Biochem. J. (1988) 254, 757–764 (Printed in Great Britain)

Dermatan sulphate proteoglycan from human articular cartilage

Variation in its content with age and its structural comparison with a small chondroitin sulphate proteoglycan from pig laryngeal cartilage

Lucia de O. SAMPAIO,* Michael T. BAYLISS, Timothy E. HARDINGHAM† and Helen MUIR
Division of Biochemistry, Kennedy Institute of Rheumatology, Hammersmith, London W6 7DW, U.K.

Low molecular mass proteoglycans (PG) were isolated from human articular cartilage and from pig laryngeal cartilage, which contained protein cores of similar size (M_r 40–44 kDa). However, the PG from human articular cartilage contained dermatan sulphate (DS) chains (50% chondroitinase AC resistant), whereas chains from pig laryngeal PG were longer and contained only chondroitin sulphate (CS). Disaccharide analysis after chondroitinase ABC digestion showed that the human DS-PG contained more 6-sulphated residues (34%) than the pig CS-PG (6%) and both contained fewer 6-sulphated residues than the corresponding high M_r aggregating CS-PGs from these tissues (86% and 20% from human and pig respectively). Cross-reaction of both proteoglycans with antibodies to bovine bone and skin DS-PG-II and human fibroblast DS-PG suggested that the isolated proteoglycans were the human DS-PG-II and pig CS-PG-II homologues of the cloned and sequenced bovine proteoglycan. Polyclonal antibodies raised against the pig CS-PG-II were shown to cross-react with human DS-PG-II. SDS/polyacrylamide-gel analysis and immunoblotting of pig and human cartilage extracts showed that some free core protein was present in the tissues in addition to the intact proteoglycan. The antibodies were used in a competitive radioimmunoassay to determine the content of this low M_r proteoglycan in human cartilage extracts. Analysis of samples from 5–80 year-old humans showed highest content (~4 mg/g wet wt.) in those from 15–25 year-olds and lower content (~1 mg/g wet wt.) in older tissue (> 55 years). These changes in content may be related to the deposition and maintenance of the collagen fibre network with which this class of small proteoglycan has been shown to interact.

INTRODUCTION

Cartilage is a specialized connective tissue with a large extracellular matrix composed of a dense network of collagen fibres (typically types II, VI, IX and XI) which entrap a high concentration of proteoglycans (PG) (Muir & Hardingham, 1986). The majority of the proteoglycans are present as high-molecular-mass molecules [M_r (1–4) × 10^6] containing chondroitin sulphate (CS) and keratan sulphate chains and form aggregates by binding to hyaluronate in association with link protein (Bayliss *et al.*, 1984; Heinegard & Paulsson, 1984; Hardingham, 1986; Muir & Hardingham, 1986). Low-molecular-mass proteoglycans of quite different structure have also been identified in cartilage (Heinegård *et al.*, 1981; Rosenberg *et al.*, 1985) which appear similar to small proteoglycans widely distributed in other non-cartilaginous tissues (Heinegård *et al.*, 1985; Vogel & Fisher, 1986; Day *et al.*, 1986). Although these proteoglycans account for only a small proportion of the total glycosaminoglycans they may be present in similar molar proportions to the large aggregating proteoglycan. In tendon, skin, sclera and cornea dermatan sulphate proteoglycans (DS-PG) have been shown to be specifically associated with collagen fibrils (Scott & Haigh, 1985) and the DS-PG from tendon has been shown to inhibit the rate of collagen fibrillogenesis *in vitro* (Vogel & Heinegård, 1985). The majority of proteoglycans in cartilage are not bound to the collagen fibres (Torchia *et al.*, 1977) but the small cartilage proteoglycan may have a specific function influencing the organization or stability of the collagen network.

Two types of small DS-PG (DS-PG-I and DS-PG-II) were isolated from bovine articular cartilage, which, although their core proteins were of similar size, appeared to be structurally distinct (Rosenberg *et al.*, 1985). Two corresponding small proteoglycans from bovine bone have also been identified (Fisher, 1985). In this study a low-molecular-mass DS-PG from mature human articular cartilage was investigated and compared with that of a non-articular cartilage from immature pig larynges, which contains no DS.

METHODS

Isolation of proteoglycans

Proteoglycans of low molecular mass were extracted and isolated essentially as decribed by Rosenberg *et al.* (1985). Human articular cartilage (from 50–60 year-old, pooled tibial, femoral and patellar cartilage from four knee joints) and pig laryngeal cartilage (from 6–9 month-

Abbreviations used: CS, chondroitin sulphate; DS, dermatan sulphate; PG, proteoglycan; PAGE, polyacrylamide gel electrophoresis.

* Present address: Escola Paulista de Medicina, Depto de Bioquimica – INFAR, Rua 3 de maio 100 – 4 andar, CP 20372 – 04044 São Paulo, Brazil.

† To whom correspondence should be addressed.

old pigs, pooled from 12 larynges) were diced in 1 mm cubes carefully avoiding all perichondrium, subchondral bone or other non-cartilaginous tissue, and extracted in 4 M-guanidine HCl containing proteinase inhibitors (Bayliss *et al.* 1984). Proteoglycans were fractionated in CsCl density gradients under associative conditions (Rosenberg *et al.*, 1985). For human articular-cartilage extracts the starting density was 1.50 g/ml and fractions of density 1.43–1.57 g/ml were taken for further purification. For pig laryngeal-cartilage extracts the starting density was 1.60 g/ml and all fractions below 1.65 g/ml were retained. For both tissues most of the large aggregating proteoglycans were in fractions above 1.57 g/ml (human) and above 1.65 g/ml (pig). The retained fractions were re-run in CsCl density gradients under dissociative conditions at a starting density of 1.45 g/ml. Fractions of densities between 1.38 and 1.48 g/ml were pooled, dialysed and freeze dried. These fractions contained only 3.9% of the total glycosaminoglycan from human articular cartilage and only 2.6% of that from pig laryngeal cartilage. They were greatly enriched in low-molecular-mass proteoglycan, but remained contaminated with proteoglycans related to the large aggregating forms.

Chromatography on DEAE-cellulose in 6 M-urea

Samples (about 300 mg) were applied in 20 ml of 6 M-urea containing 0.025 M-Tris/HCl (pH 6.8) to a column (320 mm × 28 mm) of DEAE-cellulose. Elution was in buffered 6 M-urea (as above) at 0.8 ml/min with a linear gradient of 0.2 M–0.8 M-NaCl. Column fractions (8 ml) were assayed for glycosaminoglycans using an automated dye-binding assay with Dimethylmethylene Blue (Farndale *et al.*, 1982) and they were monitored for protein at 275 nm. Fractions were pooled, dialysed and freeze dried.

Size-exclusion chromatography on TSK SW4000 in 4 M-guanidine HCl

Fractions pooled from DEAE-cellulose chromatography were further purified by size-exclusion chromatography on a column (600 mm × 7.5 mm) of TSK SW4000 in 4 M-guanidine HCl, 0.05 M-sodium acetate, 1 mM-EDTA (pH 5.8), and eluted at 0.3 ml/min. The eluant was monitored at 280 nm and fractions (0.5 ml) were collected.

Preparation of antiserum and development of radioimmunoassay

Anterisum was raised in female New Zealand White rabbits to the purified small proteoglycan from pig laryngeal cartilage as described by Ratcliffe & Hardingham (1983). Antiserum from a single rabbit with the best titre was used for subsequent experiments.

Samples of proteoglycan from human and pig preparations were ^{125}I-iodinated with chloramine-T as the oxidizing agent as described by Greenwood *et al.* (1963). The radioimmunoassay procedure used was essentially that described by Caterson *et al.* (1979) with formaldehyde-treated heat-killed *Staphylococcus aureus* (Cowan strain 1) as the immunoprecipitant. Conditions used for antiserum titration and inhibition radioimmunoassay were as previously described for antibodies to proteoglycan-binding region and link protein (Ratcliffe & Hardingham, 1983).

SDS/polyacrylamide-gel electrophoresis and immunoblotting

Electrophoresis was carried out on slab gels [5% (w/v) polyacrylamide] essentially as described by Fairbanks *et al.* (1971). Protein bands were detected by silver staining (Morrisey, 1981) and glycosaminoglycans by Toluidine Blue staining (Rosenberg *et al.*, 1985). Gels were autoradiographed using Kodak X-Omat S film and intensifying screens (Cronex Lightning Plus, DuPont). Electrophoretic transfer and immunoblotting with antiserum or antibodies was carried out as described by DeBlas & Cherwinski (1983) using nitrocellulose sheets and the bound antibodies were localized with peroxidase-conjugated second antibody. For immunoblotting on nitrocellulose the polyclonal anti-(pig cartilage proteoglycan) serum and the anti-(bovine bone proteoglycan) serum (provided by Dr. L. Fisher, National Institute of Dental Research, National Institutes of Health, U.S.A.) were used at a 1 in 300 dilution; the monoclonal anti-(bovine skin proteoglycan) antibody (provided by Dr. H. Pearson and Dr. N. Winterbottom, Department of Oral Biology, University of Alberta, Edmonton, Canada) was used at a 1 in 20 dilution of an ascites fluid; and the three monoclonal anti-(human fibroblast proteoglycan) antibodies (provided by Dr. H. Kresse, Institute of Physiological Chemistry, University of Münster, West Germany) were used at 1 in 6 (LN-1) and 1 in 20 (LN-3, LN-4) dilutions.

Characterization of proteoglycans

Samples of the protein cores were prepared by digesting the proteoglycans (5 μg) with chondroitinase ABC (0.6 mU/μg of proteoglycan) in 50 mM-Tris/HCl, 5 mM-NaF containing proteinase inhibitors (pH 8.0) for 18 h at 37 °C. To determine the relative size of the glycosaminoglycan chains attached to the proteoglycans, samples (70 μg) were incubated in 25 μl of buffer [(0.1 M-sodium acetate, 5 mM-EDTA (pH 6.0)] with 5 μg of papain for 18 h at 37 °C. The released glycosaminoglycan chains were electrophoresed on SDS/polyacrylamide gels in 7% gels and stained with Toluidine Blue (Rosenberg *et al.*, 1985). To determine the proportion of DS in the glycosaminoglycan, samples of the proteoglycan were digested with papain as above and then boiled, and the protein was precipitated with ethanol (3 vol.). The supernatant was evaporated to dryness and samples were re-dissolved in buffer [200 μl of 50 mM-sodium acetate, 5 mM-NaF (pH 7.3)] and two aliquots were digested separately with chondroitinase ABC and chondroitinase AC (5 mU/μg of proteoglycan). Digests were assayed for released unsaturated disaccharide by the thiobarbituric acid assay (Hascall *et al.*, 1972) using the unsaturated 4-sulphated disaccharide as standard. The proportion of DS in the preparation was determined from the difference in digestion with chondroitinase ABC and AC.

The proportions of 4-, 6- and non-sulphated disaccharides in the CS/DS chains was determined by h.p.l.c. analysis of the chondroitinase ABC-released disaccharides. The h.p.l.c. analysis was modified from that of Zebrower *et al.* (1986) and gave complete resolution of 6-, 4- and non-sulphated disaccharides from CS or DS, and the non-sulphated disaccharide from hyaluronate (D. G. Dunham & T. E. Hardingham, unpublished results). The proportion of each disaccharide was determined from the area under the peak monitored

Table 1. Equilibrium-density-gradient fractionation of 4 M-guanidine HCl extracts of human and pig cartilage under (*a*) associative conditions and (*b*) refractionation of A2 (human) and A2–3 (pig) under dissociative conditions in 4 M-guanidine HCl

Results are from a single large scale preparation from each source of cartilage. Protein and hexuronate (UA) were determined as described in the Methods section. DS/CS-PG II was determined by radioimmunoassay (see Fig. 4*d*). Figures in parentheses show the density range of each fraction.

	(*a*) Associative gradients				(*b*) Dissociative gradients			
	Fraction	UA	Protein (% of total)	PG-II	Fraction	UA	Protein (% of total)	PG-II
Human articular cartilage	A1 (> 1.57)	89	46	7	D1 (> 1.48)	43	11	5
	A2 (1.43–1.57)	10	42	72 →	D2 (1.38–1.48)	50	45	67
	A3 (< 1.43)	1	12	21	D3 (< 1.38)	7	44	28
Pig laryngeal cartilage	A1 (> 1.65)	87	55	3	D1 (> 1.48)	77	17	6
	A2–3 (> 1.65)	13	45	97 →	D2 (1.38–1.48)	20	29	72
					D3 (< 1.38)	3	54	22

by absorption at 230 nm and compared with standard disaccharides (Miles Laboratories).

General procedures

Hexuronate was determined by an automated procedure (Heingard, 1973) of the modified carbazole reaction (Bitter & Muir, 1962) with glucuronolactone as standard. Protein was measured by an automated modification (Heinegård, 1973) of the method of Lowry *et al.* (1951) with bovine serum albumin (fraction V) as standard (Sigma, Poole, U.K.). Radioactivity was measured in an LKB Compugamma counter.

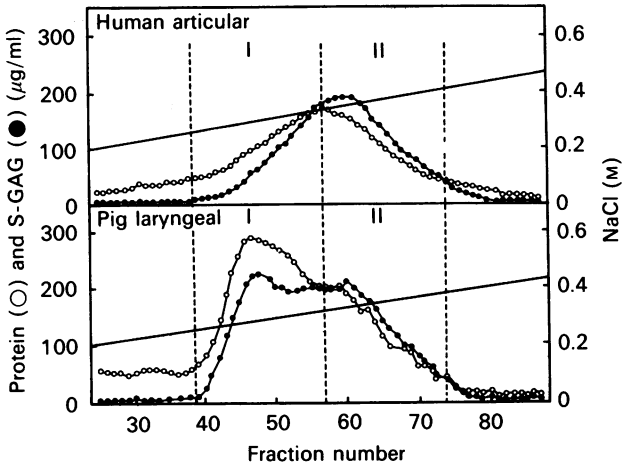

Fig. 1. Chromatography of low-molecular-mass proteoglycan fractions on DEAE-cellulose in 6 M-urea

The fractions enriched in small proteoglycans from dissociative density gradients of human articular and pig laryngeal preparations (Table 1, fractions D2) were chromatographed on DEAE-cellulose in 6 M-urea as described in the methods section. Elution was with a linear gradient of NaCl. Protein (○) and sulphated glycosaminoglycan (S-GAG, ●) were determined in each fraction. Fractions were pooled (I and II) for further purification (Fig. 2).

RESULTS

Analysis of the fractions from associative density gradients of extracts of human articular and pig laryngeal cartilage by SDS/polyacrylamide gel electrophoresis (PAGE) showed that low-molecular-weight proteoglycans were present in fractions in the middle of the gradient as reported for bovine articular cartilage (Rosenberg *et al.*, 1985). The small proteoglycans were further purified in a dissociative density gradient (starting density 1.45 g/ml), which removed more of the large aggregating proteoglycans (Table 1), and then by ion-exchange chromatography on DEAE-cellulose in 6 M-urea (Fig. 1). The pig preparations showed evidence of a bimodal distribution on DEAE-cellulose. Both fractions were re-chromatographed on a TSK SW4000 column in 4 M-guanidine HCl (Fig. 2) and the small proteoglycan was detected by SDS/PAGE analysis in fraction I. Fraction II of both preparations mainly contained larger proteoglycan components that remained at the origin on SDS/PAGE analysis.

The purified small proteoglycan preparations showed a single broad band on SDS/PAGE (Fig. 3*a*). From human articular cartilage its apparent size was M_r 70–80 kDa, whereas from pig laryngeal cartilage it was significantly larger, M_r 110–130 kDa. After digestion with chondroitinase ABC to remove most of the CS/DS chains there was a single core protein band evident in both species, which was of similar size, M_r 40–42 kDa (human) and M_r 42–44 kDa (pig) (Fig. 3*a*). After digestion of the proteoglycan with papain the released glycosaminoglycan chains gave a broad Toluidine Blue-staining band on SDS/PAGE with those from human cartilage, much smaller than those from pig (Fig. 3*b*). The smaller apparent size of the intact human proteoglycans thus mainly resulted from their containing shorter glycosaminoglycan chains.

Differential digestion with chondroitinase ABC and AC showed that the human proteoglycan contained DS as it was only 52% digested by chondroitinase AC, whereas there was no significant difference in the digestion of pig proteoglycan with the two enzymes and the pig proteoglycan therefore contained only CS chains (results not shown).

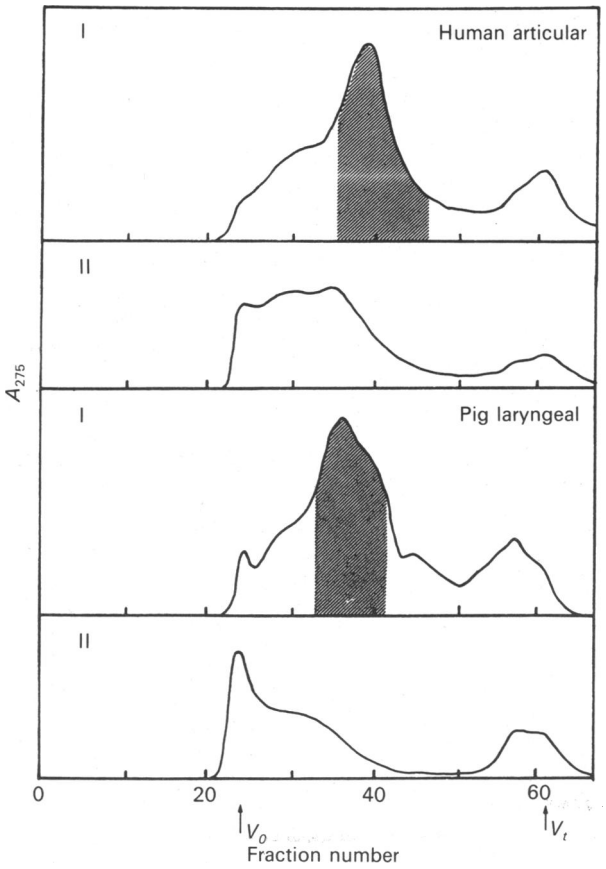

Fig. 2. Chromatography of DEAE-cellulose fractions I and II on TSK SW4000 in 4 M-guanidine HCl

Fractions I and II isolated from DEAE-cellulose chromatography of human articular and pig laryngeal preparations were chromatographed on TSK SW4000 in 4 M-guanidinium chloride as described in the Methods section. The absorbance of fractions was monitored at 275 nm. The shaded fractions were pooled for further analysis; other fractions did not contain significant amounts of the small proteoglycan. V_0 and V_t show the positions of void and total volume markers.

Analysis of the disaccharides released by chondroitinase ABC and AC (Table 2) showed that the two small proteoglycans had quite distinct patterns of sulphation. The human DS-PG contained more than five times as much 6-sulphated disaccharide compared with the pig CS-PG (34% and 6%) and its proportion of 4-sulphated disaccharide was correspondingly less (66% and 93%). Comparison of each small proteoglycan with the large aggregating CS proteoglycan from the same tissue showed that the large proteoglycan in each case contained a higher proportion of 6-sulphated disaccharide residues (human 86% and 34% and pig 20% and 6% respectively). The large proteoglycan also contained significant amounts of non-sulphated residues which were barely detectable in the small PG.

Immunochemical identity of proteoglycan core proteins

Antibodies were raised in rabbits against the small proteoglycan from pig laryngeal cartilage as it was more easily prepared and purified than that from human cartilage. The titre of the antiserum was determined separately against ^{125}I-labelled small proteoglycan from human and pig cartilage (Figs. 4*a* and *b*). Both were shown to be bound by the antibodies although the titre was stronger for the pig than for the human proteoglycan. The antiserum thus contained some antibodies that recognized common epitopes on the proteoglycans from pig and human cartilage. The extent of cross-reactivity was further tested by measuring binding with each non-radioactive antigen in competition with each radioactive antigen (Figs. 4*c* and *d*). The results showed that the human proteoglycan was only partially competitive (40%) with the antibodies that recognized the pig proteoglycan, whereas proteoglycans from both species

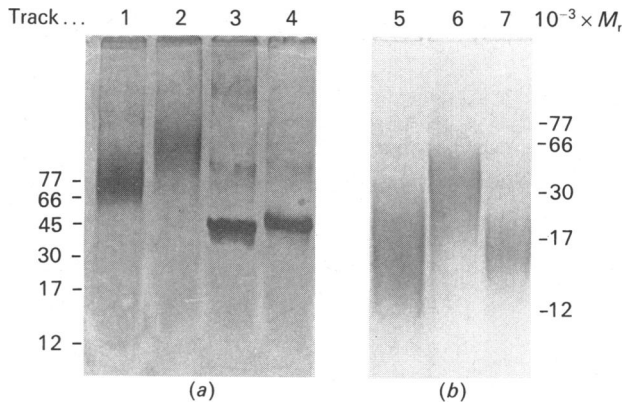

Fig. 3. SDS/PAGE of purified small proteoglycan fractions (Fig. 2); (*a*) intact proteoglycan and chondroitinase ABC digested core protein; (*b*) isolated glycosaminoglycan chains

(*a*) SDS/PAGE (5%) of the purified human (track 1) and pig (track 2) small CS/DS proteoglycans and the corresponding core proteins (human track 3, pig track 4) after chondroitinase ABC digestion. The gel was silver stained (Morrisey, 1981). (*b*) SDS/PAGE (7%) of the glycosaminoglycan chains released by papain digestion of the purified human (track 5) and pig (track 6) small DS/CS proteoglycans and of standard bovine skin DS-PG II (track 7) (supplied by Dr. Harold Pearson, Alberta, Canada). The glycosaminoglycans were stained with Toluidine Blue. The M_r of protein standards is shown for both gels.

Table 2. Disaccharide composition of the dermatan sulphate/chondroitin sulphate chains of the purified small DS/CS proteoglycans and the large CS proteoglycans from the same tissues (A1 fractions, Table 1)

C-0S, C-4S, C-6S; non-sulphated, 4-sulphated and 6-sulphated disaccharides respectively. Results are the average of two determinations.

	Human		Pig	
	DS-PG-II (% of total)	Large CS-PG (% of total)	CS-PG-II (% of total)	Large CS-PG (% of total)
C-0S	< 1	4	~1	5
C-4S	66	10	93	75
C-6S	34	86	6	20

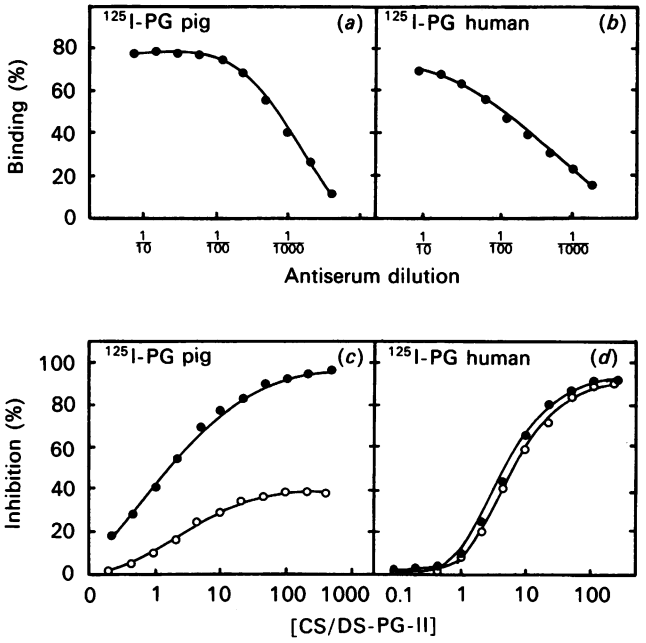

Fig. 4. Antibody titration and radioimmunoassay for the small CS/DS proteoglycans

A rabbit polyclonal antiserum raised against the small pig CS-PG was tested for its ability to precipitate (*a*) ^{125}I-labelled pig CS-PG and (*b*) ^{125}I-labelled human DS-PG. Radioimmunoassays with this antiserum at (*c*) 1 in 1000 dilution with ^{125}I-labelled CS-PG and (*d*) 1 in 350 dilution with ^{125}I-labelled DS-PG were used to determine the competitive binding of the human (○) and pig (●) CS/DS-PG preparations.

showed similar competition with the antibodies that recognized the human proteoglycan. The extent of cross-reactivity and presence of common epitopes suggested that the two proteoglycans contained closely related protein cores. Immunoblotting of the proteoglycans separated by SDS/PAGE before and after chondroitinase ABC digestion also showed that the antibodies recognized only the purified proteoglycans and their core proteins (Fig. 5).

Further evidence for the presence of homologous protein cores in these two sources of cartilage and their relationship with small proteoglycans from other species and tissue sources was obtained by testing and interaction with antibodies to other small proteoglycans (Table 3). Both were recognized by a polyclonal antiserum raised in rabbits against the small proteoglycan from bovine bone (Fisher *et al.*, 1983) and by a monoclonal mouse IgM raised against bovine skin proteoglycan (Pringle *et al.*, 1985). Of three monoclonal antibodies to human fibroblast small proteoglycan (Glössl *et al.*, 1984) all three cross-reacted with the human proteoglycan and one cross-reacted with the pig proteoglycan. The presence of many common epitopes suggests that the proteoglycans isolated from these two sources of cartilage are closely structurally related to the small CS/DS proteoglycan (PG-II) isolated from skin, bone, tendon, smooth muscle and cornea. This was further supported by tryptic peptide analysis (D. Heinegård, personal communication) which showed that many common peptide fragments were present in the two proteoglycan preparations and these corresponded to those identified in small tendon proteoglycan (PG-II) (Heinegård *et al.*, 1985).

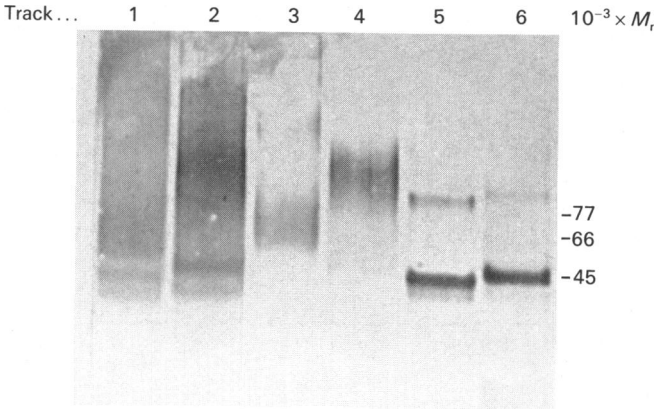

Fig. 5. SDS/PAGE and immunolocalization of the human and pig small CS/DS proteoglycan

Samples of 4 M-guanidinium chloride extracts of human (track 1) and pig (track 2) cartilage and the purified small proteoglycans (human track 3 and pig track 4) and their chondroitinase ABC digested core proteins (human track 5 and pig track 6) were electrophoresed on SDS/polyacrylamide (5%) gels. Electrophoretic transfer and immunoblotting were carried out as described in the Methods section with the anti-(pig CS-PG II) serum at 1 in 300 dilution. Antibodies were localized with peroxidase-conjugated second antibody.

Table 3. Interaction of small proteoglycans with specific antibodies

Interactions were graded from the intensity of immunoperoxidase staining, + + + very strong, + + strong, + weak, ± very weak, − no reaction.

	Human DS-PG-II	Pig CS-PG-II	Bovine skin DS-PG
Rabbit anti-pig cartilage CS-PG II	+ +	+ + +	+ + +
Rabbit anti-bovine bone CS-PG II	+	+ +	+ + +
Monoclonal anti-bovine skin DS-PG II	+ + +	+ + +	+ + +
Monoclonal anti-human fibroblast PG			
LN-1	+ +	+	+ +
LN-3	+ + +	±	±
LN-4	+ + +	−	−

With the antiserum, competitive radioimmunoassays were established for each proteoglycan which detected 1–30 μg/ml. This enabled the detailed distribution of the proteoglycans to be determined at different stages of the density gradient purification and showed that the majority of the proteoglycan was present in those fractions (see Table 1) pooled for further analysis.

Immunoblots of whole cartilage extracts electrophoresed on SDS/PAGE showed immunoreactive proteoglycan and also material of similar mobility to the

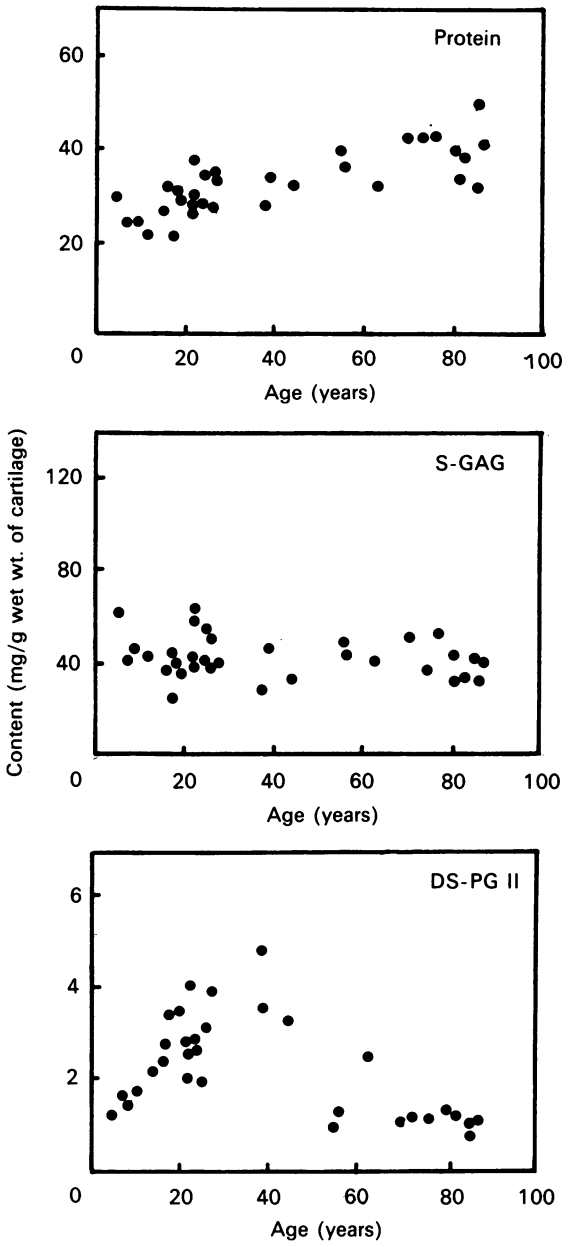

Fig. 6. Age-related changes in the DS-PG-II content of human articular cartilage

Samples ($n = 32$) of apparently normal human articular cartilage were extracted in 4 M-guanidine HCl with proteinase inhibitors and the extracts were analysed for (*a*) total protein content and (*b*) total glycosaminoglycan content; (*c*) DS-PG-II was determined using a competitive radioimmunoassay (Fig. 4*d*). All results were calculated for wet wt. of the fresh cartilage and each result is from a separate individual.

core protein. This was not only detected with the polyclonal anti-(pig PG-II) but also with the monoclonal antibody LN-1 and it was present in extracts of both human and pig cartilage. Ion-exchange chromatography on DEAE-cellulose in 6 M-urea showed that it lacked glycosaminoglycan chains as it did not bind to the column and was clearly separated from the proteoglycan which bound tightly and was eluted with 2 M-NaCl (results not shown). Both cartilages thus contained material of similar size to proteoglycan protein core which lacked the glycosaminoglycan chains.

The variation in the content of small proteoglycan in human articular cartilage was investigated by assaying extracts of cartilage (20 μm sections) from different ages (5–86 year) (Fig. 6*c*). There was considerable variation in the content (1–5 mg/g). There was an increase between 5 and 20 y, a generally high content between 20–40 y and lower levels in extracts from 55 year-olds and older. As the assay detects the proteoglycan protein core it would determine all the intact proteoglycan together with the free protein core material. This was present in extracts from all ages, but from the immunoblots there was little variation in the proportion (results not shown). Variation in content with age thus did not appear to be caused by changes in the amount of the free protein core material.

The amount of DS-PG present in extracts of human femoral head articular cartilage thus increased during skeletal development, but appeared to decline after maturity and was present at lower concentrations in older cartilage (> 55 y). This was quite different from the total glycosaminoglycan content of the same extracts which showed no age-related change, or their total protein content which showed a small increase with age (Figs. 6*a* and *b*), or from the hyaluronate content which also increased with age (Holmes *et al.*, 1988).

DISCUSSION

Investigation of the extracts from human articular cartilage and pig laryngeal cartilage showed that both contained the small proteoglycan, CS/DS-PG-II, previously identified in bovine bone, cartilage, tendon and skin and present in several other connective tissues (Heinegård *et al.*, 1985). The proteoglycans were identified by the similar size of the protein core together with the presence of common antigenic determinants. The polyclonal anti-(bovine bone PG-II) serum which showed cross-reaction with these preparations was previously used to select cDNA clones in a λgt11 expression library of bovine bone mRNA (Day *et al.*, 1987). The cDNA sequence of the expressed protein core was shown to be part of a single gene copy that is expressed in several different tissues (bone, tendon, articular cartilage, skin, smooth muscle and cornea) (Day *et al.*, 1986). The corresponding proteoglycan was cloned from a human fibroblast cDNA library and identified as PG-40 (Krusius & Ruoslahti, 1986). The cDNA sequence was homologous to the bovine PG-II sequence and the proteoglycan has been identified in human developing bone extracts (Day *et al.*, 1987). The cDNA sequences also support protein sequencing evidence which showed that the proteoglycan from bovine skin contained a single DS chain attached to a serine four residues away from the *N*-terminus of the protein core (Chopra *et al.*, 1985). Investigations of this proteoglycan from different sources have previously shown that it occurs in some tissues with DS chains, but in others with CS chains. As the conversion of CS to DS occurs after chain elongation it may depend on the expression within different cell types of the epimerase responsible for converting glucuronate to iduronate in the completed chain.

In this study comparison of the proteoglycan from two different cartilage sources showed several differences in their structure. The differences were most marked in the

attached glycosaminoglycan chains. The human PG-II contained DS chains, whereas pig PG-II contained CS, perhaps suggesting that the human articular chondrocytes contained the epimerase, but pig laryngeal chondrocytes did not. The human DS chains were also much shorter than the pig CS chains. Both were high in 4-sulphated disaccharides (66% human, 93% pig) and interestingly each contained considerably more 4-sulphated disaccharides than the large aggregating proteoglycans found in the same tissue. Chondrocytes thus appear to synthesize CS/DS chains of quite different structure on small proteoglycans compared with those they synthesize on the large aggregating proteoglycans, and chondrocytes from one source synthesize quite different glycosaminoglycan structures than those from another source.

Another small CS/DS proteoglycan has been identified in cartilage (Rosenberg *et al.*, 1985) and bone (Fisher *et al.*, 1983) based on properties of self-association (Rosenberg *et al.*, 1985), amino acid composition (Fisher *et al.*, 1983) and peptide maps (Heinegård *et al.*, 1985). This proteoglycan (PG-I) appears to be based on a different protein core and is also distinct from PG-II as it appears to be poorly immunogenic and does not react with anti-(PG-II) antibodies (Rosenberg *et al.*, 1985; Fisher *et al.*, 1983). The present results cannot exclude the presence of a small amount of this proteoglycan in the cartilage extracts as methods do not exist to detect it specifically. However, a tryptic peptide analysis of the PG-II preparations suggested minimal contamination with PG-I (D. Heinegård, personal communication).

Immunoblotting of SDS/PAGE of whole cartilage extracts not only showed PG-II to be present but also material of similar size to its protein core. It was present in extracts of both pig and human cartilage and in samples from different ages. This core protein in the extracts is unlikely to be from intracellular newly synthesized proteoglycan as guanidinium chloride is very poor at extracting intracellular material from chondrocytes in intact cartilage (Hardingham & Muir, 1972; Byers *et al.*, 1987). It may represent protein core that has failed to be glycosylated during biosynthesis. However, it may also represent a protein core from which the chain has been removed by extracellular catabolism. Vogel & Fisher (1986) have shown that mild proteolysis of purified PG-II with *Staph. aureus* V8 protease cleaved the protein core close to the *N*-terminus and released the peptide that contained the glycosaminoglycan chain. It was also shown that *Staph. aureus* V8 digestion of intact tendon cleaved the proteoglycan and released the glycosaminoglycan peptide (Vogel *et al.*, 1987) leaving a large protein core fragment bound (to the collagen) in the tissue. This fragment was shown to be capable of inhibiting collagen fibrillogenesis *in vitro* and it thus retained properties of interaction with collagen that were independent of the glycosaminoglycan chain. During the lifetime of the small DS/CS PG-II in the cartilage matrix some molecules may thus lose the *N*-terminal glycosaminoglycan peptide as a result of proteolytic cleavage, and leave a large protein core fragment in the tissue. The steady increase in the content of PG-II in cartilage (0–25 y) and its subsequent decline in older tissue may be correlated with rates of deposition and maintenance of the collagen fibril network. However, the precise role that the proteoglycan plays in fibrillogenesis *in vivo* has yet to be established.

This work was made possible by support for L. de O. S. from Fundação de Amparo a Pesquisa do Estado de São Paulo, Brasil. We also thank the Arthritis and Rheumatism Council (UK) for support. CS/DS disaccharide analysis by h.p.l.c. was carried out by D. G. Dunham. We thank Professor Dick Heinegård for carrying out a tryptic peptide analysis of the purified preparation.

REFERENCES

Bayliss, M. T., Ridgeway, G. & Ali, S. (1984) Biosci. Rep. **4**, 827–833
Bitter, T. & Muir, H. (1962) Anal. Biochem. **4**, 320–334
Byers, S., Hopkins, T. J., Kuettner, K. E. & Kimura, J. H. (1987) J. Biol. Chem. **262**, 9166–9174
Caterson, B., Baker, J., Levitt, D. & Paslay, D. J. (1979) Biol. Chem. **254**, 9369–9372
Chopra, R. K., Pearson, C. H., Pringle, G. A., Fackre, D. S. & Scott, P. G. (1985) Biochem. J. **232**, 277–279
Day, A. A., Ramis, C. I., Fisher, L. W., Gehron-Robey, P., Termine, J. D. & Young, M. F. (1986) Nucleic Acids Res. **14**, 9861–9876
Day, A. A., McQuillan, C. I., Termine, J. D. & Young, M. R. (1987) Biochem. J. **248**, 801–805
DeBlas, A. L. & Cherwinski, H. M. (1983) Anal. Biochem. **133**, 214–219
Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) Biochemistry **10**, 2606–2617
Farndale, R. W., Sayers, C. A. & Barrett, A. J. (1982) Connect. Tissue Res. **9**, 247–248
Fisher, L. W. (1985) in the Chemistry and Biology of Mineralized Tissues (Butler W. T., ed.), pp. 188–196, EBSCO Media, Birmingham, U.S.A.
Fisher, L. W., Termine, J. D., Dejter, S. W. Jr., Whitson, S. W., Yanagishita, M., Kimura, J. H., Hascall, V. C., Kleinman, H. K., Hassell, J. R. & Nilsson, B. (1983) J. Biol. Chem. **258**, 6588–6594
Glössl, J., Beck, M. & Kresse, H. (1984) J. Biol. Chem. **259**, 14144–14150
Greenwood, E. C., Hunter, W. M. & Glover, J. S. (1963) Biochem. J. **89**, 114–123
Hardingham, T. E. (1986) Rheumatology **10**, 143–183
Hardingham, T. E. & Muir, H. (1972) Biochem. J. **126**, 791–803
Hascall, V. C., Riolo, R. L., Hayward, J. & Reynolds, C. C. (1972) J. Biol. Chem. **247**, 4521–4528
Heinegård, D. (1973) Chem. Scr. **4**, 199–201
Heinegård, D., Paulsson, M., Inerot, S. & Carlstrom, C. (1981) Biochem. J. **197**, 355–366
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
Heinegård, D. & Paulsson, M. (1984) in Extracellular Matrix Biochemistry (Piez, K. & Reddi, A. eds.), pp. 277–328, Elsevier, Amsterdam
Holmes, M. W. A., Bayliss, M. T. & Muir, H. (1988) Biochem. J. **250**, 435–41
Krusius, T. & Ruoslahti, E. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 7683–7687
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
Morrisey, J. H. (1981) Anal. Biochem. **117**, 307–310
Muir, H. & Hardingham, T. E. (1986) in Copeman's Textbook of Rheumatic Diseases (Scott, J. T. ed.), pp. 177–198, Churchill Livingstone, Edinburgh
Pringle, G. A., Dodd, C. M., Osborn, J. W., Pearson, C. H. & Mosmann, T. R. (1985) Collagen Relat. Res. **5**, 23–39

Ratcliffe, A. & Hardingham, T. E. (1983) Biochem. J. **213**, 371–378
Rosenberg, L. C., Choi, H. U., Tang, L.-H., Johnson, T. L. & Pal, S. (1985) J. Biol. Chem. **260**, 6304–6313
Scott, J. E. & Haigh, M. (1985) Biosci. Rep. **5**, 71–81
Torchia, D. A., Hasson, M. A. & Hascall, V. C. (1977) J. Biol. Chem. **252**, 3617–3625
Vogel, K. G. & Fisher, L. W. (1986) J. Biol. Chem. **261**, 11334–11340
Vogel, K. & Heinegård D. (1985) J. Biol. Chem. **260**, 9298–9306
Vogel, K. G., Koob, T. J. & Fisher, L. W. (1987) Biochem. Biophys. Res. Commun. **148**, 658–663
Zebrower, M. E., Kieras, F. J. & Brown, W. T. (1986) Anal. Biochem. **157**, 93–99

Received 18 February 1988; accepted 18 April 1988