Mouse Mastocytoma Cells Synthesize Undersulfated Heparin and Chondroitin Sulfate in the Presence of Brefeldin A*

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In order to study the subcellular localization and organization of the enzymes involved in the glycosylation of the hybrid proteoglycan serglycin, mouse mastocytoma cells were metabolically labeled with [3H]glucosamine or [35S]sulfate or [3H]glucosamine in the absence or presence of brefeldin A. This drug is known to induce a disassembly of the proximal part of the Golgi complex, resulting in a redistribution of cis-, medial-, and trans-Golgi resident enzymes back to the endoplasmic reticulum, and to block the anterograde transport of proteins to the trans-Golgi network. Although the total incorporation of [3H]glucosamine into glycosaminoglycan chains was reduced to about 25% in brefeldin A-treated cells compared to control cells, both control cells and cells treated with brefeldin A synthesized heparin as well as chondroitin sulfate chains. Therefore, enzymes involved in the biosynthesis of both types of glycosaminoglycan chains seem to be present proximal to the trans-Golgi network in these cells. Chondroitin sulfate and heparin synthesized in cells exposed to brefeldin A were undersulfated, as demonstrated by ion-exchange chromatography, compositional analyses of disaccharides, as well as by a lower [35S]sulfate/[3H]glucosamine ratio compared to controls. In heparin biosynthesis, both N- and O-sulfation reactions were impaired, with a larger relative decrease in 2-O-sulfation than in 6-O-sulfation. Despite undersulfation, the heparin chains synthesized in the presence of brefeldin A were larger (30 kDa) than the heparin synthesized by control cells (20 kDa). The reduced [3H]glucosamine incorporation in brefeldin A-treated cells was partly due to decreased number of glycosaminoglycan chains synthesized, but also to the biosynthesis of chondroitin sulfate chains of smaller molecular size (8 versus 15 kDa in control cells). Brefeldin A had no effect on the glycosaminoglycan synthesis when used in a cell-free, microsomal fraction, indicating that brefeldin A does not interfere directly with the enzymes involved in the biosynthesis of glycosaminoglycans.

Most mammalian cells synthesize proteoglycans, a special group of glycoproteins containing a core protein with covalently linked glycosaminoglycan (GAG)1 side chains (1). Whereas the majority of proteoglycans are substituted with either heparan sulfates or chondroitin sulfate, some cells also synthesize hybrid proteoglycans, in which both types of GAGs are linked to the same core protein (2–4). The biosynthesis of the GAG chains takes place during the transport of the core protein from the endoplasmic reticulum through the Golgi complex. An initial polymerization product is formed, composed of repeating glucuronic acid (Glca) and hexosamine units, N-acetylgalcosamine (GlcNAc) in heparan sulfate/heparin and N-acetylgalactosamine (GalNAc) in chondroitin sulfate/dermatan sulfate. Subsequent modification involves sulfate substitution at various positions and may include C5 epimerization of GlcA to iduronic acid (IdoA) units. Based on studies of heparin biosynthesis, the enzymes responsible for the generation of a GAG chain appear to be strictly organized and tightly clustered into one or more enzyme complex(es) (5). Recently it has been suggested that the enzymes involved in the biosynthesis of chondroitin sulfate are located in the trans-Golgi network (6), whereas the enzymes involved in the biosynthesis of heparan sulfate are located in the proximal part of the Golgi apparatus (7). In both these studies brefeldin A (BFA) was used as an experimental tool to segregate biosynthetic processes occurring in the trans-Golgi network.

BFA is a fungal metabolite that has been shown to induce a disassembly of the cis-, medial-, and trans-Golgi subcompartment, followed by a fusion of these subcompartments with the endoplasmic reticulum (8). The result is a retention of secretory proteins in the endoplasmic reticulum, as well as a redistribution of enzymes normally resident in the cis-, medial-, and trans-Golgi back to the endoplasmic reticulum (9–11). In contrast, enzymes located in the trans-Golgi network are not redistributed back to the endoplasmic reticulum (12). Hence, BFA may be used to distinguish between enzymatic reactions taking place in the endoplasmic reticulum/proximal parts of the Golgi apparatus and those taking place in the trans-Golgi network.

1 The abbreviations used are: GAG, glycosaminoglycan; BFA, brefeldin A; HexA, unspecified hexuronic acid; Man, 2,5-anhydro-D-mannitol formed by reduction of terminal 2,5-anhydromannose residues with NaBH4; NSO3, N-sulfate group; OSO3, ester sulfate group (the locations of O-sulfate groups are indicated in parentheses); ΔDi-4S, 2-acetamido-2-deoxy-3-O-[(β-gluco-4-epeneprenosyluronic acid)-6-sulfo-D-galactose]; ΔDi-6S, 2-acetamido-2-deoxy-3-O-[(β-gluco-4-epeneprenosyluronic acid)-6-sulfo-D-galactose]; ΔDi-4S, 2-acetamido-2-deoxy-3-O-[(β-gluco-4-epeneprenosyluronic acid)-6-sulfo-D-galactose]; ΔDi-6S, 2-acetamido-2-deoxy-3-O-[(β-gluco-4-epeneprenosyluronic acid)-6-sulfo-D-galactose]; ΔDi-4S, 2-acetamido-2-deoxy-3-O-[(β-gluco-4-epeneprenosyluronic acid)-6-sulfo-D-galactose]; ΔDi-6S, 2-acetamido-2-deoxy-3-O-[(β-gluco-4-epeneprenosyluronic acid)-6-sulfo-D-galactose]; CHO, Chinese hamster ovary; HPLC, high performance liquid chromatography; PAPS, adenosine 3′-phosphate 5′-phosphosulfate.

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In the present study we have studied the effect of BFA on the biosynthesis of proteoglycans in mouse mastocytoma cells. These cells synthesize a hybrid form of the proteoglycan serglycin, in which both chondroitin sulfate and heparin chains are linked to the same core protein (13). Our results suggest that, in these cells, both enzymes involved in the biosynthesis of chondroitin sulfate and enzymes involved in the biosynthesis of heparin are located proximally to the trans-Golgi network. Further, we show that BFA treatment results in undersulfation of both chondroitin sulfate and heparin.

EXPERIMENTAL PROCEDURES

Materials—(15S)Sulfate (carrier-free) and n-6(6-H)Glucosamine were purchased from DuPont NEN. UDP-14C GlcA was prepared enzymatically from n-6(6-C)glucose (uniformly labeled, 320 μCi/mmol as described previously (14)). Q Sepharose, DEAE-Sephal, Sepharose CL-6B, Superose 6 (HR 10/30), Sephadex G-15, Sephadex G-50 (fine), and Sephadex G-25 (superfine) were from Pharmacia Biotech Inc. and chondroitinase ABC from Seikagaku Kogyo Co, Japan; brefeldin A was from Boehringer Mannheim. A transplanted mouse mastectomy, originally described by Furth et al. (15), was maintained in the laboratory by routine intramuscular passage every 10–12 days in the hind legs of (A/Sn × Leaden)F mice. A microsomal fraction from the tumor was prepared according to Jacobson et al. (14).

Cell Culture—Mouse mastocytoma cells were established in culture after passage through an ascites stage. Solid tumor tissue was dispersed and injected intraperitoneally into another mouse. After 12 days, cultures were established from ascites fluid and maintained in Dulbecco’s modified Eagle’s medium (Flow Laboratories) containing 4% fetal calf serum, 1 % penicillin, 100 μg/ml streptomycin, 2.5 μg/ml Fungizone, and 2 μg/ml of glutamine (all from Life Technologies, Inc.). Cultures in 50 ml flasks (Nunc, Roskilde, Denmark) reached near confluency in ~14 days and were then used in labeling experiments.

Metabolic/Radioisotope Labeling—Histamine was radioactively labeled for 5 h with 50 μCi/ml 15S sulfate or 6H Glucosamine. BFA was dissolved in ethanol and diluted to a final concentration of 1 μg/ml in standard medium. BFA was added to the cultures 15 min prior to the radioactive precursors and was present during the entire labeling period.

Isolation of Proteoglycans—The proteoglycans in the cell fraction were extracted by the addition of 4 m guanidine HCl, containing 2% Triton X-100. Alternatively, 0.05 M Tris-HCl, pH 8.0, containing 1% Triton X-100 and protease inhibitors (0.002 M EDTA, 0.001 M phenylmethylsulfonyl fluoride, 0.002 M N-ethylmaleimide, and 10 μg/ml pepstatin) was added to the cell fraction, followed by centrifugation at 600 × g for 10 min to remove nuclei. NaCl to a final concentration of 0.1 M was then added to the lysate. Unincorporated radioactive precursors and guanidine HCl were removed from the samples by gel chromatography on Sephadex G-25 (fine) columns (bed volume, 4 ml) equilibrated and eluted in phosphate-buffered saline containing 0.5% Triton X-100 and protease inhibitors (in concentrations as described above).

Enzymatic and Chemical Treatments of Proteoglycans—To release the polysaccharide chains from the peptide core of the proteoglycan, the sample was treated with 0.5 M NaOH at 4 °C for 20 h. After neutralization with 4 M HCl, the polysaccharide chains were dialyzed against water.

Galactosaminoglycans present in the proteoglycans were degraded by digestion with 0.2 unit of chondroitinase ABC/ml of 0.05 M Tris-HCl, pH 8.0, containing 0.03 M sodium acetate and 0.1 mg of bovine serum albumin (16). Prior to digestion, 100 μg of chondroitin sulfate was added as a carrier. After incubation for 15 h at 37 °C, the digest was passed through a column (1 × 200 cm) of Sephadex G-25 superfine, equilibrated with 0.2 M NH4HCO3 to separate disaccharides from undigested material. The disaccharides were freeze-dried and analyzed further by HPLC.

Depolymerization of heparin by nitrous acid deamination at pH 1.5, cleaving the polysaccharide at N-sulfated glucosamine units (17), was performed as described elsewhere (18) on material resistant to digestion with chondroitinase ABC. The 6H-labeled deamination products were reduced with NaBH4 and fractionated by gel chromatography on Sephadex G-25. Estimation of the percentage of GlcN residues carrying 6-sulfate groups was made using weighted integration of the elution profiles on Sephadex G-25 according to the following formula:

\[
\frac{\text{cpm in dis} + \text{cpm in tetras}}{\text{cpm in hexas} + \text{cpm in octas}/4} \times 100 = \text{Eq. (1)}
\]

where dis, tetras, hexas, and octas stand for di-, tetra-, hexa-, and octasaccharides, respectively.

RESULTS

Mouse mastocytoma cell cultures were metabolically labeled with 35S sulfate or 14C Glucosamine for 5 h in the absence or presence of BFA. Radiolabeled macromolecules were then isolated from the culture medium and from the cells and subjected to gel and ion-exchange chromatography. In the control cells (radiolabeled in the absence of BFA) about 10% of the proteoglycan molecules were secreted to the culture medium (2). In contrast, practically no radiolabeled proteoglycans were found in the medium fraction in the BFA treated cultures, demonstrating that BFA inhibits the secretion of macromolecules in mouse mastocytoma cells. The total incorporation of 6H Glucosamine into proteoglycans was reduced to about 25% in the BFA-treated cultures compared to the control (Fig. 1, right panel), whereas the reduction in 35S sulfate incorporation was larger, amounting to 5% of the control (Fig. 1, right panel), indicating that the proteoglycans synthesized in the presence of BFA were undersulfated. Both the control cells and the cells labeled in the presence of BFA synthesized a mixture of heparin and chondroitin sulfate. The ratio between 6H-heparin and 6H-chondroitin sulfate isolated from the control cells was about 1:1. The corresponding figure was 4:1 in the BFA-treated cultures, suggesting that BFA had a more dramatic inhibitory effect on the biosynthesis of chondroitin sulfate than on the

\[2\] While the intracellular proteoglycans most likely are of the serglycin type (23), the secreted proteoglycan probably belongs to the syndecan family, expressed in nearly all cells and tissues (24).
biosynthesis of heparin (Fig. 1, center and left panel, respectively). This was not due to different sensitivity of the two different GAG synthesizing enzymatic systems to BFA, since dose-response experiments revealed that maximum inhibitory effect on the biosynthesis of both chondroitin sulfate and heparin was obtained with 1 μg/ml BFA (Fig. 2). To exclude that the observed effect of BFA was due to direct interference of BFA with enzymes involved in the GAG synthesis, the effect of BFA on the biosynthesis of proteoglycans in a microsomal fraction from mouse mastocytoma was studied. Microsomal proteins were incubated in the presence of UDP-GlcNAc, UDP-GalNAc, and UDP-[14C]GlcA in the presence of PAPS for 30 min at 37 °C. Analysis of the 14C-labeled macromolecules (see "Experimental Procedures") demonstrated that similar amounts of the two polysaccharides were synthesized in the presence and absence of BFA (data not shown).

Polyanionic Properties of Proteoglycans and GAG Chains—[14C]Proteoglycans synthesized by microsomal proteins (see above) were also subjected to anion-exchange chromatography on Q Sepharose. Identical elution patterns were observed for proteoglycans isolated from control and BFA-treated microsomal fractions; the 14C-labeled proteoglycans were eluted in a single peak at a NaCl concentration of 0.8 M (data not shown).

When the control material from cell cultures was similarly analyzed, the 35S macromolecules were also eluted at a NaCl concentration of 0.8 M (Fig. 3, panel A). In contrast, the proteoglycans from BFA-treated cultures were eluted as a broad peak, ranging from about 0.3–0.7 M NaCl (Fig. 3, panel D).

### Table I

|                  | No brefeldin A | With brefeldin A | No brefeldin A | With brefeldin A |
|------------------|----------------|------------------|----------------|------------------|
| Heparin          | 0.26           | 0.32             | 0.53 (20 kD)   | 0.45 (30 kD)     |
| Chondroitin sulfate | 0.33         | 0.59             | 0.60 (15 kD)   | 0.73 (8 kD)      |
FIG. 4. Superose 6 gel chromatography. $^{35}$S-Labeled macromolecules isolated from the cell fraction of cells radiolabeled in the absence (panels A–E) or presence (panels F–J) of BFA were analyzed by Superose 6 gel chromatography before (panels A and F) and after treatment with nitrous acid (panels B and G), nitrous acid/alkali (panels C and H), chondroitinase ABC (panels D and I), and chondroitinase ABC/alkali (panels E and J).
Effect of Brefeldin A on Proteoglycan Synthesis

Further, both the $[^{35}S]$chondroitin sulfate (obtained after HNO$_2$ treatment) and the $[^{35}S]$heparin chains (obtained after chondroitinase ABC treatment) from BFA-treated cultures (Fig. 3, panels E and F, respectively) were eluted at a lower salt concentration than the corresponding GAG chains from control cultures (panels B and C).

Effect of BFA on Proteoglycan Size and GAG Chain Length—To determine if BFA treatment altered the size of the proteoglycans and the chondroitin sulfate and/or heparin chains, $[^{35}S]$labeled proteoglycans isolated from the cell fraction by ion exchange chromatography were analyzed by Superose 6 gel chromatography. The calculated $K_{av}$ values of the proteoglycans and GAG chains are presented in Table I. After treatment with HNO$_2$ at pH 1.5, which degrades the $[^{35}S]$labeled heparin, the intact $[^{35}S]$labeled chondroitin sulfate proteoglycans from BFA-treated cells were eluted later from the column than the chondroitin sulfate proteoglycans from control cells (Fig. 4, G and B; $K_{av} = 0.59$ and 0.33, respectively). The smaller size of the chondroitin sulfate proteoglycans from BFA-treated cells was at least partly due to a decrease in the chondroitin sulfate polysaccharide chain length; after alkali treatment of the chondroitin sulfate proteoglycans, the released chondroitin sulfate chains from BFA-treated cells were eluted at $K_{av} = 0.73$ (Fig. 4H), corresponding to a molecular mass of about 8 kDa, whereas control chondroitin sulfate chains were estimated to have a molecular mass of 15 kDa (Fig. 4C; $K_{av} = 0.60$). Also the size of the heparin proteoglycans was reduced by BFA treatment; after chondroitinase ABC treatment, the intact $[^{35}S]$labeled heparin proteoglycans from control and BFA-treated cells were eluted at $K_{av} = 0.26$ and 0.32, respectively (Fig. 4, D and I). However, $[^{35}S]$labeled heparin polysaccharide chains from BFA-treated cells, released after alkali treatment, were of larger size (Fig. 4J; $K_{av} = 0.45$; estimated molecular mass 30 kDa) than those from control cells (Fig. 4E; $K_{av} = 0.53$; estimated molecular mass 20 kDa). Hence, the proteoglycans synthesized in the presence of BFA contain a reduced number of heparin chains of larger molecular size.

The size of $[^{14}C]$polysaccharide chains synthesized by microsomal proteins was also tested by gel chromatography on Sephacry G-25. The chondroitin sulfate disaccharides were then analyzed by HPLC as described in the legend to Fig. 5. Whereas 73% of the disaccharides from control cells (Fig. 5, upper panel) were monosulfated, containing 4-O-sulfate groups, 10% were disulfated and co-eluted with the $\Delta$Di-diS$_S$ standard. The remaining 17% of the disaccharides from control cells was nonsulfated. In contrast, the BFA-treated cells (lower panel) produced low sulfated chondroitin sulfate chains, composed of about 52% nonsulfated disaccharides and about 46% monosulfated disaccharides which co-eluted with the $\Delta$Di-diS$_S$ standard. Disulfated disaccharides were virtually absent (less than 3%) in chondroitin sulfate chains from BFA-treated cells. These results demonstrate that there is a reduction in both 4- and 6-sulfation of the galactosamine units in chondroitin sulfate synthesized by mast cells cultured in the presence of BFA, compared to cells cultured without BFA.

To gain information regarding the amounts and distribution of N-sulfated glucosamine units in heparin synthesized by the mast cells grown with or without the addition of BFA, $[^{3}H]$labeled material resistant to chondroitinase ABC was lyophilized and cleaved with nitrous acid at pH 1.5 (deamination of N-sulfated regions) followed by gel chromatography on Sephadex G-25 (Fig. 6). Heparin produced by control cells was extensively depolymerized yielding di- and tetrasaccharides as the major labeled products. In contrast deamination of the
FIG. 7. Anion-exchange HPLC of 3H-labeled heparin disaccharides. 3H-Labeled heparin, isolated from cells cultured in the absence (panels A and C) or in the presence of BFA (panel B and D), was subjected to HNO₂, pH 1.5/NaBH₄ treatment (panels A and B) or N-deacetylation followed by combined HNO₂, pH 1.5-pH 3.9/NaBH₄ treatment (panels C and D). Samples of isolated disaccharides (~10 × 10⁶ cpm of 3H) were analyzed on a Parti-sil-10 SAX column eluted at a rate of 1 ml/min with KH₂PO₄ solutions of stepwise increasing concentration (--- - - - -). Most of the control material appeared as Idc_A(2-OSO₃)⁻ (panel B, broken line). The elution positions of standard disaccharides (32) are indicated by arrows: 1, nonsulfated HexA-α-Man₉; 2, GlcA-α-Man₉(6-OSO₃); 3, Idc_A-α-Man₉(6-OSO₃); 4, Idc_A(2-OSO₃)-α-Man₉; 5, Idc_A(2-OSO₃)-α-Man₉(6-OSO₃); 6, GlcA-α-Man₉(3,6-di-OSO₃). The elution positions of commercial heparin disaccharides are shown as solid lines.

**Table II.** Composition of heparin-derived disaccharides/deamination products

| Disaccharide Proportion | Without BFA | With BFA A |
|-------------------------|------------|-----------|
| GlaA/IdoA-α-Man₉         | 30.8       | 91.8      |
| GlaA-α-Man₉(6-OSO₃)     | 14.3       | 3.2       |
| Idc_A-α-Man₉(6-OSO₃)    | 11.0       | 3.3       |
| Idc_A(2-OSO₃)-α-Man₉    | 6.9        | 1.2       |
| Idc_A(2-OSO₃)-α-Man₉(6-OSO₃) | 37.0     | 0.5       |
| GlaA-α-Man₉(3,6-di-OSO₃)| ND⁴        | ND        |
| 2-OSO₃/6-OSO₃           | 0.7        | 0.24      |
| 2-OSO₃/6-OSO₃           | 0.7        | 0.24      |
| 6-OSO₃/6-OSO₃           | 0.7        | 0.058     |
| O-OSO₃/O-OSO₃           | 1.0        | 0.24      |
| O-OSO₃/O-OSO₃           | 1.7        | 0.3       |

*H-Labeled disaccharides obtained by N-deacetylation followed by deamination at pH 3.9 and 1.5 (resulting in cleavage of all glucosaminidic linkages) and reduction with NaBH₄, were analyzed by anion-exchange HPLC as described under “Experimental Procedures.”

⁴ ND, not detected.

As can be seen from Fig. 7A, small amounts of GlcA-α-Man₉(3,6-di-OSO₃) seem to be present in N-sulfated disaccharides (62% of total disaccharides; see preceding paragraph) obtained from control cells after treatment with HNO₂ at pH 1.5. When total disaccharides were analyzed (Fig. 7C), the small amount of GlcA-α-Man₉(3,6-di-OSO₃) was below the level of detection. The low level of 3-0-sulfation may be due to the transformed state of the cells. Also in established cell lines derived from this tumor, the heparin synthesized contains much less 3-0-sulfated products than commercial heparin (29).

**DISCUSSION**

While most proteoglycans contain either heparan sulfate or chondroitin sulfate, hybrids exist in which both heparin/heparan sulfate and chondroitin sulfate are linked to the same core protein (1). Serglycin, a proteoglycan found in hemopoietic cells, is unusual in that it occurs in various cells as “pure” chondroitin sulfate proteoglycan and in other cells the same core protein is substituted with heparin (23). In addition, serglycin may occur as a hybrid (3, 13). The aim of the present investigation was to study the localization and organization of the GAG-synthesizing enzymes in a cell type capable of adding both heparin and chondroitin sulfate to the serglycin core protein. Recently, mouse mastocytoma cells were shown to have this capacity (13), and these cells were therefore chosen for this study.

Incorporation of [³H]glucosamine and [³⁵S]sulfate into GAGs (Fig. 1) demonstrated that both heparin and chondroitin sulfate were synthesized by mastocytoma cells in the presence of BFA. This indicates that both chondroitin sulfate- and heparin-synthesizing enzymes are located proximal to the trans-Golgi network in these cells. Since the amount of radiolabeled GAGs synthesized in the presence of BFA was reduced, it cannot be excluded that also the trans-Golgi network in these cells contain chondroitin sulfate- and/or heparin-synthesizing enzymes. Judging from the amount of [³H]chondroitin sulfate compared to the [³H]heparin recovered after [³H]glucosamine labeling of
the cells (10 and 40%, respectively, compared to the control; Fig. 1), the chondroitin sulfate synthesis was more affected by the drug. However, the heparin chains synthesized in the presence of BFA were longer (30 kDa compared to 20 kDa in the control; Fig. 4 and Table I), whereas the chondroitin sulfate chains were shorter than those produced in the absence of BFA (8 kDa compared to 15 kDa in the control; Fig. 4 and Table I). Therefore, the reduction in number of GAG chains synthesized in the presence of BFA was roughly the same for heparin and chondroitin sulfate, amounting to 25 and 17%, respectively, compared to the control. The opposite effect of BFA on chondroitin sulfate and heparan sulfate/heparin elongation.

The presence of chondroitin sulfate-synthesizing enzymes proximal to the trans-Golgi network, as shown in the present investigation, is thus in contrast to previous results. The different location of the chondroitin sulfate-synthesizing enzymes in mastocytoma cells may tentatively suggest the existence of more than one machinery for the biosynthesis of chondroitin sulfate. This has previously been suggested for heparin/heparan sulfate bio-
synthesis, based on the identification of two genetically distinct enzymes catalyzing N-sulfation (29, 30). The difference in the N-terminal regions of these proteins may suggest that they are present in different Golgi subcompartments, since this region of the proteins contain Golgi retention signals. It is therefore possible that enzymes capable of synthesizing a certain glyco-saminoglycan are located in different Golgi compartments in different cells. If so, it would be expected that chondroitin sulfate-synthesizing enzymes also would be present in more than one variant.

The sulfation of both chondroitin sulfate (Fig. 5) and heparin (Figs. 6 and 7) was decreased in the presence of BFA. However, all the various modification reactions occurred as shown by the presence of the same disaccharide units, although in different amounts, in polysaccharides from control and BFA-treated cells. If the structural changes in heparin induced by BFA is compared with, e.g. the lowered sulfation of heparan sulfate synthesized by CHO cell mutants with a reduced N-sulfotransferase activity (31), it is apparent that the effect of BFA is more general and/or random. In the CHO cell mutant, the O-sulfate/ N-sulfate ratio is the same as in the wild type cell. This result is expected, since a N-sulfated glucosamine residue is part of the substrate recognized by the O-sulfotransferases. In the BFA-treated cells the O-sulfate/N-sulfate ratio is decreased pointing to a loss of regulation of the biosynthesis machinery, further illustrated by the larger relative decrease in 2-O-sulfation than in 6-O-sulfation.

Current views on GAG biosynthesis envisage the enzymes as part of an enzyme complex acting on the polysaccharide (5). Our results may argue against a tight association between the modification enzymes, since BFA seems to induce a less ordered and less efficient modification process. Another possibility is that the concentration of PAPS, the activated sulfate donor, may be different in the Golgi complex of control cells and the fused endoplasmic reticulum/Golgi compartment of BFA-treated cells. If less PAPS is available in BFA-treated cells, a lowered sulfation of the heparin and chondroitin sulfate should be expected. In addition, the lowered O-sulfate/N-sulfate ratio found for heparin from BFA-treated cells, and the larger relative decrease in 2-O-sulfation than in 6-O-sulfation may be explained by differences in K_m values for the different enzymes.

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