Turnover of Heparan Sulfate Depends on 2-O-Sulfation of Uronic Acids\

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To study how the pattern of sulfation along a heparan sulfate chain affects its turnover, we examined heparan sulfate catabolism in wild-type Chinese hamster ovary cells and mutant pgsF-17, defective in 2-O-sulfation of uronic acid residues (Bai, X., and Esko, J. D. (1996) J. Biol. Chem. 271, 17711–17717). Heparan sulfate from the mutant contains normal amounts of 6-O-sulfated glucosamine residues and iduronic acid and somewhat higher levels of N-sulfated glucosamine residues but lacks any 2-O-sulfated iduronic acid or glucuronic acid residues. Pulse-chase experiments showed that both mutant and wild-type cells transport newly synthesized heparan sulfate proteoglycans to the plasma membrane, where they shed into the medium or move into the cell through endocytosis. Internalization of the cell-associated molecules leads to sequential endoglycosidase (heparanase) fragmentation of the chains and eventual lysosomal degradation. In wild-type cells, the chains begin to degrade within 1 h, leading to the accumulation of intermediate (10–20-kDa) and small (4–7-kDa) oligosaccharides. Mutant cells did not generate these intermediates, although internalization and intracellular trafficking of the heparan sulfate chains appeared normal, and the chains degraded with normal kinetics. This difference was not due to defective heparanase activities in the mutant, since cytoplasmic extracts from mutant cells cleaved wild-type heparan sulfate chains in vitro. Instead, the heparan sulfate chains from the mutant were relatively resistant to degradation by cellular heparanases. These findings suggest that 2-O-sulfated iduronic acid residues in heparan sulfate are important for cleavage by endogenous heparanases but not for the overall catabolism of the chains.

Animal cells elaborate several membrane-associated proteoglycans bearing heparan sulfate, including betaglycan and one or more members of the syndecan and glypican families of proteoglycans (1–4). After their appearance on the cell surface, the proteoglycans turn over either through a shedding process or by endocytosis (as reviewed in Ref. 5). The endocytic pathway involves several sequential steps of degradation including proteolysis of the core protein, heparanase cleavage of the heparan sulfate chains, and eventually complete degradation by proteases, exoglycosidases, and sulfatases. The heparanases cleave infrequently along the chain (6), giving rise to several fragments that accumulate in lysosomes or possibly prelysosomal compartments (7–13). Some proteoglycans (e.g. those containing a glycosylphosphatidylinositol anchor) may bypass the heparanases and degrade rapidly in lysosomes without the formation of intermediate sized fragments (13).

Heparanases have been found in various cells and tissues, including human platelets (14, 15), placenta (16), mouse mastocytoma (17, 18), colon carcinoma (19), mouse melanoma (20), rat liver (21), hepatocytes (9), rat ovarian granulosa cells (10), and CHO cells (6, 7). As endo-β-galactosidases, the enzymes produce oligosaccharides with GlcUA at their reducing ends (6, 14, 22) and a glucosamine residue at their nonreducing ends. These enzymes generally require the substrate to contain N-sulfated glucosamine residues for activity, which may be located at or near the cleavage site (6, 14, 23). A less stringent requirement appears to exist for O-sulfate groups based on the utilization of different substrates (14, 18) and in vitro competition experiments with chemically modified heparins (23, 24).

To study how the pattern of sulfation might affect degradation of a heparan sulfate chain in vivo, we have examined turnover in a mutant cell line defective in 2-O-sulfation of iduronic acid residues. Altered sulfation in the mutant does not affect the overall turnover of heparan sulfate, but the defect prevents the formation of intermediate breakdown products characteristic of heparanase action. The lack of intermediates results from reduced susceptibility of the chains to cellular heparanases, suggesting that the enzymes in CHO cells prefer substrates containing 2-O-sulfated uronic acids.

EXPERIMENTAL PROCEDURES

Cell Culture—Chinese hamster ovary cells (CHO-K1) were obtained from the American Type Culture Collection (CCL-61, Rockville, MD). The cells were grown under an atmosphere of 5% CO2, 95% air and 100% relative humidity in Ham’s F-12 growth medium (Life Technologies, Inc.) supplemented with 7.5% (v/v) fetal bovine serum (HyClone Laboratories), 100 μg/ml of streptomycin sulfate, and 100 units/ml of penicillin G. Sulfate-free medium was prepared from individual components (25), substituting chloride salts for sulfate and fetal bovine serum that had been dialyzed exhaustively against phosphate-buffered saline (PBS) (26).

Pulse-Chase Experiments—Both wild-type CHO and mutant pgsF-17 cells were grown to near confluence and pulse-labeled for 1 h with 100 μCi/ml of Na235SO4 (25–40 Ci/mg; NEN Life Science Products) in sulfate free-F-12 medium. After removing the labeled medium, the cell layers were washed three times, and fresh F-12 medium supplemented with 1 mM Na235SO4 was added. In some experiments, 100 μg/ml chloroquine was included in the chase medium. At the times indicated in the figure legends, the medium was collected, and the cells were washed three times.

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¶ The abbreviations used are: CHO, Chinese hamster ovary; HPLC, high performance liquid chromatography; HGF, basic fibroblast growth factor; PBS, phosphate-buffered saline.
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times with PBS. Cell surface proteoglycans were released by treating the cells with 0.125% (w/v) trypsin for 5–10 min at 37 °C. The cells were centrifuged at 800 × g for 3 min, and the released proteoglycans were recovered in the supernatant. The pellets were designated as the intracellular pool.

**Steady-state Labeling of Heparan Sulfate—** Wild-type CHO and mutant pgp-F-17 cells were labeled to constant radiospecific activity after 24 h with Na[35S]SO4 (50 μCi/ml) in sulfate-free F-12 medium. The label of sulfate in the medium does not cause undersulfation of the chains, since CHO cells derive adequate sulfate from the catabolism of cysteine and methionine (27).

**Purification of Heparan Sulfate—** Radiolabeled glycosaminoglycan chains were isolated from various fractions in the following way. The medium, the cell surface pool, and the pellet were adjusted to 0.1 M NaOH and then neutralized with 10 M acetic acid. Chondroitin sulfate A (2 mg) and 1/4 volume of a protease solution containing 1 mg/ml Pronase (Boehringer Mannheim) in 0.24 M sodium acetate (pH 6.5) and 1.92 M NaCl were added. After overnight incubation, the solutions were diluted 5-fold with water to reduce the salt concentration to −0.1 M and applied to a 0.5-ml column of DEAE-Sephadex in a disposable polypropylene pipette tip plugged with glass wool. The column was washed with 20 mM sodium acetate buffer (pH 6.0) containing 0.25 M NaCl. Bound glycosaminoglycans were eluted with 1 M NaCl in 20 mM sodium acetate (pH 6.0) and precipitated with 4 volumes of ethanol at 4 °C (28). The pellet was dissolved in 1 ml of sodium acetate (1 M, pH 5.5) and reprecipitated with ethanol. The final material was dissolved in 20 mM Tris-HCl (pH 7.4). [35S]Chondroitin sulfate was removed by treating a sample overnight at 37 °C with 20 milliunits of chondroitinase ABC (Seikagaku). The remaining heparan sulfate was treated at 4 °C for 24 h with 0.5 M NaOH containing 1 M NaBH4 to β-eliminate the chains and reduce the terminal sugars to their corre-

Sulfate Chains—

**Cell Surface Radioiodination—** Confluent monolayers of wild-type and mutant pgp-F-17 cells were washed three times with PBS. Sodium [125I]iodide (0.5 μCi) was added in PBS with 20 units of lactoperoxidase (Sigma), and three 20-

**RESULTS**

**Mutant pgp-F-17 Accumulates Large Heparan Sulfate Chains—** Cell surface proteoglycans in CHO cells are either shed into the growth medium or taken up by endocytosis. The internalized proteoglycans undergo degradation with accumulation of intracellular oligosaccharides of intermediate (10–20 kDa) and small (4–7 kDa) size prior to complete degradation in

**CPC Degradation Assay of CHO Cell Heparanase—** Heparanase activity was partially purified (100-fold) from CHO-K1 cells as described (28). Purified [35S]heparan sulfate (~5000 cpm) was mixed with the pellet prepared from ~5 × 106 cells. After overnight incubation at 37 °C, the sample was boiled and centrifuged for 10 min at 14,000 × g to remove precipitated proteins. The supernatant was analyzed by gel filtration HPLC on a TSK G2000SW column.

**Asay for Recycling of Biotinylated Cell Surface Proteoglycans—Cell surface proteoglycans were biotinylated as described (29). The cells were first chilled on ice and then washed four times at 4 °C with a solution containing 10 mM KH2PO4 (pH 7.0), 0.137 mM NaCl, 10 mM MgCl2, and 10 mM EDTA. The cells were gently stirred at 4 °C for 30 min with 1 mg/ml of Sulfo-NHS-Biotin (Pierce) in buffer. The cells were brought to room temperature and washed once with F-12 buffer (37 °C), twice with sulfate-free F-12 medium, and then labeled with [35S]SO4 (100 μCi/ml) in sulfate-free F-12 medium for 24 h. After biotinylation, the medium was removed, and the cell layers were washed three times with PBS and incubated at 4 °C for 10 min with 1 ml of buffer containing 2% Triton X-100, 10 mM K2HPO4 (pH 7.4), 150 mM NaCl, 10 mM EDTA, 10 mM N-acetylgalactosamine, 1 mM phenylmethylsulfonyl fluoride, and 0.5 μg/ml bovine serum albumin were added to the supernatant. The sample was chromatographed on a small DEAE-Sephacel column to separate the proteoglycans from other proteins. The column was washed with 0.3 mM NaCl in the same buffer and eluted with 2.5 ml of 1 mM NaCl. A sample (0.5 ml) was diluted to 0.5 mM NaCl with water, and 30 μl of streptavidin-agarose beads (Sigma) was added. The mixtures were incubated at 4 °C overnight with end-over-end mixing. The agarose beads were then washed three times by centrifugation with a buffer composed of 0.1% Triton X-100, 50 mM Tris-HCl (pH 7.4), and 0.5 mM NaCl. An aliquot was taken for counting, and another sample was digested with chondroitinase ABC. The digestion products were removed by gel filtration, and an aliquot of the beads was counted as a measure of heparan sulfate proteoglycans containing biotin. To calculate the extent of recycling, the counts recovered on the beads were expressed relative to the counts recovered from cells that were first labeled with [35S]SO4 for 24 h and then biotinylated.

**Stable Transfection of 2-O-Sulfotransferase—** The 2-O-sulfotransferase clone, pcdNA3HS32ST, was kindly provided by H. Habuchi and K. Kimata (30). This clone contains a 1.28-kilobase pair open reading frame encoding the 2-O-sulfotransferase from CHO cells. The plasmid was stably transfected into mutant pgp-F-17 cells using Lipofectin (Life Technologies, Inc.) according to the manufacturer’s instructions. Transfectants were selected in growth medium containing 400 μg/ml G418 (effective concentration). Resistant colonies were replica-plated to pol-

**Density Gradient Fractionation—** Cell surface proteoglycans undergo degradation with accumulation of intracellular oligosaccharides of intermediate (10–20 kDa) and small (4–7 kDa) size prior to complete degradation in
lysosomes (6, 7, 28). One or more cellular heparanases catalyze the formation of these intermediate and small size fragments (6). These enzymes apparently require the presence of N-sulfated glucosamine residues in the chains, but O-sulfate groups appear to be dispensable (14, 18, 23, 24). However, much of the evidence derives from in vitro studies of partially purified enzymes with defined substrates. To test this hypothesis directly in cells, we examined the turnover of heparan sulfate chains in a CHO cell mutant (pgsF-17) defective in 2-O-sulfation of uronic acid residues. This mutant produces heparan sulfate chains with normal levels of 6-O-sulfated glucosamine residues and iduronic acid and somewhat higher levels of N-sulfated glucosamine residues (60% GlcNSO3 in the mutant versus 45% in the wild-type). In contrast, the mutant fails to make any 2-O-sulfated iduronic acid residues due to a deficiency in a 2-O-sulfotransferase (32).

Analysis of steady-state labeled [35S]heparan sulfate chains by gel filtration HPLC showed that wild-type cells contain large, intermediate, and short chains, in agreement with previous studies (6, 7). In contrast, the mutant contained only large chains that eluted near the void volume of the TSK 2000 column (Fig. 1). Their elution position on a TSK 4000 column (Kav = 0.4) indicated an Mₐ of ~8 × 10⁴ (28). The lack of intermediates in the mutant indicated that their formation somehow depended on 2-O-sulfation of the uronic acid residues. To confirm this hypothesis, mutant cells were transfected with a cDNA clone encoding the CHO 2-O-sulfotransferase (30). Stable transfectants were selected by replica plating using [125I]-bFGF blotting to detect colonies that produced cell surface heparan sulfate (32). Several colonies that bound by gel filtration HPLC of [35S]heparan sulfate obtained from mutant cells, wild-type cells, and mutant cells transfected with a CDNA encoding the 2-O-sulfotransferase. Mutant, wild-type, and transfected cells were labeled for 2 days with [35SO₄] and the [35S]heparan sulfate chains were isolated from the cells and growth media and analyzed by gel filtration HPLC (see "Experimental Procedures"). A, wild-type; ○, pgsF-17; □, transfected pgsF-17 cells.

Secretion and Turnover of Heparan Sulfate Proteoglycans Occurs Normally in the Mutant—The turnover of [35S]heparan sulfate was measured by pulse-chase experiments in which cells were labeled with [35SO₄] for 1 h and chased for different periods of time (Fig. 3). [35S]Heparan sulfate proteoglycans were isolated from the growth medium (secreted or shed material in solution), the cell surface (trypsin-releasable), and intracellular pools that remained with the cell pellet after trypsin treatment of the cells and centrifugation (see "Experimental Procedures"). The heparan sulfate chains found on cell surface proteoglycans were large in size (Mₐ ~8 × 10⁴) and decreased in amount very rapidly during the chase in both wild-type and mutant cells (Fig. 3, A and B). During the chase, chains were recovered in increasing amounts from the growth medium, but the chains remained large in size in both cells. The intracellular pool behaved very differently in mutant and wild-type cells. At the start of the experiment, most of the label was in large chains, presumably associated with newly made proteoglycans in the Golgi and proteoglycans recently internalized from the cell surface. In addition, wild-type cells contained a small amount of intermediate and small sized chains (Fig. 3E, filled circles). These fragments accumulated during the chase as the large chains decreased. The large chains in the mutant also declined with time, and a small shift in elution position occurred, suggesting slow, incomplete cleavage (Fig. 3F). Some intermediate and small sized chains accumulated as well, but the extent of cleavage was greatly reduced compared with the wild-type. Thus, the mutant produced few degradation intermediates by pulse-chase, in agreement with the steady-state labeling experiments shown in Figs. 1 and 2.

The overall extent of degradation was determined by measuring the disappearance of [35S]-pulse-labeled heparan sulfate chains from the cells and the medium. Degradation occurred with similar kinetics in mutant and wild-type cells (Fig. 4), taking place in two phases. One was rapid and accounted for about one-third of the chains labeled during the pulse, and a
second occurred more slowly. The initial rapid phase may reflect turnover of material en route to lysosomes, whereas the slow phase represents degradation of material that slowly enters the endocytic pathway from the cell surface or from the growth medium. Chloroquine inhibited degradation by 70%, consistent with the idea that degradation occurred in a low pH compartment, most likely in lysosomes (results not shown). These findings indicated that overall degradation occurred independently of 2-O-sulfation, whereas formation of intermediate and small size fragments did not.

**Heparan Sulfate Proteoglycans and Chains Are Distributed Normally in Subcellular Organelles**—The failure to generate intermediate fragments in the mutant could reflect differences in trafficking of the chains through subcellular compartments where the heparanases reside. To test this idea, we examined the internalization of proteoglycans in mutant and wild-type cells. In one method, cell surface proteins were biotinylated and then the cells were metabolically labeled with $^{35}$SO$_4$ for 24 h to detect both newly made proteoglycans and those that were sulfated after internalization from the cell surface and recycling through the Golgi (29, 34). As shown in Table I, both wild-type and mutant cells sulfated a small amount of biotinylated heparan sulfate proteoglycans, suggesting that some transfer had occurred to the Golgi compartment where sulfation takes place. About twice as much material was recovered from the mutant, but the fraction of cell surface heparan sulfate proteoglycans that recycled was quite low in both cell lines, representing $\sim$0.2% of cell surface heparan sulfate proteoglycans. Trypsin treatment of the cells prior to biotinylation did not yield any $^{35}$S-labeled biotinylated proteoglycans, indicating that the radiolabeled material did not result from biotinylation of proteoglycans inside the cells. Although recycling is a quantitatively minor process, its occurrence in both mutant and wild-type cells indicated that this part of the endocytic pathway continued in the absence of 2-O-sulfation and in fact may be somewhat accentuated when 2-O-sulfation is depressed.

![Fig. 3. $^{35}$S-heparan sulfate chains from cell surface proteoglycans, growth medium, and intracellular pools.](image1)

Wild-type and pgSF-17 cells were pulse-labeled for 1 h with $^{35}$SO$_4$ (100 $\mu$Ci/ml) and chased for 0, 1, 4, and 8 h in medium containing inorganic sulfate. $^{35}$S-Heparan sulfate proteoglycans and chains were isolated from the cell surface by trypsin treatment, from the medium, and from the intracellular pools remaining after trypsin treatment (see "Experimental Procedures"). All samples were treated with base to obtain individual chains, which were then analyzed by gel filtration HPLC (see "Experimental Procedures"). A and B, $^{35}$S-heparan sulfate from trypsin-releasable cell surface proteoglycans from wild-type and mutant cells, respectively; C and D, $^{35}$S-heparan sulfate from material secreted or shed into the growth medium from wild-type and mutant cells, respectively; E and F, $^{35}$S-heparan sulfate present in intracellular pools from wild-type and mutant cells, respectively. ●, Pulse without any chase; ○, 1-h chase; □, 4-h chase; △, 8-h chase. The arrows indicate the elution position of glycosaminoglycan standards.

![Fig. 4. Degradation of heparan sulfate.](image2)

Wild-type and mutant cells were pulse-labeled for 1 h with $^{35}$SO$_4$ (100 $\mu$Ci/ml) and chased for the indicated times. The total amount of $^{35}$S-heparan sulfate recovered from cells plus medium was measured (see "Experimental Procedures"). ●, wild-type; ○, pgSF-17.

**TABLE I**

| Strain          | Biotinylation | Experiment | cpm/culture |
|-----------------|---------------|------------|-------------|
|                 |               | I          | II          |
| Wild type       | −             | 130        | 140         |
|                 | +             | 600        | 1300        |
| Mutant F17      | −             | 50         | 270         |
|                 | +             | 1300       | 2600        |

$^{3}$The proportion of cell surface proteoglycans that were sulfated was estimated by dividing the counts labeled after biotinylation by the counts from cells labeled with $^{35}$SO$_4$ for 24 h prior to biotinylation.
We also measured the distribution of heparan sulfate proteoglycans and chains in different subcellular organelles isolated by density gradient centrifugation in Percoll. Plasma membranes were recovered in fractions 1–3 of the gradients, based on the recovery of 125I counts from cells that were sur-

membranes were recovered in fractions 1–3 of the gradients, consistent with the idea that this material represents mostly cell surface proteoglycans.

ing heparanase activity. To check this possibility, microsomal membranes of wild-type and mutant cells were mixed with large [35S]heparan sulfate chains from wild-type cells (Fig. 7A). After incubation at 37 °C for 24 h, the reaction products were analyzed by gel filtration HPLC. As shown in Fig. 7B, the extract prepared from the mutant degraded the chains to oligosaccharide fragments to the same extent as the extract prepared from wild-type cells. Furthermore, partially purified heparanase from the mutant cleaved wild-type chains to the same extent as enzyme prepared from wild-type cells. 5 Thus, the heparanase activity appeared normal in the mutant.

Heparan Sulfate Chains from the Mutant Resistant Heparanase Degradation in Vitro—Another possibility was that the heparan sulfate in the mutant was simply a poor substrate for the intracellular heparanases. To test this possibility, [35S]heparan sulfate chains from cell surface proteoglycans of mutant and wild-type cells were compared as substrates for partially purified heparanase from wild-type CHO cells. As shown in Fig. 8, chains from the mutant degraded more slowly than chains from the wild-type, and the extent of degradation was not as great.

Furthermore, when the products of the reaction were analyzed by gel filtration HPLC, intermediate and small sized fragments were less prevalent in the mutant (Fig. 9). There was greater heterogeneity in the products, and some chains did not appear to be cleaved at all. These differences suggested that the deficiency in 2-O-sulfation rendered the chains less susceptible to

4 Prior treatment of the cells with trypsin removed ~70% of the [35S] counts that eluted in the top fractions of the gradient, consistent with the idea that this material represents mostly cell surface proteoglycans.

5 C. Sanderson and K. J. Bame, unpublished observations.
heparan sulfate cleavage, which explains why intermediate and small sized oligosaccharides did not accumulate in the mutant.

**DISCUSSION**

Several important conclusions emerge from the study of heparan sulfate turnover in mutant pgsF-17. First, the depression in 2-O-sulfation of uronic acid residues in heparan sulfate decreases the susceptibility of the chains to heparanase cleavage in vitro (Figs. 8 and 9). This finding explains in part the failure of the cells to produce intermediate and small sized fragments in vitro (Figs. 1 and 2). These fragments are generally considered catabolic intermediates in the degradation of heparan sulfate chains, but the data indicate that the overall degradation of heparan sulfate to inorganic sulfate occurs independently of their formation (Fig. 3). Thus, 2-O-sulfation may play a critical role in processing of the chains, but it has little if any effect on overall degradation. This raises several interesting issues.

**Altered Susceptibility to Heparanase Cleavage**—The reduced cleavage of the chains suggests that 2-O-sulfation of the uronic acid residues plays an important role in substrate recognition by CHO heparanases. Similar enzymes have been detected from several sources, including cultured human skin fibro-

![Fig. 8. Heparan sulfate degradation by partially purified heparanase. Large [35S]heparan sulfate chains were isolated from the cell surface proteoglycans of wild-type CHO cells. At the indicated times, an aliquot of the reaction mixture was precipitated with cetylpyridinium chloride to estimate the extent of chain cleavage (see "Experimental Procedures"). ○, cleavage of wild-type [35S]heparan sulfate; ●, cleavage of pgsF-17 [35S]heparan sulfate.](image)

![Fig. 9. Gel filtration HPLC of [35S]heparan sulfate degraded by CHO heparanase. [35S]heparan sulfate from wild-type and pgsF-17 were incubated with partially purified heparanase from CHO cells as indicated in the legend of Fig. 8. The products at 0 h (○), 2 h (●), 8 h (▲), and 24 h (▲) were analyzed by gel filtration HPLC (see "Experimental Procedures"). A, products of wild-type [35S]heparan sulfate; B, products of pgsF-17 [35S]heparan sulfate.](image)
age site and the first modified residue, suggesting that multiple heparanases may exist with different specificities.

Less information is available about the H2 fragment (i.e., the fragment to the right of the cleavage site). Heparanases from multiple sources generally require N-sulfated glucosamine residues for activity, but the location of this unit relative to the cleavage site is not known with certainty. The requirement for glucosamine N-sulfation was confirmed in vivo by Bame (7), who showed that the heparan sulfate chains from a CHO cell mutant defective in GlcNAc N-deacetylase/N-sulfotransferase were relatively resistant to cleavage. The general requirement for N-sulfated glucosamine residues suggests that heparanase may prefer GlcNSO$_3$ as the nonreducing terminal residue on the H2 fragment. Supporting data for this idea derives from studies of the platelet heparanase, which will cleave heparin octasaccharides within the disaccharide unit, GlcUA-GlcNSO$_3$(3SO$_3$)$_3$ (18). Our studies of pgsF-17 suggest that the next residue should be IdoA-2SO$_3$ since the reduced level of 2-O-sulfation in the mutant diminishes cleavage of the chains. Thus, we propose that the H2 fragment has a nonreducing terminal sequence,

\[
\downarrow \text{GlcNSO$_3$-IdoA2SO$_3$-X-X}
\]

\textbf{STRUCTURE 2}

2-O-sulfation of iduronic acids therefore may help generate part of the binding site for the enzyme.

**Defective 2-O-Sulfation Alters Turnover of Heparan Sulfate in Vivo**—The altered susceptibility of heparan sulfate from the mutant to heparanase explains in part the diminution of cleavage fragments in the mutant. However, the lack of intermediate or small sized oligosaccharides is somewhat surprising given that in vitro assays showed that reduced 2-O-sulfation of the chains only led to a decrease in the rate of cleavage and not to complete resistance (Figs. 8 and 9). One way to explain these findings is to consider how proteoglycans traffic through intracellular compartments. Proteoglycans are thought to be endocytosed through endosomes and exposed to proteases, heparanases, and eventually lysosomal glycosidases and sulfatases (5). If the heparanases were located in a prelysosomal compartment, the exposure of the chains to the enzymes might be temporally limited as the chains migrate from this compartment to a lysosome. Thus, if the rate of cleavage is slow (as in the mutant) and the transit time is relatively rapid, little or no cleavage of the chains would occur.

An alternative explanation for the failure to produce few cleavage fragments is that the lack of 2-O-sulfation may change the way that heparan sulfate chains move through intracellular compartments. Bame (7) has suggested that CHO heparanases will act on native proteoglycans, liberating chains from the core protein. If cleavage of the chains from the core protein were reduced, then the proteoglycans might remain intact, which in turn might raise the possibility that the chains recycle to the cell surface as part of a proteoglycan. Recycling of uncleaved proteoglycans could raise the likelihood of their being shed, which would predict that a greater portion of material should be recovered from the growth medium in the mutant. Indeed, we have observed that a larger proportion of heparan sulfate chains are secreted from mutant cells than in the wild type, and the elevated amount of secreted material in the mutant can account for the missing intermediate and small fragments inside the cell.

**Degradation Proceeds at a Normal Rate**—Although interme-

\textsuperscript{6} X. Bai and J. D. Esko, unpublished results.

\textsuperscript{7} J. Biol. Chem.

\textsuperscript{8} Annu. Rev. Biochem.

\textsuperscript{9} J. Biol. Chem.

\textsuperscript{10} J. Biol. Chem.

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\textsuperscript{12} J. Biol. Chem.

\textsuperscript{13} J. Biol. Chem.
