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Characterization of proteoglycans synthesized by human adult glomerular mesangial cells in culture

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1. The newly synthesized proteoglycans from human adult glomerular mesangial cells labelled *in vitro* for 24 h with [^{35}S]sulphate have been characterized using biochemical and immunological techniques. 2. The following proteoglycans were identified (% of total synthesized). (i) A large chondroitin sulphate proteoglycan, CSPG-I, $M_r \sim 1 \times 10^6$ (10.6%). This proteoglycan consisted of a protein core of $M_r \sim 4 \times 10^5$ and glycosaminoglycan chains of M_r 2.5×10^4, and was present in both the cell layer and the culture medium. (ii) A major small dermatan sulphate proteoglycan, DSPG-I, M_r 3.5×10^5 (46%), which was mainly located in the culture medium. (iii) A second minor small dermatan sulphate, DSPG-II, $M_r \sim 2 \times 10^5$ (9.8%). This molecule was exclusively located in the culture medium. (iv) A large heparan sulphate proteoglycan, HSPG-I, M_r 8×10^5 (3.3%). (v) A second large heparan sulphate proteoglycan HSPG-II, $M_r \sim 6 \times 10^5$ (23%). HSPG-I and HSPG-II were extracted from both the culture medium and the cell layer. 3. Western blot analysis of the core proteins released by chondroitin ABC lyase treatment of DSPG-I and DSPG-II identified these dermatan sulphate proteoglycans as biglycan and decorin respectively. Both DSPG-I and DSPG-II had core proteins of M_r 45000. 4. The cell-layer-associated forms of CSPG-I, HSPG-I and HSPG-II were accessible to limited trypsin treatment, bound to octyl–Sepharose and could be inserted into liposomes, indicating a possible cell membrane location. 5. Pulse–chase experiments indicated that the cell-layer-associated [^{35}S]proteoglycans undergo limited metabolism to inorganic [^{35}S]sulphate, the majority of which is accounted for by the degradation of HSPG-II and to a lesser extent DSPG-I.

INTRODUCTION

Two extracellular matrices have been identified in the renal glomerulus, the glomerular basement membrane and the mesangial matrix [1,2]. The glomerular basement membrane has been investigated extensively. The mesangial matrix is less well studied, probably due to the difficulty in obtaining sufficient quantities in a pure enough state for biochemical analysis, but immunolocalization experiments indicate that it is composed of macromolecules similar to those located in the glomerular basement membrane [2]. However, the two matrices appear to differ with respect to their glycosaminoglycan (GAG) composition. Kanwar *et al.* [3] demonstrated that chondroitin sulphate proteoglycans (CSPGs) are restricted to the mesangial matrix, and Couchman and co-workers [4,5] have shown the presence of a basement-membrane-specific CSPG in the mesangial matrix of adult rats which is absent from the glomerular basement membrane. A number of glomerular diseases, including diabetic nephropathy, are accompanied by changes in the mesangial matrix, eventually leading to glomerulosclerosis [2]. Thus a precise knowledge of the composition, metabolism and function of the macromolecules that make up the mesangial matrix is important in order to understand the progress of renal glomerulosclerosis and insufficiency.

In the present study we characterize PGs synthesized by confluent cultures of human adult mesangial cells. Very recently, Klein *et al.* [6] published results of similar experiments using foetal human mesangial cells. However, the present data indicate that there are notable differences between foetal and adult human mesangial cell PGs.

MATERIALS AND METHODS

Cell culture and metabolic labelling

Human glomerular mesangial cells were established in culture from collagenase-treated glomeruli and characterized as described previously by us [7]. Cells between the 3rd and 5th passages were cultured on 35 mm. diam-plastic dishes (Falcon) and labelled with 50 μCi of [^{35}S]sulphate/ml (950–1350 Ci/mmol; Amersham) for 24 h in fresh medium containing 15% foetal calf serum and 10% normal inorganic sulphate as described for human glomerular epithelial cells [8].

Extraction and characterization of ^{35}S-labelled PGs

The [^{35}S]PGs in the culture medium (CM) and the cell layer (CL) were extracted in the presence of proteinase inhibitors, as described for human glomerular epithelial cells [8]. The extracts were buffer-exchanged on a Sephadex G-50 column (2 cm × 15 cm) equilibrated with 8 M-urea/20 mM-Bis-Tris/HCl, pH 6.0, containing 0.15 M-NaCl and 0.5% (w/v) CHAPS (urea buffer), and the labelled material eluting in the void volume was applied to a Mono Q column (Pharmacia) equilibrated in the same buffer. The total ^{35}S-labelled PGs were eluted with the same buffer containing 1.2 M-NaCl and precipitated with 90% ethanol containing 1.3% (w/v) potassium acetate in the presence of 50 μg each of CS and heparin/ml. The ^{35}S-labelled proteoglycans extracted from the CM and the CL were divided into two portions and fractionated under dissociative conditions on Sepharose CL-4B after treatment with either cold nitrous acid [9] or chondroitin ABC lyase (EC 4.2.2.4) [10,11]. CL ^{35}S-labelled PGs were fractionated by hydrophobic affinity chromatography

Abbreviations used: HS, heparan sulphate; CS, chondroitin sulphate; DS, dermatan sulphate; PG, proteoglycan; CL, cell layer; CM, culture medium; GAG, glycosaminoglycan.

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using octyl–Sepharose and inserted into liposomes as previously reported [8]. Limited trypsin treatment of cells labelled with [^{35}S]sulphate for 24 h and studies on the turnover of labelled PGs were performed as described for glomerular epithelial cells [8].

Analytical gel chromatography

Size analysis of [^{35}S]PGs was carried out on analytical columns (6 mm × 1.5 m) of Sepharose CL-4B or 6B equilibrated with 4 M-guanidine HCl/50 mM-sodium acetate, pH 6.0, containing 0.5% (v/v) Triton X-100 and 0.05% (w/v) NaN_3 (dissociative buffer). ^{35}S-labelled GAGs were isolated from the labelled PGs by either treatment with 0.05 M-NaOH/1 M-$NaBH_4$ at 45 °C for 20 h or digestion with papain (3 μg/ml) at 55 °C in 0.1 M-sodium acetate, pH 5.0, containing cysteine (0.005 M) and EDTA (0.005 M). The digests were precipitated with potassium acetate/ethanol and dried over N_2. Aliquots were reconstituted in 4 M-guanidine HCl containing 50 mM-sodium acetate (pH 6.0)/0.5% (v/v) Triton X-100 and chromatographed on an analytical column of Sepharose CL-6B eluted with the same buffer. Portions of the free GAG chains were also chromatographed on Sephadex G-50 before and after treatment with chondroitin ABC lyase or chondrotin AC II lyase (EC 4.2.2.5; Sigma) as described previously [8].

Western blotting and SDS/PAGE

PGs for Western blot analysis were purified from spent medium from confluent human mesangial cells and stored with added proteinase inhibitors. To the medium was added ~ 2×10^6 c.p.m. of [^{35}S]CSPGs prepared as above as tracer and the medium was passed over 10 ml of DEAE-Sephacel (Pharmacia) equilibrated in urea buffer (see above). The column was washed extensively with the same buffer until the A_{280} returned to the baseline level, then the PGs were eluted with 10 ml of 4 M-guanidine HCl, buffer exchanged on Sephadex G-50 (2 cm × 15 cm column) equilibrated with urea buffer, and the material eluting in the void volume was applied to a Mono Q column as described above. The PGs were then eluted with a 0.75–1.0 M-NaCl gradient in urea buffer. Fractions containing the [^{35}S]PGs were pooled, dialysed against distilled water containing proteinase inhibitors at 4 °C, freeze-dried and redissolved in 4 M-guanidine HCl dissociative buffer. The proteoglycans contained in M-I and M-II (see the Results section) were eluted from a Sepharose CL-4B column. Samples for Western blot analysis were separated in 10% gels [12] before and after digestion with chondroitin ABC lyase (proteinase-free; ICN Biochemicals), and transferred to nitrocellulose paper (Schleicher & Schuell) using a Bio-Rad Transblot apparatus as recommended by the manufacturer. PG core proteins were detected using anti-PG-SI (biglycan) antibodies (LF-15) and anti-PG-SII (decorin) antibodies (LF-30), kindly provided by Dr L. W. Fisher, National Institutes of Health, Bethesda, MD, U.S.A. Proteins were visualized using streptavidin/biotinylated alkaline phosphatase complex (Boehringer Mannheim).

Iodination with ^{125}I by the chloramine-T method [13] and SDS/PAGE of purified proteoglycans present in M-I and M-II were performed as described by Lories *et al.* [14]. After electrophoresis, the gels were stained with 0.1% Coomassie Brilliant Blue R-250 in 25% (v/v) acetic acid/50% (v/v) methanol, destained in methanol/acetic acid/water (5:10:85, by vol.) and dried under vacuum. Kodak-XAR Omat film was used for autoradiography.

RESULTS

Confluent cultures from four different human mesangial cell preparations over 24 h incorporated 3.11×10^5 c.p.m. of ^{35}S into macromolecules per 35 mm dish ($n = 10$; S.D.; ±0.62), of which 81% was present in the CM. The ^{35}S-labelled macromolecules from both the CM and the CL were chromatographed on Mono Q to separate ^{35}S-labelled PGs from other proteins and glycoproteins. The bound radiolabelled material, which accounted for 95% of the total ^{35}S-macromolecules in both the CM and the CL, was eluted with 1.2 M-NaCl and analysed for GAG composition. CS/dermatan sulphate (DS) chains accounted for 74% of the radioactivity in the CM and 60% in the CL; heparan sulphate (HS) accounted for the remainder in both compartments. Thus, overall, 72% of the ^{35}S-labelled PGs synthesized by these cells contained CS/DS chains.

When the ^{35}S-labelled CS/DS PGs extracted from the CM were chromatographed on Sepharose CL-4B (Fig. 1*a*) 25% were eluted near the excluded volume (M-I, $K_{av.}$ 0.06) while the majority (70%) was eluted later (M-II, $K_{av.}$ 0.28–0.36). The first peak was completely degraded by chondroitin ABC lyase and AC II lyase and was designated a large CSPG (CSPG-I). SDS/polyacrylamide gradient gels under reducing conditions followed by fluorography showed that CSPG-I barely entered the gel (Fig. 1*b*). Furthermore, reduction and alkylation of this

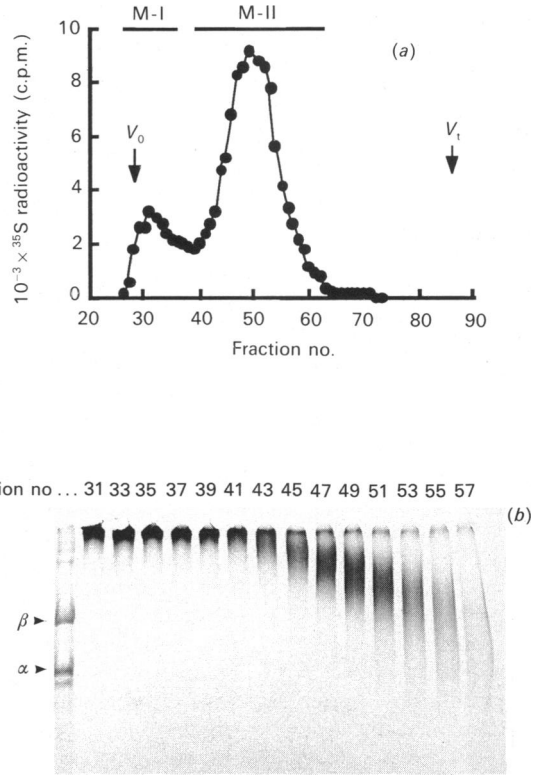

Fig. 1. Fractionation of ^{35}S-labelled CSPGs and DSPGs synthesized by human glomerular mesangial cells in culture

A labelling experiment was carried out and ^{35}S-labelled macromolecules were extracted as described in the Materials and methods section. The extracts from the CM were buffer-exchanged into 8 M-urea/20 mM-Bis-Tris/HCl (pH 6.0)/0.15 M-NaCl/0.5% (w/v) CHAPS, and the [^{35}S]PGs were separated from the protein and labelled glycoproteins by Mono Q ion-exchange chromatography. The [^{35}S]PGs were eluted with 1.2 M-NaCl, precipitated with ethanol/potassium acetate, digested with cold nitrous acid and chromatographed on an analytical dissociative Sepharose CL-4B column. Equal volumes of each fraction were taken (*a*) to determine the elution profile of the PGs and (*b*) subjected to SDS/PAGE followed by fluorography. The α- and β-chains of type III collagen are marked by arrowheads. The horizontal bars in (*a*) indicate the fractions that were pooled for further analysis.

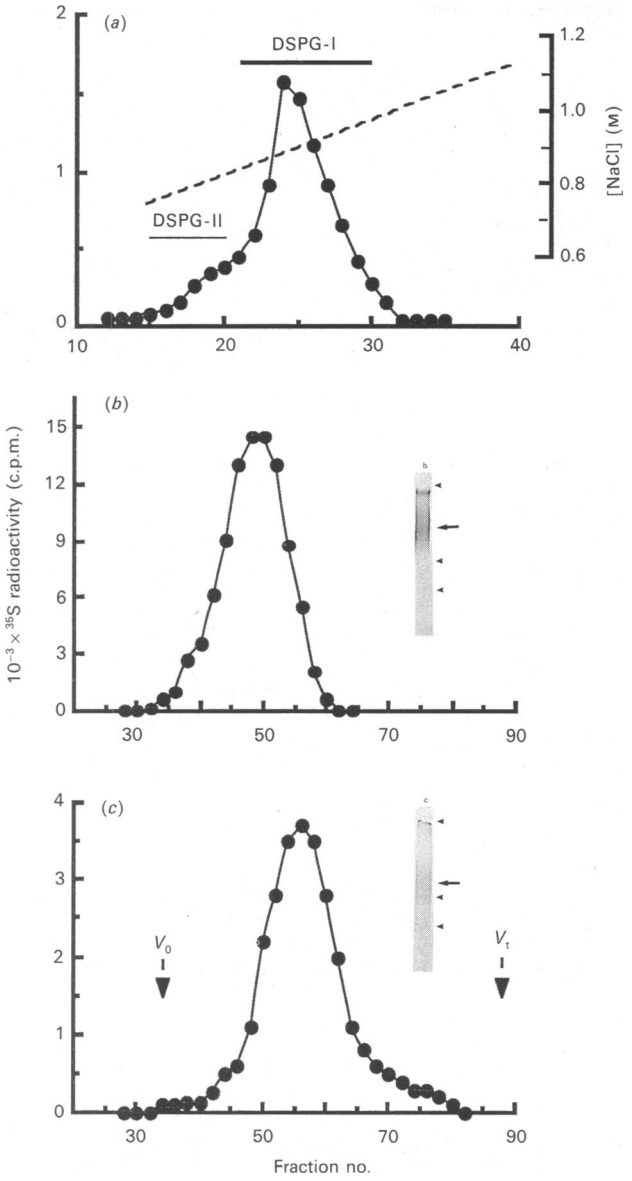

Fig. 2. Separation of ^{35}S-labelled DSPGs synthesized by human adult mesangial cells in culture

A pooled fraction M-II was prepared (see Fig. 1*a*), buffer-exchanged and separated by Mono Q ion-exchange chromatography using a salt gradient (---) generated with an f.p.l.c. system (*a*). Fractions containing DSPG-I and DSPG-II were pooled as indicated by the horizontal bars and subjected to re-chromatography on an analytical dissociative Sepharose CL-4B column: (*b*) pooled fractions containing DSPG-I ($K_{av.}$ 0.3); (*c*) pooled fractions containing DSPG-II ($K_{av.}$ 0.4). Inserts to (*b*) and (*c*) show the pooled PGs from each peak after SDS/PAGE on 3–15% gradient gels. The origin and α- and β-chains of type III collagen are marked by arrowheads, and DSPG-I and DSPG-II are marked by arrows.

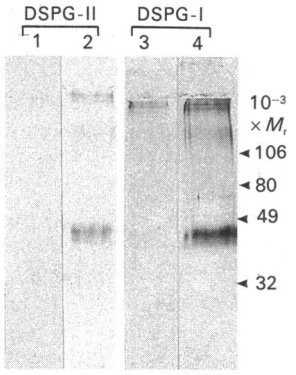

Fig. 3. Western-blot analysis of DSPGs of human adult mesangial cells

DSPG-I and DSPG-II were isolated from the spent medium from human adult mesangial cells (for details, see the Materials and methods section), incubated with (lanes 2 and 4) or without (lanes 1 and 3) chondroitin ABC lyase, and submitted to SDS/PAGE under reducing conditions, followed by electroblotting on to nitrocellulose membranes. The membranes were probed with antibodies to decorin (LF-30; lanes 1 and 2) and biglycan (LF-15; lanes 3 and 4).

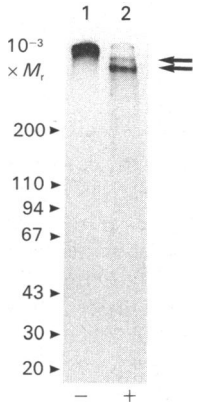

Fig. 4. SDS/PAGE of ^{125}I-labelled human mesangial cell PGs

Fraction M-I (see Fig. 1*a*) was prepared from the spent media from human adult mesangial cells in culture and radiolabelled with [^{125}I]iodide. Portions of the labelled fraction were incubated with (lane 2) or without (lane 1) chondroitin ABC lyase and submitted to SDS/gradient gel (3–15%) electrophoresis; the gels were dried and the labelled proteins were revealed by autoradiography. M_r values of core protein molecules, indicated by the arrows, were estimated from the positions of standard protein markers (indicated by arrowheads).

PG did not affect its elution profile on Sepharose CL-4B (results not shown).

The pooled material in the included peak (M-II) was completely degraded by chondroitin ABC lyase, but was 65% resistant to digestion with chondroitin AC II lyase, indicating that it contained DS as the predominant GAG. Electrophoresis of the fractions containing M-II showed the presence of two populations of ^{35}S-labelled DSPGs (see Fig. 1*b*). These PGs were further purified by a second ion-exchange chromatography step on Mono Q. When eluted with a linear gradient (0.75–1.2 M-NaCl) they separated into a minor (denoted DSPG-II) and a major (denoted DSPG-I) population (Fig. 2*a*). Digestion with chondroitin AC II lyase showed that both populations contained DS, since only 57% of DSPG-I and 25% of DSPG-II were degraded with this enzyme. Chromatography on Sepharose CL-4B under dissociative conditions (Figs. 2*b* and 2*c*) revealed that DSPG-I ($K_{av.}$ 0.3) was slightly larger than DSPG-II ($K_{av.}$ 0.4). SDS/PAGE confirmed this finding and indicated apparent M_r values of 350000 and 200000 for DSPG-I and DSPG-II respectively (see inserts in Figs. 2*b* and 2*c*).

Estimation of the M_r of CSPG and DSPG protein cores

The above results suggest that the retarded peak (M-II) contained at least two DSPGs with properties similar to those of

decorin (PG-SII) and biglycan (PG-SI). To confirm this, DSPG-I and DSPG-II were purified from the spent culture medium of confluent human mesangial cells, treated with chondroitin ABC lyase and subjected to SDS/PAGE on a 10% gel and analysed by Western blot using antibodies to biglycan and decorin. The results (Fig. 3) identify DSPG-II and DSPG-I as decorin and biglycan respectively. They are present at a ratio of about 1:4. The core protein of each PG has an M_r of 45000. Dot-blot analysis of DSPG-I with antiserum LF-30 indicated the absence of decorin. However, in contrast, similar analysis of DSPG-II with LF-15 indicated trace amounts of biglycan.

To characterize the core protein of the large CSPG, purified M-I from spent medium was iodinated with ^{125}I and submitted to SDS/PAGE under reducing conditions and autoradiography before and after treatment with chondroitin ABC lyase. Untreated samples did not penetrate the gel. Digestion of the PG with chondroitin ABC lyase yielded a major core protein band with an estimated M_r of 400000, and another minor band with an M_r of 500000 (Fig. 4). These bands did not appear after heparitinase treatment.

On gradient SDS/PAGE, ^{125}I-labelled PGs of the retarded peak (M-II) prepared from spent medium migrated as two diffuse bands with apparent M_r values of 350000 and 200000 respectively. After digestion with chondroitin ABC lyase, but not heparitinase, a ^{125}I-labelled protein of M_r 45000 was observed (results not shown). These results confirm the above Western blot experiments.

Partial characterization of mesangial cell ^{35}S-labelled HSPGs

The CM Mono Q-purified PGs were digested with chondroitin ABC lyase to remove CS and DS GAG chains, and the [^{35}S]-labelled HSPGs that remained were subjected to Sepharose CL-4B chromatography and resolved into a large ($K_{av.}$ 0.1) and small ($K_{av.}$ 0.3) species (Fig. 5). Both were susceptible to digestion with nitrous acid and they were therefore designated HSPG-I and HSPG-II. They accounted for 2% and 19% respectively of the total ^{35}S-labelled PGs synthesized by the cells. Both HSPGs contained relatively large GAG chains (for HSPG-I, M_r 65000; for HSPG-II, M_r 50000). Both of the above HSPGs were detected in the CL. The amounts of HSPGs present in the CM and CL did not allow a meaningful examination of their core proteins.

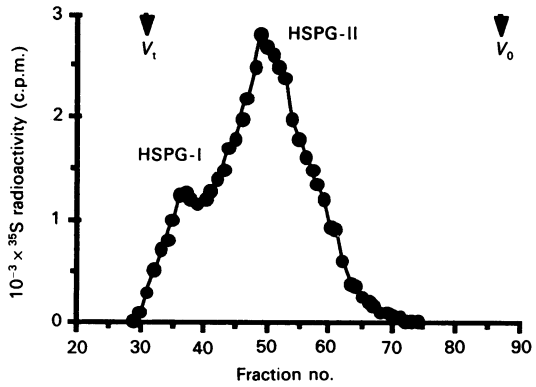

Fig. 5. Fractionation of [^{35}S]HSPGs synthesized by human adult mesangial cells in culture

The CM [^{35}S]PGs eluted from a Mono-Q ion-exchange column with 1.2 M-NaCl (see Fig. 1*a*) were treated with chondroitin ABC lyase and chromatographed on an analytical dissociative Sepharose CL-4B column.

Characterization of CL ^{35}S-labelled PGs

When the total ^{35}S-labelled PGs from the CL were analysed by Sepharose CL-4B gel-filtration chromatography under dissociative conditions, they separated into a prominent peak (Fig. 6, peak C-I) eluting near the excluded volume ($K_{av.}$ 0.06; 41% of the [^{35}S]PGs in the cell layer) and a continuum of smaller labelled molecules (Fig. 6, C-II and C-III). After alkaline borohydride treatment the [^{35}S]GAG chains derived from the nitrous acid-insensitive material from C-I were completely degraded with chondroitin AC II lyase, indicating that this fraction,

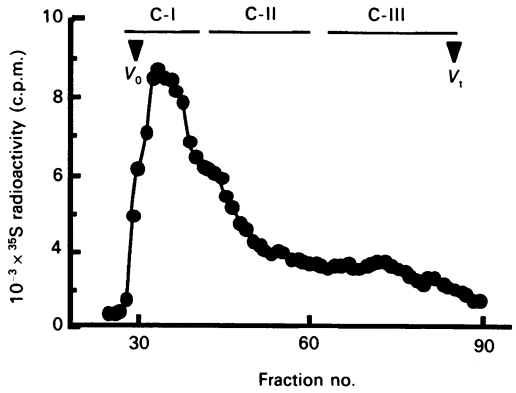

Fig. 6. Chromatography of CL [^{35}S]PGs

[^{35}S]PGs from the CL were isolated by ion-exchange chromatography as described for the CM in the legend to Fig. 1 and re-chromatographed on a column of Sepharose CL-4B under dissociative conditions. The fractions were pooled as described for further analysis (see the Results section).

Table 1. Inventory of [^{35}S]PGs synthesized by confluent cultures of human adult mesangial cells

Human adult glomerular mesangial cells in culture were labelled with [^{35}S]sulphate (50 μCi/ml) for 24 h in medium containing 15% foetal calf serum (see the Materials and methods section). ^{35}S-labelled PGs were separated from the CM and the CL as described in the text and in the legends to Figs. 1(*a*), 2 and 5. The distribution of each ^{35}S-labelled PG is expressed as a percentage of the total ^{35}S-labelled PGs synthesized by the cells. n.a., not analysed; n.d., not detected.

PG[4]	Distribution (% of total) CM	CL	$K_{av.}$	Protein core ($10^{-3} \times M_r$)
CSPG-I	7.2	3.4	0.06	400–500*
DSPG-I	40.8	5.2	0.3	45
DSPG-II	9.8	n.d.	0.4	45
HSPG-I	2.3	1.0	0.1	n.a.
HSPG-II	19.2	4.3	0.3	n.a.
CS-GAG	2.0	3.0	–	–
HS-GAG	n.d.	2.0	–	–

*Treatment of ^{125}I-labelled CSPG-I with chondroitin ABC lyase yielded two ^{125}I-labelled proteins of $M_r \sim 400000$ (major band) and 500000 (minor band).

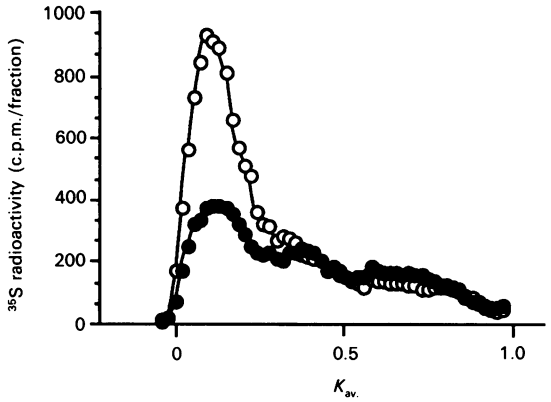

Fig. 7. Sepharose CL-4B elution profiles of the ^{35}S-labelled CL following trypsin treatment

Human adult mesangial cells were labelled with [^{35}S]sulphate for 24 h as detailed in the legend to Fig. 1, washed with phosphate-buffered saline and trypsin-treated (10 μg/ml) for 10 min at room temperature to remove the majority of the cell surface macromolecules. The cells after trypsin treatment were extracted with 4 M-guanidine HCl/4% (w/v) CHAPS and chromatographed on Sepharose CL-4B (●) as described in the legend to Fig. 6. ○, ^{35}S-labelled macromolecules extracted after incubation with buffer alone.

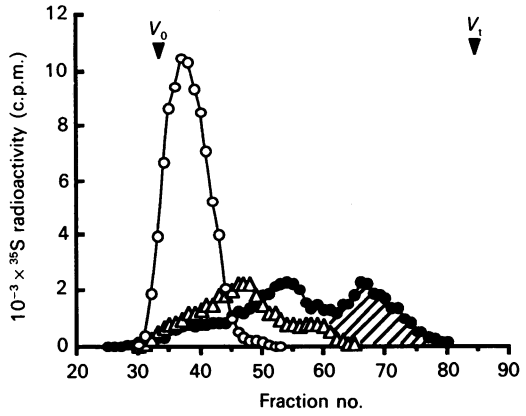

Fig. 8. Chromatography of CL ^{35}S-labelled PGs isolated on an octyl–Sepharose affinity column

Human adult mesangial cells were labelled with [^{35}S]sulphate and extracted as described in Fig. 7, and passed over a Sephadex G-50 (fine) column equilibrated in 4 M-guanidine HCl/50 mM-sodium acetate/0.15 M-NaCl for buffer exchange and removal of detergent and unincorporated isotope. The void volume was equilibrated with octyl–Sepharose and subsequently eluted with buffer alone (i.e. non-bound material) or buffer containing 0.1% or 0.5% (v/v) Triton X-100. The non-bound (●) and the 0.1% (○) and 0.5% (△) Triton X-100 eluates were chromatographed separately on a Sepharose CL-4B column under dissociative conditions. The hatched area indicates a molecule with hydrodynamic size equivalent to or smaller than that of single GAG chains.

like its CM counterpart, contained a large CSPG. Similar treatment of C-II revealed that it contained a small amount of iduronate-rich GAG chains. However, compared with the CM, the CL contained relatively small amounts of DSPGs, all of which was accounted for as DSPG-I (Table 1). The elution profile of C-III was unchanged by alkali or papain treatment (results not shown), indicating that this fraction contains single GAG chains or their degradation products.

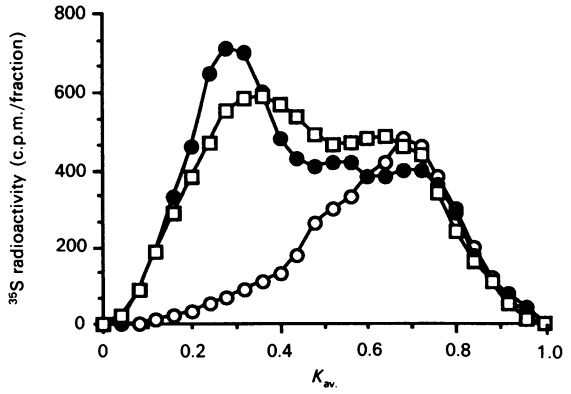

Fig. 9. Insertion of human adult mesangial CL ^{35}S-labelled PGs into liposomes

Cells were labelled with [^{35}S]sulphate and extracted as described in the legend to Fig. 7. The cell extract was passed over Sephadex G-50 (fine) equilibrated in 2% (w/v) sodium cholate/0.1 M-NaCl/20 mM-Tris/HCl, pH 7.3. A portion of the labelled material eluting in the void volume was chromatographed on a Sephacryl S-500 column (7 mm x 1.5 m) equilibrated with 4 M-urea/50 mM-Tris/HCl (pH 8.0)/0.35 M-NaCl in the absence (○) or presence (●) of 0.5% (v/v) Triton X-100. The remainder of the labelled material in the void volume was mixed with lecithin (5 mg) and liposomes were formed by dialysis against 20 mM-Tris/HCl (pH 7.3)/0.1 M-NaCl. Liposomes were chromatographed over Sephacryl S-500 equilibrated as above in the absence of 0.5% (v/v) Triton X-100 (□).

A high proportion of the large ^{35}S-labelled cell layer PG could be released by brief trypsin treatment, inferring an extracellular localization (Fig. 7). In contrast, the low-M_r material ($K_{av.} > 0.45$) was not released, suggesting that it may be intracellular and is due to single GAG chains.

To address the question as to whether the high-M_r cell-layer [^{35}S]PGs were cell- or matrix-associated, we investigated their hydrophobic properties. A total extract of the cell layer molecules was buffer-exchanged to remove detergent, chromatographed on a column of octyl–Sepharose and the non-bound and detergent-eluted material was chromatographed on Sepharose CL-4B. The unbound fraction contained predominantly material of low M_r (Fig. 8) composed of 33% HS and 66% CS/DS. The ^{35}S radioactivity contained in fractions 45–60 was composed of DSPG-I, whereas the material eluting in fractions 60–80 (Fig. 8, hatched area) corresponded to free GAG chains and their degradation products. High-M_r CL PGs were eluted from the column with buffer containing 0.1% (v/v) Triton X-100 and accounted for 50% of the total ^{35}S-molecules in the cell layer. Of these large PGs, about 70% were accounted for as CSPG-I and the remainder as HSPG-I. A more hydrophobic class of PGs could only be eluted with 0.5% (v/v) Triton X-100, and consisted mainly of HSPGs, of which HSPG-II was the major component.

When the CL molecules were exchanged into 4 M-urea/50 mM-Tris/HCl buffer, pH 8.0, containing 0.35 M-NaCl without detergent and chromatographed on Sephacryl S-500 in the same buffer, most of the high-M_r PGs were lost (Fig. 9). When the same procedure was repeated in the presence of detergent there was a full recovery of the high-M_r PGs. Finally, when the cell layer PGs were incorporated into liposomes and chromatographed in the absence of detergent, there was again a full recovery of high M_r [^{35}S]PGs (Fig. 9). When the liposomes were disrupted by the inclusion of 0.5% Triton X-100 there was again a complete recovery of [^{35}S]PGs, with a chromatography profile identical to that obtained with detergent alone (results not shown).

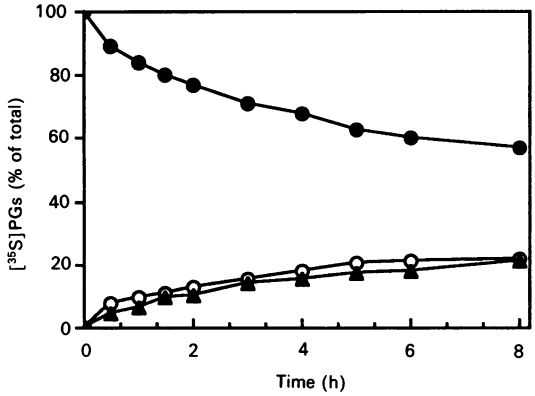

Fig. 10. Release and metabolism of CL ^{35}S-labelled PGs by human adult mesangial cells

Cells were pulse-labelled for 40 h, the culture medium removed, the cells rapidly washed with phosphate-buffered saline (X5), and the culture continued by the addition of fresh medium. At the time intervals indicated the chase medium was decanted and the CL was extracted with 4% (w/v) CHAPS/4 M-guanidine HCl. A portion of each CL and chase medium was then analysed for [^{35}S]PGs associated with the CL (●) or released into the chase medium (○) and for [^{35}S]sulphate (▲), as described in the Materials and methods section. The remainder of each CL extract and chase medium extract was used to assess the fate of individual ^{35}S-labelled PGs (see Fig. 11).

Taken together, the data from the trypsin release, octyl–Sepharose and liposome experiments indicate that a large portion of the CL high-M_r PGs have hydrophobic properties and may be intercalated via their core protein within the cell membrane. Trypsin-released material and medium-derived PGs did not bind to octyl–Sepharose; neither could they be inserted into liposomes. Moreover, PGs in the medium were recovered from Sepharose CL-4B columns with good recovery (> 90%) in the absence of detergent.

Pulse–chase experiments

The above data suggest that some newly synthesized CSPG-I and a high proportion of HSPGs are selectively retained in the cell layer, whereas the DSPGs are rapidly released into the extracellular environment. To investigate this further, pulse–chase experiments were performed. Cells were labelled for 40 h and chased for various periods of time. During an 8 h chase the loss of [^{35}S]PGs from the cell layer was accompanied by the appearance in the medium of an equal amount of radioactivity as [^{35}S]PGs (20% of the total cell layer macromolecules) and free [^{35}S]sulphate (20%) (Fig. 10). The loss of [^{35}S]PGs from the cell layer occurred predominantly during the first 90 min of the chase. Thereafter, little [^{35}S]PG was released into the culture medium or metabolized to free sulphate.

These results indicate that, as with human glomerular epithelial cells [8], loss of PGs from the mesangial cell layer proceeds by two pathways: (*a*) release of some labelled macromolecules into the extracellular environment, and (*b*) internalization and complete digestion of others with the appearance of free sulphate. However, in the mesangial cell both pathways are considerably less active than in glomerular epithelial cells.

The fate of individual PGs was also investigated (Figs. 11*a*–11*d*). In this experiment it was not possible to apportion the free [^{35}S]sulphate derived from the intracellular metabolism of individual ^{35}S-labelled PGs. However, summation of individual labelled PGs present in the CL and the chase medium over 8 h (Fig. 11, ■) allowed an estimate of the amount of each PG lost

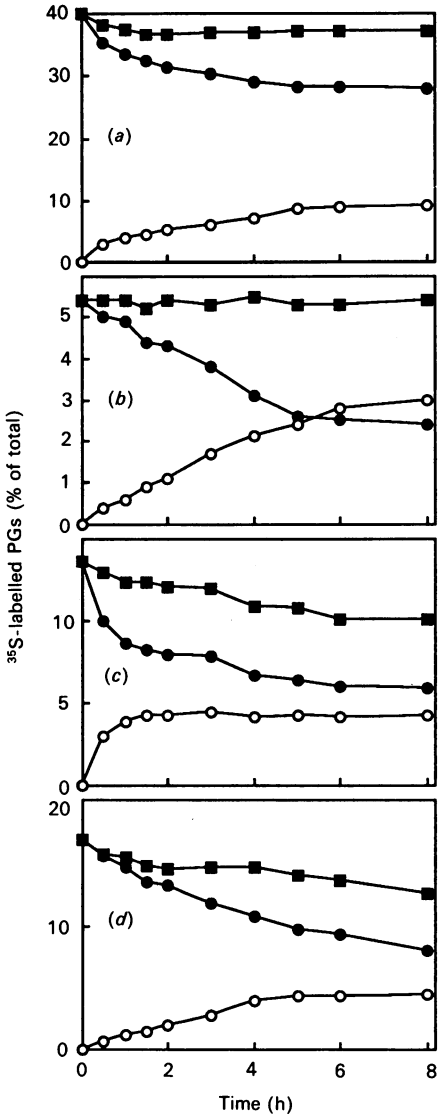

Fig. 11. Metabolism of individual ^{35}S-labelled PGs synthesized by human adult mesangial cells

Portions of the CL and chase medium from the pulse-chase experiment described in Fig. 10 were treated with either cold nitrous acid (to obtain total [^{35}S]CS/DSPGs) or digested with chondroitin ABC lyase (to obtain total [^{35}S]HSPGs) and chromatographed on a Sepharose CL-4B column under dissociative conditions to obtain (*a*) CSPG-I, (*b*) HSPG-I, (*c*) DSPG-I and (*d*) HSPG-II. ●, CL-associated PGs; ○, PGs released into the chase medium; ■, total PGs.

from the system presumably through intracellular metabolism. Thus CSPG-I (Fig. 11*a*) and HSPG-I (Fig. 11*b*) appeared to undergo very little catabolism, since loss from the CL was accompanied by the appearance of an equal amount in the chase medium. In contrast, significant amounts of the CL DSPG-I (Fig. 11*c*) and HSPG-II (Fig. 11*d*) were lost from the system, suggesting that these two PGs are metabolized to the free [^{35}S]sulphate generated during the 8 h chase period. The rate of disappearance of DSPG-I from the CL was more rapid than that of the other labelled PGs. Thus, over the first 1 h, 24% of the initial DSPG-I present in the CL was lost to the chase medium. Thereafter, there was little or no accumulation of this PG in the chase medium. However, during this period there was a continuous loss from the system, indicating that a small amount

(~ 27%) was lost to metabolism. DSPG-II was again not evident in the CL, suggesting that after synthesis this PG, like the majority of DSPG-I, is rapidly released from the cell.

DISCUSSION

In the present study the PGs synthesized in culture by human adult mesangial cells were characterized. The cells secrete a major portion (81%) of the newly synthesized [^{35}S]PGs into the medium. These consisted predominantly of CSPGs and DSPGs. At least three classes of PGs were identified which differ from one another with respect to their hydrodynamic size, core protein and GAG size. These consisted of a large CSPG (CSPG-I) and two small DSPGs (DSPG-I and DSPG-II). In addition, the cells synthesized HSPGs, the majority of which were located in the CM.

The CSPG was hydrodynamically large and contained GAG chains of average M_r 25000 attached to a core protein of M_r 400000. In addition, a large core M_r 500000 was detected. Core protein heterogeneity has been reported for a similar class of CSPGs made by vascular smooth muscle cells [15–17], aortic endothelial cells [18,19] and fibroblasts [20]. CSPG-I has properties in common with a population of aortic PGs extracted from blood vessels and secreted by cultures of vascular smooth muscle cells [17]. These large PGs consist of relatively large core proteins of M_r ~ 200000–400000, to which are attached up to 15 CS chains (M_r 20000–40000). They are able to interact with hyaluronan to form PG aggregates and thus resemble the large aggregating CSPGs in cartilage. Reduction and alkylation of the CM did not change the elution profile of CSPG-I on Sepharose CL-4B, showing that in the mesangial cell culture it was not aggregated with hyaluronic acid. The CL form of CSPG-I has hydrophobic properties which suggest that it may be intercalated with cell membranes. In contrast, the CM form does not have these properties, and together with the pulse–chase data this indicates that the cell-associated species may be processed and released into the medium.

The predominant PGs in the CM are smaller than the CSPG-I and contained iduronate-rich GAG chains. After removal of CSPG-I by gel-permeation chromatography a second Mono-Q anion exchange enabled the separation of DSPG-I and DSPG-II. Physical, biochemical and immunological properties indicate that DSPG-I and DSPG-II are related to biglycan and decorin respectively, extracellular PGs which have been isolated from several connective tissues and which are synthesized in culture by fibroblasts, smooth muscle cells and endothelium [21]. The core proteins of these DSPGs have been cloned and sequenced [22,23]. While they contain similar regions, they are the products of separate genes. The core protein of decorin (M_r 38000) contains three pairs of serine–glycine residues, one of which is substituted with a single GAG chain. Biglycan consists of a core of M_r 43000 to which two DS chains are attached. Presumably the difference in chain number allows the separation in the present work. The purified mesangial cell DSPGs had an apparent M_r of ~ 350000 (DSPG-I) and ~ 200000 (DSPG-II). Thus they are similar to the small DSPGs identified in bone [24]. In contrast, those extracted from articular cartilage [25] and isolated from cultures of bovine aortic endothelial cells [19] and rat mesangial cells [26] appear to be relatively smaller, with M_r values of ~ 100000 and ~ 220000 for decorin and biglycan respectively.

A recent report describes the PGs synthesized by human foetal mesangial cells [6]. Although they have similar properties, they differ significantly from those made by adult cells. For example, cells from adult kidneys synthesize a higher proportion of CSPGs and DSPGs (72% versus 57%) and secrete more of these into the extracellular environment (81% versus 57%). Furthermore, the foetal cells synthesize a significantly higher proportion of CSPG-I (32% versus 12%), most of which was associated with a cell membrane fraction. It is also apparent that the foetal cells also do not synthesize an HSPG equivalent to our HSPG-I or decorin. Finally, the GAG chains released from respective PGs differ in chain length. The significance of these differences is as yet unknown.

The glomerular basement membrane and the mesangial matrix, the two extracellular matrices present in the glomerulus, are maintained by the glomerular visceral epithelial and mesangial cells respectively. We and others have shown that in tissue culture, human glomerular epithelial cells derived from whole glomeruli synthesize a variety of HSPGs, including basement membrane HSPG [8,27]. In contrast, the mesangial cells synthesize only a limited amount of HSPGs. These are relatively large molecules and are clearly different from radiolabelled HSPGs isolated from cultures of glomerular epithelial cells. The core proteins of HSPG-I or HSPG-II were not investigated, but based on hydrodynamic size neither of the mesangial cell HSPGs resemble basement membrane HSPG.

The function of PGs in the mesangium is unknown, but they could play a role similar to that proposed for HSPG in the glomerular basement membrane, providing a charge barrier to plasma proteins and conferring a high degree of hydration and hence turgidity to the mesangial matrix. The mesangial accumulation of extracellular matrix in chronic renal disease, including diabetic nephropathy, glomerulosclerosis and membranous disease, is well documented [2]. PGs, particularly DSPGs, appear to be a major constituent of the mesangial matrix. The study of adult human mesangial cells provides a basis for further investigations into the involvement of mesangial cell PGs in human glomerular disease.

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REFERENCES

1. Timpl, R. (1986) Kidney Int. **30**, 293–298
2. Sterzel, R. B., Lovett, D. H., Stein, H. D. & Kashgarian, M. (1982) Klin. Wochenschr. **60**, 1077–1094
3. Kanwar, Y. S., Jakubowski, M. L. & Rosenzweig, L. J. (1983) Eur. J. Cell Biol. **31**, 290–295
4. Couchman, J. R., Caterson, B., Christner, J. E. & Baker, J. R. (1984) Nature (London) **307**, 650–652
5. McCarthy, K. J., Accavitti, M. A. & Couchman, J. R. (1989) J. Cell Biol. **109**, 3187–3198
6. Klein, D. J., Brown, D. M., Kim, Y. & Oegema, T. R. (1990) J. Biol. Chem. **265**, 9533–9543
7. Martin, J., Davies, M., Thomas, G. J. & Lovett, D. H. (1989) Kidney Int. **36**, 790–801
8. Thomas, G. J., Jenner, L., Mason, R. M. & Davies, M. (1990) Arch. Biochem Biophys. **278**, 11–20
9. Shively, J. E. & Conrad, H. E. (1976) Biochemistry **15**, 3943–3950
10. Yamagata, T., Saito, H., Habuchi, O. & Suzuki, S. (1968) J. Biol. Chem. **243**, 1523–1535
11. Oike, Y., Kimata, K., Shinomura, T. & Suzuki, S. (1980) Biochem. J. **191**, 203–207
12. Laemmli, U. K. (1970) Nature (London) **227**, 680–685
13. Greenwood, F. C., Hunter, W. M. & Glover, J. J. (1963) Biochem. J. **89**, 114–120
14. Lories, V., DeBoeck, H., David, G., Cassiman, J.-J. & Van den Berghe, H. (1987) J. Biol. Chem. **262**, 854–859
15. Chang, Y., Yanagashita, M., Hascall, V. C. & Wight, T. N. (1983) J. Biol. Chem. **258**, 5679–5688
16. Wight, T. N. & Hascall, V. C. (1988) J. Cell Biol. **96**, 167–176

17. Wight, T. N. (1989) Arteriosclerosis **9**, 1–20
18. Morita, H., Takeuchi, T., Suzuki, S., Maeda, K., Yamada, K., Eguchi, G. & Kimata, K. (1990) Biochem. J. **265**, 61–68
19. Kinsella, M. G. & Wight, T. N. (1988) J. Biol. Chem. **263**, 19222–19231
20. Yamagata, M., Yamada, K. M., Suzuki, S. & Kimata, K. (1986) J. Biol. Chem. **261** 13526–13535
21. Ruoslahti, E. (1988) Annu. Rev. Cell Biol. **4**, 229–255
22. Fisher, L. W., Termine, J. D. & Young, M. F. (1989) J. Biol. Chem. **264**, 4571–4576
23. Krusius, T. & Ruoslahti, E. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 7683–7687
24. Fisher, L. W., Hawkins, G. R., Tuross, N. & Termine, J. D. (1987) J. Biol. Chem. **262**, 9702–9708
25. Roughly, P. J. & White, R. J. (1989) Biochem. J. **262**, 823–827
26. Border, W. A., Okuda, A. S., Languino, R. & Ruoslahti, E. (1990) Kidney Int. **37**, 687–695
27. Klein, J. K., Oegema, T. R., Fredeen, T. S., Van der Woude, F., Kim, Y. & Brown, D. M. (1990) Arch. Biochem. Biophys. **277**, 389–401

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