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Review

Cell surface heparan sulfate proteoglycans and lipoprotein metabolism

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Abstract. Cell surface heparan sulfate proteoglycans are involved in several aspects of the lipoprotein metabolism. Most of the biological activities of these proteoglycans are mediated via interactions of their heparan sulfate moieties with various protein ligands, including lipoproteins and lipases. The binding of lipoproteins to heparan sulfate is largely determined by their apoprotein composition, and apoproteins B and E display the highest affinity for heparan sulfate. Interactions of lipoproteins with heparan sulfate are important for the cellular uptake and turnover of lipoproteins, in part by enhancing the accessibility of lipoproteins to lipoprotein receptors and lipases. Apoprotein B may interact with receptors without involving heparan sulfate. Heparan sulfate has been further implicated in presentation and stabilization of lipoprotein lipase and hepatic lipase on cell surfaces and in the transport of lipoprotein lipase from extravascular cells to the luminal surface of the endothelia. In atherosclerosis, heparan sulfate is intimately involved in several events important to the pathophysiology of the disease. Heparan sulfate thus binds and regulates the activity of growth factors, cytokines, superoxide dismutase and antithrombin, which contribute to aberrant cell proliferation, migration and matrix production, scavenging of reactive oxygen radicals and thrombosis. In this review we discuss the various roles of heparan sulfate proteoglycans in vascular biology, with emphasis on interactions of heparan sulfate with lipoproteins and lipases and the molecular basis of such interactions.

Key words. Heparan sulfate; lipoproteins; atherosclerosis; lipases; apoproteins; proteoglycans.

Introduction

Proteoglycans are expressed by virtually all mammalian cells and are found on cell surfaces, in the extracellular matrix and in intracellular granules [1]. The unique structural features of proteoglycans endow them with properties that may influence a wide range of biological processes. Hence, proteoglycans are of prime importance in physiological processes such as embryonic development and tissue repair, regulation of blood coagulation, cartilage function and glomerular filtration [1–4]. Several disease conditions such as cardiovascular diseases [5], cancer [6], inflammation [7], diabetes [8] and amyloidosis [9] are associated with changes in the expression of proteoglycans as well as with structural and functional alterations of their glycosaminoglycan components.

Proteoglycans have been implicated in multiple aspects of atherosclerotic disease [5]. They are involved in the clearance and metabolism of circulating lipoproteins, regulation of growth factor and cytokine action, and organization of the extracellular matrix. Proteoglycans

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are hereby linked to oxidative damage of endothelium, recruitment of monocytes and macrophages to the vascular wall, modification of lipoproteins, lipid uptake by resident macrophages, aberrant smooth muscle cell proliferation and migration, and pathological matrix production [10, 11]. Proteoglycans further promote the action of anticoagulant proteins such as antithrombin and heparin cofactor II [12], which counteract the thrombogenic predisposition of advanced atherosclerosis.

This review will mainly focus on the interactions of cell surface heparan sulfate proteoglycans with proteins important in the metabolism of lipoproteins. To illustrate the role of specific heparan sulfate-protein interactions in vascular biology, we also briefly review the activities of heparan sulfate in antithrombin binding and fibroblast growth factor (FGF) signaling. Other aspects on the role of proteoglycans in atherosclerosis have been extensively reviewed [10, 11, 13–16].

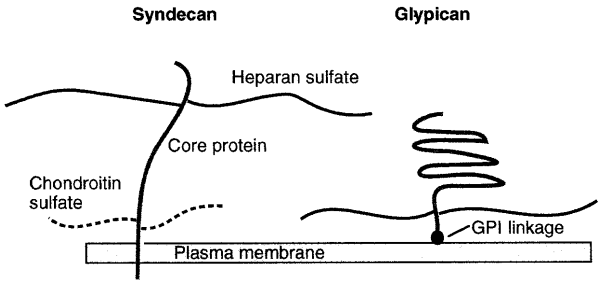

Figure 1. Syndecan and glypican families of cell surface heparan sulfate proteoglycans. Syndecans have transmembrane core proteins, whereas glypicans are bound to the cell surface via a GPI linkage. The heparan sulfate chains in syndecan are typically found at the N-terminal end of the core protein. The proximal glycosaminoglycan attachment sites, found in syndecans-1 and -3, may also bear chondroitin sulfate chains. In glypican, the heparan sulfate chains are located close to the plasma membrane, proximal to the globular part of the core protein.

Cell surface heparan sulfate proteoglycans

Proteoglycans are glycoproteins carrying one or more covalently attached glycosaminoglycan chain [1]. They are classified on the basis of the core protein primary structures that define core protein families (table 1). The major cell surface heparan sulfate proteoglycan families [1, 17] are syndecans and glypicans [fig. 1]. Additional cell surface proteins that can be substituted with heparan sulfate chains include betaglycan [18], also known as type III transforming growth factor β (TGF-β) receptor, and the hyaluronan receptor CD44 [19] (table 1). However, these 'part-time' proteoglycans may also be expressed without glycanation.

Table 1. Proteoglycan families.

Cell surface proteoglycans
Integral membrane types
Syndecans 1, 2, 3 and 4, betaglycan, CD44, CSF-1, thrombomodulin, invariant chain, NG-2
GPI-linked
Glypicans 1, 2, 3, 4, 5 and 6
Extracellular proteoglycans
Aggrecan family
Aggrecan, versican, neurocan, brevican
Small leucine-rich proteoglycan family
Biglycan, decorin, lumican, fibromodulin, collagen IX
Basement membrane proteoglycans
Perlecan, agrin
Intracellular proteoglycans
Serglycin

Syndecans

The syndecan family comprises four integral membrane proteins, named syndecans 1–4, that are characterized by homologous cytoplasmic and transmembrane domains [3, 4, 20]. The various syndecan species have different, but partially overlapping patterns of expression [3, 4]. The C-terminal cytoplasmic domains of syndecans contain two regions that are conserved between the different syndecans. The conserved regions are separated by variable domains that are heterologous between syndecans 1 and 4, but conserved within a certain syndecan type between different animal species. The syndecan cytoplasmic domains have been shown to interact with intracellular signaling molecules. They also contain tyrosine and threonine residues subject to phosphorylation (reviewed in [21]). These activities may require clustering of syndecans, induced by binding to extracellular ligands. The cytoplasmic domains interact with intracellular molecules such as protein kinase C, phosphatidylinositol 4,5-diphosphate, Src family kinases, cortactin and tubulin. The C-terminal region of the cytoplasmic domain contains an EFYA domain that has been shown to bind syntenin and CASK, PDZ domain-containing proteins that presumably link syndecans to the cytoskeleton. Different members of the syndecan family differ in their intracellular interactions and phosphorylation; for comprehensive reviews on the syndecans and intracellular signaling see [20–22]. The transmembrane domains provide the membrane attachment and mediate syndecan oligomerization [23]. The extracellular ectodomains lack disulfide bridges and are thought to be highly extended. The ectodomain sequences are highly divergent between the different syndecan species except for homology in the

glycosaminoglycan attaching regions. In syndecans-1 and -3, such regions are present both close to the plasma membrane and at the N-terminus, whereas syndecans-2 and -4 display only the proximal glycosaminoglycan attachment sites. The ectodomains are readily cleaved at protease-sensitive sites close to the plasma membrane, resulting in extracellular release of the glycosaminoglycan-containing ectodomain portion. The ectodomain of syndecan-4 also contains a region that can support cell attachment [24].

Glypicans

The glypicans [17, 25], found in at least six isoforms, are linked to the plasma membrane by a glycosyl phosphatidyl inositol (GPI) linkage that is cleavable with GPI-specific phospholipase C. Glypican core proteins are highly globular due to multiple disulfide bridges. In glypicans, the heparan sulfate chains mainly reside in the nonglobular domain close to the membrane attachment site. The different core protein structures, including the localizations of the glycosaminoglycan chains, suggest different functions for syndecans and glypicans.

Biosynthesis and structure of heparan sulfate

The biosynthesis of heparan sulfate [for reviews see 1, 26, 27] and other sulfated glycosaminoglycans occurs in the Golgi apparatus. Heparan sulfate polymerization involves transfer of alternating glucuronic acid (GlcA) and N-acetylglucosamine (GlcNAc) residues to a primer sequence GlcNAc-GlcA-Gal-Gal-Xyl, attached to a serine residue of a heparan sulfate proteoglycan core protein. The resultant $(\text{-GlcA}\beta 1,4\text{-GlcNAc}\alpha 1,4\text{-})_n$ polymer is subsequently modified by a series of reactions, in which the product of a preceding reaction generally serves as a substrate for the next reaction. The first modification step, N-deacetylation/N-sulfation of GlcNAc units, occurs in a regioselective fashion and results in the domain structure characteristic of heparan sulfate. The three domain types found in heparan sulfate polymers are (i) domains of consecutive N-sulfated disaccharide units (NS domains), (ii) domains of alternating N-acetylated and N-sulfated disaccharide units (NA/NS domains) and (iii) unmodified sequences of N-acetylated disaccharide units (NA domains) [27, 28]. The N-deacetylation/N-sulfation of GlcNAc residues is a prerequisite for the further modification reactions, which all occur in the vicinity of previously incorporated N-sulfate groups. The GlcA units are in part converted to iduronic acid (IdoA) units by C5 epimerization, and some of the IdoA residues undergo sulfation at the C2 position. Although the IdoA formation occurs in both NS and NA/NS domains, the 2-O-sulfate groups are almost exclusively confined to the former domain type. By contrast, the other main O-sulfate substituent of heparan sulfate, at C6 of GlcN residues, is found in both NS and NA/NS domains. Some heparan sulfate species are rich in 'unusual' sulfated saccharide units. For example, heparan sulfate from human cerebral cortex is abundant in 2-O-sulfated GlcA units [29], whereas endothelial cells express heparan sulfate with 3-O-sulfated $GlcNSO_3$ residues that are important for the anticoagulant activity of heparan sulfate [30].

Other glycosaminoglycans

The polymerization product of chondroitin sulfate biosynthesis is $(\text{Glc}\beta 1,3\text{-GalNAc}\beta 1,4\text{-})_n$. C5 epimerization of the GlcA residues may occur; galactosaminoglycan polymers containing IdoA residues are termed dermatan sulfate. The GalN units of these glycosaminoglycans are invariably N-acetylated. O-sulfation occurs at C4 and C6 of the GalNAc residues and also, less frequently, at C2 of the IdoA units in dermatan sulfate. Keratan sulfate differs from the heparan sulfate and chondroitin sulfate families as it lacks a hexuronic acid component but instead contains repeating $\text{-Gal}\beta 1,4\text{-GlcNAc}\beta 1,3\text{-}$ units. Both the Gal and GlcNAc units may be subject to O-sulfation at C6. Hyaluronan is a polymer of $\text{-GlcA}\beta 1,3\text{-GlcNAc}\beta 1,4\text{-}$ units. Hyaluronan is thus composed of the same sugar units as the initial polymerization product of heparan sulfate biosynthesis, but the two polymers differ with regard to the linkage between the GlcA and GlcNAc residues. Moreover, hyaluronan is not modified by epimerization or sulfation. Hyaluronan is not coupled to core proteins but occurs as a free glycosaminoglycan that is synthesized by hyaluronan synthetases suggested to be located in the plasma membrane.

Heparan sulfate-protein interactions

Most of the biological activities attributed to heparan sulfate proteoglycans involve interactions between their heparan sulfate moieties and various extracellular protein ligands. Electrostatic interactions between the negatively charged sulfate and carboxyl groups of heparan sulfate and positively charged amino acid residues in the protein component are important for these binding phenomena [26, 31]. The importance of sulfation is readily demonstrated by binding analyses with chemically desulfated heparin preparations [32, 33] and studies on heparan sulfate-dependent activities in cells treated with sodium chlorate [34]. The latter treatment inhibits cellular sulfation reactions by interfering with the formation of the sulfate donor 3′-phosphoadenosine-5′-phosphosulfate [35]. More recently, it has

Table 2. Examples of proteins that interact with heparan sulfate.

Lipoprotein lipase
Hepatic lipase
Apolipoprotein B
Apolipoprotein E
Antithrombin
Tissue plasminogen activator
Platelet derived growth factors
Fibroblast growth factors
Chemokines
Selectins
Superoxide dismutase

The table presents some heparan sulfate-binding proteins involved in the metabolism of lipoproteins and vascular biology in general.

been shown that mutations in heparan sulfate sulfotransferase genes result in severe developmental defects in the fruit fly *Drosophila melanogaster* and in mouse [36, 37].

In addition to the sulfation pattern of individual protein-binding heparan sulfate domains, factors such as the positioning of domains along the heparan sulfate polymer and the number and location of heparan sulfate chains on the core protein [38] affect the interaction properties of a proteoglycan molecule. The amount of a particular proteoglycan on the cell surface depends on its level of expression and turnover.

Most protein-binding heparan sulfate domains identified so far are relatively short sequences, 5–10 monosaccharide units in length, that reside in the NS domains of the polysaccharide [26]. However, some multimeric proteins such as interleukin 8 [39] and interferon-γ [40] bind to more complex domains, encompassing two NS sequences separated by a poorly sulfated heparan sulfate region. The protein-binding properties of the NA/NS domains, comprised of alternating N-acetylated and N-sulfated disaccharide units, are not well understood due to the lack of methods for purifying this type of heparan sulfate sequence.

A number of proteins that are important in the metabolism of lipoproteins and vascular biology are known to bind heparan sulfate (table 2), but the saccharide domains mediating the interactions have been characterized in detail only in few cases. The most thoroughly characterized protein-binding heparan sul-

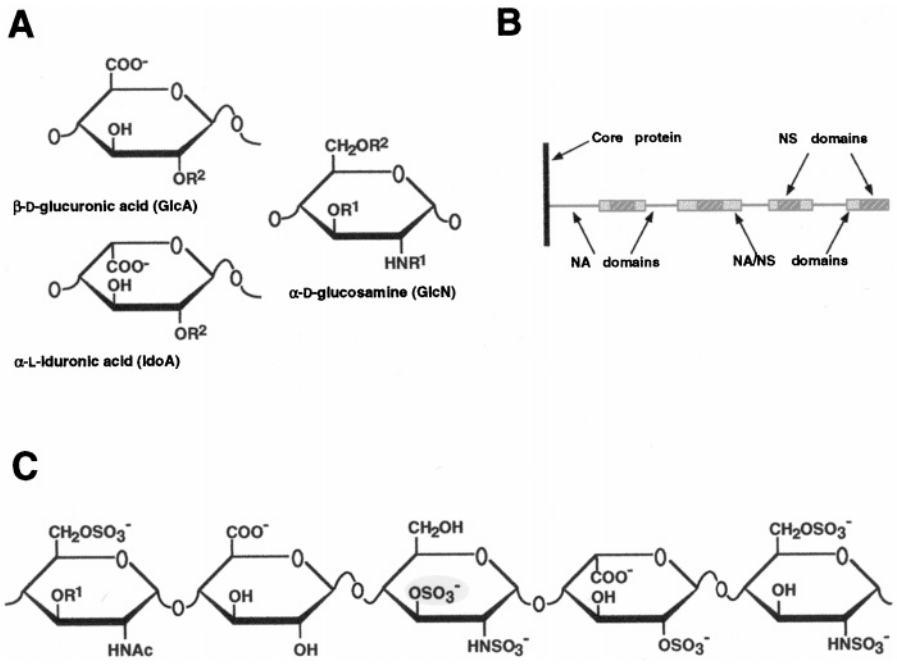

Figure 2. Structure of heparan sulfate. (*A*) Heparan sulfate is composed of disaccharide units in which GlcA, or its C5 epimer IdoA, is linked to a GlcN residue. Modifications of the initial GlcA-GlcNAc structure occur in positions indicated by R^1 (-$COCH_3$ or SO_3^-) and R^2 (-H or -SO_3^-). (*B*) Domain structure of heparan sulfate. NS domains represent sequences of consecutive N-sulfated disaccharide units, rich in IdoA residues and O-sulfate groups. NA/NS domains are composed of alternating N-acetylated and N-sulfated disaccharide units, which contain both GlcA and IdoA residues and O-sulfate groups at C6 of the GlcN residues. NA domains are largely unmodified sequences with repeating GlcA-GlcNAc disaccharide units. (*C*) Structure of the antithrombin-binding heparin/heparan sulfate pentasaccharide. The 3-O-sulfate group essential for the interaction is located in the center GlcN unit.

fate domain is the sequence interacting with antithrombin [12]. Antithrombin inhibits thrombin and several other serine proteases of the coagulation pathway, including factors IXa, Xa, XIa and XIIa. The ability of antithrombin to inhibit thrombin is greatly accelerated by heparin, which is the basis for its use as an anticoagulant drug in the clinic. The binding of heparin to antithrombin is mediated by a specific pentasaccharide sequence, hallmarked by an essential 3-O-sulfated $GlcNSO_3$ unit (fig. 2C). The pentasaccharide sequence alone is insufficient for the full thrombin-inhibiting activity of heparin, which requires instead an oligosaccharide encompassing 18 monosaccharide units. The active saccharide contains the antithrombin binding sequence and a flanking sequence that binds thrombin and thereby approximates it with antithrombin. The pentasaccharide alone is, however, able to induce a conformational change in antithrombin, increasing the inhibiting activity toward serine proteases such as factor Xa.

The antithrombin binding sequence is found in $\sim 30\%$ of the chains in commercially available heparin preparations. In endothelial cells, only a minor portion ($\sim 5\%$) of the heparan sulfate proteoglycans bind to antithrombin with high affinity [30, 41]. The factors that regulate the expression of anticoagulantly active heparan sulfate in endothelial cells are not known, but it has been shown that in the F9 embryonal carcinoma cells the proportion of such heparan sulfate species is markedly upregulated by retinoic acid and cAMP [42].

FGFs are intimately involved in the atherosclerotic process, in particular by promoting aberrant smooth muscle cell growth and migration in primary lesions and in restenosis following surgery or angioplasty [43]. Heparan sulfate binds FGFs with high affinity and is essential for FGF signaling [34, 44, 45]. The minimal domain binding to FGF-2 is an N-sulfated pentasaccharide sequence containing an essential IdoA(2-OSO_3) unit [31, 45]. The FGF receptor activation requires a longer, 10–12-mer domain that contains both 2-O- and 6-O-sulfate groups [44, 46]. Because 6-O-sulfate groups are not involved in binding of heparan sulfate to FGF-2 itself, it has been suggested that they mediate binding to the FGF receptor, resulting in formation of ternary complexes comprising FGF, FGF receptor and heparan sulfate [44].

Specific, albeit less extensively characterized structures, have also been implicated in binding of heparan sulfate to platelet-derived growth factor A chain [32], hepatocyte growth factor [47, 48], interleukin 8 [39], lipoprotein lipase [49] and FGF-1 [50, 51]. Interestingly, the FGF-1 binding heparan sulfate domain differs from that interacting with FGF-2 by the requirement of 6-O-sulfate groups, suggesting that different members of the FGF family bind to distinct heparan sulfate sequences. The structures of the protein-binding heparan sulfate domains are currently characterized in more detail by sequence analysis of heparan sulfate oligosaccharides, made possible by novel techniques involving lysosomal exosulfatases and exoglycosidases [52, 53].

Modulation of heparan sulfate structure and function

Heparan sulfate structures are known to differ between different cell and tissue types, suggesting that the biosynthesis is controlled in a tissue-specific fashion. For example, heparan sulfate from aorta displays a considerably lower degree of sulfation than species from, e.g., liver or kidney, particularly with regard to 6-O-sulfation of GlcN units [28, 29].

Heparan sulfate structures are modulated during processes such as embryonic development [54] and cell differentiation [55], aging [56], malignant transformation [57–59], macrophage-foam cell conversion [60], diabetes [61], and amyloidosis [62] in both in vivo and cell culture systems. Human aorta heparan sulfate is subject to an age-associated upregulation of GlcNSO3 6-O-sulfation that has been shown to correlate with enhanced binding of the polysaccharide to the long isoform of PDGF-AA and FGF-1, but not to FGF-2 [56]. Notably, the heparan sulfate structures between subjects within a given age group were almost identical, suggesting an interindividual conservation of the organ-specific heparan sulfate structure. Similar interindividual homogeneity has been observed in heparan sulfate from human cerebral cortex [29].

Lipoproteins

The major lipoproteins are high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low density lipoprotein (VLDL), chylomicrons (CMs), chylomicron remnants (CMRs) and lipoprotein a [Lp(a)] in addition to several intermediary forms. Multiple functions have been ascribed to the lipoproteins, their major roles being transport of lipids to appropriate target organs, energy supply of muscle tissue and energy storage in adipose tissue. The deposition of LDL and its modified forms in the vessel wall has made this molecule a subject for extensive experimental and clinical studies [43]. The interaction with proteoglycans is important in several of the steps leading to cholesterol accumulation. The cellular uptake of HDL, VLDL and CMRs has been studied with regard to the involvement of proteoglycans, and a major focus has been on the interactions between the apoproteins and proteoglycans and the roles played by lipases.

Table 3. Lipoproteins interacting with proteoglycans.

Lipoprotein	Apoproteins	Site of uptake
Chylomicrons	Apo A-I and II, apoB48 ApoC-II and III, apoE	Liver
Very low density lipoprotein (VLDL)	apoB100, apoE, ApoC-I, II and III	Peripheral tissues
Low-density lipoprotein (LDL)	apoB100	Peripheral tissues, liver, macrophages
High-density lipoprotein (HDL)	apoA, apoCII apoE	Liver

The list highlights lipoproteins most thoroughly characterized with regard to interactions with proteoglycans.

The interactions of lipoproteins with heparan sulfate are influenced by several factors. First, the different lipoproteins differ with respect to their content and composition of apoproteins (table 3). The apoproteins are exposed on the surface of the lipoprotein molecules and thereby determine to a large extent the interactions of lipoproteins with other molecules. The apoproteins with the highest affinity for proteoglycans are apoB and apoE. Second, the surface charge of LDL may undergo oxidative modulation in vivo [63], leading to altered glycosaminoglycan binding properties that will affect the cellular uptake and degradation of the lipoprotein. Third, changes at the surface of lipoprotein molecules can lead to complex formation which will have consequences for glycosaminoglycan-mediated extracellular retention and cellular uptake of the lipoprotein [13].

The cell surface proteoglycans, which for the most part carry heparan sulfate chains on their respective core proteins, clearly play a role for cellular uptake of lipoproteins. Such a role depends on the interactions of sequences in the heparan sulfate chains with protein domains, enriched in basic amino acid residues, in the different apoproteins and lipases. There are also some reports of the role played by cell surface chondroitin sulfate proteoglycans in the turnover of lipoproteins [64, 65].

Heparan sulfate and lipoprotein lipase

Lipoprotein lipase is the rate-limiting enzyme in the hydrolysis of circulating triglycerides and therefore important for the metabolism of triglyceride-rich particles such as CM and VLDL. Lipoprotein lipase belongs to a protease family, which also includes hepatic lipase, pancreatic lipase and phosphatidylserine-phospholipase A1. Recently, a novel endothelial cell-derived lipase was cloned which may be important in lipoprotein metabolism [66]. So far, only lipoprotein lipase and hepatic lipase are known as heparin-binding proteins. The lipoprotein lipase-mediated modulation and uptake of lipoproteins has been extensively studied and reviewed, including the interaction with heparan sulfate [67–69].

Biological roles of heparan sulfate-lipoprotein lipase interactions

Lipoprotein lipase is located on the luminal side of the vessel wall, where it is anchored to heparan sulfate proteoglycans. Lipoprotein lipase is active as a homodimer and contains binding sites for both heparan sulfate chains and apoproteins. Interactions between cell surface proteoglycans and lipoprotein lipase are important in several steps in lipoprotein turnover. The dimeric form of the enzyme interacts with heparan sulfate chains on the endothelial surface. The affinity of dimeric lipoprotein lipase for heparin is 6000-fold higher than that of the monomeric form [70]. This interaction is probably necessary to improve the access of the enzyme to circulating lipoprotein substrates. The lipoprotein lipase-mediated interaction with lipoprotein particles is also important to immobilize lipoproteins on the endothelial surface. Accordingly, lipoprotein lipase has a bridging function in the formation of a trimolecular complex of a lipoprotein particle, lipoprotein lipase and heparan sulfate proteoglycans. The presentation of lipoprotein lipase on the cell surface requires intact heparan sulfate proteoglycans, as mutant cells lacking functional heparan sulfate chains release the enzyme into the culture medium [71]. Furthermore, sodium chlorate-treated cells, expressing undersulfated proteoglycans, have a reduced capacity to anchor lipoprotein lipase on the surface [72]. In addition to presenting lipoprotein lipase on the surface of endothelial cells, the heparan sulfate-lipoprotein lipase interaction prolongs the time for substrate binding, thereby promoting the enzyme activity towards CM and VLDL.

Different types of interactions between heparan sulfates and lipoprotein lipase, lipoprotein particles with apoproteins, and different lipoprotein receptors are important in lipoprotein metabolism. Some of these alternatives are presented in figure 3. Similar interactions are also relevant for the uptake and turnover of lipoproteins through hepatic lipase-dependent mechanisms in the liver.

The interaction between cell surface heparan sulfate and lipoprotein lipase is important for the transport and turnover of the enzyme. Lipoprotein lipase found in the endothelia is synthesized by adipose tissue and muscle cells and transported to the apical surface of endothelial cells. The process has been shown to involve cell-associated heparan sulfate [73]. A heparan sulfate proteogly-

can with affinity for lipoprotein lipase has been purified from cultured adipocytes and shown to have characteristics of the glypicans [74]. Endothelial cells have also been shown to produce heparanase, which acts on heparan sulfate proteoglycans on the adipocyte surface [75]. It has been suggested that heparanase releases lipoprotein lipase-heparan sulfate oligosaccharide complexes that can cross the endothelial cell layer and enter to the luminal surface of the endothelium, as shown in figure 4 [75]. Complexing with heparan sulfate was also shown to increase lipoprotein lipase stability. Cell surface heparan sulfate is further involved in the receptor-mediated uptake of lipoprotein lipase. Following uptake, the enzyme is either subject to lysosomal degradation or routed back to the cell surface [76–78]. The level of heparan sulfate expression on the endothelium may thereby influence cellular lipoprotein lipase levels.

Tissue macrophages express and release lipoprotein lipase [79], which can interact with lipoproteins in the matrix of the vascular wall. These lipoprotein lipase molecules can be immobilized by heparan sulfate proteoglycans in the subendothelial matrix or on macrophage surfaces. Lipoprotein lipase bound to macrophage heparan sulfate has been shown to be important for the uptake of native and oxidized LDL [80, 81]. The generation of the lipid-loaded macrophages, also referred to as foam cells, thus involves heparan sulfate-mediated uptake of lipoproteins. The uptake mechanisms resemble those operating in endothelial cells and liver cells. Conversion of macrophages into foam cells has also been shown to affect cellular heparan sulfate metabolism. Treatment of murine J774 macrophages with acetylated LDL results in a foam cell-like phenotype. The process is accompanied by increased synthesis and release of heparan sulfate proteoglycans. In addition, the heparan sulfate structure is altered in the cells treated with acetylated LDL, and this is due to increased 6-O-sulfation of the GlcN residues [60].

The hypolipemic effect seen in the clinic after heparin treatment is due to the competition of circulating heparin with cell surface heparan sulfate for lipoprotein lipase binding. The concentration of circulating lipoprotein lipase is increased 100-fold following heparin administration [82]. Lipoprotein lipase released into the circulation is eventually eliminated through uptake in

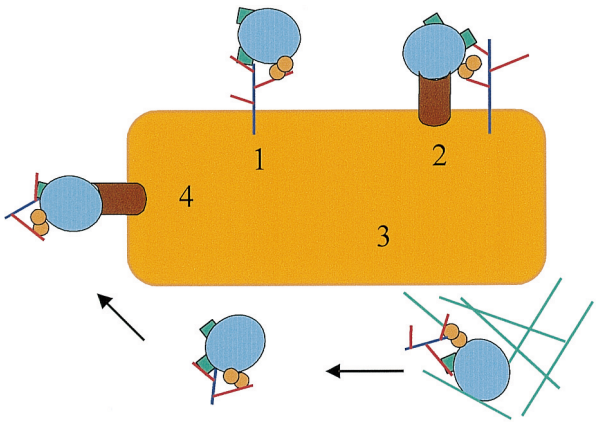

Figure 3. The role of cell surface heparan sulfate in the binding of lipoprotein to cells. The scheme shows the role of heparan sulfate for the cellular uptake of lipoproteins. (1) Cell surface heparan sulfate proteoglycan (blue core protein and red heparan sulfate chains) interact directly with lipoproteins (light blue) through the apoproteins (green squares) on the particle surface or lipoprotein lipase on the endothelial cell surface. ApoB may interact with the LDL receptor without involving heparan sulfate. (2) The cell surface heparan sulfate proteoglycan may interact with lipoproteins in concert with lipoprotein receptors (brown). Apoproteins or lipases may mediate the interaction with heparan sulfate chains. (3) Cell surface heparan sulfate proteoglycan may be shed from the cell surface. Thereafter they may interact with lipoproteins in the matrix (e.g. subendothelial or in the Space of Disse). They may also interact with secreted apoproteins or lipases. These complexes may then be released, e.g by the action of heparanases to interact with the cell surface as shown in (4). (4) Cell surface heparan sulfate proteoglycan may be shed from cell surface and then interact with lipoproteins to promote interaction with receptors for lipoproteins. Such as interaction may also be in collaboration with cell surface heparan sulfate as shown in (2).

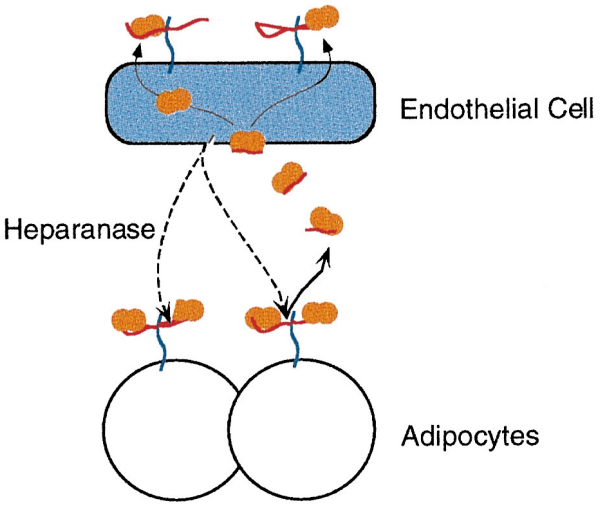

Figure 4. Proposed role of heparan sulfate and heparanase in the transport of lipoprotein lipase to the luminal endothelial surface. Lipoprotein lipase is produced by, e.g., adipocytes and immobilized by their cell surface heparan sulfate proteoglycans. Endothelial cells produce heparanase, which catalyzes endoglycosidic cleavage of the polysaccharide chains, thereby releasing lipoprotein lipase-heparan sulfate oligosaccharide complexes. The released lipoprotein lipase is taken up by endothelial cells and transported to the apical cell surface, where the enzyme becomes bound to membrane-anchored heparan sulfate proteoglycans.

the liver. Heparin and heparin fragments have been shown to increase the half-life of circulating lipoprotein lipase. It is interesting to note that the half-life of lipoprotein lipase in circulation correlates with the length of the heparin oligosaccharide [83].

Molecular basis of the heparan sulfate-lipoprotein lipase interaction

The interaction of lipoprotein lipase with heparin/heparan sulfate has been studied with the aim of defining binding sequences in both the heparan sulfate chains and the protein component. It has been shown that the interaction is dependent on both the size and the sulfation of the heparan sulfate/heparin chains. An early study showed that heparin chains with molecular weights of 10–18 kDa and dimeric lipoprotein lipase formed 1:1 complexes, whereas smaller heparin fragments formed 2:1 molar ratio complexes [84]. Some studies have addressed the interactions between lipoprotein lipase and heparan sulfate isolated from endothelial cells. In one study oligosaccharides were obtained from heparan sulfate purified from cultured bovine aortic endothelial cells and studied with regard to affinity to lipoprotein lipase. A highly sulfated decasaccharide containing exclusively repeating IdoA(2-OSO_3)-GlcNSO_3(6-OSO_3) units was the smallest fragment found to bind lipoprotein lipase [49]. Similarly, surface plasmon resonance studies suggest decasaccharides as the shortest heparin fragments that retain the ability to bind lipoprotein lipase [70]. However, other studies implicate smaller (hexasaccharide) fragments as the minimal lipoprotein lipase binding oligosaccharides [85]. More work on endothelial heparan sulfates is clearly needed to define the structural requirements for heparan sulfate-lipoprotein lipase interactions. The question of tissue-specific differences in heparan sulfate structure may be important, as suggested by the finding that circulating hepatic lipase and lipoprotein lipase differ with regard to homing to the liver [86].

Heparan sulfate-binding sequences have been identified in lipoprotein lipase fragments with affinity for heparin. A large peptide encompassing the amino acids 229–370 bound heparin, as did a fragment containing amino acids 1–392 [70, 87]. Site-directed mutagenesis of residues 281–285 with three basic amino acids greatly reduced, but did not abolish, the affinity of lipoprotein lipase for heparin-Sepharose [88], suggesting the presence of multiple heparin binding sites. The X-ray structure of pancreatic lipase has allowed the modeling of lipoprotein lipase, which reveals several exposed basic amino acids concentrated in clusters. A heparin binding region comprising four such clusters has the appropriate size to interact with an octasaccharide of a heparan sulfate chain [89].

Heparan sulfate and hepatic lipase

Hepatic lipase is responsible for the lipolysis of triglyceride-rich VLDL and CMRs. The modus operandi of hepatic lipase in terms of mediating cellular uptake of lipoproteins resembles that of lipoprotein lipase. Hepatic lipase also depends on heparan sulfate for its localization on the surface of hepatocytes. In addition, hepatic lipase has been shown by immunohistochemistry to reside in the extracellular matrix of the Space of Disse, possibly in association with syndecan and perlecan [90]. Hepatic lipase was also shown to be present on the surface of liver endothelium. Several studies have shown that cell surface heparan sulfate is important for the elimination of, e.g., CMRs from the circulation, and this subject has recently been reviewed in detail [15].

Both lipoprotein lipase and hepatic lipase hydrolyze triglycerides to free fatty acids and glycerol. However, lipoprotein lipase is activated by apoprotein C-II, whereas hepatic lipase needs other protein cofactors. Both enzymes bind heparan sulfate via their C-terminal domains, but lipoprotein lipase does so more strongly than hepatic lipase [91]. In the liver, lipoprotein lipase is located on the surface of the endothelium [92], suggesting that the two enzymes may act on the same lipoprotein substrates. The structural basis for heparan sulfate-hepatic lipase interactions has not been defined.

Hepatic lipase can bind both heparan sulfate proteoglycan and LRP (low-density lipoprotein receptor-related protein) and may therefore participate in the formation of a complex of receptor, heparan sulfate proteoglycan, CMRs and the enzyme, in parallel with the mechanisms for lipoprotein uptake involving lipoprotein lipase (see fig. 3). It has also been demonstrated that lipoprotein lipase enhances the binding of triglyceride-rich lipoproteins to LRP-heparan sulfate complexes [93, 94]. What role lipoprotein lipase plays in comparison with hepatic lipase in the hepatic uptake of CMRs remains to be established. The elimination of CMRs in the liver can be mediated through the LDL receptor on hepatocytes [95]. Another port of entry is the LRP, and the uptake through this receptor depends on the presence of cell surface heparan sulfate proteoglycan [96]. A third uptake mechanism for CMRs is through the heparan sulfate proteoglycan itself. In vivo injection of heparinase to remove liver heparan sulfate reduced the clearance of CMs, CMRs and VLDL, suggesting an important role for cell surface heparan sulfate [97]. It has also been demonstrated that HDL may be taken up by hepatocytes through mechanisms involving cell surface heparan sulfate [98]. Furthermore, syndecan antisense probes decreased the binding of CMR by 50–70% in cultured hepatocytes. Likewise, the binding was inhibited by 60% with antibodies to heparan sulfate or the LDL receptor [99]. Hence, hepatic lipase and lipo-

protein lipase, LDL receptor, LRP and cell surface heparan sulfate proteoglycan are of importance for CMR uptake in liver.

A comparison of the efficiency of the different uptake mechanisms has not been made, and it is therefore difficult to define their relative importance. Interestingly, the uptake of LDL or VLDL in HepG2 cells and fibroblasts, which is stimulated by lipoprotein lipase, has been shown to operate via the LDL receptor, and only a minor portion is taken up via the heparan sulfate proteoglycan pathway [100]. The uptake via heparan sulfate, may, however, vary with the concentration of lipoprotein and have a high capacity despite a low affinity [101].

Heparan sulfate and apoproteins

The most important heparan sulfate-binding proteins in lipoproteins are apoB and apoE. To further understand the molecular mechanisms of such interactions, it is necessary to define the protein and saccharide sequences accommodating the binding sites. Several heparin-binding sequences have been identified in apoB, apoE and apoE subtypes.

ApoB is a major apoprotein of LDL and VLDL. Heparin-binding peptides derived from apoB contain clusters of the basic amino acids arginine and lysine. Different fragments, particularly within the region 3134–3489 have been shown to bind heparin [102, 103]. More recent studies using site-directed mutagenesis and transgenic mice, harboring distinct amino acid substitutions in apoB, have provided interesting data. Proteoglycan binding, and also the LDL receptor binding, was abolished in transgenic mice with human LDL mutated in the basic amino acid rich region 3359–3369. It was, however, possible to separate the two activities. Replacement of the lysine at residue 3363 with glutamic acid resulted in loss of proteoglycan binding activity, without affecting the receptor binding [104]. The use of transgenic animals will provide further insight into apoprotein-proteoglycan interactions. The binding assays presented [104] were done using the chondroitin/dermatan sulfate proteoglycans versican and biglycan. Heparan sulfate may have additional binding sites on apoB, due to its higher affinity for protein ligands. It has also been shown that there are proteoglycan-binding sites in apoB-48, which is truncated at residue 2153 [105].

ApoE is found on CMs, CMRs, VLDL and subclasses of HDL. Both animal and cell biological studies point to the importance of interactions between cell surface heparan sulfate and apoE. The use of apoE fragments or monoclonal antibodies has allowed the identification of residues 142–147 and 243–272 [106] and 202–243 [107], as the proteoglycan binding sites. The latter fragment may be masked if apoE is lipid-associated [106]. Typically, these regions are enriched in basic amino acids. The first region (residues 142–147) has also been shown to contain the receptor binding domain [108, 109]. Different mutant forms of apoE associated with hyperlipoproteinemia may interact with heparan sulfate on cell surfaces with different efficiencies, and mutations in the 142–147 residue region gave the lowest binding [110].

The role of proteoglycans for the metabolism of apoE containing CMRs has been extensively studied and also reviewed [15 and references therein]. The major site of removal of CMRs is the liver, and proteoglycans appear to be involved in several steps of the process. First, proteoglycans on the liver endothelial surface are needed for the presentation of hepatic lipase and lipoprotein lipase. Second, the Space of Disse contains heparan sulfate proteoglycans that have been shown to bind both hepatic lipase and apoE. CMR molecules entering this compartment will be modified by interactions with these two heparin-binding proteins. The space of Disse may also function as a reservoir or filter before CMR is taken up by the hepatocytes. CMR can be taken up directly, independent of cell surface heparan sulfate, through the LDL receptor. However, the uptake through the LRP pathway depends on heparan sulfate. In addition, uptake can also be mediated directly by cell surface heparan sulfate, independent of the LDL or LRP receptors [15].

The turnover of CMR and HDL, also removed from circulation through the liver, seems to depend on heparan sulfate. Chondroitin sulfate may also play an important role in these processes [64], but further studies will be needed to define the relative importance of different proteoglycan types and classes for the lipoprotein turnover in the liver.

The importance of heparan sulfate for the uptake of lipoproteins has also been shown in fibroblasts and macrophages. The latter cell type is particularly important in the atherosclerotic process, primarily by uptake of oxidized LDL through the scavenger receptor. This process eventually leads to the development of the foam cells in the intima. The uptake of oxidized LDL in macrophages and monocytes involves heparan sulfate on the cell surface [65, 81].

Another intriguing aspect of heparan sulfate and lipoprotein metabolism is the possible involvement of apoE in Alzheimer's disease. The apoprotein E4 has been shown to be a risk factor for this disease, whereas the wild-type allele E3, which has a somewhat lower heparin binding activity, is not [111]. ApoE has furthermore been shown to be a constituent of amyloid plaques [112]. β-amyloid peptide, an important part of such deposits, is also a heparin-binding protein. In a

recent study it was shown that β-amyloid peptide decreases the apoE-mediated cellular uptake of VLDL [113]. Furthermore, the data presented indicate that the heparin-binding domain of β-amyloid peptide is the active component, most likely interfering with the apoE-mediated binding of VLDL to the LRP-heparan sulfate proteoglycan complex. Accordingly, apoE may interfere with the cellular uptake of β-amyloid peptide, possibly increasing extracellular deposition in the brain. The implication of the heparin-binding domains of apoE4 and β-amyloid peptide in the pathogenesis of Alzheimer's disease is interesting and needs to be further defined.

Heparan sulfate and superoxide dismutase

The contribution of oxidative stress to several disease conditions, including cardiovascular diseases, is being increasingly appreciated [114].

The oxidative modification, in particular of LDL, has been shown to affect both the cellular uptake and biological effects of the lipoprotein [63]. Several cellular defence mechanisms are in operation to remove reactive oxygen intermediates and products thereof. One such system is the enzyme superoxide dismutase (SOD), which exists in three isoforms. The extracellular form of SOD is a tetrameric glycoprotein containing subclasses with different affinities for heparin/heparan sulfate. The C-terminal part of the protein contains a cluster of six basic amino acids that form the major heparin binding domain [115]. The importance of this interaction is indicated by the finding that individuals with mutations in the heparin binding domain have a 10-fold increase in the plasma content of SOD [116]. Due to intracellular proteolytic processing, the extracellular form of SOD can be expressed with and without the C-terminal heparin binding domain. The ratio between the heparin binding and nonbinding SOD isoforms appears strictly regulated and differs between tissues and organs [117]. SOD without the heparin binding domain is not retained on the cell surface, but diffuses into the extracellular compartment. It has been demonstrated that the heparin-bound SOD is protected against cleavage by trypsin or plasmin, but also that the proteolytically processed SOD is still enzymatically active [118].

The oxidative processes in the endothelial cell wall have important implications for the turnover of lipoproteins, particularly LDL. A regulatory role of endothelial cell surface heparan sulfate through interactions with SOD provides a new and fascinating perspective to novel functions of proteoglycans in atherosclerotic processes. Further studies on which heparan sulfate proteoglycans are involved, and the structures of the glycosaminoglycan chains promoting such interactions, will be some of the approaches needed to widen our understanding of the role of the proteoglycans.

Heparan sulfate and inflammatory cells

One prominent feature of the atherosclerotic plaque is the accumulation of immune cells, especially macrophages. The extravasation of immune cells depends on complex processes including adhesion, chemotactic signaling, penetration of basement membrane barriers and communication with other cells. The influx of monocytes and their differentiation into macrophages, followed by uptake of oxidized LDL through the scavenger receptor are the initial events leading to the development of lipid-loaded macrophages, the foam cells [43, 119].

The expression of heparan sulfate proteoglycans on the endothelium and on activated immune cells seems to be important for these processes. It has been shown that circulating monocytes express negligible amounts of cell surface heparan sulfate [120]. However, monocytes and macrophages have been shown to upregulate syndecan expression upon appropriate stimuli [121, 122]. The expression of heparan sulfate on the surface of monocytes increases their adhesive potential and should conceivably be one of the mechanisms contributing to extravasation. Another mechanism for recruiting white blood cells to a particular area is to increase the expression of heparan sulfate on the surface of endothelial cells. Endothelial cells activated with tumor necrosis factor-α displayed increased adhesion of monocytes through heparan sulfate exposed after stimulation. The ligand on the monocytes was L-selectin [123, 124]. Other molecular interactions, including integrin and ICAM-mediated adhesion, are also involved in these processes. It is furthermore interesting to note that the heparan sulfate species on endothelial cells recognizing L-selectin is enriched in N-unsubstituted GlcN residues. These units do not seem to directly participate in the interaction, but may influence the assembly of L-selectin binding sequences during heparan sulfate biosynthesis [125].

Another intriguing aspect of heparan sulfate in the vasculature is its ability to present chemokines. Several heparin-binding chemokines are involved in the atherosclerotic processes [126]. It has been demonstrated that the chemokines macrophage inflammatory protein α-1 and Gro-α are immobilized to heparan sulfate on endothelial cells. These proteins may be important for the adhesion and migration of various classes of immune cells [127, 128]. Furthermore, several signal molecules expressed by activated immune cells, such as interferon-γ, bind glycosaminoglycans [40]. The importance of such interactions is still poorly under-

stood. The major proteoglycans carrying heparan sulfate chains for such interactions would presumably be the syndecans and the glypicans [129]. The binding to cell surface heparan sulfate of chemokines may regulate their biological activity, as has been shown for interleukin 8 [130].

Perspectives

Proteoglycans are involved in the normal and pathological lipoprotein metabolism [10, 11, 13–16, 131]. In particular, the cell biological roles of proteoglycans in relation to lipoprotein metabolism have received considerable attention, as has also the importance of chondroitin/dermatan sulfate proteoglycans in the atherosclerotic and fibrotic processes in the vascular wall [11, 13]. The molecular interactions between cell surface heparan sulfate and apoproteins and lipases have been studied to a certain extent. However, limited information is available on the specific proteoglycans and heparan sulfate structures involved in interactions with lipoproteins. The importance of such interactions has previously been discussed [132]. It is evident that heparan sulfate structures expressed are strictly regulated, both with regard to tissue differences and changes related to pathological conditions [27]. It is furthermore becoming increasingly evident that different heparan sulfate structures contain information necessary to accommodate interactions with specific protein sequences [26], and several such proteins are important in lipoprotein metabolism [14, 131].

From other studies we know that cell surface heparan sulfate proteoglycans are important for interactions between FGF and its cell surface receptor. We are also gaining further insight into the role of cell surface heparan sulfate in cell adhesion processes and the intracellular signal systems activated thereby [133, 134]. The regulation of protease inhibitors and enzymes through interactions with heparan sulfate is also being defined in molecular terms. Therefore, the role of cell surface heparan sulfate with lipoproteins and other ligands important for lipoprotein turnover needs to be characterized in further detail on the molecular level. The generation of knockout and transgenic animals [135, 136] and mutant cell lines [137] with deletions in genes coding for the proteoglycan core proteins and the glycosaminoglycan biosynthesis enzymes will undoubtedly provide us with new perspectives on heparan sulfate-protein interactions with relevance to lipoprotein metabolism. Such interactions are likely to be of great biological importance, and their more detailed understanding may have implications for treatment of patients with cardiovascular diseases.

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