. Distribution of hydrolytic activity against oxidized PtdCho among human plasma lipoprotein fractions

Lipoprotein	PLA2 activity against oxidized PtdCho
VLDL	10 ± 3.1
LDL	11 ± 6.3
Light LDL	11 ± 3.1
Heavy LDL	28 ± 12
HDL	1.0 ± 0.8

Lipoproteins were isolated from at least eight pooled plasma samples over a period of 12 months as described in the text. Enzyme assays were carried out with 200 μg of lipoprotein–protein after extraction with acetone/butanol. Values given are means ± SD of four separate assays, each in duplicate, and are expressed as nmol of fatty acid products per mg of protein.

However, the apoprotein(a) of Lp(a) (generously provided by Richard Lawn, Genentech) was inactive. The PLA2 activity associated with HDL was usually <20% of that associated with LDL. The activity, compared with known phospholipases, is considerably lower; however, this might be expected because of the difference in substrate specificity of the LDL-associated PLA2 and its potential physiological role.

Inactivation of PLA2 Activity During Oxidative Modification. We have shown previously that known inhibitors of PLA2 (pBPB and 1-bromo 2-octanone) inhibited the oxidative modification of LDL (2). These compounds are presumed to bind covalently to histidine residues at the active site of phospholipases (12–15). We have also reported that during the oxidative modification of LDL >30% of the histidine residues of apo-B are lost (11). We therefore tested the PLA2 activity of the oxidized LDL to establish whether oxidation affected the enzyme activity. Oxidation of LDL by endothelial cells or by incubation with 5 μM copper reduced the enzyme activity to <40% of the control values (Table 2). Antioxidants that inhibited the modification of LDL [e.g., butylated hydroxytoluene (BHT)] also prevented the inactivation of PLA2 activity. However, at very early stages of oxidation of LDL (for example, after only 1 or 2 hr of oxidation), a slight increase in activity was noticed in several experiments (data not shown).

Photooxidation of LDL. Rose Bengal or other dye-sensitized photooxidation has been known to affect specific amino acids, particularly histidine and methionine (16). When LDL was exposed to light in the presence of Rose Bengal there was a time-dependent inactivation of PLA2 activity (Fig. 1). Rose Bengal alone, in the absence of exposure to light, did not affect the activity. In contrast, PAF hydrolase was only reduced by ≈25% under identical conditions. PLA2 activity associated with snake venom (*Naja naja naja*; gift from E. Dennis, University of California, San Diego), known to be sensitive to histidine modification, was also considerably inactivated under these conditions. A 1-hr exposure to light in the presence of Rose Bengal resulted in almost complete loss of enzyme activity.

Amino acid analysis of the control and photooxidized LDL is given in Table 3. The amino acid composition of control LDL was comparable to that described (11). Rose Bengal-treated, light-exposed LDL was similar to control LDL except for the loss of histidine. Virtually no histidine could be detected in the photooxidized LDL. The only other amino acid that showed a decrease was methionine.

Inhibition of PLA2 Activity by pBPB. Although the inhibition of the oxidative modification of LDL by pBPB suggested an involvement of histidine, there are other possibilities. For example, pBPB also decreased the formation of TBARS.

Table 2. Inactivation of PLA2 activity during oxidative modification of LDL

LDL sample	PLA2 activity against oxidized PtdCho
Native LDL	25 ± 5.3
LDL incubated	
With endothelial cells	8.3 ± 3.0
With endothelial cells + BHT	17 ± 2.0
With Cu^{2+}	4.0 ± 1.2
With Cu^{2+} + BHT	14 ± 3.5

Oxidation of LDL was performed in the absence or presence of BHT (10 μM) by exposure to endothelial cells or 5 μM copper as described in the text with 200 μg of LDL in 2 ml of Ham's F-10 medium. After the modification incubation, LDL protein was treated with excess ice-cold acetone. Precipitated LDL protein was used for PLA2 assay. Values are means ± SD from six individual assays and are expressed as nmol of oxidized fatty acid per 2 hr per mg of protein.

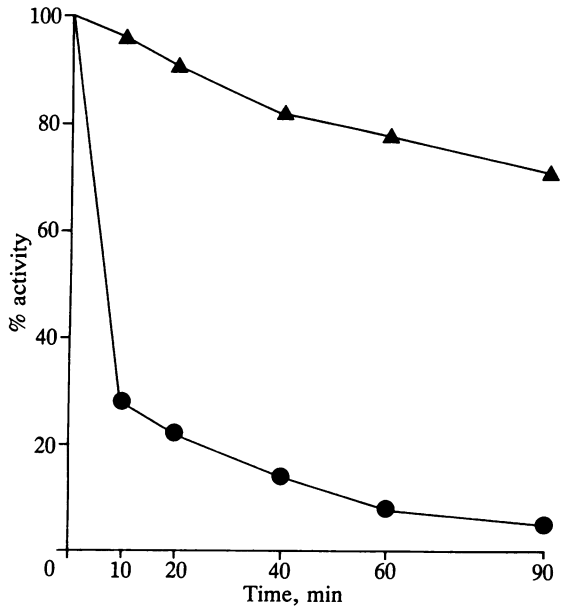

FIG. 1. Time course of Rose Bengal inactivation of LDL-associated phospholipase. Values represent averages of duplicate determinations from three different experiments. The percent of control activity remaining is shown for [^{14}C]PAF substrate (▲) and [^{14}C]PtdCho substrate (●).

While we attributed this to decreased propagation of lipid peroxidation, it is also possible that pBPB might have intrinsic antioxidant properties or interact with thiol compounds in the F-10 medium. We therefore further investigated the effect of pBPB on the PLA2 activity. First, we preincubated LDL with pBPB under conditions that would not lead to significant LDL oxidation. The LDL protein was extracted as described and the PLA2 activity was measured with oxidized PtdCho used as the substrate. As shown in Table 4, pBPB pretreatment inhibited enzyme activity. In contrast, *p*-bromoacetophenone, an inactive analog of pBPB, had no effect on PLA2 activity.

When [^{14}C]pBPB was similarly incubated with LDL, even after several washings with acetone, considerable radioactivity remained bound to the protein. SDS/PAGE of the protein followed by autoradiography revealed that almost all the radioactivity was associated with intact apo-B.

Table 3. Amino acid composition of control and Rose Bengal-treated LDL samples after exposure to light

Amino acid	% composition	
	Control	Rose Bengal treated
Thr	6.7	7.1
Ser	8.0	8.6
Pro	4.4	4.3
Gly	5.2	5.1
Ala	6.2	6.3
Val	5.4	5.4
Met	1.4	1.1
Ile	5.6	5.8
Leu	12.0	12.2
Tyr	3.3	3.3
Phe	4.9	5.0
His	2.6	0.0
Lys	7.6	7.9
Arg	3.6	3.6

LDL samples were exposed to light in the presence and absence of Rose Bengal as described. Lipids were extracted and the protein was subjected to amino acid analysis. Values given are averages from two independent samples.

Table 4. Inhibition of PLA2 activity by pBPB

LDL sample	PLA2 activity against oxidized PtdCho
Native LDL	31 ± 4.7
LDL preincubated	
With 250 nmol of pBPB	8.3 ± 1.3
With 250 nmol of BAP	34 ± 3.2

LDL (200 μg) in 0.5 ml of PBS was treated with 250 nmol of pBPB or bromoacetophenone (BAP; a structural analog of pBPB without PLA2 inhibitory properties) for 2 hr at 37°C. The samples were then extracted with ice-cold acetone to remove unreacted pBPB. The precipitated protein was used to assay enzyme activity. Values are from 10 separate determinations and are expressed as nmol of oxidized fatty acid per 2 hr per mg of protein.

In separate trials, we subjected LDL to oxidation in the presence of concentrations of pBPB (100 μM) sufficient to almost totally inhibit the formation of TBARS. BHT was also included for comparison. After 24 hr, we measured the formation of TBARS and the lipoproteins from these incubations were then delipidated and the PLA2 activity was determined with oxidized PtdCho used as substrate. As shown in Table 5, oxidation of LDL generated considerable amounts of TBARS and inhibited the PLA2 activity. As expected, BHT prevented oxidation and maintained the PLA2 activity at control levels. If pBPB had acted only as an antioxidant, it should have prevented both the generation of TBARS and loss of enzyme activity just as BHT did. Actually, pBPB inhibited the formation of TBARS, but the enzyme activity was lost, showing that (*i*) the effect of pBPB was not due exclusively to "antioxidant properties" and (*ii*) pBPB actually inhibited the enzyme activity under these conditions. The failure of other investigators (3, 4) to detect inhibition of enzyme activity may be due to use of shorter preincubation times and lower pBPB concentrations.

PLA2 Activity Distinct from PAF Hydrolase Associated with LDL. To test the possibility that apo-B itself may have PLA2 activity, LDL samples were delipidated and dissolved in buffer containing Tween 20 without heat denaturation and fractionated on SDS/polyacrylamide gel (7). Bovine serum albumin was used as an internal standard. A boiled LDL preparation was similarly subjected to electrophoresis. A vertical strip of the gel was cut and stained with Coomassie blue to locate apo-B and its fragments and albumin. Gel bands were cut, minced, and directly assayed for activity. The results, as shown in Table 6, show that high molecular weight proteins (intact apo-B and large, partially degraded fragments of $M_r > 66{,}000$) possess oxidized PtdCho hydrolytic activity compared with boiled apo-B applied to the gel as a control.

LDL was also delipidated with ethanol/ether and extracted with *N*-ethyl morpholine, as suggested by Lee *et al.* (5), a

Table 5. Inhibition of LDL oxidation by pBPB does not preserve PLA2 activity

Sample	TBARS, nmol per mg of LDL	% PLA2 activity against oxidized PtdCho
Native LDL	9.2	100
Oxidized LDL	70	38
LDL oxidized		
In presence of BHT	11	97
In presence of pBPB	11	47

LDL (200 μg of protein in 2 ml of F-10 medium) was subjected to oxidation with 5 μM copper at 37°C in the presence of 100 μM BHT or pBPB added in 5μl of warm ethanol. The control contained ethanol only. After 24 hr, proteins from the incubation medium were precipitated with excess acetone and enzyme activity was determined with oxidized PtdCho used as the substrate. Values are averages from four individual trials that varied from each other by up to 11%.

Table 6. PLA2 activity of electrophoretically separated apoprotein fractions from LDL

Sample	PLA2 activity against oxidized PtdCho			
	Trial I	Trial II	Trial III	Trial IV
Native LDL	14	38	19	24
Intact apo-B or fragments of apo-B*	6	15	17	14
Control	4.1	9.0	3.8	3.6

Controls in trials I and II are areas from the gel below albumin. Controls in trials III and IV are gels run without any samples. Each gel sample also had a "boiled" control separated simultaneously, for which the value was subtracted from the observed sample activity. Boiled LDL blanks gave 0.18 ± 0.06 nmol of oxidized fatty acid products. Results are expressed as nmol of product per 2 hr per mg of protein.

*Areas of gel corresponding to intact apo-B and large fragments of apo-B (larger than albumin).

procedure that should extract all soluble proteins. When the fractions were subjected to PLA2 assay, almost all the activity was found with the precipitated apo-B (data not shown).

DISCUSSION

The initial step in the generation of oxidized LDL is oxidation of the unsaturated fatty acid moiety of the surface PtdCho. As reported (1), the oxidized PtdCho is rapidly hydrolyzed by LDL-associated PLA2 activity, which converts ≈40% of the LDL PtdCho to lyso-PtdCho during overnight incubation. This hydrolysis of PtdCho appears to be essential for continuing oxidation of the LDL, presumably because the fatty acids released are more effective in propagation than they are if they remain attached to the glycerol backbone of PtdCho. Since it appears that only oxidized phospholipids can act as substrates and since oxidized phospholipids are continually generated in the course of the incubation, it becomes difficult to know how to assess the "specific activity" of the PLA2 involved in this complex process of LDL oxidation. Expressed per mg of total apo-B protein, the specific activity is considerably lower than that of classical PLA2 enzymes. On the other hand, if only a small domain of apo-B is responsible for enzymatic activity, the true turnover number would be considerably higher per mg of such a postulated domain.

Recently it was reported that plasma PAF hydrolyase was associated with LDL and that this enzyme might be responsible for hydrolysis of oxidized PtdCho during modification of LDL (3, 4). In this study, we provide several different lines of evidence suggesting the presence of a PLA2 activity associated with apo-B that is distinct from plasma PAF hydrolase activity. First, PLA2 activity against oxidized PtdCho is associated with apo-B-containing lipoproteins. Very little activity is associated with HDL. PAF hydrolase, in contrast, appears to be more or less evenly distributed between LDL and HDL with little or no activity in VLDL (17). Second, the substrate specificity of PAF hydrolase is quite different from that of the PLA2 activity determined in the current study. PAF hydrolase has been reported to hydrolyze only substrates that have short acyl chains of six carbons or less esterified to the 2 position (4, 18). Since the ^{14}C label in the substrate used in the present studies is in the C-1 (carboxyl) carbon of linoleic acid (containing an Ω-9 double bond), any fragmented labeled product should have at least 9 carbon atoms. Furthermore, detergents that inhibit the activity of PAF hydrolase (18) actually activated the hydrolysis of oxidized PtdCho by LDL. Third, PAF hydrolase has been shown to be insensitive to inhibition by pBPB. In the present studies we could demonstrate that labeled pBPB is bound to apo-B and that such a binding decreases PLA2 activity. We also show (Table 5) that this inhibition is not due to an antioxidant effect. The involvement of histidine is also supported by the inactivation of PLA2 activity by photooxidation of LDL in the presence of Rose Bengal. Preliminary findings indicate that diethylpyrocarbamate also inhibited activity (results not shown). Fourth, we find that extraction that would remove soluble peptides from apo-B failed to extract oxidized PtdCho hydrolytic activity. Finally, we show that apo-B itself probably has an intrinsic PLA2 activity. We separated apo-B and its larger fragments from PAF hydrolase, which has a M_r of ≈43,000 (3, 4, 18) by SDS/PAGE. Even in the presence of SDS and acrylamide the larger fragments showed PLA2 activity against oxidized PtdCho.

The presence of PLA2 activity in LDL may have biological implications. For example, if LDL surface phospholipids undergo oxidation in the plasma, such an enzyme could serve the function of eliminating the potentially toxic fatty acid products, generating lyso-PtdCho molecules on the LDL. The latter could be reesterified by the mechanism described by Subbaiah *et al.* (19), thus regenerating native LDL. The LDL-associated PLA2 activity may thus serve the role of restoring functional integrity to LDL through a deacylation–reacylation mechanism. Such an activity may also serve a function during fabrication of VLDL, possibly releasing apo-B from the microsomal membrane during its secretion. Finally, this PLA2 activity also appears to be responsible for the production of lyso-PtdCho (1), which is a monocyte chemotactic factor of LDL during oxidative modification (20). The establishment of enzyme activities associated with apo-B may provide vital information as to the function of this unique, long polypeptide that already has been shown to share amino acid homology with egg yolk vitellogenin, which in turn shares sequence homology with lipoprotein lipase (21).

We are indebted to Drs. Daniel Steinberg and Joseph L. Witztum for their advice, suggestions, and support. We also thank Dr. Nonna Kondratenko for technical assistance and Lee Hall for establishing the conditions for the total synthesis of pBPB. This work was supported by National Heart, Lung, and Blood Institute Grant HL14197.

1. Steinbrecher, U. P., Parthasarathy, S., Leake, D. S., Witztum, J. L. & Steinberg, D. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3883–3887.
2. Parthasarathy, S., Steinbrecher, U. P., Barnett, J., Witztum, J. L. & Steinberg, D. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3000–3004.
3. Stremler, K. E., Stafforini, D. M., Prescott, S. M., Zimmerman, G. A. & McIntyre, T. M. (1989) *J. Biol. Chem.* **264**, 5331–5334.
4. Steinbrecher, U. P. & Pritchard, P. H. (1989) *J. Lipid Res.* **30**, 305–315.
5. Lee, D. M., Valente, A. J., Kuo, W. H. & Maeda, H. (1981) *Biochim. Biophys. Acta* **666**, 131–146.
6. Havel, R. J., Eder, H. A. & Bragdon, J. H. (1955) *J. Clin. Invest.* **34**, 1345–1353.
7. Gabelli, C., Stark, D. G., Gregg, R. E. & Brewer, H. B. (1986) *J. Lipid Res.* **27**, 457–460.
8. Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917.
9. Patton, S. & Kurtz, G. W. (1951) *J. Diary Sci.* **34**, 669–674.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
11. Fong, L. G., Parthasarathy, S., Witztum, J. L. & Steinberg, D. (1987) *J. Lipid Res.* **28**, 1466–1477.
12. Volverk, J. J., Pieterson, W. A. & deHaas, G. H. (1974) *Biochemistry* **13**, 1446–1454.
13. Fedarho-Roberts, M., Deems, R. A., Mincey, T. C. & Dennis, E. A. (1977) *J. Biol. Chem.* **255**, 2405–2411.
14. Verheij, H. M., Boffo, M. C., Rothen, C., Bryckaert, M. C.,

Verger, R. & deHaas, G. H. (1980) *Eur. J. Biochem.* **122,** 25–32.
15. Waite, M. (1987) *The Phospholipase Handbook of Lipid Research* (Plenum, New York), Vol. 5, pp. 155–240.
16. Llorca, F. I., Iborra, J. L. & Lozano, J. A. (1983) *Photochem. Photophys.* **5,** 105–111.
17. Stafforini, D. M., McIntyre, T. M., Carter, M. E. & Prescott, S. M. (1987) *J. Biol. Chem.* **262,** 4215–4222.
18. Stafforini, D. M., Prescott, S. M. & McIntyre, T. M. (1987) *J. Biol. Chem.* **262,** 4223–4230.
19. Subbaiah, P. V. & Bagdade, J. D. (1978) *Life Sci.* **22,** 1971–1978.
20. Quinn, M. T., Parthasarathy, S. & Steinberg, D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2805–2809.
21. Baker, M. E. (1988) *Biochem. J.* **255,** 1057–1060.