Proteoglycans of human gingival epithelium and connective tissue

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Proteoglycans extracted from separated specimens of healthy human gingival epithelium and from connective tissue have been purified. The epithelial proteoglycans fractionated as a single included peak on Sepharose 4B-CL and contained heparan sulphate and dermatan sulphate glycosaminoglycans. The connective-tissue proteoglycans separated into three major populations on Sepharose 4B-CL, one of which was excluded from this gel under associative conditions (0.5 M-sodium acetate, pH 7.4). Subsequent fractionation of the excluded material under dissociative conditions (4 M-guanidinium chloride/0.05 M-sodium acetate, pH 7.4) revealed an absence of any aggregate formation of molecules within this population. The connective-tissue proteoglycans contained heparan sulphate, dermatan sulphate and chondroitin 4-sulphate, the proportions of which varied with the molecular size of the proteoglycans. Amino acid analysis of the protein cores of gingival-epithelial and connective-tissue proteoglycans revealed differences that were similar to the differences described between other types of proteoglycans such as those from skin.

The conformational structure of proteoglycans in connective tissues can influence mechanical properties, molecular transport and morphogenesis (Comper & Laurent, 1978). Consequently, tissues such as gingivae, which are subjected to continual mechanical and chemical abuse, would be expected to rely heavily upon their extracellular matrix to maintain integrity as well as an environment satisfactory for adequate cellular activity.

Despite the knowledge that the proteoglycans are integral molecules, most biochemical studies on gingivae have focused upon the glycosaminoglycan content alone of whole gingivae (Ciancio & Mather, 1971; Hiramatsu *et al.*, 1978; Sakamoto *et al.*, 1978; Embery *et al.*, 1979). Recently, glycosaminoglycans have been isolated, identified and quantified in separated specimens of gingival epithelium and connective tissue (Bartold *et al.*, 1981). Heparan sulphate appeared to be the predominant glycosaminoglycan in the epithelium, whereas dermatan sulphate was the major glycosaminoglycan component in the gingival connective tissue. Hyaluronate and chondroitin 4-sulphate were also identified in lesser amounts.

Apart from these studies concerning the glycosaminoglycan composition of gingival tissues, little consideration has been given to the intact proteoglycan molecule or the contribution of these macromolecules to the extracellular matrices of both

epithelium and connective tissue. Dziewiatkowski *et al.* (1977) have reported briefly the presence of a proteoglycan in bovine gingivae, and Embery *et al.* (1979) studied the proteoglycans of normal and inflamed human gingival tissue. Newly synthesized radiolabelled proteoglycans in human gingival epithelium and connective tissue have also been investigated by using short incubation regimes (Wiebkin *et al.*, 1979; Bartold *et al.*, 1982a).

The present study describes the extraction of the proteoglycans of human gingival epithelium and connective tissue together with observations on their structural components and gel-chromatographic behaviour.

Materials and methods

Materials

Guanidinium chloride (analytical reagent; Ajax Chemicals, Sydney, New South Wales, Australia) was purified by stirring and filtering through activated charcoal. Urea (analytical reagent; Ajax Chemicals) solutions were deionized by filtration through a mixed anion/cation ion-exchange resin. CsCl of analytical grade (Ajax Chemicals) was used in the preparation of density gradients for preparative ultracentrifugation. Papain (EC 3.4.22.2) was obtained from BDH Chemicals (Poole, Dorset, U.K.). DEAE-Sephadex A-50 and Sepharose 4B-CL

were purchased from Pharmacia (South Seas) Pty. Ltd., Sydney, New South Wales, Australia. Cellogel cellulose acetate electrophoresis strips (2.5 cm × 15 cm) were from Chemetron, Milan, Italy. Alcian Blue (E. Gurr, London S.W.14, U.K.) was used to stain electrophoresed glycosaminoglycans. *Streptomyces hyaluronidase* (EC 3.2.1.35) was purchased from Seikagaku Kogyo Co. Ltd., Tokyo, Japan. Standard glycosaminoglycans were generously given by Dr. M. B. Matthews (Department of Pediatrics, University of Chicago, Chicago, IL, U.S.A.).

Human gingivae were obtained from gingivectomy on patients who had been subjected to pre-surgical preparation and were considered clinically free from inflammation (Ramfjord, 1967; Le & Silness, 1963). After excision, the tissues were immediately placed into 10 ml of phosphate-buffered saline containing EDTA (NaCl, 6.83 g; KCl, 0.2 g; Na₂HPO₄, 1.15 g; KH₂PO₄, 0.2 g; 0.12 ml of 1% Phenol Red; EDTA, 0.372 g/litre, pH 7.2). Storage time in this solution was no longer than 60 min at room temperature.

The gingival-tissue specimens were cut into approx. 5 mm × 2 mm × 2 mm pieces and transferred to 10 ml of a similar phosphate-buffered saline but containing 10 mM-EDTA and incubated at 37°C for 60 min (Scaletta & MacCallum, 1972; McKenzie *et al.*, 1979). The epithelium could then be easily separated from the underlying connective tissue using fine dissecting forceps.

Analytical methods

Uronic acid was determined by the method of Blumenkrantz & Asboe-Hansen (1973), with D-glucuronic acid as the standard. Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard. In some cases, protein levels were read at 280 nm directly on fractions from columns.

Extraction of proteoglycans

Specimens of human gingival epithelium and connective tissue were pooled separately in acetone. In general, 1–2 g of connective tissue and 2–3 g of epithelium were the minimum dry weights from which sufficient proteoglycans could be extracted for analysis.

The proteoglycans were extracted in 4 M-guanidinium chloride/0.05 M-sodium acetate, pH 7.4, containing proteinase inhibitors [10 mM-EDTA/0.1 M-6-aminohexanoic acid/5 mM-benzamidine hydrochloride/soya-bean trypsin inhibitor (1 mg/litre)] at 4°C for 24 h (1 g of tissue/15 ml of buffer). The suspension was filtered through glass wool and the residue re-extracted in fresh buffer for a further 24 h at 4°C. The extracts were pooled and concentrated to one-tenth the original volume in a Millipore ultrafiltration unit (Pellicon filter with 10 000-mol.wt.

exclusion). The concentrated extract was then dialysed against 7 M-urea/0.05 M-Tris/HCl, pH 6.5, at 4°C for 3 days and the passed through a DEAE-Sephadex A-50 anion-exchange column equilibrated with the same 7 M-urea/0.05 M-Tris/HCl buffer. The column was eluted stepwise with (a) the starting buffer, (b) 0.15 M-NaCl in the same buffer, and (c) 2.0 M-NaCl in the same buffer (Antonopoulos *et al.*, 1974). The 2.0 M-NaCl fractions were pooled and dialysed against several changes of 0.05 M-sodium acetate, pH 7.4, containing proteinase inhibitors at 4°C for 3 days. This was followed by dialysis against deionized water at 4°C for 1 day. In some cases a precipitate formed which was separated from the rest of the dialysis residue by centrifugation (1000 g). This precipitate was insoluble in 4 M-guanidinium chloride and partially soluble in conc. 98% (v/v) H₂SO₄ acid. Uronic acid analysis revealed some uronic acid-positive material, but, owing to difficulties in resolubilization, no further analyses were performed. The dialysis residue (free from precipitates) was then freeze-dried.

Density gradient ultracentrifugation

The freeze-dried proteoglycan-rich preparation was dissolved in 4 M-guanidinium chloride/0.05 M-sodium acetate, pH 7.4, containing proteinase inhibitors and adjusted to a starting density of 1.35 g/ml with solid CsCl. Preparative ultracentrifugation was performed in a Beckman model L ultracentrifuge fitted with a type 40 rotor at 35 000 rev./min (~100 000 g) at 15°C for 48 h. The tubes were then removed, rapidly frozen, and cut into five fractions of equal volume (they were thus respectively termed D_E1, D_E2, D_E3...etc. for the epithelium and D_{CT}1, D_{CT}2...D_{CT}5 for the connective tissue (where fractions 1 were the densest one-fifth). Each fraction was then dialysed against deionized water and small portions were taken for uronic acid and protein analysis as well as for glycosaminoglycan analysis by electrophoresis.

Column chromatography

Epithelial fractions (D_E1, 2 and 3 together) and connective-tissue fractions (D_{CT}1 and 2 separately) were applied to Sepharose 4B-CL columns of 29 cm × 0.6 cm and 61 cm × 0.9 cm dimensions respectively and eluted with 0.5 M-sodium acetate, pH 7.4, or 4 M-guanidinium chloride/0.05 M-sodium acetate, pH 7.4. Both buffers contained proteinase inhibitors.

Preparation and electrophoresis of glycosaminoglycans

The remaining tissue after 4 M-guanidinium chloride extraction was digested with papain. The tissue was suspended in buffer (1 mg of tissue/10 ml of buffer) comprising 0.2 M-sodium acetate, 4 mM-

EDTA and 0.02 M-cysteine hydrochloride, pH 5.7, containing 1 mg of papain/ml at 60°C for 48 h (Bartold *et al.*, 1981). The recovered glycosaminoglycans were then redissolved in 1 ml of distilled water and portions were taken for uronic acid assay and electrophoresis.

Similarly, portions of the proteoglycan fractions obtained by density-gradient ultracentrifugation (D_E1-5 and $D_{CT}1-5$) and column chromatography on Sepharose 4B-CL were also subjected to papain digestion and glycosaminoglycan recovery.

Electrophoresis of the glycosaminoglycans was performed in 0.2 M-calcium acetate, pH 7.2, on Cellogel cellulose acetate strips at 30 V/cm for 30 min (Bartold *et al.*, 1981). Quantification was achieved, after staining of the strips with Alcian Blue (Newton *et al.*, 1974), by densitometric scanning (Breen *et al.*, 1970; Bartold *et al.*, 1981).

In some instances of electrophoretic separation, superimposition of hyaluronic acid and heparan sulphate was noted. To determine the relative proportions of each of these, hyaluronic acid was removed by digestion with *Streptomyces* hyaluronidase (Ohya & Kaneko, 1970).

Amino acid analysis of proteoglycan protein core

Samples of proteoglycan (0.2 mg) from the bottom fraction of the CsCl density gradient for both epithelium and connective tissue (D_E1 and $D_{CT}1$) were hydrolysed in 6 M-HCl for 24 h. Subsequent amino acid analysis was carried out with a Beckman model 120 C amino acid analyser.

Results

Extraction of proteoglycans

After a crude extraction of proteoglycans with guanidinium chloride, a preliminary proteoglycan preparation was obtained by ion-exchange chromatography on DEAE-Sephadex A-50. Such chromatography usually resulted in three peaks positive for both uronic acid and protein being observed (Fig. 1). The major elutable uronic acid peak for both epithelial and connective-tissue proteoglycan extracts was obtained after elution with 2.0 M-NaCl. The smaller uronic acid peak observed with the 0.15 M-NaCl eluent appeared to be hyaluronic acid (as judged by electrophoresis), with no sulphated glycosaminoglycans being detected.

Papain digestion of the residual tissue after guanidinium chloride extraction revealed some uronic acid. The 4 M-guanidinium chloride extract of epithelium and connective tissue contained 95% and 80% respectively of the total epithelial and connective-tissue glycosaminoglycans.

Fractionation of the proteoglycan-rich preparation from ion-exchange chromatography (2 M-NaCl peak) was achieved by density-gradient ultracentri-

Fig. 1. Ion-exchange chromatography of gingival proteoglycans

(a) Epithelial and (b) connective-tissue proteoglycans extracted in 4 M-guanidinium chloride/0.05 M-sodium acetate, pH 7.4, were applied to columns (1 cm × 9 cm for epithelium and 1 cm × 17 cm for connective tissue) of DEAE-Sephadex A-50 in 7 M-urea/0.05 M-Tris/HCl, pH 6.5. Elution was step-wise with (i) 7 M-urea/0.05 M-Tris/HCl; (ii) 0.15 M-NaCl in same buffer, and (iii) 2.0 M-NaCl in same buffer. Fractions of volume 1 ml were collected. The flow rate was 4 ml/h. ○, Protein; ●, uronic acid.

fugation in 4 M-guanidinium chloride at a starting density of 1.35 g/ml (Figs. 2a and 2b). The epithelial proteoglycans appeared to be more evenly distributed throughout the denser three-fifths of the gradient (D_E1 , 2 and 3) than the connective-tissue proteoglycans, which were mostly contained within the bottom two-fifths ($D_{CT}1$ and 2). Although some uronic acid was observed in the top two connective-tissue fractions ($D_{CT}4$ and 5), there was very little detectable uronic acid in the two least-dense fractions of the density-gradient fractionation of the epithelial extract. The protein profiles of material from both tissues were similar, with the greatest proportion of protein appearing in the least buoyant fractions.

Column chromatography

Column chromatography of epithelial and connective-tissue proteoglycan prepared by density-gradient centrifugation was performed on Sepharose 4B-CL eluted with 0.5 M-sodium acetate, pH 7.4.

Attempts to fractionate each of the three epithelial density-gradient fractions (D_E1 , 2 and 3) were not successful, owing to the very limited amount of material available in each. Therefore epithelial proteoglycan fractions D_E1 , 2 and 3 were pooled and then fractionated on Sepharose 4B-CL. This resulted in a single peak with a K_{av} of 0.35 being

Fig. 2. Density-gradient centrifugation of gingival proteoglycans

(a) Epithelial and (b) connective-tissue proteoglycan-rich preparations obtained from DEAE-Sephadex A-50 ion-exchange chromatography (2.0 M-NaCl peaks) were dissolved in 4 M-guanidinium chloride/0.05 M-sodium acetate adjusted to a starting density of 1.35 g/ml with CsCl. Centrifugation was at 35 000 rev./min for 48 h. The fractions thus obtained were termed D_{E1} – D_{E5} and D_{CT1} – D_{CT5} for epithelium and connective tissue respectively. ○, Protein (Folin); ●, uronic acid; . . . , density.

observed (Fig. 3). No uronic acid was detected at either V_0 or V_t of the column.

Since there were greater amounts of connective-tissue proteoglycans recovered after density-gradient centrifugation, the bottom two fractions (D_{CT1} and D_{CT2}) could be fractionated separately from Sepharose 4B-CL eluted with 0.5 M-sodium acetate, pH 7.4. The proteoglycans of fraction D_{CT1} separated into two populations after such chromatography under associative conditions (Fig. 4a). One peak was excluded from the gel (K_{av} , 0.08), whereas the other was included (K_{av} , 0.46). Quantitatively, the major proportion of these two proteoglycans was comprised of the smaller included material. Chromatography of fraction D_{CT2} also resulted in two peaks of proteoglycans being eluted, both of which were included. The larger-sized material eluted with a K_{av} of 0.31, whereas the smaller-sized material appeared more polydisperse in size with an estimated K_{av} of 0.57 (Fig. 4b).

Fig. 3. Gel chromatography of gingival-epithelial proteoglycans

Proteoglycans from the pooled epithelial density-gradient fractions D_{E1} , 2 and 3 were applied to a Sepharose 4B-CL column (0.6 cm $\times$ 29 cm) and eluted at 3 ml/h under associative conditions (0.5 M-sodium acetate, pH 7.4). Fractions (0.5 ml) were collected. V_0 was determined with Blue Dextran 2000 and V_t was determined with [35 S]sulphate.

Since some of the proteoglycans of fraction D_{CT1} were excluded from Sepharose 4B-CL under associative conditions, this fraction was also chromatographed from Sepharose 4B-CL under dissociative conditions (4 M-guanidinium chloride/0.05 M-sodium acetate, pH 7.4). A similar profile to that observed under associative conditions was obtained (Fig. 5). A small peak corresponding to larger-sized material was excluded from the gel (K_{av} , 0.14) and the bulk of the proteoglycans included within the gel (K_{av} , 0.57).

Glycosaminoglycan analyses

Papain digestion of the residual gingival epithelial and connective tissue after guanidinium treatment with chloride released the remaining glycosaminoglycans, which were identified by electrophoresis (Fig. 6) as hyaluronic acid, heparan sulphate, dermatan sulphate and chondroitin 4-sulphate. The major glycosaminoglycan that remained in the epithelial tissue was heparan sulphate (60%), whereas the major glycosaminoglycan not extracted by guanidinium chloride was hyaluronic acid (30%).

Proportional electrophoretic analysis of the constituent glycosaminoglycans of gingival epithelial and connective tissue chromatographed on Sepharose 4B-CL can be seen in Fig. 7. The epithelial proteoglycans appeared to be comprised of heparan sulphate and dermatan sulphate. No chondroitin

Fig. 4. Gel chromatography of gingival connective-tissue proteoglycans under associative conditions

Proteoglycans from the connective-tissue density-gradient fractions (a) D_{CT}1 and (b) D_{CT}2 were applied separately to columns of Sepharose 4B-CL (0.9 cm × 61 cm) and eluted at 3 ml/h under associative conditions (0.5 M-sodium acetate, pH 7.4). Fractions (1 ml) were collected. V₀ and V_t were determined as described in Fig. 3.

sulphate or hyaluronic acid was detected. On the other hand, the glycosaminoglycans of the connective-tissue proteoglycans corresponded to heparan sulphate, dermatan sulphate and chondroitin 4-sulphate. No hyaluronic acid was detected.

Quantitatively, heparan sulphate was the major glycosaminoglycan of epithelial proteoglycans (80%), whereas dermatan sulphate was the major glycosaminoglycan of the connective-tissue proteoglycans (50%).

The glycosaminoglycan composition of each of the peaks obtained by column chromatography of

Fig. 5. Gel chromatography of gingival connective-tissue proteoglycans under dissociative conditions

Proteoglycans from the D_{CT}1 density-gradient fraction were applied to a Sepharose 4B-CL column (0.6 cm × 29 cm) and eluted at 3 ml/h under dissociative conditions (4 M-guanidinium chloride/0.05 M-sodium acetate, pH 7.4). Fractions (1.0 ml) were collected. V₀ and V_t were determined as described in Fig. 3.

the connective-tissue proteoglycan fractions D_{CT}1 and D_{CT}2 on Sepharose 4B-CL was also analysed by electrophoresis after papain digestion (Fig. 8). In each peak all three glycosaminoglycans, heparan sulphate, dermatan sulphate and chondroitin 4-sulphate, were observed. However, there were some quantitative differences between each of these fractions. The largest connective-tissue proteoglycan excluded Sepharose 4B-CL under associative conditions, D_{CT}1 (*K*_{av} 0.08), contained predominantly dermatan sulphate and chondroitin 4-sulphate. The lower-molecular-weight fractions D_{CT}1 (*K*_{av} 0.46) and D_{CT}2 (*K*_{av} 0.31) contained dermatan sulphate as the predominant glycosaminoglycan, with smaller proportions of heparan sulphate and chondroitin 4-sulphate. The lowest-molecular-weight fraction, D_{CT}2 (*K*_{av} 0.57), contained predominantly dermatan sulphate, with very little heparan sulphate and chondroitin sulphate being detected.

Amino acid analysis

Amino acid profiles were obtained for protein cores of proteoglycans in the densest fractions of the density-gradient preparations of gingival epithelial and connective tissue (D_E1 and D_{CT}1). The profiles obtained from such analyses are listed in Table 1. In both tissue preparations, glutamic acid was the major contributor for both epithelium and connective tissue. Aspartic acid and glycine were also predominant contributors to the overall amino acid compositions of these proteoglycans. Methionine was characteristically a minor component. Overall,

Fig. 6. Densitometric scans of cellulose acetate electrophoresis of glycosaminoglycans released by papain from the tissue residue

(a) Standard glycosaminoglycans; HA, hyaluronic acid; HS, heparan sulphate; DS, dermatan sulphate; ChS-4, chondroitin 4-sulphate. (b) Epithelial papain digest, glycosaminoglycans (HA, 22.7%; HS, 59.5%; DS, 8.9%; ChS-4, 8.9%). (c) Epithelial papain digest, glycosaminoglycans treated with *Streptomyces hyaluronidase*. (d) Connective-tissue papain digest, glycosaminoglycans (HA, 35.9%; HS, 10.2%; DS, 23.1%; ChS-4, 30.8%). (e) Connective-tissue papain digest, glycosaminoglycans

Fig. 7. Densitometric scans of cellulose acetate electrophoresis of papain-digested gingival proteoglycans prepared for chromatography on Sepharose 4B-CL

(a) Standard glycosaminoglycans: HA, hyaluronic acid; HS, heparan sulphate; DS, dermatan sulphate; ChS-4, chondroitin 4-sulphate. (b) Epithelial proteoglycans (HA, 0.00%; HS, 80%; DS, 20%; ChS-4, 0.0%). (c) Connective-tissue proteoglycans (HA, 0.0%; HS, 11.5%; DS, 50.0%; ChS-4, 38.5%). The glycosaminoglycans were obtained by papain digestion of an aqueous extraction of epithelial and connective-tissue proteoglycans obtained from ion-exchange chromatography and density-gradient centrifugation. Electrophoretic conditions were the same as for Fig. 6. The proportions of the glycosaminoglycans are expressed as percentages of total glycosaminoglycans.

treated with *Streptomyces hyaluronidase*. Samples (5 μ l) of glycosaminoglycans were applied in a 0.5 cm line at the cathode and electrophoresed in 0.2 M-calcium acetate, pH 7.2, at 30 V/cm for 30 min. Strips were stained with Alcian Blue and scanned at 600 nm. Proportions of glycosaminoglycans are expressed as percentages of total glycosaminoglycans.

Fig. 8. Densitometric scans of cellulose acetate electrophoresis of the glycosaminoglycan component of various connective-tissue proteoglycans fractionated on Sepharose 4B-CL

(a) Standard glycosaminoglycans: HA, hyaluronic acid; HS, heparan sulphate; DS, dermatan sulphate; ChS-4, chondroitin 4-sulphate. (b) Fraction D_{CT}1; peak K_{av} 0.08 (HA, 0.0%; HS, 32.0%; DS, 32.0%; ChS-4, 35.0%). (c) Fraction D_{CT}1; peak K_{av} 0.46 (HA, 0.0%; HS, 11.5%; DS, 50.0%; ChS-4, 38.5%). (d) Fraction D_{CT}2; K_{av} 0.57 (HA, 0.0%; HS, 9.0%; DS, 82.5%; ChS-4, 8.5%). After chromatographic fractionation of the D_{CT}1 and D_{CT}2

Table 1. Amino acid composition of gingival-epithelial and connective-tissue proteoglycans

Proteoglycans from the D_E1 and D_{CT}1 fractions of the density gradient were analysed for amino acid composition after hydrolysis in 6M-HCl on a Beckman model 120 C amino acid analyser. Values from the data of Oldberg *et al.* (1979) and Damle *et al.* (1982) are included for comparison. Abbreviation used: ND, not determined.

	Content (residues/1000 residues)			
	Epi-thelium	Con-nective tissue	Heparin sulphate proteoglycan*	Dermatan sulphate proteoglycan†
Asx	110	113	125	125
Thr	46	52	78	42
Ser	90	66	142	65
Glx	194	129	161	104
Pro	50	86	77	72
Gly	119	120	100	76
Ala	79	77	57	49
†-Cys	ND	ND	ND	ND
Val	47	48	33	66
Met	3	Trace	15	6
Ile	31	43	34	56
Leu	62	93	68	127
Tyr	21	20	19	23
Phe	27	27	16	29
Lys	53	63	41	87
His	10	11	12	23
Arg	38	52	21	32
Trp	ND	ND	ND	ND

* Results from Oldberg *et al.* (1979).

† Results from Damle *et al.* (1982).

the epithelial proteoglycans appeared to be characterized by a relatively higher serine and glutamic acid content, whereas the connective-tissue proteoglycan contained greater proportions of proline, leucine and arginine.

Discussion

Proteoglycans have been isolated and partially characterized after extraction by guanidinium chloride from separated human gingival epithelium and connective tissue. The epithelial proteoglycans appeared to be comprised of only two glycosaminoglycans, namely heparan sulphate and dermatan sulphate. This finding is surprising, since chondroitin 4-sulphate is also a major contributor to the

fractions, portions from each peak were subjected to papain digestion and the glycosaminoglycans were then electrophoresed. The electrophoretic conditions were the same as for Fig. 6. The proportions of the glycosaminoglycans are expressed as percentages of total glycosaminoglycans.

overall sulphated glycosaminoglycan composition of gingival epithelium. Indeed, previous studies (Bartold *et al.*, 1981) indicate that chondroitin 4-sulphate contributes approx. 20% of the total epithelial glycosaminoglycans. In the present study the papain digestion of the residual epithelial tissue, after guanidinium chloride extraction, revealed some chondroitin 4-sulphate together with hyaluronic acid, heparan sulphate and dermatan sulphate remaining in the tissue. The contribution of chondroitin sulphate to the overall glycosaminoglycan content not extracted by guanidinium chloride was estimated to be 9%. Therefore, since approximately one half of the chondroitin 4-sulphate content of gingival epithelium was extracted by guanidinium chloride, it would appear that this chondroitin 4-sulphate was lost during the purification steps of ion-exchange chromatography and density-gradient centrifugation. This finding highlights the serious problem of recovery of these molecules when working with tissues, such as gingival epithelium, which contain extremely small quantities of proteoglycans.

Indeed, barely sufficient proteoglycans were extracted from human gingival epithelium to allow chromatography on a Sepharose 4B-CL column under associative conditions.

The profiles obtained from such chromatography of epithelial proteoglycans revealed a single symmetrical peak that was completely included in the gel (K_{av} , 0.53). This is in direct contrast with our previous studies (Wiebkin *et al.*, 1979; Bartold *et al.*, 1982a) in which a small proportion of epithelial proteoglycans were demonstrated to be excluded from Sepharose 2B-CL. Such differences may be related to the different experimental procedures adopted. In the previous studies, incorporation *in vitro* of [35 S]sulphate into newly synthesized proteoglycans was measured. Under such conditions it appears that a high-molecular-weight proteoglycan is synthesized that is excluded from Sepharose 2B-CL. The proportion of very-high-molecular-weight material excluded from Sepharose 2B-CL in the [35 S]sulphate proteoglycan studies was in fact never more than 20% of the total extracted proteoglycan.

Associative chromatography on Sepharose 4B-CL of the connective-tissue proteoglycans revealed at least three different proteoglycan species on the basis of molecular size. Glycosaminoglycan analysis of these three groups revealed heterogeneity in which dermatan sulphate was the predominant component of the smaller-sized proteoglycan fractions. The larger-sized proteoglycans appeared to contain increased proportions of heparan sulphate and chondroitin sulphate. For example, D_{CT1} proteoglycans of K_{av} , 0.08 comprise almost equal proportions of heparan sulphate, dermatan sulphate and

chondroitin 4-sulphate, whereas D_{CT1} proteoglycans of K_{av} , 0.31 and D_{CT2} with K_{av} , 0.46 contained less heparan sulphate, proportionally more dermatan sulphate and slightly more chondroitin 4-sulphate than the D_{CT1} of K_{av} , 0.08. The fraction containing the smallest-sized proteoglycans studied, D_{CT2} (K_{av} , 0.57), contained predominantly dermatan sulphate, with heparan sulphate and chondroitin 4-sulphate being relatively minor contributors. Such findings demonstrate a general polydispersity of gingival connective-tissue proteoglycans not only with respect of size but also glycosaminoglycan composition. Furthermore, gingival proteoglycans appear to be different from other proteoglycans from similar sources. For example, proteoglycans extracted from skin (Damle *et al.*, 1979) and periodontal ligament (Pearson & Gibson, 1982) are claimed to contain only chondroitin sulphate or only dermatan sulphate glycosaminoglycan side chains respectively. However, the various gingival proteoglycan fractions reported in the present study appear to be comprised of more than one glycosaminoglycan species.

The previous observation that gingival connective-tissue proteoglycans are not dissociated by 4M-guanidinium chloride (Wiebkin *et al.*, 1979) was confirmed by the present study. The D_{CT1} fraction could be separated into two populations (K_{av} , 0.14 and 0.57) under dissociative conditions, which were considered to be similar to the two populations observed under associative conditions (K_{av} , 0.08 and 0.31), since the proportion of larger-sized material remained constant.

The amino acid profiles of both epithelial and connective-tissue proteoglycans revealed some differences in their overall composition. However, with the exception of glutamic acid, these differences were small. A consideration of the amino acid composition of certain proteoglycans indicates some characteristics specific for gingival proteoglycans. For example, predominantly heparan sulphate proteoglycans appear to contain high proportions of aspartic acid, serine, glutamic acid and glycine (Oldberg *et al.*, 1979). On the other hand, predominantly dermatan sulphate proteoglycans (Damle *et al.*, 1982) have elevated amounts of aspartic acid, glutamic acid and leucine. Such differences in amino acid composition of different proteoglycans are highlighted in the gingival-epithelial and connective-tissue proteoglycans respectively of the present study (Table 1). Epithelial proteoglycan, which appeared to be comprised of predominantly heparan sulphate, was high in the amino acids aspartic acid, serine, glutamic acid and glycine, whereas the connective tissue proteoglycans, which contained greater amounts of dermatan sulphate, were higher in aspartic acid, glutamic acid, glycine and leucine amino acid residues.

It is clear from the present and other studies (Wiebkin *et al.*, 1979; Bartold *et al.*, 1982a) that the proteoglycan composition of human gingival epithelial and connective tissue is different. Such macromolecules are important in maintaining tissue integrity in the gingivae, which are subjected to continual chemical and mechanical abuse. For example, in the extracellular matrix of gingival epithelium there are two large macromolecular populations, namely hyaluronic acid (Bartold *et al.*, 1982b) and the proteoglycans. Since the actual space available in the highly cellular epithelium for occupancy by such macromolecules is very small, and complex molecular arrangements have been described (Wiebkin *et al.*, 1979), one could expect the effective concentration of these molecules to be high. Consequently these putative intercellular molecular interactions may specifically regulate the diffusion and molecular-exclusion effects characteristic of this tissue. Furthermore, a less complex stoichiometry of the connective-tissue proteoglycans may render them more susceptible to enzymic degradation. Consequently these differences could explain, in part, the observation of tissue destruction occurring in connective tissue before epithelium in the early stages of periodontal disease (Page & Schroeder, 1976).

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