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Influence of collagen lattice on the metabolism of small proteoglycan II by cultured fibroblasts

Hermann GREVE,*‡ Petra BLUMBERG,* Gerd SCHMIDT,* Wolfgang SCHLUMBERGER,† Jürgen RAUTERBERG† and Hans KRESSE*§
*Institute of Physiological Chemistry and Pathobiochemistry, University of Münster, Waldeyerstrasse 15, D-4400 Münster, and †Institute of Arteriosclerosis Research at the University of Münster, Domagkstrasse 3, D-4400 Münster, Federal Republic of Germany

Small dermatan sulphate proteoglycan II from cultured human skin fibroblasts interacts with type I collagen *in vitro* and *in vivo*. When fibroblasts are maintained in a type I collagen lattice the proteoglycan remains exclusively within the lattice, and its association with fibrils can be demonstrated immunocytochemically. On the basis of [^{35}S]sulphate incorporation, small proteoglycan II comprises about 80% of total proteoglycans secreted by cells in monolayer culture. In a collagen lattice, fibroblasts down-regulate its synthesis to the level of large chondroitin sulphate/dermatan sulphate and of heparan sulphate proteoglycans, the synthesis of which remains unaffected. Compared with the product from monolayer cultures, small proteoglycan II from collagen gels contained a longer polysaccharide chain which is characterized by a larger proportion of disulphated and a smaller proportion of monosulphated glucuronic acid-containing disaccharides. The half-life varied between 60 and 110 h. It is suggested that the compositional differences between the proteoglycan from monolayer cultures and from cells in a collagen lattice are related to the slower intracellular trafficking of the proteoglycan under the latter culture conditions.

INTRODUCTION

Fibroblasts in monolayer cultures represent a valuable model for studying several aspects of proteoglycan metabolism. However, the use of such systems is questionable if the turnover and interactions of proteoglycans with extracellular-matrix components are to be studied. In monolayer, the lower surface of the cells interacts with an immobilized substratum, whereas the upper side is in contact with a large volume of liquid medium that is replaced at regular intervals. Three-dimensional collagen lattices have been shown to provide a much more appropriate environment for fibroblasts. The cells are able to contract the gels to form a very dense matrix resembling authentic connective tissue (Bell *et al.*, 1979). After a few days, they adopt a more bipolar morphology and the cytoskeletal organization typical of fibroblasts *in vivo* (Tomasek *et al.*, 1982). Indeed, a condensed fibroblast-populated collagen lattice, with keratinocytes added to its surface, has been used as skin graft in experimental animal studies (Bell *et al.*, 1981).

Compared with monolayer cultures, the metabolism of fibroblasts becomes profoundly modulated under the influence of the three-dimensional extracellular matrix. It has been shown, for example, that the expression of types I and III collagen is markedly decreased (Nusgens *et al.*, 1984; Paye *et al.*, 1987; Mauch *et al.*, 1988), whereas an increase in α1(VI) and α2(VI) collagen mRNAs was found (Hatamochi *et al.*, 1989). We are interested in a ubiquitously distributed small chondroitin sulphate/dermatan sulphate proteoglycan, named small proteoglycan II (DS-PG II) (Heinegård *et al.*, 1985; Rosenberg *et al.*, 1985) or decorin (Yamaguchi & Ruoslahti, 1988), which binds to the 'd' band of collagen fibrils in non-mineralized connective tissue (Scott & Orford, 1981; Scott & Haigh, 1985) and inhibits fibrillogenesis of type I and II collagens (Vogel *et al.*, 1984; Hedbom & Heinegård, 1989). Other investigators, however, found that small proteoglycan from uterine cervix did not influence the rate of fibrillogenesis, but had an effect on the topographical arrangement between the collagen fibrils (Uldbjerg & Danielsen, 1988). Binding of the proteoglycan to fibronectin located on the surface of cultured fibroblasts was also observed (Schmidt *et al.*, 1987). The proteoglycan is the predominant proteoglycan species secreted by cultured fibroblasts, and its biosynthesis, secretion and endocytosis have been studied in considerable detail (Glössl *et al.*, 1983, 1984; Hoppe *et al.*, 1985; Greve *et al.*, 1988). In light of the proposed role of the proteoglycan in fibrotic processes and wound healing, we wanted to study its metabolism under the more physiological condition of a fibroblast-populated collagen lattice. The results of this study are presented here.

EXPERIMENTAL

Cell culture

Human skin fibroblasts from juvenile or adult human donors were cultivated in modified Eagle's Minimum Essential Medium with Earle's salts, supplemented with non-essential amino acids, antibiotics and 10% (v/v) fetal-bovine serum (Boehringer, Mannheim, Germany) as described by Cantz *et al.* (1972). Confluent cultures between passages 5 and 10 were used for the experiments.

For the preparation of collagen lattices, acid-soluble type I collagen from calf skin (Sigma, Deisenhofen, Germany) was dissolved in 16.7 mM-acetic acid (3.3 mg/ml), centrifuged at 16000 *g* for 1 h, and stored frozen in 1 ml portions until used. A 675 μl portion of 1.78-fold concentrated culture medium (Eagle's Minimum Essential Medium or Waymouth MAB 87/3 medium; see below) was mixed with 150 μl of fetal-bovine serum, 75 μl of 0.1 M-NaOH and 450 μl of collagen in a 35 mm-diam. bacteriological plastic dish (Becton–Dickenson), and then 150 μl of trypsin-treated fibroblasts suspended in serum-free medium was

Abbreviations used: DS-PG II, small dermatan sulphate proteoglycan II; PBS, phosphate-buffered saline.
‡ Present address: Hoffmann-La Roche A.G., D-7889 Grenzach-Whylen, Federal Republic of Germany.
§ To whom correspondence should be addressed.

rapidly added. The number of cells added per dish was 1.5×10^5–3×10^5; the number had been determined in a haemocytometer. After 6 h, the gels were gently detached from the walls and the bottom of the dishes by shaking or by passing a curved-tip Pasteur pipette around the perimeter of the gels. Media were exchanged every second day.

Metabolic labelling

Monolayer cultures and fibroblast-populated collagen lattices of diameter between 8 and 10 mm were incubated in the presence of [^{3}H]leucine (sp. radioactivity 2.55 TBq/mmol; Amersham–Buchler, Braunschweig, Germany) and/or [^{35}S]sulphate (carrier-free, Amersham-Buchler) exactly as described by Glössl *et al.* (1984). Small proteoglycan in the media was quantified by immune precipitation with rabbit antibodies adsorbed to Protein A–Sepharose (Sigma) as described by Glössl *et al.* (1984). Briefly, the media were made 70% saturated with $(NH_4)_2SO_4$, and the precipitate was extracted, in the presence of protease inhibitors, with 25 mM-Tris/HCl, pH 7.2, containing 0.5% sodium deoxycholate, 0.5% Triton X-100 and 0.5 M-NaCl. After centrifugation (10000 *g*-min), the extracts were sequentially treated with control IgG-coated Protein A–Sepharose and with Protein A–Sepharose coated with IgG from a monospecific antiserum against DS-PG II core protein. It had been ascertained that the immune reaction was at least 90% complete. The collagen lattices were washed with 2 × 1.5 ml of 25 mM-Tris/HCl, pH 7.2, containing 0.15 M-NaCl, and disintegrated by incubation for 5 h at 37 °C with 200 μl of this buffer containing additionally 25 BTC-U (1 BTC-U liberates 1 nmol of leucine equivalent/min from non-denatured tendon at 37 °C and pH 7.2) of collagenase (Advance Biofactures, Lynbrook, NY, U.S.A.) and 10 mM-$CaCl_2$. Cells were then removed by centrifugation (10000 *g*-min), and the supernatant was immune-precipitated after adding an equal volume of 0.2 M-Tris/HCl, pH 7.2, containing 1 M-NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.2 M-6-hexanoic acid, 20 mM-EDTA, 20 mM-*N*-ethylmaleimide and 10 mM-benzamidinium hydrochloride. Control incubations with purified [^{3}H]leucine-labelled small proteoglycan from monolayer cultures indicated that treatment with collagenase did not cause degradation of the core protein. During the course of the experiments, it was found that the collagen lattice could be disintegrated within 15 min by treatment at 45 °C with 50 BTC-U of collagenase and intermittent vortex-mixing under otherwise identical conditions. Cell viability as determined by Trypan Blue exclusion was > 90%.

For studies on the interaction *in vitro* of DS-PG II and collagen, [^{35}S]methionine-labelled proteoglycan was obtained as follows. Monolayer cultures of fibroblasts (growth area 150 cm^2) were incubated for 14 h in the presence of 15 MBq of [^{35}S]methionine (sp. radioactivity 51 TBq/mmol; Amersham-Buchler) under analogous conditions (methionine-free medium) as described for the preparation of [^{3}H]leucine-labelled proteoglycans (Glössl *et al.*, 1984). DS-PG II was purified by an $(NH_4)_2SO_4$-precipitation step, followed by anion-exchange chromatography (Hausser *et al.*, 1989).

Glycosaminoglycan analysis

The glycosaminoglycan chains of DS-PG II were isolated by a β-elimination reaction and characterized by chromatography on a Sephacryl S-300 column as described previously (Greve *et al.*, 1988). The disaccharide composition of [^{35}S]sulphate-labelled dermatan sulphate was determined after parallel digestions with chondroitin ABC and AC lyase (Seikagaku Kogyo, Tokyo, Japan) as stated previously (Rauch *et al.*, 1986). The proportions of disulphated and monosulphated disaccharides were obtained after high-voltage electrophoresis on Whatman no. 3MM paper in 5% acetic acid/0.5% pyridine, pH 3.5, for 25 min at 60 V/cm and measuring the radioactivity in 1 cm paper segments. Monosulphated disaccharides were separated on a Partisil-PAC column (Whatman, Maidstone, Kent, U.K.) as described by Zebrower *et al.* (1986). Proteoglycans not reactive towards DS-PG II antibodies were dialysed against water and subjected to alkaline borohydride treatment before glycosaminoglycan purification by chromatography on Dowex 1-X2 (200–400 mesh) (Greve *et al.*, 1988). The fraction of glycosaminoglycans resistant toward chondroitin ABC lyase was subsequently subjected to HNO_2 deamination at pH 1.5 (Shively & Conrad, 1976), followed by neutralization with 2 M-NaOH. After both the enzymic and the chemical degradation steps, respectively, the samples were evaporated to dryness, and the radioactivity solubilized by 70% (v/v) ethanol was determined. Uronic acids were quantified as described by Bitter & Muir (1962).

Interactions of type I collagen and DS-PG II *in vitro*

The interaction of type I collagen with DS-PG II and DS-PG II core protein was studied by a modification (Schmidt *et al.*, 1987) of the procedure of Engvall & Perlmann (1971) and by direct measurements of the binding of radioactive samples. In both assays, stock solutions of type I collagen (3.3 mg/ml) in 16.7 mM-acetic acid were diluted with 20 mM-$NaHCO_3$, pH 9.6, immediately before coating of microtitre plates. DS-PG II core protein was obtained by incubation of the proteoglycan with 50 m-units of chondroitin ABC lyase in the presence of protease inhibitors (Rauch *et al.*, 1986), followed by dialysis against distilled water. Control incubations were performed in the presence of heat-inactivated enzyme (30 min at 70 °C). Polyclonal affinity-purified rabbit antibodies against DS-PG II core protein (170 μg of protein/ml; Voss *et al.*, 1986) were diluted 500-fold with phosphate-buffered saline (PBS; 137 mM-NaCl/2.7 mM-KCl/16.1 mM-Na_2HPO_4/1.9 mM-KH_2PO_4), pH 7.4, containing 30 g of BSA/litre and 0.1% Tween 20. The second antibody, goat anti-(rabbit IgG)–peroxidase conjugate (Sigma), was also diluted 500-fold with this buffer. Colour development was with 200 μl of 1 mM-2,2′-aminobis-(3-ethylbenzthiazolinesulphonic acid) and 740 μM-H_2O_2 in 50 mM-sodium citrate, pH 4.0. Coating the microtitre plates with DS-PG II (up to 1.25 μg/ml) or equivalent amounts of core protein indicated that the latter antigen gave rise to a 1.4-fold greater colour development than the former after analogous incubations with first and second antibodies. Use of [^{35}S]methionine-labelled ligands (see below) indicated that similar amounts of DS-PG II and of core protein were bound by the microtitre plates.

Binding studies were also performed in the presence of [^{35}S]methionine-labelled DS-PG II and core protein under otherwise identical conditions. The radioactive ligand was added to a stock solution of unlabelled DS-PG II and core protein, respectively. Bound material was quantitatively solubilized by incubating each microtitre well for 2 h at 37 °C with 6000 USP units of papain (0.2 mg; Merck, Darmstadt, Germany) in 200 μl of 0.1 M-sodium acetate buffer, pH 6.5, containing 5 mM-EDTA and 5 mM-cysteine hydrochloride.

Immunocytochemistry

Small pieces of collagen lattices were pre-fixed with 0.1% glutaraldehyde and 2% paraformaldehyde in PBS for 30 min, washed and incubated with primary antibody solution (17 μg of protein in PBS) for 16 h at room temperature. After six washes with PBS for 6 h, the lattices were incubated with Protein A coupled to colloidal gold (12 nm diameter) (Roth *et al.*, 1982); Frens, 1973) for 16 h at room temperature. Lattices were again washed extensively in PBS for 6 h at room temperature, and post-fixed with Karnovsky's reagent (Karnovsky, 1965) for 2 h

at 4 °C. Control samples were incubated with Protein A–gold alone. The collagen lattices were then post-fixed with 1.3 % OsO_4 in 0.1 M-collidine buffer (pH 7.3). Samples were dehydrated in ethanol, cleared in propylene oxide, and embedded in Epon 812 (Luft, 1961). Ultrathin sections were double-stained with uranyl acetate and lead citrate, and examined with a Philips EM 410 electron microscope at 60 kV.

Other methods

SDS/polyacrylamide-gel electrophoresis (Laemmli, 1970) followed by fluorography (Bonner & Laskey, 1974) was performed as described previously.

RESULTS

Interaction *in vitro* of DS-PG II with type I collagen

To study the influence of type I collagen on the metabolism of DS-PG II, we wanted to study first the interaction of DS-PG II from fibroblast secretions *in vitro*. In an e.l.i.s.a. test system, both intact DS-PG II and glycosaminoglycan-free core protein derived therefrom bound to immobilized type I collagen in a dose-dependent manner (Fig. 1). Taking into account the 1.4-times greater reactivity of the antibodies towards the glycosaminoglycan-free core protein, this result suggests that the presence of the dermatan sulphate chain facilitates the interaction between the proteoglycan and type I collagen. This contrasts with the binding behaviour towards fibronectin, where greater amounts of immunoreactive core protein than of the proteoglycan were bound at all doses tested (Schmidt *et al.*, 1987).

The conclusion that the presence of dermatan sulphate chains facilitates the binding is valid only if the antibody response towards both proteoglycan and core protein is either unaffected or altered to the same extent upon binding of the ligands to type I collagen. We have therefore measured additionally the interaction of [^{35}S]methionine-labelled ligands with type I collagen under otherwise identical conditions. In these experiments, too, there was less binding of dermatan sulphate-free core protein than of intact DS-PG II at all doses tested (Fig. 2). However, full saturation of binding ws not observed even at the highest ligand concentration. Doubling the incubation time from 90 min to 180 min led to a 1.3-fold increase in the amount of bound DS-PG II, whereas the amount of bound core protein increased 1.5-fold. This finding would be compatible with the assumption that the differences in the binding of the two ligands are not the result of a decreased binding capacity of type I collagen for dermatan sulphate-free core protein.

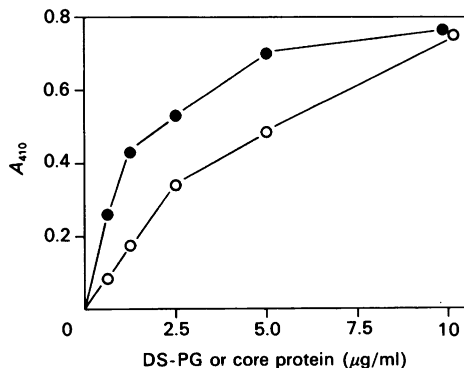

Fig. 1. Binding of DS-PG II (●) and of its glycosaminoglycan-free core protein (○) to type I collagen

Microtitre wells were coated with acid-soluble type I collagen (10 µg/ml) as described in the Experimental section. Binding of DS-PG II and of equivalent amounts of its glycosaminoglycan-free core protein was quantified by use of affinity-purified polyclonal antibodies against core protein. Identical blank values were observed when either the antigen or the first or second antibody was omitted.

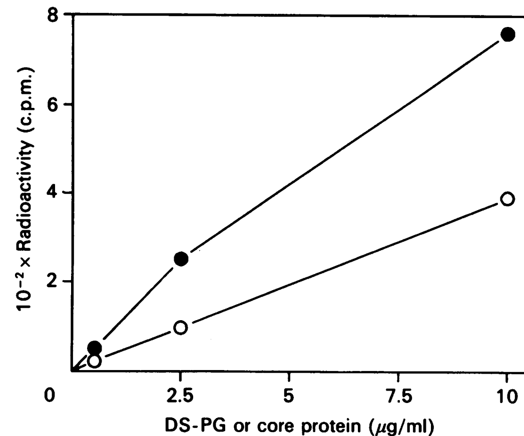

Fig. 2. Binding of [^{35}S]methionine-labelled DS-PG II (●) and of its glycosaminoglycan-free core protein (○) to type I collagen

Miocrotitre wells were coated with type I collagen as described in the legend of Fig. 1. Unlabelled DS-PG II or core protein was mixed with labelled material to give 15000 c.p.m./10 µg of DS-PG II (or the equivalent amount of core protein). For the determination of bound material, the papain digests from eight wells were combined. Less than 50 c.p.m. was found when the wells had been coated with BSA instead of type I collagen and 10 µg of DS-PG II or core protein/ml was used as ligand.

DS-PG II production in collagen lattices

Pre-embedding immunocytochemistry of collagen lattices at the electron-microscopic level indicated that fibroblasts produced sufficient amounts of DS-PG II within 6 days to decorate the collagen fibrils with this proteoglycan (Fig. 3). Quantitative determination of [^{3}H]leucine-labelled DS-PG II indicated that more than 90 % of the secreted proteoglycan remained within the collagen lattice from the first day onward (Schlumberger *et al.*, 1989). With time, however, there was a dramatic decline in its biosynthesis (Fig. 4). In three additional analyses of day-6 cultures, production of [^{35}S]sulphate-labelled DS-PG II was 4–9 % of that of analogously treated monolayer cultures when referred to the cell number. A surprisingly low DS-PG II production on day 1 was also found in two independent experiments, but this phenomenon was not investigated further.

Although a toxic effect of a component within the collagen lattice on DS-PG II biosynthesis cannot rigorously be excluded, it is noteworthy that the decline in [^{35}S]sulphate incorporation was specific for DS-PG II. Only minor changes in the production of other proteoglycan types were found (Fig. 4). When the [^{35}S]sulphate incorporation was studied in an analogous experiment using monolayer cultures, there was an almost continuous decrease from day 1 (100 %) to day 8 (78 %) in the amount of total ^{35}S radioactivity incorporated. The proportion of DS-PG II of total proteoglycans did not vary significantly with time and was between 81 and 87 %.

Structure of DS-PG II

SDS/polyacrylamide-gel electrophoreses revealed that the size of the DS-PG II core protein was not altered upon maintenance of fibroblasts in a collagen lattice (Fig. 5). Thus core-protein fragments could not be detected by immunoprecipitation methods, and the core protein was substituted with either two or three asparagine-bound oligosaccharides. The latter statement

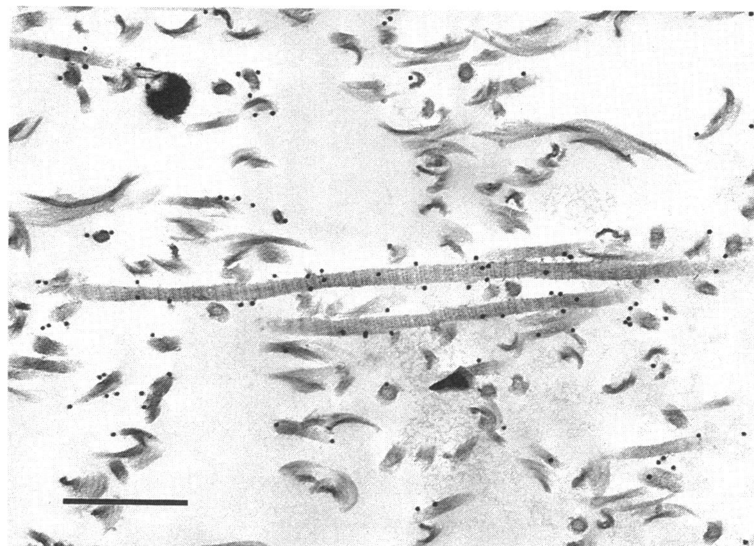

Fig. 3. Immunocytochemical localization of DS-PG II in collagen lattices

Fibroblasts (1.5×10^5) were maintained in collagen lattices for 6 days before immunocytochemical labelling with affinity-purified antibodies against DS-PG II core protein. Bar represents 0.5 μm.

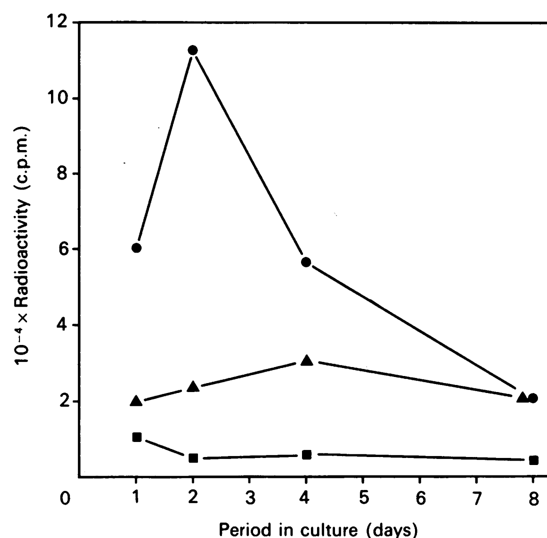

Fig. 4. Time-dependence of proteoglycan synthesis

Fibroblasts (3×10^5 cells) were maintained in collagen lattices for 24, 48, 96 or 192 h, and then metabolically labelled for a further 4 h in the presence of 0.74 MBq of [^{35}S]sulphate/ml. ●, DS-PG II; ▲, large chondroitin sulphate/DS-PG; ■, heparan sulphate-PG.

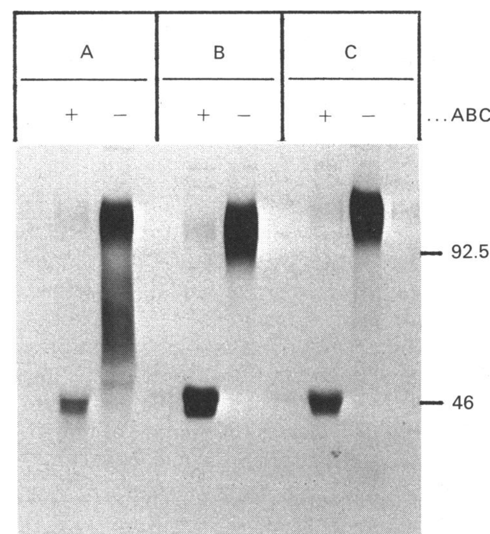

Fig. 5. Fluorograph of DS-PG II

Fibroblasts (3×10^5 cells) were maintained in collagen lattices (A, C) or in monolayer culture (B) for 8 days and then labelled for 6 h with [^{3}H]leucine (4.4 MBq/ml) and [^{35}S]sulphate (1.48 MBq/ml). DS-PG II was immune-precipitated either after treatment of the lattice with 450 μl of 16.7 mM-acetic acid for 15 min, followed by neutralization (A), or after $(NH_4)_2SO_4$ precipitation of the culture medium (B) and of the collagenase digest (C) respectively. Samples of the proteoglycan were digested with chondroitin ABC lyase (ABC) before SDS/polyacrylamide-gel electrophoresis and fluorography. The mobilities ($10^{-3} \times M_r$) of phosphorylase *b* and ovalbumin are indicated at the right-hand side.

was confirmed by tunicamycin treatment (result not shown). Interestingly, a 5–6-fold higher dose of the inhibitor was needed to produce a similar effect to that found in monolayer fibroblasts (Glössl *et al.*, 1984). Solubilization of the lattice by acetic acid caused some fragmentation of the proteoglycan and was therefore not used in other experiments.

Fig. 5 also shows that the intact proteoglycan extracted from collagen lattices moved more slowly than the proteoglycan from monolayer cultures during gel electrophoresis. Such behaviour would be expected if there was an increase in size of the dermatan sulphate chain. Therefore the size of the glycosaminoglycan chain was determined, after a β-elimination reaction, by chromatography on a calibrated Sephacryl S-300 column (Greve *et al.*, 1988). Dermatan sulphate from monolayer cultures was eluted with a mean $k_{av.}$ value of 0.135, which corresponds to an M_r-37000 polymer. The glycosaminoglycan chains obtained from DS-PG II in collagen lattices, however, were regularly found to be larger in size (M_r 40000; $k_{av.} = 0.125$).

In addition to the increased glycosaminoglycan chain size, the copolymeric structure of the chain was also altered. The data in Table 1 indicate that during formation of the glycosaminoglycan

Table 1. Glycosaminoglycan composition of DS-PG II

Fibroblasts were maintained in collagen lattices or in monolayer cultures as described in the legend of Fig. 5. Secreted DS-PG II from the lattice and from the medium of monolayer cultures was obtained after incubation for 15 h in the presence of 1.48 MBq of [^{35}S]sulphate/ml. Chondroitin lyase-generated disaccharides from free glycosaminoglycan chains were quantified as described in the Experimental section. For calculations, the radioactivity was divided by the number of sulphate ester groups. Mean values and ranges of data from three independent experiments are given.

	Content (% of total)	
	Collagen lattice	Monolayer
Dermatan sulphate-derived monosulphated disaccharide	78 (76–80)	63 (61–68)
Dermatan sulphate-derived disulphated disaccharide	10 (7–12)	6 (4–7)
Chondroitin 4-sulphate-derived disaccharide	14 (9–17)	24 (17–26)
Chondroitin 6-sulphate-derived disaccharide	3 (2–4)	7 (4–9)

chain in collagen lattices a greater proportion of D-glucuronic acid residues became epimerized to L-iduronic acid residues than during glycosaminoglycan synthesis in monolayer cultures. In connection with the decreased proportion of 6-sulphated disaccharides, it can therefore be stated that DS-PG II from the collagen lattice is even more typically of dermatan sulphate type than is the proteoglycan from monolayer cultures.

Secretion and turnover of DS-PG II

The enzymic solubilization of collagen lattices at 45 °C occurred within such a short time that it was possible to obtain an estimate of the secretion kinetics of DS-PG II by pulse–chase labelling of cells with [^{3}H]leucine under the two different culture conditions. The data provided in Fig. 6 suggest that the proportion of exocytosed DS-PG II of the total proteoglycan synthesized was considerably smaller in gel-cultured than in monolayer-cultured fibroblasts. In two independent experiments more than 50% of the core-protein-associated radioactivity was not secreted within a chase period of 3 h. However, the amount of DS-PG II-associated radioactivity was so small that a distinction between intracellular and cell-surface-bound proteoglycan could not be made. Analogously performed experiments using [^{35}S]sulphate as radioactive precursor suggested that it was predominantly the mature proteoglycan that remained cell-associated. Monolayer cultures behaved differently. Half of the pulse-labelled core protein was secreted within less than 1.5 h.

The turnover of [^{3}H]leucine- and [^{35}S]sulphate-labelled DS-PG II in collagen lattices was determined in a final set of experiments. In three separate experiments, there was a parallel decline of the ^{3}H and the ^{35}S radioactivity in DS-PG II, indicating a coupling of the degradation of the protein and the glycosaminoglycan moieties of the proteoglycan. Straight lines were obtained in semi-logarithmic plots. The calculated half-life times, however, varied considerably, between 62 h and 110 h. The only variable in the design of the experiment was the cell strain used. Fig. 7 provides the data of an experiment where a half-life of 90 h was obtained. In all experiments, 50% of the cell-associated ^{3}H radioactivity disappeared within 30–40 h.

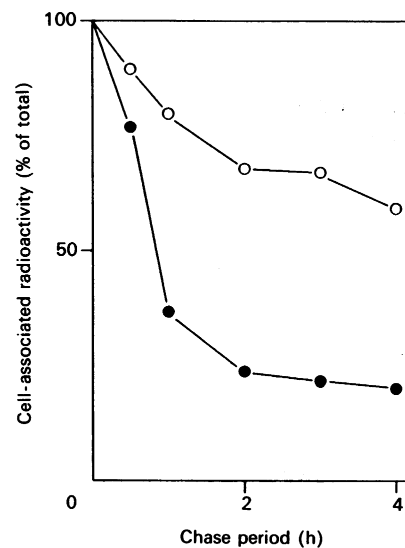

Fig. 6. Secretion of DS-PG II

Fibroblasts (2.5 × 10^{5} cells) were maintained either as a monolayer or in a collagen lattice for 4 days. The cultures were pulse-labelled for 15 min with 2 ml of medium containing 11 MBq of [^{3}H]leucine. The same medium was used sequentially for labelling the monolayer and the collagen-lattice cultures respectively. Chase media and the collagenase buffer contained 0.2 mM-leucine. The period of collagenase digestion (15 min at 45 °C) was included in the chase time. The distribution of DS-PG II at the end of the pulse-labelling period could not be determined, but it was supposed from previous experiments with monolayer cultures that all the labelled DS-PG II core protein would remain cell-associated during the pulse-labelling period (Glössl *et al.*, 1984). At the end of the chase period, DS-PG II was quantified in the culture medium, the collagenase buffer and the cell extract. Total DS-PG II radioactivity of collagen lattice cultures (○) was 1300–2050 c.p.m., and that of monolayer cultures (●) 22000–28000 c.p.m.

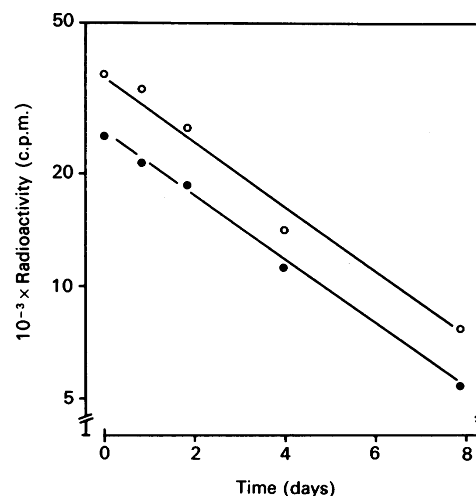

Fig. 7. Decay of DS-PG II-associated radioactivity: ○, ^{3}H; ●, ^{35}S

Fibroblasts (2.5 × 10^{5} cells) were cultured in a collagen lattice for 5 days before labelling in the presence of [^{3}H]leucine (1.5 MBq/ml) and [^{35}S]sulphate (0.37 MBq/ml) for 6 h. The cultures were then maintained in the chase medium containing 0.2 mM-leucine and 2 mM-Na_2SO_4 for the times indicated before collagenase treatment at 37 °C.

DISCUSSION

The results described in this paper provide a further example of the profound modulation of metabolism occurring upon an alteration of the extracellular matrix. In previous studies it had already been established that iduronic acid-rich dermatan sulphate proteoglycans accumulate within a collagenous extracellular matrix when skin fibroblasts (Gallagher *et al.*, 1983) or vascular smooth-muscle cells (Lark & Wight, 1986) are cultured on top of collagen gels. In the present study, fibroblasts were incorporated into the collagen lattice, thereby causing a shrinkage of the gel in all three dimensions. Embedded in this collagen lattice, fibroblasts produced sufficient amounts of DS-PG II to decorate the collagen fibrils. Although it is evident from the literature that the binding of DS-PG II to collagen is through its core protein (Vogel *et al.*, 1984), the presence of the dermatan sulphate chain seems to facilitate this interaction, as indicated by the results of the immunoassays.

It had never been possible to achieve a metabolic equilibrium between secretion and endocytosis of proteoglycans in monolayer cultures. We have shown previously that fibroblasts can ingest DS-PG II by receptor-mediated endocytosis, whereby the core protein serves as ligand of cell-membrane proteins (Glössl *et al.*, 1983; Hausser *et al.*, 1989). In the collagen lattice, a specific decline in the biosynthesis of DS-PG II was observed. This decreased synthesis is considered to be counterbalanced by a decreased rate of endocytosis, as judged by the relatively long half-life of DS-PG II of about 4 days. In monolayer cultures, interactions of the core protein with other core-protein-binding macromolecules, e.g. with type I collagen or core-protein-specific antibodies, were shown to interfere with the endocytosis of the proteoglycan (Schmidt *et al.*, 1990). Binding of DS-PG II to collagen fibrils would therefore be a means to decrease the need for DS-PG II biosynthesis. In accordance with this notion are the results of previous studies showing that there is only a very slow turnover of DS-PG II in bovine tendon, where the proteoglycan is also bound to collagen fibrils (Koob & Vogel, 1987). The mechanism of the down-regulation of the production of DS-PG II cannot be deduced from the present data. Mechanical confinement of the fibroblasts could represent one important factor, since DS-PG II biosynthesis was decreased to a similar extent upon culturing fibroblasts in an alginate gel and in a collagen lattice (Schlumberger *et al.*, 1989). For fibroblasts maintained in an alginate gel, the DS-PG II concentration in the vicinity of the cells should be lower than in the collagen lattice, since DS-PG II is not retained by alginate. A simple feedback mechanism between the extracellular proteoglycan concentration and the rate of proteoglycan biosynthesis therefore does not seem to operate.

A second noteworthy result of this study concerns the compositional changes of the dermatan sulphate chain that occur upon cultivation in a collagen lattice. In light of the retardation of the kinetics of secretion of the proteoglycan, it is tempting to speculate that there is a prolonged exposure to chain-elongating enzymes and to uronic acid C-5 epimerase. Unfortunately, the methods to degrade the collagen gel without destroying the cells are still not working rapidly enough for a sufficiently precise investigation of the kinetics of post-translational modifications during pulse–chase labelling experiments. Other possibilities which should be taken into consideration include changes in the level of the respective concentrations of nucleotide sugars and of phosphoadenylyl sulphate.

A further unresolved problem concerns the mechanism and the consequences of the proteoglycan accumulation within or at the surface of lattice-cultivated fibroblasts. It seems possible that fibroblasts could accumulate intracellularly a certain quantity of proteoglycan as a reservoir to cope with rapidly occurring needs when they are kept under tissue-like conditions. One could also speculate that this accumulation is a feature of the mechanisms leading to the down-regulation of DS-PG II biosynthesis. An enrichment of plasma-membrane-associated DS-PG II, on the other hand, could be expected if the distribution and/or the mobility of the endocytosis receptor would be altered in a cell surrounded by collagen fibrils.

In summary, the results described in this paper clearly demonstrate the useful application of fibroblast cultures in collagen lattices. It is not yet known how exactly the model resembles an authentic connective tissue with regard to the metabolism of DS-PG II. Nevertheless, the present model could be used for further studies on the kinetics of the interactions of different matrix components and on the factors affecting the metabolic equilibrium of these components.

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REFERENCES

Bell, E., Ivarsson, B. & Merrill, C. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 1274–1278
Bell, E., Ehrlich, H. P., Buttle, D. J. & Nakatsuji, T. (1981) Science **211**, 1052–1054
Bitter, T. & Muir, H. M. (1962) Anal. Biochem. **4**, 330–334
Bonner, W. M. & Laskey, R. A. (1974) Eur. J. Biochem. **46**, 83–88
Cantz, M., Kresse, H., Barton, R. W. & Neufeld, E. F. (1972) Methods Enzymol. **28**, 884–897
Engvall, E. & Perlmann, P. (1971) Immunochemistry **8**, 871–874
Frens, G. (1973) Nature (London) Phys. Sci. **241**, 20–22
Gallagher, J. T., Gasiunas, N. & Schor, S. L. (1983) Biochem. J. **215**, 107–116
Glössl, J., Schubert-Prinz, R., Gregory, J. D., Damle, S. P., von Figura, K. & Kresse, H. (1983) Biochem. J. **215**, 295–301
Glössl, J., Beck, M. & Kresse, H. (1984) J. Biol. Chem. **259**, 14144–14150
Greve, H., Cully, Z., Blumberg, P. & Kresse, H. (1988) J. Biol. Chem. **263**, 12886–12892
Hatamochi, A., Aumailley, M., Mauch, C., Chu, M.-L., Timpl, R. & Krieg, T. (1989) J. Biol. Chem. **264**, 3494–3499
Hausser, H., Hoppe, W., Rauch, U. & Kresse, H. (1989) Biochem. J. **263**, 137–142
Hedbom, E. & Heinegård, D. (1989) J. Biol. Chem. **264**, 6898–6905
Heinegård, D., Björne-Persson, A., Cöster, L., Franzén, A., Gardell, S., Malmström, A., Paulsson, M., Sandfalk, R. & Vogel, K. (1985) Biochem. J. **230**, 181–194
Hoppe, W., Glössl, J. & Kresse, H. (1985) Eur. J. Biochem. **152**, 91–97
Karnovsky, M. J. (1965) J. Cell Biol. **27**, 137A–138A
Koob, T. J. & Vogel, K. G. (1987) Biochem. J. **246**, 589–598
Laemmli, U. K. (1970) Nature (London) **227**, 680–685
Lark, M. W. & Wight, T. N. (1986) Arteriosclerosis **6**, 638–650
Luft, J. H. (1961) J. Biophys. Cytol. **9**, 409–414
Mauch, C., Hatamochi, A., Scharfetter, K. & Krieg, T. (1988) Exp. Cell Res. **178**, 493–503
Nusgens, B., Merrill, C., Lapiere, C. & Bell, E. (1984) Collagen Relat. Res. **4**, 351–364
Paye, M., Nusgens, B. V. & Lapiere, C. M. (1987) Eur. J. Cell Biol. **45**, 44–50
Rauch, U., Glössl, J. & Kresse, H. (1986) Biochem. J. **238**, 465–474
Rosenberg, L. C., Choi, H. U., Tang, L.-H., Johnson, T. L., Pal, S., Webber, C., Reiner, A. & Poole, A. R. (1985) J. Biol. Chem. **260**, 6304–6313
Roth, J., Bendayan, M. & Orci, L. (1982) in Techniques in Immunocytochemistry (Bullock, G. R. & Petrusz, P., eds.), pp. 107–133, Academic Press, London
Schlumberger, W., Thie, M., Rauterberg, J., Kresse, H. & Robenek, H. (1989) Eur. J. Cell Biol. **50**, 100–110
Schmidt, G., Robenek, H., Harrach, B., Glössl, J., Nolte, V., Hörmann, H., Richter, H. & Kresse, H. (1987) J. Cell Biol. **104**, 1683–1691
Schmidt, G., Hausser, H. & Kresse, H. (1990) Biochem. J. **266**, 591–595
Scott, J. E. & Haigh, M. (1985) Biosci. Rep. **5**, 71–81
Scott, J. E. & Orford, C. R. (1981) Biochem. J. **197**, 213–216

Shively, J. W. & Conrad, H. E. (1976) Biochemistry **15**, 3932–3942
Tomasek, J. J., Hay, E. D. & Fujiwara, K. (1982) Dev. Biol. **92**, 107–122
Uldbjerg, N. & Danielsen, C. C. (1988) Biochem. J. **251**, 643–648
Vogel, K. G., Paulsson, M. & Heinegård, D. (1984) Biochem. J. **223**, 587–597
Voss, B., Glössl, J., Cully, Z. & Kresse, H. (1986) J. Histochem. Cytochem. **34**, 619–625
Yamaguchi, Y. & Ruoslahti, E. (1988) Nature (London) **336**, 244–246
Zebrower, M. E., Kieras, F. J. & Brown, W. T. (1986) Anal. Biochem. **157**, 93–99

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