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A biochemical analysis of human periodontal tissue proteoglycans

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Proteoglycans synthesized by periodontal (gingival, periodontal ligament, dental follicle) fibroblasts were analysed by SDS/polyacrylamide and agarose gel electrophoresis after being labelled with radioactive sulphate. Medium, cell membrane and extracellular matrix fractions were analysed separately. Samples were treated with chondroitinase AC, chondroitinase ABC, heparitinase or a combination of chondroitinase ABC and heparitinase before electrophoretic separation of proteoglycans. Antibodies to versican and decorin were used to identify these molecules by Western immunoblots. For steady-state metabolic radiolabelling of fibroblasts, medium and cell membrane fractions contained about equal proportions of radiolabelled proteoglycans (about 43%), whereas less radioactivity (about 14%) was found in proteoglycans of the matrix fraction. Periodontal fibroblasts produce six major proteoglycans: versican, a high-molecular-mass chondroitin sulphate proteoglycan (CSPG); decorin, a dermatan sulphate proteoglycan (DSPG); a membrane-associated heparan sulphate proteoglycan (HSPG); two medium- or matrix-associated HSPGs; and a 91 kDa membrane-associated CSPG. Variation in decorin molecular size was observed in mass cultures of fibroblasts. Similar polydispersity in molecular size of decorin was seen in several clones established from one mass culture.

INTRODUCTION

Connective-tissue matrix is composed of several extracellular-matrix (ECM) macromolecules such as collagens, elastin, fibronectin, hyaluronan and proteoglycans (PGs) (Reddi, 1984). PGs are glycoconjugates containing a protein core in which Ser-Gly sequences serve as attachment sites to one or more glycosaminoglycan (GAG) side chains (Gallagher *et al.*, 1986; Hassell *et al.*, 1986; Poole, 1986; Ruoslahti, 1988, 1989; reviewed by Gallagher, 1989; Wight, 1989). GAGs vary in molecular size, nature, epimerization and sulphation within the same PG. PGs have a number of functions in the ECM and on the cell surface. They play a role in connective tissue matrix formation, growth regulation, cell adhesion, as cell surface matrix receptors, and they also bind certain growth factors (Ruoslahti, 1989).

The principal PGs of soft connective tissue have recently been characterized through cDNA cloning of the core protein sequences. Decorin (previously known as PGII) and biglycan (PGI) are two closely related PGs with apparent molecular masses of 120 and 200 kDa respectively and protein cores of 45 kDa with clear differences in their *N*-terminal sequences. Decorin contains one GAG chain whereas two GAG chains may be attached to biglycan (Krusius & Ruoslahti, 1986; Fisher *et al.*, 1989). A complete sequence for the large chondroitin sulphate proteoglycan (CSPG), versican, synthesized by fibroblasts has been published (Zimmermann & Ruoslahti, 1989). The molecule contains about 10–12 GAG chains attached to a large core protein which also contains several functional domains such as lectin-like and epidermal growth factor-like domains.

Periodontal connective tissues (gingiva and periodontal ligament) form a supporting system which links human teeth to the surrounding bone (Birkedal-Hansen, 1982). Several PGs have been isolated from human and bovine periodontal tissues (reviewed by Larjava, 1984; Bartold, 1987; Rahemtulla, 1992). Human gingival fibroblasts have been shown to synthesize several types of PG, but these PGs have not been fully characterized. We have demonstrated that gingival fibroblasts secrete into the culture medium decorin which is larger than its counterpart synthesized by skin fibroblasts, and this difference is due to the larger GAG chains (Larjava *et al.*, 1988). In order to gain further information on the PGs synthesized by periodontal cells, we have carried out an extensive investigation of newly synthesized PGs by several fibroblast cell lines obtained from healthy human periodontium. In addition, we have studied the distribution of PGs in different culture compartments (medium, cell membranes, ECM). The results show that human periodontal fibroblasts express at least six PGs: decorin, which is found exclusively in the medium; heparan sulphate proteoglycan (HSPG) and a 91 kDa CSPG-like molecule which are present in the cell membrane fraction; and two large HSPGs which are present only in the fibroblast ECM extracts. The large CSPG, versican, is shared by the various culture compartments.

MATERIALS AND METHODS

Materials

Benzamidine hydrochloride, phenylmethanesulphonyl fluoride, *N*-ethylmaleimide, trypsin and EDTA were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Carrier-free [^{35}S]sulphate was obtained from Amersham (Arlington Heights, IL, U.S.A.). Cell culture plastic ware was from Falcon (Oxnard, CA, U.S.A.) and cell culture media and other cell culture chemicals were from Gibco Chemical Co. (Grand Island, NY, U.S.A.). Chondroitinases ABC, AC, heparitinase and secondary antibodies were purchased from ICN Biomedicals (Costa Mesa, CA, U.S.A.). Kodak X-Omat AR films were from Kodak Eastman Co. (Rochester, NY, U.S.A.). Fluoro-Hance and Safety-Solve were obtained from Research Product International Corp. (Mount Prospect, IL, U.S.A.). Zeta-Probe membrane and chemicals for agarose-gel electrophoresis (and SDS/PAGE) were from Bio-Rad (Richmond, CA, U.S.A.). All other chemicals were of reagent grade and were purchased from Sigma Chemical Co. or Fisher Scientific Co. (Springfield, NJ, U.S.A.).

Abbreviations used: ECM, extracellular matrix; GAG, glycosaminoglycan; PG, proteoglycan; CSPG, chondroitin sulphate proteoglycan; HSPG, heparan sulphate proteoglycan; FCS, fetal calf serum; PBS, phosphate-buffered saline; CMF, cell membrane fraction.

Table 1. Distribution of [^{35}S]sulphate label in medium, CMF and ECM

Periodontal fibroblasts were seeded at equal densities and cultured for 72 h, after which the cells were radiolabelled with 50 μCi of [^{35}S]sulphate/ml for 48 h. Radioactivity in the non-dialysable medium fraction, 2% Triton X-100 extract (CMF) and 2% SDS extract of the remaining matrix (ECM) was determined (for details see the Materials and methods section). Results ± S.D. from nine different periodontal cell lines are shown.

Fraction	10^{-6} × Radioactivity (c.p.m./flask)	Percentage distribution
Medium	1.83 ± 0.80	42.6 ± 5.9
CMF	1.95 ± 1.27	43.0 ± 11.8
ECM	0.54 ± 0.32	14.4 ± 7.5

Tissue culture

Gingival fibroblast cultures were established from healthy gingiva using the explant technique, a method previously used for establishment and culture of gingival fibroblasts (Larjava *et al.*, 1988). Periodontal ligament fibroblast cell lines were established by explanting periodontal membrane tissue from extracted teeth as previously described (Rose *et al.*, 1987), and dental follicle fibroblasts were established from the follicle surrounding the crown of an unerupted tooth. Cells were routinely cultured in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 1 mM-sodium pyruvate, antibiotics (50 μg of streptomycin/ml, 50 i.u. of penicillin/ml) and 10% heat-inactivated fetal calf serum (FCS). Twelve different cell lines obtained from passages 5–15 were used in the present study.

A normal human gingival fibroblast culture was used as a source for fibroblast clones. The cells were cloned at the fourth passage using either limited dilution or cloning ring techniques (Freshney, 1983) in Opti-MEM 1 supplemented with 10% FCS. The best growing clones were cultured for further analysis. Seven clones (passages 6–7) were selected for the analysis of PG synthesis. The cell morphology of the clones was evaluated by light microscopy after staining.

Labelling and gel electrophoresis of PGs

Fibroblasts were seeded at equal cell densities (30000/cm^2), and cultured for 72 h. Before radiolabelling, the cells were rinsed once with phosphate-buffered saline (PBS) and Opti-MEM 1 medium without serum and containing 50 μCi of [^{35}S]sulphate/ml was added to the cells. Cells were labelled to steady state for 48 h and then processed for analyses of PG expression.

Proteinase inhibitors (1:20 of a solution containing 0.5 M-EDTA, 40 mM-benzamidine hydrochloride and 0.4 M-*N*-ethylmaleimide, and 1:200 of 0.6 M-phenylmethanesulphonyl fluoride solubilized in methanol) were added to the media and the mixture was extensively dialysed against cold distilled water, freeze-dried and stored until further analyses. The cell layer was

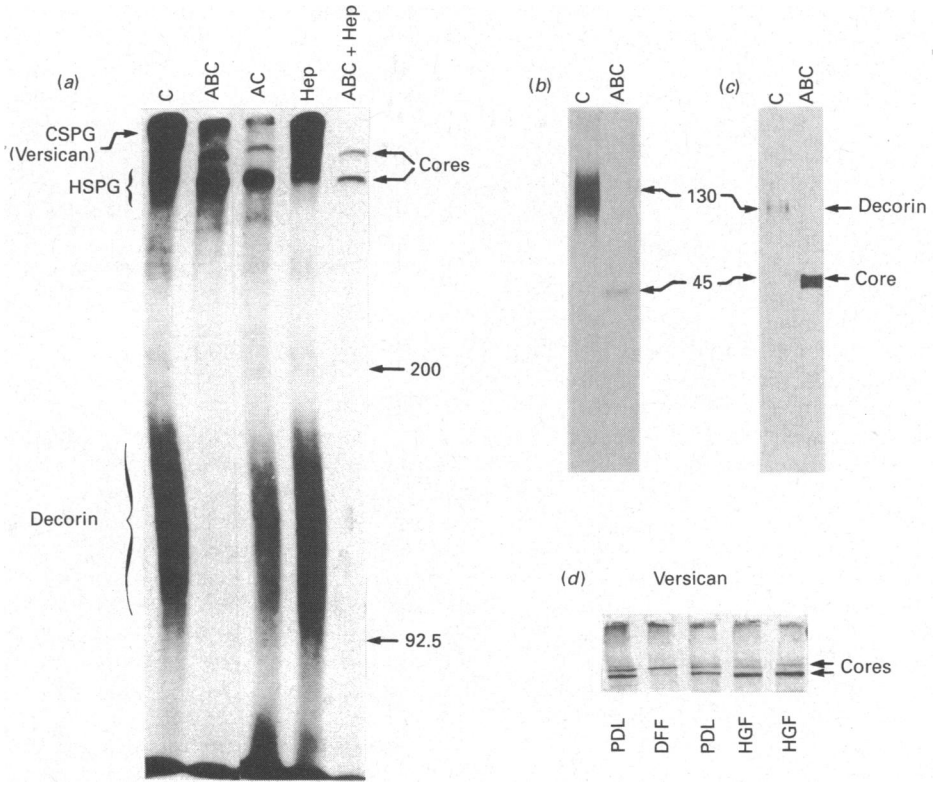

Fig. 1. Analysis of medium PGs

(*a*) SDS/PAGE (5% resolving gel) runs of $^{35}SO_4$-labelled medium PGs expressed by human gingival fibroblasts. Cells were labelled for 48 h in Opti-MEM and the medium was extensively dialysed in the presence of proteinase inhibitors. Portions of the samples were treated with the control buffer alone (C), chondroitinase ABC (ABC), chondroitinase AC (AC), heparitinase (Hep) or with a combination of chondroitinase ABC and heparitinase. Molecular-mass markers (kDa) are indicated on the right, and the positions of versican, HSPG and decorin on the left. Versican core proteins visible after chondroitinase treatments are marked on the right. (*b*) Treatment of immunoprecipitated decorin with control buffer (C) and chondroitinase ABC (ABC). (*c*) Western immunoblots of decorin before (C) and after chondroitinase ABC (ABC) treatment. (*d*) Western immunoblots of versican in periodontal ligament (PDL), dental follicle (DFF) and human gingival (HGF) fibroblast medium fractions. Samples were treated with chondroitinase ABC before electrophoresis.

washed several times with a large volume of PBS, and the cell membrane fraction (CMF) was extracted on ice for 10 min with 2% Triton X-100 in PBS containing the same dilution of the stock proteinase inhibitors as indicated above. The remaining ECM was rinsed several times with PBS and extracted by scraping with a rubber policeman into 10 mM-sodium phosphate buffer, pH 7.0, containing 2% SDS, 10% glycerol, 0.003% Bromophenol Blue (SDS buffer). In some experiments the ECM was treated with either chondroitinase ABC or heparitinase (see below) before extraction with the SDS buffer.

Media samples, CMF and ECM samples were treated with the following specific enzymes to characterize the various radiolabelled PGs: chondroitinase AC (1 unit/ml), chondroitinase ABC (1 unit/ml) and heparitinase (20 units/ml). The samples and the enzymes were dissolved in 50 mM-Tris/HCl and 30 mM-sodium acetate, pH 7.4, and the mixture was incubated overnight at 37 °C. Enzyme digestions were performed in the presence of proteinase inhibitors, which were added at the concentrations mentioned above. After digestion, the samples were electrophoresed either on SDS/polyacrylamide gels (Laemmli, 1970) with 4% stacking and 5 or 7.5% resolving gels, or on 0.75% agarose gels (Säämänen *et al.*, 1989). The SDS/polyacrylamide gels were fixed, enhanced, dried and exposed to X-ray films for autoradiography. Agarose gels were air-dried and directly exposed to X-Omat autoradiography film. Standard PGs for agarose-gel electrophoresis were prepared from bovine sclera (versican, decorin) using DEAE-cellulose chromatography, caesium chloride density-gradient centrifugation and molecular-sieve chromatography (Cöster & Fransson, 1981). Biglycan was a gift from Dr. Annukka Säämänen (University of Kuopio, Kuopio, Finland). PG standards were stained with 0.02% Toluidine Blue in 3% acetic acid (Säämänen *et al.*, 1989).

Western immunoblotting and immunoprecipitation

PGs after SDS/PAGE and agarose-gel electrophoresis were electrotransferred to Zeta Probe membranes, and the filters were blocked overnight with 1% non-fat dry milk in PBS at room temperature. Immunodetection was performed using polyclonal antipeptide antibodies to decorin (Krusius & Ruoslahti, 1986) and monoclonal antibodies to versican (Rahemtulla *et al.*, 1988; Takagi *et al.*, 1990) at a dilution of 1:200 in PBS containing 1% non-fat dry milk. After 2 h, the filters were rinsed several times with PBS, and 1:500 dilutions of horseradish peroxidase-conjugated anti-rabbit or anti-mouse immunoglobulins were added and the filters incubated for 2 h at room temperature. After several washes with PBS, the filters were developed with diaminobenzidine as substrate.

Immunoprecipitation of decorin was performed exactly as described previously (Larjava *et al.*, 1988), with peptide antibodies to decorin (Krusius & Ruoslahti, 1986). Immunoprecipitates were treated with control buffer alone or with chondroitinase ABC as described above before electrophoretic fractionation.

RESULTS

Analysis of radiolabelled PGs by SDS/PAGE

In the present study, the PGs were labelled in serum-free Opti-MeM 1 containing high sulphate concentrations. This technique resulted in good labelling and the incorporation of radioactive sulphate was equal to conditions when 10% FCS is used in the culture medium without compromising the well-being of the cultures, a phenomenon sometimes seen with some cell lines when cells are labelled in serum-depleted conditions. Sulphate-containing labelling medium also permits epimerization of glucuronic acid to iduronic acid at the normal rate (Silbert *et al.*, 1986). When periodontal fibroblasts, seeded at equal densities, were radiolabelled at steady state with [^{35}S]sulphate, approximately equal amounts of radiolabelled PGs (43%) were found in

Table 2. Distribution of [^{35}S]sulphate label in various PGs of different cell culture compartments

Cells were radiolabelled as described briefly in Table 1 and in detail in the Materials and methods section. PGs were electrophoresed on both SDS/PAGE polyacrylamide and agarose gels and identified by treatment with specific glycosidases and immunodetection. Autoradiograms were analysed by densitometry. Percentage distribution is given for each PG type in all fractions. The sum of each fraction is 100%. The estimation of percentage distribution of the total radiolabel in these cultures is based on data from Table 1.

Fraction	Percentage of different PGs in each fraction	Percentage of total label in culture
Medium (clones)		
Decorin	55	–
Versican + HSPGs	45	–
Medium (mass cultures)		
Decorin	48	21
Versican	26	11
HSPGs	26	11
CMF		
91 kDa PG	8	4
Versican	19	8
HSPGs	73	31
ECM		
Versican	42	6
HSPGs	58	8

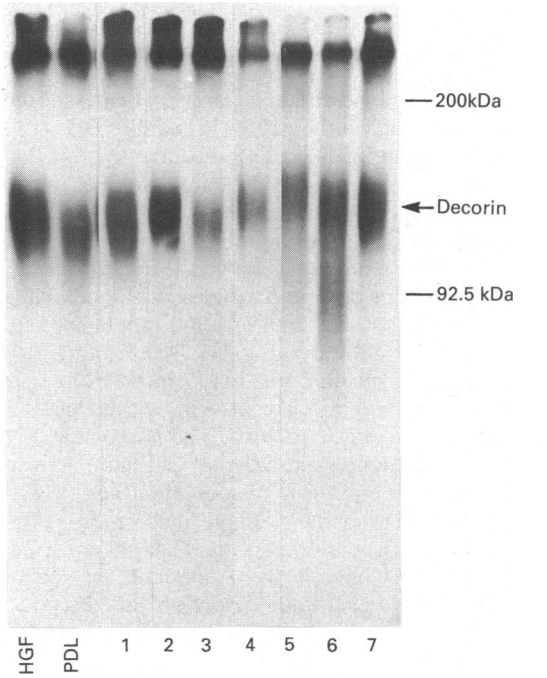

Fig. 2. Heterogeneity of decorin synthesized by cloned gingival fibroblasts

Seven clones (numbers 1 to 7) were labelled with radioactive sulphate for 48 h after which medium samples were analysed by SDS/PAGE (7.5% resolving gel). The positions of the molecular-mass markers and decorin are shown on the right. PGs produced by mass cultures of human gingival fibroblasts (HGF) and periodontal ligament fibroblasts (PDL) are shown on the left for comparison.

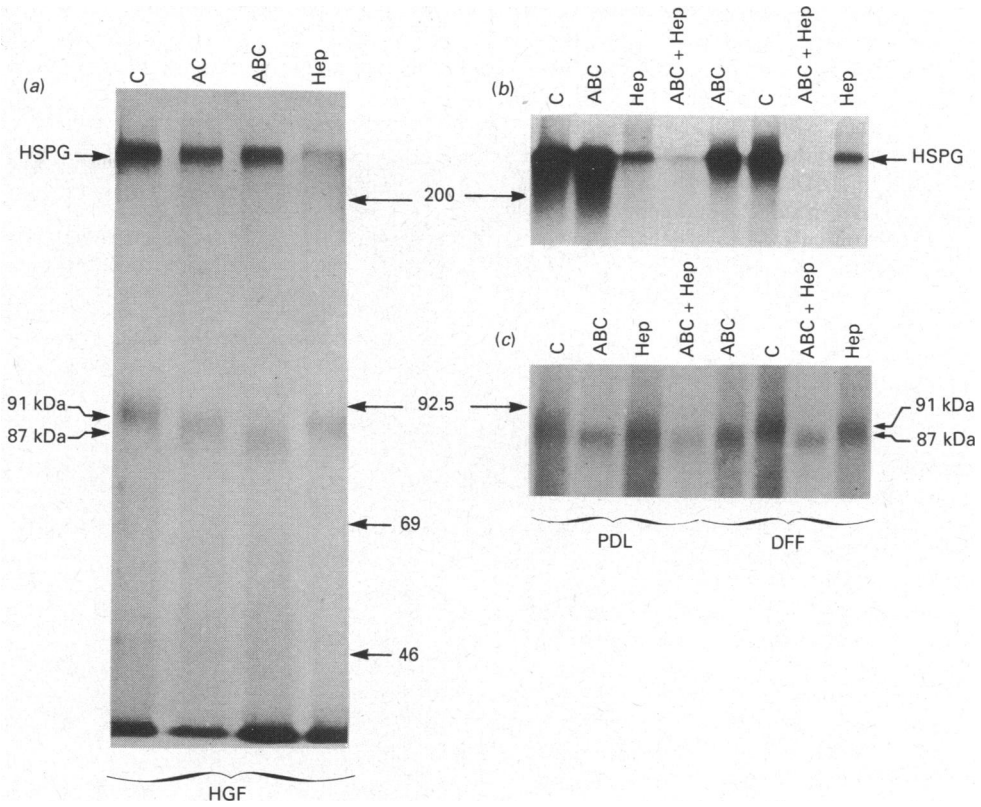

Fig. 3. CMF PGs of periodontal fibroblasts

Cells were labelled with $^{35}SO_4$-containing Opti-MEM for 48 h, after which the medium was removed. After several washes with PBS, the cell membranes were extracted for 10 min on ice with 2% Triton X-100 in PBS containing a mixture of proteinase inhibitors. CMF PGs were analysed by SDS/PAGE. Analyses of three different periodontal fibroblast lines are shown: (*a*) human gingival fibroblasts (HGF); (*b*), (*c*) periodontal ligament fibroblasts (PDL) and dental follicle fibroblasts (DFF). Molecular-mass markers (kDa) are indicated by arrows. Extracts were treated with control buffer alone (C), chondroitinase AC (AC), chondroitinase ABC (ABC), heparitinase (Hep), or with a combination of chondroitinase ABC and heparitinase. Major PGs and various forms of 91 kDa PGs are indicated with arrows.

the medium and membrane fractions, whereas the matrix fraction contained significantly less (14%) (Table 1).

The medium fraction. The medium fraction of periodontal fibroblasts labelled to steady state contained two major radiolabelled components, when analysed by SDS/PAGE: one band with an electrophoretic migration of 130 kDa and another wide band which did not enter the gels (Fig. 1*a*). The 130 kDa band was identified as decorin on the basis of its mobility in the gels, sensitivity to chondroitinase ABC, immunoprecipitation (Fig. 1*b*) and immunoblotting (Fig. 1*c*). Decorin comprised about half the PGs in the medium fraction (Table 2). The upper band was reduced to two large core proteins when digested with either chondroitinase AC II or ABC. The proportion of the two core proteins was different in different cell lines. This large PG was identified as versican by immunoblotting (Fig. 1*d*) and by comparing the properties with previously published data (Krusius *et al.*, 1987). In addition to the versican molecules, the upper band also contained a small amount of HSPG which was digestible with heparitinase. No clear differences in PG profiles were seen between gingival and periodontal ligament fibroblast cell lines.

Immunoprecipitation analyses of decorin revealed polydispersity among several of the cell lines studied, as observed earlier (Larjava *et al.*, 1988). We have analysed PGs of ten different periodontal cell lines by electrophoresis of labelled media samples on SDS/polyacrylamide gels followed by autoradiography. In all cases we have observed that decorin showed slightly varying molecular mass, the difference between the highest and the lowest being approx. 20 kDa. We then tested the hypothesis that this phenomenon is related to the clonal selection of fibroblast cultures. One normal gingival fibroblast mass culture was cloned and the seven best growing clones were cultured further. The clones differed in cell morphology, some being epitheloid in shape whereas others were spindle-shaped or stellate types. The clones were labelled with [^{35}S]sulphate, and media samples were analysed by SDS/PAGE. All these cell lines synthesized and secreted decorins that were of variable molecular mass. The relative molecular size of the decorin was variable and the molecular mass ranged over about 20 kDa (Fig. 2).

Cell membrane fraction. The cell membrane fraction, which had been extracted with 2% Triton X100, contained one major high-molecular-mass (about 250–300 kDa) sulphate-labelled band which did not enter the resolving gel (Fig. 3*a* and 3*b*). Treatment of this band with heparitinase released approximately 73% of the total radiolabel (Table 2). This result implies that this fraction consisted of a high-molecular-mass PG bearing HS chains. A small amount of the radiolabel remaining in the top band was resistant to heparitinase treatment but was digested by the combined treatment and chondroitinase ABC. This material was later shown to be versican associated with the membranes (see Fig. 5). All the cell lines studied contained very similar PGs in the CMF (Fig. 3*a* and 3*b*).

Longer exposures of CMF samples to X-ray films revealed an additional radiolabelled band which migrated into the SDS/polyacrylamide gel, with a relative molecular size of 91 kDa (Fig. 3*a* and 3*b*). This fraction was partially characterized by digestion

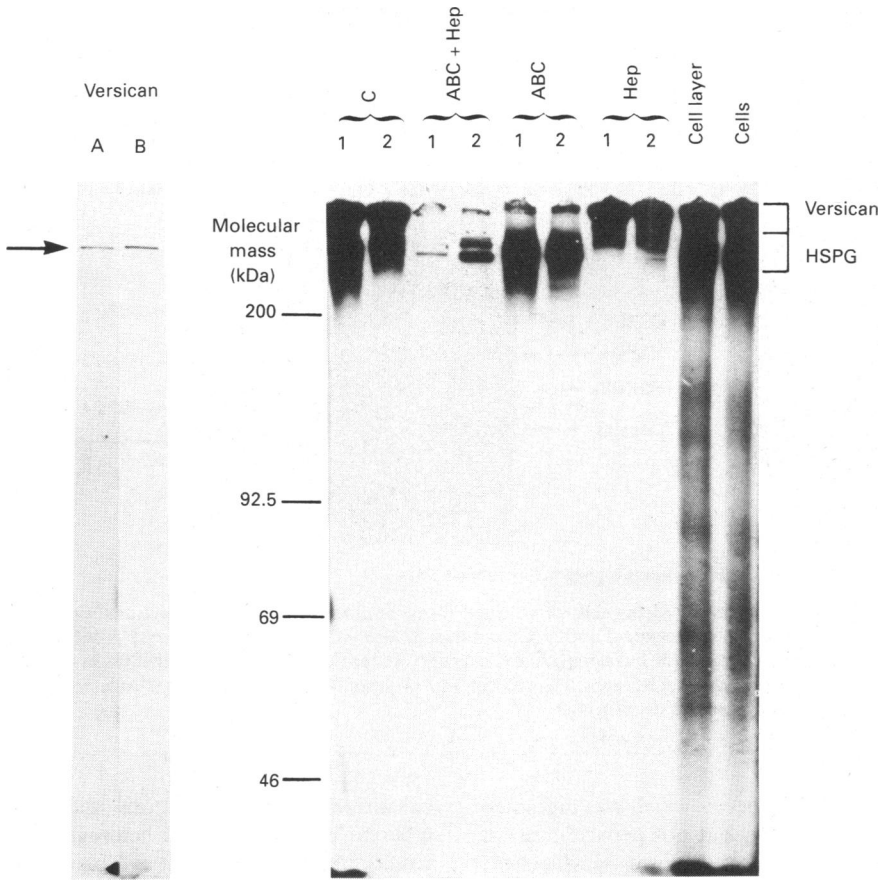

Fig. 4. ECM PGs of periodontal fibroblasts

Cells were first labelled for 48 h with $^{35}SO_4$, after which the medium and CMF were removed. After several washes with PBS, ECM proteoglycans were treated *in situ* with control buffer alone (C), chondroitinase ABC (ABC), heparitinase (Hep) or both chondroitinase ABC and heparitinase. After digestion, PGs in the matrix (1) and those released during the incubation (2) were analysed by SDS/PAGE using 7.5% resolving gels. For comparison, trypsin-treated cells (cells) and the whole cell layer containing both ECM and CMF (cell layer) were run. Samples of the combination treatments (ABC + Hep) were also analysed by Western blots (B) using monoclonal antibodies against versican and the blot was autoradiographed (A).

with the specific glycosidases. This material was resistant to heparitinase but was susceptible to chondroitinase ABC or chondroitinase AC and contained about 8% of the total radioactivity in the membrane fraction (Table 2). After the enzyme digestion, most of the radioactivity was lost and the remaining material migrated further into the gel, with a relative mobility 3–4 kDa less than the non-enzyme-treated sample. On the basis of the observation that this fraction is susceptible to chondroitinase ABC and chondroitinase AC in the presence of proteinase inhibitors, we propose that it is a CSPG. The 91 kDa fraction obtained from all the different cell lines (gingival, periodontal ligament and dental follicle) was partially sensitive to chondroitinase ABC but not to heparitinase (Fig. 3*a* and 3*c*).

ECM fraction. ECM PGs were studied after first removing the CMF by 2% Triton X-100 treatment and rinsing with PBS. The remaining ECM was treated *in situ* with buffer alone, chondroitinase ABC, heparitinase or both (Fig. 4). In electrophoretic separation of ECM PGs, the radiolabelled material was localized in one major area at the top of the resolving gel (Fig. 4). The lower part of this material was degraded by heparitinase treatment and the upper part was digested by chondroitinase ABC. The material released by the control buffer alone was found to contain high-molecular-mass PGs probably weakly associated with the ECM (Fig. 4, lane C2). Chondroitinase ABC and combined treatments revealed two large core proteins (Fig. 4, lane ABC+Hep 2) similar to those seen in the medium fraction (Fig. 1*a*). They were identified as versican core proteins by Western blotting (Fig. 4, lane B). HSPG in the ECM fraction contained slightly more radioactivity than versican (Table 2).

We also analysed the PG patterns of the whole cell layer and trypsin-treated cells by SDS/PAGE. The results of these experiments indicate that about 7% of the label within the cell layer was associated with the trypsin-treated cells presumably representing an intracellular pool of PGs and cell surface trypsin-insensitive PGs. Several high-molecular-mass bands were also seen.

Analysis of PGs by agarose-gel electrophoresis

All the fractions studied contained very large PGs which were not properly resolved by SDS/PAGE. We therefore analysed them by 0.75% agarose-gel electrophoresis which separates PGs with a molecular mass higher than that of decorin (Fig. 5).

Using this technique, we found that the CMF contained three major radiolabelled bands. The lower band was identified as free GAG chains of mainly DS type on the basis of its similar electrophoretic behaviour to GAG standards and sensitivity to chondroitinase ABC. The major wide band contained HSPGs which were sensitive to heparitinase but not to chondroitinase. These membrane HSPGs were slightly different in size from those found in the matrix and medium (HSPG 1+2). The CMF

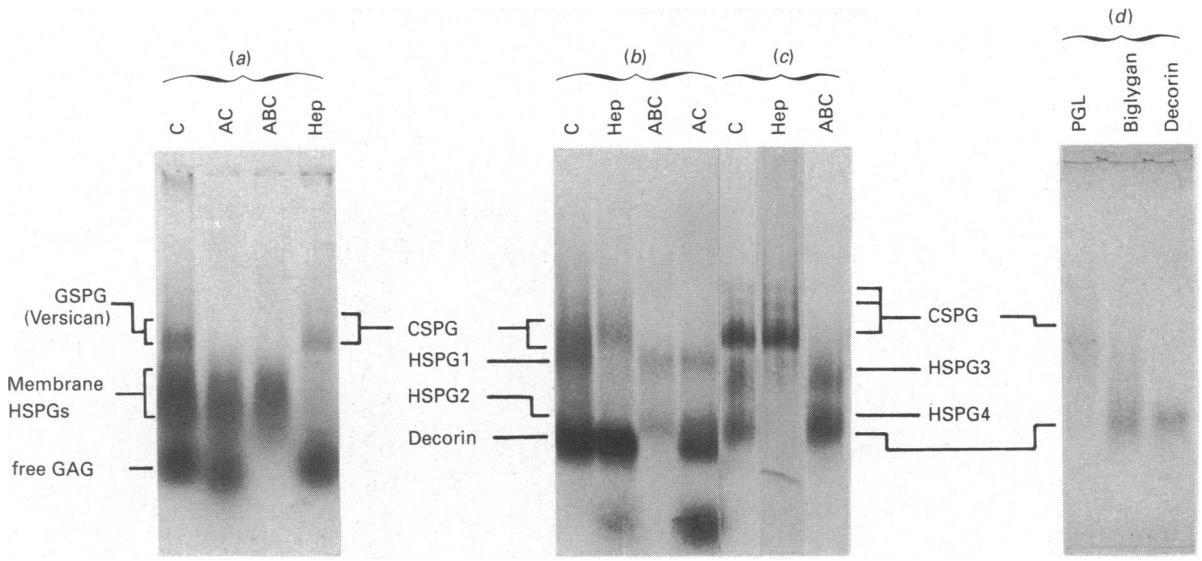

Fig. 5. Agarose-gel electrophoresis (0.75% gels) of human gingival fibroblast PGs

Cells were labelled with [^{35}S]sulphate for 48 h and the cell membrane (*a*), medium (*b*) and ECM (*c*) fractions were isolated as described in the Materials and methods section. Treatments with control buffer (C), chondroitinase AC (AC), chondroitinase ABC (ABC) and heparitinase (Hep) are shown. Bands that are chondroitinase-resistant but heparitinase-sensitive are marked as membrane HSPGs in (*a*), HSPG1 and HSPG2 in (*b*) and HSPG3 and HSPG4 in (*c*). Chondroitinase ABC-sensitive decorin and versican (CSPG) are indicated together with standard proteoglycans stained with Toluidine Blue (PGL, biglycan and decorin) (*d*).

also contained a very large versican species which was digestible by both chondroitinase AC and ABC but not heparitinase. In this area two bands were frequently seen, the lower of which was always the predominant band in the CMF. These bands were tentatively identified as versican because (1) they were chondroitinase-sensitive, (2) antibodies against the large versican reacted with bands in this area in Western immunoblots, and (3) the versican standard migrated to the same position.

The medium fraction contained four clearly distinct radiolabelled bands. They were identified as versican, HSPGs (1+2) and decorin on the basis of their sensitivity to enzymic degradation by chondroitinases and heparitinase.

The ECM fraction was composed of three major bands of which versican predominated (Fig. 5). Two minor bands, which were also chondroitinase ABC-digestible, were seen on SDS/PAGE. These bands migrated into the gels more slowly than the versican (Fig. 5). Two HSPGs (3+4) were clearly detectable on the autoradiographs. These bands were chondroitinase ABC-resistant but heparitinase-sensitive.

DISCUSSION

Several previous investigations have demonstrated the presence of CS/DSPGs and HSPGs in gingiva and periodontal ligament, but, their characterization has been rather limited (Bartold, 1987). In the present report, we have attempted to prepare an inventory of the PGs expressed by several fibroblast cell lines of periodontal tissues. Our data show that the PGs synthesized by these fibroblasts are very similar to those found in skin fibroblast cultures (Schmidtchen *et al.*, 1990). There are, however, some differences, suggesting a tissue-specific expression of the various types of PG.

When periodontal fibroblasts were labelled to steady state at equal cell densities, two major PGs, decorin and versican, were found in cell culture secretions. Decorin is the major type expressed by skin and other fibroblasts in culture (Glössl *et al.*, 1984; Rauch *et al.*, 1986). Media from several periodontal fibroblast cell lines were analysed and decorin was found to constitute 40–60% of the total radiolabelled PG. Decorin from different cell lines showed heterogeneity in its molecular mass, confirming previous results. This has been attributed to the difference in the DS chain length (Rauch *et al.*, 1986; Larjava *et al.*, 1988). It is well established that periodontal fibroblasts are composed of clones of cells that differ in their proliferative potential, production of ECM components, and in their response to exogenous agents (reviewed by Schor & Schor, 1987; Larjava, 1987). In order to elucidate whether the molecular heterogeneity of decorin observed in the different cell lines was due to clonal selection of the cell lines, several clones were established from a single mass culture and the synthesis of decorin was studied. Among the clones examined, some 20 kDa difference in decorin molecular mass was found. It is obvious therefore that the difference in decorin molecular mass in mass cultures of fibroblasts is based on the clonal selection of fibroblasts during cell culture. It does not, however, rule out the existence of tissue-specific differences in decorin expression.

Decorin was only found in the cell culture medium in this study. Previously it has been shown that decorin co-localizes with fibronectin fibrils in the ECM of fibroblasts (Schmidt *et al.*, 1987). It is possible that a small amount of decorin is also present in our cell culture system but that it was not detectable by the radiolabelling techniques used in this study. Interaction between decorin and fibronectin could play a role in cell adhesion by inhibiting fibronectin-mediated cell attachment (Rosenberg *et al.*, 1986; Ruoslahti, 1989).

Biglycan was not found in gingival fibroblast cultures. This observation is consistent with other studies where the absence of biglycan was noted in the periodontal tissue cells (Fisher *et al.*, 1989). However, biglycan expression can be up-regulated in gingival fibroblasts by transforming growth factor-β (Kähäri *et al.*, 1991). In other human tissues such as cartilage, biglycan expression is high in the fetal but low in adult tissues (Melching & Roughley, 1989). If this is the case for periodontal tissues, then it is not surprising to find low or undetectable expression of biglycan in our cell lines which originated from tissue obtained from adult donors.

Versican is the large CSPG produced by fibroblasts and proposed to have affinity with other cell surface and ECM components (Ruoslahti, 1989). Our studies have demonstrated that periodontal fibroblasts synthesize at least two versican species which differ in molecular size. At present we do not have any information on whether the two large CS bands in SDS/polyacrylamide and agarose gels are products of different mRNAs expressed by fibroblasts (Krusius *et al.*, 1987) or differently glycosylated forms of the same core, as is the case for decorin (Rauch *et al.*, 1986; Larjava *et al.*, 1988). The major species of versican in both medium and matrix fractions had similar molecular mass to the related large PG isolated from bovine sclera with an estimated molecular mass of 1.2 million Da. Interestingly, in agarose gels two minor CSPG forms were detectable in the ECM fraction. It is probable that these molecules present differently glycosylated forms of versican. Variable glycosylation could modify the affinity of the various active domains present on the versican molecule (Ruoslahti, 1989). It is also unclear why the expression of the two versican species present in the medium fraction was variable, nor do we know whether these two forms possess different functional properties.

Multiple HSPGs are found on the fibroblast cell surface and in the ECM (Gallagher *et al.*, 1986; Heremans *et al.*, 1989, 1990; Lories *et al.*, 1989; Märynen *et al.*, 1989; Schmidtchen *et al.*, 1990). In the present report we found HSPGs associated with the cell membranes and an additional two to four species that were associated with the medium and ECM compartment. All these HSPGs were large, over 200 kDa, and could be separated only by agarose-gel electrophoresis. The multiple forms of small HSPGs found in skin fibroblast cultures were not found by our analyses. The techniques used in the present investigation were, however, unlikely to detect small HSPGs. Some but not all of the small molecular forms may be degradation products. Recently, a low-molecular-mass HSPG has been cloned and called glypican. This 64 kDa proteoglycan is anchored in the membrane by a hydrophobic interaction of the diacylglycerol moiety of inositol phospholipid (David *et al.*, 1990).

Another fibroblast cell-membrane-associated HSPG (fibroblast syndecan) has also been cloned (Märynen *et al.*, 1989). Its transmembrane and cytoplasmic domains are homologous to the classical syndecan of mouse mammary epithelial cells (Mali *et al.*, 1990). The extracellular domain is, however, completely different. It is conceivable that at least one of the two membrane HSPGs found in the periodontal fibroblasts is related to this syndecan molecule. The two ECM HSPGs synthesized by periodontal fibroblasts were large, between 300 kDa and 10^6 kDa. The largest one may be the HSPG with 400 kDa core protein characterized in the skin fibroblast ECM (Heremans *et al.*, 1989, 1990) which was shown to be similar to the basement membrane low-density HSPG, perlecan (Hassell *et al.*, 1986).

In the membrane fraction we also found a CSPG with a molecular mass of about 91 kDa. It incorporated radiolabelled sulphate and was decreased by about 3–4 kDa in relative molecular size by chondroitinase treatment in the presence of a mixture of proteinase inhibitors. Therefore it is unlikely that this decrease was caused by proteinases present in the incubation mixture. This 91 kDa CSPG could be related to the small CSPG found in lung and skin fibroblasts (David *et al.*, 1989; Schmidtchen *et al.*, 1990). Both are of similar size and are only found in the detergent extracts of cells. Furthermore, all PGs of this size may be related to the new PG family member, CD44, also called Hermes antigen or ECM receptor III (Jalkanen *et al.*, 1988, 1992; Gallatin *et al.*, 1989; Picker *et al.*, 1989). In other studies (H. Larjava, L. Häkkinen & L. Koivisto, unpublished work), we have observed that CD44 is synthesized by periodontal fibroblasts and can be immunoprecipitated by use of specific antibodies. The molecular mass and electrophoretic properties after chondroitinase digestion of CD44 are similar to the 91 kDa CSPG found in this study. CD44 was originally characterized as lymphocyte homing receptor but later found to be associated with cell surfaces of various cell lines including fibroblasts. It is present in two forms, one about 90 kDa and the other 200 kDa. It, like syndecan, serves as a matrix receptor (Jalkanen *et al.*, 1992), but in some cell lines it functions as a hyaluronic acid receptor (Aruffo *et al.*, 1990).

In summary, we have identified the following PGs expressed by fibroblasts isolated from human periodontium. Two major CSPGs and DSPGs (versican and decorin) were found in the matrix and medium compartment. Large HSPGs were found associated with the membrane fraction together with one CSPG, a 91 kDa molecule. ECM and medium contained two to four HSPGs which were distinct from those found in the membrane fraction.

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