Proteoglycans in polarized epithelial Madin–Darby canine kidney cells

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Madin–Darby canine kidney (MDCK) cells were cultured on polycarbonate filters to study the synthesis and sorting of proteoglycans in polarized epithelial cells. Two strains of MDCK cells were used. MDCK I cells resemble distal tubule epithelial cells, and MDCK II cells share some characteristics with proximal tubule cells. Both strains were grown to confluency and labelled with [³⁵S]sulphate for 24 h. The apical and basolateral media and the cell fractions were harvested and analysed by DEAE ion-exchange chromatography. A large portion of the [³⁵S]sulphate-labelled macromolecules bound strongly to the ion-exchange columns, and could be eluted in three distinct peaks. The latest eluting peak was demonstrated to contain almost exclusively chondroitin sulphate, whereas peak 2 contained mostly heparan sulphate, demonstrated by using chondroitinase ABC and nitrous acid (pH 1.5) respectively to depolymerize the [³⁵S]glycosaminoglycan chains. Peak 1 contained negligible amounts of proteoglycans. Large differences could be observed in proteoglycan sorting in MDCK I and II cells. Strain I secreted approx. 67 % of the proteoglycans to the apical side and 17 % to the basolateral side. The cell fraction contained 17 % of the proteoglycans after 24 h of labelling. In contrast, 19 % of the proteoglycans were

sorted to the apical side of MDCK II cells and 61 % to the basolateral side, whereas the cell fraction contained 20 %. Furthermore, the level of [³⁵S]proteoglycan biosynthesis (apical and basolateral media and cell fraction total) was higher in MDCK I cells than in strain II. Based on the amount of material degraded by chondroitinase ABC and nitrous acid respectively, and the total amounts of [³⁵S]proteoglycans recovered from the cells, it was calculated that the MDCK I strain synthesized approx. 56 % chondroitin sulphate and 44 % heparan sulphate. In contrast, the MDCK II strain synthesized 69 % heparan sulphate and 31 % chondroitin sulphate. To further identify the [³⁵S]proteoglycans synthesized by MDCK I and II cells, antibodies against perlecan, versican and syndecan were used. The antibody against mouse syndecan did not cross-react with any of the proteoglycans produced in MDCK I or II cells. Both MDCK I and II cells expressed perlecan; 57–61 % could be recovered from the basolateral fractions and 18–34 % from the apical medium. Versican was also found in both MDCK I and II cells. Compared with perlecan, a larger percentage of versican (43–53 %) was found in the cell fractions.

INTRODUCTION

Proteoglycans are acidic macromolecules composed of glycosaminoglycan chains attached covalently to a protein core. They appear to be synthesized by all types of cells, and show a wide tissue distribution [1,2]. The highest concentration of proteoglycans can be found in the extracellular matrix, but these acidic macromolecules are also important constituents of cell surfaces and intracellular granules and vesicles. Proteoglycans have been implicated in a wide range of cellular processes [3], and are important for such diverse phenomena as the structural integrity of extracellular matrices, binding of lipolytic enzymes on the endothelial wall and the storage and transport of growth factors. The type of proteoglycan in the extracellular matrix differs between tissues. In cartilage the predominant species is aggrecan, whereas the major constituent in the basement membranes of the kidney is perlecan. Differences in tissue distributions of distinct proteoglycans are most probably due to differences in biological functions.

One of the basic functions of the kidneys is to filter the waste products of the blood and hence to regulate the concentrations of body fluid constituents. The basement membrane of the glomeruli is important for proper kidney filtration function, and structural changes to this particular matrix and to the mesangial matrix are contributing factors to kidney dysfunctions seen in pathological states such as, for example, diabetes [4]. Several

studies have addressed the question of proteoglycan biosynthesis in both the glomerular and the mesangial matrices. The presence of basement-membrane-like heparan sulphate proteoglycans (HSPGs) and versican-related proteoglycans has been demonstrated in cultured epithelial cells [5,6] and mesangial cells [7] from human glomeruli. In experimentally induced glomerulonephritis, elevated levels of the small connective-tissue-type proteoglycan decorin have also been shown [8]. Electron microscopy studies of the kidney have revealed the presence of HSPG with a regular pattern in the basement membrane of kidney epithelial cells [9]. However, it has also been demonstrated that the glomeruli contain large chondroitin sulphate proteoglycans (CSPGs) [10]. There is evidently a large repertoire of proteoglycans present in the glomeruli, contributed from epithelial, endothelial and mesangial cells. Most of those deposited into the basement membrane will be synthesized by both the epithelial cells and the endothelial cells [11].

In studies of epithelial cells there has been increasing use of the technique of cell culture in two-chamber systems where the cells are grown on semi-permeable supports. This system has the advantage of inducing the correct polarization of the epithelial cells, giving rise to an experimental separation of the basolateral and apical domains of the plasma membrane. The Madin–Darby canine kidney (MDCK) cell line has been widely used to study the synthesis and polarized expression of such different molecules as the amyloid β -peptide [12], hepatocyte growth factor [13],

mannose 6-phosphate receptor [14] and integrins [15]. Limited information is available on the sorting of proteoglycans in cultured polarized cells, be it in culture dishes or on semi-permeable filters. The aim of the present study was to use polarized MDCK cells to characterize proteoglycan synthesis and sorting to the apical and basolateral sides of the epithelial monolayer. Furthermore, we have made use of two MDCK strains with some differences in functional properties. By certain criteria the original MDCK strain II has been shown to resemble proximal tubule epithelial cells, whereas MDCK strain I cells are more closely related to the distal collecting duct segments [16]. Our studies show that these two strains show different patterns of HSPG and CSPG expression, and also differ in the sorting of these macromolecules to the apical and basolateral sides of the cells.

EXPERIMENTAL

Materials

BSA, *N*-ethylmaleimide, ϵ -aminocaproic acid, PMSF, 2,4-dinitrophenylalanine, Dextran Blue and Triton X-100 were all from Sigma Chemical Co., St. Louis, MO, U.S.A. Chondroitin ABC lyase (EC 4.2.2.4) was purchased from Seikagaku Kogyo Co. (Tokyo, Japan). Protein A-Sepharose 4B, DEAE-Sephacel and Sephadex G-50 Fine and Superfine were obtained from Pharmacia LKB Biotechnology Inc., Uppsala, Sweden. [35 S]Sulphate, 14 C-labelled molecular mass standards and Amplify were from Amersham.

Cell cultures

MDCK cell strains I and II were established on polycarbonate filters (Costar Transwell; pore size 0.4 μ m; diameter 24.5 mm) at a density of 10^6 cells per filter, and were used for labelling 3–4 days later. The cells were routinely cultured in Dulbecco's modified Eagle's medium with 5% (v/v) fetal calf serum, 1% penicillin/streptomycin and 2 mM L-glutamine (all from Gibco Ltd., Paisley, Scotland, U.K.). However, for labelling with [35 S]sulphate, cells were seeded in sulphate-free RPMI-1640 (Gibco) without penicillin and streptomycin and containing only 2% fetal calf serum, in order to reduce the level of unlabelled sulphate. The cells acquire the same morphology and trans-epithelial resistance with this medium as with the Dulbecco's modified Eagle's medium described above. After 3 days in this medium the cells were labelled for 24 h with 0.1 mCi/ml [35 S]sulphate in both the apical and the basolateral media. The apical and basolateral media volumes were typically 1.6 ml and 2.6 ml respectively.

Both MDCK I and II cells polarize and establish epithelial monolayers when cultured on filters. An intact monolayer can be demonstrated by measuring the transepithelial resistance with Millicell-ERS equipment (Millipore Corp., Bedford, MA, U.S.A.). In all experiments MDCK I cells had a transepithelial resistance above $3000 \Omega \cdot \text{cm}^2$ and that of MDCK II cells was above $200 \Omega \cdot \text{cm}^2$. It has been shown that the numbers of confluent filter-grown MDCK I and II cells differ. On nitrocellulose filters there were 2.66×10^6 ($\pm 7\%$) MDCK I cells and 3.8×10^6 ($\pm 7.5\%$) MDCK II cells per filter, corresponding to a filter area per cell of 118 mm^2 and 82.7 mm^2 respectively [17]. In our studies we have used polycarbonate filters. MDCK I cells have a filter area per cell of 124 mm^2 on this particular filter [18] and MDCK II cells have an area of 91 mm^2 per cell [19], in close agreement with numbers obtained on nitrocellulose filters. The difference between MDCK I and II cells in transepithelial

resistance is not due to the different number of cells per filter, but to the phosphorylation levels of tight-junction proteins [20].

35 S-labelled macromolecules

After labelling, the medium fractions were harvested and an equal volume of 8 M guanidine/4% Triton X-100/0.1 M sodium acetate buffer, pH 6.0, was added. The cell fraction was solubilized in 4 M guanidine/2% Triton X-100/0.05 M sodium acetate buffer, pH 6.0. To measure the level of [35 S]sulphate incorporated into [35 S]macromolecules, 50 μ l aliquots from each fraction were run on Sephadex G-50 Superfine spin columns, and the amount of radioactivity in the run-through fractions was determined in a scintillation counter after the addition of scintillation fluid. Alternatively, 1 ml of each fraction was applied to a 4 ml column of Sephadex G-50 Fine in 0.05 M Tris/HCl, pH 8.0/0.15 M NaCl. The first 1 ml eluting after application was discarded; the next 1.5 ml was collected and an aliquot of this was counted for radioactivity in the scintillation counter. The latter method was primarily used for preparative purposes, and to exchange guanidine with a buffer compatible with enzyme digestions and ion-exchange chromatography.

Ion-exchange chromatography

35 S-labelled macromolecules from cell and medium fractions obtained by Sephadex G-50 Fine chromatography were subjected to preparative ion-exchange chromatography. The samples were loaded on to the columns (4 ml gel volume) in 0.05 M Tris/HCl, 0.15 M NaCl and washed with the same buffer. Thereafter a gradient extending from 0.15 to 1.5 M NaCl in 0.05 M Tris/HCl was applied. Fractions of 2 ml were collected from the start of the chromatography. Aliquots of the fractions were counted for radioactivity in a scintillation counter. Fractions containing peaks of 35 S-labelled material were pooled and dialysed against water at 4 °C with a mixture of protease inhibitors containing 10 mM EDTA, 1 mM ϵ -aminocaproic acid, 1 mM *N*-ethylmaleimide and 1 mM PMSF. Dialysed samples were frozen until further analysis.

Enzyme treatment

Samples from medium and cell fractions pooled after DEAE ion-exchange chromatography were treated with chondroitin ABC lyase. Approx. 10000 c.p.m. of each sample was incubated with 0.01 unit of enzyme in 0.05 M Tris/HCl, pH 8.0, containing 0.025 M sodium acetate and 25 μ g/ml BSA. Samples were incubated at 37 °C overnight and thereafter subjected to gel chromatography on columns of Sephadex G-50 Superfine. Markers for the void and total volumes of the gel were Dextran Blue and 2,4-dinitrophenylalanine respectively. Fractions of 1 ml were collected and the radioactivity was counted in a scintillation counter. The percentage of material degraded by the enzyme, and hence representing chondroitin/dermatan sulphate, was calculated from the pooled amount of c.p.m. in the void volume and the fractions preceding the total volume of the gel, and compared with the elution profile of untreated material.

HNO₂ treatment

The amount of heparan sulphate in the different samples was determined by Sephadex G-50 Superfine gel chromatography, as for the chondroitinase ABC-treated samples, following nitrous acid treatment at pH 1.5, as described by Shively and Conrad [21].

SDS/PAGE

³⁵S-labelled proteoglycans isolated by DEAE ion-exchange chromatography were subjected to SDS/PAGE before and after chondroitinase ABC or nitrous acid treatment. The samples were boiled in sample buffer with 1 % SDS and applied to precast 4–20 % gradient gels from Novex (Encinitas, CA, U.S.A.). Standards used were ¹⁴C-labelled rainbow standards from Amersham. After electrophoresis the gels were fixed, treated with Amplify (Amersham), dried and subjected to autoradiography using Fuji Medical X-ray film (Fuji, Japan).

Immune precipitations

MDCK cells were labelled with [³⁵S]sulphate for 24 h, after which the apical and basolateral media and the cell fractions were harvested. The cell fraction was solubilized directly into 0.05 M Tris/HCl, pH 7.5, with 1 % Nonidet P-40, 2 mM EDTA, 150 mM NaCl and 35 µg/ml PMSF. The three fractions from both MDCK I and II cells were incubated with four different antibodies. A polyclonal antibody against versican was provided by Dr. E. Ruoslahti, La Jolla Cancer Research Foundation, La Jolla, CA, U.S.A. The antibody had been preabsorbed against control CHO cells, fetal calf serum–Sepharese and *Escherichia coli* bacterial extract. Rabbit antiserum against mouse perlecan was a gift from Dr. J. R. Hassell, University of Pittsburgh, PA, U.S.A. A monoclonal antibody (281-2) against mouse syndecan raised in the rat was given to us by Dr. M. Jalkanen, Center for Biotechnology, Turku, Finland. Finally, as a control we used a rabbit antibody raised against the bovine mannose 6-phosphate receptor, provided by Dr. Bernard Hoflack, European Molecular Biology Laboratory, Heidelberg, Germany.

The antibodies were incubated with the different fractions at 4 °C overnight in lysis buffer, to which 5 mM MgSO₄ had been added to reduce unspecific binding of free [³⁵S]sulphate. The samples were then incubated with Protein A–Sepharese, pre-washed with PBS containing 1 % BSA, to reduce unspecific binding to the beads. The samples were washed and finally run on 4–20 % Novex gradient gels under non-reducing conditions. After electrophoresis, bands were visualized by autoradiography. Some autoradiograms were scanned using a Molecular Dynamics Laser Densitometer.

RESULTS

³⁵S-labelled macromolecules

Initial experiments were performed to determine the capacity of MDCK I and II cells to express [³⁵S]sulphated macromolecules. The levels of expression of such molecules were measured in the cell fraction and in the apical and basolateral media after exposure of the cells to [³⁵S]sulphate for time periods from 2 to 24 h. With the MDCK I cells there was a time-dependent increase in the amount of ³⁵S-labelled macromolecules in the cell fraction up to 24 h (with the exception of the 8 h time point) (Figure 1a). The release of such molecules into the two medium compartments was low during the first 4 h of incubation. After this, however, there was a time-dependent increase in the levels of [³⁵S]macromolecules in both the apical and basolateral media. With MDCK II cells there was also a time-dependent increase in the levels of [³⁵S]macromolecules both in the cell fraction and in the apical and basolateral media (Figure 1b).

MDCK I cells are derived from the original MDCK II cells [16]. In the latter strain, the percentage of macromolecules labelled with [³⁵S]sulphate and recovered in the cells and the basolateral medium represented approx. 70 % of the total after 24 h of labelling, whereas the apical medium contained 30 %.

With the MDCK I strain, in contrast, the apical fraction contained 60 % (mean of three separate experiments), and the basolateral medium and cell fraction together contained 40 % of the ³⁵S-labelled macromolecules. There is, accordingly, a marked difference in the synthesis and secretion patterns of ³⁵S-labelled macromolecules between the two strains of MDCK cells used in this study.

DEAE ion-exchange chromatography

³⁵S-labelled macromolecules from the cell fraction and the apical and basolateral media of MDCK I and II cells were subjected to ion-exchange chromatography. From Figure 2 it is evident that the majority of the [³⁵S]macromolecules in both the apical and the basolateral media and the cell lysate represent proteoglycans, which are typically eluted at high NaCl concentrations from DEAE columns. In the experiment shown there is only a very small amount of macromolecules from the apical medium eluting in the early fractions from the ion exchanger. This was, however, found to vary somewhat; in three experiments less than 10 % was eluted in the early apical fractions, but in one experiment approx. 40 % was eluted in these fractions. In both the apical and basolateral media and the cell fraction, the material eluting after the start of the NaCl gradient was reproducibly resolved into three major peaks. In order to analyse this material in more detail, fractions representing the three different peaks were pooled, dialysed and subjected to further analyses (see below). When corresponding material from MDCK II cells was subjected to DEAE ion-exchange chromatography the elution profiles were similar to those shown for MDCK I cells in Figure 2. A comparison of the amounts of [³⁵S]macromolecules recovered in the three major peaks eluting after the start of the NaCl gradient (fraction 1 being the first and fraction 3 the latest eluting) showed that fraction 1 (non-proteoglycan fraction; see below) represented 24 % ($n = 3$) of the ³⁵S-labelled macromolecules in MDCK II cells and 10 % ($n = 3$) in MDCK I cells.

A polycarbonate filter (4.7 cm²) will contain 3.8×10^6 MDCK I cells or 5.7×10^6 MDCK II cells at confluence (see the Experimental section). Total proteoglycan synthesis was approx. 50 % higher per filter with MDCK I than with MDCK II cells (mean of four experiments). Accordingly, per cell, MDCK I cells synthesized approximately twice as much [³⁵S]proteoglycan as did MDCK II cells during a 20 h incubation.

[³⁵S]Glycosaminoglycans

Pooled material from peaks 1, 2 and 3 obtained after preparative DEAE ion-exchange chromatography of MDCK I cell ³⁵S-labelled material was subjected to chondroitinase ABC treatment followed by SDS/PAGE. Figure 3 shows that the migration pattern of apical fractions 1 and 2 was virtually unaffected by enzyme treatment; accordingly, these fractions do not contain chondroitin sulphate-related glycosaminoglycans. In contrast, material in fraction 3 was completely depolymerized after chondroitinase ABC treatment. Fraction 1 contained several sulphated proteins, which were not analysed further. The flow-through fractions also contained sulphated proteins.

When the basolateral medium and cell fractions obtained by DEAE ion-exchange chromatography were subjected to the same treatment, a very similar pattern emerged. Fractions 1 and 2 were resistant to enzyme treatment, whereas fraction 3 material was almost completely depolymerized by chondroitinase ABC.

Aliquots of the material subjected to enzyme digestion were also treated with nitrous acid, pH 1.5, which is known to depolymerize heparin and heparan sulphate-related glycosaminoglycans [21]. The various fractions were then analysed by

Figure 1 Incorporation of [³⁵S]sulphate into macromolecules by (a) MDCK I and (b) MDCK II cells

MDCK I and II cells were labelled with [³⁵S]sulphate, and apical and basal media and cell fractions were chromatographed on Sephadex G-50 Fine columns. Material eluting in the void volume was quantified. The differences in volumes in the various fractions were corrected for, and the results are expressed on a per well basis. Experiments with both cell lines were done in duplicate ($n = 3$) with similar results.

SDS/PAGE. Fractions 1 and 3 after DEAE ion-exchange chromatography of the apical medium were resistant to nitrous acid treatment, whereas fraction 2 was almost completely depolymerized (Figure 4). The same pattern was also observed for the basolateral medium fractions and for the fractions obtained from the cell lysates. Hence in both the apical and basolateral media, and in the cell fraction, the material eluting in peak 2 after the start of the NaCl gradient was HSPG, whereas peak 3 contained mainly chondroitin/dermatan sulphate proteoglycans.

The intensity of the bands obtained by autoradiography after SDS/PAGE both with and without the treatment described above differed (compare apical fraction 2 with and without

enzyme; Figure 3). There are, accordingly, limitations to the use of this method for the quantitative determination of the amounts of chondroitin [³⁵S]sulphate and heparan [³⁵S]sulphate. Material from the apical and basolateral media and cell fractions resolved into three DEAE-binding peaks was therefore run on Sephadex G-50 columns after chondroitinase ABC or nitrous acid treatment for more quantitative measurements. The material recovered from the first of the three major peaks after ion-exchange chromatography was resistant to both chondroitinase ABC and nitrous acid treatments, whereas peak 2 material was almost completely depolymerized by nitrous acid but was resistant to chondroitinase ABC treatment. In contrast, peak 3 material was almost completely degraded by chondroitinase

Figure 2 DEAE ion-exchange chromatography

The apical (a), basolateral (b) and cell (c) fractions from MDCK I cells labelled with [^{35}S]sulphate were subjected to ion-exchange chromatography following G-50 Fine gel chromatography. The columns were washed in 0.05 M Tris/HCl, pH 8.0, with 0.15 M NaCl. The start of the salt gradient, extending from 0.15 to 1.5 M NaCl in the same buffer, is indicated with arrows. The separation of the material eluting after the NaCl gradient into three peaks was evident in four different experiments.

ABC but was not susceptible to depolymerization by nitrous acid. Peak 2 contained $80.0 \pm 5.5\%$ heparan sulphate ($n = 6$) and peak 3 contained $72.5 \pm 8.0\%$ ($n = 6$) chondroitin sulphate (results not shown). This confirms the data presented in Figures 3 and 4. The sum of material degraded by chondroitinase ABC and nitrous acid does not account for 100% of the proteoglycans present in either MDCK I or MDCK II cells. Typically, 10% or less of the ^{35}S -labelled material from both MDCK I and II cells eluting in fractions 2 and 3 from the DEAE columns was found to be resistant to both nitrous acid and chondroitinase ABC treatment. It cannot be excluded that the cells synthesize minor amounts of keratan sulphate. The levels of hyaluronan synthesis have not been addressed in this study.

Similar results were obtained when apical and basolateral media and cell lysates from MDCK II cells were subjected to preparative DEAE ion-exchange chromatography followed by nitrous acid or chondroitinase ABC treatment and SDS/PAGE (results not shown).

Figure 3 SDS/PAGE of ^{35}S -labelled macromolecules from MDCK I cells after chondroitinase ABC treatment

^{35}S -labelled macromolecules from the apical fraction of MDCK I cells, corresponding to the three peaks eluted from DEAE ion-exchange columns after the salt gradient (see Figure 2), were subjected to SDS/PAGE under non-reducing conditions both prior to and after treatment with chondroitinase ABC (C-ABC) as indicated. The molecular masses of protein standards (lane S) are indicated. One experiment representative of three is shown.

Figure 4 SDS/PAGE of ^{35}S -labelled macromolecules from MDCK I cells after nitrous acid treatment

^{35}S -labelled macromolecules recovered from the apical fraction of MDCK I cells after DEAE ion-exchange chromatography in three distinct peaks were subjected to SDS/PAGE before and after nitrous acid treatment (HNO_2) as indicated. The migration distances of the protein standards (lane S) are indicated. The same results were obtained in three separate experiments.

[^{35}S]HSPGs and [^{35}S]CSPGs in MDCK cells

From the data presented, it is clear that both MDCK I and II cells synthesize HSPGs and CSPGs. From the DEAE ion-exchange chromatography data presented in Figure 2 and the gel electrophoresis data in Figures 3 and 4, we conclude that peaks 2 and 3 (see Figure 2) from all three fractions (apical and basolateral media and cell lysates) contained almost exclusively proteoglycans. By pooling the amount of radiolabelled material recovered from the void and included fractions after Sephadex G-50 Superfine gel chromatography, both before and after nitrous acid and chondroitinase ABC treatment, it is possible to obtain estimates of the amounts of HSPGs and CSPGs recovered in the apical and basolateral medium and cell fractions in both

Table 1 Proteoglycans in MDCK I and MDCK II cells

The percentages of [^{35}S]HSPG and [^{35}S]CSPG in the apical (Api), basolateral (Baso) and cell fractions were calculated on the basis of elution profiles of [^{35}S]proteoglycans after Sephadex G-50 gel chromatography, following chondroitinase ABC and HNO_2 treatment. The values are means $\pm$ range from two separate experiments. The percentages of total [^{35}S]proteoglycans in the three fractions were calculated from the amount of material in peaks 2 and 3 obtained by DEAE ion-exchange chromatography (see Figure 2). The values are means $\pm$ range from three separate experiments. The percentages of [^{35}S]perlecan and [^{35}S]versican were determined by scanning densitometry after immunoprecipitation, SDS/PAGE and autoradiography. The values are means $\pm$ range from two different experiments.

	Content (%)					
	MDCK I			MDCK II		
	Api	Baso	Cell	Api	Baso	Cell
HSPG	19 $\pm$ 4	45 $\pm$ 4	42 $\pm$ 4	72 $\pm$ 3	86 $\pm$ 7	80
CSPG	81 $\pm$ 7	55 $\pm$ 4	52 $\pm$ 2	18 $\pm$ 4	11 $\pm$ 4	14 $\pm$ 6
Total	67 $\pm$ 11	17 $\pm$ 7	17 $\pm$ 7	19 $\pm$ 3	61 $\pm$ 14	20 $\pm$ 11
Perlecan	34 $\pm$ 3	57 $\pm$ 3	9 $\pm$ 6	28 $\pm$ 3	61 $\pm$ 6	14 $\pm$ 4
Versican	34 $\pm$ 2	22 $\pm$ 1	43 $\pm$ 1	18 $\pm$ 7	29 $\pm$ 1	53 $\pm$ 6

MDCK I and II cells. From Table 1 it is evident that MDCK I cells secreted 67 % of the proteoglycans to the apical side of the cell and 17 % to the basal side. The dominant apical fraction contained 81 % CSPGs. Of the total [^{35}S]proteoglycans synthesized, CSPGs represented 56 % and HSPGs 44 %. MDCK II cells secreted only 19 % of the [^{35}S]proteoglycans synthesized into the apical medium; 61 % were secreted into the basal medium and 20 % were recovered in the cell fraction. In this cell line the level of HSPG biosynthesis was higher than in MDCK I cells. In the apical medium, HSPGs accounted for 72 % of the proteoglycans, whereas the cell fraction and the basolateral medium contained 80 and 86 % HSPGs respectively. In MDCK II cells, accordingly, HSPGs represented 69 % and CSPGs 31 % of the total [^{35}S]proteoglycans synthesized.

Cell-associated proteoglycans can be associated with either the apical or the basolateral plasma membrane, or with intracellular compartments. Preliminary studies with subcellular fractionation of MDCK I and II cells, where the plasma membrane, the Golgi apparatus and the endosomes/lysosomes can be recovered in three distinct fractions [22], revealed that approx. 65 % of the [^{35}S]proteoglycans were associated with the plasma membrane. This experimental approach does not distinguish between the apical and basolateral plasma membrane domains.

Immunological characterization of [^{35}S]proteoglycans

To characterize further the HSPGs and CSPGs synthesized by MDCK I and II cells, antibodies were obtained against perlecan, versican and syndecan and used in immune precipitations. It was possible, using an anti-perlecan antibody, to precipitate [^{35}S]proteoglycans from the apical, basolateral and cell fractions from both MDCK I and II cells (Figures 5a and 5b). The control antibody used in this experiment was against the mannose 6-phosphate receptor, and was negative in all fractions with the exception of a small degree of reactivity in the cell fraction from MDCK I cells. Scanning of the gels after autoradiography, and adjusting for the volume differences in the three fractions, revealed that in MDCK I cells 34 % of the perlecan was in the apical medium, 57 % was in the basal medium and 9 % was in the cell fraction. With the MDCK II cells, 28 % was in the apical medium, 61 % in the basal medium and 14 % in the cell fraction

Figure 5 SDS/PAGE after immunoprecipitation with anti-perlecan antibodies

Apical (Api) and basolateral (Baso) media and cell fractions from ^{35}S -labelled MDCK I (a) and II (b) cells were immunoprecipitated with an antiserum against perlecan (P). An antibody against the mannose 6-phosphate receptor was used as a control (C). Precipitated material from each fraction was subjected to SDS/PAGE and autoradiography. Lane S contains molecular mass standards. One experiment representative of three is shown.

(see Table 1). The same volumes were applied to the gel from the two different MDCK strains. A comparison of the amounts of perlecan precipitated showed that MDCK II cells (apical, basal and cell fraction total) contained approx. twice as much perlecan as MDCK I cells.

The CSPG species isolated from both MDCK I and II cells have a high molecular mass, as judged from their migration patterns on SDS/PAGE (Figure 3). To determine whether any of this CSPG could represent versican, an anti-versican polyclonal antibody was used with the apical, basolateral and cell fractions from both MDCK I and II cells. Immunoprecipitated material was run on SDS/PAGE. All three fractions from both cell types contained material that was recognized by the anti-versican antibody (Figure 6). Antibodies against mouse syndecan-1 were used with parallel fractions. No ^{35}S -labelled material could be precipitated with this antibody (results not shown), most probably because it does not cross-react with canine syndecan. The amounts of versican recovered from the various fractions were also determined by densitometry scanning. The values obtained were adjusted for the differences in volume between the fractions.

Figure 6 SDS/PAGE after immunoprecipitation with anti-versican antibodies

Fractions from the apical (A), basolateral (B) and cell (C) fractions of ^{35}S -labelled MDCK I and II cells were precipitated with an antibody against versican. Precipitated material from the three fractions of both cell strains was subjected to SDS/PAGE and autoradiography. Antibodies against mouse syndecan were used as a negative control (not shown). Lane S contains molecular mass standards. The same results were obtained in three different experiments.

As can be seen in Table 1, 34 % of the versican precipitated was in the apical fraction, 22 % was in the basolateral fraction and 43 % was in the cell fraction from MDCK I cells. In MDCK II cells the corresponding values were 18, 29 and 53 % in the apical, basolateral and cell fractions respectively. Versican is, accordingly, found mostly in the apical and cell fractions, and less in the basolateral fractions in both MDCK I and II cells. The recovery of versican from the MDCK I strain was calculated to be approx. 50 % higher per filter than from MDCK II cells.

DISCUSSION

Proteoglycan biosynthesis has been studied in several different types of kidney cells. A major focus has been on the role of proteoglycans in relation to the construction and function of the basement membrane in the glomeruli [9,11]. By dye staining and immunohistochemistry it has been demonstrated that the glomerular basement membrane contains HSPGs [23–25]. By use of cultured epithelial cells from human glomeruli the presence of several HSPG species has been demonstrated, including the basement membrane type [5,6]. The major part of the HSPG synthesized by epithelial cells seems to be destined for the basement membrane [26]. The proteoglycan profile of the mesangial matrix differs from that of the glomeruli basement membrane. In addition to HSPGs the presence of decorin, biglycan [27] and versican has been demonstrated in the mesangial matrix [7]. The levels of decorin and biglycan in mesangial cells have been shown to increase during pathological conditions such as glomerulonephritis [8]. Proteoglycans in the basement membrane are derived from both epithelial and endothelial cells. The contribution from the epithelial cells can be analysed in further detail by measuring the basolateral sorting of proteoglycans *in vitro*.

In the present paper we have made use of polycarbonate filters for the polarized growth of MDCK cells. The aim of our studies has been to gain further insight into proteoglycan expression and sorting in polarized epithelial cells. A limited number of studies have addressed the issue of proteoglycan synthesis and sorting in detail. It has been demonstrated that regulated and constitutive

secretion of glycosaminoglycans in a mouse pituitary cell line show distinctly different patterns, particularly with reference to dependence on protein synthesis [28]. Furthermore HSPG, detected with an antibody against kidney basement membrane proteoglycan, is sorted predominantly to the basolateral side of cultured MDCK cells. Laminin is sorted only to the basolateral side [29]. MDCK cells transfected with mouse syndecan sorted this proteoglycan to the basolateral side, but deletion of the last 12 amino acids of the cytoplasmic tail abrogated the basolateral sorting, and syndecan was transported to both the apical and basolateral sides of the cells [30]. Finally, it has been demonstrated in other epithelial cells that the sorting of HSPG to the basolateral side is dependent on trimeric G proteins [31].

The results presented here clearly show that HSPG, recognized by a polyclonal antiserum against perlecan, is found mainly on the basolateral side of MDCK I and II cells. We could, however, also detect perlecan on the apical side of both MDCK I and II cells. The apical secretion of HSPGs, which are to a major extent recovered from the basolateral side of epithelia, has also been reported in other studies with both MDCK and other epithelial cells [29,31]. Whether this is a reflection of the *in vitro* systems or indicates possible functions for perlecan or perlecan derivatives on the apical side of the cells is not known. In colon carcinoma cells perlecan has been shown to be localized at the cell surface, to which it can be linked via fatty acids [32]. Perlecan is, however, predominantly recovered from the basolateral medium. This sorting pattern most probably reflects the importance of perlecan as a constituent of the basement membrane of epithelial cells.

Syndecan is most probably also present in the basolateral and cellular fractions, but the antibody used does not cross-react with the canine species. By using the same antibody against mouse syndecan it has been shown in the developing mouse kidney that this proteoglycan is associated with the kidney epithelial cells [33]. Other studies using biochemical approaches have shown that there are at least two different HSPGs present in cultured human epithelial cells [5,6]. On the basal side of the cells syndecan will represent a membrane-intercalated form, whereas perlecan is typically found in the basement membrane of epithelial cells. Perlecan is therefore likely to be found mostly as a released product in the basolateral medium under *in vitro* conditions. Syndecan has, however, also been shown to be shed from the plasma membrane [34]. Both of these HSPGs are therefore likely to be found in the basal medium. HSPGs were also recovered from the apical medium, and perlecan could be immunoprecipitated from this fraction.

The results presented show that both MDCK I and II cells express versican, and that a large proportion of this is contained in the cell fraction. This is in contrast to perlecan, most of which was found in the basolateral medium. By immunohistochemistry CSPG has been shown to be present in rat kidney in the Bowman's capsule and the tubular basement membrane, but not in the glomeruli basement membrane [10]. In future studies it will be of interest to study the biosynthesis and sorting of other chondroitin/dermatan sulphate proteoglycans such as decorin and biglycan, as these have been implicated in kidney pathology [8].

Two major differences between MDCK I and II cells were found with regard to proteoglycan expression and sorting. First, the MDCK I strain synthesized almost equal amounts of CSPG and HSPG (56 and 44 % respectively), whereas the MDCK II strain synthesized more HSPG (69 %). Secondly, MDCK I sorted the major portion (67 %) of the proteoglycans to the apical side of the cells, whereas MDCK II cells sorted 61 % to the basolateral side. MDCK I cells have been shown by several criteria to resemble cells in the distal tubules, whereas strain II

cells display characteristics of proximal tubule epithelial cells [16]. One striking difference between these two strains of MDCK cells is the fact that confluent MDCK I cells have a higher transepithelial resistance than MDCK II cells [2,35]. The differences in proteoglycan synthesis and sorting between MDCK I and II cells reported here may be related to the different functional properties of the two cell lines.

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