Synthesis of Corneal Keratan Sulfate Proteoglycans by Bovine Keratocytes in Vitro*

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Keratan sulfate proteoglycans (KSPGs) are the major proteoglycans of the cornea and are secreted by keratocytes in the corneal stroma. Previous studies have been able to show only transient secretion of KSPG in cell culture. In this study, cultures of bovine keratocytes were found to secrete the three previously characterized KSPG proteins into culture medium. Reactivity with monoclonal antibody I22 demonstrated substitution of these proteins with keratan sulfate chains. KSPG constituted 15% of the proteoglycan metabolically labeled with [35S]sulfate in keratocyte culture medium. This labeled KSPG contained keratan sulfate chains of 4700 Da compared to 21,000 Da for bovine corneal keratan sulfate. Labeled keratan sulfate from cultures contained nonsulfated, monosulfated, and disulfated disaccharides that were released by digestion with endo-β-D-galactosidase or keratanase II. Nonsulfated disaccharides were relatively more abundant in keratan sulfate than in corneal keratan sulfate. These results show that cultured bovine keratocytes maintain the ability to express all three of the known KSPG proteins, modified with keratan sulfate chains and sulfated on both N-acetylgalosamnine and galactose moieties. KSPG made in vitro differs from that found in vivo in the length and sulfation of its keratan sulfate chains. The availability of cell cultures secreting corneal keratan sulfate proteoglycans provides an opportunity to examine biosynthesis and control of this important class of molecules.

Keratan sulfate proteoglycans (KSPGs)† represent the major class of proteoglycans in the corneal stroma (1). KSPGs are thought to play an important role in corneal structure and physiology, particularly in the maintenance of corneal transparency (2). These molecules consist of keratan sulfate chains attached to proteins via hybrid-type N-linked linkage oligosaccharides (3, 4), a linkage structure that differs from that of the O-linked keratan sulfate of cartilage and brain proteoglycans (5), making these compounds unique in both structure and their abundance in the cornea. Three different corneal KSPG core proteins have been identified. All are members of a family of leucine-rich proteins that contains several other common proteoglycans (6–8). The KSPG proteins are distributed in a number of tissues in addition to cornea (8–10). In noncorneal tissues the KSPG proteins are modified with oligomeric N-acetyllactosamine (i.e. short nonsulfated keratan sulfate) rather than the long, highly sulfated glycosaminoglycan chains characteristic of corneal KSPG (11, 12). Corneal KSPGs can, therefore, be understood to be a group of widely distributed extracellular proteins that exhibit a unique tissue-specific form of glycosylation.

Corneal KSPGs are secreted by keratocytes, the predominant cell type of the corneal stroma (13). Synthesis of normal KSPG molecules appears to be highly regulated in these cells. During development, migrating neural crest cells do not begin to secrete sulfated KSPG until their arrival in the stroma (14, 15). In healing corneal wounds, rejecting corneal grafts, and other pathologic conditions of the cornea, sulfated KSPG is reduced or has an altered structure (16–22). Initial studies of keratocytes maintained in cell culture reported that little or no synthesis of the keratan sulfate glycosaminoglycan characteristic of normal cornea KSPG occurs in vitro (23–27). More recent studies have shown that some keratocyte cultures synthesize the KSPG core proteins but do not add sulfated keratan sulfate to this protein (28, 29). In the present study, we examined cultured bovine keratocytes and found that these cells do, in fact, secrete sulfated KSPG, but the keratan sulfate chains of this compound are shorter and less highly sulfated than those of normal corneal KSPG.

EXPERIMENTAL PROCEDURES

Materials—Bovine corneal KSPG (70P fraction) (30), arterial lumican (11), and endo-β-D-galactosidase (31) were prepared as described previously. Purification of monoclonal antibody I22 against keratan sulfate and of affinity-purified antibodies against KSPG proteins also was described elsewhere (14, 32). Purified bovine corneal keratan sulfate, keratanase (keratan sulfate 1,4,6-β-D-galactosidase, EC 3.2.1.103 from Pseudomonas sp.), and keratanase II (keratan sulfate endo-β-N-acetylgallosaminidase from Bacillus sp.) were purchased from Seikagaku America Inc. (Rockville, MD). For clarity, keratanase is referred to as keratanase I according to the convention of Ernst et al. (33).

Methods—Central portions of fresh corneas from slaughter-aged steers were incubated in 0.2% trypsin in (NaCl, 0.137 M; KCl, 5 mM; Na2HPO4, 1 mM; KH2PO4, 1 mM; glucose, 60 mM, pH 7.2) for 10 min at 37 °C, then epithelial and endothelial cells were removed by scraping in cold saline. The stroma was rinsed, minced, and incubated in Dulbecco’s modified Eagle’s medium/F12 medium (Sigma, catalog no. D9605) with 10% fetal bovine serum (Life Technologies, Inc.) and antibiotics (penicillin, streptomycin, 100 μg/ml; gentamicin, 50 μg/ml, amphotericin B, 2.5 μg/ml) in 5% CO2 at 37 °C. Culture medium was changed weekly until keratocyte outgrowth became confluent (3–5 weeks). Cultures were maintained in closed 75-cm2 plastic flasks in an air atmosphere at 37 °C and were passaged 1:2 after confluency by trypsinization. Cells were stored frozen in liquid nitrogen in the fourth passage.

For KSPG production, confluent cultures (passage 6–10) were transferred into Dulbecco’s modified Eagle’s medium/F12 medium in 0.1%
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horse serum (Life Technologies, Inc.) with antibodies, insulin, 5 μg/ml, selenium, 5 ng/ml, and transferrin, 5 μg/ml (ITS, Collaborative Research Inc., Lexington, MA). Keratan sulfate was metabolically labeled by addition of carrier-free H3SO4, 100 μCi/ml for 3 days. Culture medium was collected and combined with a rinse of the cell layer in buffered saline. This soluble fraction contained all the detectable KSPG and was used as the source for KSPG for purification and analysis.

Immunological detection of KSPG used solid phase ELISA and dot blotting similar to those described previously (10, 12, 17) with minor modifications. ELISA assays were carried out in Immulon II 96-well plates (Dynatech Laboratories Inc., Chantilly, VA) using TTTBS (0.15 M NaCl, 0.02 M Tris- HCl, pH 7.4, 0.01% Tween 20, 0.1% thimerosal) for rinses, and TTTBS with 1% BSA for antibody solutions. Samples were analyzed in triplicate, and specific binding was determined by subtracting binding obtained using primary antibodies. Dot blotting of affinity-purified corneas was carried out on nitrocellulose as described previously (18) using luminol detection (9). Film images were digitized using a 12-bit CCD camera and quantified with NIH Image software (available at http://rsb.info.nih.gov/nih-image).

Immune precipitation of KSPG from culture medium used protein G-agarose (Pierce). The washed beads were incubated with an equal volume of antiserum for 4 h at room temperature, then washed by centrifugation three times in 1% BSA in TTTBS. Antibody-loaded beads were used immediately or stored 4°C for up to 1 week. Twenty micro centrifugation three times in 1% BSA in TTTBS. Antibody-loaded beads were used immediately or stored 4°C for up to 1 week. Twenty micro-centrifugation three times in 1% BSA in TTTBS. Antibody-loaded beads were used immediately or stored 4°C for up to 1 week. Twenty micro-centrifugation three times in 1% BSA in TTTBS. Antibody-loaded beads were used immediately or stored 4°C for up to 1 week. Twenty micro-centrifugation three times in 1% BSA in TTTBS. Antibody-loaded beads were used immediately or stored 4°C for up to 1 week. Twenty micro-centrifugation three times in 1% BSA in TTTBS. Antibody-loaded beads were used immediately or stored 4°C for up to 1 week. Twenty micro-

temperature. 100 μl of 1 x unlabeled NaBH4, were added for 2 h to complete reduction. Borate was removed by repeated drying from methanol in a stream of nitrogen. The reduced oligosaccharides were analyzed on a 1 x 50-cm column of TSK-HW40S gel (TosoHaas, Montomeryville, PA) eluted in 0.1 M NH4HCO3 at 0.5 ml/min at room temperature. Di- and tetrasaccharides released by hyaluronidase digestion of chondroitin sulfate were used as standards. The radioactive disaccharides in each preparation were collected, dried, and analyzed by high pH anion exchange chromatography using a Dionex PA-100 column eluted with 20 mM NaOH at 1 ml/min. A gradient of NaCl from 0 to 0.2 M NaCl at 5 to 20 min, then to 1.0 M NaCl at 35 min, eluted the sulfated disaccharides. Fractions of 0.5 min were collected, and 1H and 35S radioactivity was determined by scintillation counting.

RESULTS

Initial experiments determined that conditioned medium from cultures of bovine keratocytes contained proteins recognized by antibodies to corneal KSPG. Fig. 1 shows immunoblotting of the KSPG antigens from culture medium after electrophoretic separation by SDS-PAGE. The KSPG from culture medium (lane 3) was heterogeneous in size (70 to >100 kDa), larger than arterial lumican, a nonsulfated form of KSPG protein (lane 2), but smaller than the KSPG from cornea (lane 1). Unconditioned medium (lane 4) had no detectable KSPG.

Three KSPG core proteins from corneal stroma can be separated by ion exchange chromatography and SDS-PAGE after removal of their modifying keratan sulfate chains with endo-beta-galactosidase (6). Concentrated keratocyte-conditioned culture medium was treated with endo-beta-galactosidase, then proteins in the medium were separated by Mono Q ion exchange chromatography. As shown in Fig. 2A, the KSPG antigens, detected by ELISA, were separated into two somewhat heterogeneous components, similar in distribution to the core proteins of purified corneal KSPG (Fig. 2B). Immunoblotting of the KSPG proteins in the pooled peak fractions from these columns is shown in Fig. 3. Purified corneal KSPG proteins from the A region of both columns contained 46- and 36-kDa proteins (lanes 1 and 3). These proteins were previously identified as proteins 37A (keratan) and 25. In lanes 2 and 4, proteins in the B fractions from both keratocyte culture medium and purified corneal KSPG separated as a single 48-50-kDa protein, previously shown to be lumican (protein 37B) (6). These results indicate that all three KSPG proteins previously identified in cornea are secreted by stromal keratocytes into the culture medium.

Sulfation of corneal KSPGs allows separation of these molecules from the noncorneal forms of the KSPG proteins by ion exchange HPLC. Fig. 4A shows separation of corneal KSPG by gradient elution from a Mono Q ion exchange column. In Fig. 4B, the nonsulfated form of KSPG from artery (arterial lumican) was found to elute at lower salt concentrations than did...
Corneal KSPG. Fig. 4C shows that Mono Q chromatography under the same conditions separated KSPG antigens from cell culture medium into two major components, one eluting at an ionic strength similar to arterial lumican and one eluting at higher salt concentrations, slightly before corneal KSPG.

The data in Figs. 1 and 4 indicate that some of the KSPG in keratocyte-conditioned culture medium is larger and some more highly charged than the nonsulfated form of these molecules. These physical characteristics suggest that a portion of the KSPG protein secreted in cell culture contains keratan sulfate. Keratan sulfate in these molecules was confirmed by the KSPG protein secreted in cell culture contains keratan sulfate and its higher molecular weight component (K_{av} = 0.2) and about 15% as a smaller (K_{av} = 0.76) component. The larger 35S-labeled component was found to be 90% sensitive to chondroitinase ABC, whereas the smaller component was not sensitive to chondroitinase (data not shown). KSPG antigens (Fig. 6A, open circles) eluted in the region of the smaller, chondroitinase-resistant component. KSPG proteins immune-precipitated from labeled culture medium eluted at the same volume as the smaller proteoglycan peak (Fig. 6B). Similarly, KSPG isolated

![Diagram A](Image 102x581 to 245x729)

**Fig. 2. Separation of KSPG core proteins by ion-exchange chromatography.** Conditioned medium (50 ml) from keratocyte cultures (A) and nonconditioned medium containing 100 μg of purified corneal KSPG (B) were concentrated by ultrafiltration to 1 ml, dialyzed against 0.1 m Tris-phosphate, pH 6.8, and treated with 0.01 unit/ml endo-β-galactosidase overnight at 4 °C. The KSPG core protein in 0.2 ml of each digest was separated on a 1-m Mono Q fast protein liquid chromatography (FPLC) ion exchange column with a gradient of NaCl in 6 m urea, and then KSPG core proteins in fractions were detected by ELISA with anti-KSPG antibodies as described under "Experimental Procedures." Fractions in the shaded areas (designated A and B) were collected and concentrated for analysis by SDS-PAGE.

**Fig. 3. Immunoblotting of separated core proteins.** Pooled fractions of KSPG proteins from Fig. 2 were separated by SDS-PAGE, transferred to nitrocellulose, and detected with antibody against KSPG core proteins. Lanes 1 and 2 are from conditioned culture medium. Lanes 3 and 4 are from purified KSPG. Pool A (Fig. 2) is shown in lanes 1 and 3. Pool B is shown in lanes 2 and 4. The size of the upper bands was determined to be 48–50 kDa based on standard mobilities and the lower bands approximately 36 kDa.

![Diagram B](Image 350x512 to 512x729)

**Fig. 4. Ion-exchange HPLC of intact KSPG.** Purified corneal (A) and arterial (B) KSPG (25 μg) in 1 ml of culture medium and 1 ml of medium from keratocyte cultures (C) were separated by Mono Q anion exchange chromatography, and ELISA detection of the eluted KSPG antigens was carried out in a 1:200 dilution of the eluted fractions as described under "Experimental Procedures." The dashed line in panel C shows the profile of the eluting salt gradient.

**Fig. 5. ELISA detection of KSPG.** A “sandwich” ELISA requiring simultaneous binding of KSPG to anti-core protein antibodies and to I22 monoclonal antibody against sulfated keratan sulfate was carried out as described previously (10, 12) with modifications as described under "Experimental Procedures." A, purified corneal KSPG, open circles; purified arterial KSPG, closed circles. B, keratocyte cell culture medium, closed circles; keratocyte medium treated with endo-β-galactosidase (0.010 unit/ml for 16 h at 4 °C), open triangles; nonconditioned medium, open circles.
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by differential alcohol fractionation produced a labeled component of a size identical to the immune-precipitated KSPG (Fig. 6C). Ion exchange in combination with alcohol precipitation was used to purify labeled KSPG for further analysis.

Treatment of 35S-labeled KSPG with proteinase K released a macromolecular material (Figs. 7, A and B) that was shown to be keratan sulfate by its sensitivity to endo-β-galactosidase (Fig. 7C) and keratanase I (Fig. 7D). Endo-β-galactosidase hydrolyzes both sulfated and nonsulfated keratan sulfate moieties, but keratanase I requires sulfation of glucosamine of the hydrolyzed disaccharide (33). The extent of the degradation of keratan sulfate from cell culture by keratanase I demonstrates that the sulfate label in these molecules is a component of keratan sulfate disaccharides and not associated with residual core proteins.

Fig. 6. Gel filtration analysis of proteoglycans from keratocyte cultures. Proteoglycans from culture medium were isolated by DEAE-chromatography after 3 days of labeling with [35S]sulfate as described under “Experimental Procedures.” A, labeled proteoglycans were separated on a 1 × 30-cm Superose 6 HR gel filtration column (Pharmacia) in 6 M urea, 0.1 M NaCl, 0.02 M Tris-HCl, pH 8, and radioactivity was determined by scintillation counting (closed circles). KSPG antigens in the fractions were determined by dot blot of the fractions onto nitrocellulose and detection with anti-KSPG antibodies (open circles). B, KSPG antigens were immune precipitated from the labeled purified proteoglycan as described under “Experimental Procedures,” then separated by Superose 6 chromatography as in A. C, KSPG was separated from the purified proteoglycan by fractional alcohol precipitation (described under “Experimental Procedures”) then chromatographed as for A and B. V0 and V1 mark the elution volumes of blue dextran and K2Fe(CN)6, respectively.

Further characterization of this sulfation was carried out by analysis of the disaccharides released after endo-β-galactosidase or keratanase II treatment. Oligosaccharides from the digests were 3H-labeled by reduction with 3H-sodium borohydride and then separated by gel filtration. As shown in Fig. 8A (solid circles), about two thirds of the sulfated products released by endo-β-galactosidase digestion eluted at a volume similar to disaccharide standards, and the remaining one third eluted as tetrasaccharides. Nonspecific tritium labeling was present in unidentified components smaller than the disaccharide standards, but specific labeling was primarily found associated with the disaccharide fractions (open circles). Tritiated digestion products from purified corneal keratan sulfate (Fig. 8C) also were primarily disaccharides. In contrast to endo-β-galactosidase, keratanase II cleaves keratan sulfate only at sites of sulfated glucosamine moieties (36). The sulfated products produced by keratanase II digestion of keratan sulfate labeled in culture (Fig. 8C, closed circles) consisted of about 80% disaccharides and about 20% of a component eluting between the di- and tetrasaccharide standards. Specific labeling with tritium was associated mostly with the disaccharide fraction from keratan sulfate from culture (Fig. 8C, open circles) and from cornea (Fig. 8D).

Fig. 7. Keratan sulfate chains of 35S-KSPG. KSPG, metabolically labeled with [35S]sulfate and purified by ion exchange chromatography and alcohol fractionation, was analyzed on a 1 × 30-cm Superose 12 HR gel filtration column (Pharmacia), eluted in 0.2 M NaCl, 0.02 M Tris-HCl, pH 8, as intact molecules (A), or after digestion with proteinase K (B), endo-β-galactosidase (C), or keratanase I (D) as described under “Experimental Procedures.” V0 and V1 mark the elution volumes of blue dextran and K2Fe(CN)6, respectively.
Digested with endo-β-galactosidase (A and B) or keratanase II (C and D), and the resultant oligosaccharides were labeled by reduction with 3H-NaBH4 as described under “Experimental Procedures.” The labeled oligosaccharides were separated by gel filtration chromatography on a 1 × 50-cm column of TSK-HW40S gel in 0.1 M NH4HCO3. Closed circles show keratan sulfate disaccharide peaks were obtained from keratocyte keratan sulfate (Fig. 9), one eluting at a salt concentration similar to the disaccharide fractions of the keratan sulfate proteoglycan molecules contained all three of the core proteins previously identified in corneal KSPG. Attached to these proteins were keratan sulfate glycosaminoglycan chains containing nonsulfated, monosulfated, and disulfated N-acetylgalactosamine moieties of the keratan sulfate in vivo.

Keratanase II cleaves keratan sulfate at sites of sulfated N-acetylgalactosamine, irrespective of sulfation of the galactose moieties; therefore, digests should contain only monosulfated and disulfated disaccharides. As predicted, two sulfated disaccharide peaks were obtained from keratocyte keratan sulfate (Fig. 9D), one eluting at a salt concentration similar to the monosulfated disaccharide released by endo-β-galactosidase and one at a higher salt concentration. 3H-Labeled disaccharides from both keratocyte and corneal keratan sulfate eluted at positions similar to the two sulfated disaccharides (Fig. 9, E and F). The 3H/35S ratios of the two peaks are consistent with the second peak having two sulfates per disaccharide. The ratio of 3H-monosulfated to disulfated disaccharides in this digest was 6:1 for both keratocyte and corneal keratan sulfate. The presence of mono- and disulfated disaccharides in keratan sulfate from culture medium demonstrates the ability of keratocytes to sulfate both galactose and N-acetylgalactosamine moieties of the keratan sulfate in vitro.

The size of the keratan sulfate chains from KSPG made in vitro was compared using gel filtration to that of corneal keratan sulfate. As shown in Fig. 10B, bovine corneal keratan sulfate was quite heterogeneous with a size range of 10 to 50 kDa and a median estimated at 21 kDa based on the elution volumes of standard sulfated polymers. The labeled keratan sulfate from culture medium (Fig. 10A) was estimated as 4.7 kDa under the same conditions.

The experiments presented here characterize the KSPG secreted by continuous cultures of bovine keratocytes. These proteoglycan molecules contained all three of the core proteins previously identified in corneal KSPG. Attached to these proteins were keratan sulfate glycosaminoglycan chains containing non-sulfated, monosulfated, and disulfated N-acetylgalactosamine disaccharides. The keratan sulfate chains were smaller and less highly sulfated than those isolated in vivo.

The presence of keratan sulfate in these molecules was documented in several ways. (a) Some of the KSPG molecules from culture were larger and some more highly charged than the non-sulfated KSPG of artery, suggesting the presence of glycosaminoglycan chains. (b) KSPG from cultures reacted with a keratan sulfate-specific monoclonal antibody and anti-core protein antibodies simultaneously demonstrating association of the proteins with keratan sulfate. (c) The antigenic keratan sulfate and purified 35S-labeled keratan sulfate were both sensitive to three different keratan sulfate-specific endoglycosidases. (d) Chromatographic properties of the disaccharides released by glycosidase digestion were identical to disaccharides released from authentic corneal keratan sulfate.

**DISCUSSION**

The presence of keratan sulfate in these molecules was documented in several ways. (a) Some of the KSPG molecules from culture were larger and some more highly charged than the non-sulfated KSPG of artery, suggesting the presence of glycosaminoglycan chains. (b) KSPG from cultures reacted with a keratan sulfate-specific monoclonal antibody and anti-core protein antibodies simultaneously demonstrating association of the proteins with keratan sulfate. (c) The antigenic keratan sulfate and purified 35S-labeled keratan sulfate were both sensitive to three different keratan sulfate-specific endoglycosidases. (d) Chromatographic properties of the disaccharides released by glycosidase digestion were identical to disaccharides released from authentic corneal keratan sulfate.
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The finding that cultured cells can express corneal keratan sulfates in a stable manner is a novel and potentially useful discovery. More than two decades ago Conrad and Dorfman (24) showed that secretion of highly sulfated keratan sulfate ceased in chick corneas in organ culture in 48 h and that in primary keratocytes the keratan sulfate secretion disappeared after six hours in culture. Since that report absence of keratan sulfate synthesis by cultured keratocytes has been confirmed repeatedly (23–29). Mammalian corneas in culture appear to secrete keratan sulfate for somewhat longer periods of time, in one case up to 2 weeks in culture; however, as with chick keratocytes, biosynthesis of keratan sulfate was lost rapidly when the cells were separated from the stromal matrix (37–39). The success of the current study at identifying stable corneal keratan sulfate synthesis in vitro after so many previous attempts is probably the result of a number of factors. Methodology clearly played some part. Earlier studies often employed extensive dialysis of protease-treated proteoglycans, a technique that probably resulted in loss of much of the keratan sulfate due to its small size. It is also clear that there are species differences. Recent studies with chick keratocytes demonstrated synthesis of a glycosylated KSPG that was sensitive to endo-β-galactosidase but not to keratanase I, a clear indication that the carbohydrate chains made by chick keratocytes were not sulfated (29). A second study found that human keratocytes secrete very little KSPG protein (26). The species from which the keratocyte cultures are derived may, therefore, be a determining factor in their ability to secrete keratan sulfate on a long term basis.

The keratan sulfate made in keratocyte cultures was sulfated on both galactose and glucosamine moieties; consequently these cells probably express all of the enzymes required for normal keratan sulfate biosynthesis. The KSPG molecules made in the cultures, however, were markedly different from those made in vivo. The relative proportion of nonsulfated disaccharides was higher in keratan sulfate from cell culture than in corneal keratan sulfate and the overall chain length of the keratan sulfate made in vitro was much shorter. Oben et al. (40) have developed a model of corneal keratan sulfate that proposes each chain contains four nonsulfated disaccharides proximal to the linkage region followed by 10–12 monosulfated disaccharides. The nonreducing terminus of each chain contains a domain of variable length composed primarily of disulfated disaccharides (40). In a keratan sulfate molecule of median size, 24 disulfated disaccharides are found, producing a molecular weight of $M_r$ 20,000. Based on a molecular size of 4700 Da and the ratio of hydrolysis products from Figs. 8 and 9, we estimate that the keratan sulfate chains made by bovine keratocytes contain 4 nonsulfated disaccharides, 4 or 5 monosulfated disaccharides, and 1–2 disulfated disaccharides. These molecules may therefore start with a “standard” nonsulfated region but have less than half the typical monosulfated disaccharide and a greatly truncated disulfated domain compared to the typical corneal keratan sulfate molecule. The results of this analysis suggest that bovine keratocytes in culture produce the “constant” (nonsulfated) portion of the typical keratan sulfate chain but do not extend the “variable” (sulfated) region of the chain as efficiently as they do in vivo. Keller et al. (41) suggest a correspondence between elongation of the keratan sulfate chain and the rapidity of its sulfation during biosynthesis. Accordingly, the differences between keratan sulfate proteoglycans produced in vitro and those found in the normal cornea might arise by the alteration of a single step in the biosynthetic pathway, possibly a decrease in the activity of one of the glycosyltransferase enzymes.

Our results present the first cell culture system exhibiting a stable expression of corneal keratan sulfate in amounts sufficient to be purified and characterized. The presence both mono- and disulfated disaccharides indicates that all of the enzymatic facilities are present in these cells for production of normal keratan sulfate. This finding is significant because extracts of keratocytes is difficult to obtain from intact cornea due to the low cell number and the extreme durability of the tissue. Previous studies involving purification and characterization of the enzymes involved in keratan sulfate synthesis have been limited. The cell culture system described here can provide a useful tool for analysis of the molecular mechanisms that control biosynthesis of these important molecules.

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