Glycosaminoglycan Production by Bovine Aortic Endothelial Cells Cultured in Sulfate-depleted Medium*

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Donald E. Humphries†, Cynthia K. Silbert‡, and Jeremiah E. Silbert§§

From the †Connective Tissue Research Laboratory, Veterans Administration Outpatient Clinic, the Veterans Administration Medical Center, and the ‡Department of Medicine, Harvard Medical School, Boston, Massachusetts 02108

Bovine aortic endothelial cells were cultured in medium containing [3H]glucosamine and concentrations of [35S]sulfate ranging from 0.01 to 0.31 mM. While the amount of [3H]hexosamine incorporated into chondroitin sulfate and heparan sulfate was constant, decreasing concentrations of sulfate resulted in lower [35S]sulfate incorporation. Sulfate concentrations greater than 0.11 mM were required for maximal [35S]sulfate incorporation. Chondroitin sulfate was particularly sensitive so that the sulfate to hexosamine ratio in [3H]chondroitin [35S]sulfate dropped considerably more than the sulfate to hexosamine ratio in [3H]heparan [35S]sulfate. Sulfate concentration had no effect on the ratio of chondroitin 4-sulfate to chondroitin 6-sulfate. The ratios of sulfate to hexosamine in cell-associated glycosaminoglycans were essentially identical with the ratios in media glycosaminoglycans at all sulfate concentrations.

DEAE-cellulose chromatography confirmed that sulfation of chondroitin sulfate was particularly sensitive to low sulfate concentrations. While cells incubated in medium containing 0.31 mM sulfate produced chondroitin sulfate which eluted later than heparan sulfate, cells incubated in medium containing less than 0.04 mM sulfate produced chondroitin sulfate which eluted before heparan sulfate and near hyaluronic acid, indicating that many chains were essentially unsulfated. At intermediate concentrations of sulfate, chondroitin sulfate was found in very broad elution patterns suggesting that most did not fit an "all or nothing" mechanism. Heparan sulfate produced at low concentrations of sulfate eluted with narrower elution patterns than chondroitin sulfate, and there was no indication of any "all or nothing" sulfation.

Most, if not all, cells produce proteoglycans as cellular components or as materials deposited in extracellular matrix. Although these proteoglycans are usually highly sulfated, there are instances when undersulfation occurs. Patients with Lowe's syndrome produce undersulfated chondroitin sulfate (1, 2), and homozygous brachymorphic mice have cartilage which contains undersulfated chondroitin sulfate (3). Cells cultured in the presence of monensin or β-xylloses have been found to produce undersulfated chondroitin sulfate (4–6), presumably due to effects on transport and substrate inefficiency, respectively. Embryonic connective tissue has also been reported to make undersulfated chondroitin sulfate (7). Heparan sulfate produced by a rat hepatoma cell line (8) and a transformed fibroblast cell line (9) has been reported to be undersulfated relative to control cells, possibly due to impaired deacetylation.

One possible cause of low proteoglycan sulfation could be a limitation in the amount of inorganic sulfate available to the cells. This has been examined with organ cultures of chick embryo cartilage (10, 11). When incubation media contained low amounts of sulfate, undersulfation occurred, resulting in a mixture of glycosaminoglycans consisting of some unsulfated chondroitin chains and some fully sulfated chondroitin chains. This suggested an "all or nothing" sulfation pattern as has been described earlier for cell-free synthesis of chondroitin sulfate (12). Undersulfation was reported to be restricted to matrix chondroitin sulfate; chondroitin sulfate associated with the cells was reported to be almost completely sulfated even when formed at the lowest sulfate concentration examined (10). However, the work was performed with whole pieces of epiphyseal cartilage, raising a possibility that the variation in sulfation might be due to variations of sulfate access to different areas of the cartilage.

There has also been a report concerning undersulfation of chondroitin sulfate and dermatan sulfate in human skin fibroblasts obtained from normal patients and patients with Lowe's syndrome when these cells were cultured in media containing a low sulfate concentration (1). In contrast to the undersulfation in cartilage, the cell-associated glycosaminoglycans were reported to be affected extensively, while media glycosaminoglycans were only slightly undersulfated. However, the characterization and distribution of glycosaminoglycans found in these skin fibroblasts was quite different from that previously reported by us and others (13–16). For example, there was almost no cell surface heparan sulfate produced. Thus, interpretation is difficult.

While these reports with cartilage and skin fibroblasts clearly indicate that sulfate concentration is important in determining the degree of chondroitin sulfation, many aspects of this requirement remain unanswered. The sulfate concentrations used in these studies included concentrations that approached or were higher than that found in serum and were also higher than the concentrations that have been used in many studies concerning the formation of 35S-proteoglycans in cell cultures. Nevertheless, analysis of disaccharides obtained by digestion with chondroitin lyase demonstrated a considerable undersulfation, even at high sulfate concentrations. Furthermore, the reported inconsistencies in sulfation between cellular and extracellular chondroitin sulfate raises questions concerning differences in sulfation between classes.

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† To whom correspondence and reprint requests should be addressed: Veterans Administration Outpatient Clinic, 17 Court St., Boston, MA 02108.
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or types of proteoglycans and the mechanisms by which these differences could occur. Because of these inconsistencies and unanswered questions, we were interested in determining whether cells other than chondrocytes or fibroblasts would have similar sulfate concentration requirements for cellular and extracellular chondroitin sulfate and whether heparan sulfate would be affected in the same way.

We chose to examine the sulfate requirements for production of proteoglycans by bovine aorta endothelial cells in culture, because these cells synthesize large amounts of proteoheparan sulfate in addition to the large amounts of chondroitin sulfate proteoglycans. Furthermore, a larger proportion of the proteoglycans are cell-associated, in contrast to cartilage and skin fibroblasts where most of the proteoglycans are extracellular. We have found that the sulfate requirements for production of proteoheparan sulfate are considerably different from the requirements for chondroitin sulfate and that the pattern of chondroitin sulfate at low sulfate concentration is quite different from that previously reported for cartilage chondroitin sulfate. In addition, we have found that sulfate requirements for cell-associated proteoglycans are essentially the same as the requirements for extracellular proteoglycans. This is not in agreement with either of the earlier studies.

MATERIALS AND METHODS

RPMI 1640 and "sulfate-free" RPMI 1640 medium in which MgCl₂ was substituted for MgSO₄, were purchased from GIBCO. Carrier-free H₂³⁵SO₄, [³H]highglucosamine (39 Ci/mmole), and Aquassure, liquid scintillation counting mixture were from New England Nuclear. Chondroitin ABC lyase, chondroitin AC II lyase, and the standard disaccharides, ADi-6S, ADi-4S, and ADi-OS were obtained from Miles (Elkhart, IN). Chondroitin 4-sulfate, chondroitin 6-sulfate, and hyaluronic acid were purchased from Sigma (St. Louis, MO). Chondroitin from Viobin (Monticello, IL). Sephadex and Sepharose were obtained from Pharmacia. DEAE-cellulose and Whatman paper were purchased from Whatman.

Formation and Isolation of [³H,³⁵S]-Glycosaminoglycans—Endothelial cells, obtained from calf aorta by the standard technique of collagenase digestion, were grown in RPMI 1640 containing 20% fetal calf serum. Cells were grown to confluence (approximately 2.5 × 10⁶ cells) and preincubated in 2 ml of serum-free and "sulfate-free" RPMI 1640 medium for 1 h before the addition of [³H]glucosamine and various concentrations of [³⁵S]sulfate (at constant specific activity). After 5 h, the medium was removed and cells were washed with 1 ml of phosphate-buffered saline.

The labeled glycosaminoglycans were isolated from other labeled materials and precursors by treating the cells or medium sequentially with pancreatic (1 mg/ml, 16 h, 37 °C) and NaOH (0.1 M, 2 h, 37 °C) before desalting on a Sephadex G-100 column (0.7 × 50 cm) with 0.5 M ammonium bicarbonate as eluant. ¹ The excluded fractions were pooled, lyophilized, and redissolved in H₂O. Cell-associated glycosaminoglycans were still contaminated with other [³H]hexosamine-labeled material. Therefore, these samples were applied to a DEAE-

¹ A preliminary report has been published (17).

² The abbreviations used are: ADi-6S, 2-acetamido-2-deoxy-3-O-(β-D-Glc-4-enepyranosyluronic acid)-6-O-sulfo-d-galactose; ADi-4S, 2-acetamido-2-deoxy-3-O-(β-D-Glc-4-enepyranosyluronic acid)-4-O-sulfo-d-galactose; ADi-OS, 2-acetamido-2-deoxy-3-O-(β-D-Glc-4-enepyranosyluronic acid)-d-galactose.

³ Alkaline elimination was routinely performed without borohydride. This provided for degradation by "peeling" of much of the glycoprotein-derived contaminating 3-substituted [³H]galactosamine from oligosaccharides which otherwise would chromatograph on DEAE-cellulose near some of the nonsulfated nitric acid-treated heparan oligosaccharides. The peeling reaction however does not ordinarily affect the xylose of the proteoglycan linkage region since the xylose is substituted at the 4-position rather than the 3-position required for peeling (18). Multiple experiments performed on [³H,³⁵S]-glycosaminoglycans obtained by pancreatin treatment alone have provided patterns on DEAE-cellulose indistinguishable from those where pancreatin treatment was followed with alkali treatment.

RESULTS

Endothelial cells were labeled in 2 ml of serum-free medium containing [³H]glucosamine (14 × 10⁶ cpm/ml) and [³⁵S]sulfate (65 × 10⁶ cpm/mmol) at concentrations ranging from 0.003 mM to 0.3 mM. In addition, cells were labeled with [³H]glucosamine with no added [³⁵S]sulfate. [³H,³⁵S]-Glycosaminoglycans were obtained by treatment of the [³H,³⁵S]-proteoglycans with pancreatin and alkali as described under "Materials and Methods." Aliquots of the medium [³H,³⁵S]-glycosaminoglycans were then treated with chondroitin ABC lyase and the disaccharide degradation products (approximately 60% of the labeled material) were analyzed by paper chromatography. Results are shown in Fig. 1. As expected, with decreasing concentrations of [³⁵S]sulfate, there were decreasing amounts of [³H,³⁵S]ADi-6S and [³H,³⁵S]ADi-4S, and a concomitant increase in the amount of [³H]ADi-OS. At 0.3 mM [³⁵S]sulfate, 15% of the labeled ADi-6S was found to be [³H]ADi-OS. The ratios of [³H,³⁵S]ADi-4S to [³H,³⁵S]ADi-6S (measured by [³⁵S]) were similar at all concentrations of [³⁵S]sulfate. Chondroitin ABC lyase treatment of medium [³H,³⁵S]-glycosaminoglycans which had been previously treated with chondroitin AC lyase indicated that there was essentially no dematan sulfate (data not shown).

The ratio of [³⁵S] to [³H] in the isolated sulfated disaccharides was very close to 1 to 1 at 0.3 mM [³⁵S]sulfate, but decreased as the [³⁵S]sulfate concentration was lowered, so that the ratio was only 0.5 to 1 when 0.01 mM [³⁵S]sulfate was added. This indicated that the [³⁵S]sulfate was "diluted" with small amounts of sulfate derived from the cells or in the RPMI 1640 medium prior to the addition of [³⁵S]sulfate, resulting in lower specific activities of [³⁵S]sulfate. From this isotope dilution of 50% at 0.01 mM, we estimated that the concentration of the unlabeled sulfate from whatever source to be approximately 0.01 mM. Therefore, we added 0.01 mM sulfate to our calculation of total sulfate content for each incubation and have adjusted our results accordingly.

The [³H]hexosamine and [³⁵S]sulfate incorporated into [³H]chondroitin [³⁵S]sulfate and [³H]heparan [³⁵S]sulfate at the various sulfate concentrations are shown in Table I. Incorporation of [³H]hexosamine into both medium and cell-associated chondroitin sulfate and into medium and cell-associated heparan sulfate remained constant at varying concentra-
sulfate, indicating that there were no differences in the amounts of \(^{3}H\)-glycosaminoglycans produced. In contrast, \(^{35}S\) sulfate incorporation into chondroitin sulfate and heparan sulfate decreased as sulfate concentrations were lowered. Concentrations of sulfate greater than 0.31 mM did not result in increased \(^{35}S\) sulfate incorporation (data not shown).

With lower concentrations of sulfate, incorporation of \(^{35}S\) sulfate into \([\text{H}]\)chondroitin \(^{35}S\) sulfate was reduced much more than incorporation into \([\text{H}]\)heparan \(^{35}S\) sulfate. When the concentration of sulfate was reduced from 0.51 mM to 0.02 mM, there was a drop in the ratio of sulfate/hexosamine incorporated into medium chondroitin sulfate from 1.0 to 0.13, while this ratio decreased only from 0.67 to 0.27 in the case of heparan sulfate. Thus, the sulfate/hexosamine ratio for chondroitin sulfate decreased more than 7-fold, while the ratio for heparan sulfate decreased less than 3-fold. The ratios of sulfate to hexosamine incorporated into cell layer glycosaminoglycans were essentially identical with the ratios in the medium. For this reason, only the medium was analyzed further.

\(^{3}H,^{35}S\)-Glycosaminoglycans isolated from the medium were chromatographed on DEAE-cellulose to determine if low sulfate concentrations resulted in glycosaminoglycans that were all partially undersulfated or, alternatively, if the products contained a mixture of some totally sulfated glycosaminoglycans and some essentially unsulfated glycosaminoglycans as had been reported for cartilage chondroitin sulfate (10). The results are shown in Fig. 2. At 0.31 mM sulfate, the \([\text{H}]\) heparan \(^{35}S\) sulfate and \([\text{H}]\)chondroitin \(^{35}S\) sulfate were almost totally separated from each other. The former eluted (fractions 40 to 58) between the hyaluronic acid and chondroitin sulfate standards, while the latter eluted (fractions 58 to 78) slightly later than the standard chondroitin 4-sulfate. The \(^{3}H,^{35}S\)-glycosaminoglycans produced at lower concentrations of sulfate eluted progressively earlier. None of the \(^{3}H,^{35}S\)-glycosaminoglycans produced at 0.02 mM sulfate were found in the area of fully sulfated chondroitin sulfate, but significant amounts eluted after hyaluronic acid indicating there was still some sulfation.

Labeled samples were then degraded with nitrous acid or chondroitin ABC lyase prior to chromatography on DEAE-cellulose in order to provide elution profiles of \([\text{H}]\)chondroitin \(^{35}S\) sulfate and \([\text{H}]\)heparan \(^{35}S\) sulfate, respectively. The DEAE-cellulose chromatograms of \([\text{H}]\)chondroitin \(^{35}S\) sulfate obtained in this fashion are shown in Fig. 3. The \([\text{H}]\) chondroitin \(^{35}S\) sulfate formed by cells grown in 0.31 mM sulfate eluted (fractions 52 to 78) slightly later than the chondroitin 4-sulfate standard. The nitrous acid degradation products from the \([\text{H}]\)heparan \(^{35}S\) sulfate eluted (fractions

![FIG. 1. Paper chromatograms of disaccharides from \([\text{H}]\) chondroitin \(^{35}S\) sulfate. Labeled glycosaminoglycans formed by cells incubated in medium containing \([\text{H}]\)glucosamine and varying concentrations of \(^{35}S\) sulfate were mixed with chondroitin, chondroitin 4-sulfate, and chondroitin 6-sulfate and degraded with chondroitin ABC lyase prior to chromatography on paper in 1-butanol-acetic acid:1 M ammonium acetate:2:3:1. Strips of 1 cm were eluted and analyzed for \(^{35}S\) sulfate and \([\text{H}]\)hexosamine. Migration of the degradation products, ADi-6S, ADi-4S, and ADi-oS, is shown at the bottom.](image)

### Table I

Production of \([\text{H}]\)chondroitin \(^{35}S\) sulfate and \([\text{H}]\)heparan \(^{35}S\) sulfate in media and cell-associated fractions of endothelial cells grown in medium containing varying concentrations of sulfate

<table>
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<th>Sulfate concentration</th>
<th>(\mu \text{M}^{*})</th>
<th>(\text{cpm} \times 10^{3})</th>
<th>HexN</th>
<th>(\text{nmol}^{*})</th>
<th>(\text{SO}_{4})</th>
<th>(\text{SO}_{4}/\text{HexN})</th>
<th>(\text{cpm} \times 10^{3})</th>
<th>(\text{nmol}^{*})</th>
<th>(\text{SO}_{4})</th>
<th>(\text{SO}_{4}/\text{HexN})</th>
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<td></td>
<td>1.44</td>
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*Each concentration was adjusted to include the 0.01 mM unlabeled sulfate calculated to be present in the "sulfate-free" medium (see text). The calculated nanomoles of sulfate incorporated was adjusted similarly.

* Nanomoles of hexosamine (HexN) incorporated was based on the known specific activity of \(^{35}S\) sulfate and the 1:1 ratio of sulfate:hexosamine in chondroitin sulfate at the highest sulfate concentration (Fig. 1).
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**FIG. 2.** DEAE-cellulose chromatograms of $^3$H,$^35$S-glycosaminoglycans. Labeled glycosaminoglycans formed by cells incubated in medium containing $[^3]$H-glucosamine and varying concentrations of $[^35]$S-sulfate were applied to a DEAE-cellulose column (0.7 x 4 cm) equilibrated in 0.05 M NaOAc, pH 5.5. Labeled glycosaminoglycans were eluted with a logarithmic gradient of 0 to 1 M LiCl, 0.05 M NaOAc, pH 5.5, starting at fraction 10. Hyaluronic acid and chondroitin sulfate were added as standards. Fractions of 0.5 ml were collected and aliquots were analyzed for $[^35]$S-sulfate (□) and $[^3]$H-hexosamine (○).


The DEAE-cellulose chromatograms of $[^3]$H-heparan $[^35]$S-sulfate are shown in Fig. 4. The $[^3]$H-heparan $[^35]$S-sulfate formed by endothelial cells cultured in 0.31 mM sulfate eluted (fractions 40 to 60) between the hyaluronic acid and chondroitin sulfate standard. The $[^3]$H-heparan $[^35]$S-sulfate formed with the lower sulfate concentrations eluted progressively

**FIG. 3.** DEAE-cellulose chromatograms of $[^3]$H chondroitin $[^35]$S-sulfate. Labeled glycosaminoglycans formed by cells incubated in medium containing $[^3]$H-glucosamine and varying concentrations of $[^35]$S-sulfate were treated with nitrous acid and applied to a DEAE-cellulose column (0.7 x 4 cm) equilibrated in 0.05 M NaOAc, pH 5.5. Labeled chondroitin sulfate was eluted with a logarithmic gradient of 0 to 1 M LiCl, 0.05 M NaOAc, pH 5.5, starting at fraction 10. Hyaluronic acid and chondroitin sulfate were added as standards. Fractions of 0.5 ml were collected and aliquots were analyzed for $[^35]$S-sulfate (□) and $[^3]$H-hexosamine (○).

**FIG. 4.** DEAE-cellulose chromatograms of $[^3]$H heparan $[^35]$S-sulfate. Labeled glycosaminoglycans formed by cells incubated in medium containing $[^3]$H-glucosamine and varying concentrations of $[^35]$S-sulfate were treated with chondroitin ABC lyase and applied to a DEAE-cellulose column (0.7 x 4 cm) equilibrated in 0.05 M NaOAc, pH 5.5. Labeled heparan sulfate was eluted with a logarithmic gradient of 0 to 1 M LiCl, 0.05 M NaOAc, pH 5.5, starting at fraction 10. Hyaluronic acid and chondroitin sulfate were added as standards. Fractions of 0.5 ml were collected and aliquots were analyzed for $[^35]$S-sulfate (□) and $[^3]$H-hexosamine (○).
earlier and over a somewhat broader area. Even at 0.02 mM [35S]sulfate, cells produced [3H]heparan [35S]sulfate which eluted predominantly (fractions 30 to 48) after hyaluronic acid indicating considerable sulfation. The early eluting materials (fractions 12 to 18) were the [H,35S]-disaccharide degradation products of chondroitin ABC lyase digestion. Chondroitin ABC lyase digestion of medium from cells cultured in lower sulfate concentrations demonstrated progressively more undersulfated disaccharide (fractions 4 to 8) and progressively less sulfated disaccharides (fractions 12 to 18).

Comparison of these results to those of Table I and Fig. 3 confirmed our original conclusion that sulfation of heparan sulfate was conserved relative to sulfation of chondroitin sulfate. The continuous distribution and gradual shift to earlier elution patterns indicated that essentially all of the heparan sulfate chains were partially undersulfated. Thus, there was no suggestion of an “all or nothing” sulfate pattern for any of the heparan sulfate. This appeared to be different from the sulfation of chondroitin sulfate where there was a suggestion of an “all or nothing” sulfation character for at least a small portion of the glycosaminoglycan chains.

**DISCUSSION**

The results presented in this study indicated that relatively high concentrations of sulfate (higher than 0.1 mM for chondroitin sulfate) were required to produce maximal sulfation of proteoglycans in endothelial cells. Decreasing sulfate concentrations resulted in the production of undersulfated chondroitin sulfate and undersulfated heparan sulfate, although the chondroitin sulfate was affected to a much greater extent (Table I). At 0.31 mM sulfate, heparan sulfate had only 71% as much sulfation as chondroitin sulfate. At 0.02 mM sulfate, however, the heparan sulfate had 2 times more sulfation. Analysis of the undersulfated chondroitin sulfate revealed that as the amount of unsulfated disaccharide increased, the ratio of the remaining chondroitin 4-sulfate and chondroitin 6-sulfate disaccharides remained constant (Fig. 1).

DEAE-cellulose chromatography confirmed that the glycosaminoglycans produced at low concentrations of sulfate were undersulfated and that chondroitin sulfate was most affected (Figs. 2–4). Chondroitin sulfate formed in low concentrations of sulfate eluted in a broad distribution pattern with a suggestion that there might be a small degree of “all or nothing” sulfation (Fig. 3). However, heparan sulfate formed at low sulfate concentrations eluted with a narrower distribution than that of chondroitin sulfate, indicating partial sulfation of all the chains (Fig. 4). As proposed from the sulfate/hexosamine ratios, [3H]heparan [35S]sulfate formed at low sulfate concentrations had elution profiles indicating a much higher degree of sulfation than that of chondroitin sulfate.

In all of these studies, there were small amounts of sulfate present in the “sulfate-free” culture medium since a small percentage of the chondroitin sulfate and a fair percentage of the heparan sulfate were still sulfated, even when no [35S] sulfate was added. This is also apparent from the [35S]/[H] ratios in the chondroitin sulfate disaccharides obtained by chondroitinase digestion of chondroitin sulfate formed with varying concentrations of [35S]sulfate (Fig. 1). From the decrease in the specific activity of the [35S]sulfate in these disaccharides, we estimated that this concentration was 0.01 mM and could be due to a small amount of sulfate in the “sulfate-free” RPMI 1640 medium or in the cells. Calculation of the amount of sulfate expected from the lot analysis of reagent grade medium components indicated that at least 0.005 mM would be expected, but may have been higher in the tissue culture grade components used for the “sulfate-free” RPMI 1640 medium. Cellular sulfate pools, sulfate produced from sulfur-containing amino acids (or sulfate produced by metabolism of minor amounts of sulfated substances in the cells) could represent other sources. However, an experiment with medium that was deficient in cysteine and methionine, as well as sulfate, did not result in a further decrease in the degree of glycosaminoglycan sulfation even though it did result in an overall diminution of proteoglycan synthesis. Cells labeled with [3H]glucosamine in sulfate-depleted medium for short sequential intervals of time did not result in progressive undersulfation, indicating the absence of a significant intracellular sulfate pool.

Our results on the sulfate requirements for maximal sulfation of chondroitin sulfate are in partial agreement with work done earlier on sulfate requirements in cartilage (10, 11). However, we found essentially 100% sulfation of chondroitin at higher concentrations of sulfate, while the earlier work indicated incomplete sulfation even at the higher concentration. Furthermore, our findings do not agree with the “all or nothing” sulfation mechanism previously suggested, since most of the endothelial cell chondroitin sulfate formed at low sulfate concentrations was partially sulfated. In contrast to the earlier work on cartilage (10, 11) and cultured skin fibroblasts (1), we found that the cell-associated chondroitin sulfatase had essentially the same degree of undersulfation as the chondroitin sulfate found in the media. Thus, our uniform results are consistent with a single mechanism for sulfation of all forms of chondroitin sulfate produced by a single cell type.

Our results indicated that sulfation of heparan sulfate is quite different from sulfation of chondroitin sulfate. Conservation of the sulfation of heparan sulfate relative to chondroitin sulfate could be explained by distribution of the enzymes for biosynthesis of heparan sulfate and chondroitin sulfate into two separate compartments with different efficiencies for adenosine 3'-phosphate, 5'-phosphosulfate transport. However, a more likely explanation would be differences in K_m between the chondroitin sulfate sulfotransferases and the various heparan sulfate sulfotransferases (N-sulfotransferase, GlcNAc-6-sulfotransferase, iduronate-2-sulfotransferase). Indeed, the K_m, with regard to adenosine 3'-phosphate, 5'-phosphosulfate for the N-sulfotransferase for heparin and heparan sulfate have been reported to be approximately 20 μM and 50 μM, respectively (21, 22), while we have found the K_m for sulfation of chondroitin sulfate to be approximately 500 μM. It is also interesting to note that patients with Lowe's syndrome secrete undersulfated chondroitin sulfate fragments, but heparan sulfate is fully sulfated (2). The absence of any “all or nothing” sulfation mechanism in heparan sulfate also suggests that there might be some differences between the way heparan and chondroitin are sulfated.

Our studies confirm that sulfate concentration is an important determinant in the degree of proteoglycan sulfation. Furthermore, the amount of sulfate required for maximal sulfation approaches the lower end of the range found in normal human serum (0.22 to 0.43 mM) (23). It is also apparent from our studies that inadequate concentrations of sulfate with in vitro experiments will result in distortion of the structures of chondroitin sulfate and heparan sulfate and will lead to misleading conclusions concerning incorporation of [35S]sulfate into proteoglycans.

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