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The Proteoglycan Decorin Links Low Density Lipoproteins with Collagen Type I*

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Decorin is a small dermatan sulfate-rich proteoglycan which binds to collagen type I *in vitro* and *in vivo*. In atherosclerotic lesions the contents of low density lipoprotein (LDL), decorin, and collagen type I are increased, and ultrastructural studies have suggested an association between LDL and collagen in the lesions. To study interactions between LDL, decorin, and collagen type I, we used solid phase systems in which LDL was coupled to a Sepharose column, or in which LDL, decorin, or collagen type I was attached to microtiter wells. The interaction between LDL and decorin in the fluid phase was evaluated using a gel mobility shift assay. We found that LDL binds to decorin by ionic interactions. After treatment with chondroitinase ABC, decorin did not bind to LDL, showing that the glycosaminoglycan side chain of decorin is essential for LDL binding. Acetylated and cyclohexanedione-treated LDL did not bind to decorin, demonstrating that both lysine and arginine residues of apoB-100 are necessary for the interaction. When collagen type I was attached to the microtiter plates, only insignificant amounts of LDL bound to the collagen. However, if decorin was first allowed to bind to the collagen, binding of LDL to the decorin-collagen complexes was over 10-fold higher than to collagen alone. Thus, decorin can link LDL with collagen type I *in vitro*, which suggests a novel mechanism for retention of LDL in collagen-rich areas of atherosclerotic lesions.

Deposition of low density lipoproteins (LDL)[1] in the extracellular matrix of the arterial intima is an important step in the development of atherosclerosis (1). LDL interacts with the various components of the extracellular matrix. These components include proteoglycans (PG), elastin, and collagen. Binding of LDL to PG has been characterized in detail (2). Interaction between LDL and elastin has also been reported (3). In contrast, interaction between native LDL and collagen has received little attention (4, 5).

Collagen is the major connective tissue component of atherosclerotic arteries, comprising up to 40% of the total protein in fibrous plaques and 60% in advanced lesions (6). Most of the collagen (50–75%) in a normal artery or in a diseased intima is of type I (7–9). Immunofluorescence microscopy has located LDL along collagen fibers in atherosclerotic lesions (10, 11). Electron microscopic and immunoelectron microscopic studies have also suggested an association between LDL and collagen in the arterial intima (12–14).

Decorin (15) is a small PG having a core protein with a molecular mass of 45 kDa and a single dermatan sulfate-rich side chain. Decorin has been shown to bind to and modify the fibrillar structure of collagen type I (16, 17), and importantly, to co-localize with collagen type I in primary atherosclerotic plaques (18).

The present study concerns the interaction between native LDL and collagen type I in the absence and presence of decorin. In the absence of decorin, there was no significant interaction between LDL and collagen type I. However, when decorin was first allowed to bind to the immobilized collagen, significant amounts of LDL were bound to the decorin-collagen complexes formed. Thus, decorin can link LDL with collagen type I.

EXPERIMENTAL PROCEDURES

Materials—[1,2-^{3}H]Cholesteryl linoleate, *t*-butoxycarbonyl-L-[^{35}S] methionine, *N*-hydroxysuccinimidyl ester (^{35}S labeling reagent), and *N*-succinimidyl[2,3-^{3}H]propionate (^{3}H-Bolton-Hunter reagent) were from Amersham; acid-soluble collagen type I from calf skin, ϵ-amino caproic acid, bovine serum albumin (BSA), and heparin from Sigma; EDTA, SDS, and acetic anhydride from Merck; CM-Sepharose, DEAE-Sepharose, HiTrap Q-columns, Hi-Trap *N*-hydroxysuccinimidyl ester-activated column, Superose 6 HR 10/30 columns, and dextran sulfate from Pharmacia Biotech; Celite 545 (acid-washed) and 1,2-cyclohexanedione from Fluka; chondroitinase AC and ABC, chondroitin-6-sulfate, and dermatan sulfate from Seikagaku, and microtiter plates (Combiplate 8, Enhanced Binding) from Labsystems (Helsinki, Finland). NuSieve GTG-agarose was from FMC BioProducts, phenylmethylsulfonyl fluoride from Boehringer Mannheim, the Schrynel nylon filter from Zürcher Beuteltuchfabrik AG, a 5-μm NH_2 (0.3 × 25 cm) column from Spherisorb, and Dulbecco's phosphate-buffered saline from Life Technologies, Inc. The cholesteryl ester transfer protein was a kind gift from Drs. C. Ehnholm and M. Jauhiainen, National Public Health Institute, Helsinki, Finland.

Isolation, Characterization, and Modification of Decorin—Proteoglycans were isolated from bovine fetal skin essentially as described (19). Briefly, PG were extracted from the skin at 4 °C with 7.8 M urea, 0.15 M NaCl, 5 mM EDTA, and 25 mM Tris, pH 6.5, in the presence of 5 mM ϵ-aminocaproic acid, 0.2 mM phenylmethylsulfonyl fluoride, and 0.02% (w/v) NaN_3 (extraction buffer). After extraction, the mixture was filtered through a Schrynel 48-μm nylon filter and centrifuged at 100,000 × *g* for 60 min. The supernatant was batch-adsorbed with CM-Sepharose. The CM-Sepharose was removed by centrifugation at 4 °C, and the supernatant was adsorbed with DEAE-Sepharose. The Sepharose was packed into a column (5 × 30 cm), and the bound material was eluted with extraction buffer containing 1 M NaCl. The peaks at 280 nm were collected, diluted with the extraction buffer without NaCl to give a final NaCl concentration of 0.15 M, and loaded on a HiTrap Q column (5 ml) equilibrated with the extraction buffer. PG were eluted with a linear gradient of 0.15–1.0 M NaCl in the extraction buffer (120 ml) at a flow rate of 2 ml/min, dialyzed against water, and lyophilized. The disaccharide composition of the PG was analyzed by high performance liquid chromatography using a 5-μm NH_2 column after treatment of the PG with chondroitinase ABC and AC (20). The PG preparation contained 68% of dermatan sulfate, 3% of chondroitin 6-sulfate, and 29% of chondroitin 4-sulfate. The composition of the PG preparation was analyzed by N-terminal sequencing. A fraction corre-

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[1] The abbreviations used are: LDL, low density lipoproteins; apoB-100, apolipoprotein B-100; BSA, bovine serum albumin; CL, cholesteryl linoleate; GAG, glycosaminoglycan(s); HDL, high density lipoproteins; PG, proteoglycan(s).

sponding to 6 μg of total protein was subjected to Edman degradation, using a Procise 494 protein-sequencing system. Released phenylthiohydantoin-derivatives were quantified using model 610 software. This revealed that the sample contained over 90% of decorin and less than 10% of biglycan, and no other sequences were found. The isolated decorin was found to have an anticoagulant capacity in citrated human plasma, as previously characterized (21). ^{35}S-Decorin was prepared by labeling the core protein of decorin with ^{35}S labeling reagent by the Bolton-Hunter procedure (22). Chondroitinase ABC-treated decorin was prepared by incubating 60 μg of ^{35}S-decorin with 75 milliunits of chondroitinase ABC in 160 μl of Dulbecco's phosphate-buffered saline containing 100 μg/ml BSA at 37 °C for 18 h. The amounts and concentrations of decorin are expressed in terms of protein.

Isolation and Modification of Lipoproteins—Human LDL (d = 1.019–1.050 g/ml) and HDL_3 (d = 1.125–1.210 g/ml) were isolated from plasma of healthy volunteers by sequential ultracentrifugation (23). The LDL was labeled with [^{3}H]cholesteryl linoleate ([^{3}H]CL-LDL) as described previously (24). Apolipoprotein B-100 (apoB-100) of LDL and apolipoproteins of HDL_3 were tritiated by the Bolton-Hunter procedure (22) with *N*-succinimidyl[2,3-^{3}H]propionate to yield ^{3}H-LDL and ^{3}H-HDL_3, respectively. LDL was acetylated by treatment of [^{3}H]CL-LDL with acetic anhydride (25). Cyclohexanedione-LDL was prepared by treatment of [^{3}H]CL-LDL with 1,2-cyclohexanedione (26). The amounts and concentrations of LDL are expressed in terms of protein.

Adsorption of Proteins to Microtiter Wells—A suspension of collagen was obtained by dissolving 1 mg/ml collagen type I in 0.1 M acetic acid followed by dialysis against 67 mM phosphate buffer, pH 7.2, for 2–3 days at 4 °C (27). For collagen binding experiments, microtiter wells were coated with 50 μl of either a suspension of collagen type I (50 μg/ml) or of BSA (5 mg/ml) in 67 mM phosphate buffer, pH 7.2, by incubation at 37 °C overnight (27). For decorin binding experiments, microtiter wells were coated with 50 μl of either decorin (10 μg/ml) or BSA (5 mg/ml) in Dulbecco's phosphate-buffered saline by incubation at 37 °C overnight. For LDL binding experiments, microtiter wells were coated with 50 μl of either LDL (100 μg/ml) or BSA (5 mg/ml) in Dulbecco's phosphate-buffered saline by incubation at 37 °C for 2 h. Nonspecific binding sites in the wells were blocked by incubation at room temperature for 1 h with 100 μl of buffer A (50 mM NaCl, 2 mM $CaCl_2$, 2 mM $CaCl_2$, 10 mM HEPES, pH 7.4, and 5 mg/ml BSA).

Microtiter Well Binding Assay—Binding of radiolabeled LDL or HDL_3 to microtiter wells coated with decorin, binding of radiolabeled decorin to microtiter wells coated with LDL, and binding of radiolabeled LDL to microtiter wells coated with collagen type I was studied by incubating the compound to be tested in 50 μl of buffer A at room temperature for 2 h in the respective wells. In some experiments various amounts of sulfated polysaccharides were added with radiolabeled LDL. Wells coated with BSA served as controls for nonspecific binding. Unbound compounds were removed by aspirating the incubation medium, and the wells were then rinsed three times with 100 μl of buffer A. The bound compounds were then detached by incubating the wells twice with 100 μl of 10% SDS at room temperature for 30 min, the SDS solutions were pooled, and the liberated compounds were quantified by scintillation counting. In some experiments, collagen-coated or BSA-coated microtiter wells were first incubated in the absence or presence of ^{35}S-decorin (50 μl) for 2 h, after which the wells were washed, and the ability of ^{3}H-LDL to bind to the decorin-collagen complexes formed was studied as described above.

Affinity Chromatography of Decorin on LDL Affinity Column—LDL was coupled to a *N*-hydroxysuccinimidyl ester-activated HiTrap column (1 ml) according to the manufacturer's instructions. Decorin and ^{35}S-decorin (20 μl samples) were analyzed on the LDL affinity column by elution with a linear gradient of NaCl (0–250 mM in 10 min) in buffer containing 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 10 mM HEPES, pH 7.4, at a flow rate of 1 ml/min. Elution of decorin was monitored at 280 nm. In some experiments, 500-μl fractions were collected and analyzed for their radioactivities. Chromatography was performed with a SMART system from Pharmacia.

Gel Mobility Shift Assay—Affinity of LDL for decorin was measured by the gel mobility shift assay, essentially as described previously by Camejo *et al.* (28). Briefly, 0.4 μg of ^{35}S-decorin was mixed with 0–50 μg of LDL in 20 μl of buffer containing, 140 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 5 mM HEPES, pH 7.2. After incubation for 1 h at 37 °C, 3 μl of glycerol was added, and 10-μl aliquots from each tube were applied to slots in 0.5% agarose submarine gels. The gels, approximately 3 mm in thickness, were made of NuSieve-agarose dissolved in buffer containing 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 5 mM HEPES, pH 7.4. The gels were electrophoresed for 2 h at 60 V, fixed overnight in 0.1% cetylpyridinium bromide, dried, and autoradiographed.

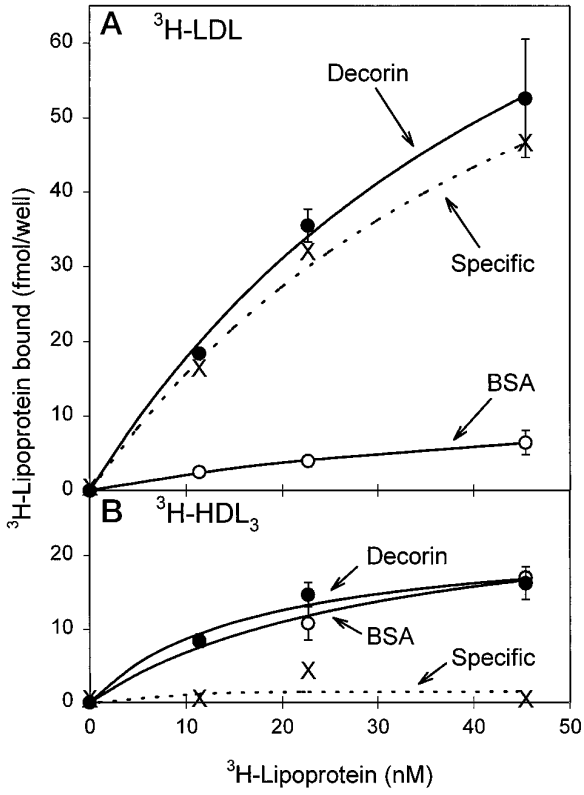

FIG. 1. **Binding of LDL (*A*) and HDL_3 (*B*) to decorin.** Microtiter wells coated with decorin or BSA were incubated for 2 h at room temperature with the indicated concentrations of ^{3}H-LDL (*A*) or ^{3}H-HDL_3 (*B*) in 50 μl of buffer A. The wells were washed, and the bound lipoproteins were detached with SDS, and quantified by scintillation counting. Specific binding of lipoproteins to decorin (×) was calculated by subtracting the amounts of lipoproteins bound to the BSA-coated wells from the total amounts of lipoproteins bound to the decorin-coated wells. Values are means of triplicate incubations ± S.D.

Other Assays—Protein was determined by the procedure of Lowry *et al.* (29) with bovine serum albumin as standard.

RESULTS

Interaction between LDL and Decorin—To study the ability of LDL to bind to decorin, ^{3}H-LDL was added to microtiter wells coated with decorin. As shown in Fig. 1*A*, increasing the concentrations of LDL led to binding of progressively increasing amounts of LDL to the immobilized decorin. No such increase in LDL binding was observed in the albumin-coated wells. When ^{3}H-HDL_3 was used, only insignificant binding to decorin was observed (*Panel B*).

To study the interaction between decorin and LDL, 10 μg of ^{35}S-decorin was applied to an LDL affinity column and then eluted with a linear NaCl gradient. As shown in Fig. 2, 96% of the applied decorin bound to the LDL, and the bound decorin was released from the LDL at approximately 100 mM NaCl in a NaCl gradient, suggesting that decorin was bound to LDL via ionic interactions. In accord with this result, we found that, in the microtiter well binding assay, increasing concentrations of NaCl progressively decreased the binding of ^{35}S-decorin to LDL-coated microtiter wells (not shown).

To find out whether LDL and decorin would interact in physiologic ionic conditions, we studied the ability of LDL to retard the electrophoretic mobility of decorin on agarose gel after LDL and decorin had been incubated in a buffer with an ionic strength of 0.15. Fig. 3 is an autoradiograph of an agarose gel electrophoresis of ^{35}S-decorin incubated with increasing amounts of LDL. It is evident that LDL was able to retard the electrophoretic mobility of decorin, showing that LDL and

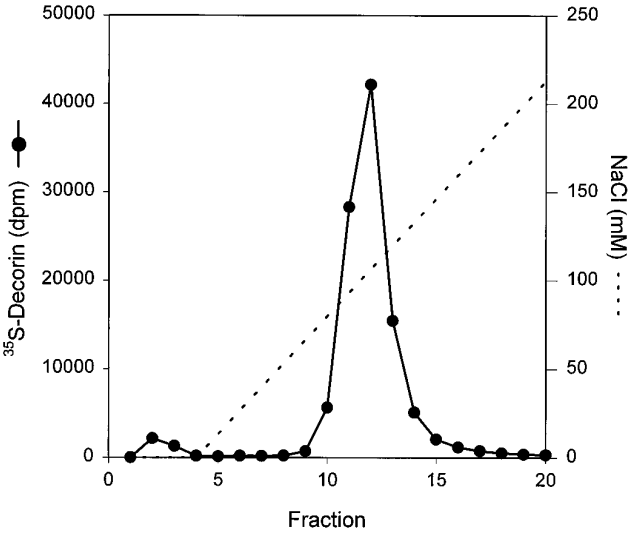

FIG. 2. **Affinity chromatography of ^{35}S-decorin on LDL column.** 10 μg of ^{35}S-decorin was injected into an LDL column in buffer containing 2 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM HEPES, pH 7.4, and eluted with a NaCl gradient in the same buffer at a flow rate of 1 ml/min. Fractions of 500 μl were collected and analyzed for their radioactivities.

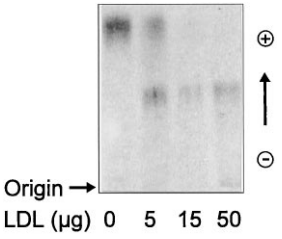

FIG. 3. **Electrophoresis of ^{35}S-decorin on agarose gel.** 0.4 μg of ^{35}S-decorin was incubated for 1 h at 37 °C with the indicated amounts of LDL in 20 μl of buffer containing 140 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 5 mM HEPES, pH 7.2. The samples were electrophoresed on a 0.5% agarose gel as described under "Experimental Procedures," and radiolabeled decorin was detected by autoradiography.

decorin had interacted in the physiologic ionic conditions used.

To study whether LDL binds to the core protein or to the glycosaminoglycan (GAG) side chain of decorin, we treated ^{35}S-decorin with chondroitinase ABC to yield a decorin preparation having the core protein but lacking the GAG chain, and then compared the abilities of native and chondroitinase ABC-treated ^{35}S-decorin to bind to LDL-coated microtiter wells. As shown in Fig. 4, chondroitinase ABC-treated decorin lacked the ability to bind to LDL, suggesting that LDL binds to the GAG side chain of decorin. To confirm this finding we studied the ability of LDL to retard the electrophoretic mobility of chondroitinase ABC-treated ^{35}S-decorin. We found that similar concentrations of LDL that were able to retard the electrophoretic mobility of LDL (shown in Fig. 3) had no effect on the electrophoretic mobility of the decorin core protein (not shown), demonstrating that the GAG chain is indeed essential for binding of decorin to LDL. Treatment of LDL with chondroitinase ABC did not affect the interaction between LDL and decorin (not shown).

To study the role of the positively charged amino acids of apoB-100 in the interaction between LDL and decorin, [^{3}H]CL-LDL was modified either by acetylation or cyclohexanedione treatment to block the lysine and arginine residues of apoB-100, respectively. We found that either modification abolished the binding of LDL to decorin (Fig. 5). Thus, both the lysine and the arginine residues of apoB-100 are essential for the interaction between LDL and decorin, a result consistent with previous studies on the interaction between LDL and PG of other types (30).

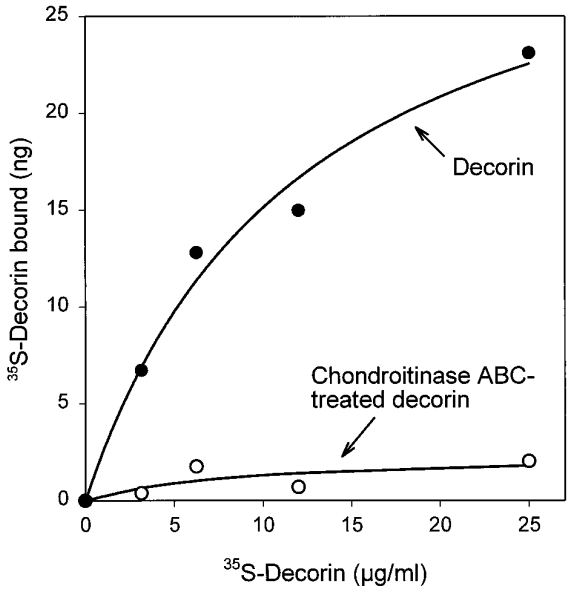

FIG. 4. **Binding of native and chondroitinase ABC-treated decorin to LDL.** Microtiter wells coated with LDL or BSA were incubated for 2 h at room temperature with the indicated concentrations of native or chondroitinase ABC-treated ^{35}S-decorin in 50 μl of buffer A. The wells were washed, and the bound ^{35}S-decorin was detached with SDS and quantified by scintillation counting. The data points indicate specifically bound decorin, which was calculated by subtracting the amounts of decorin bound to BSA-coated wells from the amounts of decorin bound to LDL-coated wells. Values are means of triplicate incubations.

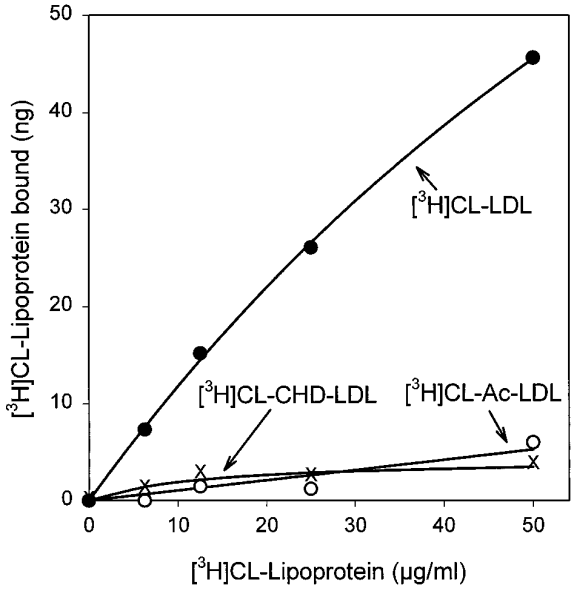

FIG. 5. **Binding of native, acetylated, and cyclohexanedione-treated LDL to decorin.** Microtiter wells coated with decorin or BSA were incubated for 2 h at room temperature with the indicated concentrations of native, acetylated, or cyclohexanedione-treated [^{3}H]CL-LDL in 50 μl of buffer A. The wells were washed, and bound [^{3}H]CL-lipoproteins were detached with SDS and quantified by scintillation counting. The data points indicate specifically bound LDL calculated by subtracting the amounts of LDL bound to the BSA-coated wells from the amounts of LDL bound to the decorin-coated wells. Values are means of triplicate incubations. *[3H]CL-CHD-LDL* denotes cyclohexanedione-treated [^{3}H]CL-LDL, and *[3H]CL-Ac-LDL* denotes acetylated [^{3}H]CL-LDL.

To assess the degree of specificity in the interaction between LDL and decorin, we compared the abilities of soluble sulfated

polysaccharides and decorin to inhibit the binding of ^{3}H-LDL to immobilized decorin. As expected, all studied sulfated polysaccharides were able to inhibit the binding (Fig. 6). More specifically, dextran sulfate and heparin, two molecules with the highest negative charge densities, were most effective, and chondroitin 6-sulfate, dermatan sulfate, and decorin which have lower negative charge densities, were less effective. These results suggest that the other tested negatively charged molecules bind to the same regions in apoB-100 of LDL as decorin.

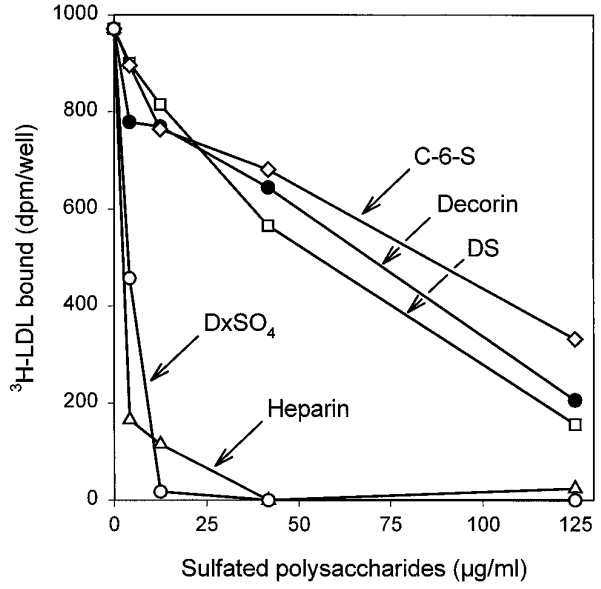

FIG. 6. **Displacement of LDL from immobilized decorin by soluble sulfated polysaccharides.** Microtiter wells coated with decorin or BSA were incubated for 2 h at room temperature with 10 μg/ml of ^{3}H-LDL and the indicated concentrations of sulfated polysaccharides in 50 μl of buffer A. The wells were washed, and the bound lipoproteins were detached with SDS, and quantified by scintillation counting. Specific binding of LDL to decorin was calculated by subtracting the amounts of LDL bound to the BSA-coated wells from the total amounts of LDL bound to the decorin-coated wells. Values are means of triplicate incubations. Abbreviations: *C-6-S*, chondroitin 6-sulfate; *DS*, dermatan sulfate; *DxSO$_4$*, dextran sulfate.

Interaction between LDL and Collagen Type I in the Presence of Decorin—To study the interaction between LDL and immobilized collagen type I in the absence or presence of decorin, collagen-coated microtiter wells were incubated with ^{35}S-decorin, the wells were washed, and the ability of ^{3}H-LDL to bind to collagen or decorin-collagen complexes formed was assessed. As shown in Fig. 7*A*, in the absence of decorin the ability of LDL to bind to immobilized collagen was negligible. However, when collagen-coated wells had been incubated with decorin, which led, on average, to binding of 100 ng of decorin to collagen per well, binding of LDL to the decorin-collagen complexes formed at each LDL concentration tested was more than 10-fold higher than binding to collagen alone. Finally, we studied the ability of ^{3}H-LDL to bind to immobilized collagen as a function of the amount of decorin bound to collagen (Fig. 7*B*). It appeared that the greater the amount of decorin bound to collagen, the greater was the amount of LDL bound to the decorin-collagen complexes.

DISCUSSION

The present results show that decorin can link LDL and collagen type I *in vitro*. In this linkage, the positively charged amino acids of apoB-100 bind to the negatively charged GAG chain of decorin, and, as shown by others (16, 31), the core protein binds to collagen type I. The novelty of this "sandwich" is the association between LDL and collagen via a proteoglycan.

LDL is known to bind to various glycosaminoglycans and proteoglycans (2). This (relatively weak) interaction is electrostatic, and depends on the binding of some of the positively charged amino acids of two types, lysine and arginine, of apoB-100 to the negatively charged sulfate groups of the GAG (30). The segments of apoB-100 that are involved in the binding between LDL and GAG have been identified (32). When we compared different GAG and sulfated polysaccharides for their ability to block the binding of LDL to immobilized decorin, we

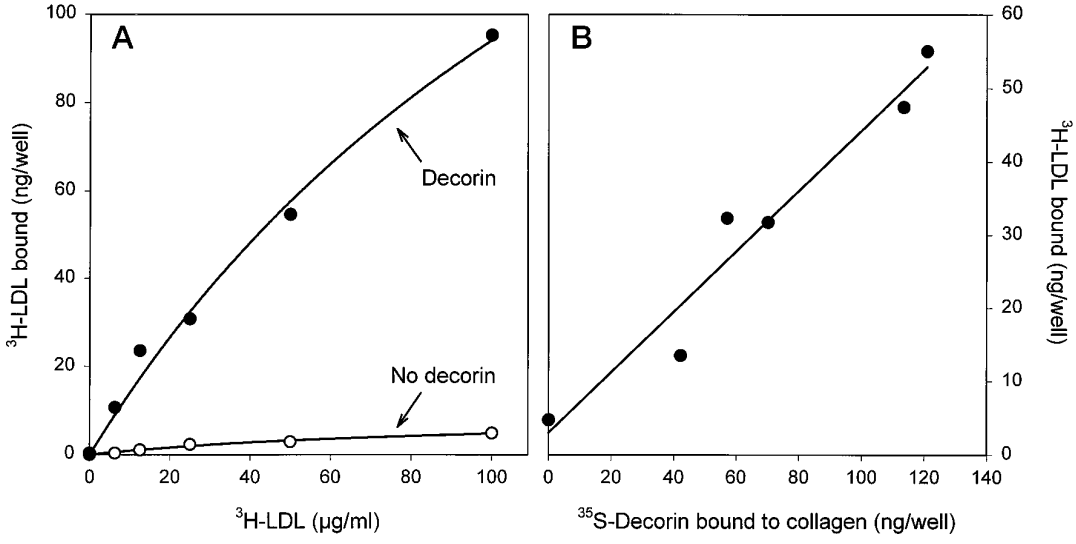

FIG. 7. **Binding of LDL to collagen type I in the absence or presence of decorin (*A*), and as a function of the amount of decorin bound to collagen (*B*).** *Panel A,* microtiter wells coated with collagen or BSA were incubated in the absence or presence of 100 μg/ml ^{35}S-decorin (50 μl) in buffer A for 2 h, and the wells were washed to remove unbound ^{35}S-decorin. The indicated concentrations of ^{3}H-LDL in buffer A were added to the wells, incubated for 2 h, and bound ^{3}H-LDL and ^{35}S-decorin were detached with SDS, and quantified by scintillation counting. The amount of ^{35}S-decorin bound to collagen was 121 ± 12 ng/well (mean ± S.D.). *Panel B,* increasing concentrations of ^{35}S-decorin (0–100 μg/ml) in buffer A were incubated in the wells coated with collagen or BSA, the wells were washed, and the ability of ^{3}H-LDL (50 μg/ml; 50 μl) in buffer A to bind to the wells was studied, as described above. Bound ^{3}H-LDL and ^{35}S-decorin were detached with SDS, and quantified by scintillation counting. All incubations (in *Panels A* and *B*) were carried out at room temperature. The data points indicate the amounts of specifically bound LDL or decorin, which were calculated by subtracting the amounts of LDL or decorin bound to BSA-coated wells from the amounts of LDL or decorin bound to collagen-coated wells. Values are means of triplicate incubations.

found that soluble heparin and dextran sulfate were more effective than decorin, dermatan sulfate, and chondroitin 6-sulfate, which were equally effective. This is in good agreement with previous work showing that the strengths of binding of GAG and sulfated polysaccharides to LDL in descending order are: heparin > dermatan sulfate > heparan sulfate > chondroitin 4-sulfate (33).

Of the sulfated polysaccharides listed above, the two with the highest affinity for LDL are dextran sulfate and heparin, which also have the highest degree of sulfation. Indeed, the degree of sulfation of a GAG is known to be critical for LDL binding. Thus, LDL binds to chondroitin 6-sulfates that have oversulfated regions, but not to a chondroitin 6-sulfate that lacks regions of oversulfation (34). Camejo *et al.* (34) finding that proliferating smooth muscle cells produced GAGs with increased chain length that showed increased affinity for LDL, proposed that LDL binding depends on the chain length of the GAG (28). Vijayagopal *et al.* (35) showed that cholesterol-enriched smooth muscle cells also produced PG of increased size that had a stronger affinity for LDL. However, in that report the degree of sulfation of the GAGs was also studied, and was found to be increased. It is thus possible that the strength of binding of GAG to LDL is more intimately related to the increased sulfation than to the increased length of the GAG, a possibility which is consistent with a previous suggestion by Sambandam *et al.* (34).

The interaction between decorin and collagen type I has been studied extensively, and recently a scheme of interaction has been proposed (36). Is decorin unique in its ability to link LDL with collagen type I? Decorin belongs to a family of structurally related extracellular matrix proteoglycans and glycoproteins which also includes biglycan (37), fibromodulin (38), lumican (39), keratocan (40), and chondroadherin (41). There is strong evidence that, of these molecules, decorin (42) and fibromodulin (43) bind to collagen type I, but whether biglycan does so is unclear (44). Furthermore, only decorin and biglycan have chondroitin/dermatan sulfate chains that potentially bind LDL, whereas the other molecules listed either lack the GAG chain or have a keratan sulfate chain which, in arteries, is not sulfated or only lightly sulfated (45). Therefore, theoretically, only decorin and biglycan can link LDL with collagen type I. However, biglycan is located pericellularly and decorin is located in collagen-rich areas of the arterial intima (18), where, potentially, it can link LDL with collagen type I. Thus, in light of present knowledge, the only small PG capable of linking LDL with collagen type I is decorin.

The arterial intima has been shown to contain, besides collagen type I, also collagen types III, IV, V, VI, and VIII (46). Of these, decorin binds to types I (43), V (47), and VI (48) *in vitro*, and co-localizes with collagen types I and III in atherosclerotic plaques (18). Whether all these collagen types are important for retention of lipid in the atherosclerotic plaque is not known. Interestingly, two other molecules in atherosclerotic lesions, fibronectin (49, 50) and thrombospondin (51), have also been shown to bind decorin.

The arterial intima contains, besides decorin, a large variety of proteoglycans (52). These include basement membrane PG (*e.g.* perlecan), cell surface heparan sulfate PG (*e.g.* the syndecan family and glypicans), versican, small dermatan sulfate-rich PG (decorin, biglycan), small keratan sulfate PG (fibromodulin, lumican, and keratocan), and heparan sulfate PG on elastin. The findings that, of the pig (53) and human (34) aortic PG, the fractions that most effectively interacted with LDL were those containing the small dermatan sulfate-rich PG (*i.e.* decorin and biglycan) support the idea that LDL binds to decorin even in the presence of other PG in the arterial intima.

Taken together, the current finding that decorin links LDL with collagen type I, and the previous demonstrations that the amounts of both collagen (6) and decorin (18) are increased in arterial lesions, suggest that the collagen fibers in the lesions, when covered with decorin, are capable of retaining significant amounts of LDL.

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