Selective Effects of Sodium Chlorate Treatment on the Sulfation of Heparan Sulfate

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We have analyzed the effect of sodium chlorate treatment of Madin-Darby canine kidney cells on the structure of heparan sulfate (HS), to assess how the various sulfation reactions during HS biosynthesis are affected by decreased availability of the sulfate donor 3′-phosphoadenosine 5′-phosphosulfate. Metabolically [3H]glucosamine-labeled HS was isolated from chlorate-treated and untreated Madin-Darby canine kidney cells and subjected to low pH nitrous acid cleavage. Saccharides representing (i) the N-sulfated domains, (ii) the domains of alternating N-acetylated and N-sulfated disaccharide units, and (iii) the N-acetylated domains were recovered and subjected to compositional disaccharide analysis. Upon treatment with 50 mM chlorate, overall O-sulfation of HS was inhibited by ~70%, whereas N-sulfation remained essentially unchanged. Low chloride concentrations (5 or 20 mM) selectively reduced the 6-O-sulfation of HS, whereas treatment with 50 mM chlorate reduced both 2-O- and 6-O-sulfation. Analysis of saccharides representing the different domain types indicated that 6-O-sulfation was preferentially inhibited in the alternating domains. These data suggest that reduced 3′-phosphoadenosine 5′-phosphosulfate availability has distinct effects on the N- and O-sulfation of HS and that O-sulfation is affected in a domain-specific fashion.

The multiple biological activities of heparan sulfate (HS) depend largely on interactions of the polysaccharide with diverse protein ligands, including enzymes, enzyme inhibitors, growth factors, matrix components, and microbial proteins (1–4). The proteins bind primarily to sulfated domains that are assembled during HS biosynthesis in the Golgi apparatus (2, 4). HS chain formation is initiated by addition of alternating D-glucuronic acid (GlcUA) and N-acetyl-D-glucosamine (GlcNAc) residues to the priming sugar sequence GlcNAc-GlcUA-

Gal-Gal-Xyl, that is linked to serine residues in HS proteoglycan core proteins. The subsequent modification of the nascent (GlcUA-GlcNAc), polymer, particularly the pattern of sulfation, determines the binding specificity of a given HS species to various proteins (2, 4). The first modification step, N-deacetylation/N-sulfation of GlcNAc residues, occurs in a regioslective fashion and results in the domain design characteristic of HS. The three domain types are (i) sequences of consecutive N-sulfated disaccharide units (NS domains), (ii) sequences of alternating N-acetylated and N-sulfated disaccharide units (NA/NS domains) and (iii) repeats of N-acetylated disaccharide units (NA domains) (2, 4). The further modification reactions all occur in the vicinity of previously incorporated N-sulfate groups. GlcUA residues are in part converted to l-iduronic acid (IdoA) residues by C5 epimerization, and some of the IdoA units undergo 2-O-sulfation. Although IdoA formation occurs in both NS and NA/NS domains, 2-O-sulfation appears to be largely restricted to the former domain type (5). By contrast, the other major O-sulfate substituent, at C6 of GlcN residues, is found both in NS and NA/NS domains, as well as in the portions of NA domains that are vicinal to N-sulfate groups of neighboring domains. Consequently, more than 50% of all 6-O-sulfate groups in a HS polymer may be found outside the NS domains (5). Rarely, O-sulfation occurs also at C2 of GlcUA units and at C3 of GlcNSO3 units (2, 4).

In HS biosynthesis, the product of a given modification reaction generally serves as a substrate for the next reaction. Although this substrate specificity constrains the number of possible structures occurring in HS, the type and extent of sulfation vary between HS species from various cell (6, 7) and tissue (5, 8) types. HS structures also undergo modulation during processes such as development (9, 10), aging (11), and various disease conditions, including diabetes (12) and amyloidosis (13), particularly with regard to 6-O-sulfation of the GlcN residues. At least in some cases, such modulation seems to involve a particular HS domain type. For example, we have found an age-dependent increase in GlcN 6-O-sulfation preferentially of the NS domains in human aorta HS (11). On the other hand, studies with malignantly transformed cells suggest an association between transformation and altered 6-O-sulfation of NA/NS domains (14, 15). Interestingly, the HS 6-O-sulfotransferases are known to present in at least three genetically distinct isoforms (4), raising the possibility that the modulation might involve controlled action of distinct 6-O-sulfotransferase species.

To better understand the control of HS sulfation, we have studied the effects of chlorate treatment on the production of HS by MDCK cells. Chlorate competitively inhibits the formation of 3′-phosphoadenosine 5′-phosphosulfate (PAPS) (16), the high energy sulfate donor in cellular sulfation reactions. The

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sulfation pathway involves transport of sulfate to the cytosol, its reaction with ATP to form adenosine 5'-phosphosulfate, and phosphorylation of adenosine 5'-phosphosulfate to form PAPS (17, 18). PAPS in then transported into the Golgi apparatus and used in sulfate transfer to acceptor structures. Culture of cells in chlorate-supplemented medium has been widely used in experimental work addressing HS functions (19–21), but the effects of chlorate treatment on the various HS sulfation reactions have not been studied in detail. It has, however, been shown that chlorate treatment of fibroblasts predominantly reduced the formation of O-sulfate rather than N-sulfate groups (22), suggesting that chlorate may affect distinct sulfation reactions to different extents.

We here show, by detailed assessment of HS structure in chlorate-treated and control MDCK cells, that chlorate differentially down-regulates the various HS sulfation reactions, in the overall order of 6-O-sulfation > 2-O-sulfation > N-sulfation. We further demonstrate that chlorate treatment has selective effects on the 6-O-sulfation of different HS domains, such that the 6-O-sulfation of NA/NS domains is affected to a lesser extent than the 6-O-sulfation of NA/NS and NA domains.

MATERIALS AND METHODS

Purification of HS

Cell Culture and Labeling—MDCK cells (strain II) were maintained at 37 °C in Dulbecco's modified Eagle's medium (Bio-Whittaker, Ver- viers, Belgium) supplemented with 5% fetal calf serum (Life Technologies, Inc.), 2 mM l-glutamine, 100 units/ml penicillin, and 50 μg/ml streptomycin sulfate (23). For experiments, cells were seeded on polycarbonate filters (4.7 cm²; pore size, 0.4 μm; Type 3412; Costar, Cambridge, MA) kept in glass dishes containing 90 ml of culture medium. The cultures reached confluence after 3–4 days, after which the filters (many) in tightly sealed glass tubes at 100 °C for 4 h (27). Hydrazine was added to the cell monolayer on the filter in its holder (apical medium). Metabolic labeling in the absence or presence of chlorate (0–50 mM) was carried out in glucose-free Dulbecco's modified Eagle's medium (Life Technologies, Inc.), supplemented with 2% fetal calf serum, 2 mM glutamine, and 100 μCi/ml [3H]GlcN (Amersham Pharmacia Biotech). After 24 h, the apical and basolateral media were collected, and loose cells were pelleted by centrifugation. The media supernatants were brought to 4 ml guanidine-HCl, 2% Triton X-100 in 0.1 M sodium acetate, pH 6.0. The basolateral media fractions, which have been shown to contain >75% of secreted HS proteoglycans in cultures of polarized MDCK cells (23), were subjected to isolation and characterization of HS as described below.

Isolation of Radiolabeled HS—Labeled macromolecules in the culture media were separated from free radioactivity by chromatography on a 4-ml column of Sephadex G-50 (Amersham Pharmacia Biotech) in 0.05 M Tris-HCl, pH 8.0, containing 0.15 M NaCl. Proteoglycans were purified by chromatography on a column of DEAE-Sephacel (Amersham Pharmacia Biotech) eluted with a linear gradient of NaCl (0.15–1.5 M) in 0.05 M Tris-HCl, pH 8.0. Proteoglycan fractions were pooled, dialyzed against water, and lyophilized. DEAE-purified proteoglycans (each sample representing material from six 4.7-cm² filters) were treated with 1 unit of chondroitinase ABC (EC 4.2.2.4; Seikagaku Corporation, Tokyo, Japan) in 0.04 M Tris-HCl, pH 7.8, 0.04 M sodium acetate containing 1 mg/ml bovine serum albumin for 4 h at 37 °C. HS chains were thereafter liberated from their protein cores by alkaline β-elimination (treatment with 0.5 N NaOH at 4 °C overnight) followed by neutralization of the samples by careful addition of 4 M acetic acid. Previous control experiments involving biologically 35S-labeled heparin-like polysaccharide showed no loss of sulfate groups to occur under these conditions (cf. Ref. 24). HS chains were recovered by DEAE chromatography as follows: samples were diluted 5-fold with water and passed through a 2-ml column of DEAE-Sephacel equili- brated with 0.2 M NH₄HCO₃. Columns were washed in a stepwise manner with 0.2 M NH₄HCO₃, 0.25 M NaCl and again with 0.2 M NH₄HCO₃, followed by elution of bound HS chains with 2 M NH₄HCO₃. The eluates were freeze-dried and stored at ∼20 °C until further analysis.

Characterization of Intact HS Chains

To assess the purity of the HS preparations, aliquots of [3H]HS were treated with HNO₂ at pH 1.5 (see below). The HNO₂-treated and untreated samples were chromatographed on a column of Superose 12 (1 × 30 cm; Amersham Pharmacia Biotech) in 1 M NaCl, 0.05 M Tris-HCl, pH 8.0, at a flow rate of 0.5 ml/min. Fractions of 0.5 ml were collected and analyzed for radioactivity by scintillation counting. The charge density of HS was studied by anion exchange chromatography. Samples of [3H]HS were applied to a 2-ml column of DEAE- Sephaloc equilibrated with 0.05 mM LiCl, 0.05 mM sodium acetate, pH 4.0. Bound HS was eluted with a linear gradient of 0.05–1.5 M LiCl in 0.05 M sodium acetate, pH 4.0. Fractions of 0.5 ml were collected and analyzed for radioactivity.

To study the hydrodynamic size of HS, samples of [3H]HS were applied to a column of Superose 6 (1 × 30 cm; Amersham Pharmacia Biotech), calibrated with hyaluronan and heparin fragments of defined size as described previously (25). The column was run in 1 M NaCl, 0.05 M Tris-HCl, pH 7.4, at a flow rate of 0.5 ml/min. Fractions of 0.5 ml were collected and analyzed for radioactivity. The occurrence of N-unsulfated GlcNAc units was studied by treating samples of [3H]HS with HNO₂ at pH 3.9 (see below), followed by chromatography on Superose 6.

Characterization of HS Domains

Analysis of N-Substitution Pattern—Samples of [3H]HS were treated with HNO₂, pH 1.5, reagent in a volume of 0.5 ml for 10 min at room temperature (26), after which the pH was raised to ∼9 by addition of 2 M Na₂CO₃. This treatment causes deaminative cleavage of the polysaccharides at GlnNSO₃ units, which are concomitantly converted into anhydromannosidase residues. These were further reduced to 2,5-anhydro- mannotol (αManR) units with NaBH₄ (5 mg/ml). After 3 h, the excess NaBH₄ was destroyed by acidifying the samples (to pH ∼4) by addition of 4 M acetic acid followed by neutralization with 4 M NaOH. For analysis of the depolymerization pattern, the cleavage products were chromatographed on a column of Bio-Gel P-10 (1 × 150 cm; Bio-Rad) in 1 M NaCl, 0.05 M Tris-HCl, pH 7.4, 0.01% Triton X-100 at a flow rate of 2.3 ml/h. Fractions of 1.2 ml were collected and analyzed for radioactivity. The N-sulfation degree of the GlcNAc residues was calculated as described previously (14).

Compositional Disaccharide Analysis of HS Domains—For preparation of saccharide material representing the NS, NA/NS, and NA domains, the products of the HNO₂, pH 1.5, cleavage were applied to a column of Sephadex G-15 (1 × 190 cm; Amersham Pharmacia Biotech). Fractions containing disaccharides (corresponding to NS domains) were pooled, desalted by lyophilization, and subjected to compositional disaccharide analysis (see below) to determine the relative hexa- and tetrasaccharide fractions (which represent NA/NS and NA domains, respectively). The cleavage products were chromatographed on a column of Superdex 30 (1.6 × 80 cm; Amersham Pharmacia Biotech) calibrated with standard heparin oligosaccharides and run in 0.5 M NH₄HCO₃. The tetrasaccharide and >-hexasaccharide fractions were pooled, desalted by lyophilization, and lyophilized and lyophilized. 2.3 ml of 30% hydrazine (Fluka), 1% (w/v) hydrazine sulfate (Merck, Darmstadt, Germany) in tightly sealed glass tubes at 100 °C for 4 h (27). Hydrazine was removed by repeated evaporation and intermittent addition of water by following the last eluate by dissolution of the samples on a Sephadex G5 column in 0.2 M NH₄HCO₃. N-Deacetylated saccharides were then treated with HNO₂ at pH 3.9 (26) for 10 min at room temperature, after which the solution was neutralized by addition of Na₂CO₃ and reduced with NaBH₄ as described for the HNO₂, pH 1.5, procedure. This treatment causes cleavage at GlcNH₂⁺ units generated by the N-deacetylation process. The resultant disaccharides were desalted by chromatography on a column of Sephadex G-15 in 0.2 M NH₄HCO₃, lyophilized, and subjected to anion exchange HPLC.

RESULTS

Chlorate Treatment Reduces HS Sulfation—Confluent MDCK cell cultures were metabolically radiolabeled with [3H]GlcN in the presence or absence of sodium chlorate. Proteoglycans were recovered from the conditioned media by 2 U. Lindahl, unpublished data.
DEAE chromatography, followed by isolation of \(^{3}\text{H}\)HS as described under “Materials and Methods.” The purified \(^{3}\text{H}\)HS preparations were quantitatively degraded into lower molecular weight species by treatment with HNO\(_{2}\) at pH 1.5, as shown by gel chromatography on Superose 12 (data not shown), and thus did not contain any contaminating \(^{3}\text{H}\)-labeled macromolecules. To monitor the effect of chlorate on HS sulfation, samples of \(^{3}\text{H}\)HS from control and chlorate-treated cells were chromatographed on an anion exchange column of DEAE-Sephacel. The HS samples were quantitatively retained by the column, but they required different concentrations of LiCl for their elution. The peak elution positions of HS from control cells and from cells treated with 5 mM chlorate corresponded to 0.85 M LiCl, whereas HS synthesized in the presence of 20 or 50 mM chlorate emerged at lower LiCl concentrations (0.73 and 0.53 M, respectively; Fig. 1). These data suggest that chlorate inhibits HS sulfation in a dose-dependent fashion in MDCK cells.

Previous studies with microsomal fraction from mastocytoma tumors suggest that sulfation of the growing heparin polymer promotes its further elongation (30). On the other hand, chlorate treatment has been reported to increase HS chain length in cultured adipocytes (31). To assess the effects of chlorate treatment on HS chain length in MDCK cells, we subjected \(^{3}\text{H}\)HS from chlorate-treated and control cells to gel chromatography on Superose 6. The peak \(K_{\text{av}}\) for HS from control cells was 0.40, corresponding to a molecular mass of \(-35\text{ kDa}\) (Fig. 2). HS species from chlorate-treated cells were eluted earlier than those from control cells (Fig. 2), indicating, in agreement with previous data (31), that such treatment results in longer HS chains. These findings further exclude the possibility that the shifts in elution positions observed on DEAE chromatography (Fig. 1) were due to differences in HS chain length.

The N-Substitution Pattern Is Preserved in HS from Chlorate-treated Cells—To examine whether chlorate treatment affected the N-sulfation of HS, samples of \(^{3}\text{H}\)HS were cleaved by deamination with HNO\(_{2}\), and the patterns of depolymerization were analyzed by gel chromatography on Bio-Gel P10. At pH 1.5, HNO\(_{2}\) induces selective cleavage of the hexosaminidic bonds adjacent to GlcNSO\(_{3}\) residues (26). NS domains thus yield disaccharides, whereas the occurrence of one or more GlcNAc residues between the \(N\)-sulfated disaccharides results in the formation of tetrasaccharides (representing NA/NS domains) and longer oligosaccharides (NA domains). The deamination products were separated into oligosaccharides ranging from 2 to \(\geq12\) monosaccharide units in length (Fig. 3), and the proportions of the different species were used to calculate the degrees of \(N\)-sulfation of the HS polymers (14). The four preparations showed essentially similar degrees of GlcN \(N\)-sulfation, corresponding to \(-40\%\) of the disaccharide units, thus indicating that the \(N\)-deacylation/\(N\)-sulfation of GlcNAc residues was highly resistant to chlorate treatment. These findings further suggest that the differences in charge density seen upon DEAE chromatography of the same HS samples (Fig. 1) would be due to variation in \(O\)-sulfation. Although treatment with 5 or 20 mM chlorate did not affect the proportions of NS, NA/NS, and NA domains, HS from cells treated with 50 mM...
chlorate showed somewhat reduced proportions of NS domains (11% as compared with 16% in HS from control cells) and \( \geq 12\)-mer NA domains but was more abundant in 6–8-mer NA domains (Fig. 3). Furthermore, HS from cells treated with 50 mM chlorate was partially depolymerized upon treatment with HNO\(_2\) at pH 3.9, indicating the presence of N-unsubstituted GlcN units that had presumably resulted from GlcNAc deacetylation without subsequent N-sulfation during HS biosynthesis. Size assessment of the cleavage products by gel chromatography on Superose 6 suggested that the number of N-sulfation remained essentially unaltered, the high dose chlorate treatment induced limited redistribution of the N-sulfate groups along the HS polymer and some formation of GlcNH\(_3\) \(_N\) units.

Differential Effects of Chlorate Treatment on 2-O- and 6-O-Sulfation of NS Domains—The effects of chlorate treatment on the O-sulfation of NS domains were assessed by anion exchange HPLC analysis of the disaccharide fractions isolated following HNO\(_2\), pH 1.5, treatment of \(^{3}H\)HS samples (Fig. 4), as described under “Materials and Methods.” Quantification of the labeled disaccharides thus obtained from the control sample indicated heavy O-sulfation, the di-O-sulfated IdoA(2-OSO\(_3\))-GlcNSO\(_3\)(6-OSO\(_3\)) species constituting \( \geq 80\% \) of all disaccharide units. Additional disaccharide constituents included the mono-O-sulfated GlcUA-GlcNSO\(_3\)(6-OSO\(_3\)), IdoA-GlcNSO\(_3\)(6-OSO\(_3\)), and IdoA(2-OSO\(_3\))-GlcNSO\(_3\) species as well as non-O-sulfated HexA-GlcNSO\(_3\) units, in proportions ranging from 5 to 17% of all disaccharide units (Fig. 4; Table I). Although chlorate treatment, as expected, decreased the overall O-sulfation in a dose-dependent fashion, the 2-O- and 6-O-sulfation reactions were differentially affected (Fig. 4; Table I). At the lower concentrations tested (5 and 20 mM), chlorate treatment reduced the proportions of disaccharide units containing 6-O-sulfated GlcNSO\(_3\) residues without significantly affecting the 2-O-sulfation of IdoA. By contrast, treatment with 50 mM chlorate appreciably reduced both 2-O- and 6-O-sulfation and led to a dramatic (>80%) loss of the di-O-sulfated IdoA(2-OSO\(_3\))-GlcNSO\(_3\)(6-OSO\(_3\)) disaccharide units (Fig. 4; Table I). These findings suggest that the 6-O-sulfotransferases(s) that act on GlcNSO\(_3\) residues of NS domains are more readily affected by chlorate treatment than is the IdoA 2-O-sulfotransferase. Altogether, the data show that GlcN 6-O-sulfation is impaired at a relatively low (20 mM) chlorate concentration, whereas GlcNAc N-deacetylation/N-sulfation resists even 50 mM chlorate.

Domain-specific Effect of Chlorate on HS Sulfation—Previous analyses of HS preparations from various bovine tissues have shown that 2-O-sulfate groups tend to be restricted to NS domains, whereas 6-O-sulfation involves also NA/NS and NA domains (at the border to NS or NA/NS domains) (5). We therefore studied the influence of chlorate treatment on the 6-O-sulfation of NA/NS and NA domains. Fractions of tetrasaccharide constituents in the 6-O-sulfated NS domains were analyzed for radioactivity. The sizes of the cleavage products are indicated (as number of monosaccharide units) in the top panel.
Heparan Sulfate Biosynthesis in Chlorate-treated Cells

Compositional disaccharide analysis of NS, NA/NS, and NA domains of HS from chlorate-treated and untreated MDCK cells

The disaccharide composition of NS, NA/NS, and NA domains was analyzed as described under “Materials and Methods” and in the legends to Figs. 4 and 5. The proportions of O-sulfate groups were calculated from the disaccharide composition data. ND, not detected.

The disaccharide composition of NS, NA/NS, and NA domains was analyzed as described under “Materials and Methods” and in the legends to Figs. 4 and 5. The proportions of O-sulfate groups were calculated from the disaccharide composition data. ND, not detected.

| Domain | Chlorate | Deamination product | Type of O-sulfation |
|--------|----------|---------------------|---------------------|
|        | mM      | %*                  | Sulfate groups/100 disaccharide units | Total OSO3 |
| NS     | 0 5     | 11 5                | 17 62               | 97 78 157 |
|        | 5 10    | 10 3                | 24 53               | 77 66 143 |
|        | 20 25   | 7 5                 | 29 46               | 75 58 133 |
|        | 50 70   | 13 25               | 30 10               | 40 51 91  |
| NA/NS  | 0 78    | 13 11               | 1 1                 | 2 21 23 |
|        | 5 91    | 11 11               | 2 2                 | 4 21 25 |
|        | 50 95   | 11 9                | ND ND               | ND 5 5 |
| NA     | 0 94    | 11 9                | ND ND               | ND 9 9 |
|        | 5 100   | ND 3                | ND ND               | ND 6 6 |
|        | 20 100  | ND 3                | 1 ND                | 1 3 4  |
|        | 50 100  | ND 3                | ND ND               | ND ND ND |

a Percentage of all disaccharides from the respective domain type.

b Sulfate groups per 100 disaccharide units within each domain type.

In summary, our data show that chlorate treatment may drastically reduce overall O-sulfation of HS (up to ~70%; Fig. 7A) without significantly affecting N-sulfation. Whereas reduction in O-sulfation involved both 2-O- and 6-O-sulfate groups, the former components were preferentially affected at higher chlorate concentrations. The results further indicate that the reduction in 6-O-sulfation occurs in a domain-specific manner, in the order NA domains > NA/NS domains > NS domains (Figs. 5 and 6).

DISCUSSION

We have used graded sodium chlorate treatment of MDCK cells along with structural analysis of metabolically labeled HS to gain information on the regulation of HS sulfation in an intact cell system. A model summarizing the results is shown in Fig. 7. The different sulfation reactions differed markedly in susceptibility to chlorate treatment. Thus, N-sulfation was essentially unaffected even in the presence of 50 mM chlorate, whereas 6-O-sulfation was progressively depressed with increasing chlorate concentration (Fig. 7A). By contrast, 2-O-sulfation was appreciably inhibited only at chlorate concentra-

FIG. 5. Compositional disaccharide analysis of NA/NS and NA domains. Samples of [3H]HS from untreated and chlorate-treated (50 mM) cells were subjected to deaminative cleavage by HNO2 at pH 1.5. Saccharides corresponding to NA/NS domains (4-mers) and NA domains (>4-mers) were recovered by gel chromatography, N-deacetylated, and reacted with HNO2 at pH 3.9. The resultant disaccharides were separated by anion exchange HPLC (see under “Materials and Methods” and legend to Fig. 4). Insets show enlargements of the chromatograms over the time period for elution of O-sulfated disaccharide species. The peaks are numbered as follows: (1), HexA-aManR; (2), GlcA-aManR(6-OSO3); (3), IdoA-aManR(6-OSO3); (4), IdoA(2-OSO3)-aManR(6-OSO3); (5), IdoA(2-OSO3)-aManR(6-OSO3).
Heparan Sulfate Biosynthesis in Chlorate-treated Cells

The degree of 6-O-sulfation in HS from control cells is set as 100%.

2-O-sulfotransferase and GlcNSO₃ 6-O-sulfotransferase are 0.2 (32) and 0.44 µM (33), respectively, whereas the corresponding value proposed for N-deacetylase/N-sulfotransferase (NDST) from mastocytoma cells is much higher ($K_m$ 40 µM) (34). Although these values would suggest a higher sensitivity of NDST than of O-sulfotransferases toward chlorate inhibition, it should be noted that the data reflect widely different assay conditions used for measuring the PAPS affinities (see Ref. 32 for discussion). Indeed, HS biosynthesis in the intact cell is presumably catalyzed by membrane-bound enzymes that are believed to act in processive fashion (2), and attempts at reproducing individual steps of this process with exogenous substrates must be evaluated with care. Furthermore, the NDSTs and 6-O-sulfotransferases are present in at least three isoforms each (4) that may have different PAPS affinities and saccharide substrate specificities. For example, heparin-producing mast cells express predominantly NDST-2 (35), and transfection of 293 kidney epithelial cells with NDST-2 leads to production of HS with more extended NS domains (36), thus raising the possibility that this NDST isoform may be involved in the generation of “heparin-like” NS sequences. The substrate specificities of the different 6-O-sulfotransferase isoforms (see Ref. 4) are so far unknown, but they may potentially differ with regard to NS, NA/NS, or NA domain targets. In such a case, the 6-O-sulfotransferase isoform(s) sulfating NA and NA/NS domains would be more readily affected by the reduced PAPS availability than the isoform that sulfates the NS domains.

The observed chlorate effects could also reflect differences in the mechanisms of PAPS uptake between various Golgi compartments, assuming that different sulfotransferases occur at distinct Golgi loci. At present, only scattered information is available concerning the Golgi localization of the enzymes involved in HS biosynthesis. The cytoplasmic and transmembrane regions of NDSTs 1–3 diverge (37), suggesting that they may reside in different Golgi compartments. NDST-1 was recently localized to the trans-Golgi network in NDST-1 transfected murine fibroblasts (38), such that at least some N-sulfation would occur as a late Golgi event. On the other hand, mastocytoma cells treated with Brefeldin A (which blocks transport from Golgi to the trans-Golgi network) produce heparin with N-, 2-O- and 6-O-sulfate groups (39), indicating that these sulfotransferase activities are also expressed proximal to the trans-Golgi network.

Culture of cells in sodium chloride-supplemented media has been widely used to study the role of sulfated glycosaminoglycans in a variety of cellular activities (19–21). The degree of undersulfation achieved by the chlorate treatment depends on the concentrations of chloride and sulfate ions in the culture medium, because chlorate inhibits PAPS formation by competing with sulfate ions for binding to ATP-sulfurylase (40). In the present study, the cells were grown in medium with normal sulfate supplementation, thus presumably explaining the finding that HS sulfation was only partially inhibited despite the high dose (50 mM) chlorate treatment. Our present findings, that chlorate treatment affects different HS sulfation reactions to different extents, raise the possibility that the altered HS-dependent activities seen in chlorate-treated cells may reflect selective changes in HS structures (unless a complete block of HS sulfation has been achieved). We have previously shown that HS from MDCK cells treated with 50 mM chlorate is unable to bind fibroblast growth factor-1 but binds fibroblast growth factor-2, whereas HS from untreated cells binds avidly to both fibroblast growth factor species (41). Chlorate treatment thus can be used as a tool to discriminate between the HS binding specificities of different protein ligands. Further studies should be conducted to study the effects of chlorate on different types of cells having potentially different sets of sulfotransferases (4) and different organization of the biosynthetic assembly lines in the Golgi.

The possible role of PAPS availability in the control of HS biosynthesis in vivo is not understood. Interestingly, mRNA for PAPS synthetase, the human PAPS synthesizing enzyme, with both ADP sulfurylase and adenosine 5’-phosphosulfate kinase activities, is expressed at highly different levels in distinct tissues, pointing to tissue variability in the PAPS production capacity (17). In fact, PAPS concentrations found in different tissues have been reported to vary between 4 and 80 nmol/g of tissue and are considerably lower than that of UDP-GlcUA in liver (200 nmol/g) (40). A potential additional regulatory step is translocation of PAPS from the cytosol to the Golgi lumen, through the action of a specific antiporter protein (18). The
findings of the present study indicate that such variability may affect not only the overall degree of sulfation of HS chains, but also the fine structural details contributed by various O-sulfate groups.

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