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Glycosaminoglycan-lipoprotein interaction

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Glycosaminoglycans (GAGs) bound to various proteoglycans (PGs) present in the cardiovascular system have been proposed to perform a wide range of functions. These include conferring viscoelastic properties; interacting with and modulating growth factors and enzymes; and as receptors and co-receptors in lipoprotein metabolism. Binding of apoB-100 lipoproteins, particularly low density lipoproteins (LDL), to GAGs of extracellular matrix PGs in arteries has been proposed to be an initiating event in development of atherosclerosis. This study was initiated with the aim of getting an overview of the binding patterns of different lipoprotein subclasses with individual GAG categories. We thus evaluated the interaction of lipoproteins with GAGs commonly found in the cardiovascular system using a gel mobility-shift assay developed for this purpose. The same procedure was used to measure lipoproteins binding to metabolically [^{35}S]-labeled whole PGs prepared from three cell types, arterial smooth muscle cells, THP-1 macrophages and from HepG2 cells. The effect of GAG composition on PGs on lipoprotein binding was evaluated by enzymatic degradation of the carbohydrate chains. Heparan sulfate was found to bind beta very low density lipoproteins (β-VLDL) and a chylomicron remnant model (β-VLDL+apoE), but not LDL. Dermatan sulfate was found to bind LDL, but not β-VLDL or the chylomicron remnant model. Chondroitin sulfate and heparin were found to bind all lipoproteins tested (LDL, β-VLDL and β-VLDL+apoE) although with different affinities. We can conclude that each lipoprotein subclass tested binds a specific assortment of the GAGs tested. The observations made contribute to the understanding of new and complex mechanisms by which carbohydrate and lipid metabolism may be linked.

Keywords: **glycosaminoglycan, lipoprotein, atherosclerosis, proteoglycan, gel mobility-shift, LDL, VLDL**

Abbreviations: **GAG, glycosaminoglycan; PG, proteoglycan; LDL, low density lipoproteins; VLDL, very low density lipoproteins; EMEM, Eagles minimal essential medium; PMA, phorbol 12-myristate 13-acetate.**

Introduction

Proteoglycans (PGs) are important components of the extracellular environment of all tissues. Proteoglycans are proteins that possess glycosaminoglycans, GAGs, attached to specific serine residues flanked by glycines in the protein core. Glycosaminoglycans are long, unbranched, polycarbohydrates consisting of disaccharide units. Different classes of GAGs comprise different disaccharide repeating units. For example, chondroitin has a repeating unit of glucuronic acid and N-acetyl galactosamine, whereas heparan sulfate has repeating units of glucuronic acid and N-acetyl glucosamine. Contrary to the common notion that GAGs are monotonous, with a single repeating structural motif, we now know that these sugar polymers display vast structural diversity. For instance, heparan sulfate also contains significant amounts of iduronic acid (both non-sulfated and 2-O-sulfated) as well as up to 50% of N-sulfated glucosamine. Different N-acetyl/N-sulfate ratios are typically found for heparan sulfate and heparin. Chondroitin sulfate and dermatan sulfate are only N-acetylated. Discrete structural forms of GAGs generated by complex patterns of sulfation and epimerization are found in specific tissues. These complex patterns are also influenced by aging and disease [1,2]. Furthermore, the degree of structural diversity that is possible for GAGs is remarkable as there can be up to 16 steps involved in their biosynthesis [3–5]. Glycosaminoglycans are connected to the protein via a linkage tetra saccharide [GlucA-Gal-Gal-Xyl] that is unique in PGs. Also, within this region diversity arises from the substitution of sulfate and phosphate groups [6,7]. Developmental geneticists have been surprised to find in the last few years that genes that affect growth and morphogenesis often turn out to encode individual PGs or GAG biosynthetic enzymes [4,5]. This is probably a reflection of the fact that PGs serve as growth-factor co-receptors [8] as well as a reservoir for cytokines and growth factors [9].

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Proteoglycans are well-known components of extracellular matrices, including cartilage, basement membranes and connective tissue. However, they are also found in abundance on the cell surface. In the cardiovascular system each compartment has a specific set-up of PGs. Proteoglycan-lipoprotein interactions are mediated by electrostatic interactions between positively charged regions of apolipoproteins on the lipoprotein and negative charges, mainly from carboxyl- and sulfate groups on the GAG chains [10]. Thus, mainly heparan sulfate PGs on endothelial cells bind very low density lipoproteins (VLDL) and lipoprotein lipase in capillaries. Lipoprotein lipase digests VLDL, and free fatty acids are released to the circulation. Heparan sulfate PGs on liver cells facilitate binding and internalization of chylomicron remnant particles that are found in the circulation postprandially [11]. Lipoprotein lipase has also been proposed to function as an apolipoprotein that allows the accumulation of VLDL in the liver by binding both to the lipid moiety of VLDL and to heparan sulfate PGs [12]. In the extracellular matrix of the arterial wall mainly chondroitin sulfate PGs bind low density lipoproteins (LDL). However, the physiological significance of LDL binding in the arterial wall remains to be established. Deposition of LDL in arteries has been proposed to be an initiating factor in the development of atherosclerosis [13–15]. Decorin can bind small dense LDL via its GAG side chain and at the same time the core protein of decorin attaches to collagen fibers. As a consequence LDL becomes stuck in the matrix, and the lipid content in the vessel wall is enhanced [16].

Many aspects of lipoprotein-proteoglycan interaction are not fully understood and therefore there is a need for further studies. The distribution of individual PGs in different tissues and the classification of PGs are formidable tasks as they vary widely in size and number of glycosaminoglycan chains. A somewhat rational classification can be achieved by organizing related proteoglycans into families. A complicating factor is that the glycosaminoglycan composition on an individual proteoglycan may change depending on growth conditions or cell age [7,17]. We restricted this study to GAGs that are the most common in the cardiovascular system, i.e. chondroitin sulfate, dermatan sulfate and heparan sulfate. The binding of lipoproteins to each of the above GAGs was estimated individually. Previous studies included the immobilization of GAGs on agarose beads [18], precipitation of lipoprotein-glycosaminoglycan complexes [19] or ELISA-based assays [15].

An assay that has the obvious advantages that the binding can take place in solution and at near physiological salt concentration is the gel mobility-shift assay [17,20]. The gel mobility-shift assay was used here to estimate binding properties of LDL, β-VLDL, and β-VLDL+apoE (a chylomicron remnant model [11], Figure 1) to different commercially available GAGs. The assay was also used to estimate the binding of LDL to PGs synthesized by smooth muscle cells, by macrophages and by hepatic cells. This is a thorough study of GAG-lipoprotein interaction from a cardiovascular perspective with gel mobility-shift assay.

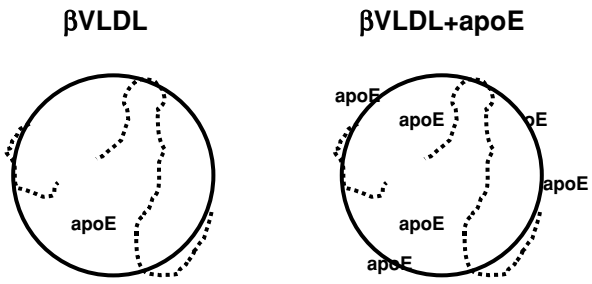

Figure 1. Simplified cartoon of β-VLDL and β-VLDL+apoE. The surface of the β-VLDL particle is covered by phospholipids and free cholesterol. Circumventing the particle and imbedded in the lipid core is one large apolipoprotein B molecule (hatched) per particle. In addition the β-VLDL particle contains apolipoproteins A and C (not shown) and apoE. The chylomicron remnant model used here, β-VLDL+apoE, is enriched in apoE.

Materials and methods

Materials

Chondroitin sulfate C (80% chondroitin-6-sulfate and 20% chondroitin 4-sulfate, from shark cartillage) and dermatan sulfate (chondroitin sulfate B, from pig skin) were purchased from Seikagaku Kogyo Co. (Tokyo, Japan). Heparin (from porcine intestinal mucosa) was purchased from Leo Pharmaceutical Products (Ballerup, Denmark). Papain, heparan sulfate (from bovine intestinal mucosa), chondroitinase ABC, chondroitinase AC, phorbol 12-myristate 13-acetate (PMA) and HEPES were from Sigma (St. Louis, MO). Heparitinase was from ICN (Aurora, OH). NuSieve 3:1 agarose was from FMC BioProducts (Rockland, ME). Disposable PD 10 columns (Sephadex G-25), HiTrap Q columns and [^{35}S]-sulfate were from Amersham Biosciences (Uppsala, Sweden). Trypsin and cell culture media were from Biowhittaker (Verviers, Belgium). Fetal bovine serum was from Biochrom KG (Berlin, Germany). Human blood serum was from young healthy donors. Human recombinant apolipoprotein E_3 ($apoE_3$) was purchased from PanVera (Madison, WI). All other chemicals were from Merck (Darmstadt, Germany).

Lipoproteins

LDL ($d = 1.019$ to 1.063 g/ml) was isolated from fresh human plasma by using differential ultracentrifugation. It was stored and its integrity was checked as described [21]. The LDL from two donors was pooled and stored up to two weeks at 4°C. β-VLDL from rabbits fed 1% cholesterol for 1–5 weeks was isolated and combined with $apoE_3$ as in [22]. Before use, potential free $apoE_3$ was removed from the β-VLDL+apoE complexes by size exclusion chromatography on PD 10 columns. A

simplified cartoon of β-VLDL versus β-VLDL+apoE is shown in Figure 1.

Cell culture

HepG2 cells and THP-1 cells were bought from American Type Culture Collection, ATCC, (Manassas, VA) and were subcultured by standard procedures. The HepG2 cells were grown in Eagles minimum essential medium (EMEM) with 10% (v/v) FBS with the addition of glutamine, sodium pyruvate and antibiotics. THP-1 cells were grown in suspension in RPMI 1640 supplemented with 10% FBS with the addition of glutamine, sodium pyruvate, antibiotics and 50 μmol/L β-mercaptoethanol. Differentiation was induced by exposing THP-1 cells to medium containing 0.16 μmol/L PMA (no β-mercaptoethanol). After 4 h incubation unattached cells were removed, and attached cells were incubated for 3 days (with medium containing 0.16 μmol/L PMA) to become fully differentiated macrophages before use. Untransformed mycoplasma-free human arterial smooth muscle cells were cultured as previously described [23].

Biolabeling of glycosaminoglycans

Biolabeling of proteoglycans from tissue culture was done as previously described [24,25]. Each cell type was kept in the appropriate medium as described above. [^{35}S]-sulfate labeled GAGs were obtained by exposing cultured cells to fresh medium with the addition of 67 μCi [^{35}S]-sulfate for 72 h before harvest. At harvest the cultures were in a sub-confluent state. Only GAGs synthesized during the 72 h incubation period were [^{35}S]-sulfate labeled with this procedure. Proteoglycan core proteins were not labeled. The culture medium from each incubation was used for PG preparation as described below.

Proteoglycan preparation

Secreted PGs were isolated from the culture medium. The medium was collected and Complete Mini protease inhibitors (Roche, Mannheim, Germany) were added. The sample was dialyzed against binding buffer containing 8 M UREA, 2 mM EDTA, 0.5% Triton X-100, and 20 mM Tris-HCl, pH 7.5, at 4°C during 48 h. A HiTrap Q (5 ml) column was equilibrated with binding buffer, and the sample was applied at 5 ml/min. The column was washed with 0.25 M NaCl to remove weakly bound glycoproteins and unincorporated [^{35}S]-sulfate. The remaining bound material was eluted with a linear salt gradient: 0.25–2 M NaCl in binding buffer. Fractions of 2.5 ml were collected. Total counts in each fraction were determined by liquid scintillation counting. The fractions containing proteoglycans were pooled and dialyzed against H_2O at 4°C, lyophilized, and then dissolved in a small volume of H_2O and stored at −20°C until use.

Glycosaminoglycan analysis

Digestion and electrophoresis of glycosaminoglycans were carried out as previously described [26]. In brief, isolated [^{35}S]-sulfate biolabeled PGs were treated with papain (5 IU/mg PG protein) at 65°C for 4 h in order to completely digest PG core proteins. Liberated GAGs were precipitated in 2 volumes of 95% EtOH, pelleted by centrifugation (3500 rpm, 15 min.), and dried in *vacuo*. Dried GAGs were dissolved in 20 mM Tris-HCl, pH 7.4, 0.15 M NaCl, divided and subjected to treatment with 50 U/l chondoitinase ABC, AC, or no enzyme at 37°C overnight. After treatment with these lyases, all samples were supplemented with $CaCl_2$ to 10 μM and split in halves. One half was subjected to treatment with 10 U/l heparitinase I at 37°C overnight. After the final lyase treatment, the GAGs were recovered after EtOH precipitation, dried as before and dissolved in electrophoresis buffer. The GAG preparations were fractionated by electrophoresis in 0.5% (w/v) agarose in 0.05 M 1,3-diaminopropane acetate buffer, pH 9.0, at 150 V for 1 h. Gels were fixed with 0.1% cetylpyridinium chloride in water for 2 h. The dried gels were exposed to Fuji X-ray film. Bands on autoradiograms were identified by comparison with stained bands of simultaneously electrophoresed chondroitin sulfate, dermatan sulfate, and heparan sulfate references.

Gel mobility shift assay

Gel mobility shift experiments to evaluate proteoglycan-lipoprotein interaction were performed as reported [20]. In brief the same quantities of biolabeled PGs, based on equivalent [^{35}S]-sulfate content or GAG content (1.6 μg/ well was used), were gently mixed with increasing amounts of lipoproteins in a buffer containing 10 mM HEPES, pH 7.4, 140 mM NaCl, 5 mM $CaCl_2$, 2 mM $MgSO_4$ in 1.5 ml Eppendorf tubes. The mixing was accomplished by inverting the tube several times, and the mixture was incubated for 1 h at room temperature. It is important not to vortex the mixture as that may cause aggregation of lipoproteins. 1–2 mm thick 0.7% agarose gels were cast on the hydrophilic side of gel-bond film (FMC BioProducts, Rockland, ME) in 10 mM HEPES, pH 7.2, 2 mM $CaCl_2$, 4 mM $MgSO_4$. A gelcomb was used to make wells. The samples in 10% glycerol were loaded on the gel. Electrophoresis was performed in a horizontal electrophoresis system (Bio-Rad, Hercules, CA) submerged under buffer (10 mM HEPES, pH 7.2, 2 mM $CaCl_2$, 4 mM $MgSO_4$) at 60 V. In the first ten minutes, no circulation was used (in order to let the samples enter the gel). Then re-circulation of the buffer and cooling of the circulating buffer was used. Electrophoresis continued for two hours. Then the gels were fixed for 2 h in 0.1% cetylpyridinium chloride dissolved in 70% EtOH [27] and air dried. Evaluation of the gels was performed by autoradiography or by staining the gels for glycosaminoglycans (toluidine blue). Densitometric evaluations were carried out with Bio-Rad Molecular Imager system and evaluated with the Quantity One program software (Bio-Rad, Hercules, CA). Graph Pad Prism software (San

Diego, CA) was used to fit one site hyperbola binding curves to the data.

Results

The binding of lipoproteins (LDL, β-VLDL, and β-VLDL+apoE) to commercially available chondroitin sulfate, dermatan sulfate, heparan sulfate and heparin was applied to gel mobility-shift assay. The results were evaluated by staining the gels for GAGs (toluidine blue) and scanning the bands (densitometry) (Figure 2a). The sensitivity of the toluidine blue staining of the GAGs was a limiting factor in the assay and a contributor to variation between duplicate samples (Figure 2). When metabolically [^{35}S]-labeled material (intact PGs) and autoradiography were used instead of toluidine blue staining, the variation between duplicate samples was generally smaller (Figure 3). It was also observed that heparan sulfate and dermatan sulfate were stained less intensively than heparin and chondroitin sulfate by toluidine blue. A summary of the binding affinity estimates achieved with the gel mobility-shift assay and toluidine blue staining (Figure 2) is given in Table 1. Under the experimental conditions heparan sulfate did not bind LDL (Figure 2b). Dermatan sulfate bound LDL to some extent, however a fit of the one-site binding curve to the data was unobtainable. Heparin and chondroitin sulfate both bound LDL in a saturable manner. The dissociation constant, K_D, for heparin binding of LDL was 271 $\pm$ 187 nM and for chondroitin sulfate binding of LDL 342 $\pm$ 206 nM. Heparan sulfate and heparin both appeared to bind β-VLDL in a saturable manner, with similar affinity. Chondroitin sulfate appeared to bind little β-VLDL (Figure 2c) but saturable binding in the concentration range used could not be clearly shown. Dermatan sulfate did not bind β-VLDL. Chondroitin sulfate, heparan sulfate and heparin all appeared to bind the chylomicron remnant model

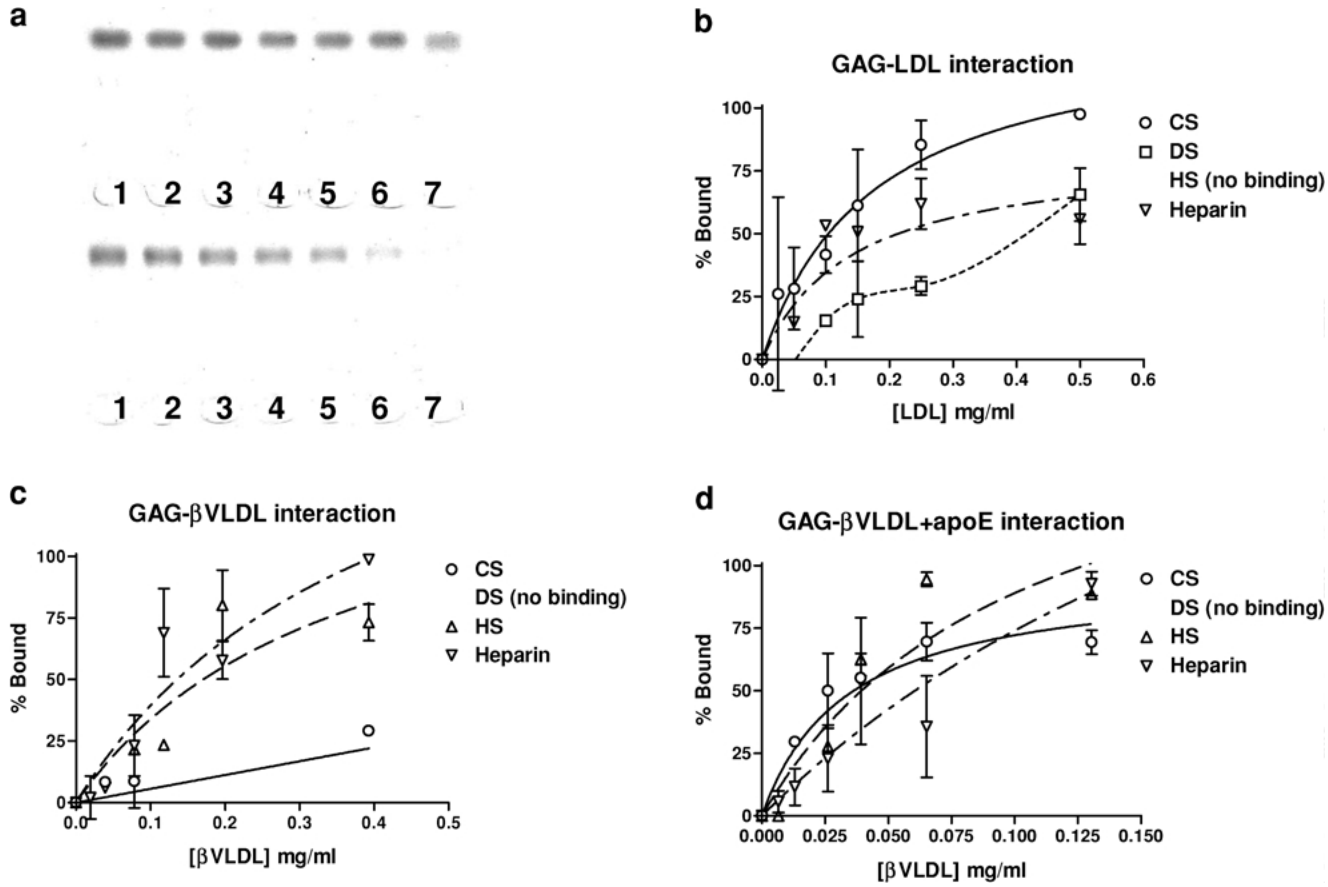

Figure 2. Binding of lipoproteins to glycosaminoglycans. Panel a: LDL in the concentrations 0 (lanes 1), 0.025 (lanes 2), 0.05 (lanes 3), 0.10 (lanes 4), 0.15 (lanes 5), 0.25 (lanes 6) and 0.5 (lanes 7) mg/ml were incubated with dermatan sulfate (upper part) or chondroitin sulfate (lower part), electrophoresed (upwards in the figure), stained and developed as described in methods section. A single run is shown. Data from duplicate samples were used to construct the corresponding binding isotherms in panel b. The same types of experimental data were used to compare binding affinities for chondroitin sulfate (CS, circle, solid line), dermatan sulfate (DS, square, short hatched), heparan sulfate (HS, pyramid, long hatched) and heparin (triangle, short-long hatched) to LDL (panel b), β-VLDL (panel c) and β-VLDL+apoE (panel d). Please note the difference in scale on the abscissa between panels b, c and d. Absence of symbols indicate that the average value was less than zero. Where applicable binding isotherms were calculated and are listed in Table 1. Each experiment was repeated two or more times with consistent results.

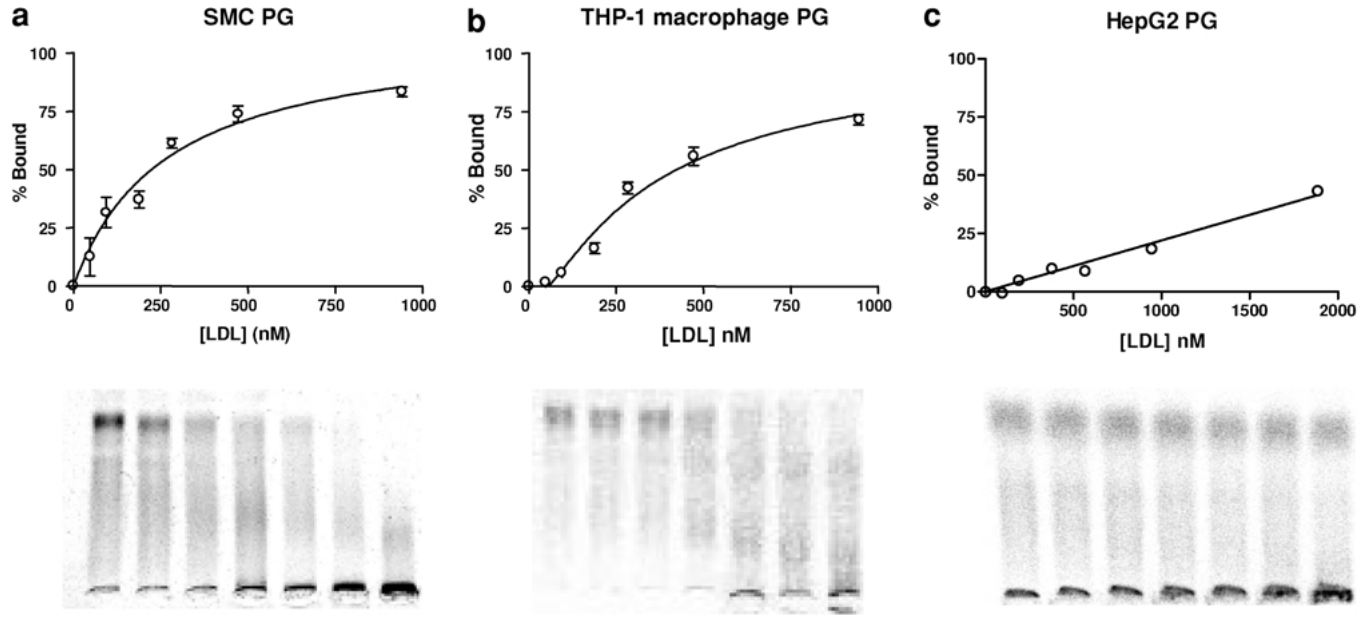

Figure 3. Binding of LDL to whole proteoglycans. The indicated amounts of LDL were incubated with [^{35}S]-labeled proteoglycans isolated from smooth muscle cells, SMC, (panel a), THP-1 macrophages (panel b) and HepG2 cells (panel c). The electrophoretic mobilities of the proteoglycan-LDL mixtures were evaluated by electrophoresis and autoradiography. The average of duplicate determinations ± range is plotted in panels a and b. Results from a single determination are plotted in panel c. Autoradiography pictures of gel mobility-shift of single samples where the amount of added LDL increases from left to right are displayed below each graph. Each experiment was repeated two or more times with consistent results. Please note the difference in scale on the abscissa in panel c.

(β-VLDL+apoE) (Figure 2d). Dermatan sulfate did not bind β-VLDL+apoE. The affinities of heparan sulfate and heparin for β-VLDL+apoE were 2–4 times higher than for β-VLDL alone (Table 1). The total binding of β-VLDL and β-VLDL+apoE to heparan sulfate was similar. The same was true for heparin. Chondroitin sulfate bound β-VLDL+apoE in a saturable manner and appeared to have the highest affinity for β-VLDL+apoE of the glycosaminoglycans tested (Figure 2d). It should be noted that the binding of chondroitin sulfate to β-VLDL was not pronounced (Figure 2c).

Metabolically [^{35}S]-labeled preparations of PGs secreted from three cell types, smooth muscle cells, THP-1 macrophages and HepG2 cells were isolated. These cell types are interesting from a cardiovascular perspective. Smooth muscle cells synthesize and secrete the majority of the extracellular matrix proteins including PGs that are present in the arterial wall [28]. Foam cells that are present in atherosclerotic plaques originate from macrophages that have accumulated massive amounts of lipid [29]. Also macrophages secrete substantial amounts of PGs [30]. Hepatic cells control lipoprotein metabolism by synthesis of VLDL and uptake of all lipoprotein subclasses, at least in part, through pathways involving PGs [11]. All three cell types were non-confluent and were added an equal amount of [^{35}S]-sulfate radioactivity and metabolically labeled for the same period of time. The amount of [^{35}S]-labeled PGs received from each cell type with this approach could be expected to

Table 1. Estimated binding affinities for lipoprotein-GAG interaction. Abbreviations as in Figure 1

	LDL		*β-VLDL*		*Chylomicron*	
	K_D *(nM)*	B_t *(nmol)*	K_a *(mg/ml)*	B_t *(mg)*	K_a *(mg/ml)*	B_t *(mg)*
CS	342	134	Unable to calculate		0.034	97
DS	Unable to calculate		No binding		No binding	
HS	No binding		0.36	154	0.10	181
Heparin	271	83	0.42	205	0.28	281

KD = dissociation constant, Bt = total binding, Ka = apparent dissociation constant: Where the binding was low a fit of saturable binding curves to the data was unobtainable.

vary. However cell type-specific differences in GAG composition and size, should be clear.

The resulting qualitatively different PG preparations were tested for binding with LDL. Please note that here it was the binding to intact PGs that was evaluated and that for each cell-type it would be a mixture of whatever GAG-containing PGs it secreted. Proteoglycans secreted from smooth muscle cells and from THP-1 macrophages both interacted readily with LDL and displayed affinities in a similar range (Figure 3a, $K_D = 268 \pm 52$ nM and Figure 3b, $K_D = 181 \pm 141$ nM). Proteoglycans secreted from HepG2 cells on the other hand displayed diffuse and non-saturable binding to LDL in the concentration range used (Figure 3c). It becomes more difficult in practice to increase the LDL concentration beyond 2000 nM, and we conclude that the affinity of proteoglycans secreted from HepG2 cells for LDL is low.

In a previous study the GAG composition of smooth muscle cell PGs, isolated under identical conditions from the same source that was used here, was determined by enzymatic digestion, agarose electrophoresis and autoradiography [26]. Secreted PGs from smooth muscle cells were found to contain about 80% chondroitin sulfate (digestable both with chondroitinase AC and chondroitinase ABC), only 6% dermatan sulfate (resistant to chondroitinase AC, but digestable with chondroitinase ABC) and 14% heparan sulfate (digestable with heparitinase, not sensitive to chondroitinases). Similar results were obtained with the [^{35}S]-sulfate labeled smooth muscle cells PGs used here (Figure 4a). The same analyses gave results in the same range for PGs secreted from THP-1 macrophages (Figure 4b). They were estimated to contain about 87% chondroitin sulfate, 8% dermatan sulfate and only 5% heparan sulfate. Proteoglycans secreted from HepG2 cells appeared to contain almost exclusively heparan sulfate and only trace amounts of chondroitin sulfate or dermatan sulfate (Figure 4c).

Discussion

Previous studies indicated differences in affinities of different lipoprotein subclasses for GAGs. However these studies were mainly done with one type of GAG [11,31,32] or included severe methodological drawbacks such as interaction under non-physiological (low salt) conditions [18,31,32]. To our knowledge no comparison has been made of VLDL, VLDL remnants and LDL binding to GAGs and PGs from vascular cells. We used rabbit β-VLDL pre-incubated with recombinant human apoE$_3$ as a model for chylomicron remnants (Figure 1). This is an established model for remnant particles [11,33]. Since LDL only contains one protein (apolipoprotein B-100) we were able to calculate dissociation constants for the binding isotherms in Figure 2b (Table 1) and Figure 3. Beta-VLDL contains several apolipoproteins; therefore we were restricted to calculating apparent dissociation constants for the binding isotherms in Figure 2c and d (Table 1). It should be emphasized that the structure of individual GAGs and their binding properties may widely differ depending on animal or tissue source. All commercial GAG preparations used in this study were of non-human origin.

This study focuses on the interaction of GAGs with lipoproteins. *In vivo* of course the GAGs are part of intact PGs. Thus for instance the intact PGs isolated from different cellular sources and investigated in Figure 3 for each cell type constitute a mixture of different PGs. The actual mixture of whole PGs in each PG preparation in Figure 3 and the potential for interaction also of the core proteins with lipoproteins are aspects that were left out here. This is a limitation.

We found that PGs secreted from smooth muscle cells or from THP-1 macrophages that both contained chondroitin sulfate as their main GAG constituent (Figure 4a and b) appeared to bind LDL in a saturable manner (Figure 3a and b). This is consistent with the finding that commercially available chondroitin sulfate also binds LDL in a saturable manner (Figure 2b).

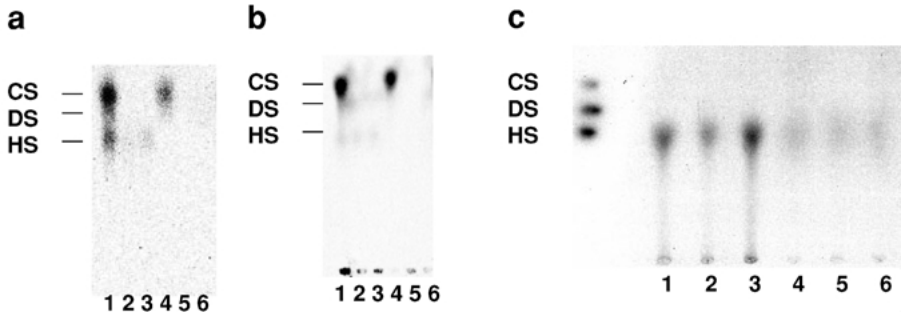

Figure 4. Gel electrophoresis of biolabeled glycosaminoglycans prepared by papain digestion of proteoglycans secreted by smooth muscle cells (panel a), THP-1 macrophages (panel b) and HepG2 cells (panel c). Autoradiographs of agarose gels of glycosaminoglycans incubated in buffer (lanes 1), in chondroitinase AC (lanes 2), in chondroitinase ABC (lanes 3), in heparitinase (lanes 4), in chondroitinase AC and heparitinase (lanes 5) and in chondroitinase ABC and heparitinase (lanes 6) are shown. The electrophoretic mobilities of chondroitin sulfate (CS), dermatan sulfate (DS) and heparan sulfate (HS) are indicated to the left in each panel. In panel c samples were applied in every second well. Enzymatic digestion of the proteoglycan preparations used in Figure 3a, b and c are shown.

In fact, the affinities estimated for LDL binding to commercially available chondroitin sulfate ($K_D = 342$ nM), to PGs secreted from smooth muscle cells ($K_D = 268$ nM) or from THP-1 macrophages ($K_D = 181$ nM) are all in a similar range. Analogously PGs secreted from HepG2 cells that appeared to contain almost only heparan sulfate and only trace amounts of other GAGs (Figure 4c) did not bind LDL in a saturable manner, and the affinity for LDL appeared to be low (Figure 3c). This is consistent with the lack of affinity for LDL found with commercially available heparan sulfate (Figure 2b). The estimated total binding of β-VLDL and the chylomicron model (β-VLDL+apoE) to heparan sulfate and heparin remained similar despite higher affinity for β-VLDL+apoE. This indicates that the increased charge density in the chylomicron model (versus VLDL alone) may increase the affinity to a limited number of binding sites for β-VLDL that are available on heparan sulfate and heparin. The results obtained are an indication that local variation in GAG composition could have profound effects on interactions with individual lipoprotein compartments.

In Figure 4c, lanes 2 and 3 seemed to indicate that digestion of PGs secreted from HepG2 cells with chondroitinase AC actually decreased the intensity of the GAG band (heparan sulfate) while chodroitinase ABC digestion did not. We have no explanation for this paradox. It could be an error inherent to the method, but it does not affect our conclusion from Figure 4c; inability to detect substantial amounts of chondroitin sulfate or dermatan sulfate in PGs secreted by HepG2 cells.

The extra apoE in the chylomicron model (β-VLDL+apoE, Figure 1) appears to confer new binding sites (detectable binding) for structural components of chondroitin sulfate whose affinity for VLDL without additional apoE is low. The physiological significance in the binding of remnant particles to chondroitin sulfate remains elusive. Chondroitin sulfate appears mainly to be present in tissues that are not exposed to postprandial bursts of large lipoproteins (VLDL and chylomicron remnants). Blood vessels are lined with endothelial cells that are covered with heparan sulfate proteoglycans, and liver appears to contain mainly heparan sulfate proteoglycans (Figure 4c). Mainly LDL (and HDL) are small enough to penetrate the endothelial monolayer and be exposed to chondroitin sulfate-rich proteoglycans in the arterial intima [34–36]. Therefore it is possible that large lipoproteins (VLDL and chylomicron remnants) do not normally interact with extracellular matrix rich in chondroitin sulfate. Such exposure could be envisioned only after endothelial denudation.

The fact that heparan sulfate did not bind LDL in the gel mobility shift assay but bound β-VLDL and the chylomicron remnant model (β-VLDL + apoE) with fairly high affinity (Figure 1, Table 1) is interesting. Heparan sulfate on endothelial cells interacts with β-VLDL (for lipolysis) and lets LDL pass by in the circulation. Heparin contains additional structural components that allows it to bind LDL. However, heparin as such is not a prominent intima component except in mast cells [37]. In contrast, the liver appears equipped to capture β-VLDL and remnant particles rapidly, and heparan sulfate proteoglycans in the liver have been proposed to play a role in the sequestering of remnant particles [11,38]. Low density lipoproteins on the other hand are probably allowed to circulate until they are eventually taken up by the LDL receptor in liver and in other tissues.

We have made several proteoglycan preparations from the smooth muscle cells used in this study and from THP-1 macrophages. The composition of secreted proteoglycans are mainly in the range described ([26], Figure 4a and b), which is 80% or more chondroitin sulfate content. In a previous study we observed that addition of non-esterified fatty acids to the culture medium increased the content of mRNA for selected PGs and inreased the chain length of GAGs in smooth muscle cells [25]. This resulted in increased capacity of secreted proteoglycans to bind LDL. Infusion of fatty acids into muscle was proposed to increase the flux of carbohydrate through the hexosamine pathway [39]. The end products in the hexosamine pathway, UDP-GlucNAc and UDP-GalNAc, are building blocks for the synthesis of GAG chains of PGs. Thus in muscle, at least in theory, lipid uptake may affect GAG synthesis.

Proteoglycans from HepG2 cells show a greater variability than the experimental smooth muscle cell cultures. We have isolated secreted proteoglycans from HepG2 cells with from almost 100% heparan sulfate (Figure 3c) down to about 30% heparan sulfate [40]. We do not know what factors determine the composition of the proteoglycans produced by HepG2 cells, but we suspect that factors such as the density of cells and time in culture could be important. HepG2 cells are known, under certain growth conditions, to switch from an embryonic state, where they produce fetuin as their major protein, to an adult-like state where albumin is the major secreted protein [41,42]. Such transitions could be expected to be accompanied by changes in the GAG composition of the PGs produced as PGs are important in growth and in embryonic development. Murine liver proteoglycans appear to contain more dermatan sulfate than proteoglycans from HepG2 cells (human hepatoma) [40]. We previously found that addition of insulin to the culture medium increased the chondroitin sulfate content of proteoglycans secreted from HepG2 cells and induced changes that resulted in a decreased affinity for chylomicron remnant particles [40]. This was consistent with alterations found in liver proteoglycans isolated from hyperinsulinemic Zucker rats versus healthy controls [40]. The condition may through impaired remnant clearance contribute to the dyslipidemia of insulin resistance and type 2 diabetes. These observations and the results presented here illustrate how carbohydrate and lipid metabolism may be linked in a very complex spider web through multiple secretory pathways.

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