pathway of oligosaccharide assembly and determine how such changes affect function. We recently showed that when mannosamine was added to canine kidney cell cultures, it caused the formation of unusual and truncated lipid-linked oligosaccharides which could still be utilized as glycosyl donors. The mannosamine was incorporated into these lipid-linked oligosaccharides and this apparantly prevented the addition of 1,2-linked mannoses to these structures. Interestingly enough, the mannosamine-containing oligosaccharides were still transferred to protein. Mannosamine also prevents the assembly of the glycan portion of the GPI-anchor, and thus blocks the attachment of protein to this structure. In this case also, mannosamine is incorporated into the glycan, probably in the second mannose position and prevents addition of the third mannose. In terms of *N*-linked oligosaccharides, a number of "alkaloid-like" compounds have been isolated, mostly from plants, or synthesized chemically and these compounds inhibit the glycosidases that are involved in the trimming of the $Glc_3Man_8(GlcNAc)_2$-protein to produce various types of complex structures. A number of these compounds (i.e., swainsonine, castanospermine, deoxynojirimycin, deoxymannojirimycin) have been well studied, but several new α-mannosidase inhibitors are of considerable interest. These compounds are kifunensine which inhibits mannosidase I, mannostatin which inhibits mannosidase II and mannoamidrzone which is a more general mannosidase inhibitor (Supported by NIH grant HL 17783).

S1.6

Substrate Specificities of Glycosyltransferases Involved in the Formation of Heparin and of *E. Coli* K5 Capsular Polysaccharide

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The GlcA and GlcNAc transferases catalyzing the initial polymerization reaction in the biosynthesis of heparin are assayed by measuring the transfer of radiolabelled monosaccharide units from the appropriate UDP-sugars to the nonreducing ends of exogenous oligosaccharide acceptors. Such acceptors were derived from *E. coli* K5 capsular polysaccharide which has the same repeating disaccharide unit as the heparin precursor polysaccharide.

Subsequent to polymerization of the heparin precursor, a series of modification reactions occur, involving *N*-deacetylation/*N*-sulfation of the GlcNAc units, C5 epimerization of the D-GlcA to L-IdoA units and *O*-sulfation at various positions. Concomitant sulfation promotes elongation of the heparin-precursor polysaccharide in a microsomal fraction from a heparin-producing mouse mastocytoma (Lidholt *et al.* (1989) *Biochem. J.* **261**, 999-1007). Accordingly, partially *N*-sulfated oligosaccharides were found to serve as substrates for the microsomal GlcA and GlcNAc transferases (Lidholt, K. and Lindahl, U. (1992) *Biochem J.* **287**, 21-29).

An octasaccharide with the structure $[GlcA\text{-}GlcNAc]_3$-GlcA-$aMan_R$ ($aMan_R$, anhydromannitol) derived from the *E.coli* capsular polysaccharide, was partially *N*-deacetylated (by hydrazinolysis) and *N*-sulfated (reaction with trimethylamine-sulfurtrioxide). The corresponding heptasaccharides were obtained by β-D-glucuronidase digestion of the octasaccharides. Amongst the variously *N*-substituted heptasaccharides available, a GlcNAc-GlcA-$GlcNSO_3$-GlcA-$GlcNSO_3$-GlcA-$aMan_R$ structure was clearly favored as acceptor for the mastocytomal GlcA-transferase. The GlcNAc-transferase, tested with the corresponding octasaccharide substrates, was less selective with regard to *N*-substituent pattern. These findings imply specific coupling between the chain elongation and *N*-sulfation steps during the biosynthetic process.

The same partially *N*-sulfated oligosaccharides were tested as substrates for glycosyltransferases in a membrane preparation from *E. coli* K5. GlcA and GlcNac transfer reactions were found similar to those implicated in heparin biosynthesis, and with similar recognition of *N*-substituents in the acceptor saccharide structures.

S1.7

Biosynthesis of Heparin/Heparan Sulfate: Copurification of GlcNAc and GlcA Transferases Suggests Two Enzyme Activities in One Protein

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The biosynthesis of heparin/heparan sulfate is initiated by the formation of polysaccharide chains, composed of alternating GlcA and GlcNAc units. Subsequent modification reactions, presumably coupled to the polymerization reaction (Lidholt, K and Lindahl, U (1992) *Biochem J.* **287**, 21 – 29), involve *N*-deacetylation/*N*-sulfation of the GlcNAc units, C5 epimerization of the GlcA unit to IdoA units and *O*-sulfation at various positions (Lindahl *et al.(1986) Trends Biochem. Sci.* **11**, 221 – 225).

The GlcA and GlcNAc transferase activities catalyzing the polymerization reaction can be assayed in solubilized systems, by measuring the transfer of radiolabeled monosaccharide units from the appropriate UDP-sugars to exogenous oligosaccharide acceptors. Such acceptors were derived from *E.Coli* K5 capsular polysaccharide which has the same repeating disaccharide unit as the heparin/heparan sulfate precursor polysaccharide.

Previous studies using CHO (Chinese hamster ovary) cells showed that a single mutation impedes both the GlcNAc and the GlcA transferase reactions (Lidholt *et al.* (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 2267 – 2271), suggesting that the two catalytic activities may be expressed by the same protein.

Purification of the GlcNAc and GlcA transferases from solubilized, heparin-producing mouse mastocytoma tissue showed that the two activities appeared together in all steps tested. The transferases bound to DEAE-Sephacel, Reactive Yellow-Sepharose, UDP-hexanolamine-agarose and 3′, 5′-ADP-agarose. The two enzyme activities also remained associated through gel filtration.

Search for a more easily accessible source of the glycosyltransferases showed appreciable GlcNAc- and GlcA-transferase activities in solubilized form, in bovine serum. The two activities were copurified through consecutive chromatographies on: Reactive Red-Sepharose, ConA-Sepharose, UDP-hexanolamine-agarose, BlueSepharose and Superose 12 and through chromatofocusing, with an apparent ~200 000-