Phospholipase A$_2$ Modification of Low Density Lipoproteins Forms Small High Density Particles with Increased Affinity for Proteoglycans and Glycosaminoglycans*

(Received for publication, June 7, 1999, and in revised form, June 24, 1999)

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The presence of a lipoprotein profile with abundance of small, dense low density lipoproteins (LDL), low levels of high density lipoproteins (HDL), and elevated levels of triglyceride-rich very low density lipoproteins is associated with an increased risk for coronary heart disease. The atherogenicity of small, dense LDL is believed to be one of the main reasons for this association. This particle contains less phospholipids (PL) and unesterified cholesterol than large LDL, and the apoB-100 appears to occupy a more extensive area at its surface. Although there are experiments that suggest a metabolic pathway leading to the overproduction of small, dense LDL, no clear molecular model exists to explain its association with atherogenesis. A current hypothesis is that small, dense LDL, because of its higher affinity for proteoglycans, is entrapped in the intima extracellular matrix and is more susceptible to oxidative modifications than large LDL. Here we describe how a specific reduction of approximately 50% of the PL of a normal buoyant LDL by immobilized phospholipase A$_2$ (PLA$_2$) (EC 3.1.1.4) produces smaller and denser particles without inducing significant lipoprotein aggregation (<5%). These smaller LDL particles display a higher tendency to form nonsoluble complexes with proteoglycans and glycosaminoglycans than the parent LDL. Binding parameters of LDL and glycosaminoglycans and proteoglycans produced by human arterial smooth muscle cells were measured at near to physiological conditions. The PLA$_2$-modified LDL has about 2 times higher affinity for the sulfated polysaccharides than control LDL. In addition, incubation of human plasma in the presence of PLA$_2$ generated smaller LDL and HDL particles compared with the control plasma incubated without PLA$_2$. These in vitro results indicate that the reduction of surface PL characteristic of small, dense LDL subfractions, besides contributing to its small size and density, may enhance its tendency to be retained by proteoglycans.

The low density lipoproteins (LDL)$^1$ with density 1.019 to 1.063 g/ml in humans are characterized by the presence of a major apolipoprotein, the apoB-100, and a variable lipid complement. This operationally defined density range can contain subclasses of particles of different size and density caused by dissimilar contents of core and surface lipids (1, 2). Much interest is focused on the metabolic and genetic factors leading to the presence of LDL profiles in which the majority of particles centers around small, dense particles (LDL-III) with diameters of 25.5 to 24.2 nm and densities between 1.040 and 1.060 g/ml, the phenotype B. Subjects with such phenotype have significantly higher susceptibility to CHD, when compared with subjects that have most of their LDL as large particles (LDL-I and LDL-II) with diameters between 25.5 and 27.5 nm and densities between 1.025 and 1.040 g/ml, the phenotype A (3–5). Excess LDL-III is a marker of insulin resistance and type II diabetes, and it occurs almost always in combination with moderate hypertriglyceridemia, above 2 mm or 180 mg/dl, and low levels of HDL (6, 7). Because its association with CHD, this lipoprotein pattern has been also termed the atherogenic lipoprotein phenotype (ALP) (8). The susceptibility to develop the ALP appears associated with genes in chromosomes 11, 15, and 16, and heredity controls about 50% of its expression. The rest of the expression is dependent on hormonal and life style factors (3).

The metabolic reasons for the appearance of an excess of LDL-III are under intense exploration, and several steps in the lipolytic conversion of VLDL and intermediate density lipoproteins have been postulated to be responsible for its production in conjugality with the action of cholesterol ester transport protein. One hypothesis, supported on metabolic studies, proposes that overproduction of large TG-rich VLDL causes an increase in the exchange of TG for cholesterol esters (CE) between this particle and LDL leading to a temporary increase of TG in LDL. The resulting TG-rich LDL become a good substrate for the action of hepatic lipoprotein lipase, which also has a phospholipase activity, and possibly for the endothelial lipoprotein lipase. This produces a small dense particle with a reduced content of core CE and of the surface components PL and UC (9). However, when subfractions of LDL with increasing density are isolated from normal subjects a similar trend in decreased content of surface UC and PL and core CE per

$^*$ This work was supported by the Swedish Society for Medical Research Project 970133, the Swedish Heart and Lung Foundation Projects 61538 and 63503, the Swedish Medical Research Council Projects 4531, King Gustaf Vs 80 years foundation, and AstraZeneca, Mölndal, Sweden. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

$^1$ The abbreviations used are: LDL, low density lipoproteins; HDL, high density lipoproteins; TG, triglyceride; VLDL, very low density lipoproteins; CHD, coronary heart disease; PL, phospholipids; UC, unesterified cholesterol; PLA$_2$, phospholipase A$_2$; GAG, glycosaminoglycans; PG, proteoglycans; ALP, atherogenic lipoprotein profile; CE, cholesterol esters; OCS, chondroitin 6-sulfate; NEFA, nonesterified fatty acids, BHT, butylated hydroxytoluene; BSA, bovine serum albumin; CSPG, chondroitin sulfate proteoglycans.
apob-100 particle is observed (2, 9). Therefore the phenotype B can be considered the result of exaggerated accumulation in plasma of small, dense particles that may exist at lower concentration in normal men and women with the A phenotype. Two, nonexclusive, hypotheses have been considered to be responsible for the increased atherogenicity of small, dense LDL and its association with cardiovascular disease. One is its preferential entry and retention in the arterial wall, especially at sites of lesion development. This is supported by in vitro experiments (10, 11). The other, also based on in vitro results, suggests that small, dense LDL is more susceptible to oxidative and hydrolytic modification than buoyant, large LDL once entrapped in the arterial intima at sites of lesion progression (12, 13).

The reasons why small, dense LDL are better retained in lesions than buoyant LDL are not clear. However, our laboratory showed ex vivo that smaller subfractions of human LDL bind with higher affinity to human arterial chondroitin sulfate proteoglycans (CSPG). The smallest LDL showed a significantly lower content in surface PL and UC than large LDL. Owing to the lower content in the surface lipids, the apo-B-100 was estimated to have up to 45% larger area to cover at the surface monolayer (2, 9, 14). However, the apparent causality for CSPG results mainly in a lower content of PL and UC at the intima may contribute to its atherogenicity. As men-

The reaction was stopped by removing the PLA2-agarose by a brief centrifugation at 10,000 rpm. After centrifugation at 20 °C for 46 h, the supernatants were removed and the pellets dissolved in 10 mM HEPES buffer at double the amount suggested by the manufacturer. The cholesterol was measured according to the CHOD-PAP method with reagents bought from Roche Molecular Biochemicals (Hoffmann-La Roche, New Jersey). The absorbance was measured at 500 nm with a UV-visible detector (Jasco UV-970, Jasco International Co., Ltd., Japan). Data were integrated with a Chromo 4L chromatography data system (Gynkotek GmbH, Germany). The distribution of lipoproteins was continuously measured as cholesterol and phospholipid distribution profiles were measured with a size exclusion high performance liquid chromatography system, SMART, with a Supersose 6 PC 3:230 column (Amersham Pharmacia Biotech). The chromatographic system was linked to an air-segmented continuous flow system for on-line post-derivatization analysis of the total cholesterol and phospholipids using enzymatic colorimetric reagents. The SMART system was connected to a sample injector (Gina 50, Gynkotek HPLC, Garmeling, GmbH). Elution buffer consisted of 0.01 M Tris, 0.03 M NaCl, pH 7.40, and the flow rate was 35 μl/min. The on-line flow system was equipped with a peristaltic pump, flow rate 0.7 ml/min, and a coil for 8 min of incubation at 37 °C. The absorbance was measured at 500 nm with a UV-visible detector (Jasco UV-970, Jasco International Co., Ltd., Japan). Data were integrated with a Chromo 4L chromatography data system (Gynkotek GmbH, Germany).

The distribution of lipoproteins was continuously measured as cholesterol and phospholipids by using the enzymatic reagents reconstituted in buffer at double the amount suggested by the manufacturer. The cholesterol was measured with a kit from Roche Molecular Biochemicals (cholesterol MPRI 1442341) and phospholipids with a kit from Wako Chemicals (phospholipids B 990–54009 E). Each run lasted 60 min, and the sample size used was 10 μl. The integrated area is expressed in molar concentrations.

LDL were re-isolated, after incubation with or without PLA2, by D2O ultracentrifugation as described below. All lipid classes in LDL were analyzed with a modification of the high performance liquid chromatography developed by Homan and Anderson (21). The column was an Alltech, Allsphere Si, 5 μm, 150 × 4.6 mm. In the first solvent n-heptane was used instead of tetrahydrofuran. The detector was an evaporative light scattering 500 from Alltech (Deerfield, IL). The external standards were purchased by Lardaud Fine Chemicals (Sweden). The high performance liquid chromatography system was from Gynkotek GmbH, equipped with a Chromo 4L chromatography data system (Gynkotek GmbH, Germany).

**LDL Precipitation Assay**—Analysis of interactions between PLA2-LDL and GAG at low ionic strength was done essentially as described (16, 22). Briefly, LDL (1 mg/ml) modified by PLA2 for the indicated time periods was diluted to 0.1 mg of apoB/ml with buffer 10 mM HEPES, 2 mM CaCl2, 4 mM MgCl2, pH 7.2. This gives a final NaCl concentration of 14 mM. Four μg of GAG was added to 100 μl of LDL, and the samples were incubated for 30 min at room temperature. The precipitate formed was collected by centrifugation at 10,000 rpm for 30 min at 4 °C. The supernatants were removed and the pellets dissolved in 10 mM HEPES buffer, pH 7.4, containing 1 mM NaCl. The cholesterol in the pellets was measured according to the CHOD-PAP method with reagents bought from Roche Molecular Biochemicals. In experiments with proteoglycans (decorin and biglycan), the LDL was diluted with buffer 10 mM HEPES, 4 mM CaCl2, 2 mM MgCl2, 20 mM NaCl, pH 7.4. This gives a higher final NaCl concentration (32 mM); otherwise the same protocol was used as for the GAG.

**Gel Mobility Shift Assay**—Analysis of interactions between PLA2-LDL and CSPG at physiological salt concentrations was done with the following modification of a described procedure (23, 24). First, the PLA2-LDL and native LDL were reisolated by ultracentrifugation using a 10 mM HEPES buffer, pH 7.4, prepared with D2O with a final density of 1.082 g/ml. After centrifugation at 20 °C for 3 h at 260,000 × g (rotor TLA 120.2, Optima TLX Ultracentrifuge, Beckman), the top fraction containing LDL was collected by aspiration. A constant amount of CSPG (0.5 μg) was incubated for 1 h at room temperature with increasing concentrations of LDL in a final volume of 20 μl of 10 mM HEPES buffer, pH 7.4, containing 140 mM NaCl, 4 mM CaCl2, 2 mM MgCl2. Two μl of glycerol was added, and the samples were loaded on a 0.7% agarose gel (NuSieve 3:1) prepared with running buffer containing 10 mM HEPES, 2 mM CaCl2, and 4 mM MgCl2. pH 7.4. Electrophoresis was run for 2 h, and after air-drying the gel was stained with 0.015% Oil Red O and 0.06% Fat Red B in the fix solution.
PLA2-agarose by a brief centrifugation at 10,000 rpm for 20 h. The reaction was stopped by removing the PLA2-agarose incubated at 37 °C for the indicated time points. The generation of NEFA was monitored by measuring the release of NEFA using a commercial kit (NEFA-C, Wako Chemicals). Aliquots (0.7 ml) from the reaction were collected. The activity of PLA2 was measured using the release of NEFA using a commercial kit (NEFA-C, Wako Chemicals). No detectable amounts of NEFA were quantified as a function of the incubation time, and the supernatant was collected. The generation of NEFA was reduced, we attributed this mobility change to some residual material that remained LDL-bound. No difference in mobility was detected between native LDL (kept at 4 °C) and LDL incubated without PLA2. Isoelectric focusing did not show any significant change in the isoelectric point comparing native LDL and LDL incubated with PLA2 for 3 and 14 h (data not shown). To rule out the possibility of oxidation of the lipoproteins, the content of conjugated dienes was analyzed at 234 nm in the PLA2-LDL and compared with native LDL. No increase in the content of conjugated dienes was found in the PLA2-LDL (data not shown). This is, however, expected since the LDL solutions always contained 10 μM BHT.

To evaluate the effect of PLA2 hydrolysis of LDL-PL on its density, the lipoproteins were fractionated using density gradient centrifugation in D2O. The cholesterol content in the collected fractions was determined, and representative gradient profiles are shown in Fig. 3. In the experiment presented in Fig. 3 the peak density of LDL increased from 1.036 to 1.041 g/ml after 3 h PLA2 modification and to 1.046 g/ml after 14 h. There was no indication of aggregation based on turbidity measurements at 450 nm in any of the collected fractions (data not shown). The denser, PLA2-modified LDL were also of smaller size than native LDL. In the nondenaturing polyacrylamide gel electrophoresis in Fig. 4, the size of the lipoprotein particles decreased from 27 to 26.6 nm and to 26.5 nm after incubation with PLA2 for 3 and 14 h, respectively. No aggregated lipoproteins were observed in the gel. The 17 nm band in each lane represents thyroglobulin that was used as internal standard and added together with the sample buffer prior to loading the gel.

The degree of possible LDL aggregation due to PLA2 lipolysis was further analyzed using size exclusion chromatography. Control LDL and LDL incubated with PLA2 for 14 h were applied to a Superose 6 column, and the elution profiles were measured as cholesterol, and phospholipid content was continuously recorded. Representative elution profiles are shown in Fig. 5. To indicate the resolution of the SMART system, human plasma profiles are also included in the figures. In the PLA2-LDL, there was only a slight tendency of LDL aggregation, and after repeated injections using different PLA2-LDL preparations it could be concluded that the degree of aggregation was consistently low (<5%). The phospholipid profiles (Fig. 5B) show a reduction of about 50% in the phospholipid content in the LDL incubated with PLA2 for 14 h compared with the control. On the other hand, there is an increase in the content of conjugated dienes found in the PLA2-LDL-PL (Fig. 4). The size of the lipoprotein particles decreased from 27 to 26.6 nm and to 26.5 nm after incubation with PLA2 for 3 and 14 h, respectively. No aggregated lipoproteins were observed in the gel. The 17 nm band in each lane represents thyroglobulin that was used as internal standard and added together with the sample buffer prior to loading the gel.

Results

Incubation of LDL with PLA2 at physiological conditions results in hydrolysis of LDL phospholipids. The PLA2-derived NEFA were quantified as a function of the incubation time, and the results are shown in Fig. 1. Incubation of LDL without PLA2-agarose did not generate any detectable amounts of NEFA under the conditions used in this study. The electrophoretic mobility of the PLA2-LDL was compared with LDL incubated in parallel without PLA2. As shown in Fig. 2 the mobility of the PLA2-LDL was slightly modified and the lipoprotein migrated as a more diffuse band. Although the amount of BSA used should sequester most of the NEFA produced, we attributed this mobility change to some residual NEFA that remained LDL-bound. No difference in mobility was detected between native LDL (kept at 4 °C) and LDL incubated without PLA2. Isoelectric focusing did not show any significant change in the isoelectric point comparing native LDL and LDL incubated with PLA2 for 3 and 14 h (data not shown). To rule out the possibility of oxidation of the lipoproteins, the content of conjugated dienes was analyzed at 234 nm in the PLA2-LDL and compared with native LDL. No increase in the content of conjugated dienes was found in the PLA2-LDL (data not shown). This is, however, expected since the LDL solutions always contained 10 μM BHT.

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of choline in the albumin fraction supporting a role of albumin as a lysophosphatidylcholine transporter.

A lipid analysis of LDL before and after incubation with or without PLA2 for 14 h showed that the action of PLA2 is highly specific and almost only degrades the PC component present in the LDL (Fig. 6). The lipoproteins used for the analysis were reisolated from the reaction buffer by D2O ultracentrifugation. After incubation of LDL for 14 h with PLA2 about 50% of the PC was degraded. The lipid content in native LDL and LDL incubated for 14 h at 37 °C without PLA2 (Fig. 6, Ctrl-LDL) was similar.

The hydrolysis of LDL-PL by PLA2 caused an increase in the affinity of the lipoprotein for GAG and PG when measured by LDL precipitation at low ionic strength or by gel mobility shift assay at near to physiological ionic conditions. Fig. 7 shows the increase of complex formation of the PLA2-LDL with C6S in correlation to the degree of PL hydrolysis. Incubation of native LDL with C6S under these conditions led only to formation of small amounts of nonsoluble complexes. Experiments using dermatan sulfate gave similar results (data not shown). PLA2-modified LDL incubated with the proteoglycans decorin and biglycan also formed more nonsoluble complexes than native LDL (Fig. 8). More nonsoluble complexes were formed with biglycan than with decorin probably due to the higher content of GAG in biglycan compared with decorin (two and one CS/DS chain, respectively). Gel mobility shift assay was used to study the interactions of PLA2-LDL and C6S at conditions in which reversible complexes are formed. PLA2-modified lipoproteins were incubated with C6S, and the complexes formed were
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Disappearance of three independent incubations.

error bars indicate the positive value of the standard deviation of three independent incubations.

pellet was measured according to the CHOD-PAP method with reagents from Roche Molecular Biochemicals. Each bar represents the average, and the error bars indicate the positive value of the standard deviation of three independent incubations.

Similarly, the parameters analyzed by proton NMR spectroscopy were average lipoprotein sizes, total TG, total cholesterol, VLDL TG, LDL particle concentration, and LDL and HDL cholesterol. Table II summarizes the results from the NMR analysis. The average LDL and HDL sizes decreased after incubation of the plasmas with PLA$_2$, whereas the average VLDL size did not change significantly. There were no differences in total TG, VLDL TG, LDL cholesterol, and LDL particle concentration between plasma incubated with or without PLA$_2$. However, there was a statistically significant decrease in the total cholesterol, and it appeared to be due to a decrease in HDL cholesterol specifically. By using the proton NMR technology, it is possible to divide the total VLDL fraction in 6 discrete subclases, with the largest being VLDL6 and then in descending order to VLDL1. In the same way LDL and HDL is divided into 3 and 5 subclases, respectively. Fig. 9 shows the relative distribution of the subfractions after incubation of plasma with PLA$_2$. The data are presented as percentage of each subclass in relation to the total lipoprotein class. It can be observed that there is a shift in the LDL fractions toward smaller particles after incubation with PLA$_2$ compared with the control, especially the level of LDL3 decreases and LDL1 increases. Similar results were found in the HDL fractions where the significant changes were found in subclass HDL4 and HDL2, respectively. No clear differences were observed in the VLDL fractions.

### DISCUSSION

The molecular or metabolic reasons for the association between increased risk of CHD and the presence of high concentrations of small, dense LDL remain to be established. It is not obvious why a reduced number of PL and UC molecules at the surface monolayer and an altered exposure of the apoB-100 could be more atherogenic than the situation with a larger LDL. One possibility is the dissimilar affinity of different LDL binding sites than large LDL. These nonreceptor associated binding sites are made mostly of heparan sulfate PG at the cell surface. Olsson et al. (28) carried out competition experiments in which the LDL binding to fibroblasts was competed with synthetic peptides with specific sequences of apoB-100 that are responsible for PG binding (29). They showed that small, dense LDL requires higher concentrations of the competing peptides than more buoyant LDL for its detachment from the fibroblast surface (28). Galeano et al. (30) recently found that small, dense LDL has a reduced affinity for the LDL receptor and an increased affinity for nonreceptor associated binding sites than large LDL. These nonreceptor associated binding sites are made mostly of heparan sulfate PG at the cell surface. These researchers concluded that the atherogenicity of small, dense LDL could be related to a decreased hepatic clearance and to a higher anchoring to proteoglycans of the extracellular matrix in arteries. Results with rabbit aortic segments also indicate that small, dense LDL is better retained in the extracellular intima than more buoyant LDL (11). Increased residence time or retention of LDL is an early marker of atherosclerotic lesion progress (31–33).
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Human plasma from nine overnight fasting healthy donors was diluted with phosphate-buffered saline and incubated without (control) or with PLA₂-agarose for 20 h at 37 °C. The generation of NEFA was determined with a commercial kit (NEFA-C, Wako), and the lipoprotein profiles were analyzed by proton NMR spectroscopy. Statistical analyses were performed by Student’s paired t test to determine the significance of change in the differences between control and PLA₂-modified plasma. A statistical difference was considered for \( p < 0.05 \).

| Parameter                           | Median (min–max) | Median (min–max) | Mean of difference |
|-------------------------------------|------------------|------------------|--------------------|
| NEFA (mM)                           | 0.074 (0.026–0.143) | 0.221 (0.122–0.369) | −0.151*             |
| VLDL size (nm)                      | 41.9 (33.6–61.7)  | 40.8 (33.7–52.7)  | 1.94 NS             |
| LDL size (nm)                       | 21.6 (20.9–22.0)  | 21.3 (20.7–22.0)  | 0.21†               |
| HDL size (nm)                       | 9.1 (8.7–10.0)    | 8.7 (8.1–9.5)     | 0.46                |
| Total TG (mg/dl)                    | 74.9 (52.1–202.9) | 77.1 (62.0–201.4) | −1.50 NS            |
| VLDL TG (mg/dl)                     | 30.8 (14.3–139.1) | 32.1 (15.3–141.3) | 2.33 NS             |
| Total cholesterol (mg/dl)           | 216.5 (185.1–349.9) | 215.5 (181.9–229.5) | 7.96*               |
| LDL cholesterol (mg/dl)             | 156.2 (117.5–251.4) | 162.7 (117.0–233.6) | 2.70 NS             |
| LDL particle concentration (nm)     | 1600 (1163–2490)  | 1669 (1159–2375)  | −25.11 NS           |
| HDL cholesterol (mg/dl)             | 53.7 (29.8–67.7)  | 44.4 (24.2–66.9)  | 8.81*               |

\( a^* p < 0.001. \)

\( b^* NS, not significant. \)

\( c^* p < 0.01. \)

\( d^* p < 0.05. \)

Our laboratory found that human arterial PG can discriminate LDL subfractions, binding more efficiently those small, dense LDL that were poor in PL and UC at its surface monolayer. These subfractions have therefore more surface area for the apoB-100 to cover (14). The results presented here indicate that the reduction of PC in the surface monolayer of LDL by PLA₂ in vitro reduces the size and increases the density of the particle to values close to those observed in LDL-III. By using the data from the lipid analysis of PLA₂-LDL presented in Fig. 6 and knowing that each particle contains at most one apoB-100 molecule, it is possible to estimate the surface area covered by the apoB-100. The calculations and assumptions needed for this estimation have been outlined in detail previously (14). The data presented in this study show that a reduction of about 50% of the PC content after PLA₂ modification of LDL would, according to these calculations, result in a 25% decreased surface area. Such new free area should be covered by a more extended apoB-100 in the PLA₂-LDL assuming also that there is no reorganization of the polar phase/nonpolar phase lipid class distribution (2, 14).

The changes in surface area covered by PL are associated with an increase in the affinity of the PLA₂-LDL for PG and GAG. This interaction depends strongly on ionic interactions between arginine- and lysine-rich segments of the apoB-100 with the sulfated groups of the GAG (29). When LDL is incubated with PLA₂ in the presence of excess albumin, there is little change on its surface-charge balance (34). Recently, Örní et al. (35) showed, using NMR techniques, that a rapid hydrolysis of LDL-PL by PLA₂ caused a substantial increase in the exposure of normal lysine residues but not of active lysines per apoB-100. Under their experimental conditions the authors showed that PLA₂ modification induced aggregation of LDL particles. As a consequence the number of active lysines per LDL aggregate increased and caused a stronger binding to PG columns. In our experiments we could only detect a very low degree of lipoprotein aggregation (<5%). However, the enzyme treatment caused an increase in the affinity for PG and GAG, evaluated by two methods (Figs. 7 and 8 and Table 1). Our results are in line with a recent report demonstrating that LDL as well as lipoprotein(a) show an increased binding to subendothelial matrix after PLA₂ modification without lipoprotein aggregation (36).

LDL subfractions that are poor in PL and UC exist in plasma, and hypothetically the described increased affinity for intima PG may cause its retention in this compartment. Once there, the increased residence time may give the opportunity for oxidative and hydrolytic modifications of LDL that may condition its further processing by cells (37, 38). This possibility is supported by studies describing how PLA₂-modified LDL is more susceptible to free radical-mediated oxidation (39, 40). Additionally, secretory nonpancreatic PLA₂, active toward LDL, is present in the extracellular matrix of human lesions (41–44). This enzyme may further reduce the surface components of LDL in the intima, causing increased affinity for the matrix PG and leading to aggregation (35, 38). This hypothesis is supported by reports showing that apoB-containing particles isolated from normal human arterial intima have a reduced PC content (45). Also, the particles and aggregates from human lesions show a reduced content of PG and appear enriched in sphingomyelin (46, 47). A similar result was obtained during the analysis of apoB-containing particles from lesions of Watanabe rabbits (48).

The action of phospholipases on the surface monolayer of LDL depends on the surface concentration of the substrates and on the surface pressure of the monolayer (49, 50). The surface monolayer of LDL appears to have a high lateral pressure that does not allow the association of other apolipoproteins but apoB-100 (2, 51). It is possible that a reduction of PC by PLA₂ or hepatic lipase in plasma or by PLA₂ in the intima may potentiate the action of other enzymes like sphingomyelinase because of the increase in the surface concentration of sphingomyelin and the reduction in surface pressure. Recent results about the increased hydrolysis of the sphingomyelin of LDL retained in the intima of rabbit aorta after treatment with human nonpancreatic secretory PLA₂ support these ideas (52). These results indicate that a further reduction in the intima of the LDL surface-polar components by lipases could make its entrapment irreversible by the aggregation and increased affinity for extracellular PG (35).

Biglycan and decorin are proteoglycans present in the arterial wall (53). Recently, biglycan was reported to be found in lipid-enriched areas of human coronary atherosclerotic lesions co-localized with apoB-100 (54). Additionally, in vitro experiments have shown that biglycan and decorin are able to interact with apoB-100-containing lipoproteins (54, 55). In the present study we demonstrate that hydrolysis of PL in LDL by PLA₂ increased significantly the capacity of LDL to form non-soluble complexes with biglycan and decorin.

The potential role of secretory PLA₂ in atherosclerosis has recently been studied in transgenic mice expressing human secretory group IIA PLA₂ (56, 57). It was demonstrated that these animals exhibited significant atherosclerotic lesions both
modify the pathogenesis of atherosclerosis by enhancing their trapping in the artery wall.

Acknowledgments—We thank Dr. Johannes Hulte and Aira Lidell for their help with LDL particle size determinations.

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