Heparan Sulfate Oligosaccharides Require 6-O-Sulfation for Promotion of Basic Fibroblast Growth Factor Mitogenic Activity*

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The interaction of heparan sulfate (HS) with basic fibroblast growth factor (bFGF) is influential in enabbling the growth factor to bind to its cell surface tyrosine kinase receptor. In this study, we have investigated further the structural properties of HS required to mediate the activity of bFGF in a mitogenic assay. We have prepared a library of heparinase III-generated HS oligosaccharides fractionated by both their size (dp6-dp12) and sulfate content. The ability of these oligosaccharides to activate bFGF in a mitogenic assay was then correlated with their length and disaccharide composition. All octa- and hexasaccharide fractions tested were unable to activate bFGF. Dodeca- and decasaccharide fractions were found to contain both activating and non-activating oligosaccharides, and showed a clear correlation between total sulfate content and the level of activatory activity. Disaccharide analysis of a range of dodeca- and decasaccharide fractions showed that both activating and non-activating oligosaccharides were composed mainly of N-sulfated and IdoA(2S)-containing disaccharides. The only significant difference between activating and non-activating oligosaccharides was the content of 6-O-sulfated disaccharides, in particular the disaccharide IdoA(2S)α1,4GlcNSO3(6S). These results show that there is a requirement for 6-O-sulfation of N-sulfated glucosamine residues, in addition to the 2-O-sulfation of IdoA, for the promotion of bFGF mitogenic activity by naturally occurring HS oligosaccharides. Analysis of the structure-activity relationships in the dodecasaccharide fractions in particular, suggests that a minimum bFGF activation sequence exists which is dependent on the positioning of at least one 6-O-sulfate group.

Basic fibroblast growth factor (bFGF)1 is a member of a large family of polypeptides. It is found in a wide variety of mammalian tissues, and has been shown to influence a variety of cellular processes such as proliferation, migration, and differentiation (1–3). The FGFs act primarily through high affinity tyrosine kinase receptors (4), although, in addition, their activity is modulated by and largely dependent on lower affinity HS proteoglycan receptors (5–7). The mechanism by which HS promotes bFGF action is not clearly understood, with several hypotheses having been proposed. These include the proposition that HS binding confers a conformational change on bFGF, which in turn promotes binding to its high affinity receptor (5). Another model suggests that HS acts as a bridge by simultaneously binding both the growth factor and its tyrosine kinase receptor (8–10). A further model, based on the ability of heparin to oligomerize bFGF, suggests that heparin/HS presents dimers of the growth factor to FGF receptors, to facilitate the receptor dimerization required for signal transduction (7, 11–14). A mechanism akin to that involved in human growth hormone action has also been proposed (15, 16), in which a monomeric complex of HS and bFGF induces receptor dimerization through two distinct receptor binding interfaces on the growth factor (17, 18).

Structurally defined oligosaccharides from heparin/HS have been shown to bind strongly to bFGF (8, 19–23). These oligosaccharides were found to be rich in IdoA(2S)-containing disaccharides, with the affinity for bFGF increasing with oligosaccharide length and IdoA(2S) content. The main disaccharide repeat units of these oligosaccharides are IdoA(2S)α1,4GlcNSO3(6S) in HS and heparin, respectively. Recent x-ray crystallographic studies of bFGF complexed with heparin saccharides have shown the fundamental role played by IdoA(2S)α1,4GlcNSO3 in HS and heparin, respectively. The minimum length of the biological activity with bFGF.

Studies relating to the ability of heparin/HS oligosaccharides to activate bFGF in biological assays have been contradictory, both in terms of the minimum oligosaccharide length required for activation, and the role that specific sulfate groups play in mediating the response. The minimum length of the biologically active oligosaccharides has been variously reported as di- and trisaccharides (25), hexasaccharides (26), octasaccharides (7), decasaccharides (22, 27), and dodecasaccharides (8, 23). The presence of 2-O-sulfated IdoA has been shown to be an absolute requirement for the ability of HS oligosaccharides to bind bFGF, as well as for the promotion of the growth factor’s mitogenic activity. The role of 6-O-sulfate groups, however,
remains unclear. Chemically produced, selectively 6-O-desulfated heparins which still bound bFGF strongly were unable to activate bFGF in biological assays (8, 10), which suggests a role for 6-O-sulfation in mediating biological activity. Others studied, however, have suggested that 6-O-sulfation has no direct role, with the main contributors to biological activity being 2-O-/N-sulfates and the carboxyl group of IdoA (23, 27).

To date, bFGF activation studies using heparin/HS-derived oligosaccharides have relied on size separation and affinity chromatography to generate material for assessment of biological activity. In this study, we have generated a library of HS oligosaccharides separated on the basis of molecular size and content/pattern of sulfation but not bFGF affinity. We have then correlated their ability to activate bFGF in a biological assay with their disaccharide composition.

EXPERIMENTAL PROCEDURES

Materials—Cell culture media and horse serum were obtained from Life Technologies, Inc. (Paisley, United Kingdom). Human recombinant bFGF was supplied by R & D Systems (Abingdon, Oxon, UK) and TCS (Botolph Claydon, Buckingham, UK). Porcine mucosal HS was obtained from Biosources (Oss, The Netherlands). Heparinases I, II, and III were supplied by Grampian Enzymes (Aberdeen, UK). Sephadex G-25 was obtained from Pharmacia Biotech (St. Albans, Herts, UK). [methyl-3H]Thymidine (3H)glucosamine was supplied by DuPont NEN (Hounslow, UK). Bio-Gel P-4 and P-2 were obtained from Bio-Rad (Hemel Hempstead, Hertfordshire, UK). ProPac PA1 analytical columns were purchased from Dionex (Camberley, Surrey, UK). TSK3000PW, TSK3000SW, and TSK2000SW columns were purchased from Phenomenex (Macclesfield, Cheshire, UK). All other reagents were supplied by BDH-Merk LTD (Lutterworth, Leicester, UK).

Preparation of HS Oligosaccharides—Porcine mucosal HS (200 mg) was exhaustively digested by heparinase III (50 mU) in 1 ml of 100 mM sodium acetate, 0.5 mM calcium acetate buffer, pH 7.0, for 24 h at 37 °C. The progress of the enzyme was monitored by absorbance at 232 nm, and further additions of enzyme were made until digestion was complete. The digestion products were then size separated using a Bio-Gel A-5m column (25–50 mm, 3.5×10−3 cm) and further additions of enzyme were made until digestion was complete. The digest products were then size separated using a Bio-Gel P-6 column (170×1.5 cm). Samples were eluted at a flow rate of 6 ml/h in 0.5 mM ammonium bicarbonate and 1-ml fractions collected. Peaks were detected by measuring the absorbance of fractions at 232 nm, were established by comparison with authentic disaccharides, detected by SAX HPLC of the sized oligosaccharide mixtures were applied to two ProPac PA1 columns connected in series (TSK3000PW 30×0.75 cm, TSK3000SW 60×0.75 cm, and TSK2000SW 60×0.75 cm). The columns were eluted with 0.5 mM NaCl at a flow rate of 0.6 ml/min, and the elution profiles monitored by absorbance at 232 nm, 0.005 absorbance units at full scale. Retention times were determined for each oligosaccharide fraction and the void and total volumes measured using blue dextran and potassium dichromate.

Strong Anion-exchange HPLC of Disaccharides—The HS oligosaccharide fractions were reduced to disaccharides by complete depolymerization with a mixture of heparinases I, II, and III followed by Bio-Gel P-2 chromatography as described previously (19). The resulting disaccharides were then resolved by HPLC on a ProPac PA1 analytical column (25×0.48 cm, Dionex). The column was first equilibrated with Milli Q water (adjusted to pH 4.0 with HCl), samples were then injected and the disaccharides resolved using a two-stage NaCl gradient (0 to 0.12 M in 90 min followed by 0.12 to 1.0 M in 45 min) in Milli Q water, pH 4.0. The elution positions of specific disaccharides, detected by absorbance at 232 nm, were established by comparison with authentic standards.

RESULTS

HS Dependence on bFGF Activation of BAF-32 Cells—BAF-32 cells are a lymphoblastoid line which have been transfected with the specific FGF receptor FGFR1 (7). These cells are thought to be largely devoid of cell surface HS, and only respond to bFGF in the presence of added HS or heparin. The absence of cell surface HS proteoglycans was confirmed by examination of the GAG components synthesized by BAF-32 cells, in which the only detectable material was chondroitin sulfate (results not shown). Fig. 1A shows a typical dose response of these cells to bFGF in the presence and absence of heparin (1 μg/ml). The maximum activity at a fixed heparin concentration of 1 μg/ml was achieved at 50 ng/ml bFGF, with levels 5–6-fold higher than basal [3H]thymidine incorporation and 4–5-fold higher than with 50 ng/ml bFGF alone. The
amount of intact HS required to induce the maximum response with a fixed level of bFGF (10 ng/ml) was also determined (Fig. 1B). It can be seen that maximum stimulation of bFGF occurs at intact HS concentrations of 250 ng/ml. No significant inhibitory effect was observed up to HS concentrations of 1 μg/ml.

**Isolation of Heparinase III Generated HS Oligosaccharides**—In order to study the role of oligosaccharide length and sulfation pattern in bFGF activation, a comprehensive library of HS fragments was constructed. Heparinase III-generated HS oligosaccharides from porcine mucosal HS were resolved on the basis of saccharide length by Bio-Gel P-6 chromatography (Fig. 2). Fractions were pooled as indicated, and freeze-dried prior to SAX chromatography. Oligosaccharides of dp14 and larger were not processed further due to a lack of resolution on size separation. Size defined fractions (dp6-dp12) were subsequently resolved according to their charge by application to twin ProPac PA1 analytical columns (250 × 4.8 mm connected in series), and elution with a linear gradient of NaCl (0.04–2 M in 60 min) in Milli Q, pH 3.0. Elution profiles for the SAX separations of the deca- and dodecasaccharides (dp10 and dp12) are shown in Fig. 3 (profiles for the hexa- and octasaccharides are not shown, see below). Peaks were pooled as indicated and assigned designations according to oligosaccharide length and elution position from SAX chromatography. It should be noted at this stage, that each pooled fraction was found by polyacrylamide gel electrophoresis to be heterogeneous and comprised of several oligosaccharide species, presumably of identical length with a very similar or identical total sulfate content.

**Response of BAF-32 Cells to bFGF and Heparinase III Generated HS Oligosaccharides**—Oligosaccharide fractions obtained by SAX chromatography of size-defined oligosaccharide mixtures (dp6-dp12) were tested for activity in the BAF cell assay described previously using a fixed suboptimal concentration of bFGF (10 ng/ml). Results are expressed as a percentage of a positive control, this being the maximum [3H]thymidine incorporation obtained with bFGF (10 ng/ml) and a high level of intact HS chains (1 μg/ml), with the level of zero activity being [3H]thymidine incorporation with bFGF alone. The results showed no activation with any sample in which the oligosaccharide length was less than decasaccharide, i.e. octa- and hexasaccharides (dp8 and dp6, results not shown). Dose-response curves for the various individual deca- and dodecasaccharide fractions, however, showed the presence of both activating and non-activating oligosaccharides (Fig. 4). Fig. 4A shows that one of the decasaccharides (dp10D) had activity approaching the level obtained for bFGF and intact HS chains (greater than 90% control). The remaining two activating fractions, dp10C and dp10B, showed relatively lower levels of activity (approximately 50 and 30% of control levels, respectively). It was also of significant interest that one non-activating fraction dp10A was identified which not only failed to activate bFGF but positively inhibited basal bFGF activity. Interestingly there is a clear correlation between the SAX elution position and the ability of an oligosaccharide to promote bFGF activity.

The data obtained from the dodecasaccharide (dp12) fractions (Fig. 4B) showed that three of the oligosaccharide fractions tested were able to promote the activation of bFGF (dp12E, dp12F, and dp12G), with fractions dp12F and dp12G approaching the maximum levels obtained with intact chains (greater than 90% of the positive control), and the third oligosaccharide fraction (dp12E) showing approximately 70% of control activity. Four non-activating/inhibitory dodecasaccharides—dp12A, dp12B, dp12C, and dp12D were also identified. No further effects upon activity, either stimulatory or inhibitory, were observed when oligosaccharides were used at concentrations up to 40 μg/ml (results not shown). Again it was significant that the three activating fractions eluted later from the SAX column than the non-activating ones which, as seen previously with the decasaccharide fractions, points to a role for content of sulfation in governing the activation of bFGF. However, the activating fraction dp12E eluted only slightly later...
HS oligosaccharides. Activation of bFGF mitogenic activity was measured by [3H]thymidine incorporation into BAF-32 cells treated with bFGF (10 ng/ml) and increasing concentrations of the indicated HS oligosaccharide fractions. A slight elevation in the [3H]thymidine incorporation levels taken from one of two combined decasaccharide experiments are cell suspension 3,371 cpm, and bFGF (10 ng/ml) alone. Typical [3H]thymidine incorporation for bFGF (10 ng/ml) alone. Typical [3H]thymidine incorporation for bFGF (10 ng/ml) plus intact HS (1 μg/ml) was 15,153 ± 1,308 cpm.

than the non-activating fraction dp12D, suggesting that sulfation pattern as well as content could be a significant determinant of bioactivity.

**Determination of Oligosaccharide Length**—One important concern when relating the above differences in activity to varying sulfate content within each size-defined group of oligosaccharide fractions was the absolute confirmation of their number of constituent disaccharide units. This was achieved by performing analytical gel permeation HPLC on all the oligosaccharide fractions, the respective Kav values were then plotted against elution time from the SAX chromatography profiles (Fig. 5). The plot shows clear non-overlapping groupings of sized oligosaccharide fractions. A slight elevation in the Kav values is observed as SAX elution time increases within each sized group of oligosaccharides. This is due to the increase in hydrodynamic volume associated with the increased sulfate content. The results clearly show that the oligosaccharide fractions of interest are of the expected size, and with the individual elution profiles in every case showing no detectable cross-contamination with oligosaccharides of a different size (results not shown).

**Disaccharide Composition of HS Oligosaccharide Fractions**—Analyses of the disaccharide compositions of the decasaccharides (dp10) (Table I) showed them to be enriched in the 2-O-sulfated hexuronic acid containing-disaccharides ΔHexA(2S)α1,4GlcNSO3 and ΔHexA(2S)α1,4GlcNSO3(6S), in which ΔHexA(2S) would most probably correspond to IdoA(2S) in the intact oligosaccharide (19, 29), and not GlcA(2S), which has been shown to be a rare component of HS (30). Interestingly, when the overall content of 6-O-, 2-O-, and N-sulfates is calculated for each decasaccharide fraction, no significant differences were seen in 2-O-sulfate and N-sulfate content. However, a positive and very marked correlation between increasing 6-O-sulfate content and maximum bFGF activation was observed (Fig. 6A). Increases were observed with all 6-O-sulfate-containing disaccharides, i.e. ΔHexAα1,4GlcNAc(6S), ΔHexAα1,4GlcNSO3(6S), and ΔHexAβ1,4GlcNSO3(6S), with the disaccharide IdoAα1,4GlcNSO3(6S) being the main consistent contributor to the overall 6-O-sulfate increase with its level rising 5-fold between dp10A and dp10D.

Dodecasaccharide (dp12) fractions showed broadly similar disaccharide compositions to that seen previously for the decasaccharides, with the main components being IdoA(2S)-containing disaccharides (Table II). However, overall 6-O-sulfate content of the dodecasaccharide fractions analyzed was less than that seen in the decasaccharides. Again a general correlation between increasing total 6-O-sulfate content and elevated biological activity was observed (Fig. 6B) although the trend was less marked than in the decasaccharides. Once more, the major disaccharide change which could be consistently related to activity was in IdoA(2S)α1,4GlcNSO3(6S) content, with an approximate 3-fold increase between the non-activating/inhibitory dp12A and the activating dp12G. Interestingly the switch between an activating dp12E fraction and non-activating dp12D fraction was only accompanied by a small increase in IdoA(2S)α1,4GlcNSO3(6S) content (Table II) with the activating dp12E fraction containing on average less than one of these units. Total 2-O- and N-sulfate levels varied slightly but showed no consistent correlation with activity over the entire range of dodecasaccharide fractions tested.

The combination of biological activity and compositional data clearly shows that a high content of IdoA(2S)α1,4GlcNSO3 disaccharides within a HS oligosaccharide, in itself does not ensure that a HS oligosaccharide has the ability to activate bFGF. Instead the data suggests, that in addition there is a...
pivotal role for 6-O-sulfation in promoting the biological activity of bFGF.

**DISCUSSION**

In this study, we have produced a library of heparinase III-generated HS oligosaccharides differing in their length and sulfate content. When co-introduced with bFGF into a heparin/HS-dependent mitogenic assay, these oligosaccharides showed an activity cut-off below a certain length, this being below decasaccharides. Interestingly, a number of deca- and dodecasaccharide fractions (dp10A, dp12A, B, C, and D) were found to be unable to stimulate bFGF activity and indeed suppressed the basal activity of bFGF on the BAF-32 cells (Fig. 4). Preliminary studies also indicate that these oligosaccharides (dp10A, dp12A, B, C, and D) have inhibitory activity against heparinase-mediated activation of bFGF (results not shown). A clear correlation was also observed between total sulfate content and bFGF stimulatory activity, in particular with decasaccharides (Figs. 3 and 4), with levels of activity for some of the oligosaccharide fractions approaching that achieved by the intact parent HS chains (dp10D, dp12F, and dp12G).

Heparinase III is generally believed to cleave linkages of the type GlcNR(6S)α1,4GalCA with resistant oligosaccharides having the general formula \(\text{HexA}α1,4(\text{GlcNSO}_3(6\text{S})\alpha1,4\text{GlcNR})\), with R being Ac or SO₃ (31). However, some reports have recently suggested that the enzyme acts more widely on the linkage GlcNR(6S)α1,4GlcA/IdoA (32, 33), thereby producing fragments enriched in contiguous sequences of IdoA(2S)-containing disaccharides. Indeed the presence of heparinase III-resistant oligosaccharides of the type \(\text{HexA}α1,4\text{GlcNSO}_3\text{HexA}α1,4\text{GlcA/IdoA}\) has been confirmed by heparinase I and nitrous acid degradation of heparin III-resistant domains (19). The disaccharide analyses of our oligosaccharide fractions (Tables I and II) suggests therefore, that they contain an internal repeat of IdoA(2S)-containing disaccharides (IdoA(2S)α1,4GlcNSO₃ and/or IdoA(2S)α1,4GlcNSO₃(6S)). A positive correlation between bFGF stimulatory activity and sulfate content was indicated previously from the SAX elution profile. However, the disaccharide analyses of the decasaccharides clearly show that biological activity increases with 6-O-sulfate content (Fig. 6A), in particular an increase in the specific decasaccharide IdoA(2S)α1,4GlcNSO₃ (6S) (Table I), with no appreciable changes in either 2-O- or N-sulfate content. Dodecasaccharide fractions (dp12) also showed a similar positive correlation between activity and increased 6-O-sulfate content (Fig. 6B), again the major consistent contributor to this was the trisulfated disaccharide (Table II).

Our results suggest that the previously identified, high affinity bFGF-binding tetradecasaccharide sequence (oligo-H) from fibroblast HS, devoid of 6-O-sulfates and comprising of an internal repeat of five IdoA(2S)α1,4GlcNSO₃ disaccharides (19), will be unable to promote bFGF activation of the tyrosine kinase receptor. These results are supported by others who have shown that intact de-6-O-sulfated heparins were unable to restore bFGF-induced mitogenesis of chlorate-treated Swiss 3T3 fibroblasts (8), and were ineffective in promoting binding of bFGF to a soluble form of FGFR-1 (10). These conclusions have, however, been contested, since partial loss of 2-O-sulfate groups of IdoA residues occurs along with extensive de-6-O-
Heparinase III-resistant dodecasaccharides (dp12) were previously fractionated by SAX-HPLC and pooled as shown in Fig. 3B. These were then depolymerized using a combination of heparinases, and the resulting disaccharides were resolved by SAX-HPLC (see “Experimental Procedures”) and quantified. Also shown is the number of N-sulfate and 2- and 6-O-sulfate groups/oligosaccharide.

| Disaccharide structure | dp12A | dp12B | dp12C | dp12D | dp12E | dp12F | dp12G |
|------------------------|-------|-------|-------|-------|-------|-------|-------|
| 1 HexA-GlcNAc           | 19.4  | 5.1   | 16.1  | 15.8  | 11.1  | 7.6   | 7.7   |
| 2 HexA-GlcNAc(6S)       | 2.6   | 8.8   | 4.3   | 6.5   | 6.3   | 7.9   | 12.1  |
| 3 HexA-GlcNSO3          | 15.8  | 23.0  | 12.1  | 14.2  | 16.4  | 12.1  | 9.9   |
| 4 HexA-GlcNSO3(6S)      | 4.9   | 3.9   | 2.4   | 5.5   | 4.8   | 6.9   | 10.2  |
| 5 HexA(2S)-GlcnSO3     | 48.9  | 40.7  | 53.4  | 49.5  | 45.9  | 43.5  | 39.2  |
| 6 HexA(2S)-GlcnSO3(6S) | 7.6   | 7.5   | 6.3   | 8.5   | 8.5   | 13.1  | 21.3  |
| 7 HexA(2S)-GlcNAc       | 0.8   | 11.0  | 5.5   | ND*   | 2.4   | 0.7   | ND    |

| 6-O-SO3/oligosaccharide | 0.89  | 1.2   | 0.77  | 1.23  | 1.46  | 2.16  | 2.59  |
| 2-O-SO3/oligosaccharide | 3.48  | 3.55  | 3.91  | 3.48  | 3.68  | 3.93  | 3.6   |
| N-SO3/oligosaccharides  | 4.62  | 4.5   | 4.45  | 4.66  | 4.81  | 5.03  | 4.8   |

* ND, not determined.

sulfation. This concern was in part supported by Ishihara and co-workers (27), who showed that oligosaccharides rich in IdoA(2S)-1,4GlcNSO3 disaccharides, derived from de-6-O-sulfated heparin, were biologically active. However, it should be noted that these deca- and dodecasaccharides still contained the trisulfated disaccharide IdoA(2S)-1,4GlcNSO3(6S) at average levels of 12 and 14%, respectively (27). Yet, in a more recent study utilizing partially desulfated intact heparins, this group went on to demonstrate that 6-O-sulfation is necessary for heparin to promote bFGF-induced cell growth, and that about 60% of the 6-O-sulfate groups could be removed without appreciable loss of activity (34). Likewise, chemically N-sulfated acharan sulfate, composed of IdoA(2S)-1,4GlcNSO3 repeat units, failed to activate bFGF (35). Walker and co-workers (23) reported that biological activity was correlated most closely to an oligosaccharide’s content of N- and 2-O-sulfate groups, with no role being clearly established for 6-O-sulfate groups. However, their most active dp16 oligosaccharide preparation did contain noticeable amounts of IdoA(2S)- containing HS oligosaccharides. The results of this study show that in the deca- and dodecasaccharides fractions tested, the bFGF promoting activity increases with the number of 6-O-sulfate groups present. Indeed the results with the decasaccharides suggest that an average of two of the three IdoA(2S)-1,4GlcNSO3 disaccharides present should be 6-O-sulfated in order to stimulate bFGF activity to the level obtained with intact HS. Also it should be noted that intermediate plateau levels of activity are seen in the decasaccharide fractions dp10B and dp10C (Fig. 4A). Since each oligosaccharide fraction is still a mixture of several species, it is likely that the differing overall levels of activity are attributable to the relative amounts of activating and inhibitory oligosaccharides present. If therefore the inhibitory action of an oligosaccharide is due to a lack of 6-O-sulfation in specific position(s), then the increased activity of the more 6-O-sulfated oligosaccharide fractions could be a reflection of the increasing occurrence of 6-O-sulfate groups in specifically required positions. This view is strengthened by data obtained from the dodecasaccharide fractions, in which once again a trend between content of IdoA(2S)-1,4GlcNSO3(6S) and bFGF promoting activity was seen. However, inspection of their compositions shows that in the activating fractions an average of 1.11 of the probable four IdoA(2S)-1,4GlcNSO3 disaccharides present are 6-O-sulfated. In the non-activating dodecasaccharides this figure is reduced to an average of 0.45; the difference between the activating dp12E fraction and non-activating dp12D is even smaller at 0.79 and 0.51 trisulfated disaccharides per fraction, respectively. These relatively small changes in 6-O-sulfate content thus result in a dramatic switch between non-activating and activating behavior (Fig. 4B, Table II). We believe that the most likely explanation for this is an exact requirement for the positioning of one or more 6-O-sulfate groups within the dodecasaccharides.

In conclusion, to our knowledge this is the first study that has shown a direct correlation between the 6-O-sulfate content of naturally occurring HS oligosaccharides and their ability to promote bFGF activity. Both activating and non-activating oligosaccharides were shown to be comprised of a similar 2-O- and N-sulfated disaccharide backbone with only activating oligosaccharides containing significant quantities of 6-O-sulfate. Furthermore, evidence is presented to suggest that in the activating oligosaccharides the position of 6-O-sulfate groups is critical, with perhaps just one specifically positioned 6-O-sulfate being required for promoting bFGF-induced mitogenic activity. These oligosaccharide domains enriched in IdoA(2S)-containing disaccharides have been shown to be a common structural element in HS, and are thought to be the result of a carefully regulated mechanism of HS biosynthesis. The enzymes which control the addition of 6-O-sulfate groups within these domains could be an important control point, which would allow cells to modify their response to bFGF by a controlled variation in the 6-O-sulfation pattern of cell surface HS.

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