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Heparan Sulfate Proteoglycans of the Cardiovascular System

Specific Structures Emerge But How Is Synthesis Regulated?

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Introduction

The cell surfaces and surrounding extracellular matrix of the cardiovascular system possess large quantities of heparan sulfate proteoglycans (HSPGs)[1] (1). These highly charged macromolecules consist of different core proteins with covalently linked heparan sulfate chains (HS) of varying monosaccharide sequence which serve as critical mediators of biologic processes (2, 3). For example, these components are involved in regulating mesodermal cell fate, positioning of the heart, vasculogenesis and angiogenesis after ischemic injury, interactions of cells with adhesive proteins and blood vessels, proliferation of smooth muscle cells during atherogenesis, metabolism of lipoproteins, nonthrombogenic characteristics of endothelial cells, etc. (4–9). Detailed investigations over the past decade have defined the structures of HSPGs, uncovered the molecular mechanisms by which these components carry out their diverse functions, and revealed that specific monosaccharide sequences of HS are required for interaction with biologic targets. In this review, we outline the current state of our knowledge about the structure and the biosynthesis of HSPGs as well as describe interactions of these components with growth factors, enzymes, and protease inhibitors. These observations provide a conceptual framework for elucidating the roles of HSPGs in other biologic systems.

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1. *Abbreviations used in this paper:* AT, antithrombin; CS, chondroitin sulfate; FGFs, fibroblast growth factors; GAG, glycosaminoglycan; GlcA, glucuronic acid; GlcA 2S, 2-*O*-sulfated glucuronic acid; GlcNAc, *N*-acetylglucosamine; GlcN(Ac/S), *N*-acetyl or *N*-sulfated glucosamine; GlcNAc 6S, 6-*O*-sulfated *N*-acetylglucosamine; GlcNS 3S, 3-*O*-sulfated *N*-sulfated glucosamine; HexA, unspecified uronic acid, either GlcA or IdoA; HS, heparan sulfate chains; HSPGs, heparan sulfate proteoglycans; IdoA, iduronic acid; IdoA 2S, 2-*O*-sulfated iduronic acid; LPL, lipoprotein lipase; NST, *N*-deacetylase/*N*-sulfotransferase; 6-OST, 6-*O*-sulfotransferase. For all sequences given, the linkages and configurations are →4-D-GlcApβ1→, →4-D-GlcNp(Ac/S)α1→, and →4-L-IdoApα1→.

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Structure and biosynthesis of HSPGs

The syndecan, glypican, and perlecan core protein families constitute the major HSPGs generated within the cardiovascular system (Fig. 1). The syndecan core protein family contains four human or murine members as well as a *Drosophila* homologue. Syndecan family members exhibit an extracellular region with multiple glycosaminoglycan (GAG) attachment sites and a protease dibasic cleavage sequence, a homologous membrane spanning region, and a short highly conserved cytoplasmic tail with four tyrosine residues at fixed positions (for review see reference 10). The sequences of the extracellular regions of syndecan family members are quite divergent except for the GAG attachment and putative cleavage sites, suggesting that this domain may function as a potentially cleavable protein scaffold onto which GAGs are attached. The residues of the extracellular and transmembrane regions of syndecan family members allow homo-oligomerization which can permit the cytoplasmic tail to interact with and activate protein kinase C (11, 12). The cytoplasmic tails of syndecan family members also interact with either intracellular microfilaments or focal adhesion, depending upon the core protein (13). In the former instance, a specific tyrosine residue appears to be involved in regulating this association (14). The phosphorylation of intracytoplasmic serine residues and possibly tyrosine residues may play a critical role in the above process (15). Syndecan family core proteins exhibit cell type–specific distributions with vascular endothelial cells/smooth muscle cells expressing syndecans-1, -2, and -4 with predominant targeting to basolateral surfaces. The glypican core protein family is comprised of four human or murine members as well as the *Drosophila* homologue *dally*. (Recent data reveal the glypican family to be even larger [Saunders, S., S. Paine-Saunders, and A.D. Lander, manuscript submitted for publication].) Glypican family members posses an extracellular region with GAG attachment sites, 14 invariant cysteine residues, which stabilize a highly compact tertiary structure, and a COOH-terminal GPI anchor. The extracellular regions of the different family members are quite similar, which suggests that these areas may be involved in important cellular functions such as binding to ligands or interaction with Golgi components to direct glycanation. Glypican family core proteins are selectively expressed on different cell types with only glypican-1 present on vascular endothelial cells/smooth muscle cells (for review see reference 16). These core proteins are mainly targeted to apical surfaces which are partially dependent upon the extent of glycanation (17). Perlecan represents the final class of core protein to be considered with only a single human or murine family member, but multiple splice variants are possible (18). The intact

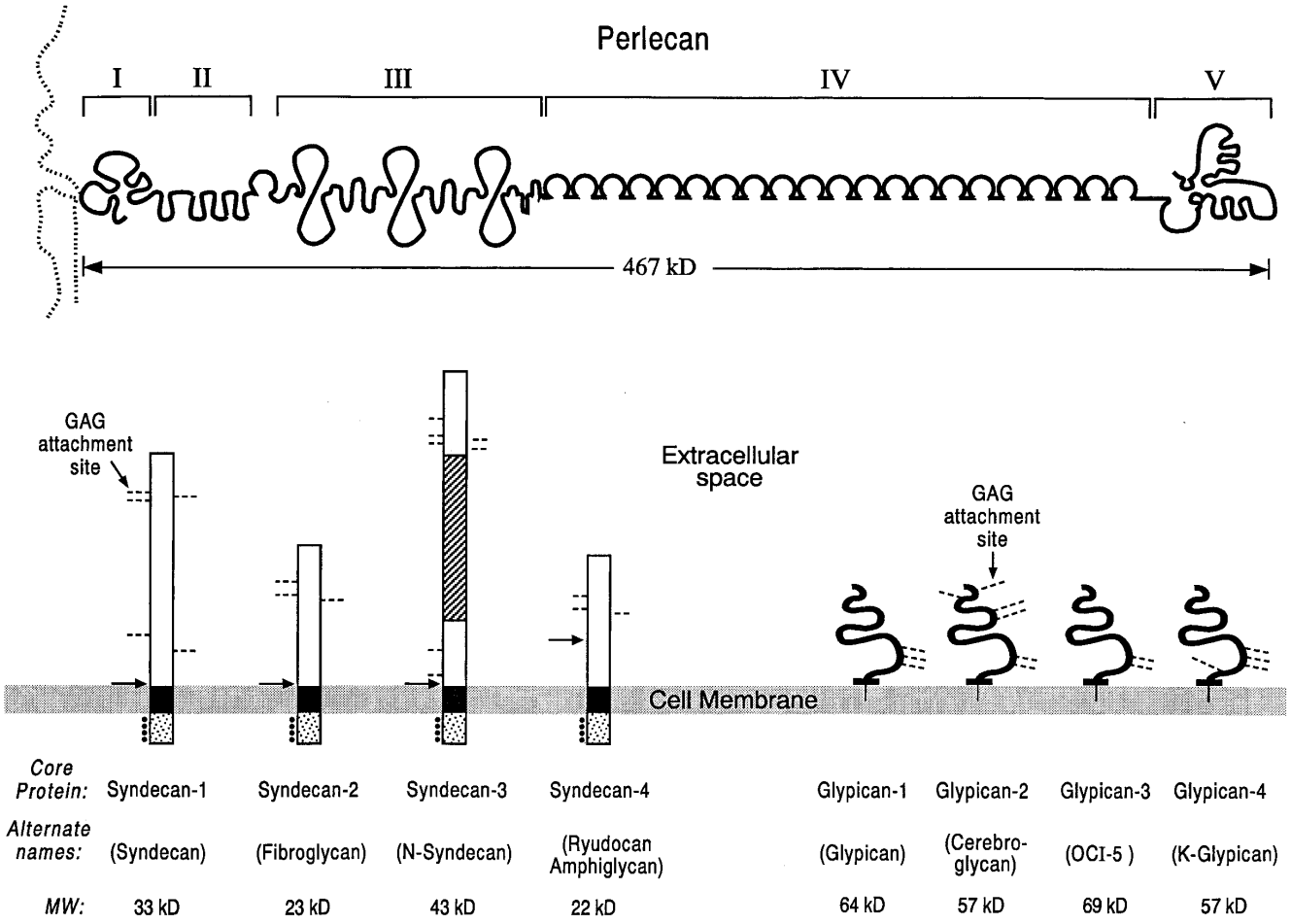

Figure 1. Major HSPG core protein families. Displayed are the human members of the membrane spanning syndecans, the GPI anchored glypicans, and the matrix localized perlecan (467 kD). Potential and identified GAG attachment sites are indicated by dotted lines. For the syndecans, indicated are the homologous transmembrane domain (*black*) and intracellular domain (*stipple*) with conserved tyrosines (*dots*), as well as potential dibasic cleavage site (*arrows*) and the Thr, Ser, Pro rich domain of syndecan-3 (*crosshatch*). The structure of perlecan is also displayed and includes a region for HS attachment (*I*), as well as domains which are homologous to the LDL receptor (*II*), laminin short arm (*III*), N-CAM (*IV*), and laminin A globular end (*V*).

core protein contains five separate regions with a variety of intriguing structural motifs decorated by posttranslational modifications such as GAG chains, O-linked nonsulfated oligosaccharides as well as long chain fatty acids. Perlecan is secreted by multiple cell types including vascular endothelial cells/smooth muscle cells, and interacts with collagens, laminin, and other components within the basement membrane (19).

The biosynthetic mechanism of core protein glycanation has been extensively investigated over the past decade, but only partially defined. The glycanation of core proteins is initiated by four specific enzymes which generate a unique linkage region tetrasaccharide (20). The tetrasaccharide acceptor site is represented by a Ser-Gly (Ala)-X-Gly (Ala) sequence with subsequent attachment of HS, rather than chondroitin sulfate (CS), favored by multiple acidic amino acids at a distance of seven to nine residues, the occurrence of tryptophan residues in close proximity, and the presence of adjacent tracts of Ser-Gly repeats (for review see reference 21). However, the relative intracellular concentrations of metabolic intermediates required for HS versus CS biosynthesis, and the overall structures of core proteins also play a critical role in specifying the linkage of a particular GAG. The attachment of HS to glypican family members is unusually selective, frequently approaching 100%; the linkage of HS to the NH_2 terminus of perlecan is highly specific frequently approximating 80% with CS linked to the remaining sites, whereas the coupling of HS to syndecan family members is favored averaging 60% with the remaining sites decorated with CS (22–24). Under certain conditions, occasional acceptor sites in the above core proteins may be unsubstituted.

The fine structure of HS attached to core proteins is established by a complex biosynthetic pathway (Fig. 2). The generation of HS is initiated by HS copolymerase which sequentially transfers glucuronic acid (GlcA) and *N*-acetylglucosamine (GlcNAc) residues from their respective sugar nucleotides to the tetrasaccharide linkage region to produce GAGs with $\sim$ 100 disaccharide units of $\rightarrow$4-D-GlcApβ1$\rightarrow$4-D-GlcNpAcα1$\rightarrow$. The homogenous copolymer is structurally altered at rare GlcNAc residues by *N*-deacetylase/*N*-sulfotransferase (NST), which replaces *N*-acetyl groups with *N*-sulfate groups, with the process

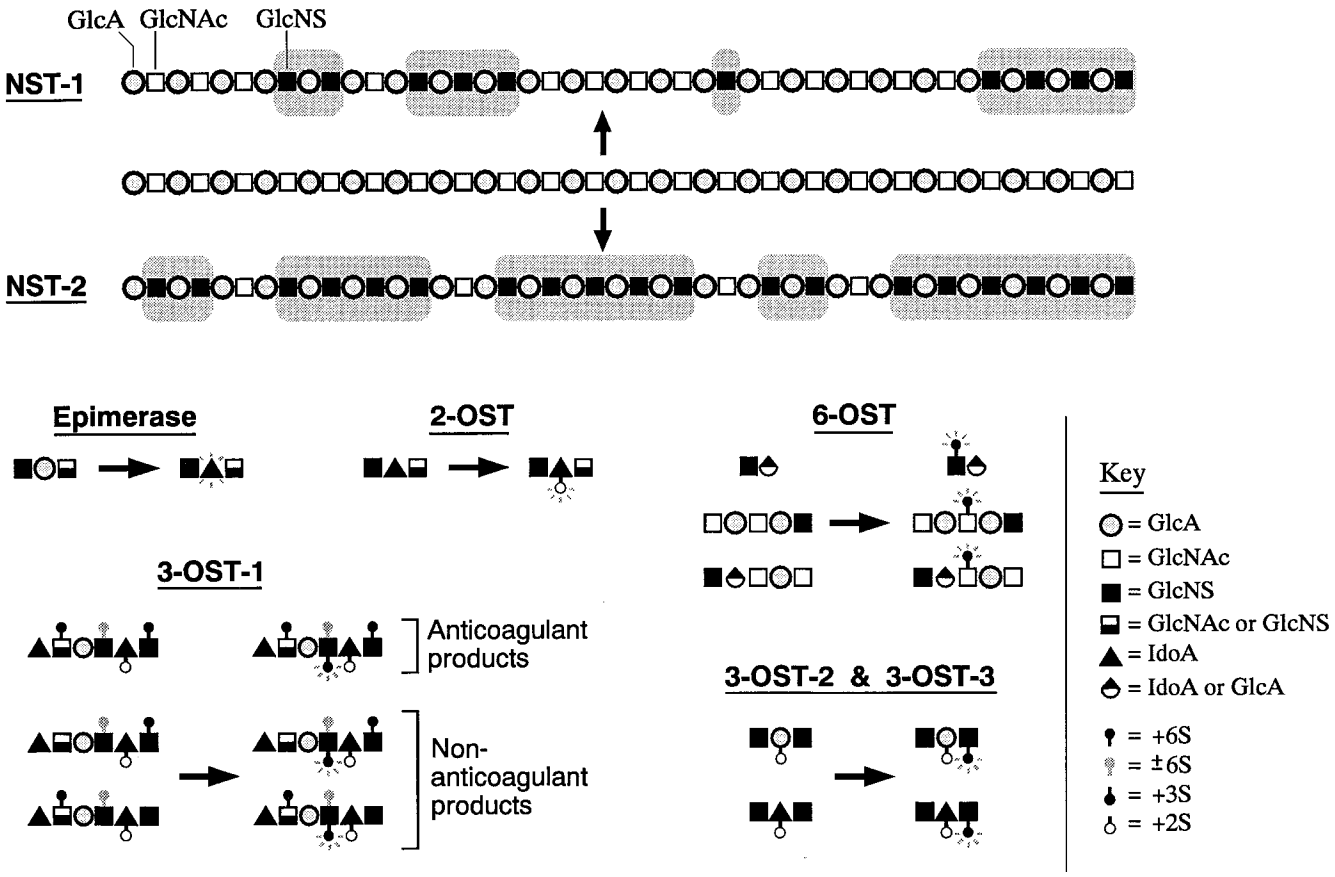

Figure 2. Biochemical specificities of heparan biosynthetic enzymes. Specificities are based on biochemical activities of cell extracts (epimerase, 2-OST, 6-OST) (for review see reference 25) or from expression of cloned cDNAs of NST (87, 88) and 3-OST isoforms (Liu, J., N.W. Shworak, J.J. Schwartz, L.M.S. Fritze, and R.D. Rosenberg; and Zhang, L., N.W. Shworak, J. Liu, and R.D. Rosenberg, manuscripts in preparation). Shading indicates blocks of GlcNS generated by NST isoforms.

spreading in both directions to generate modified domains of ~ 5 disaccharide units separated by relatively unmodified regions of ~ 18 disaccharide units. The C5 glucuronosyl/iduronosyl epimerase then catalyzes transformation of occasional D-GlcA residues to L-iduronic acid (IdoA) residues if *N*-sulfate groups are present at the immediate upstream position. The equilibrium distribution of this reaction lies far in the direction of GlcA but epimerization is favored by sulfation of a IdoA. The iduronosyl 2-*O*-sulfotransferase (2-OST) then sulfates selected IdoA residues (creates IdoA 2S) provided that *N*-sulfated glucosamine (GlcNS) groups are located at the immediate upstream position and 6-*O*-sulfated glucosamine [GlcN(Ac/S) 6S] groups are absent from the immediate downstream position. The glucuronosyl 2-*O*-sulfotransferase also sulfates rare GlcA residues (creates GlcA 2S) which prevent subsequent epimerization to IdoA. The glucosaminyl 6-*O*-sulfotransferase (6-OST) then frequently sulfates GlcN(Ac/S) residues provided that *N*-sulfate groups are present at either the immediate upstream or downstream positions (extensively reviewed in reference 25). Finally, the glucosaminyl 3-*O*-sulfotransferase (3-OST-1) sulfates occasional GlcNS and GlcNS 6S residues (creates GlcNS 3S and GlcNS 3S 6S, respectively) predominantly when →HexA (GlcA or IdoA)→GlcN(Ac/S)±6S 6S→GlcA→ is present in the immediate upstream position and the downstream location is filled by an →IdoA 2S→ GlcNS→. Thus, the above enzymatic reactions which generate distinct HS fine structures represent, at the very minimum, a partially ordered biochemical pathway with clear cut precursor/product relationships.

Until recently, all biosynthetic enzymes were postulated entities based upon the biochemical activities of cell lysates. During the past 4 yr, virtually all of these proteins have been purified and several have been molecularly cloned (26–32). The available data on the initial and final sulfation enzymes in the biosynthetic pathway provide intriguing clues about potential mechanisms for generating HS with regions of defined monosaccharide sequence. The primary structures and biochemical specificities of murine/human NSTs and 3-OSTs have been determined. We note that NST-1 and NST-2 were cloned from hepatic cells and mast cells, respectively, but are also expressed in other cell types (30–32). The two enzymes exhibit alternate specificities that initially lead to a varying extent of *N*-sulfation of HS (i.e., NST-1 generates ~ 40% *N*-sulfation of HS whereas NST-2 produces ~ 80% *N*-sulfation of HS), which subsequently results in different patterns of sulfation/epimerization (Fig. 2). Murine/human 3-OST-1 have also been molecularly cloned and homologous regions used to isolate human cDNA clones for 3-OST-2, and 3-OST-3 which are 60% homologous to human 3-OST-1 (Shworak, N.W., J. Liu, L.M.S. Fritze, J.J. Schwartz, L. Zhang, and R.D. Rosenberg, manuscript submitted for publication, and Shworak, N.W., J. Liu, L. Zhang, and R.D. Rosenberg, manuscript in preparation). The

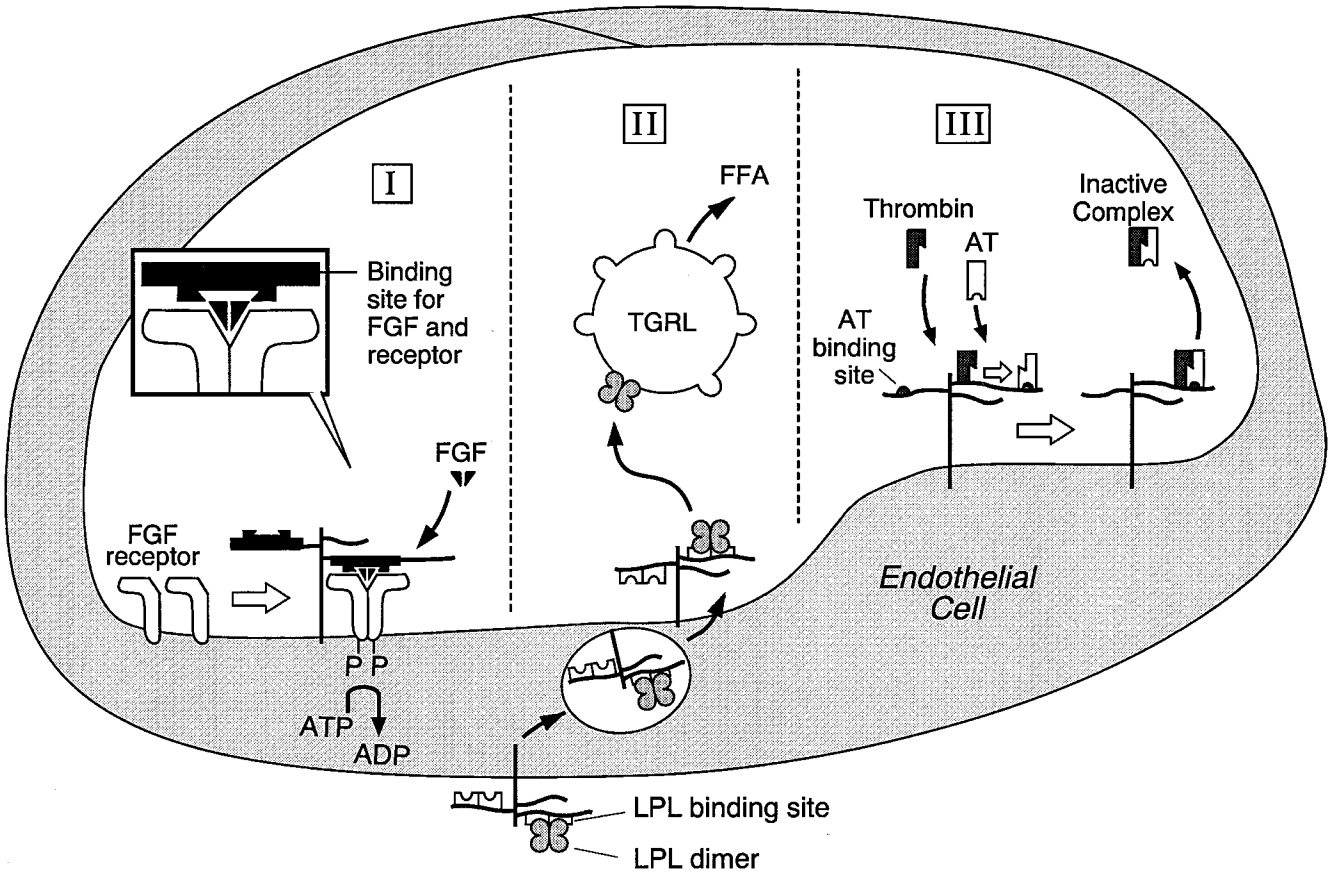

Figure 3. Distinct HS structures allow HSPGs to regulate multiple events within a single cardiovascular cell type. HSPGs are schematically depicted as a core protein with three attached HS chains. As described in the text, discrete HS sequences regulate (*I*) FGF signaling, (*II*) the presentation of LPL to triglyceride-rich lipoproteins (*TGRL*) to generate FFA, and (*III*) neutralization of coagulation proteases by AT.

3-OSTs have been expressed, and the sites of sulfation have been determined. The results demonstrate that 3-OST-1 sulfates GlcNS residues when the upstream uronic acid is GlcA, whereas 3-OST-2 and 3-OST-3 sulfate GlcNS residues when the upstream uronic acid is IdoA 2S or GlcA 2S (Fig. 2). The specificities of these enzymes are also dependent upon additional residues in the neighborhood of the site of sulfation. Based upon these data, we speculate that isoforms of biosynthetic enzyme exist in different cell types which sulfate HS at specific sites based upon the surrounding monosaccharide sequence and hence are capable of producing GAGs with regions of defined structure. The regulation of the concentrations of these enzymes, provided that they serve as limiting components in a biosynthetic pathway, would establish particular levels of HS with regions of defined monosaccharide sequence.

Growth factors and HSPGs

Fibroblast growth factors (FGFs), vascular endothelial growth factor, heparin binding EGF, the Wnts, interleukin-3, GM-CSF, and IFN-gamma form tight complexes with HSPGs (for review see reference 33). These mitogens, differentiation factors, and cytokines are critically involved in early development, angiogenesis, thrombosis, and atherosclerosis of the cardiovascular system. In the section below, we describe the regulation of FGF signaling which serves as a paradigm for elucidating the role of HSPGs in modulating the function of other growth factors/cytokines.

The HSPGs are required for high-affinity binding of FGFs to their receptors (34, 35) (Fig. 3). Syndecans-1, -2, and -4 as well as glypican-1, possess HS which carry out this function. The crystal structures of FGF-2 and heparin oligosaccharides reveal specific interactions between asp^{28}, arg^{121}, lys^{126}, and gln^{135} of the growth factor and the IdoA 2-*O*-sulfate and GlcN *N*-sulfate residues as well as other carboxyl groups within the oligosaccharide (36, 37). The binding of HS or heparin to FGF-2 leads to the dimerization of growth factor without significant conformational alterations, as documented by cross-linking experiments and crystallographic studies (36–38). HS or heparin also interacts with FGFR1 (39, 40). The HS binding domain of FGFR1 is positioned between the first and second IgG repeats of the extracellular domain; antibodies generated against this sequence block FGF-2 interactions, and mutations of this region drastically reduce binding of growth factor. The minimal structure of the FGF-2 binding site of HS is represented by the pentasaccharide HexA→GlcNS→HexA→GlcNS→IdoA 2S (41–43). The critical importance of the IdoA 2S residue has been confirmed by showing that Chinese hamster ovary mutants lacking this modification cannot bind to or respond to growth factor (44). However, the smallest oligosaccharide required to generate a mitogenic signal with added FGF-2 is significantly larger than that needed for growth factor binding (38). Experiments using selectively modified GAGs demonstrate that a signaling sequence is required which includes both IdoA 2S and GlcNS 6S groups (45). Indeed, the minimal binding and

signaling sequence must be present on a dodecasaccharide to induce mitogenesis with no activity noted if the same sequences are located on a decasaccharide. The two sequences appear to be necessary for binding to both growth factor and receptor.

Based upon the above data, it is surmised that HSPGs bearing specific binding and signaling sequences complex with FGF-2 to induce growth factor dimer formation and also interact directly with FGFR1 (34, 35, 39, 40). The end result is transient dimerization of the receptor which facilitates phosphorylation of cytoplasmic tails, assembly of intracellular components, and mitogenesis (46, 47). Additional monosaccharide sequences may also be required to generate stably phosphorylated higher order receptor complexes that lead to an increased strength of cell signaling (46). The regulation of FGFs by cell surface HSPGs is also likely to be modulated by extracellular and intracellular events. On the one hand, the FGFs interact with basement membrane perlecan which reduces the quantities of growth factor binding to receptor but also provides a ready reservoir for rapid mobilization (48). On the other hand, the binding of the cytoplasmic tails of syndecans to cytoskeletal elements and protein kinase C may modulate the response to FGF signaling (11, 12). Finally, we note that the structures of HS required to initiate a mitogenic response with FGF-1 and FGF-4 are different from those described above (45). Thus, it appears likely that specific monosaccharide sequences of HS may differentially regulate the biologic effects of FGF family members within the cardiovascular system.

Surprising results have emerged from genetic screens in *Drosophila* that underscore the importance of growth factor regulation by HSPGs at the whole animal level. The Wnts are growth factors known to generate complexes with HS and play an important role in development. The seven membrane spanning Frizzled gene family has been identified recently as the long-sought Wnt receptors, and in vitro depletion of HS from *Drosophila* cells inhibits binding of Wnt 1 to Frizzled as well as suppresses cell signaling (49, 50). A genetic screen to identify new components in the Wnt signaling pathway generated mutant flies with deletions of NST that exhibit a severe developmental malfunction of this system (51). Using a similar approach, saturation mutagenesis was used to identify new effectors involved in cell division patterning in *Drosophila*, which uncovered *dally* mutants with abnormalities in M phase progression (52). The sequencing of wild-type *dally* cDNA reveals an open reading frame extremely homologous to glypican with 14 cysteine residues and several GAG attachment sites as well as a putative GPI anchor. It appears likely that *dally* is a core protein that bears HS and is required for the binding of growth factor needed for normal cell division.

Lipoprotein metabolism and HSPGs

The metabolism of lipoproteins is partially regulated by HSPGs through interactions with lipoprotein lipase (LPL) and apolipoproteins B and E (apoB and apoE). LPL is the rate-limiting enzyme for hydrolysis of triglycerides in very low density lipoproteins as well as chylomicrons and therefore controls the delivery of fatty acids to tissues. In the section below, we describe the modulation of this pathway by HSPGs which provide a conceptual framework for considering the effects of GAGs on other cell surface bound enzymes in the cardiovascular system.

The primary site of action of LPL is the luminal surface of capillary endothelial cells where the enzyme is anchored to HSPGs (53) (Fig. 3). The core proteins involved in this interaction have not been completely defined but the available data are compatible with a major role for syndecan 1 (54). The enzymatically active dimeric LPL binds 6,000-fold more tightly than inactive monomeric enzyme to HSGPs (55). This interaction allows dimeric LPL to be localized on the endothelial cell surface whereas monomeric enzyme enters the blood and is cleared by the liver. The binding of LPL to HSPGs fails to appreciably alter the conformation of the enzyme or its ability to interact with lipoproteins. Thus, LPL is targeted to the appropriate locale to carry out its biologic role without perturbing structural features needed for optimal function. The localization of LPL to the endothelial cell surface may also involve a 116-kD NH_2-terminal region processed form of apoB (NTAB) which contains separate binding domains for LPL and HS. The endothelial cell expression of NTAB may alter the interactions of LPL with HSPGs or apoB containing lipoproteins. The apical anchoring of LPL exposes the enzyme to circulating triglyceride-rich lipoproteins, and the presence of apoE may facilitate binding to endothelial cell HSGPs. The surface bound LPL attaches to lipoproteins, possibly by complexing to apoB, with the cofactor apoCII activating the enzyme. During lipolysis, LPL can dissociate from cell surfaces which makes it unclear whether hydrolysis of triglycerides takes place at the cell surface or within the capillary lumen (for an extensive review see reference 56). Surprisingly, LPL is synthesized predominantly in myocytes and adipocytes, and presumably exchanges between HSPGs of the extracellular matrix to reach the basolateral surface of endothelial cells. At this latter site, the enzyme bound to carrier HSPGs is internalized and transcellularly transported to the apical surface (57). LPL complexed with HSPGs of cell surfaces and extracellular matrix can simultaneously interact with lipoproteins, and such nonenzymatic bridging may affect lipoprotein localization and metabolism. On the one hand, retention of LDL and VLDL within the subendothelial space is dependent on the presence of both HSPGs and LPL (58). On the other hand, LPL-dependent degradation of lipoproteins is initiated through uptake by several distinct receptors and, in specific cell types, HSPGs may constitute a major, functionally independent internalization pathway (59–61).

The interaction of HS with dimeric LPL leads to the formation of a 1:1 stoichiometric complex which depends upon specific groups of the enzyme and GAG. Based upon the crystallographic structure of the homologous pancreatic lipase, the front surface of LPL is thought to contain the active site, whereas the back surface is believed to possess four regions of clustered basic residues (62). In particular residues 279–282 and 292–304 (human LPL numeration) show homology to the heparin-binding consensus sequences -X-B-B-X-B-X- and -X-B-B-B-X-X-B-X-, which are comprised of basic (B) and small neutral (X) amino acid residues (63). Mutational analysis demonstrates that Arg^{279}, Arg^{280}, Arg^{282}, Arg^{296}, Arg^{297}, as well as unspecified sites within residues 404–430, are required for binding to HS (64, 65). The sulfate groups of HSPGs are critical for interaction with LPL since the reduction in levels of PAPS, the biochemical sulfate donor, greatly decreases the binding of enzyme to the cell surface (66). In an attempt to define the critical oligosaccharide sequence, HS fragments were subjected to LPL affinity chromatography, which yielded de-

casaccharide that represented 2% of the initial HS mass. These decasaccharides constitute the minimum size capable of binding dimeric enzyme as efficiently as HS, and are represented by the sequences (IdoA 2S→GlcNS 6S→)$_5$ and (IdoA 2S→GlcNS 6S→)$_3$ GlcA→GlcNS 6S→IdoA 2S→GlcNS 6S (67, 68). Detailed biochemical investigations reveal that ionic bonding to LPL only requires 10 of the 20 available negative groups, with nonelectrostatic interactions accounting for 40% of the total binding energy (55). Thus, more in depth structural analyses are required to establish the exact set of ionic and nonionic groups involved in the interaction of HS with LPL.

Blood coagulation and HSPGs

The blood coagulation cascade consists of a series of linked inactive precursor–serine protease transformations which generate thrombin, thereby activating platelets as well as producing fibrin. The various natural anticoagulant mechanisms of the blood vessel wall oppose the action of the blood coagulation cascade and hence prevent thrombotic events. In the section below, we describe the endothelial cell anticoagulant heparan sulfate pathway which accelerates inhibition of coagulation proteases by a circulating plasma protease inhibitor and provides a framework for considering how HSPGs with regions of defined monosaccharide sequence might be generated in other biologic systems.

The plasma protease inhibitor antithrombin (AT) slowly inactivates thrombin (T), Factor Xa, and other coagulation proteases by formation of enzyme–AT complexes (69) (Fig. 3). The binding of the active center of coagulation proteases to the reactive site of AT induces a partial insertion of the amino terminal of the protease inhibitor into its interior, which arrests cleavage of the reactive site bond and traps the enzyme in a stable complex. Heparin induces a conformational alteration in AT by binding to two sets of positively charged residues, and in certain cases augments interactions of protease inhibitor with free enzyme as well as enhances enzyme reactivity. These molecular events dramatically accelerate enzyme–AT complex generation. Once generation of the interaction product is completed, heparin dissociates and then catalyzes additional rounds of enzyme neutralization by plasma inhibitor (69–71; for reviews see references 72 and 73). The affinity fractionation of heparin by AT revealed a small subpopulation of GAG with the unique sequence: →HexA→GlcN(Ac/S) 6S→GlcA→GlcNS 3S 6S→IdoA 2S→GlcNS 6S→ which is mainly responsible for binding protease inhibitor and accelerating Factor Xa inhibition (74–79). The evaluation of individual residues demonstrate that the 6-*O*-sulfate group on residue 2 and the 3-*O*-sulfate group on residue 4 act in a concerted fashion to bind AT and accelerate Factor Xa neutralization (78, 79). The downstream flanking disaccharide, →IdoA 2S→GlcNS 6S→, is also of importance in this process (80). Two additional heparin domains of unknown structure are required for neutralization of thrombin, as well as the remaining coagulation proteases other than Factor Xa. Cultured cloned endothelial cells synthesize small amounts of anticoagulant HSPGs with covalently linked HS possessing the AT binding site, which accelerates enzyme–inhibitor complex formation. The physiologic role of endothelial cell anticoagulant HSPGs has been defined by perfusing rat hindlimbs with T and AT, and showing that T–AT complex generation is dramatically accelerated by a heparan sulfate vessel wall component. The in vivo location of anticoagulant HSPGs was ascertained by light and EM level autoradiography which revealed small amounts of anticoagulant HSPGs on the luminal surface of endothelial cells with much larger quantities deposited in the subendothelial space (for review see reference 81). Thus, it appears likely that coagulation enzyme–AT interactions are dramatically accelerated in normal and damaged blood vessels by anticoagulant HSPGs.

The HS biosynthetic pathway generates limiting amounts of anticoagulant HSPGs with regions of defined structure that contain the AT binding site, and also produces the more abundant nonanticoagulant HSPGs with regions of varying structure that carry out other biologic functions. It has been argued that the amounts of anticoagulant HSPGs generated could not be formed by a completely random process and that the anticoagulant HS biosynthetic mechanism must be ordered. However, the anticoagulant HSPGs are produced by a complex pathway with multiple enzymatic components, and until recently regulation of this process remained obscure. The analyses of cell mutants, created by overexpression of the syndecan-4 core protein or chemical exposure, revealed that anticoagulant HS generation requires a pathway-specific component present in limiting amounts (82, 83). The rate-limiting step was identified by establishing a cell-free system using microsomal conversion activity from wild-type microsomes, and radiolabeled HSPG precursor from microsomes of mutants blocked in anticoagulant HSPG generation (84). The addition of these two extracts results in the production of large amounts of anticoagulant HSPG. Further investigation demonstrated that the concentrations of microsomal conversion activity in many different cell types predicts the cellular levels of anticoagulant HSPG generated. The treatment of radiolabeled wild-type microsomal and cell surface HSPGs with excess microsomal conversion activity transforms a maximum of 35% of total HSPGs into anticoagulant HSPG. This extent of conversion should be contrasted to the existing levels of cell surface anticoagulant HSPG, which average $\sim$ 1–5% of total HSPGs (84). These investigations provide convincing evidence that microsomal conversion activity represents the limiting intracellular component that transforms small amounts of an HSPG precursor population into anticoagulant HSPGs.

The above assay system was then used to isolate the limiting factor which proved to be the long sought glucosaminyl 3-*O*-sulfotransferase (3-OST-1, see previous section) (29). This enzyme is present in trace amounts, as compared with other HS biosynthetic enzymes, and sulfates six sites per GAG chain to generate either anticoagulant or nonanticoagulant HS. Detailed analyses show that the sulfated sequence in both precursor populations is a tetrasaccharide of similar structure (Fig. 2). Surprisingly, anticoagulant and nonanticoagulant HS differ mainly in the downstream disaccharide which must contain →IdoA 2S→GlcNS 6S→ to interact tightly with AT. The invariant presence of this disaccharide in AT binding sites of anticoagulant HS tagged with 3-*O*-sulfated glucosamine and the absence of this disaccharide in the alternate sites of nonanticoagulant HS labeled with 3-*O*-sulfated glucosamine reveal an ordered biosynthetic pathway for assembling precursor populations. Thus, the HS biosynthetic enzymes other than 3-OST-1 generate two types of precursors with differing monosaccharide sequences that are sulfated by 3-OST-1 to a limited extent to produce anticoagulant and nonanticoagulant HS. Indeed, the selective overexpression of 3-OST-1 in two different nonendothelial cell types dramatically augments the levels of anticoagulant HS. Therefore, to a major extent, the generation of

anticoagulant HS is controlled by the intracellular levels of a 3-OST isoform which sulfates specific sites in precursor GAGs depending upon the neighboring monosaccharide sequence. We note that 3-OST-2 or 3-OST-3, neither of which appears to be present in endothelial cells, sulfates precursor HS at other sites and hence produces GAGs with potential interaction sites for biologic targets other than AT. It is of interest that HS modified by the latter 3-OST isoforms has been identified in the glomerular basement membrane where it has been hypothesized to be involved in regulating permeability to proteins (85, 86).

Conclusions

We have summarized recent advances in our knowledge of HSGPs, which demonstrate that covalently linked HS with regions of defined monosaccharide sequence interact with receptors and proteins to regulate different biologic functions. Indeed, given the various cellular processes thought to be controlled by GAGs, one should anticipate the existence of numerous discrete HS sequences. However, it remains unclear how the biosynthetic mechanism is able to generate and specifically regulate the concentrations of each of these components. Based upon available data, we speculate that the various classes of sulfotransferases exist as multiple isoforms, each able to recognize and modify slightly different monosaccharide sequences. Cell type–specific expression of these isoforms would then dictate the synthesis of a particular array of GAGs. The actual levels of a given HS population with regions of defined monosaccharide sequence could then be regulated by controlling the concentrations/activities of key enzymes present in limiting amount. The validation of this hypothesis, or the development of alternative models, will require improved approaches for separating HS into functionally discrete components, more powerful methods for sequencing GAGs, and the availability of enzyme cDNAs to allow genetic manipulation of biosynthetic pathways. Recent progress in these three areas suggests that the mechanisms for controlling the diversity of HSPGs will be delineated over the next few years. Success in this endeavor should provide us with novel insights into the regulation of different biologic functions, and lead to the development of new therapeutic approaches for cardiovascular disorders.

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