A New Model for the Domain Structure of Heparan Sulfate Based on the Novel Specificity of K5 Lyase*

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Elucidation of the molecular structure of heparan sulfate (HS) is the key to understanding its functional versatility as a co-receptor for growth factors and morphogens. We have identified and exploited the novel substrate specificity of the coliphage K5 lyase in studies of the domain organization of HS. We show that K5 lyase cleaves HS principally within non-sulfated sequences of four or more N-acetylated disaccharides. Uniquely, sections comprising alternating N-acetylated and N-sulfated units are resistant to the enzyme, as are the highly sulfated S domains. Spacing of the K5 lyase cleavage sites (~7–8 kDa) is similar to that of the S domains. On the basis of these findings, we propose a refined model of the structure of HS in which N-acetylated sequences of four to five disaccharide units (GlcNAc-GlcUA)₄–₅ are positioned centrally between the S domains. The latter are embedded within N-acetylated and N-sulfated sequences, forming extended regions of hypervariable sulfation distributed at regular intervals along the polymer chain. K5 lyase provides a means of excision of these composite sulfated regions for structural and functional analyses.

Heparan sulfate (HS) is a linear polysaccharide found in all animal tissues, where it occurs as a cell surface or extracellular matrix proteoglycan (1, 2). Proteoglycans interact with a variety of protein ligands (3), through which they regulate a wide range of biological activities, including developmental processes (4, 5), angiogenesis (6), blood coagulation (7), cell adhesion (8), and tumor metastasis (9).

HS biosynthesis is a very complex process (for a review see Ref. 10); the initial product of chain polymerization is the polysaccharide precursor heparan, made up of repeating disaccharide units of 1,4-linked GlcNAc and β1,4-linked glucuronic acid (GlcUA). Subsequent modifications result in the formation of clusters of sulfated residues at intervals along the chain. These are the so-called S domains, separated by intervening regions of intermediate or low sulfation that include unmodified disaccharides (11). The initial step in the transition from heparan to HS is catalyzed by N-deacetylation/N-sulfotransferase enzymes, which typically convert 40–50% of the GlcNAc residues to N-sulfoglucosamine (GlcNS). Subsequently, GlcUAs adjacent to these GlcNS units are frequently epimerized to form iduronate (IdoUA). The resulting GlcNS/IdoUA-rich domains are then the primary substrates for the O-sulfotransferases that can add sulfate groups to C-2 of IdoUA and C-6 and C-3 of GlcNSO₃. Not all potential monosaccharide substrates become O-sulfated, and this leads to considerable variation in sulfation patterns within the S domains. Additionally, alternating sequences made up of GlcNS- and GlcNAC-containing disaccharides (NA/NS domains) are also found in the HS chain (12), and 6-O-sulfate groups frequently occur within these regions including GlcNAc (17). A significant proportion of the heparan chain fails to be modified in any way, and thus extended sequences of GlcNAc-GlcUA disaccharides (NAc domains) still exist in the mature HS.

To date, most of the proteins for which an HS-binding sequence has been identified interact with the heterogeneous S domains. Given the unmodified nature of the NAc domains, it is thought that this type of sequence has little or no interaction with protein ligands and merely acts to space the S domains correctly. Little is known about the relative positioning of the NA/NS domains within the polymer chain or the role they play in ligand binding, although they are thought to occur primarily at the borders between S domains and NAc domains, i.e. forming transition zones between the two.

In some notable instances, e.g. antithrombin III (13), fibroblast growth factor-1 (14, 15), and fibroblast growth factor-2 (16, 17), the interactions between protein and the S domains are dependent on a critical sequence of sugars carrying essential sulfate modifications. For some proteins, the saccharide requirements for ligand binding are less well defined, although the length and the general composition of the binding sequences have been characterized, i.e. a preference for C-6 sulfation over C-2 or N-sulfation, in the case of both hepatocyte growth factor/scatter factor (18) and vascular endothelial growth factor (19). Recently, the C-terminal endostatin fragment of collagen XVIII has been proposed to bind to S domains interrupted by one GlcNAc residue (20), and with specific requirements for sulfation at C-6 of amino sugars (21). In a third family of HS-binding proteins, the region of HS found to interact with the ligand is more extended, encompassing typically two S domains, including the intervening regions of lower sulfation. This kind of interaction is a particular characteristic of the multimeric cytokines, e.g. platelet factor-4 (22), interleukin-8 (23), and interferon-γ (24).
K5 Lyase Digestion of HS

Methods

Preparation of Intact HS Chains—Maintenance, radiolabeling, and extraction of HS chains from cultures of NIH-3T3 fibroblasts were carried out as described previously (32). Briefly, the culture medium from cells radiolabeled with [3H]glucosamine was digested with Pronase, and glycosaminoglycans were recovered by DEAE-Sephalac. Contaminating galactosaminoglycans were removed by digestion with chondroitinase ABC. Subsequent Sepharose CL-6B gel filtration yielded intact HS chains.

Establishment of the K5 Lyase Incubation Conditions—Cleavage of the K5 polysaccharide by K5 lyase is through a β-elimination mechanism, which generates oligosaccharides terminating with a Δ4,5-unsaturated uronic acid at the non-reducing end (33). This enables the extent of degradation to be followed by monitoring the increase in absorbance at 232 nm. With 0.5 mg/ml K5 polysaccharide and 10 μg of enzyme, the rate of degradation is initially very rapid, before leveling off and continuing at a much slower rate (data not shown). After 2 h the digestion was complete, because addition of more enzyme resulted in no further increase in the UV absorbance.

Specific Enzyme Digestions of HS—Previous studies have indicated that K5 lyase has activity over a broad pH range from 5.5 up to 9 (31), with an optimum at pH 8.5. Digests were therefore performed in a total volume of 1 ml of 25 mM Tris acetate, pH 8.5, with 10 μg of enzyme. Heparinase I or III digests of HS were performed in a total volume of 1 ml of 0.1 mM sodium acetate, 0.1 mM calcium acetate, pH 7.0, with 2 mIU of enzyme. All enzyme digestions were for 16 h at 37 °C. Digestion products were analyzed by gel filtration chromatography on a Bio-Gel P10 column eluted with 0.25 mM NH4HCO3 at a flow rate of 4 ml h⁻¹ or a Sepharose CL6B column (1.5 × 70 cm) eluted with 0.2 M NH4HCO3 at a flow rate of 12 ml h⁻¹.

Strong Anion-exchange HPLC—Large K5 lyase-resistant oligosaccharides, which eluted in the void volume of a Bio-Gel P10 column (1.5 × 170 cm), were further resolved using strong anion-exchange (SAX) HPLC. Samples were applied to a ProPac PA-1 column (4.6 × 250 mm) pre-equilibrated with MilliQ water adjusted to pH 3.5 with HCl. Elution was effected with a biphase NaCl gradient of 0–0.3 M NaCl over 7 min, followed by 0.3–1.1 M NaCl over 120 min, at a flow rate of 1 ml min⁻¹. Fractions of 0.5 ml were collected and aliquots monitored for radioactivity. Relevant fractions were pooled, concentrated, and desalted on a PD10 column eluted with water and then evaporated to dryness on a centrifugal evaporator.

Disaccharide Analysis—HS samples were completely digested with a combination of heparinas I–III (2 mIU of each) and analyzed for their disaccharide compositions, by SAX-HPLC, as described previously (34).

Preparation of Modified Glycosaminoglycans—Completely desulfated heparin was produced by the sequential de-N-sulfation (35), re-N-sulfation (36), de-O-sulfation (37), and de-N-sulfation (38) of heparin. Disaccharide analysis revealed the main disaccharide present to be the unsulfated UA-GlcN (99%), with the remaining trace being UA-GlcNac (0.7%)

N-Sulfated K5 polysaccharide was kindly supplied by Dr. P. Oreste at Glycodies (Milan, Italy). Disaccharide analysis revealed its sole disaccharide component to be UA-GlCNac.

Nitric Acid Scission—Nitric acid deaminative cleavage of HS was carried out at pH 1.5 using a ratio of nitric acid to sample of 2:1 (v/v) (37). After incubation at room temperature for 30 min, the reaction was stopped by neutralization with 2 M Na2CO3.

RESULTS

K5 Lyase Digestion of HS, Comparison with Heparinase I and Heparinase III—HS was digested with either K5 lyase, heparinase I, or heparinase III and fractionated on Sepharose CL-6B (Fig. 1) or Bio-Gel P10 (Fig. 2) to determine the size of the products. Digestion of HS with K5 lyase caused a shift in peak elution position on Sepharose CL-6B from a Kav of 0.36–0.76 (Fig. 1, A and B), corresponding to a change in molecular mass from ~45 kDa for intact HS to 7.5 kDa for K5 lyase-digested chains (by reference to the calibration curve of Wasteson (38)). Heparinase I-digested chains eluted with a similar size (~7.5 kDa) (Fig. 1C), whereas the products of a heparinase III digest eluted predominantly in the total volume of the column (i.e., ~< 2.5 kDa) (Fig. 1D).

A more detailed comparison of the sizes of the digested fragments can be seen in the Bio-Gel P10 profiles (Fig. 2).
Intact HS elutes in the void volume (Fig. 2A). After digestion with K5 lyase (Fig. 2B), a series of small peaks representing oligosaccharides ranging in size from dp2 up to dp20 were resolved, though the majority of the oligosaccharides still eluted in the void volume. A similar profile was produced for heparinase I-degraded HS, although the degree of depolymerization was less than that observed with K5 lyase (Fig. 2C). As expected from previous studies (16, 32), digestion by heparinase III cleaved almost entirely to 0% at dp2 to 50% at dp12. Beyond dp14 the increase in sulfated disaccharides was much less pronounced and eventually appears to plateau at around 55%.

Location of N-Sulfated Disaccharides in K5 Lyase-generated HS Oligosaccharides—Information on the positioning of the N-sulfated disaccharides within K5 lyase-generated oligosaccharides of size dp4–10 (see Fig. 2B) was obtained by deamidative cleavage with low pH nitrous acid, followed by Bio-Gel P-10 chromatography (Fig. 4). The sizes of the nitrous acid cleavage products, and their relative proportions, indicates the likely positioning of the N-sulfated units.

Cleavage of K5 Polysaccharide by K5 Lyase—Previous studies (33) on K5 lyase reported that the main degradation products from its action upon K5 polysaccharide were dp6–dp10. However, smaller oligosaccharides were detected when HS was digested with the lyase (Fig. 2B). Therefore, the degradation of K5 polysaccharide was re-examined.

Bio-Gel P10 separation of K5 lyase-digested K5 polysaccharide yielded a range of oligosaccharides from dp2 to dp8 (Fig. 5).
Filtration column (1.5 × 75 cm), were chromatographed on a Bio-Gel P10 gel (B, C, A), and dp10 (B) with low pH nitrous acid. Fractions were monitored for radioactivity.

These sizes were confirmed by re-chromatography of pooled fractions and comparison of their elution positions with those of sized HS oligosaccharides.

Fig. 4. Nitrous acid degradation profiles of K5 lyase-resistant, dp6–10 HS oligosaccharides. Oligosaccharides corresponding to dp6 (A), dp8 (B), and dp10 (C), which had been produced by digestion of HS with K5 lyase (see Fig. 2B), were chromatographed on a Bio-Gel P10 gel filtration column (1.5 × 75 cm) before (---) and after (——) incubation with low pH nitrous acid. Fractions were monitored for radioactivity.

Fig. 5. Gel filtration profiles of K5 polysaccharide before and after digestion with K5 lyase. K5 polysaccharide before (A) and after (B) digestion with K5 lyase was chromatographed on a Bio-Gel P10 gel filtration column (1.5 × 75 cm). The eluent was monitored for UV absorbance at 232 nm.

These results show that the K5 lyase can degrade contiguous N-acetylated oligosaccharides as small as a dp6, but relatively ineffectively. Its minimal effective substrate is more likely to be N-acetylated disaccharides. The proportion of disaccharides, derived from alternating NA/NS disaccharides) demonstrates that the K5 enzyme does not degrade the NA/NS domains. In addition, the absence of species dp ≥10, and the minor content of dp8s, indicates that contiguous sequences of ≥4 N-acetylated units are the major substrates for the K5 lyase enzyme in the HS chain as well as in the K5 polysaccharide.

To analyze further the range of HS species resistant to K5 lyase, the resistant fraction (i.e., material in the Volusion on SAX-HPLC column (Fig. 7). Because of the highly heterogeneous nature of this enzyme-resistant material, it did not clearly resolve into discrete species. The majority instead eluted as a broad peak spanning the range 0.5–1 M NaCl. This was arbitrarily split into 10 fractions (as shown), and each was analyzed for its disaccharide composition (Table I).

As might be expected, the percentage of both the non-sulfated (UA-GlcNAc) and the major mono-sulfated (UA-GlcNS) disaccharides gradually fell as the overall charge of the oligosaccharides increased. These units were replaced mainly by the fully sulfated UA(2S)-GlcNS(6S) disaccharide. Most interesting, there were almost no changes in UA-GlcNAc(8S) and UA(2S)-GlcNAc contents across these fragments and only very modest differences in UA-GlcNS(8S) and UA(2S)-GlcNS disaccharides.

Information on how the lengths of the S domains alter across the SAX-HPLC profile of the K5 lyase-resistant HS fragments was obtained by digesting selected fractions (Fig. 8, fractions 1, 3, 5, and 7) with heparinase III, followed by Bio-Gel P10 chromatography (Fig. 8, A–D).

In fraction 1, 29% of the sequences compose S domains (i.e., fragments dp ≥4 after heparinase III digestion), and these are nearly all dp4 in length (Fig. 8A). As the S-HPLC elution time increases, longer and more abundant S domains are observed, in agreement with the effect of sulfation upon SAX retention. For example, in fraction 7 the S domains ranged from dp4 to dp16 in size (Fig. 8D).

Changes in the distribution of the N-sulfated and N-acetylated disaccharides in and around the S domains in these fragments were also examined by their susceptibility to cleavage with low pH nitrous acid, as analyzed by Bio-Gel P-10 chromatography (Fig. 8, E–H). The nitrous acid scission profiles of each pool were quite similar (Fig. 8, E–H), in contrast to the heparinase III digestion profiles (Fig. 8, A–D). The major products from all fractions were dp4, indicative of NAVNS sequences. The proportion of disaccharides, derived from contiguous N-sulfated sequences, generally increases through the range from fractions 1 to 7, mirroring the increase in S domain content (compare Fig. 8, A–D and E–H). Sequences containing two consecutive N-acetylated disaccharide units (i.e., dp6) make up 14–19% of each fraction, with sequences of three such units (i.e., dp8) making up 3–9%.
Finally, we determined the size of SAX-HPLC-derived fractions 2, 4, 6, and 8 (Fig. 7) by Sepharose CL-6B gel filtration chromatography. Most surprising, all the fractions eluted with a similar broad size distribution of ~3–12.5 kDa, centered around an average of 7.5 kDa (data not shown). This is similar to the main peak seen in the K5 lyase digest of the parent HS (Fig. 1B).

DISCUSSION

In this study we show for the first time that the bacteriophage K5 lyase can degrade HS. This degradation excises a novel range of oligosaccharides distinct from those that can be produced with current tools for HS degradation. Structural analysis of these fragments has given us new insights into the domain structure of HS.

The K5 polysaccharide is identical in structure to the HS precursor, heparan, prior to its modification by epimerization and sulfation. K5 polysaccharide is readily degraded by K5 lyase to yield a mixture of oligosaccharides ranging from dp2 to dp8. This is in contrast to the findings of a previous study (33) where the predominant products were dp6 to dp10. A possible explanation may be that in the present work a pure, recombinant soluble form of the enzyme was used, whereas previously the intact bacteriophage was the source of an immobilized enzyme. The preference shown by K5 lyase for a substrate comprising at least four disaccharides, and its inability to degrade down to predominantly disaccharide products, suggests a strong tendency toward an endolytic rather than exolytic action.

The K5 lyase displays a very strict structural specificity. It does not degrade hyaluronan (an isomer of K5 polysaccharide), demonstrating that it will only cleave at an α1–4- and not a β1–4-hexosaminidic linkage. Similarly, its inability to degrade N-sulfated K5 polysaccharide indicates the antagonistic effect of N-sulfates, even when in combination with GlcUA residues. This in itself demonstrates that the very first modification in HS biosynthesis, namely the removal of the N-acetyl group and its replacement by an N-sulfate, is enough to introduce resistance to the enzyme. In addition, the almost complete failure of the enzyme to cleave a fully desulfated, re-N-acetylated heparin (comprising mainly GlcNAc α1–4 IdoUA repeat units) indicates the absolute importance of GlcUA residues for enzyme cleavage. The substrate preference of K5 lyase is reflected in its degradative action upon HS. Digestion of HS gives rise to a wide range of saccharides, ranging in size from dp2 to >26. Compositional analyses of these fragments (Fig. 3) indicate that sulfation within HS prevents cleavage by the enzyme, as predicted from the pattern of resistance shown by the substrates discussed above. As the length of the K5 lyase-resistant fragments increases, the proportion of modified constituent disaccharides also increases, reaching a maximum at approximately dp14 (Fig. 3). This indicates that fragments dp ≈ 14 do not just contain progressively longer S domains but must possess other motifs such as UA-GlcNS disaccharides.

Disaccharide analysis of small K5 lyase-resistant fragments from HS revealed that most dp6–10 species contain a single N-sulfated disaccharide. We have analyzed the positioning within the fragments of this N-sulfated unit. The major nitrous acid cleavage products of dp6–10 fragments are dp4s (Fig. 4). This suggests that the N-sulfated disaccharide is positioned internally. It is likely that these K5-lyase released fragments originate from regions within the intact HS chains where an isolated N-sulfated disaccharide is present within long sequences of N-acetylated disaccharides. No alternating NA/NS-type sequences were found within the dp6–10 size fractions.

The elution position of a small proportion of each dp6–10 pool was unaffected by nitrous acid treatment (Fig. 4). However, these contained only N-acetylated disaccharides (data not shown). Considering that complete K5 lyase degradation of K5 polysaccharide yields products as large as dp8, it is not surprising that nitrous acid-resistant fragments of similar length are generated during K5 lyase digestion of HS.

We have also analyzed the much larger K5 lyase-resistant fragments of HS that were recovered from the void volume of the Bio-Gel P10 gel filtration column (Fig. 2). This showed that 16% of disaccharides within these fragments could be excised by nitrous acid in the form of dp6 and dp8 species. No K5 lyase-resistant sequences were found that contained more than consecutive GlcNAc units, even though N-acetylated sequences of ≥dp8 comprise 27% of the parent HS (Fig. 6A). In contrast, 60% of the nitrous acid scission products from the large K5 lyase-generated fragments comprised tetrasaccharides and thus came from alternating NA/NS sequences, whereas 21% were disaccharides originating from S domains (Fig. 6B). Both the tetrasaccharides and disaccharides are more highly represented in the K5 lyase-resistant fragments than in the parent HS (Fig. 6). Overall these findings confirm that the K5 lyase cleaves outside of the modified regions of the HS chain, excising fragments mainly composed of both NA/NS- and S domains, indicating the close proximity of the two within the intact HS chain.

Table 1

| Pool number | Disaccharide as % total disaccharides |
|-------------|--------------------------------------|
| 1           | 2                                     |
| 2           | 3                                     |
| 3           | 4                                     |
| 4           | 5                                     |
| 5           | 6                                     |
| 6           | 7                                     |
| 7           | 8                                     |
| 8           | 9                                     |
| 9           | 10                                    |
| 10          | Total pool                            |

Each of the 10 pooled fractions from the SAX-HPLC profile in Fig. 7 were analyzed for their disaccharide compositions by SAX-HPLC (see “Methods”).

Fig. 7. Charge fractionation of the large K5 lyase-resistant HS oligosaccharides. K5 lyase-resistant HS fragments eluting in the void volume of Bio-Gel P10 (see Fig. 2B) were chromatographed on a ProPac PA-1 SAX-HPLC column (see “Methods”). Fractions were monitored for radioactivity.
We have shown that there is a considerable degree of size and charge heterogeneity within the long K5 lyase-resistant HS fragments as revealed by fractionation on SAX-HPLC (Fig. 7). As the overall charge carried by these fragments increased, analysis of their heparinase III cleavage products showed that this was primarily because of an increase in the range and size of S domains found within them (Fig. 8, A–D). The appearance of larger S domains correlated with an increasing content of the tri-sulfated disaccharide, UA(2S)-GlcNS(6S), suggesting that larger S domains are sulfated to a greater extent than shorter ones, confirming previous sequencing data on these domains (32, 39).

Although it is not formally proven that the NA/NS regions flank S domains, it is the case that in the nitrous acid analyses, tetrasaccharides originating from NA/NS domains were only detected in conjunction with disaccharides originating from S domains (Figs. 6 and 8). This does suggest that the two domains are in close proximity, if not contiguous, within the HS chain, fitting the description of NA/NS domains as transition zones.

Previously, the isolation and identification of presumed transition zone sequences in HS, and their relationship with the better characterized S domains, were hampered by the lack of a scission method that only cleaved HS in the unmodified regions of the chain. Available scission techniques either disrupted the transition zones (heparinase III), the S domains (heparinase I), or both (low pH nitrous acid). K5 lyase now meets this need and has enabled us to show that S domains and NA/NS domains can be isolated in tandem in the same oligosaccharide. Despite the increases in length and sulfation of S domains in subfractions of progressively higher charge densities, the level of O-sulfation in the transition zones, as revealed by the content of both UA-GlcNAc(6S) and UA-GlcNS(6S), was remarkably consistent (Table I). This indicates that O-sulfation in the NA/NS and S domains is independently regulated with variations centered principally on the S domains, at least in the case of the 3T3 cell-derived HS studied here. Moreover, the increase in S domain length in oligosaccharides of higher charge density coincided with a reduction in the amount of NA/NS domains as revealed by nitrous acid scission (note the increase in content of dp2 peak derived from S domains, compared with dp4 from NA/NS domains in Fig. 8). This suggests that, during the biosynthesis of HS, long S domains are formed from fusion of shorter ones with loss of intervening transition zone sequences.

K5 lyase and heparinase I clearly possess radically different substrate specificities. However, both enzymes leave transition zones intact. K5 lyase excises oligosaccharides in which intact S domains remain linked to their flanking transition zones, whereas heparinase I excises oligosaccharides in which intact NAc domains are attached to transition zones. Both enzymes also degrade intact HS from an average molecular mass of ~45 kDa to an average of 7.5 kDa and generate products of similar size range and distribution. These findings imply that the distances between K5 lyase cleavage sites within the HS chain are broadly

![Image](https://example.com/image1.png)

**Fig. 8.** Analysis of S domain length and nitrous acid susceptibility across the range of charge-fractionated, large K5 lyase-resistant HS fragments. SAX-HPLC separated fractions of large K5 lyase-resistant HS fragments were pooled as shown in Fig. 7 and then digested with either heparinase III (A–D) or low pH nitrous acid (E–H), before chromatography on a Bio-Gel P10 gel filtration column (1.5 × 160 cm). Fractions were monitored for radioactivity. A and E, pool 1; B and F, pool 3; C and G, pool 5; and D and H, pool 7.

![Image](https://example.com/image2.png)

**Fig. 9.** A refined model of HS domain structure. An ordered structure is proposed for HS in which the polymer chain is characterized by an alternating arrangement of composite sulfated regions and long N-acetylated sequences; the latter are interspersed with occasional solitary GlcNS residues. The domain organization is such that the distances between the main K5 lyase cleavage sites (NAc regions) are similar to the spacing of the heparinase I cleavage sites (the S domains). Thus the unmodified NAc sequences are positioned centrally between the most highly modified regions (i.e. the S domains). This must reflect a tightly regulated mechanism of polymer biosynthesis. The composite sulfated domains are structurally complex being composed of S domains flanked by transition zone sequences (NA/NS domains), each with their own distinctive patterns of N- and O-sulfation. The model represents an average structure, and clearly there will be chain-to-chain variation in domain spacing within any HS population.
similar to the spacing of the heparinase I sites, being equivalent to an average distance of 16–18 disaccharides.

The foregoing results enable some significant additions to be made to the model we originally proposed for the structure of HS (40). The original model showed a series of S domains distributed in a relatively uniform manner along the HS chain and separated by non-sulfated regions; the S domain spacing of ∼16–18 disaccharides was based on the average size of heparinase I-resistant fragments and is confirmed by the findings in the present paper (Fig. 2). We can now add to this model the following new information. (a) The NA/NS domains are in close proximity to, and most likely positioned on both sides, of the S domains to form “composite” regions of sulfation. (b) The longest sequences of unmodified N-acetylated disaccharides (NAc domains of dp ≥8) are located centrally between the S domains; thus, within the constraints of the overall molecular organization of HS, the extended unmodified sections are maximally separated from the most highly modified regions. (c) Solitary N-sulfated disaccharides are present at low frequency within predominantly unmodified sections; these were identified by nitrous acid scission in K5-resistant fragments of dp6–10 (Fig. 4).

These new findings are incorporated into the new refined model depicted in Fig. 9. In this model an ordered polymeric structure is shown in which the S domains and the long NAc domains (i.e. heparinase I and K5 lyase cleavage sites, respectively) are arranged in an alternating and equidistant manner along the polymer chain. The model represents an average structure, and there will be some variations in domain spacing within any HS population. The S domains are embedded in transition zones to form long and complex segments of sulfated sugar residues that are likely to comprise the major protein recognition regions in HS-protein interactions. Uniquely, K5 lyase provides a means of excision for these composite regions of sulfation and thus, for the first time, enables their structure-activity relationships to be evaluated.

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