Structural and Sequence Motifs in Dermatan Sulfate for Promoting Fibroblast Growth Factor-2 (FGF-2) and FGF-7 Activity*

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Glycosaminoglycans have been implicated in the binding and activation of a variety of growth factors, cytokines, and chemokines. In this way, glycosaminoglycans are thought to participate in events such as development and wound repair. In particular, heparin and heparan sulfate have been well studied, and specific aspects of their structure dictate their participation in a variety of activities. In contrast, although dermatan sulfate participates in many of the same biological processes as heparin and heparan sulfate, the interactions of dermatan sulfate have been less well studied. Dermatan sulfate is abundant in the wound environment and binds and activates growth factors such as fibroblast growth factor-2 (FGF-2) and FGF-7, which are present during the wound repair process. To determine the minimum size and sulfation content of active dermatan sulfate oligosaccharides, dermatan sulfate was first digested and then separated by size exclusion high pressure liquid chromatography, and the activity to facilitate FGF-2 and FGF-7 was assayed by the cellular proliferation of cell lines expressing FGFR1 or FGFR2 IIIb. The minimum size required for the activation of FGF-2 was an octasaccharide and for FGF-7 a decasaccharide. Active fractions were rich in monosulfated, primarily 4-O-sulfated, disaccharides and iduronic acid. Increasing the sulfation to primarily 2/4-O-sulfated and 2/6-O-sulfated disaccharides did not increase activity. Cell proliferation decreased or was abolished with higher sulfated dermatan sulfate preparations. This indicated a preference for specific dermatan sulfate oligosaccharides capable of promoting FGF-2- and FGF-7-dependent cell proliferation. These data identify critical oligosaccharides that promote specific members of the FGF family that are important for wound repair and angiogenesis.

Components of the extracellular matrix are broken down following injury or inflammation. These extracellular matrix products in turn participate in various phases of the wound repair process. Following injury, proteoglycans (PG)1 and their glycosaminoglycan (GAG) chains are released and become soluble and abundant in fluids collected from wounds (1). Dermatan sulfate (DS) is the most common GAG in the skin and is estimated to comprise between 36 and 78% of the total sulfated GAG in wound fluid samples (1). DS consists of repeating disaccharide units of N-acetylgalactosamine and glucuronic acid (GlcA)-linked β1→4 and β1→3, respectively. Similar to modifications in heparin and heparan sulfate (HS) but distinct from the other chondroitin sulfates (CS), the GlcA of DS undergoes epimerization of the C-5 carbon, resulting in iduronic acid (IdoUA). In addition, sulfation modifications can occur at the 2-position of the uronic acid and at the 4- or 6-position of the N-acetylgalactosamine residue.

GAGs and their PG core proteins participate in a variety of functions during wound healing, including binding multiple growth factors and promoting their activities (1–8). Of these activities, the fibroblast growth factor (FGF) family has been shown to participate in several of the major steps, including inflammation, repair, and regeneration (9). FGF-2, also known as basic FGF, has been implicated in the recruitment of inflammatory cells during the inflammatory stage of wound healing. In addition, FGF-2 signals endothelial cell and fibroblast proliferation and may be involved in endothelial cell migration. FGF-7, or keratinocyte growth factor, contributes to epithelial cell regeneration. Studies suggest that FGF-7 can act in a paracrine fashion in which stromal cells release FGF-7, and the target epithelial cells express the FGF-7 receptor (25).

One of the most studied interactions occurs between FGF-2 and HS. It has been proposed (9) that HS binds FGF-2 on the cell surface, thereby protecting FGF from degradation and creating a reservoir of growth factor. It has also been shown (5, 6) that HS is required for the interaction of FGF-2 and its receptor. For HS and FGF-2, detailed work has described the sequence of HS required for FGF-2 binding and cellular activation. For binding and promoting FGF-2 activity, 2-O-sulfation and N-sulfation are required on the HS chain. In addition, the presence of iduronic acid is important for activity. The minimum oligosaccharide for binding FGF-2 is an octasaccharide, although longer oligosaccharides are required for promoting FGF-2 activity. These studies showed that the presence of specific sequences in HS is critical for activity with FGF-2. They further suggest that such sequences are not produced randomly but are generated as a consequence of the regulation of GAG-producing and GAG-modifying enzymes (6). In contrast, limited work exists for sequence specificities for other types of GAGs, notably DS.

Similar to heparin and HS, DS will bind and promote FGF-2- and FGF-7-dependent cellular proliferation (1, 7). These occur through the activation of the mitogen-activated protein kinase growth factor; KGF, keratinocyte growth factor; MWCO, molecular weight cutoff; HPLC, high pressure liquid chromatography.
signalizing pathway (7). Using DS sources that varied in their size and sulfation patterns, it was found that the ability of DS to bind and activate FGF-7-dependent cell proliferation varies depending on the DS source (7). However, because these DS preparations were heterogeneous and contained DS of various size and sulfation patterns, a determination of the specific sequence required for FGF-7 activation has yet to be elucidated. The present study set out to purify DS fractions based on their size and to determine what sequence specificities of DS are required to activate FGF-2- and FGF-7-dependent cell proliferation. The results identified a minimal size and indicated that active DS fractions contain primarily 4-O-sulfated disaccharides and a high degree of IdoUA residues.

**Experimental Procedures**

**Media and Reagents**

DS from porcine intestinal mucosa (DS-03122) was purchased from Celeus Laboratories, Inc. (Cincinnati, OH). DS isolated from *Styela plicata* and *Ascidia nigra* was a generous gift from Mauro S. G. Pavao (Universidade Federal do Rio de Janeiro, Brazil). Chondroitin sulfate AC (~70% A and 30% C) (catalog no. C-5829) and heparin (catalog no. H-3393), chondroitinase ABC (catalog no. C-3667), chondroitinase AC (catalog no. C-2780), chondroitinase B (catalog no. C-8058), heparinase I (catalog no. H-2519), and heparinase II (catalog no. H-6512) were purchased from Sigma. Spectrum SpectraSpher cellulose ester MWCO 1000 and MWCO 5000 dialysis membranes were purchased from Fisher Scientific. FGF-7 and FGF-2 and anti-human RGD/GAG/FGF-7 (catalog no. AF-251-NA) and anti-human basic FGF (catalog no. AF-233-NA) neutralizing antibodies were purchased from R&D Systems (Minneapolis, MN). Mouse lymphocyte BaF3 cells stably transfected with FGFFR2 IIIb, designated Ba/F/KGFR, and specific for FGF-7 were selected and grown as described previously (7, 10). F32 cells expressing FGRF1 and specific for FGF-2 were grown as described previously (1, 11). The Blyscan kit for analysis of PG and GAG was obtained from Accurate Chemical Scientific Corporation (Westbury, NY).

**Preparation of GAG Fractions**

**Digestion**—For large scale fraction preparations, 50 mg of lyophilized GAG was resuspended in 500 μl of chondroitinase AC digestion buffer (50 mM Tris and 60 mM sodium acetate, pH 8.0) and 0.5 unit of chondroitinase AC. The digestion was carried out at 37 °C for 16 h, and then the preparation was boiled for 10 min to remove enzyme activity and filtered through a 0.45-μm polyvinylidene difluoride membrane for HPLC. The reaction was lyophilized down to a total of 200 μl, which were injected as two separate runs of 100 μl each.

**HPLC Purification**—100-μl samples were injected and analyzed using a Superdex peptide HR 10 × 300-cm column (Amersham Biosciences) attached to an AKTA purifier HPLC system (Amersham Biosciences). The run profile consisted of running 50 mM phosphate, 150 mM NaCl, pH 7.0, buffer at a flow rate of 0.5 ml/min. 500-μl fractions were collected using an automatic fraction collector. The void volume eluted typically at fraction 15, with fractions of interest ranging from 17 to 35 before the total volume of the column was reached.

**Diagnosis**—Following fractionation, fractions were combined from the multiple runs and lyophilized down to a total volume of 500 μl. Fractions were dialyzed using a CE MWCO 500 or 1000 membrane at 4 °C in water, which was changed once daily for a total of 3 days. Samples were then lyophilized down to a pellet.

**Carbazole Assay**—Quantification of uronic acid content by the carbazole assay was used to determine the final fraction concentration as described previously (12).

**GAG Desulfation**

100 mg of GAG was first subjected to cation exchange using the Dowex 50 WX8 (Bio-Rad) to exchange the Na+ for H+. Neutralized with pyridine, and lyophilized. The sample was then resuspended in two tubes, each consisting of 50 mg of GAG with 2 ml of 90% MeSO, 10% H2O, and boiled for 6 h. The samples were then diluted to a total volume of 10 ml, and four PD-10 columns (Amersham Biosciences) were used to desalt 2.5 ml of the desulfated GAG. Each column was eluted with 3.5 ml of 10% EtOH, and the samples were lyophilized. The desulfated GAGs were then subjected to digestion, HPLC purification, dialysis, and uronic acid analysis, as described above.

**Cellular Proliferation Assay**

Ba/F/KGFR cells were selected for 1 week in heparin and FGF-1 as described previously (7). Following selection, Ba/F/KGFR and F32 cells were expanded in T75 flasks and allowed to grow for 3 days before they were set up in an experiment. The growth medium consisted of RPMI 1640, 10% fetal calf serum, 1-glutamine (2.92 mg/ml), penicillin/streptomycin (100 units/ml and 50 μg/ml, respectively), and 10% interleukin-3-conditioned medium. Before cells were used in an experiment, they were washed three times in 50 ml of phosphate-buffered saline to remove all traces of growth medium before seeding at a final concentration of 5000 cells/well. Experiments were set up in a 384-well plate (Costar, Fisher Scientific) with a total volume of 25 μl/well. If present, FGF was added to FGF-7 at a final concentration of 5 ng/ml were added to the cells before seeding them in the plate. All dilutions of cells and GAG samples were carried out in non-conditioned medium (RPMI 1640, 10% fetal calf serum, 1-glutamine, penicillin/streptomycin) that did not contain factors necessary for the cells to proliferate. Cells were incubated for 3 days at 37 °C prior to performing a non-radioactive cell proliferation assay (Promega Corp., Madison, WI).

**Iduronic Acid and Glucuronic Acid Content of Fractions**

Individual fractions that had been separated as described above were verified by repeat analysis on the Superdex peptide HR 10 × 300-cm column. Then, portions of these fractions were resuspended in chondroitinase AC buffer (50 mM Tris, 60 mM sodium acetate, pH 8.0) and digested with 250 million units of chondroitinase AC or resuspended in 15 μl of chondroitinase B buffer (50 mM Tris, 60 mM sodium acetate, 0.05% bovine serum albumin, pH 8.0) and digested with 1 unit of chondroitinase B. The digested fractions were then injected separately onto the same peptide column, and the analysis was repeated to determine product size.

**Disaccharide Analysis**

The Glycotechnology Core at the University of California, San Diego, performed all disaccharide analyses. The GAG samples were digested completely using chondroitinase ABC. The disaccharides were purified from the enzyme, and any large, undigested GAG samples were separated by a Millipore Microcon centrifugal filter device, using a MWCO 10,000 membrane (Fisher Scientific). The analysis and separation of the disaccharides was achieved by using reverse phase ion pair chromatography (C18 TosoHaas ODS-120T column, 4 μm particle size), with post-column derivatization based on the method described previously by Sakai et al. (13).

**Mouse and Human Wound Fluid**

Wound fluid was collected from sterile tubes inserted into the backs of mice as described previously (14). Human wound fluid was collected postoperatively from patients, as described previously (1). PGs/GAGs were isolated from wound fluid by anion exchange purification (1). The concentration of PGs/GAGs in wound fluid was determined by Blyscan analysis as described previously (1).

**Results**

**FGF-dependent Cell Proliferation Requires a Minimum Size DS Oligosaccharide for Activation**—In the presence of DS, FGF-2- and FGF-7 activate FGFR1 and FGFR2 IIIb, respectively, but the structure of the DS oligosaccharides necessary for this activation has not been defined. To determine the minimum DS oligosaccharide size required for promoting receptor activation, crude high molecular weight porcine DS preparations (primarily 4-O-sulfated) were partially digested with chondroitinase AC, cleaving the glucuronic acid bonds and producing DS oligosaccharides of various lengths. These oligosaccharides were then separated by size exclusion liquid chromatography, and the concentration of each size fraction was determined by carbazole assay. Each DS fraction (2 μm) was added to Ba/F/KGFR or F32 cells in the presence or absence of FGF-7 and FGF-2, respectively. Ba/F/KGFR cells express FGFR2 IIIb, and F32 cells express FGFR1. Both cell lines did not express cell surface GAG and were dependent on their respective FGF receptor for proliferation in the absence of interleukin-3. This analysis demonstrated that the smallest DS oligosaccharide sufficient to promote at least a 2-fold increase...
in cell proliferation with FGF-7 was a decasaccharide, although optimum activity was achieved with slightly longer oligomers (Fig. 1a). For FGF-2, octasaccharides of DS did have activity, although longer oligomers resulted in optimum activity (Fig. 1b). To verify that these observations were growth factor-dependent, FGF-blocking antibodies were used to inhibit FGF-dependent cell proliferation. Anti-human KGF/FGF-7 antibody blocked FGF-7-dependent cell proliferation (Fig. 1c), and anti-basic FGF antibody blocked FGF-2-dependent cell proliferation (Fig. 1d) to base line levels similar to GAG alone or to the growth factor alone (data not shown).

4-O-Sulfation of the DS Oligosaccharides Is Required for Promoting FGF-dependent Cell Proliferation—Previous work has shown that the degree of GAG sulfation and in some cases the specific sulfation sequence are critical for biological activity (4, 7, 15–17). To confirm that the sulfation of DS plays a role in the activation of FGF-dependent cellular proliferation, crude porcine DS was desulfated (18, 19), digested, purified by size, and assayed as described for Fig. 1. Desulfated DS fractions of all sizes had no activity in promoting FGF-7- or FGF-2-dependent cell proliferation, compared with sulfated DS fractions (a 14-mer is shown in Fig. 2).

It was next asked what type of sulfation is important for promoting FGF activity. Table I shows the types of DS used in the experiments, with their disaccharide sulfation content shown as a percentage of the total GAG. DS isolated from porcine intestinal mucosa and containing primarily (~90%) 4-O-sulfated disaccharides was included in these experiments and used to make sulfated and desulfated fractions. Two types of DS isolated from Ascidacea, S. plicata and A. nigra, with primarily disulfated disaccharides, 2/4-O- and 2/6-O-sulfated, respectively, were also evaluated (17, 20). In addition, disaccharide analysis was also performed on DS isolated from mouse and human wound fluid, which was shown previously to be active in promoting FGF-2- and FGF-7-dependent cell proliferation (1, 7). The sulfation content of wound fluid GAG was similar to that of the commercially available porcine intestinal DS. These reagents provided the opportunity to compare the activation of cellular proliferation by a primarily 4-O-sulfated DS, a DS containing 75% 2/4-O-sulfated disaccharides, 2/4-O- and 2/6-O-sulfated, and a DS containing almost 98% 2/6-O-sulfated disaccharides.

Analysis of these DS preparations showed that the presence of primarily 4-O-sulfated DS had greater activity with FGF-7 than the more highly sulfated DS (Fig. 3a). 2/4-O-sulfated DS had very little activity, and 2/6-O-sulfated DS had a decrease compared with 4-O-sulfated DS. For FGF-2, the differences among 4-O-sulfated, 2/4-O-sulfated, and 2/6-O-sulfated DS were not as great as for FGF-7, although the more highly sulfated DS had slightly less activity than the 4-O-sulfated DS (Fig. 3b). The addition of highly sulfated DS to the 4-O-sulfated DS preparations did not inhibit activation of FGF-2 or FGF-7 (Fig. 3).

Because heparin can also stimulate FGF-7- and FGF-2-dependent cell proliferation, it was imperative that activity resulting from the presence of heparin in the DS preparations be ruled out. Therefore, heparin contamination was determined in
Each DS preparation by disaccharide analysis. The concentration of heparin detected in a 2-μM porcine DS preparation was 0.011 μM. In independent experiments, the minimum concentration of heparin required to stimulate FGF-7-dependent cell proliferation was 0.013 μM, and this response was 20% of the response seen with DS. S. plicata DS had 0.016 μM heparin contamination in a DS preparation of 50 μg/ml, although this preparation did not stimulate the cells to proliferate. A. nigr a DS contained 0.38 μM heparin in a DS preparation of 50 μg/ml. Because A. nigr a contained heparin at a concentration that might confound interpretation of DS results, all DS samples were treated additionally with heparin lyases. Heparin lyase treatment did not abolish stimulation of FGF-7- or FGF-2-dependent cell proliferation by the DS preparations, although digestion with chondroitinase ABC cleaved both GlcA and IdoUA bonds and abolished activity (data not shown).

IdoUA-rich DS Fractions Facilitate FGF-7-dependent Cell Proliferation—The content of IdoUA and GlcA in 4-O-sulfated DS was evaluated in preparations that were purified by size exclusion liquid chromatography to dodecasaccharides. This DS oligosaccharide was divided and digested separately with either chondroitinase AC to cleave GlcA bonds or chondroitinase B to cleave IdoUA bonds and then analyzed again by size exclusion liquid chromatography to determine what type of uronic acid was present in the DS dodecasaccharide. Digestion with chondroitinase AC resulted in the production of an 8-mer and a 4-mer (Fig. 4a, dashed line) from the original 12-mer fraction (Fig. 4a, solid line). When the 12-mer fraction (Fig. 4b, solid line) was digested with chondroitinase B, the resulting products were all disaccharides (Fig. 4b, dashed line).

Previous data have suggested that crude preparations of DS but not CS A/C are able to bind and promote FGF-2 and FGF-7 activity (1, 7). To further evaluate dependence on the IdoUA residue in purified DS or CS A/C oligosaccharides, both DS and CS were partially digested using chondroitinase AC and purified by size. An IdoUA-containing 14-mer derived from DS had greater activity in promoting FGF-7-dependent cell proliferation than did the CS-derived 14-mer (Fig. 5a). However, the DS 14-mer and the CS 14-mer at higher GAG concentrations had comparable activity in the activation of FGF-2-dependent cell proliferation (Fig. 5b). Additional digestion of the 14-mer with chondroitinase ABC eliminated FGF-2- and FGF-2-dependent proliferation.

DISCUSSION

PG and in particular their GAG chains have the ability to participate in a wide variety of functions. They can act as structural components in the extracellular matrix or modify signaling information from a diverse number of soluble and cell surface molecules. In recent years, the specificity of some GAG interactions has been shown to depend on the size, particular sulfation patterns, and monosaccharide identity of the GAG chain, negating theories that GAG involvement is a nonspecific process. GAGs bind to and participate in the signaling of several different growth factors, including members of the FGF family. In detailed studies (5, 6, 21), the binding sequences of HS and FGF-2, HS, DS, and hepatocyte growth factor/scatter factor (2, 8, 22), and DS and heparin cofactor II (16, 23, 24) have shown that specific and sometimes unusual sequence components are required for the GAG to bind and activate a growth factor. The present study set out to determine the structural characteristics of the DS chain that are necessary and sufficient to promote FGF-2- and FGF-7-dependent cellular proliferation.

Using cell lines engineered to express receptors selective for FGF-2 and FGF-7 and devoid of cell surface heparan sulfate, our data indicated that the minimum DS size requirement for FGF-2 was an octasaccharide and for FGF-7 a decasaccharide, although optimum activity was achieved with slightly larger oligosaccharides. Because these fractions were isolated based on size exclusion HPLC, each fraction collected was not completely homogenous. Each fraction contained primarily one size of DS but also contained some slightly larger and slightly smaller oligosaccharides. Therefore, size determination was an approximation. However, based on the total profile of size exclusion HPLC data and analysis after additional digestions, these fractions appeared to be most representative of the oligosaccharide length predicted by comparison to known standards. For example, the 12-mer DS fraction produced after chondroitinase digestion was composed of two size fractions, an 8-mer and a 4-mer. Both fractions contain all IdoUA residues because digestion with chondroitinase B resulted in only disaccharides. In addition, some DS 12-mers did not contain any GlcA residues but were composed of all IdoUA residues, as shown by digestion of the 12-mer with chondroitinase B. These data contradict some previous data showing that DS does not bind or activate FGF-2 (5), although the data confirm other reports (1, 7). This may be explained partly by different DS sources containing various sizes and sulfation patterns.

Sulfation of DS is critical for activity as shown by experiments in which DS was first desulfated and then fractionated and assayed by cell proliferation. Fractions lacking sulfation were not capable of inducing FGF-dependent cell proliferation.

![Graph](http://www.jbc.org/)

**FIG. 2.** Sulfation is required for DS-promoted FGF-dependent cell proliferation. 100 mg of sulfated DS was desulfated, digested with chondroitinase AC, and separated by size exclusion liquid chromatography. The graph compares desulfated and sulfated DS 14-mer. a, DS 14-mer (2 μM) was added to BaF3/FGFR cells with no FGF-7 (open bars) or with a final concentration of 5 ng/ml FGF-7 (filled bars). b, DS 14-mer (2 μM) was added to F32 cells with no FGF-2 (open bars) or with a final concentration of 5 ng/ml FGF-2 (filled bars). For both experiments, data are represented as the mean ± S.D. of triplicate determinations.
A highly monosulfated DS (90% 4-O-sulfated) was found to have activity in both FGF-2 and FGF-7 systems, but increasing the sulfation to primarily disulfated 2/4-O-sulfated DS did not increase activity. Unfortunately, because of limitations in the availability of some DS sources and the amounts of DS required to fractionate and purify DS oligosaccharides, smaller fractions of some DS and CS preparations were unable to be produced. This was the case for the 2/4-O-sulfated DS isolated from *S. plicata*. For these studies, experiments were limited to using crude, heterogeneous populations. Therefore, differences based on size might have been a factor in the interpretation of the data. The size of ascidian-derived GAGs may have been too large to act effectively. However, partially digesting without fractionating the crude 2/4-O-sulfated DS preparation did not result in increased activity, suggesting that inactivity was not because of size but the presence of specific 2/4-O-sulfation.

**TABLE I**

| Pig   | *Styela plicata* | *Ascidia nigra* | Mouse WFGAG | Human WFGAG |
|-------|------------------|-----------------|-------------|-------------|
| 0-Sulfated | 1.4              | UD              | UD          | 20.1        | 14.0        |
| 2-Sulfated | UD              | UD              | UD          | UD          | UD          |
| 4-Sulfated | 92.1             | 23.0            | UD          | 77.9        | 59.0        |
| 6-Sulfated | UD              | UD              | 2.2         | UD          | 25.5        |
| 2/4-Sulfated | 6.5              | 75.6            | UD          | UD          | UD          |
| 2/6-Sulfated | UD              | UD              | 97.8        | UD          | UD          |
| 4/6-Sulfated | UD              | UD              | 1.9         | 1.4         | UD          |
| 2/4/6-Sulfated | UD              | UD              | UD          | UD          | UD          |

**FIG. 3.** Influence of DS sulfation patterns on FGF-dependent cell proliferation. DS that differed in sulfation patterns (4-O-sulfated pig-derived DS, 2/4-O-sulfated *S. plicata*-derived DS, and 2/6-O-sulfated *A. nigra*-derived DS) were analyzed for their ability to promote FGF-dependent cell proliferation. *a*, 50 µg/ml total DS (~2 µM) was added to BaF/KGFR cells with no FGF-7 (open bars) or with a final concentration of 5 ng/ml FGF-7 (filled bars). *b*, DS (50 µg/ml each) was added to F32 cells with no FGF-2 (open bars) or with a final concentration of 5 ng/ml FGF-2 (filled bars). In *a* and *b*, the first three experimental conditions contain only 50 µg/ml DS source. The final two experimental conditions contain two types of DS (25 µg/ml each), totaling 50 µg/ml DS, to determine whether one type of DS inhibited another. For both experiments, data are represented as the mean ± S.D. of triplicate determinations.
The commercial sources of DS used in these experiments were of similar sulfation content, primarily 4-O-sulfated, as was the DS isolated from wound fluids. Some differences in sulfation content existed between the human and mouse samples, which may be partially accounted for by differences in collection; human wound fluid was collected from patients postoperatively, whereas mouse wound fluid was collected by inserting sterile tubing into the subcutaneous space of the mouse back. Significant differences in activity have not been seen to result from these preparations.

To determine the IdoUA and GlcA content of the DS oligosaccharides, a DS fraction was digested by both chondroitinase AC and B. Chondroitinase AC treatment of a DS 12-mer, or dodecasaccharide, resulted in a peak corresponding to an octasaccharide and a tetrasaccharide, whereas treatment with chondroitinase B resulted in a peak corresponding only to a disaccharide. In Fig. 4b, the chondroitinase B enzyme and buffer and DS disaccharides had an overlapping retention time of 15.3. Because no original or resulting intermediate peaks appeared following digestion with chondroitinase B, it can be concluded that the majority of the uronic acids in the DS fractions were IdoUA. There appeared to be some GlcA present as shown in Fig. 4a, which suggests approximately one GlcA residue for every four to five IdoUA residues. The digestion of other fractions supports this conclusion; for example, the digestion of longer DS oligosaccharides resulted in three peaks corresponding to two GlcA residues within the chain (data not shown). In addition, these data argue that the fractions were relatively homoogenous in size and uronic acid content.

To determine whether the presence of the IdoUA residues in DS was critical to promoting FGF activity, the ability of CS preparations containing only GlcA residues was compared with DS. Consistent with previous reports, crude DS did not have much activity in either the FGF-2 or FGF-7 assay (1, 5, 7). However, fractionating CS into smaller oligosaccharides increased their activity in the FGF-2 system, although activity increased only slightly in the FGF-7 system. The CS preparation consisted of ~4% unsulfated, 56% 4-O-sulfated, and 40% 6-O-sulfated CS disaccharides. FGF-7 preferred DS and therefore IdoUA residues at all concentrations of DS. Alternatively, FGF-2 preferred the IdoUA residues in DS at lower GAG concentrations, whereas at higher GAG concentrations both DS and CS had comparable activity in inducing FGF-2-dependent cell proliferation. Therefore, although the presence of IdoUA residues in the DS appears to be more critical for FGF-7 than for FGF-2 at higher concentrations, we cannot rule out that a more highly sulfated CS preparation, similar to the active DS (90% 4-O-sulfated), would have greater activity in the FGF assays. We also cannot rule out the presence of some DS contamination in the CS preparations. However, a similar experiment comparing crude DS and crude CS at varying concentrations revealed that DS had very little activity in either the FGF-7 or FGF-2 system at both low and higher GAG concentrations, indicating that size or an inhibitory fraction may be a factor in the observed results.

Because heparin is active in FGF-7- and FGF-2-dependent cell proliferation, it was imperative that activity resulting from heparin contamination be ruled out. Although some small contamination of heparin was found in all preparations, the abundance necessary to stimulate proliferation was too low, and digestion with chondroitinase ABC but not heparin lyases resulted in a decrease in cell proliferation. Therefore, the contribution of heparin or HS contamination to the results discussed here was negligible.

This study set out to determine DS sequence specificities that are critical to promoting the activity of FGF-2 and FGF-7. The presence of these growth factors is relevant to several events in the wound repair process. Following skin injury, DS becomes soluble and exists as a heterogeneous population of oligosaccharides in the wound. This investigation determined that DS oligosaccharides that can enable FGF-2 and FGF-7 activity are rich in 4-O-sulfation and IdoUA. The presence of more highly sulfated DS, specifically 2/4-O-sulfated DS or fragments smaller than octasaccharides and decasaccharides, does not promote FGF-2 and FGF-7 activity, respectively. This suggests that the modification of these elements in DS synthesis or breakdown could influence FGF activity. This, in turn, would influence the repair process. Future investigation into whether a change in GAG size, sulfation, or uronic acid content contributes to abnormalities seen in non-healing wounds will be of great interest in determining the factors that participate in these pathological events.

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