

Transport of UDP-Galactose into the Golgi Lumen Regulates the Biosynthesis of Proteoglycans*

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The lumen of the Golgi apparatus is the subcellular site where galactose is transferred, from UDP-galactose, to the oligosaccharide chains of glycoproteins, glycolipids, and proteoglycans. The nucleotide sugar, which is synthesized in the cytosol, must first be transported into the Golgi lumen by a specific UDP-galactose transporter. Previously, a mutant polarized epithelial cell (MDCKII-RCA⁺) with a 2% residual rate of transport of UDP-galactose into the lumen of Golgi vesicles was described (Brandli, A. W., Hansson, G. C., Rodriguez-Boulant, E., and Simons, K. (1988) *J. Biol. Chem.* 263, 16283–16290). The mutant has an enrichment in glucosyl ceramide and cell surface glycoconjugates bearing terminal *N*-acetylglucosamine, as well as a 75% reduction in sialylation of cell surface glycoproteins and glycosphingolipids.

We have now studied the biosynthesis of galactose containing proteoglycans in this mutant and the corresponding parental cell line. Wild-type Madin-Darby canine kidney cells synthesize significant amounts of chondroitin sulfate, heparan sulfate, and keratan sulfate, while the above mutant synthesizes chondroitin sulfate and heparan sulfate but not keratan sulfate, the only proteoglycan containing galactose in its glycosaminoglycan polymer. The mutant also synthesizes chondroitin 6-sulfate rather than only chondroitin 4-sulfate as wild-type cells. Together, the above results demonstrate that the Golgi membrane UDP-galactose transporter is rate-limiting in the supply of UDP-galactose into the Golgi lumen; this in turn results in selective galactosylation of macromolecules. Apparently, the K_m for galactosyltransferases involved in the synthesis of linkage regions of heparan sulfate and chondroitin sulfate are significantly lower than those participating in the synthesis of keratan sulfate polymer, glycoproteins, and glycolipids. The results also suggest that the 6-*O*-sulfotransferases, in the absence of their natural substrates (keratan sulfate) may catalyze the sulfation of chondroitin 4-sulfate as alternative substrate.

tween cells and their environment (1, 2). They have been implicated to play a role in cell-cell (3) and cell-matrix interactions (4), organization of basement membranes (5), control of macromolecules' diffusion (6), and also interactions with a variety of ligands such as growth factors, hormones, and neurotransmitters (7).

In most GAGs,¹ the repeating disaccharide units are composed of one amino sugar and one uronic acid, the only exception being keratan sulfate in which galactose replaces the sugar acid. Most GAGs are attached to serine of the core protein by a tetrasaccharide of xylose-galactose-galactose-glucuronic acid (8, 9). Keratan sulfate is an exception; keratan sulfate I, from cornea, is *N*-linked to proteins and keratan sulfate II, from skeletal tissues, is *O*-linked via serine or threonine to *N*-acetylgalactosamine (10).

The biosynthesis of proteoglycans is a post-translational event and takes place in the lumen of the endoplasmic reticulum and Golgi apparatus (11, 12). Most sugar nucleotides involved in these glycosylation steps are synthesized in the cytoplasm of the cell and require specialized transporters to translocate them to their site of synthesis within the lumen of these organelles (13).

Because nucleotide sugar transporters may play important roles in the control of biosynthesis of many glycoconjugates, we searched for a model where the *in vivo* relevance of these transporters could be demonstrated. A Madin-Darby canine kidney strain II cell line resistant to *Ricinus communis* agglutinin was previously isolated and characterized (14). The *in vitro* biochemical defect leading to the altered phenotype was determined to be a 98% deficiency in the rate of transport of UDP-galactose into Golgi vesicles; transport of UDP-GlcNAc and CMP-sialic acid was similar to that into wild-type cells. The levels of activity of galactosyltransferases and sialyltransferases were the same in mutant and wild-type cells. The defect resulted in enrichment of cell surface glycoconjugates bearing terminal *N*-acetylglucosamine and of glucosylceramides, both endogenous acceptors for galactose. UDP-galactose is also crucial in the assembly of the linkage regions of virtually all proteoglycans; therefore this MDCK II cell mutant can be used

Proteoglycans are complex macromolecules consisting of a protein core to which glycosaminoglycans are covalently linked. Their strategic localization in the plasma membrane and extracellular matrix makes them important intermediates be-

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¹ The abbreviations used are: GAGs, glycosaminoglycans; CS, chondroitin sulfate; HS, heparan sulfate; KS, keratan sulfate; Gal, galactose; Gal6S, galactose 6-sulfate; GlcNAc6S, 2-deoxy-2-acetamido-6-*O*-sulfo-D-glucose; Di6S, disaccharide 6-sulfate, GlcNAc6S-Gal; ΔU-GalNAc4S, *O*-(β-D-enopyranosyluronic acid)-(1-3)-2-deoxy-2-acetamido-D-galactose-4-*O*-sulfate; ΔU-GalNAc6S, *O*-(β-D-enopyranosyluronic acid)-(1-3)-2-deoxy-2-acetamido-D-galactose-6-*O*-sulfate; GlcNS, 2-sulfoamino-2-deoxy-D-glucose; ΔU-GlcNS, *O*-(enopyranosyluronic acid)-(1-4)-2-sulfoamino-2-deoxy-D-glucose; ΔU-GlcNS6S, *O*-(enopyranosyluronic acid)-(1-4)-2-sulfoamino-2-deoxy-D-glucose-6-*O*-sulfate; ΔU-GlcNAc6S, *O*-(enopyranosyluronic acid)-(1-4)-2-deoxy-2-acetamido-D-glucose-6-*O*-sulfate; ΔU,2S-GlcNS6S, *O*-α-L-ido-(enopyranosyluronic acid 2-*O*-sulfate)-(1-4)-2-sulfoamino-2-deoxy-D-glucose-6-*O*-sulfate; MDCK, Madin-Darby canine kidney.

as a tool to investigate the consequences of limited availability of UDP-Gal within the Golgi lumen in the biosynthesis of different glycosaminoglycan chains. We found that limiting the availability of UDP-galactose selectively inhibits the biosynthesis of keratan sulfate; this in turn results in changes in the sulfation pattern of chondroitin sulfate.

MATERIALS AND METHODS

Glycosaminoglycans and Enzymes—Dermatan sulfate (from pig skin) and chondroitin 4- and 6-sulfate (from whale and shark cartilage, respectively) were purchased from Seikagaku America. Heparan sulfate from bovine pancreas was prepared at Opocrin Research Laboratory, Modena, Italy (15). Heparitinases I and II were prepared from induced *Flavobacterium heparinum* (16). Chondroitinase ABC and bovine cornea keratan sulfate were purchased from Sigma. Keratanase from *Pseudomonas* sp. and keratanase II from *Bacillus* sp. were purchased from Seikagaku America.

Cells—MDCK strain II cells (MDCK II) and the *R. communis* agglutinin-resistant strain of MDCK II, MDCK-RCA^r, were obtained from Dr. Enrique Rodriguez-Boulton, Department of Cell Biology and Anatomy, Cornell University Medical College, New York. Both cell lines were maintained in Earle's minimum essential medium, 5% fetal calf serum, 110 units/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine at 37 °C and 5% CO₂. Cells were free of mycoplasma (17).

Radiolabeling of Cells and Isolation of Glycosaminoglycans—Monolayer cultures in 25-cm² Falcon dishes were radiolabeled for 48 h with 150 µCi/ml H₂³⁵SO₄ in 5 ml of minimal essential medium lacking sulfate (Joklik's MEM, Life Technologies, Inc.) supplemented with calcium. Alternatively, cells were radiolabeled with 10 µCi of [³H]glucosamine/ml for 48 h (18). At the end of the incubation period, the medium was removed and the cells were washed two times, each with 5 ml of phosphate-buffered saline. The washes were combined with the culture medium. Cells attached to the flask were solubilized with 1 ml of 3.5 M urea in phosphate-buffered saline, pH 7.4. Twenty-µl aliquots were utilized to estimate protein content. Proteoglycans from cells and medium were precipitated with 3 volumes of ethanol at -20 °C in the presence of chondroitin 4-sulfate as carrier. An aliquot (5 µl) from these proteoglycans were analyzed by agarose gel electrophoresis and the rest was incubated with 6 mg/ml Pronase in 0.05 M Tris-HCl, pH 8.0, at 37 °C for 18 h. Following the incubation, the mixture was boiled for 7 min at 100 °C. Radioactive GAGs were purified on a 0.5-ml DEAE-Sephacel column (2.9 × 0.6 cm) equilibrated in water. The column was sequentially washed with 2.5 ml of water, 0.2 M NaCl, and 2 M NaCl. Fractions of 0.5 ml were collected and the radioactivity in each fraction was measured. GAGs eluted in the latter fraction were analyzed by agarose gel electrophoresis in 1,3-diaminopropaneacetate buffer, pH 9.0, as described previously (19). Following electrophoresis, GAGs were precipitated in the gel with 0.2% cetyltrimethylammonium bromide for 1 h. The gel was dried, stained with toluidine blue, and exposed to x-ray film for 24 h.

Isolation of Keratan Sulfate—Keratan sulfate radiolabeled with [³⁵S]sulfate or [³H]glucosamine (approximately 200,000 cpm) was separated from other glycosaminoglycans by incubation with a crude extract (10 µg of protein) from *F. heparinum* (16). The incubation was done in 0.05 M ethylenediamine acetate buffer, pH 7.0, at 30 °C for 18 h. This extract contains a mixture of enzymes capable of digesting heparan sulfate, chondroitin sulfate, dermatan sulfate, heparin, and hyaluronic acid. Keratan sulfate is the only GAG resistant to these enzymes (20). Intact keratan sulfate was then separated from small products through a Centricon 10 filter (Amicon).

Enzymatic Digestion of Glycosaminoglycans and Product Identification—Approximately 70,000 cpm of ³⁵S-GAGs were incubated with 0.04–0.1 unit of specific enzymes such as chondroitinase ABC, keratanase, and keratanase II. These were used according to the manufacturer's instructions. Heparitinase I and II incubations were done as described previously (16). After incubation, the products were separated on Whatman No. 1 descending paper chromatography in isobutyric acid: 1 N NH₄OH (5/3, v/v) for 24 h (16). Nonradioactive products were visualized with silver nitrate staining while radioactive ones were detected by autoradiography.

RESULTS

Keratan Sulfate is Synthesized in Wild-type but not Mutant MDCK II Cells—To determine which glycosaminoglycans are synthesized by wild-type and RCA^r-mutant MDCK II cells, monolayer cultures were incubated for 48 h with radiolabeled

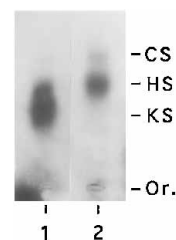


FIG. 1. **Labeling of glycosaminoglycans with [³H]glucosamine.** Approximately 50,000 cpm of total cellular GAGs labeled with [³H]glucosamine were applied to an agarose gel slab in 1,3-diaminopropaneacetate buffer, pH 9.0. After electrophoresis, GAGs were precipitated in the gel with 0.2% cetyltrimethylammonium bromide for 1 h. The gel was then incubated with autoradiography enhancer and water, each for 30 min. Standard GAGs were visualized with toluidine blue. The gel was dried and labeled GAGs were visualized on x-ray film after exposure for 5 days at -70 °C. Lanes 1 and 2, intracellular GAGs from wild-type cells and RCA^r-mutant cells, respectively. Or, origin.

sulfate. Radiolabeled GAG chains were obtained, separated on agarose gel electrophoresis, and quantitated by autoradiography. Both wild-type and mutant cells synthesize sulfate radiolabeled GAGs comigrating with standard chondroitin sulfate and heparan sulfate. In addition, wild-type cells appear to synthesize a glycosaminoglycan which migrates slower than heparan sulfate and will be shown below to be keratan sulfate. The percent of intracellular GAGs which were HS, CS, and KS was 53, 6, and 41%, respectively, while that of GAGs secreted into the medium was 68% HS, 19% CS, and 13% KS.

To further characterize GAGs from wild-type and mutant MDCK cells, cultures were incubated with tritiated glucosamine for 48 h; GAGs were obtained and separated as described for the sulfate-labeled cells. As can be seen in Fig. 1, one broad radioactive band, comigrating with standard heparan sulfate, was observed in both wild-type and mutant cells. In addition, a broad band, migrating slower than standard heparan sulfate was observed in wild-type but not mutant cells.

The experiments described below were designed to demonstrate that the above described slower migrating GAG, containing glucosamine and sulfate, is indeed keratan sulfate. Because standard keratan sulfate from cornea partially comigrates with heparan sulfate in the above agarose gel system, ³⁵S-GAGs from the medium and cells were treated with a crude fraction of enzymes from *F. heparinum* as described under "Materials and Methods." This fraction contains enzymes which degrade chondroitin sulfate, heparan sulfate, heparin, dermatan sulfate, and hyaluronic acid. Keratan sulfate is resistant (20) and can be adequately separated from the others GAGs. As can be seen in Fig. 2, both medium and cells from wild-type MDCK cells contained GAGs which appeared to be resistant to enzymes from *F. heparinum*. These GAGs were absent in mutant cells (Fig. 2). The broad migration pattern of KS after treatment with extract from *F. heparinum* is probably not the result of incomplete digestion of HS because the pattern did not change following repeated addition of extract. We hypothesize that there are two distinct populations of KS; by analogy to KS from cornea, regions that are highly fucosylated or sulfated and are closer to the linkage region appear to be more resistant to keratanases (30).

Conclusive evidence that the component resistant to the action of crude extract of *F. heparinum* is indeed keratan sulfate was obtained with two specific keratanases. Keratanase from *Pseudomonas* sp. (21) specifically cleaves keratan sulfate yielding saturated disaccharides with galactose at the reducing end. No cleavage occurs when galactose is sulfated in position 6. Keratanase from *Bacillus* sp. (22) specifically cleaves keratan sulfate by hydrolyzing 1,3-β-glucosaminidic residues linked to galactose. For cleavage the enzyme requires that glucosa-

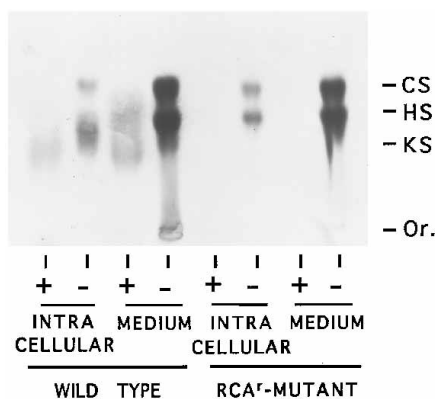


FIG. 2. Digestion of ^{35}S -glycosaminoglycans with a crude mixture of enzymes from *F. heparinum*. Total ^{35}S -GAGs (20,000–80,000 cpm) were digested with (+) or without (–) a crude fraction of enzymes from *F. heparinum* (see “Materials and Methods”). After incubation at 30 °C for 18 h the mixture was applied to an agarose gel slab in 1,3-diaminepropaneacetate buffer, pH 9.0. After electrophoresis, GAGs were precipitated with 0.2% cetyltrimethylammonium bromide for 1 h. The gel was dried and standard GAGs were stained with toluidine blue. Radioactive GAGs were visualized on x-ray film after 9 days at room temperature.

mine be sulfated in the 6-*O*-position but acts independently of the sulfate at the 6-*O*-position of the galactose linked to glucosamine.

[^{35}S]Keratan sulfate, synthesized by wild-type cells, was obtained as described under “Materials and Methods.” Aliquots from the medium and intracellular material were then treated separately with or without keratanases from *Pseudomonas sp.* and *Bacillus sp.*; samples were then analyzed by agarose gel electrophoresis and paper chromatography. As seen in Figs. 3a (medium) and 4a (intracellular material), keratanases degraded approximately 50–60% of GAGs comigrating with keratan sulfate, compared to controls incubated without enzymes.

Treatment of radiolabeled keratan sulfate from wild-type cells and medium with keratanase from *Pseudomonas sp.* showed three main products following paper chromatography (Figs. 3b and 4b, lane 1). The fastest moving product comigrates with a degradation product from bovine cornea keratan sulfate, previously identified as GlcNAc,6S-Gal (21). The second fastest migrating radioactive product (B), apparently not present in bovine cornea keratan sulfate, is hypothesized to be a trisulfated tetrasaccharide, GlcNAc,6S-Gal,6S-GlcNAc,6S-Gal, containing one internal galactose 6-sulfate, which renders it resistant to the action of this keratanase. The slowest migrating product (D) is hypothesized to be GlcNAc,6S-Gal,6S-GlcNAc,6S-Gal,6S-GlcNAc,6S-Gal based on its resistance to the above keratanase and previously described mobility in this system (20). The identity of the above oligosaccharides was not further established. Radioactive products comigrating with GlcNAc,6S-Gal and product B were also observed when [^3H]glucosamine-labeled KS was digested with the above keratanases (not shown).

Digestion of the above radiolabeled keratan sulfate with keratanase from *Bacillus sp.* (Figs. 3b and 4b, lane 2), resulted in formation of two major products, with the same migration as those found when standard keratan sulfate from bovine cornea was treated with this enzyme. The action of this keratanase on intracellular keratan sulfate generated, besides the products found in cornea, additional products (Fig. 4b, lane 2). The radioactivity at the origin is most likely undigested keratan sulfate also observed previously in the gel of Figs. 3a and 4a.

Structural Analyses of Chondroitin Sulfate from Wild-type and Mutant MDCK II Cells—The structure of chondroitin sulfate synthesized by wild-type and mutant cells was analyzed

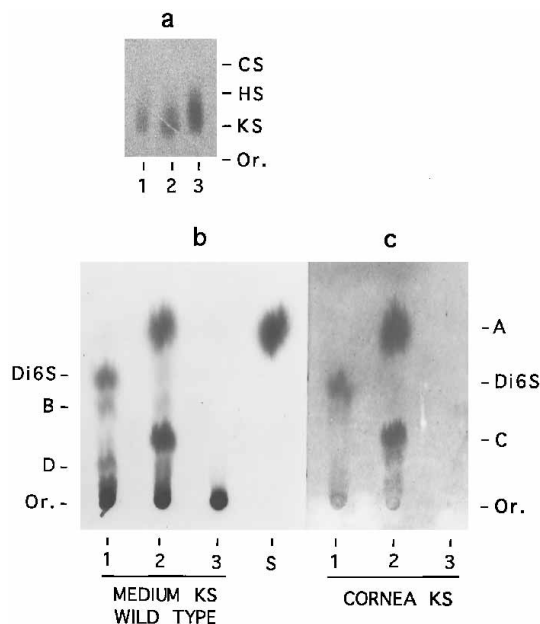


FIG. 3. Digestion of [^{35}S]keratan sulfate from the medium with specific keratanases. Approximately 70,000 cpm of [^{35}S]keratan sulfate, isolated as described under “Materials and Methods,” together with standard keratan sulfate, were digested with either 0.04 unit of keratanase from *Pseudomonas sp.* (lanes 1) or 0.002 unit of keratanase II from *Bacillus sp.* (lanes 2) or buffer alone (lanes 3). 4,000 cpm from each incubation were subjected to agarose gel electrophoresis (a). The remaining sample was subjected to descending paper chromatography on Whatman No. 1 in isobutyric acid, 1 M NH_4OH 5:3 (v/v) for 24 h (b and c). Labeled products released by keratanase from *Pseudomonas sp.* (b, lane 1) and *Bacillus sp.* (b, lane 2) were visualized on x-ray film after 5 days at room temperature. b, lane 3, control incubation with labeled KS and buffer alone. S: [^{35}S]SO $_4$; in this solvent system, sulfate and *N*-acetylglucosamine-6-sulfate comigrate. A, putative GlcNAc,6S; Di6S, GlcNAc,6S-Gal; B, putative; GlcNAc,6S-Gal,6S-GlcNAc,6S-Gal; C, unknown; D, putative GlcNAc,6S-Gal,6S-GlcNAc,6S-Gal,6S-GlcNAc,6S-Gal. Products from standard keratan sulfate were detected with silver nitrate (c).

following incubation of ^{35}S -GAGs from the medium and cells with chondroitinase ABC (Fig. 5). All samples showed radiolabeled products comigrating with previously characterized $\Delta\text{U}(1\text{--}3)\text{GalNAc}4\text{S}$ from standard chondroitin 4-sulfate. The disaccharide was also a substrate for chondro-4-sulfatase but not for chondro-6-sulfatase (not shown). This is consistent with the presence of chondroitin 4-sulfate in this original radioactive sample.

An unexpected product was detected in the mutant but not in the wild-type cells. It represents 13% of the mutant disaccharides and comigrates with a degradation product from standard chondroitin 6-sulfate, previously characterized as $\Delta\text{U}(1\text{--}3)\text{GalNAc}6\text{S}$. The disaccharide was also a substrate for chondro-6-sulfatase but not for chondro-4-sulfatase (not shown). The slowest migrating radioactive product (A) is a tetrasaccharide, disaccharide disulfate, or a mixture of these, based on previous degradation structures of chondroitin sulfate from whale cartilage (23).

Enzymatic Fragmentation of Heparan Sulfate—Heparan sulfate was analyzed by the combined action of two specific enzymes from *F. heparinum* (16) on ^{35}S -GAGs from wild-type and mutant cells and respective media. Heparitinase I is specific for *N*-acetyl or *N*-sulfate glucosaminido-glucuronic acid linkages. The enzyme acts only on heparan sulfate regions where the *N*-acetyl or *N*-sulfate glucosamine is not sulfated at the 6-position; heparitinase II acts preferentially upon glucosaminido-glucuronic acid linkages where *N*-acetyl or *N*-sulfate glucosamine is sulfated at the 6-position. The combined action of these

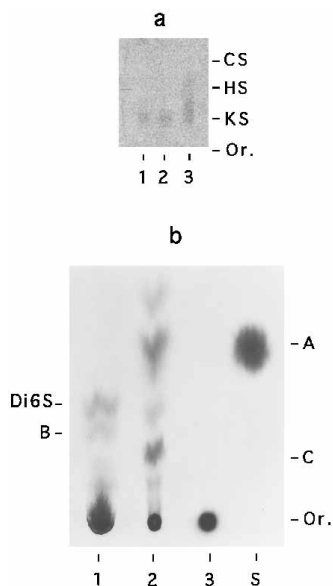


FIG. 4. Digestion of intracellular [^{35}S]keratan sulfate with specific keratanases. Approximately 70,000 cpm of [^{35}S]keratan sulfate was incubated with keratanase from *Pseudomonas* sp. (lanes 1), *Bacillus* sp. (lanes 2), or buffer alone (lanes 3) and then subjected to electrophoresis (a) or chromatography (b) as described in the legend of Fig. 3. Or, origin; A, putative GlcNAc,6S; Di6S, GlcNAc,6S-Gal; B, putative GlcNAc,6S-Gal,6S-GlcNAc,6S-Gal; C, unknown. S, [^{35}S]SO $_4$.

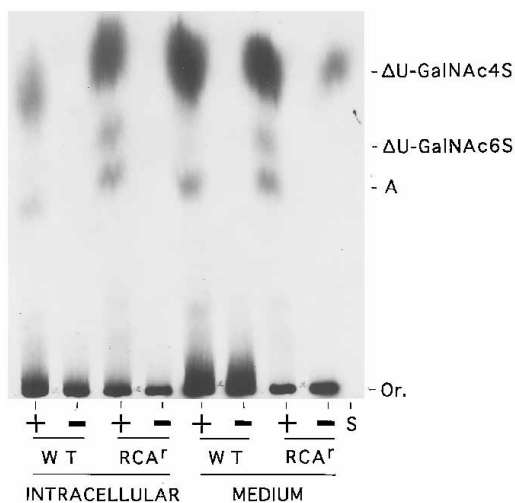


FIG. 5. Digestion of ^{35}S -glycosaminoglycans with chondroitinase ABC. Approximately 30,000 cpm of total intracellular or medium ^{35}S -GAGs, together with standard chondroitin 4-sulfate, were incubated with (+) or without (-) 0.02 units of chondroitinase ABC as described by the manufacturer. After incubation, the mixture was subjected to paper chromatography as described in the legend of Fig. 3. S, [^{35}S]SO $_4$. $\Delta\text{U-GalNAc4S}$, unsaturated disaccharide 4-sulfate; $\Delta\text{U-GalNAc6S}$, unsaturated disaccharide 6-sulfate; A, putative unsaturated tetrasaccharide; Or, origin.

two enzymes gave rise to four radioactive products (not shown), each comigrating with degradation products from standard heparan sulfate (from bovine pancreas) and previously identified as (in slower to faster migrating order) $\Delta\text{U},2\text{S-GlcNS},6\text{S}$, $\Delta\text{U-GlcNS},6\text{S}$, $\Delta\text{U-GlcNS}$, and $\Delta\text{U-GlcNAc},6\text{S}$. The formation of unsaturated, trisulfated disaccharides after heparitinase II indicates a high content of 2-sulfated iduronic acid. No difference was found between heparan sulfate disaccharides from wild-type and mutant cells.

DISCUSSION

Three important conclusions can be drawn from the above studies: (a) wild-type MDCK cells synthesize keratan sulfate in

culture; (b) in mutant MDCK cells that are highly deficient in transport of UDP-galactose into the Golgi lumen, the biosynthesis of keratan sulfate as well as glycoproteins and glycolipids are highly reduced while that of heparan sulfate and chondroitin 4-sulfate is virtually intact; and (c) the above mutant cells show a change in the sulfation pattern of chondroitin 4-sulfate with additional sulfation in the 6-O-position. The above shown selective biosynthesis of galactose containing glycoconjugates, together with previous studies (14, 24–27), demonstrate that limited availability of UDP-galactose in the lumen of the Golgi results in biosynthesis of chondroitin sulfate and heparan sulfate, both glycoconjugates with galactose in the linkage region; at the same time there is a marked decrease in the biosynthesis of glycoproteins, glycolipids, and keratan sulfate, the latter a proteoglycan which contains galactose in its glycosaminoglycan polymer. One likely explanation for this is that the K_m for galactosyltransferases involved in the biosynthesis of the linkage region of proteoglycans such as chondroitin sulfate and heparan sulfate is significantly lower than other galactosyltransferases resulting in preferential synthesis of these proteoglycans over other galactose containing glycoconjugates. Unfortunately, direct proof for this hypothesis is difficult to establish as physiologic apparent K_m measurements require the use of physiologic substrate intermediates, a problem in GAG biosynthesis. In addition, reactions *in vitro* require exogenous detergents, another source of difficulties in interpreting physiologic parameters. The possibility that galactosylation of different galactose-containing proteoglycans occurs in different regions of the Golgi apparatus and that there is differential transport of UDP-galactose into these hypothetical compartments cannot be ruled out although there is no current evidence that would support such hypothesis.

Mutant Chinese hamster ovary cells with a 90–98% deficiency in transport of UDP-galactose into Golgi vesicles have been described (24–26); these cells showed a corresponding decrease in galactose and sialic acid containing glycoproteins and glycosphingolipids. Heparan sulfate and chondroitin sulfate biosynthesis appeared to be normal (27). Keratan sulfate was not detected in wild-type or mutant Chinese hamster ovary cells (27).

The occurrence of keratan sulfate in wild-type MDCK cells and its absence in the above mutants deserves some attention. This particular proteoglycan does not occur widely as heparan sulfate and chondroitin sulfate, for example. Depending on the tissue it originates, significant structure variability has been reported including chain length, degree of branching and sulfation, as well as content of fucose, sialic acid, and *N*-acetylglucosamine (28, 29). Keratan sulfate of wild-type MDCK cells appear to have similarity with that from cornea. Significant portions of both of these were resistant to keratanase I and II. In a previous study, it was described that keratanase from *Pseudomonas* sp. is inactive when galactose is sulfated in the 6-position (21), and also when *N*-acetylglucosamine is fucosylated (30) in both cases preventing digestion of adjacent non-sulfated galactose. It is possible that the resistance to keratanase in keratan sulfate from MDCK cells may not solely be due to sulfation of galactose, but also to fucosylation of *N*-acetylglucosamine, or both.

The additional sulfation of chondroitin 4-sulfate in position 6, in RCA $^{-}$ -mutant MDCK II cells, was a major surprise, but one may speculate that in the absence of galactose incorporation into the keratan sulfate polymer, the galactose-6-O-sulfotransferase for keratan sulfate, without a substrate in the Golgi lumen, may utilize chondroitin sulfate galactosamine as an acceptor. This hypothesis is supported by the fact that purified chondroitin 6-sulfotransferase can sulfate not only

chondroitin sulfate but also keratan sulfate (31).

Together, the above results strongly suggest that nucleotide sugar transporters in the Golgi membrane are limiting in their supply of substrates to the Golgi lumen; this has not only quantitative effects as to the types of macromolecules being synthesized, but also qualitative. Therefore, it will be important to further understand the regulatory role of these transporters in the biosynthesis and modifications of Golgi luminal glycoproteins, proteoglycans, and glycosphingolipids.

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