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The core proteins of large and small interstitial proteoglycans from various connective tissues form distinct subgroups

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Large and small proteoglycans were separately isolated from a number of connective tissues and compared to determine the extent of structural similarity. This was studied by enzyme-linked immunosorbent assays and by the peptide patterns obtained when ^{125}I-labelled proteoglycans were digested with trypsin. All the large proteoglycans, i.e. from tendon, sclera, cartilage and aorta, appear to contain the structure typical for the hyaluronic acid-binding region, both shown by enzyme-linked immunosorbent assay and by content of peptides unique for this region. These proteoglycans also share other structural features of the protein core, as indicated by immunological cross-reactivity and similar peptide patterns. The large proteoglycans from aorta in addition show the presence of unique structures both upon immunoassay and with regard to peptide pattern. Among the small proteoglycans two groups can be identified, although amino acid composition and protein core sizes are grossly similar. One group consists of the small proteoglycans from aorta and cartilage having similar peptide maps and showing immunological cross-reactivity in enzyme-linked immunosorbent assay. The other distinctly different group consists of the small proteoglycans from bone, cornea, sclera and tendon, which among them show identity in enzyme-linked immunosorbent assay and similar peptide patterns. Proteoglycans from the two groups, however, show partial immunological cross-reactivity.

In recent years a large number of apparently different proteoglycans have been isolated. Some, such as those containing heparan sulphate, appear to have a major function at the cell surface (Oldberg *et al.*, 1979; Kjellén *et al.*, 1981). Other proteoglycans, containing chondroitin sulphate or dermatan sulphate, are primarily interstitial, i.e. localized in the extracellular matrix (for references see Heinegård & Paulsson, 1984). It should be stressed, however, that there also appear to exist dermatan and/or chondroitin sulphate proteoglycans intercalated in the cell membrane and, on the other hand, heparan sulphate proteoglycans in the extracellular matrix, particularly in basement membrane (Hassel *et al.*, 1980).

A fundamental body of knowledge on the structure of the interstitial proteoglycans has been obtained from studies of the large proteoglycans in cartilage. These molecules have M_r values of 1×10^6–3×10^6, but they can form large aggregates with hyaluronic acid (Hardingham & Muir, 1972). The central core protein contains side-chain substituents of chondroitin sulphate, keratan sulphate, *O*-linked oligosaccharides and *N*-linked oligosaccharides (for references see Heinegård & Paulsson, 1984). One large portion of the core protein contains all the glycosaminoglycan chains, i.e. the chondroitin sulphate-rich and keratan sulphate-rich regions (Heinegård & Axelsson, 1977), and the other major portion of the proteoglycan, the hyaluronic acid-binding region (Heinegård & Hascall, 1974), contains few substituents, i.e. only a few *N*-linked oligosaccharides (Lohmander *et al.*, 1980).

Recent data show that there are two types of aggregating cartilage proteoglycans, differing in electrophoretic mobility on agarose/polyacrylamide gels, in amino acid composition and with regard to side-chain constituents. Their protein cores show differences, as indicated by peptide patterns of trypsin digests (Heinegård *et al.*, 1985*a*).

Other proteoglycans present in cartilage include a population of non-aggregating large proteo-

glycans, apparently distinct from those aggregating (Heinegård & Hascall, 1979) as judged from amino acid composition, a limited immunological cross-reactivity and a smaller number of side chains per protein.

A fourth population of proteoglycans in cartilage appears to be completely unrelated to the other three. These molecules are much smaller, having an M_r of only 76000 (Heinegård *et al.*, 1981). They contain one or two chondroitin sulphate side chains that are considerably larger than those of the aggregating proteoglycans, i.e. weight-average M_r values of about 35000 compared with 17000–18000. The core protein has an amino acid composition distinct in its very high content of leucine, aspartic acid/asparagine and glutamic acid/glutamine (Heinegård *et al.*, 1981). It also contains some oligosaccharide substituents (Heinegård *et al.*, 1981).

Other tissues contain predominant small proteoglycans with many structural features similar to those of the small cartilage proteoglycans. Thus M_r values of less than 100000 have been found for those from bone (Franzén & Heinegård, 1984), sclera (Cöster & Fransson, 1981) and tendon (Anderson, 1975; Vogel & Heinegård, 1985). These proteoglycans all have a protein core with high contents of leucine, aspartic acid/asparagine and glutamic acid/glutamine, being substituted with one or two very large chondroitin sulphate or dermatan sulphate chains as well as with oligosaccharides. Similar small proteoglycans have been described in periodontal ligament (Pearson & Gibson, 1982), cervix (Uldbjerg *et al.*, 1983), aorta (Salisbury & Wagner, 1981; S. Gardell, unpublished work), skin (Damle *et al.*, 1982) and cornea (Axelsson & Heinegård, 1975). Typically, the protein core prepared by chondroitinase digestion of all of these proteoglycans has a mobility on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis corresponding to an apparent M_r of about 45000–48000 when compared with globular standard proteins.

Many of these tissues also contain large proteoglycans with structural features resembling those of aggregating proteoglycans from cartilage. Examples are proteoglycans from aorta (Salisbury & Wagner, 1981; S. Gardell, unpublished work), tendon (Anderson, 1975; Vogel & Heinegård, 1985), sclera (Cöster & Fransson, 1981), periodontal ligament (Pearson & Gibson, 1982) and skin (Damle *et al.*, 1982). These proteoglycans have high contents of serine, glycine and glutamic acid/glutamine. They all contain smaller glycosaminoglycan side chains with weight-average M_r values of less than 20000. The side chains are chondroitin sulphate, and possibly in some cases dermatan sulphate with a high glucuronic acid/iduronic acid ratio. Additional substituents are a large number of *O*-linked oligosaccharides. Furthermore a proportion of these proteoglycans appear to have the capacity to interact with hyaluronic acid, important for the formation of proteoglycan aggregates (for references see Heinegård & Paulsson, 1984).

The present study was undertaken to show that there are indeed groups of proteoglycans with very similar, if not identical, core proteins, although the type of side chain may vary.

Materials and methods

Preparation of proteoglycans and fragments thereof

Proteoglycans were extracted from bovine cartilage, cornea, tendon, sclera, bone and aorta and purified by using standard procedures as described elsewhere (Axelsson & Heinegård, 1975; Heinegård & Hascall, 1979; Cöster & Fransson, 1981; Franzén & Heinegård, 1984; Heinegård & Paulsson, 1984; Heinegård *et al.*, 1985*a*). The preparation of corneal proteoglycans used was 50P, i.e. the one containing dermatan sulphate (Axelsson & Heinegård, 1980).

These procedures relied on extraction of proteoglycans with 4M-guanidinium chloride, containing proteinase inhibitors, and subsequent purification by CsCl-density-gradient centrifugation, ion-exchange chromotagraphy in 7M-urea on DEAE-cellulose eluted with salt gradients and gel chromatography. Hyaluronic acid-binding region and chondroitin sulphate peptides (A1.T.A1.CB.6B3) were prepared as described by Heinegård & Axelsson (1977). In all cases, purity was checked by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, where none of the samples contained detectable non-proteoglycan proteins even when large amounts of sample were applied.

Antibody production

Antibodies were raised in rabbits. Approx. 0.5mg of proteoglycan was dissolved in 0.5ml of 0.15M-NaCl/5mM-sodium phosphate buffer, pH7.4, and mixed with an equal volume of Freund's complete adjuvant. Immunization was done subcutaneously in the neck of 6-month-old rabbits. Booster doses of equal amounts of proteoglycans in Freund's incomplete adjuvant were given monthly until sufficient antibody titre was obtained, usually after one or two boosters. Rabbits were bled every 14 days by venesection in the ear. Antibody titre was checked by enzyme-linked immunosorbent assay as described below.

Immunoassay

Enzyme-linked immunosorbent assay by the procedure of Engvall & Perlman (1971), with some

modifications (Heinegård *et al.*, 1985*b*), was performed both for checking antibody titre and for measuring immunological cross-reactivity as reflected by the capacity of proteoglycans from different sources to inhibit in a particular assay.

It has previously been found important to use poly(vinyl chloride) micro-titre plates rather than such of polystyrene when the enzyme-linked immunosorbent assay was performed with proteoglycan antigens (Heinegård *et al.*, 1985*b*). In the present work we used Dynatech M29 (Dynatech Laboratories, Alexandria, VA, U.S.A.), which in general gives good performance. Before assay all antigens were digested with chondroitinase ABC. Samples at 1 mg/ml in 0.1 M-Tris/HCl/0.1 M-sodium acetate buffer, pH 7.3, were digested with 0.01 unit of chondroitinase ABC for 4 h at 37°C. Plates were coated with 200 μl of antigen, dissolved in 4 M-guanidinium chloride/5 mM-sodium acetate buffer, pH 5.8, at concentrations of 0.1 μg/ml (for hyaluronic acid-binding region) and 1 μg/ml (for proteoglycans), as indicated in the Figure legends for each particular experiment. After incubation overnight at room temperature in a humidified chamber, the plates were extensively rinsed with 0.15 M-NaCl/5 mM-sodium phosphate buffer, pH 7.4, also containing 0.05% (w/v) Tween 20. First antibody (200 μl) in appropriate dilutions in 0.15 M-NaCl/5 mM-sodium phosphate buffer, pH 7.4, containing 0.05% (w/v) Tween 20 was added. After incubation for 60 min at room temperature the plates were rinsed and incubated with second antibody, i.e. pig anti-(rabbit IgG) antibody (200 μl) conjugated to alkaline phosphatase (Orion Chemicals, Turku, Finland) at a dilution of 1:200. After 60 min at room temperature the plates were rinsed and incubated with substrate solution (200 μl), i.e. *p*-nitrophenyl phosphate (Sigma Chemical Co.) (1 mg/ml) in 1 M-diethanolamine buffer, pH 10, containing 1 mM-$MgCl_2$. The absorbance at 405 nm was measured with a Multiscan photometer (Flow Industries) immediately after substrate addition and again after a 60 min incubation at room temperature. The increase in colour yield was taken as the value of enzyme activity. The titre of first antibody giving an absorbance value of about 1 was used for inhibition assays. In these assays samples or standards diluted with incubation buffer were mixed with an equal volume of a dilution of the first antibody. The samples were incubated overnight at room temperature in polystyrene micro-titre plates, and 200 μl portions of the mixture were added to the coated wells in place of first antibody. Subsequent incubations were done as described above. All analyses were done in triplicates. In all cases inhibition curves for the reference, i.e. the antigen against which the antibodies were directed, and the antigens to be compared were determined by incubating the samples in the same micro-titre plate.

^{125}I-labelling

Proteoglycan samples (or hyaluronic acid-binding region) were labelled with ^{125}I by using the chloramine-T procedure essentially as described by Greenwood *et al.* (1963) with samples dissolved in 6 M-urea. The specific radioactivity of the proteoglycans was 5000–20000 c.p.m./ng.

Peptide mapping

^{125}I-labelled samples (50000 c.p.m.) were mixed with 0.1 M-Tris/HCl buffer, pH 8.0, to give a final concentration of 10 mM-Tris/HCl. Then 0.5 μl of a 1 mg/ml solution of 1-chloro-4-phenyl-3-tosylamidobutan-2-one ('TPCK')-treated trypsin (Sigma Chemical Co.) in the Tris buffer was added. Digestion was for 4 h at 37°C.

Two-dimensional thin-layer high-voltage electrophoresis in pyridine/acetate buffer, pH 6.4, was done on silica plates (Merck, Darmstadt, West Germany) in a Savant (New York, NY, U.S.A.) electrophoresis chamber. A plate (20 cm × 20 cm) was divided into two equal 10 cm × 20 cm strips, and two samples to be compared were each applied to one such strip and simultaneously electrophoresed for 40 min with 50 V/cm. After the plate had been dried, chromatography in the second dimension was developed with butanol/pyridine/acetic acid/water (6:5:3:1, by vol.) as described by Bates & Perham (1975). Autoradiography was done by simply leaving the plate on top of an envelope containing a sheet of AGFA-GEVAERT OSRAY M3 X-ray film for 2–3 days.

Electrophoresis on agarose/polyacrylamide gels

Samples were dissolved in 1% (w/v) sodium dodecyl sulphate at 1 mg/ml. Portions containing 20–50 μg of proteoglycan were diluted with an equal volume of 0.02% Bromophenol Blue/60% (w/v) sucrose dissolved in electrophoresis buffer, pH 6, and electrophoresed on agarose/polyacrylamide slab gels essentially as described by McDevitt & Muir (1971). The procedure was modified such that the gels were cast in buffer containing 0.1% (w/v) Triton X-100 (D. Heinegård, unpublished work).

Results and discussion

The proteoglycan preparations used in this study have been extensively characterized previously, with regard to molecular mass, size of glycosaminoglycan side chains, carbohydrate composition and amino acid composition. Their amino acid compositions are given in Tables 1 and 2.

Table 1. *Amino acid compositions of large proteoglycans*

Amino acid	Amino acid composition (residues/1000 residues)				
	Cartilage				
	Chondroitin sulphate-rich (Heinegård *et al.*, 1985*a*)	Keratan sulphate-rich (Heinegård *et al.*, 1985*a*)	Aorta (S. Gardell, unpublished work)	Tendon (Vogel & Heinegård, 1985)	Sclera (Cöster & Fransson, 1981)
Aspartic acid	72	67	94	68	96
Threonine	62	58	103	63	58
Serine	139	119	125	125	92
Glutamic acid	138	151	151	163	138
Proline	96	104	63	95	83
Glycine	136	123	109	122	110
Alanine	68	72	69	66	65
Cysteine	—	—	—	—	8
Valine	57	62	55	61	62
Methionine	—	—	—	—	—
Isoleucine	41	41	35	40	41
Leucine	77	74	57	75	88
Tyrosine	21	24	11	13	16
Phenylalanine	33	38	32	29	43
Histidine	13	12	23	28	19
Lysine	20	21	39	20	45
Arginine	28	33	34	32	37

Table 2. *Amino acid compositions of small proteoglycans*

Amino acid	Amino acid composition (residues/1000 residues)					
	Cartilage (Heinegård *et al.*, 1981)	Aorta (S. Gardell, unpublished work)	Tendon (Vogel & Heinegård, 1985)	Sclera (Cöster & Fransson, 1981)	Cornea	Bone (Franzén & Heinegård, 1984)
Aspartic acid	129	136	145	123	136	128
Threonine	39	44	46	49	47	45
Serine	66	79	108	68	77	76
Glutamic acid	98	100	108	122	103	105
Proline	72	57	82	74	68	77
Glycine	71	82	102	84	83	86
Alanine	50	55	63	54	56	49
Cysteine	19	—	—	9	—	—
Valine	53	59	33	59	54	52
Methionine	1	—	—	7	—	—
Isoleucine	55	51	33	55	57	58
Leucine	143	137	112	115	125	128
Tyrosine	28	27	24	15	22	29
Phenylalanine	34	36	30	34	34	32
Histidine	36	30	26	25	30	31
Lysine	64	61	61	76	77	69
Arginine	44	46	27	32	31	36

The compositions of the large proteoglycans show large similarities, both with regard to amino acid compositions (Table 1) and with regard to high contents of galactosaminoglycans, primarily chondroitin sulphate. Furthermore, in some cases, a high content of *O*-linked oligosaccharides has also been demonstrated (Heinegård *et al.*, 1985*a*; Vogel & Heinegård, 1985). The agarose/polyacrylamide-gel electrophoresis of these indeed demonstrates further similarities (Fig. 1). All the samples appear to be slightly heterogeneous, containing components that differ somewhat in electrophoretic mobilities. The migration is, however, in all cases similar to that of the aggregating proteo-

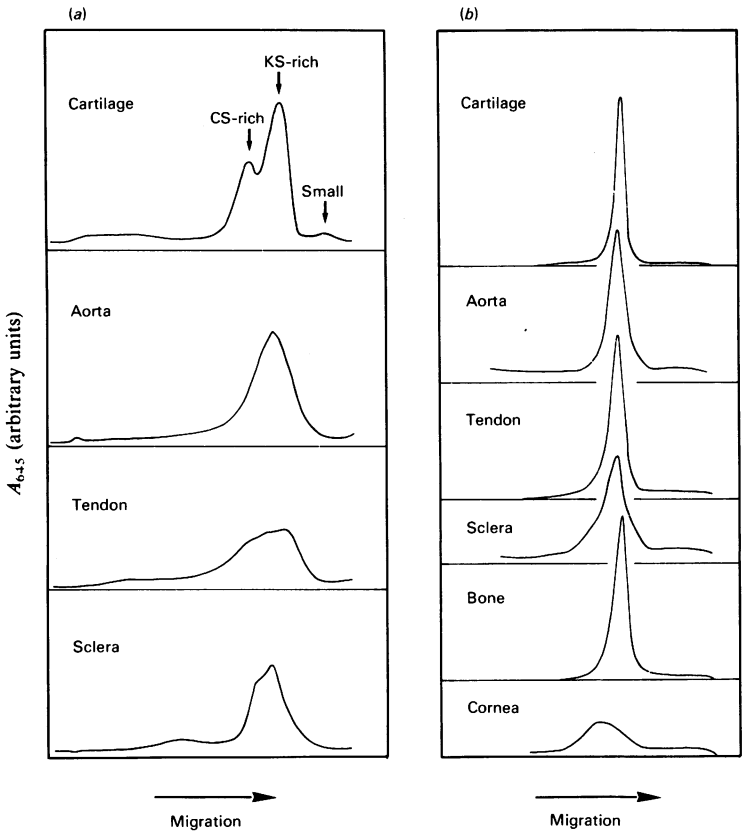


Fig. 1. *Agarose/polyacrylamide-gel electrophoresis of proteoglycans*

Large proteoglycans (*a*) and small proteoglycans (*b*) at amounts of 20 μg and 30 μg respectively were electrophoresed on agarose/polyacrylamide gels. The tissue of origin is indicated in the Figure. The sample of large proteoglycans from cartilage represents a mixture containing both chondroitin sulphate (CS)-rich, keratan sulphate (KS)-rich and small proteoglycans.

glycans from cartilage (Heinegård *et al.*, 1985*a*). Interestingly, the proportions of the two components vary somewhat from one tissue to another.

The compositions of the small proteoglycans are also rather similar within the group, but distinct from that of the large proteoglycans (Table 2). The small proteoglycans contain large galactosaminoglycan side chains, have a core protein with an apparent M_r of 45000–50000 and contain oligosaccharides. There are, however, also differences. Some, like the one from cartilage, do not contain sialic acid, and those from cartilage and aorta have somewhat higher leucine contents than the others, indicating the presence of subgroups of related small proteoglycans. Agarose/polyacrylamide-gel electrophoresis showed that all the small proteoglycans had a similar electrophoretic mobility (Fig. 1), although those from cornea gave a somewhat different pattern. It is known, however, that bovine corneal proteoglycans have a very low content of sulphate (Axelsson & Heinegård, 1975), possibly causing a higher degree of polydispersity with regard to charge density and therefore electrophoretic mobility.

In summary, the data suggest that there may be more than one type of small proteoglycans, although all are similar according to several structural parameters.

Large proteoglycans

Immunological cross-reactivity. To study the presence of subgroups of the proteoglycans further, an immunochemical approach was taken. It should be stressed that all antibodies used appear to be directed against determinants in the core protein, since digestion of the proteoglycans with papain eliminated their antigenicity. Antibodies were available to a mixture of the large cartilage proteoglycans (A1.D1 fraction), to the large sclera proteoglycans and to large aorta proteoglycans. Since the two aggregating proteoglycans from cartilage have been separated and purified (Heine-

gård *et al.*, 1985*a*), each could be used as the antigen in assays employing an antibody raised against a mixture. Altogether four assays, i.e. for the cartilage chondroitin sulphate-rich, for the cartilage keratan sulphate-rich, for the sclera large, and for the aorta large proteoglycans were developed, all showing good sensitivity (Fig. 2). Dilutions of the following antigens were tested for capacity to compete in all the assays: chondroitin sulphate-rich and keratan sulphate-rich proteoglycans from cartilage, large sclera, large aorta and large tendon proteoglycans as well as chondroitin sulphate peptides from cartilage proteoglycans. In most cases the preparations showed almost complete immunological identity, demonstrated by the similar slopes of the inhibition curves (Fig. 2). Notably, however, only the aorta proteoglycan reacted in the assay for this proteoglycan, indicating that these antibodies were directed against sites not present in the other proteoglycans. Interestingly, the aorta proteoglycan gave inhibition curves with a slope similar to that of the antigen used to raise the respective antibody in all the other assays, showing that the aorta proteoglycans also contain epitopes identical with those of the other proteoglycans. There were, however, also some other differences. The sclera proteoglycan, in the assay for cartilage proteoglycans, showed a different slope than the others, and the tendon proteoglycan in the assay for the one from sclera also gave a somewhat different slope. In summary, however, the striking feature of the large proteoglycans is that they appear to share a large proportion of the antigenic sites, showing that, at least in part, they are of similar structure. Indeed, they also appear to contain similar hyaluronic acid-

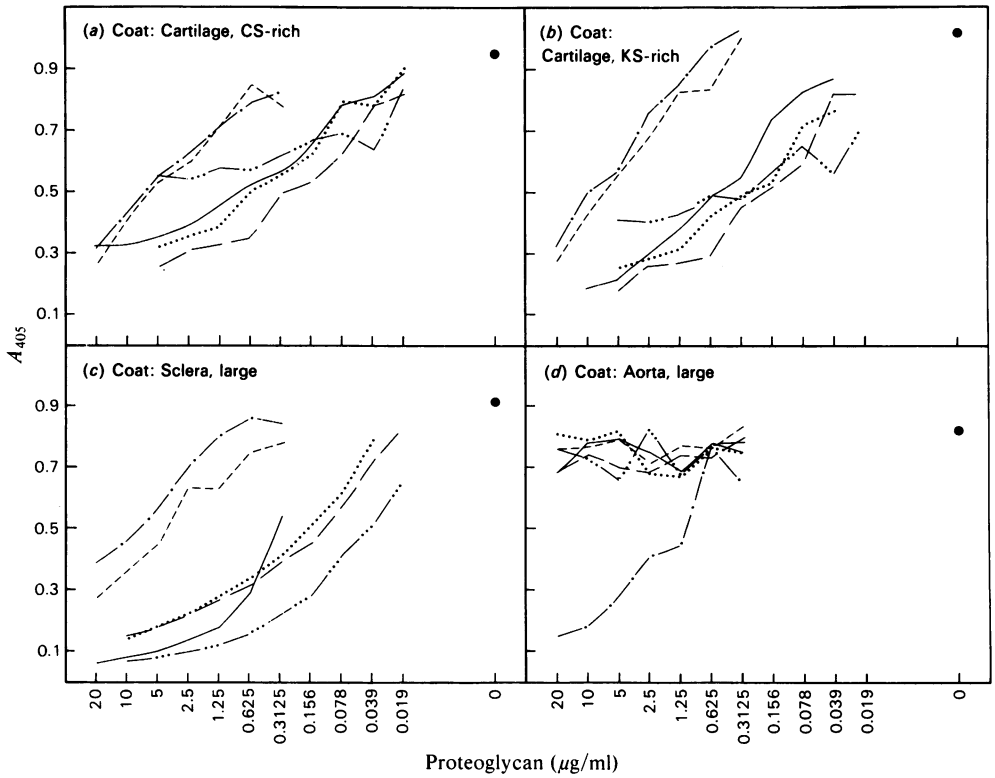


Fig. 2. *Immunological cross-reactivity of large proteoglycans*

Proteoglycans, i.e. cartilage, chondroitin sulphate-rich (*a*) at 1 μg/ml, cartilage keratan sulphate-rich (*b*) at 1 μg/ml, large sclera (*c*) at 1 μg/ml and large aorta (*d*) at 1 μg/ml, were coated on poly(vinyl chloride) micro-titre plates. First antibody, raised against aggregating proteoglycans from cartilage at a dilution of 1:1000 (*a* and *b*), raised against large sclera proteoglycan at a dilution of 1:1600 (*c*) or raised against the large aorta proteoglycan at a dilution of 1:100 (*d*), was used and the assays of dilutions of chondroitinase ABC digested proteoglycans were performed as described in the Materials and methods section. —·—·—, Aorta, large proteoglycan; ···—···—, sclera, large proteoglycan; ———, tendon, large proteoglycan; ·····, cartilage, chondroitin sulphate (CS)-rich proteoglycan; — — —, cartilage, keratan sulphate (KS)-rich proteoglycan; – – – – –, chondroitin sulphate peptides (A1.TA1.CB.6B3, in Heinegård & Axelsson, 1977) prepared from trypsin digests of cartilage proteoglycans. Zero inhibition is indicated by a ● symbol.

binding regions. Several of the preparations of large proteoglycans contain molecules capable of interacting with hyaluronic acid to form aggregates (Salisbury & Wagner, 1981; Heinegård *et al.*, 1985*a*; Vogel & Heinegård, 1985; S. Gardell, unpublished work). In support, an assay for the isolated hyaluronic acid-binding region of bovine nasal-cartilage proteoglycans gave parallel inhibition curves for all the antigens (Fig. 3), showing that the antigenicity and therefore the structure of this region is similar in the proteoglycans. The poor overall inhibition observed depends on the presence of antibodies against neoepitopes first exposed after isolation of the hyaluronic acid-binding region by trypsin digestion (Wieslander & Heinegård, 1979), seen in the present study with proteoglycans from nasal cartilage.

Peptide pattern. In the enzyme-linked immunosorbent assay only some structures in the molecules, i.e. those that are antigenic, will be detected. Therefore, although the extensive cross-reactivity shows that portions of the protein core are similar from one molecule to the other, there may nevertheless be other less antigenic regions of the protein core that are dissimilar. A more complete comparison of regions of the protein core of proteoglycans can, however, be obtained by way of studies of peptide patterns after proteinase treatment of the proteoglycan. The low content of protein, combined with the high content of strongly polyanionic glycosaminoglycans of proteoglycans, does, however, present difficulties in traditional peptide separations by thin-layer high-voltage electrophoresis followed by t.l.c. In order to detect the peptides by staining with ninhydrin, such large amounts of material have to be applied that separations are much impaired. Therefore in the present study we chose to label proteoglycans with ^{125}I and limit the study to such radioactive peptides that could be detected with autoradiography. The large number of labelled peptides observed, however (see below), should make the patterns representative. The sensitivity in this case is increased several thousandfold, and only a few micrograms of material is required for the whole procedure, including labelling with chloramine-T.

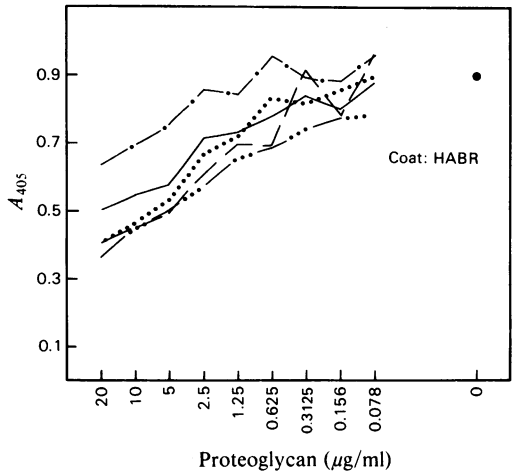


Fig. 3. *Immunoassay of hyaluronic acid-binding region in proteoglycans*

Bovine nasal-cartilage proteoglycan hyaluronic acid-binding region (HABR) at 0.1 μg/ml was coated on poly(vinyl chloride) micro-titre plates. Dilutions of chondroitinase ABC-digested proteoglycans were assayed for contents of hyaluronic acid-binding region by using an antibody (dilution 1:20000) raised against isolated hyaluronic acid-binding region. Curve forms are as defined in Fig. 2 legend.

The two populations of aggregating cartilage proteoglycans gave similar peptide patterns, although a few peptides differed indicating minor structural differences (Fig. 4). Some of the peptides in both preparations were derived from the hyaluronic acid-binding region, shown for reference (Fig. 4). Interestingly, many of the same peptides were also identified in the large proteoglycans from sclera and tendon, corroborating the structural similarity of these molecules (Fig. 5). The large aorta proteoglycan showed a very different pattern (Fig. 4), as would be expected from the immunochemical data. The presence of a structurally very similar hyaluronic acid-binding region in these proteoglycans was also demonstrated, as indicated schematically in Fig. 5, where the blackened spots represent such peptides. The large non-aggregating proteoglycans from cartilage showed an entirely different peptide pattern, indicating their different nature.

The data, then, indicate that large proteoglycans containing both a hyaluronic acid-binding region and other related peptide sequences are found in many tissues. There are, however, also differences, most markedly in relative quantities of the peptides. These different proportions of identical peptides in the proteoglycans may indicate that there are repetitive sequences of the core protein or, simply, may represent an artifact of the labelling procedure used.

Small proteoglycans

Immunological cross-reactivity. Antibodies directed against the small cartilage, the small tendon, the small sclera and the bone proteoglycans were available and were used to develop sensitive enzyme-linked immunosorbent assay for the respective proteoglycan (Fig. 6). It is of note that none of the large proteoglycans react in the assay for the small proteoglycan from the same tissue.

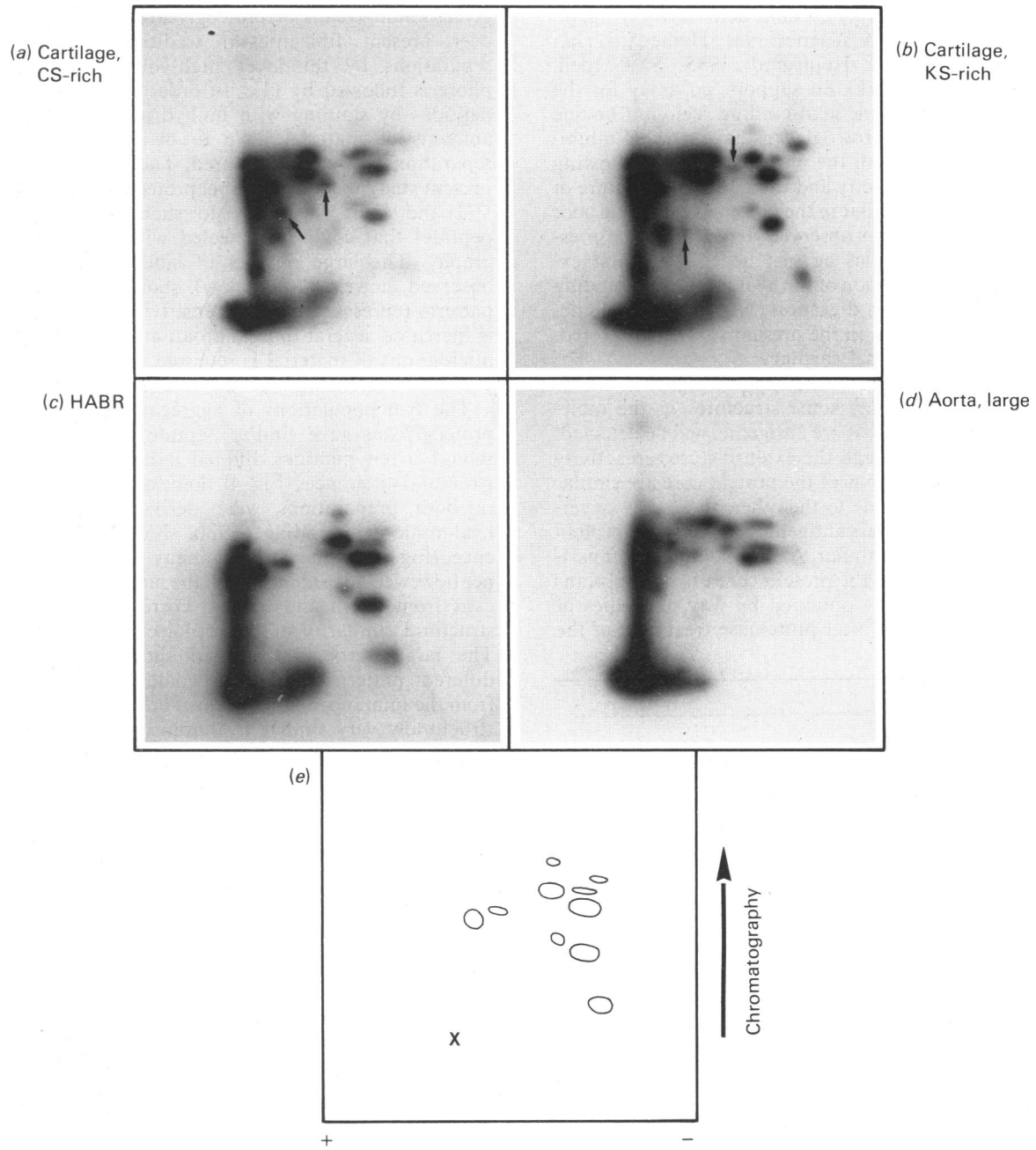


Fig. 4. *Peptide patterns of trypsin-digested* ^{125}*I-labelled large proteoglycans from cartilage and aorta*
Peptide patterns of trypsin-digested proteoglycans were developed on thin-layer plates of silica gel essentially as described by Bates & Perham (1975). The + and − signs denote polarity during electrophoresis and the X denotes the starting point. The samples were (*a*) cartilage, chondroitin sulphate (CS)-rich, (*b*) cartilage, keratan sulphate (KS)-rich, (*c*) hyaluronic acid-binding region (HABR) for reference and (*d*) aorta, large proteoglycans. Those peptides present in the isolated hyaluronic acid-binding region and identifiable in the other proteoglycans are schematically shown in (*e*). The arrows indicate peptides only present in one of the cartilage proteoglycans.

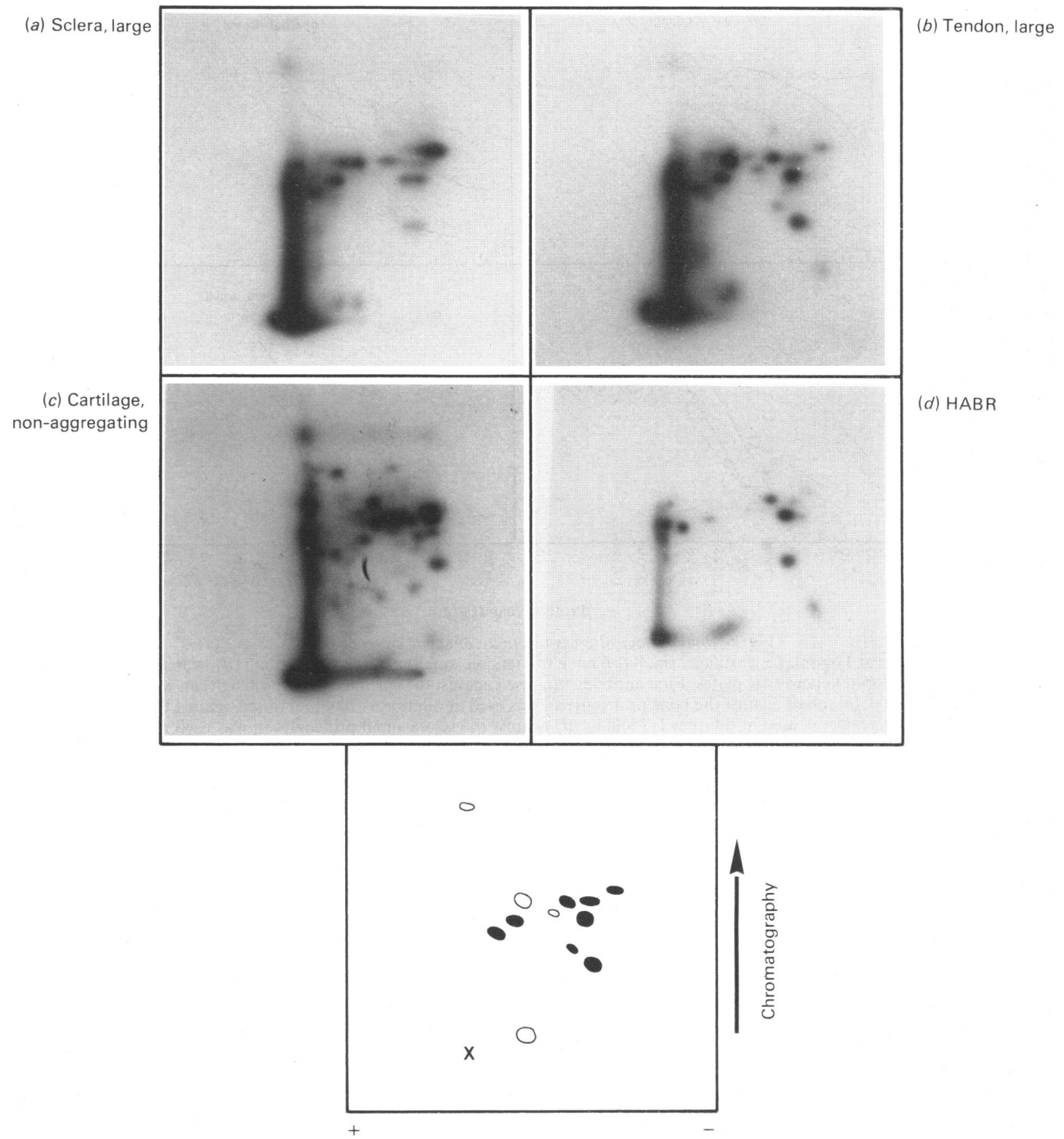


Fig. 5. *Peptide patterns of trypsin-digested ^{125}I-labelled large proteoglycans from sclera, tendon and cartilage, non-aggregating*

Peptide patterns of trypsin-digested proteoglycans were developed on thin-layer plates of silica gel essentially as described by Bates & Perham (1975). The + and − signs denote polarity during electrophoresis and the X denotes the starting point. The samples were (*a*) sclera, large, (*b*) tendon, large, (*c*) cartilage, non-aggregating, proteoglycans and (*d*) isolated hyaluronic acid-binding region (HABR) for reference. Those peptides identified in both the sclera and the tendon proteoglycan and common to cartilage, aggregating, proteoglycans are schematically shown in (*e*), where the filled spots indicate those peptides derived from the hyaluronic acid-binding region.

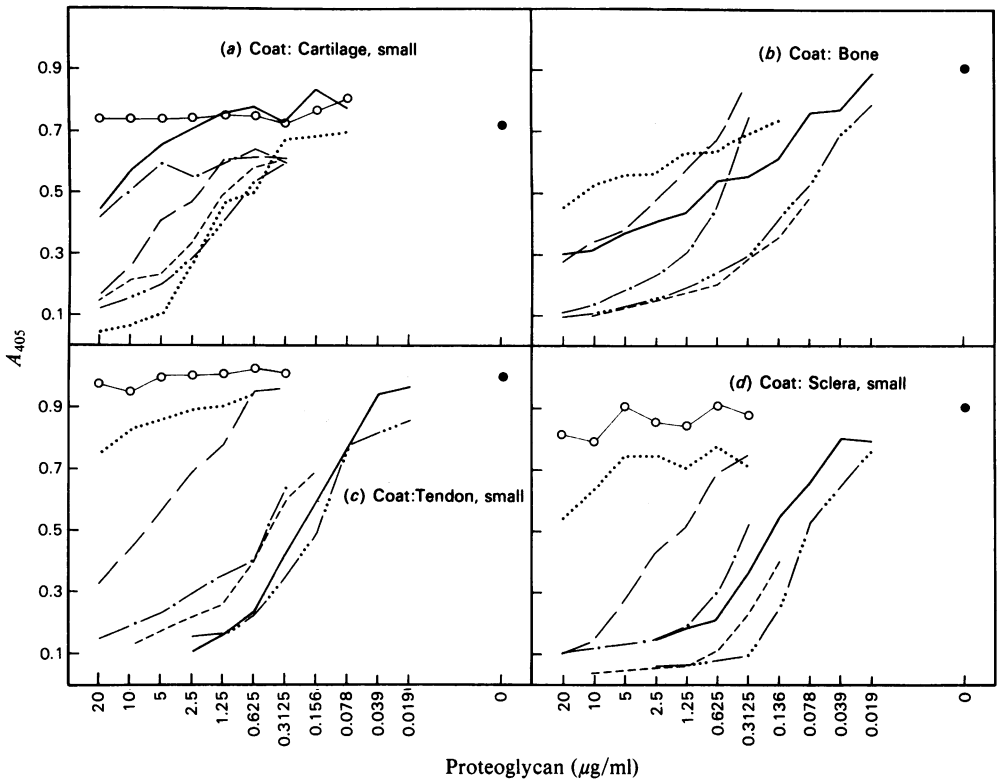


Fig. 6. *Immunological cross-reactivity of small proteoglycans*
Proteoglycans at 1 μg/ml, i.e. cartilage, small (*a*), bone (*b*), tendon, small (*c*), and sclera, small (*d*), were coated on poly(vinyl chloride) micro-titre plates. First antibody (*a*) raised against the cartilage small proteoglycan was used at dilution 1:1500, (*b*) raised against the bone proteoglycan was used at dilution 1:2000; (*c*) raised against the tendon small proteoglycan was used at dilution 1:2500, or (*d*) against the sclera small proteoglycan was used at dilution 1:1600. The assays for dilutions of chondroitinase ABC-digested small proteoglycans were done as described in the Materials and methods section. Proteoglycans were:·····, cartilage, small; — — —, aorta, small; ———, tendon, small; —·—·—, cornea, dermatan sulphate proteoglycan referred to as 50 P (Axelsson & Heinegård, 1975); —···—···, sclera, small; – – – – –, bone. For reference, dilutions of chondroitinase ABC-digested large proteoglycans (○—○) from cartilage (*a*), from tendon (*c*) and from sclera (*d*) are included. Zero inhibition is indicated by a ● symbol.

Neither do any of the small proteoglycans react in an assay for hyaluronic acid-binding region (results not shown). It is apparent, then, that the small proteoglycans are quite different in nature, further verified by peptide patterns, discussed below. All of the small proteoglycans tested, i.e. cartilage, aorta, tendon, cornea, sclera and bone, showed capacity to inhibit in all assays (Fig. 6). Except for the one from cartilage, the inhibition curves had similar slopes indicating immunological identity. Those from bone, cornea, sclera and tendon showed very similar inhibition on a weight basis, but the one from aorta gave less than 10% of this inhibition, indicating that only a small proportion of the molecules carried the antigenic determinant. In the assay for the small cartilage proteoglycan, however, the aorta proteoglycan gave a similar degree of inhibition, whereas the other proteoglycans gave less pronounced inhibition. Taken together, these data indicate that there are at least two groups of closely related small proteoglycans, on one hand those from aorta and cartilage and on the other hand those from bone, cornea, sclera and tendon.

Peptide pattern. Further support for the presence of two groups of molecules was obtained from the peptide patterns (Figs. 7 and 8). The small proteoglycans from cartilage and aorta gave very similar patterns (Fig. 7), with at least ten peptides found in both, schematically shown in the Figure. On the other hand, the patterns observed for the small proteoglycans from bone, cornea, sclera and tendon were rather similar to each other but quite distinct from those of aorta and cartilage proteo-

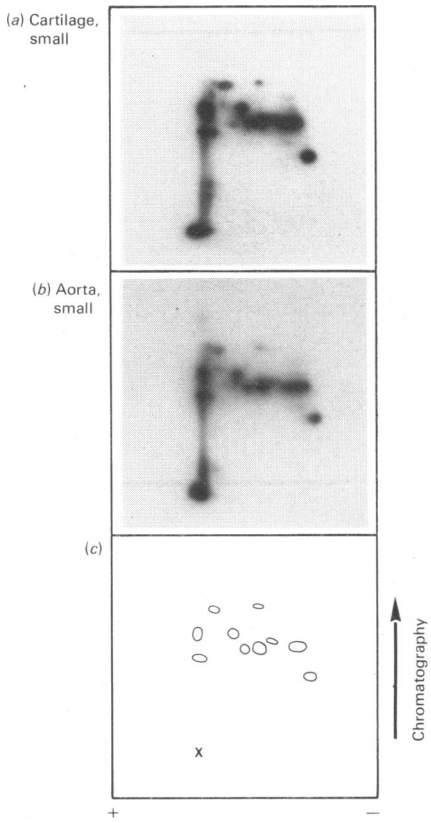


Fig. 7. *Peptide patterns of trypsin-digested ^{125}I-labelled small proteoglycans from cartilage and aorta*

Peptide patterns of trypsin-digested proteoglycans were developed on thin-layer plates of silica gel essentially as described by Bates & Perham (1975). The + and − signs denote polarity during electrophoresis and the X denotes the starting point. The samples were (*a*) cartilage, small, and (*b*) aorta, small proteoglycans. For reference those peptides identified in both preparations are schematically shown in (*c*).

glycans. In this case also more than ten peptides were found to be present in all four preparations. All the peptide patterns of the small proteoglycans were different from those of the large proteoglycans.

General discussion

Both the immunological cross-reactivities and the chemical data from the peptide mapping show that there are two groups of small proteoglycans, the molecules within each group having very similar core proteins. Interestingly, chemical data show that the small proteoglycan from cartilage and aorta have somewhat higher leucine contents than do the other ones. The chemical differences are consistent with the grouping of the molecules in two classes. It is known, however, that they differ with respect to glycosaminoglycan substituent, being either chondroitin sulphate in the cartilage (Heinegård *et al.*, 1981) and in the bone (Franzén & Heinegård, 1984) proteoglycans or dermatan sulphate with high contents of iduronic acid in the aorta (S. Gardell, unpublished work), sclera (Cöster & Fransson, 1981) and tendon (Vogel & Heinegård, 1985) proteoglycans, and the corneal proteoglycan contains sparsely sulphated dermatan sulphate chains with an intermediate iduronic acid content (Axelsson & Heinegård, 1975). All of these proteoglycans contain very large galactosaminoglycan side chains compared with the large proteoglycans. It appears that it is not the structure of the core protein that determines the type of side chain, but rather the synthetic machinery of the cell of origin, possibly its content of the necessary epimerase.

To probe further the identity of the core proteins of the proteoglycans in the various groups, sequence data or at least quantitative peptide patterns representing all peptides are required. The presented data, however, do allow us to suggest that there are classes of proteoglycans with similar core proteins, containing side-chain substituents of either chondroitin sulphate or dermatan sulphate. It appears that the core proteins in the molecules of a class are not identical, only closely similar.

Since much of the character of the proteoglycan is determined by the core protein, we suggest a nomenclature for these proteoglycans (Table 3), stating group, PG-LA (large aggregating) for the large proteoglycans and PG-Sm (small) for the small ones, possibly subdividing the latter into two groups, PG-SmI and PG-SmII representing the type found in cartilage and aorta (PG-SmI) and those in bone, cornea, sclera and tendon (PG-SmII), tissue of origin and possibly nature of glycosaminoglycan side chains, where all chains containing iduronic acid are called dermatan sulphate.

The identification of large aggregating proteoglycans in many tissues is interesting. It is possible that a major difference in structure between those from cartilage and those from fibrous connective tissues is the relative proportion between *O*-linked oligosaccharides and keratan sulphate. Only the cartilage proteoglycans have to date been conclusively shown to contain keratan sulphate. Interestingly, the proportion of large proteoglycans is higher in tissues exposed to compressive load, i.e. cartilage, distal compared with proximal flexor tendon (Vogel & Heinegård, 1985) and aorta. It is possible that the synthesis of these proteoglycans increases in response to mechanical forces.

Another interesting group of proteoglycans are

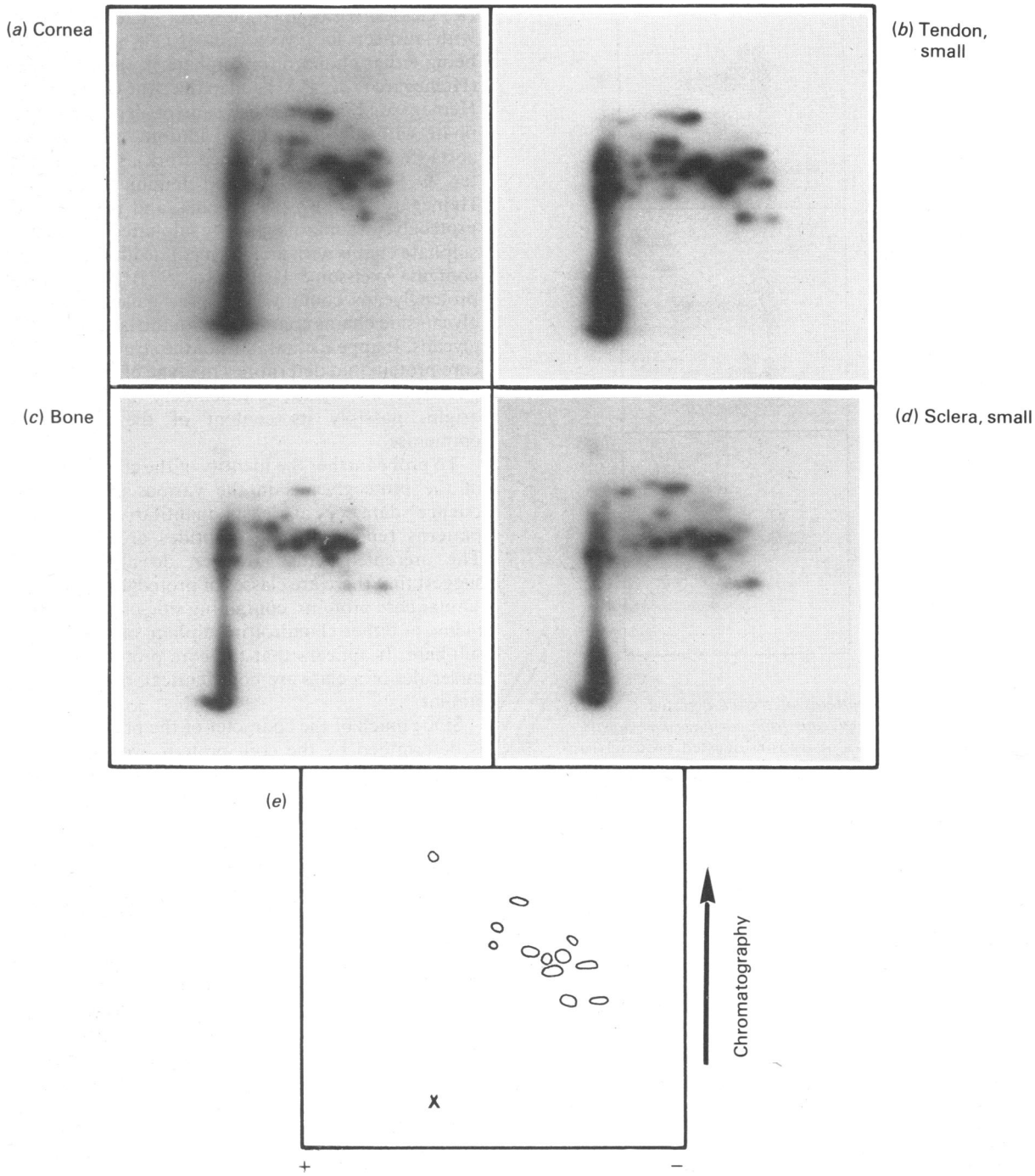
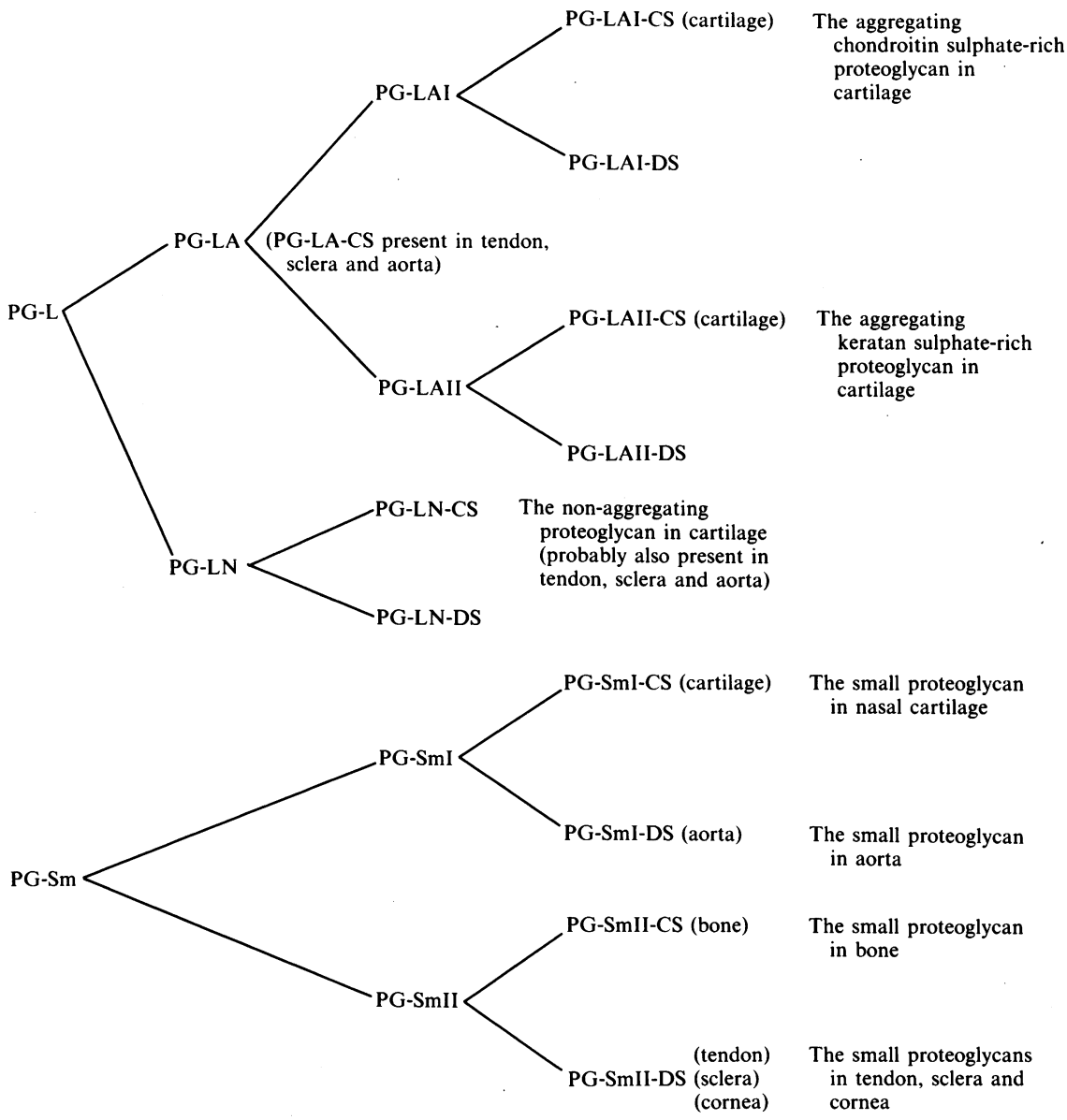


Fig. 8. *Peptide patterns of trypsin-digested ^{125}I-labelled small proteoglycans from cornea, tendon, bone and sclera*
Peptide patterns of trypsin-digested proteoglycans were developed on thin-layer plates of silica gel essentially as described by Bates & Perham (1975). The + and − signs denote polarity during electrophoresis and the × denotes the starting point. The samples were (*a*) cornea, dermatan sulphate proteoglycan, referred to as 50 P (Axelsson & Heinegård, 1975), (*b*) tendon, small proteoglycan, (*c*) bone, major proteoglycan, and (*d*) sclera, small proteoglycan. Those peptides identified in all four samples are schematically shown in (*e*).

Table 3. *Suggested nomenclature for interstitial galactosaminoglycan-containing proteoglycans*
Proteoglycans that have not been subgrouped should be referred to as PG-L (large) and PG-Sm (small) respectively. Subgroups can be introduced by adding numbers as indicated.



the large non-aggregating proteoglycans identified in nasal cartilage (Heinegård & Hascall, 1979). Problems encountered in identifying this group of proteoglycans in other tissues are different stabilities of the interaction of different proteoglycans with hyaluronate, possibly an effect of minor structural differences perhaps involving disulphide bridges, as well as other modifications of the proteoglycans in the matrix. Furthermore, it is likely that the cartilage non-aggregating proteoglycan is a mixture of distinct molecules with fragments of the aggregating proteoglycans. The identification of this type of proteoglycan in fibrous connective tissues requires better techniques for their fractionation.

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References

Anderson, J. C. (1975) *Biochim. Biophys. Acta* **379**, 444–455

Axelsson, I. & Heinegård, D. (1975) *Biochem. J.* **145**, 491–500

Axelsson, I. & Heinegård, D. (1980) *Exp. Eye Res.* **31**, 57–66

Bates, D. L. & Perham, R. N. (1975) *Anal. Biochem.* **68**, 175–184

Cöster, L. & Fransson, L.-Å. (1981) *Biochem. J.* **193**, 143–153

Damle, S. P., Cöster, L. & Gregory, J. D. (1982) *J. Biol. Chem.* **257**, 5523–5527

Engvall, E. & Perlman, P. (1971) *Immunochemistry* **8**, 871–874

Franzén, A. & Heinegård, D. (1984) *Biochem. J.* **224**, 59–66

Greenwood, F. C., Hunter, W. M. & Glover, J. S. (1963) *Biochem. J.* **89**, 114–123

Hardingham, T. E. & Muir, H. (1972) *Biochim. Biophys. Acta* **279**, 401–405

Hassel, J. R., Robey, P. G., Barrach, H.-J., Wilczek, J., Rennard, S. I. & Martin, G. R. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4494–4498

Heinegård, D. & Axelsson, I. (1977) *J. Biol. Chem.* **252**, 1971–1979

Heinegård, D. & Hascall, V. C. (1974) *J. Biol. Chem.* **249**, 4250–4256

Heinegård, D. & Hascall, V. C. (1979) *J. Biol. Chem.* **254**, 927–934

Heinegård, D. & Paulsson, M. (1984) in *Extracellular Matrix Biochemistry* (Piez, K. & Reddi, H., eds.), pp. 278–328, Elsevier, New York

Heinegård, D., Paulsson, M., Inerot, S. & Carlström, C. (1981) *Biochem. J.* **197**, 355–366

Heinegård, D., Wieslander, J., Sheehan, J., Paulsson, M. & Sommarin, Y. (1985*a*) *Biochem. J.* **225**, 95–106

Heinegård, D., Inerot, S. & Lindblad, G. (1985*b*) *Scand. J. Lab. Clin. Invest.* in the press

Kjellén, L., Pettersson, I. & Höök, M. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5371–5375

Lohmander, S., DeLuca, S., Nilsson, B., Hascall, V. C., Caputo, C. B., Kimura, J. H. & Heinegård, D. (1980) *J. Biol. Chem.* **255**, 6084–6091

McDevitt, C. & Muir, H. (1971) *Anal. Biochem.* **44**, 612–622

Oldberg, Å., Kjellén, L. & Höök, M. (1979) *J. Biol. Chem.* **254**, 8505–8510

Pearson, C. H. & Gibson, G. J. (1982) *Biochem. J.* **201**, 27–37

Salisbury, B. G. J. & Wagner, W. D. (1981) *J. Biol. Chem.* **256**, 8050–8057

Uldbjerg, N., Malmström, A., Ekman, G., Sheehan, J., Ulmsten, U. & Wingerup, L. (1983) *Biochem. J.* **209**, 497–503

Wieslander, J. & Heinegård, D. (1979) *Biochem. J.* **179**, 35–45

Vogel, K. & Heinegård, D. (1985) *J. Biol. Chem.* in the press