

Heparin Biosynthesis

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Abstract Heparin and heparan sulfate share the same polysaccharide backbone structure but differ in sulfation degree and expression pattern. Whereas heparan sulfate is found in virtually all cells of the human body, heparin expression is restricted to mast cells, where it has a function in storage of granular components such as histamine and mast cell specific proteases. Although differing in charge and sulfation pattern, current knowledge indicates that the same pathway is used for synthesis of heparin and heparan sulfate, with a large number of different enzymes

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taking part in the process. At present, little is known about how the individual enzymes are coordinated and how biosynthesis is regulated. These questions are addressed in this chapter together with a review of the basic enzymatic steps involved in initiation, elongation, and modification of the polysaccharides.

Keywords GAGosome • Golgi • Heparan sulfate • Heparin • Heparin biosynthesis • Mast cell

1 Introduction

Heparin is found in mast cell granules where it interacts with histamine, proteases, and inflammatory mediators. The negative charge of the polysaccharide, due to a high degree of sulfate substitution, is important for its ability to bind the other granule constituents. Storage, retention, and in some cases activation of these components are most likely the main functions of mast cell heparin. The sulfate groups of the heparin molecule are added during biosynthesis, which occurs in the Golgi compartment of the mast cell. Here, the heparin chains are simultaneously elongated and modified by a large number of enzymes.

Heparin chains are attached to serglycin. In addition to mast cells, this protein is found also in other hematopoietic cells as well as in endothelial cells. However, in these cells the serglycin core protein is substituted with chondroitin sulfate instead of heparin (Kolset and Tveit 2008). The physiological role of heparin was long believed to be control of blood coagulation, since this is a potent pharmacological effect of the polysaccharide (Petitou et al. 2003). However, the localization of endogenous heparin in mast cells, and not in blood, makes it unlikely to fulfill this function.

Heparan sulfate (HS) contains the same polysaccharide backbone as heparin and is also sulfated. The major difference between the two is the degree of modification, heparin being more heavily sulfated than HS (Fig. 1b). Also, HS isolated from different tissues and cell types differ in structure when compared to each other (Ledin et al. 2004), but the overall sulfation degree of all these HS species is much lower than that of heparin (Gallagher and Walker 1985). In contrast to heparin, HS is produced by virtually all cells of the human body. HS chains are attached to a variety of core proteins that are either secreted to the extracellular space (e.g., perlecan, agrin, and collagen XVIII) or associated with the cell surface (syndecans and glypicans). The four members of the syndecan core protein family are trans-membrane proteins, while the six glypicans are linked with the plasma membrane through a GPI-anchor (Fig. 1a).

Current knowledge indicates that the same biosynthesis pathway is used for heparin and HS biosynthesis. Why then, do mast cells make heparin while other cells synthesize HS?

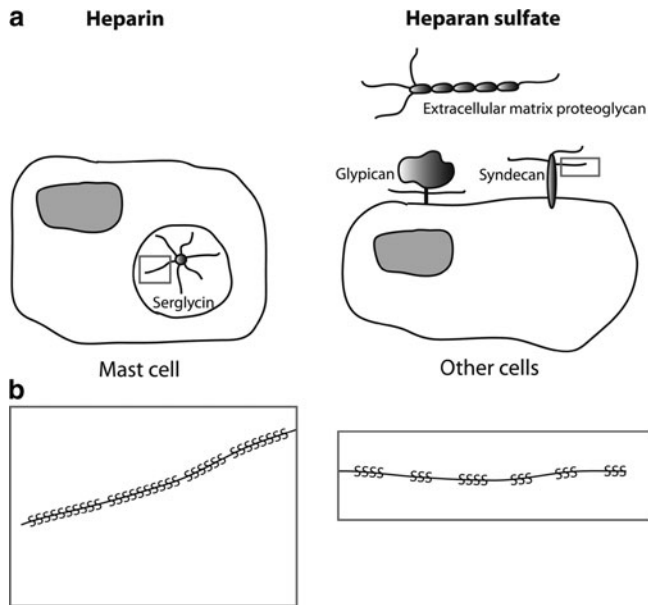


Fig. 1 (a) Mast cells produce highly sulfated heparin attached to the serglycin core protein. The heparin proteoglycan is stored in mast cell granules. Other cells synthesize less negatively charged heparan sulfate, which is found at the cell surface as glypican and syndecan proteoglycans, and in the extracellular matrix. (b) A blow-up of the boxes in panel a, illustrating the difference in sulfation pattern between heparin and hepan sulfate

2 Heparin and Heparan Sulfate Biosynthesis

As mentioned above, heparin and HS share the same basic structure, consisting of N-acetyl-D-glucosamine (GlcNAc) and glucuronic acid (GlcA) units that are partly modified by epimerization of GlcA to iduronic acid (IdoA) and by sulfation at different positions of mainly GlcNAc and IdoA residues. The synthesis is a rapid process, as shown for heparin produced by mouse mastocytoma microsomes. Polymerization and modification of a polysaccharide chain was estimated to be completed in ~1 min (Hook et al. 1975; Lidholt et al. 1989).

Below follows an overview of the different steps in heparin/HS synthesis, followed by a section in which the enzymes responsible for the different reactions are described (Fig. 3).

2.1 Initiation

The biosynthesis of heparin and HS begins with the formation of a tetrasaccharide linkage region (-GlcA-Gal-Gal-Xyl-). This process is catalyzed by four enzymes

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MMQKLLKCSRLVLALALILVLESSVQGYPTQRARYQWVRC 40
NPDSNSANCLEEKGPMFELLPGESNKIPRLRTDLFPKTRI 80
QDLNRIFPLSEDYSGSGFGSGSGSGSGSGSGFLTEMEQDY 120
QLVDESDAFHDNLRSLDRNLPSDSQDLGQHGLEEDFML 158

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Fig. 2 Human serglycin amino acid sequence (UniProtKB accession P10124). The glycosaminoglycan attachment site is *underlined*

adding individual monosaccharides sequentially to the growing glycosaminoglycan (GAG) chain. A xylose residue from UDP-xylose is first transferred to the hydroxyl group of a serine residue on the core protein (Fig. 2). Only certain serines, defined by Ser–Gly residues flanked by acidic residues, are selected for GAG attachment (Esko and Zhang 1996). The serglycin protein contains a stretch of repetitive Ser–Gly residues that can be decorated with heparin chains, making the protein densely glycosylated. This repetitive sequence is conserved in different species, although the number of Ser–Gly units varies. Notably, this sequence is more than twice as long in rat as in, for example, mouse and human.

The attachment of xylose is followed by a stepwise transfer of two galactose and one glucuronic acid residues. These units may be modified by phosphorylation (xylose) and/or sulfation (galactose units). In vitro studies have shown that the linkage region enzymes are sensitive to these modifications (Gulberti et al. 2005; Tone et al. 2008). Phosphorylation/sulfation of the tetrasaccharide linker may thus be a way of regulating GAG synthesis.

Formation of the linkage region is identical in heparin/HS and chondroitin sulfate (CS) synthesis. The crucial point in determining whether a heparin/HS chain or a CS chain will be attached to the linkage tetrasaccharide is the addition of the next monosaccharide. While a N-acetylgalactosamine residue will initiate CS elongation, addition of GlcNAc results in HS/heparin formation. The enzymes responsible for transferring this first hexosamine to the tetrasaccharide linkage region are unique to this reaction step and do not take part in the subsequent polymerization process. It has been suggested that O-sulfation of the Gal residues may lead preferentially to CS synthesis (Ueno et al. 2001).

2.2 Elongation

Upon addition of the first GlcNAc residue, the heparin/HS chain is elongated by addition of alternating glucuronate and N-acetyl-glucosamine residues from their respective UDP-sugars. The final products are extended polysaccharides, heparin chains from different sources being in the range of $M_r = 60,000$ – $100,000$ Da (Robinson et al. 1978). Commercially available heparins are processed and their molecular weights range between $\sim 7,000$ and $25,000$ Da. In comparison with newly synthesized heparin, heparan sulfate chains are generally shorter ($M_r = 22,000$ – $45,000$ Da) (Lyon et al. 1994).

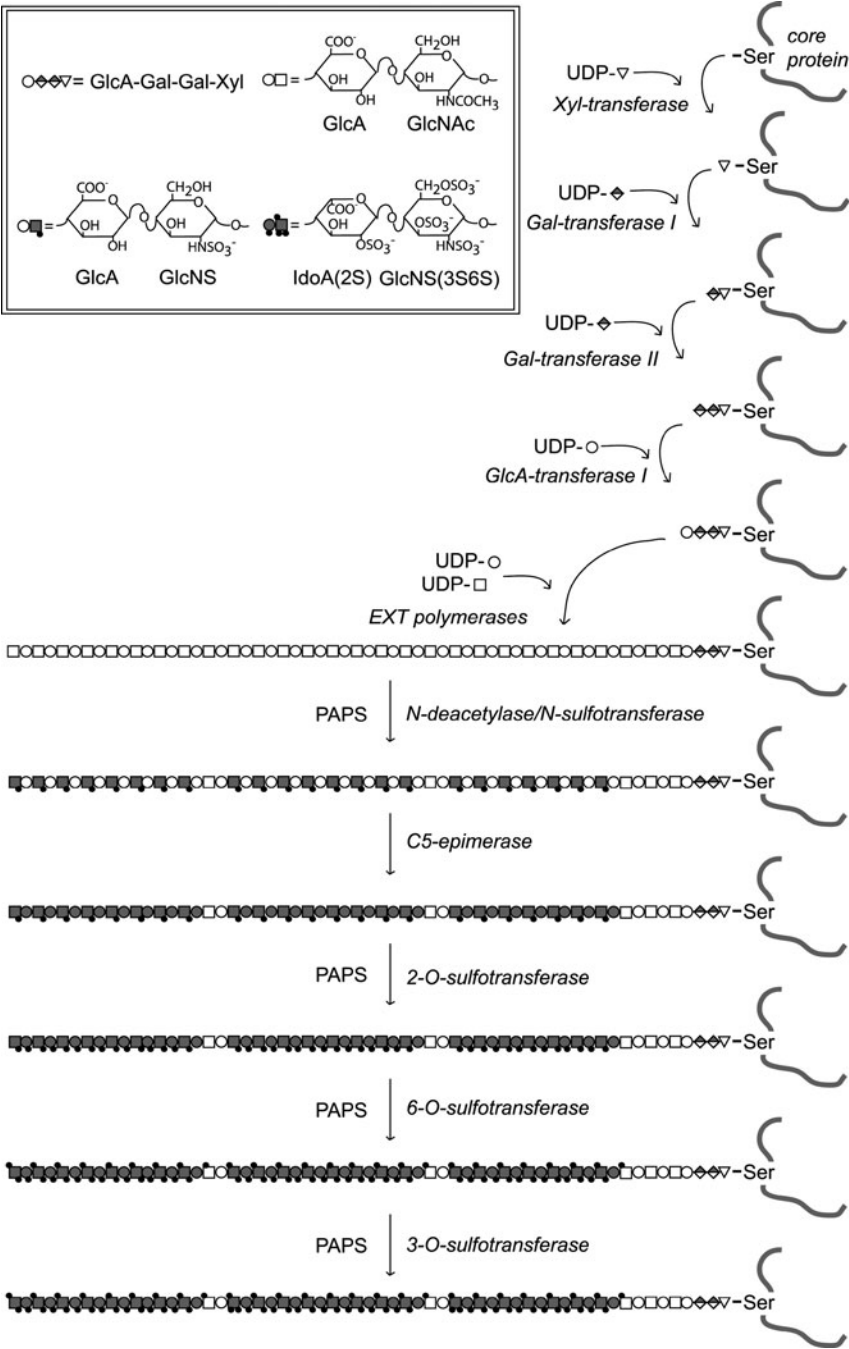


Fig. 3 Heparin/heparan sulfate biosynthesis. The growing polysaccharide is attached to a serine residue in a core protein. Different UDP-sugars and PAPS are used as substrates and each

2.3 Modification

As the HS/heparin chain grows, it is modified by a set of various enzymes (Esko and Lindahl 2001). HS chains are only partly modified, with the modifications occurring in clusters, resulting in polysaccharide chains having regions that are highly sulfated interspersed with unmodified regions. Heparin is more heavily sulfated, containing 80–90% N-sulfated glucosamine, whereas about 30–60% of the glucosamine residues in HS are N-sulfated (Gallagher and Walker 1985; Lyon et al. 1994). D-Glucuronic acid residues adjacent to N-sulfated glucosamine can be epimerized to L-iduronic acid followed by 6-O-sulfation of GlcNAc and 2-O-sulfation of IdoA and, more rarely, of GlcA. Sulfate groups can also be found at the C3-position of GlcNAc, although this modification is not very common. The occurrence of GlcNH₂ residues has also been reported (Westling and Lindahl 2002).

3 The Enzymes

All enzymes taking part in HS/heparin biosynthesis have been cloned. They are transmembrane proteins (with the exception of 3-O-sulfotransferase-1) with the enzymatically active domain located in the Golgi lumen, where HS/heparin synthesis takes place.

3.1 Linkage Region Enzymes

Transfer of the first xylose residue to the core protein is performed by a xylosyltransferase. Two highly similar isoforms, XylT1 and XylT2, with tissue-specific expression patterns exist in mammals (Gotting et al. 2007). Although the XylT2 was cloned already in 2000 (Gotting et al. 2000), its enzyme activity was not demonstrated until several years later when three independent papers on the subject were published (Schon et al. 2006; Cuellar et al. 2007; Voglmeir et al. 2007). It has long been discussed whether xylosylation takes place in the endoplasmic reticulum or in the Golgi compartment. However, by using fluorescently tagged

Fig. 3 (continued) individual reaction step is catalyzed by a specific enzyme. Symbols used for individual monosaccharides are explained in the box to the *upper left*. After synthesis of the linkage region, the polymerase complex composed of EXT1 and EXT2 add alternating units of glucuronic acid and N-acetylglucosamine to the nonreducing end of the chain. In the presence of the sulfate donor 3'-phosphoadenosine 5'-phosphosulfate (PAPS), a series of modifications takes place, beginning with N-deacetylation and N-sulfation of the original N-acetylglucosamine units. N-Deacetylation/N-sulfation is followed by epimerization of glucuronic acid to iduronic acid and finally ending with stepwise O-sulfation of the sugars, including 2-O-sulfation of the uronic acid and 6-O- and 3-O-sulfation of the glucosamine

xylosyltransferases, Schon and colleagues could determine localization of both enzymes to the early cisternae of the Golgi apparatus (Schon et al. 2006).

Although the xylosyltransferases are Golgi-located transmembrane proteins, xylosyltransferase enzyme activity can be detected also in cell culture supernatants and in human body fluids (see references in Gotting et al. 2007). However, the substrate for these enzymes, UDP-xylose, is not found extracellularly, and the function of secreted enzyme is not known. Also, other glycosaminoglycan biosynthesis enzymes are secreted (Nagai et al. 2007). Could proteolytic cleavage of the enzymes be a way of regulating GAG biosynthesis by rapidly downregulating enzyme activity in the Golgi compartment?

The enzymes responsible for the subsequent steps of the linkage region formation, galactosyltransferase-I (GalT1), galactosyltransferase-II (GalT2), and glucuronyltransferase (GlcAT1), occur as single isoforms and have been shown to be located to the medial Golgi (Bai et al. 2001; Pinhal et al. 2001).

Addition of the first GlcNAc residue to the GlcA of the heparin/HS linkage region is performed by enzymes with GlcNAcT-I activity, in contrast to polymerization of the HS chain, which is dependent on enzymes with GlcNAcT-II activity. Two members of the exostosin gene family, Exostosin-like 2 (EXTL2) and EXTL3, have been shown to possess GlcNAc-TI activity, thus being capable of adding the first GlcNAc unit to the heparin/HS chain (Kitagawa et al. 1999; Kim et al. 2001). While EXTL2 possesses only GlcNAcT-I activity, EXTL3 is capable of transferring GlcNAc also in the polymerization reaction. Another member of the exostosin-like gene family, EXTL1, could possibly take part in elongation of heparin/HS chains since it has GlcNAc-TII activity (Kim et al. 2001). However, little is known about the individual contribution of exostosin-like enzymes to HS/heparin biosynthesis in vivo.

3.2 Polymerases

HS/heparin polymerization is carried out by EXT1 and EXT2 (Zak et al. 2002). These enzymes were first characterized as tumor suppressors since heterozygous mutations in the genes encoding the enzymes are responsible for the development of benign skeletal tumors in patients with hereditary multiple exostoses (HME) (McCormick et al. 1999). Both enzymes have been shown to have dual enzyme activities in vitro, GlcA-TII and GlcNAc-TII (Lind et al. 1998; McCormick et al. 2000; Busse and Kusche-Gullberg 2003), but the EXT2 polymerizing activity is weak. While EXT1 alone is able to polymerize HS chains in vitro, EXT2 does not seem to have this capacity (Busse and Kusche-Gullberg 2003). It has been suggested that the role of EXT2 in HS biosynthesis is to act as a chaperone for EXT1 (Wei et al. 2000). Consistent with this idea, there is evidence suggesting that the localization of EXT1 in the Golgi compartment depends on expression of EXT2 (McCormick et al. 2000). It has been demonstrated that the two enzymes form a hetero-oligomeric complex and that this dimer probably represents the biologically

relevant form of the heparin/HS polymerization unit (Kobayashi et al. 2000; McCormick et al. 2000; Senay et al. 2000; Busse and Kusche-Gullberg 2003; Kim et al. 2003). The EXT enzymes may also be part of larger enzyme complexes, sometimes referred to as GAGosomes (Esko and Selleck 2002), see below.

3.3 *Modification Enzymes*

Mammalian HS/heparin modification enzymes, with the exception of C5-epimerase and 2-O-sulfotransferase, exist in several isoforms with four NDSTs, three 6-O-sulfotransferases and seven 3-O-sulfotransferases reported. For an overview of substrate preferences for the different enzymes, see Lindahl and Li (2009).

NDSTs have a key role in HS biosynthesis as further modifications occur mainly in N-sulfated regions. However, 6-O-sulfate groups can be found also in cells lacking N-sulfated HS, showing that N-sulfation is not an absolute prerequisite for other modifications at all occasions (Holmborn et al. 2004). The NDSTs are bifunctional enzymes, responsible for both N-deacetylation and N-sulfation of GlcNAc residues in HS chains (Grobe et al. 2002). The N-sulfotransferase domain has been located to the carboxyl part of the protein and has been crystallized (Berninsone and Hirschberg 1998; Kakuta et al. 1999). The three-dimensional structure of the deacetylase domain is not known, but expression of a truncated form of NDST2 (A66-P604) resulted in a protein with retained N-deacetylase activity, indicating that the domain is located closer to the N-terminal than is the N-sulfotransferase active site (Duncan et al. 2006). Studies on cells expressing NDST1 mutants lacking either N-deacetylase or N-sulfotransferase activity have shown that the two reaction steps do not have to be performed by the same NDST molecule. Instead, two separate NDST molecules can work together, one performing the N-deacetylation reaction and the other transferring the sulfate group (Bengtsson et al. 2003).

Four vertebrate NDST isoforms have been identified and cloned, NDST1–NDST4 (Hashimoto et al. 1992; Eriksson et al. 1994; Orellana et al. 1994; Kusche-Gullberg et al. 1998; Aikawa and Esko 1999; Aikawa et al. 2001). NDST1 and NDST2 transcripts are found in most tissues both during the embryonic stage and in adult mice, whereas NDST3 and NDST4 show more restricted mRNA expression (Kusche-Gullberg et al. 1998; Aikawa et al. 2001; Pallerla et al. 2008). However, transcription levels may not necessarily correlate with translation levels. In fact, as discussed further below, expression of the NDST isoforms may be both translationally and posttranslationally regulated (Grobe and Esko 2002).

In addition to N-sulfated and N-acetylated glucosamine residues, low levels of N-unsubstituted glucosamine can also be detected in HS/heparin preparations (Westling and Lindahl, 2002). Such residues have been suggested to be generated by the N-deacetylase activity of NDST enzymes without subsequent N-sulfation. GlcNH₂ residues have been identified in mouse embryonic stem cells lacking both NDST1 and NDST2 (Holmborn et al. 2004) as well as in tissues of NDST3

knockout mice (Pallerla et al. 2008). It is possible that all NDST isoforms under certain conditions have the capacity to generate GlcNH₂. Alternatively, such residues are formed through other, so far unknown, mechanisms.

After N-sulfation, the C5-epimerase acts to transform some of the GlcA residues into IdoA by epimerization of the C5 carboxyl group. GlcA residues may be recognized as substrates if they are linked to an N-sulfated unit at the nonreducing end (Jacobsson et al. 1984). IdoA residues are thus confined to N-sulfated domains.

2-O-Sulfation is closely associated with epimerization. Most IdoA units are sulfated at the C2 position, whereas 2-O-sulfated GlcA residues are rare (Rong et al. 2001). In cerebral cortex, however, this type of modification is more abundant (Lindahl et al. 1995). 2-O-Sulfation of IdoA units is largely confined to contiguous N-sulfated domains, although 2-O-sulfated IdoA is occasionally found also adjacent to N-acetylated GlcN residues (Rong et al. 2001). The 2-O-sulfotransferase, like the C5-epimerase, only occurs in one single isoform.

There are three enzymes catalyzing the 6-O-sulfotransferase reaction, 6OST1-3 (Habuchi et al. 2000). The occurrence of a 6-OST-2 splice variant has also been reported (Habuchi et al. 2003). The three isoforms differ slightly regarding expression pattern and substrate preferences, but are all able to modify both GlcNAc and GlcNS residues in different sequence settings (Jemth et al. 2003; Smeds et al. 2003).

A 3-O-sulfate group is a crucial component of the antithrombin-binding pentasaccharide of heparin. The high affinity binding of this pentasaccharide to antithrombin results in a conformational change of the protein and enhanced interaction with thrombin leading to inhibition of blood coagulation (Petitou et al. 2003). The involvement of 3-O-sulfate groups in this interaction, as well as in HS binding to the herpes simplex gD protein, where the uncommon 3-O-sulfated GlcNH₂ residues are recognized (Shukla et al. 1999), indicates that 3-O-sulfation probably is dedicated to interactions involving very specific HS structures. Notably, there are seven isoforms of the enzyme responsible for 3-O-sulfation, also suggesting a crucial role for this type of HS modification. Although the 3-O-sulfotransferase-1 has been suggested to be the most critical isoform for producing the antithrombin-binding HS sequence, ablation of the gene in mice does not result in a procoagulant phenotype (Shworak et al. 2002). Instead, genetic-background-dependent lethality and intrauterine growth retardation are observed, but the cause of these abnormalities is not yet known.

3.4 *Postbiosynthesis Endosulfatases*

Postsynthetic modification of HS also occurs, performed by two endosulfatases, Sulf1 and Sulf2, located at the cell surface (Lamanna et al. 2007). These sulfatases act after extracellular translocation of the GAG chains, by cleaving off 6-O-sulfate groups in the internal part of the polysaccharide chain. Whether heparin, exposed in

the extracellular space after mast cell degranulation, is modified by the Sulfs is not known. However, the preferred substrate of the Sulfs is an internal trisulfated disaccharide, abundant in heparin.

3.5 Which Enzyme Isoforms are Present in Mast Cells?

So far, only a few studies have dealt with mast cell expression of heparin/HS biosynthesis enzymes. Obviously, all enzymes responsible for the formation of the linkage region and polymerization of the polysaccharide chain can be expected to be expressed by mast cells. However, it is not known whether xylosyltransferase-1 or -2 (or both) are responsible for the initiation of the heparin chain and which of the EXTL enzymes that participate in heparin formation. From studies of mouse mastocytoma NDSTs, it is clear that NDST2 is the dominating NDST isoform, present at high concentration, while NDST1 transcript is barely detected (Kusche-Gullberg et al. 1998). Accordingly, connective tissue type mast cells from mice deficient in NDST2 lack sulfated heparin, show abnormal morphology and contain reduced amounts of histamine and mast cell proteases (Forsberg et al. 1999; Humphries et al. 1999). The altered morphology and decreased levels of inflammatory mediators are also seen in mice deficient in serglycin, where no proteoglycans are found in the intracellular granules (Abrink et al. 2004).

Heparin contains both iduronic acid and 2-O-sulfate groups and hence the single isoform enzymes C5-epimerase and 2-O-sulfotransferase must be expressed by mast cells. Analysis of glycosaminoglycans isolated from mouse ears, where mast cells are abundant, showed no difference in heparin composition when mice deficient in 6-OST-1 were compared to wild-type mice. Thus, heparin 6-O-sulfation preferentially relies on 6-OST-2 and/or 6-OST-3, at least in skin mast cells (Habuchi et al. 2007). An immortalized mouse mast cell line has been shown to express 3-O-sulfotransferase-1 (Shworak et al. 1997), but it has not been studied whether also other 3-O-sulfotransferase isoforms are expressed.

4 Why Do Mast Cells Form Heparin When Other Cells Synthesize Heparan Sulfate?

As mentioned above, modifications of HS chains are made in clusters resulting in regions that are highly sulfated (NS-domains) interspersed with nonsulfated regions (NA-domains) and with intermediately sulfated regions (NA/NS-domains) usually surrounding the NS-domains (Gallagher 2001). The sulfated regions are not completely modified at all possible sites, resulting in specific patterns depending on cell type and developmental stage (David et al. 1992; Lindahl et al. 1995; Maccarana et al. 1996; Brickman et al. 1998; van Kuppevelt et al. 1998; Jenniskens

et al. 2000, 2002; Allen and Rapraeger 2003; ten Dam et al. 2003; Ledin et al. 2004; Warda et al. 2006). Notably, very little structural variation is seen when HS from the same tissue but from different individuals is compared (Lindahl et al. 1995; Ledin et al. 2004), suggesting that HS biosynthesis is a highly regulated process. Heparin, on the contrary, lacks the pattern of alternating NA- and NS-domains and can be characterized as a more or less continuous NS-domain.

4.1 Enzyme Abundance

How is the domain pattern in HS formed, and why do not all positions available for sulfation become modified in HS biosynthesis? In contrast to DNA in protein synthesis, there is no template to determine the design of the final glycosaminoglycan product. Instead, expression levels of the individual enzymes are obviously an important factor. By regulating the abundance of the enzymes/isoenzymes, at transcriptional or translational levels, or by changing their turnover, different HS/heparin modification patterns may be obtained. As mentioned above, when the NDST2 gene is knocked out in mice, the mast cells are abnormal and lack heparin (Forsberg et al. 1999), although HS from different other tissues of these animals appears unaffected (Ledin et al. 2004). It can therefore be concluded that NDST2 is the NDST isoform mainly responsible for heparin synthesis. When instead mice devoid of NDST1 are analyzed for HS structure, a dramatic reduction of N-sulfation in various tissues, including liver, is observed (Ledin et al. 2006), indicating that N-sulfation in HS producing cells relies mostly on NDST1. However, the question of how the difference in expression levels of the isoforms is regulated remains to be answered.

4.2 Regulation of Enzyme Expression

The knowledge of transcriptional control of HS biosynthesis enzymes is scarce, but the NDST2 gene has been shown to be under regulation of the GA-binding protein, which is a transcription factor (Mori et al. 2001). Mice carrying a mutation in the *mi* allele express abnormal *mi* transcription factor. This results in decreased amounts of NSDT2 protein in skin mast cells as a consequence of disturbed nuclear localization of the GA-binding protein, which normally binds to a GGAA motif in the 5'-untranslated region of NDST2.

Regulation of the NDST proteins probably also occurs at the translational level as suggested by differential expression of constructs in which the different NDST 5'-untranslated regions were ligated to a reporter gene (Grobe and Esko 2002). Evidence for translational regulation of HS biosynthetic enzymes in *Drosophila melanogaster* has also been presented (Bornemann et al. 2008). In addition, post-translational modifications such as glycosylation could play a role as has been shown for one of the chondroitin sulfate sulfotransferases (Yusa et al. 2005). In fact,

glycosylation of NDST protein affects its enzyme activity (Carlsson and Kjellén, unpublished).

4.3 GAGosome Composition

The GAGosome model (Esko and Selleck 2002), suggesting close proximity of the enzymes in a physical complex, also offers a tentative explanation for regulation of HS/heparin modification. Here, the ability of the individual enzymes to associate with the other enzymes/components of the GAGosome as well as their relative concentration will be important (Fig. 4). Supporting the hypothesis, the HS polymerases EXT1 and EXT2 are known to function as a complex (Kobayashi et al. 2000; McCormick et al. 2000; Senay et al. 2000). Moreover, physical association has been observed between XylT and GalT (Schwartz 1975) and between GlcA C5-epimerase and IdoA 2-O-sulfotransferase (Pinhal et al. 2001). Recently, interaction between EXT2 and NDST1 was also reported (Presto et al. 2008). In this study, it was also shown that the expression levels of EXT polymerases can affect the amount of NDST1 protein in cells, in turn influencing HS structure.

Based on our studies of liver HS structure in NDST1- and NDST2-deficient mouse embryos, we previously suggested that NDST1 is preferentially incorporated into the GAGosomes (Ledin et al., 2006). In a control liver, where similar amounts of NDST1 and NDST2 are expressed, the GAGosomes will contain NDST1 and HS will be produced (Fig. 4). In mast cells which express lots of NDST2 compared to NDST1 (Kusche-Gullberg et al. 1998), NDST2 will be the dominating NDST isoform incorporated into the GAGosomes resulting in heparin production (Fig. 4).

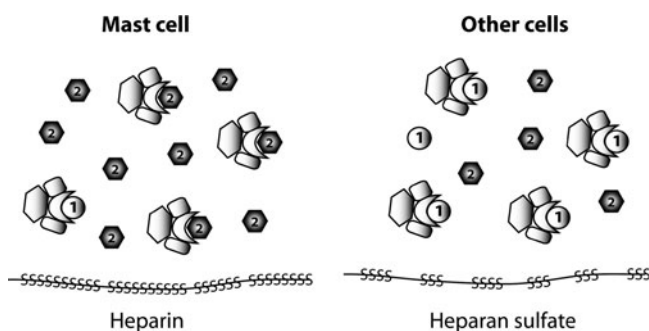


Fig. 4 A tentative model to explain why mast cells make heparin while other cells synthesize heparan sulfate. In most cells, NDST1 and NDST2 are expressed at similar levels, but NDST1 is more readily incorporated into the GAGosome. The NDST1 containing enzyme complexes synthesize heparan sulfate. In heparin-producing mast cells, NDST2 expression is massive, whereas NDST1 transcript is barely detected. Despite its lower affinity for the GAGosome, NDST2 can now be incorporated into GAGosomes, resulting in heparin production

4.4 *Control of Substrate Levels*

Access to the different substrates needed for HS/heparin biosynthesis, i.e. UDP-sugars (UDP-GlcNAc and UDP-GlcA) and the sulfate donor PAPS is also an obvious point of regulation.

4.4.1 UDP-Sugars

Formation of glycosidic linkages between monosaccharides is an energetically unfavorable process, which requires coupling of the monosaccharides to high-energy nucleotides through reaction with UTP. The resulting UDP-sugars can then be used in glycoconjugate synthesis such as GAG formation. While synthesis of UDP-sugars takes place in the cytosol, GAG synthesis occurs in the lumen of ER and Golgi compartments. The activated sugar donors must thus be translocated into these organelles. This is mediated through the action of membrane energy-independent nucleotide sugar antiporters, which shuffle nucleotide sugars into the organelles while simultaneously transporting nucleotide monophosphates back to the cytosol (Berninsone and Hirschberg 2000; Caffaro and Hirschberg 2006). Obviously, altering the concentration of available UDP-sugars by regulating either synthesis or transport of the UDP-sugars may influence HS/heparin biosynthesis.

4.4.2 PAPS: The Sulfate Donor

3'-phosphoadenosine 5'-phosphosulphate (PAPS) is the universal sulfate donor for all biochemical sulfotransferase reactions. For sulfation reactions taking place in the Golgi compartment, as in heparin/HS biosynthesis, inorganic sulfate must be transported into the cell, transformed into its activated form, PAPS, and be translocated into the Golgi where it is used as a substrate. Cellular uptake of sulfate is performed by a number of transmembrane antiporter and symporter molecules (ul Haque et al. 1998). A spectrum of recessively inherited disorders affecting bone and cartilage development have been related to mutations of genes encoding such sulfate transporters (Superti-Furga et al. 1996).

In the cytosol, PAPS is synthesized from inorganic sulfate and ATP in a two-step reaction by PAPS synthase (PAPSS), a bifunctional enzyme containing both an ATP sulfurylase domain and an APS kinase domain needed for the reaction. Two isoforms exist in vertebrates, PAPSS1 and PAPSS2 (Fuda et al. 2002; Strott 2002; Venkatachalam 2003). The expression of the two isoforms differs, with PAPSS1 being found ubiquitously, while PAPSS2 has a more restricted expression pattern (Fuda et al. 2002).

The PAPS synthases are localized to the cytosol and, unexpectedly, to the nucleus. After synthesis, PAPS utilized in HS and heparin sulfation is transported into the Golgi compartment by PAPS transporters, of which two have been identified in humans (Kamiyama et al. 2003, 2006). PAPS transporters probably

act by an antiport mechanism, but the antiporter molecule has so far not been identified. PAP or 5'-AMP are possible candidates (Frederick et al. 2008).

Recent results in our laboratory indicate that the PAPS concentration may be a critical factor for regulation of NS-domain length (Carlsson et al. 2008). In the absence of PAPS, NDST catalyzes limited and seemingly random N-deacetylation of GlcNAc residues. In the presence of PAPS, the NDST enzymes work in a processive manner adding sulfate groups to contiguous disaccharides creating NS-domains, the length of which depends on the concentration of PAPS.

5 Past, Present, and Future

Studies of heparin biosynthesis have been ongoing since 1960s, when Jeremiah Silbert started to study how mast cell granule fraction incorporated radioactively labeled UDP-sugars into a polysaccharide that was degradable with heparinase (see refs. 9 and 10 in Silbert 2009). Many more important articles on heparin biosynthesis have come from this lab (see Silbert 2009). In the seventies, Ulf Lindahl began his investigations of heparin biosynthesis using microsomal fractions prepared from a transplantable mouse mastocytoma (see Lindahl 2000). In this system, the order of the modification reactions was worked out, and it was demonstrated that the substrate specificities of the modification enzymes to a large extent regulated the final structure of the polysaccharide. The enzymes taking part in the biosynthesis reactions of both heparin and HS biosynthesis are now all known and have been cloned through the efforts of several labs, including that of Ulf Lindahl (Lindahl and Li 2009). Much work still remains, e.g. to understand how the enzymes are assembled in the Golgi compartment, the nature of the potential GAGosome, and how enzyme expression is regulated. With this knowledge, we may in the future be able to influence both heparin and HS biosyntheses *in vivo*. In addition, it may be possible to construct biosynthetic machineries consisting of selected recombinant enzymes able to synthesize heparin or HS oligosaccharides of desired structure.

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