

Proteoglycans isolated from dissociative extracts of differently aged human articular cartilage: characterization of naturally occurring hyaluronan-binding fragments of aggrecan

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Proteoglycans extracted with 4 M guanidinium chloride from young (mean 20 years) or old (mean 79 years) macroscopically normal human articular cartilage were separated by density gradient centrifugation and Q-Sepharose chromatography and characterized by gradient gel SDS/PAGE and immunodetection before and after removal of glycosaminoglycan chains. The extracts contained two large populations of aggrecan, a population of small N-terminal aggrecan fragments, as well as decorin, biglycan and fibromodulin. The distribution of all these species in density gradient fractions has been determined. The large aggrecan populations comprised four different chondroitin sulphate-bearing core proteins while the population of smaller fragments comprised eight different components. The two smallest fragments (35 and 42 kDa), identified as the first globular domain of aggrecan (N-terminal) (G1) and containing no glycosaminoglycan, were detected only in extracts of old cartilage. A

55 and a 70 kDa fragment of G1 were present in both keratan sulphate-containing and non-keratan sulphate-containing forms. Four other fragments, each containing keratan sulphate epitopes, were identified and these contained either G1 epitopes (one 95 kDa species), or G1 and G2 epitopes (three species). These results have suggested that proteolytic processing at the N-terminus is more extensive than has previously been recognized and raises the possibility that more than one proteinase may be involved in aggrecan degradation *in vivo*. With the exception of the two smallest G1 fragments, the repertoire of proteoglycan fragments found in young and old human articular cartilage is essentially the same, although the relative abundance of various species differed. The older tissue contains a larger proportion of C-terminally truncated aggrecan fragments and a significantly decreased content of decorin and biglycan.

INTRODUCTION

Aggrecan is a major structural component of cartilage and represents 5–10% of tissue wet weight [1]. The heterogeneity of aggrecan that is observed in extracts of human articular cartilage [2–4] is thought to result from proteolytic processing of the molecule, and, indeed, specific cleavage sites located within the interglobular domain [5–9] and the chondroitin sulphate domain [10,11] have been detected. Proteolytic processing in the interglobular domain between the first globular domain of aggrecan (N-terminal) (G1) and the second globular domain of aggrecan (G2) is a key event in aggrecan turnover. It releases large chondroitin sulphate-containing fragments that are rapidly lost from the tissue [12], and small G1 fragments that retain hyaluronan-binding capacity and accumulate with age *in vivo* [13,14]. The presence of hyaluronan-binding G1 fragments in cartilage has been well documented [13,15,16]; however they have not previously been characterized in terms of their relative size or number.

The aim of the present study is to identify and characterize proteoglycans and proteoglycan fragments that require dissociative conditions for extraction (approximately 70%) and to compare the relative abundance of the different species in old and young tissue. The results provide new information on the range of G1-containing aggrecan products that accumulate in cartilage with age and indicate that aggrecan turnover may result from the

actions of more than one proteinase, or a proteinase with more than one catalytic activity.

EXPERIMENTAL

Tissue and materials

Full-depth slices of human articular cartilage were removed from femoral heads of six cadavers of individuals aged 18, 22, 67, 77, 83, and 90 years, within 12 h of death. All specimens appeared macroscopically normal. Tissues were stored at -60°C until processing. Hyaluronan from human umbilical cord was from Sigma, St. Louis, MO, U.S.A. Chondroitinase ABC was from ICN, Costa Mesa, CA, U.S.A. and *endo*- β -galactosidase (*Bacteroides fragilis*; EC 3.2.1.103) was from Boehringer, Mannheim, Germany. All other chemicals and materials have been described elsewhere [17].

Extraction of proteoglycans

Prior to extraction specimens were divided into age groups designated young (18 and 22 year-old specimens) and old (68, 77, 83, 90 year-old specimens). The two pools were extracted and analysed separately. Samples were sectioned at 20 μm on a freezing cryostat and 10 g portions of each pool were extracted with PBS in the presence of proteinase inhibitors (5 mM phenylmethanesulphonyl fluoride/5 mM benzamide/5 mM 6-amino-

Abbreviations used: G1, first globular domain of aggrecan (N-terminal); G2, second globular domain of aggrecan; GuHCl/SA, 4 M guanidinium chloride/50 mM sodium acetate, pH 5.8; mAb, monoclonal antibody.

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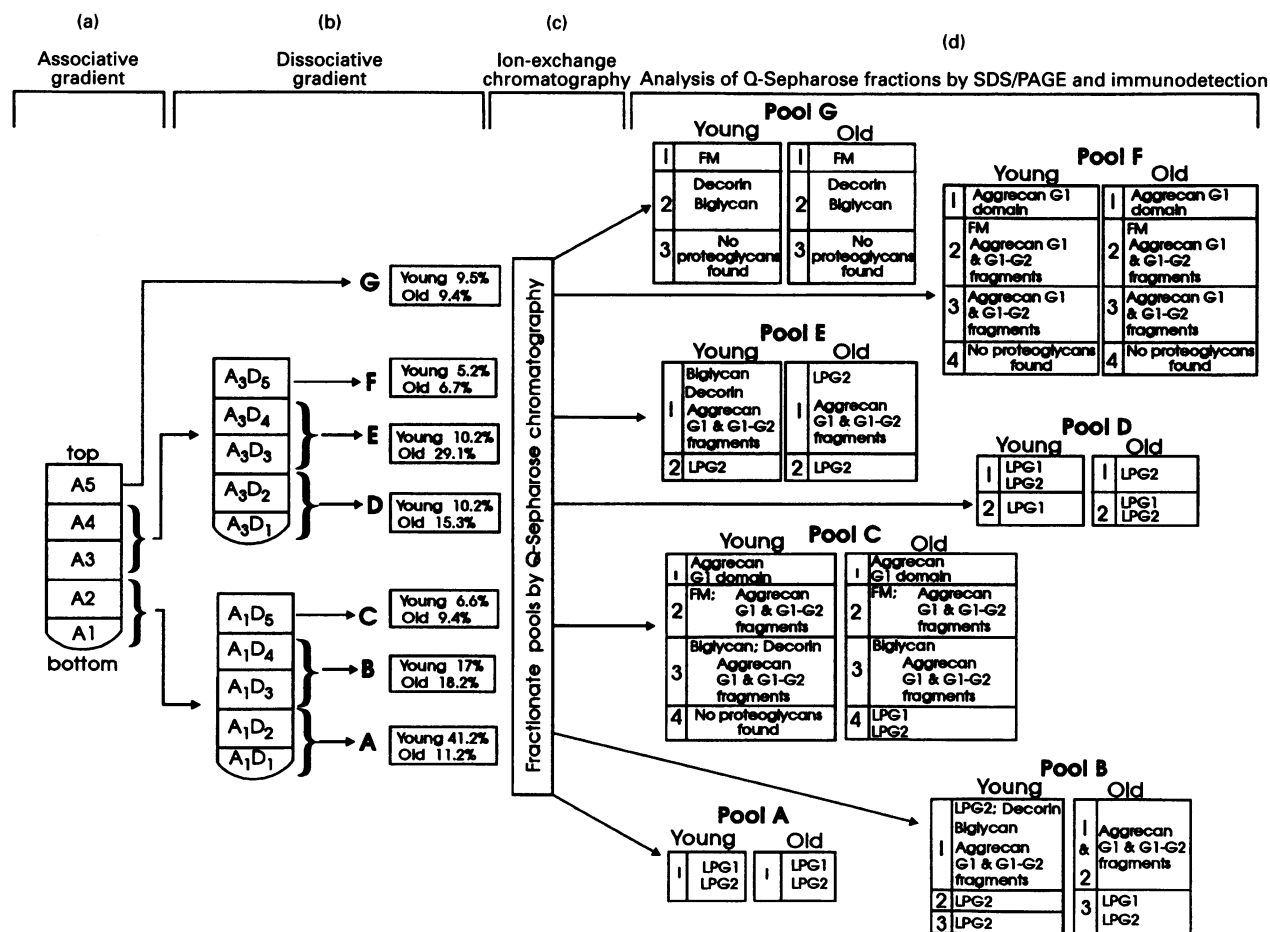


Figure 1 Schema of the experiment

Schema of the fractionation of proteoglycans from 4 M GuHCl/SA extracts of human articular cartilage by successive density gradient centrifugations under associative (a) and dissociative (b) conditions. Yields in pools separated by centrifugation are given as the percentage of total dry weight extracted (boxed values in b). The presence of proteoglycans and their fragments in the different fractions separated by Q-Sepharose chromatography of density gradient-separated pools is summarized in part (d). This part presents qualitative results only; for quantitative differences between young and old cartilage, refer to the text. FM, fibromodulin; LPG1 and LPG2, large populations of aggrecan.

hexanoic acid/5 mM *N*-ethylmaleimide/10 mM EDTA). After PBS extraction the tissue was recovered by centrifugation and washed, then re-extracted with 4 M guanidinium chloride (GuHCl)/50 mM sodium acetate (SA), pH 5.8, with proteinase inhibitors. Residual tissue remaining after extraction was solubilized with papain [18].

Density gradient centrifugations and Q-Sepharose chromatography

Hyaluronan (50 µg/ml) was added to proteoglycans in the GuHCl/SA extracts and the extracts were dialysed at 4 °C for 24 h against 9 vol. of 0.1 M sodium acetate, pH 6.8, containing proteinase inhibitors. The dialysed extracts were adjusted to 1.5 g/ml by the addition of solid CsCl [19] and centrifuged at 200000 *g* in a VTi50 vertical rotor (Beckman) at 8 °C for 24 h. The resulting gradients were fractionated into five equal parts designated A₁ to A₅ from the bottom of the tubes (Figure 1). Aliquots of each fraction were dialysed against distilled water and assayed for sulphated glycosaminoglycans and protein (Figure 2). The A₅ fraction at the top of the gradient was dialysed and freeze-dried while the two bottom fractions (A₁+A₂) and the two middle fractions (A₃+A₄) were pooled for further fractionation by dissociative gradient density centrifugation. The

pools were adjusted to dissociative conditions by the addition of solid GuHCl to 4 M and the density was adjusted to 1.5 g/ml for centrifugation as before. The dissociative gradients were divided into five equal parts designated D₁ to D₅, assayed for sulphated glycosaminoglycan and protein (Figure 2), and the two bottom fractions (D₁+D₂) and the two middle fractions (D₃+D₄) were pooled. The resulting pools were designated by capital letters as indicated in Figure 1. Each pool was dialysed against distilled water, freeze-dried and weighed. Further fractionation of proteoglycans in these pools was achieved by chromatography on Q-Sepharose eluted with a linear salt gradient from 0.1 to 1 M NaCl in a buffer that contained 7 M urea [17]. Fifty milligrams of dry material was applied to the Q-Sepharose column unless otherwise stated. Fractions pooled after Q-Sepharose chromatography were dialysed against distilled water, freeze-dried and weighed. Q-Sepharose-separated pools were designated by numbers, as indicated in Figure 3.

Gel electrophoresis and immunodetection

SDS/PAGE was performed as described [20] using either 1.6–16% or 4–16% gradient gels [21]. Stains-all dye was prepared by dissolving 10 mg of dye in 5 ml of formamide, adding 35 ml

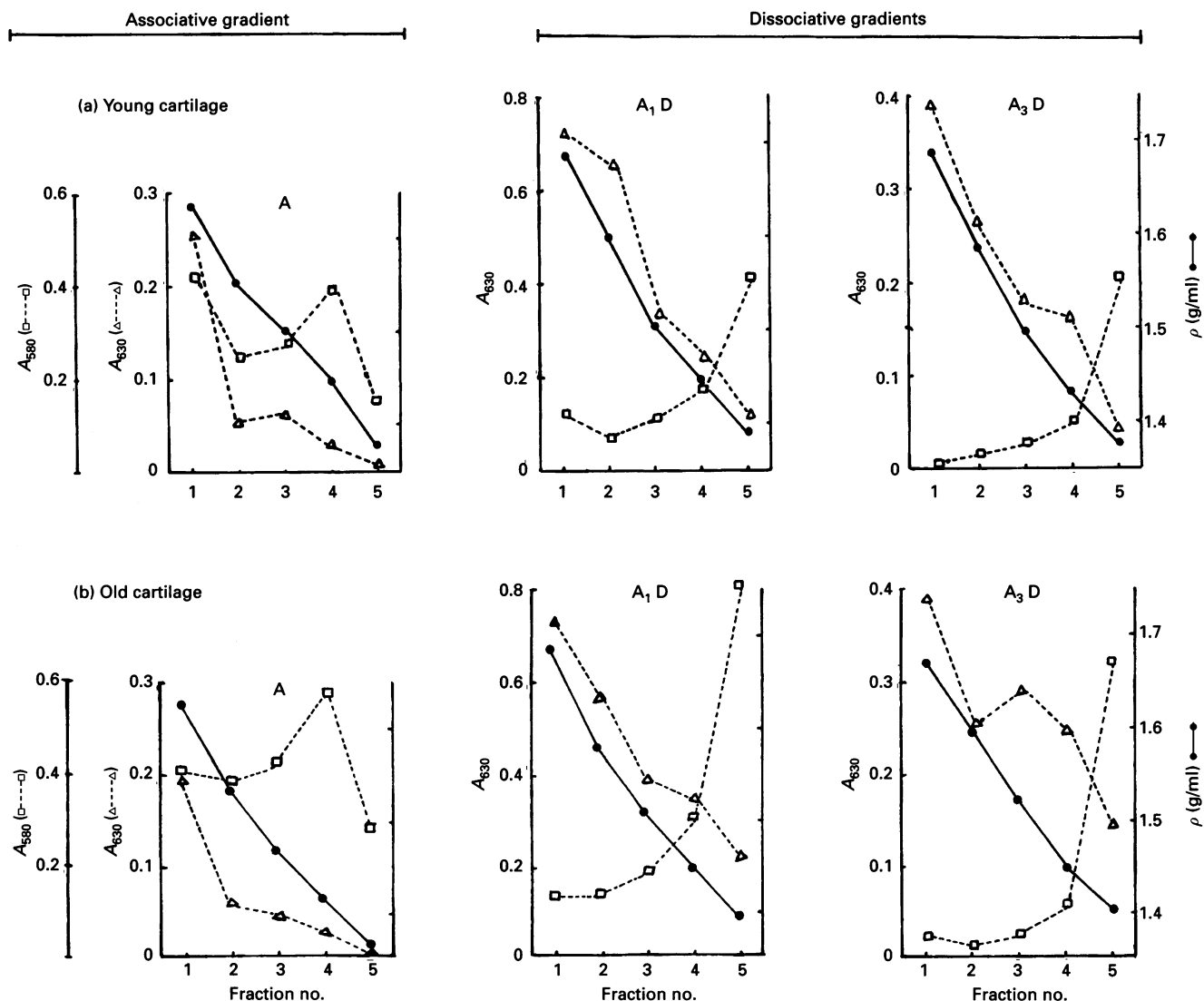


Figure 2 CsCl-density gradient centrifugation of GuHCl/SA extracts of human articular cartilage

Proteoglycans present in GuHCl/SA extracts of human articular cartilage derived from young (a) or old (b) adults were fractionated by centrifugation under associative conditions (A) in the presence of exogenous hyaluronan (50 $\mu\text{g}/\text{ml}$). Fractions $A_1 + A_2$ and $A_3 + A_4$ were pooled as shown in Figure 1 and re-centrifuged under dissociative conditions (A_1D and A_3D , respectively). Aliquots of fractions were dialysed against distilled water and assayed for sulphated glycosaminoglycan (A_{630}) and protein content (A_{580}). The dissociative fractions were pooled as shown in Figure 1.

of isopropanol and 1 ml of 3 M Tris/HCl, pH 8.8, and making the volume to 200 ml with distilled water. Gels were stained overnight after removing SDS, and destained in 40% methanol (v/v)/5% glycerol (v/v)/0.1 M Tris/HCl, pH 8.8, then air-dried between two cellophane sheets. All handling of Stains-all stained gels was done in the dark. The gels were loaded with equal amounts of sulphated glycosaminoglycan and therefore the relative amounts of material in the different pools could not be directly compared.

Immunodetection with monoclonal 5-D-4 (anti-keratan sulphate), monoclonal 3-B-3 that recognizes neo-epitopes generated by chondroitinase ABC digestion of chondroitin 6-sulphate, polyclonal LF-15 (anti-biglycan), polyclonal LF-30 (anti-decorin), polyclonal anti-G2 domain and polyclonal anti-fibromodulin has been described previously [17]. A polyclonal anti-G1 antiserum raised against a purified G1-domain of human

aggrecan was a gift from Dr. Tim Hardingham (Kennedy Institute of Rheumatology, London, U.K.).

Enzyme treatments and analytical procedures

Chondroitinase ABC digestion (0.01 units/100 μg of proteoglycan) was done in 50 mM Tris/acetate buffer, pH 8.0, at 37 $^{\circ}\text{C}$ for 6 h. *Endo*- β -galactosidase digestion (0.005 units/100 μg of proteoglycan) was done in a 50 mM sodium acetate, pH 5.8, at 37 $^{\circ}\text{C}$ overnight. The buffers contained 0.2 mg/ml BSA, 10 mM EDTA, 10 mM *N*-ethylmaleimide and 5 mM phenylmethane-sulphonyl fluoride. The digests were stopped by adding SDS/PAGE sample buffer and boiling for 5 min. The concentration of sulphated glycosaminoglycans was determined using the 1,9-Dimethylmethylene Blue assay [18,22] with chondroitin sulphate

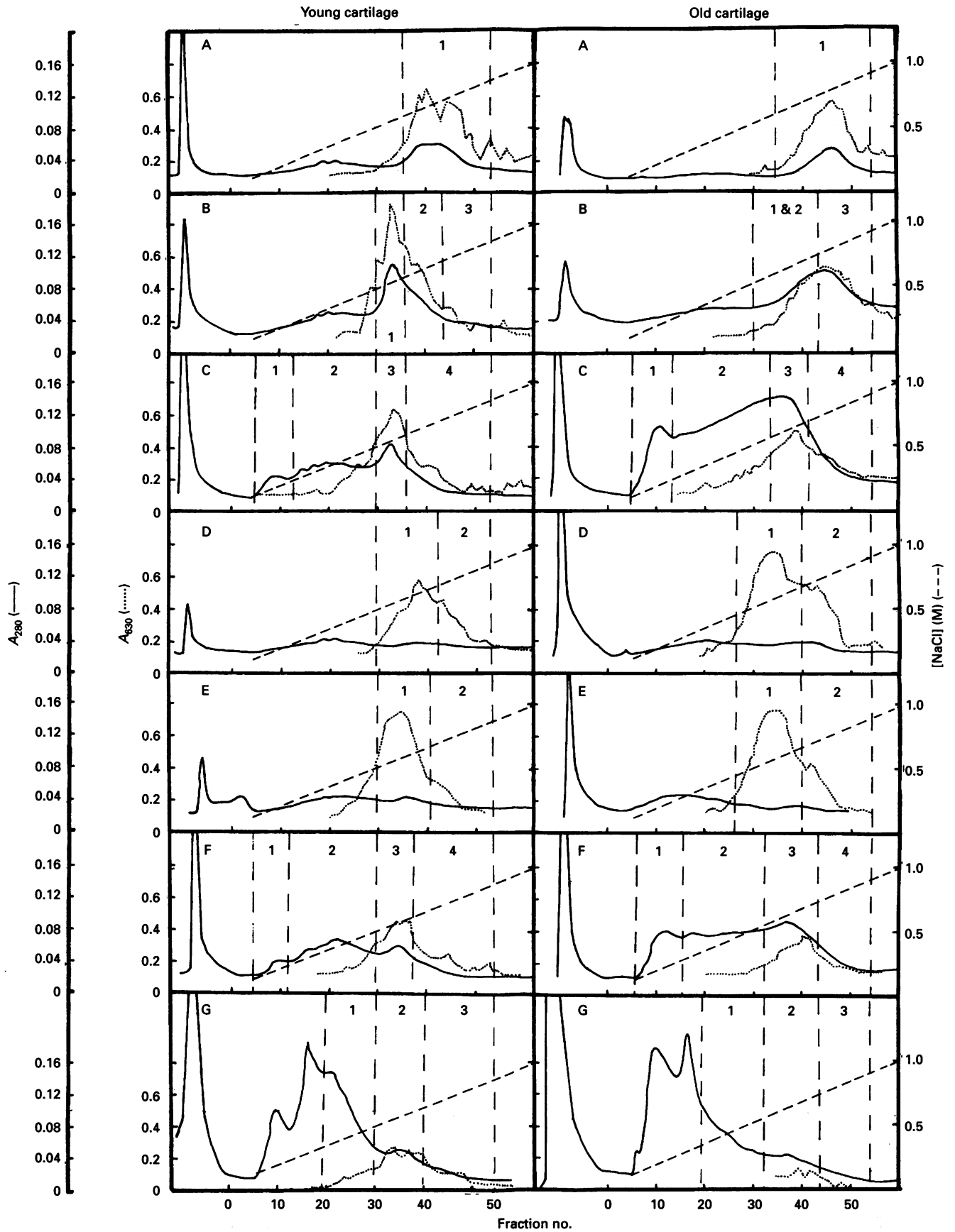


Figure 3 For legend see opposite

standards. Proteins were assayed using the procedure of Bradford [23], adapted for automated use with an e.l.i.s.a. reader.

RESULTS

Isolation and separation of proteoglycans

Human articular cartilage derived from young and old adults was extracted successively with PBS then with 4 M GuHCl/SA and the content of sulphated glycosaminoglycan in the extracts was determined. Approximately 9% of total glycosaminoglycan content was extracted with PBS from both young and old tissue. Subsequent extraction with 4 M GuHCl/SA yielded 70% and 67% of total glycosaminoglycans from young and old cartilage, respectively; the remainder (21% and 24%) was present in the papain-solubilized residue. The total mass extracted with GuHCl/SA from both samples was similar (68.6 mg dry wt./g wet wt. and 67.0 mg dry wt./g wet wt. of young and old cartilage). The characterization of proteoglycans present in the PBS extracts has been described previously [17]. A flow diagram summarizing the experimental protocol described in the present paper is shown in Figure 1. Proteoglycans in the dissociative extracts were fractionated by associative and dissociative density gradient centrifugation (Figures 1a and 1b) and the content of protein and sulphated glycosaminoglycan in each fraction was determined (Figure 2). The yields of dry material recovered from the density gradient-separated pools after dialysis and lyophilization were calculated for each pool as the percentage of the total dry weight and are presented in Figure 1 (boxes in part b).

The old cartilage contained a significantly higher proportion of medium-density proteoglycans (Figure 1b, pools D + E + F, 51.1% old compared with 25.6% young). Most high-density proteoglycan aggregates ($A_1 + A_2$) from young tissue were recovered as monomers at the same density under dissociative conditions (Figure 1b, pool A, 41.2% young compared with 11.2% old), whereas most high-density aggregates from old tissues were recovered at medium densities in dissociative gradients (Figure 1b, pool B). Another major difference between extracts of young and old cartilage was the marked high protein content in both A_1D_5 and A_3D_5 fractions from old tissue compared with corresponding fractions from young tissue (Figure 2). In summary, the distribution of the high- and medium-density proteoglycan aggregates and monomers following density gradient centrifugation reflected the finding that cartilage proteoglycans with reduced glycosaminoglycan content accumulate in tissue with age.

Density gradient-separated pools were further fractionated by Q-Sepharose chromatography (Figure 3). Several striking qualitative differences between the chromatographic profiles of the young and old tissue pools were observed. (1) The proteins that accumulated in the D_5 fractions (pools C and F) of old cartilage were eluted from Q-Sepharose at low ionic strength. The same peaks were present in young tissue extracts but were much less abundant (Figure 3, panels C and F). (2) The high- and medium-density monomers from old cartilage (pools A and B) eluted from Q-Sepharose at a higher ionic strength compared with

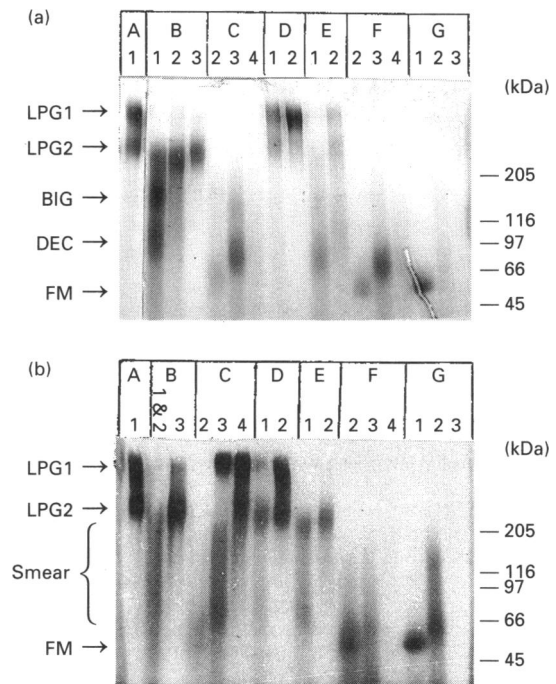


Figure 4 Analysis of Q-Sepharose chromatography-fractionated proteoglycans by SDS/PAGE

Fractionated proteoglycans derived from extracts of young (a) or old (b) human articular cartilage were electrophoresed on 1.6–16% polyacrylamide gradient gels and stained with Stains-all dye. BIG, biglycan; DEC, decorin; FM, fibromodulin. Designation of electrophoretic tracks corresponds to the designation of pools separated by Q-Sepharose chromatography (refer to Figure 3).

proteoglycans from the corresponding pools isolated from young cartilage (Figure 3, panels A and B). (3) The main proteoglycan peak that was present in pool B from extracts of young cartilage [Figure 3, pool B(1)] was not present in pool B isolated from the old cartilage. This peak was shown to represent two small proteoglycans biglycan and decorin (see below). (4) The A_5 fractions from young and old cartilage contained two protein peaks that were eluted from Q-Sepharose at low ionic strength (Figure 3, panel G); the proportional content of these peaks differed in young and old cartilage. Based on their electrophoretic mobilities under reducing and non-reducing conditions these peaks may represent serum albumin (first peak) and COMP (cartilage oligomeric matrix protein [24]; second peak); however no further characterization was done.

The Q-Sepharose fractions from young and old cartilage extracts were pooled comparably (except for pool B where the profiles differed markedly). The pools were numbered as shown and proteoglycans present in each pool were further separated by SDS/PAGE (Figure 4) followed by immunodetection with specific antibodies.

Figure 3 Chromatography of density gradient centrifugation-separated pools on Q-Sepharose

Portions of material recovered in pools that were separated by density gradient centrifugations were applied to Q-Sepharose and eluted with a linear salt gradient. Approx. 50 mg of dry material was applied to the column, except for pools C, F and G (young) and pools F and G (old) where the total amount of isolated material was applied. The eluate was monitored for protein (A_{280}) and assayed for sulphated glycosaminoglycan (A_{630}). Panels A–G correspond to the density gradient pools shown in Figure 1. The Q-Sepharose fractions were pooled as shown and designated by numbers. Comparable density gradient pools from young and old cartilage have been pooled in the same way, with the exception of pool B, where the profiles of eluting proteoglycans for the extracts of young and old cartilage totally differed.

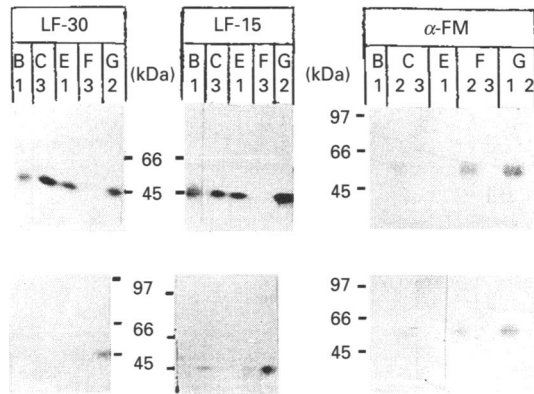


Figure 5 Immunodetection of small proteoglycans

Protein cores of deglycosylated small proteoglycans from young (upper panel) or old (lower panel) tissue were separated by SDS/PAGE, transferred to nitrocellulose and immunodetected with LF-30 (anti-decorin), LF-15 (anti-biglycan) and α -FM (anti-fibromodulin) antibodies as indicated. Core proteins detected with LF-30 and LF-15 were digested with chondroitinase ABC. Core proteins detected with anti-fibromodulin antibodies were digested with *endo*- β -galactosidase. Designation of electrophoretic tracks corresponds to the designation of pools separated by Q-Sepharose chromatography (refer to Figure 3).

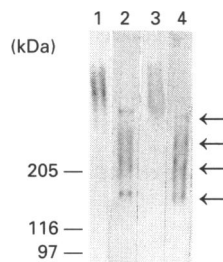


Figure 6 Immunodetection of large populations of aggrecan with mAb 3-B-3 before and after enzymic deglycosylation

Large proteoglycans present in pools A of young (tracks 1 and 2) and old (tracks 3 and 4) tissue extracts before (tracks 1 and 3) or after (tracks 2 and 4) deglycosylation with chondroitinase ABC and *endo*- β -galactosidase were separated by SDS/PAGE on 1.6–16% gradient gels, transferred to nitrocellulose and immunodetected with 3-B-3 mAb. To allow detection with 3-B-3 mAb, blocked immunoblots of native proteoglycans (tracks 1 and 3) were treated with chondroitinase ABC to generate the 3-B-3 neo-epitopes before incubation with the antibody.

Distribution of small proteoglycans in density gradients

Three small leucine-rich proteoglycans, decorin, biglycan and fibromodulin were identified in cartilage extracts by SDS/PAGE and immunoblotting. The fractionation pattern of decorin and biglycan showed marked differences between extracts of young and old tissue. Decorin and biglycan from young cartilage were found in all three pools that resulted from density gradient centrifugation under associative conditions ($A_1 + A_2$, $A_3 + A_4$ and A_5) and after dissociative fractionation they distributed between medium- and low-density pools (i.e. mainly pools B, C, E and F, Figure 4a). Decorin and biglycan core proteins were identified by specific immunodetection in pools B, C, E and G (Figure 5). The highest content of decorin and biglycan was found in young tissue extracts in pools B and C where they eluted from Q-Sepharose as conspicuous peaks of sulphated glycosaminoglycan that were absent in the corresponding pools from old tissue (Figure 3). The extracts of old human cartilage contained much lower proportions of both biglycan and decorin

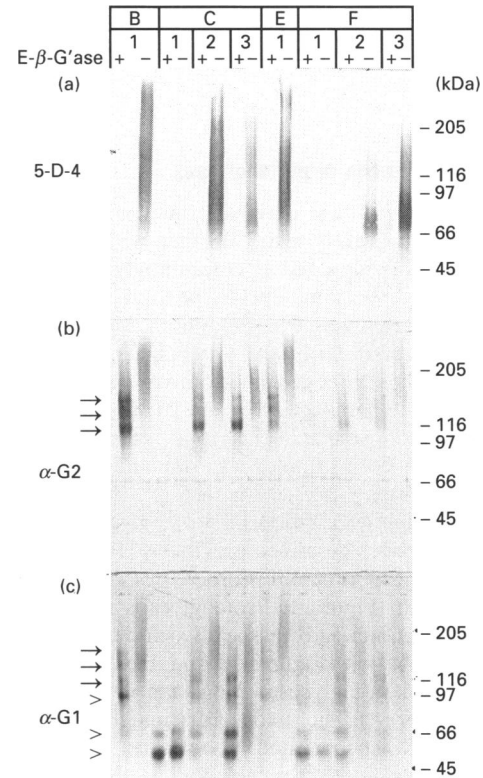


Figure 7 Identification of small fragments of aggrecan carrying keratan sulphate chains

Small fragments of aggrecan were identified in the extracts of the young cartilage by immunodetection with anti-keratan sulphate 5-D-4 mAb (a), anti-G2 domain (b), and anti-G1 domain (c) antibodies, before (–) and after (+) digestion with *endo*- β -galactosidase (*E*- β -G'ase). Proteoglycans were separated on 4–16% polyacrylamide gradient gels, transferred to nitrocellulose and immunodetected with antibodies as indicated. The arrows and arrowheads mark core proteins discussed in the text that were recognized by anti-G2 and anti-G1 antisera respectively. Designation of electrophoretic tracks corresponds to the designation of pools separated by Q-Sepharose chromatography (refer to Figure 3).

and significant amounts of each were only identified by immunoblotting in pool G (Figure 5).

Most of fibromodulin was present in pool G but, similarly to decorin and biglycan, also fibromodulin was recovered in all the associative pools ($A_1 + A_2$, $A_3 + A_4$, A_5) and subsequently in the D_5 fractions isolated from fractions $A_1 + A_2$ and $A_3 + A_4$ (pools C and F, Figures 4 and 5) from extracts of young as well as old cartilage. It eluted from Q-Sepharose before decorin and biglycan at 0.3 to 0.5 M NaCl and was identified in Stains-all stained gels as a conspicuous pink and relatively sharp band of molecular mass 55 kDa. Its identification as fibromodulin was verified by immunostaining before and after *endo*- β -galactosidase treatment (results not shown).

Large populations of aggrecan

Two large populations of aggrecan (LPG1 and LPG2 [17]) were present in extracts of young and old cartilage and were resolved by SDS/PAGE in large-pore gradient gels. They were present as major components of both high- and medium-density fractions in associative and dissociative gradients. Both populations contained chondroitin 6-sulphate (Figure 6) and keratan sulphate (results not shown). Since other proteoglycans present in the

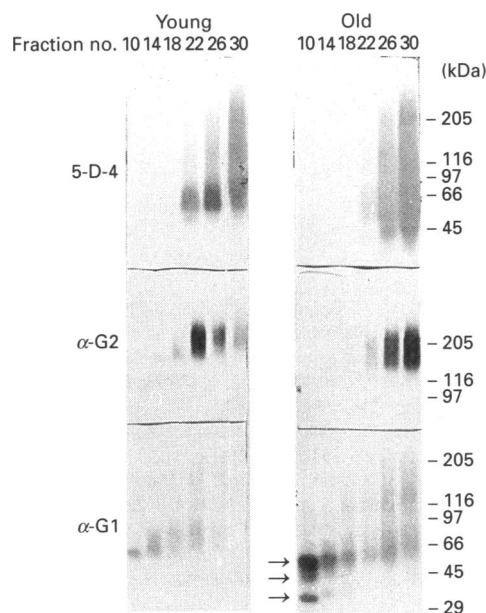


Figure 8 Immunodetection of low-buoyant-density aggrecan fragments in fractions separated by Q-Sepharose chromatography

Aliquots of chromatographic fractions of pools C (refer to Figure 3) were separated by SDS/PAGE on 1.6–16% polyacrylamide gradient gels, transferred to nitrocellulose and immunodetected with anti-keratan sulphate (5-D-4 mAb), anti-G2 domain, and anti-G1 domain antibodies, as indicated. Three G1 fragments present in pool C1 from extracts of the old cartilage are marked with arrows.

GuHCl/SA extracts did not contain sufficient amounts of chondroitin 6-sulphate to allow detection with 3-B-3 monoclonal antibody (mAb), this antibody was used to identify specifically protein cores derived from these large proteoglycans after their deglycosylation with chondroitinase ABC and *endo*- β -galactosidase. Four major core proteins were detected in pool A from young as well as old cartilage (Figure 6, arrows). The apparent molecular mass of these core proteins was approximately 380, 320, 240 and 180 kDa. The smallest core protein (180 kDa) was probably the most prevalent one as it could be detected with Coomassie Blue R after electrotransfer to a poly(vinylidene difluoride) membrane, whereas the other core proteins were only detected by immunostaining.

Small fragments of aggrecan

The same medium- and low-density pools that in young cartilage extracts contained clearly separated bands of biglycan and decorin (Figure 4a, pools B, C, E, F), in old cartilage contained proteoglycans that were visualized on SDS/PAGE as broad, pinkish smears (decorin and biglycan stained blue) that spanned a continuum of molecular masses from 60 to approx. 200 kDa (Figure 4b, brace). These components stained poorly with Alcian Blue, did not stain with Coomassie Blue, and were detected only in gels stained with Stains-all dye. They did not react with the 3-B-3 mAb after treatment of immunoblots with chondroitinase ABC but reacted strongly with the 5-D-4 mAb. They were present mainly in extracts of old tissue, and indeed their accumulation in ageing cartilage accounted for the increased proportion of protein in the D_5 fractions (Figure 2) that was eluted from Q-Sepharose at low ionic strength (Figure 3, pools C1, C2, F1 and F2). However, a small proportion of these same components was also identified in extracts of young cartilage as

5-D-4-reactive material that co-migrated on SDS/PAGE with decorin and biglycan. These proteoglycans have been identified by immunodetection (Figures 7 and 8) as aggrecan fragments derived from the N-terminal end of the molecule.

After *endo*- β -galactosidase treatment to remove keratan sulphate chains, a broad G2-containing band was resolved into three bands with apparent molecular masses of 110, 140 and 160 kDa (Figure 7b, arrows). Although the relative proportion of bands in individual pools varied, each of the three bands was always present. The anti-G1 antiserum reacted with the same bands (Figure 7c, arrows) indicating that these fragments contained both the G1 and G2 domains. Three other bands with molecular masses of 55, 70 and 95 kDa (Figure 7c, arrowheads) stained only with the anti-G1 antiserum and were present in both the old and young tissue extracts.

In addition to these G1 and G1–G2 fragments, extracts of old cartilage contained two smaller G1 bands that were not found in young tissue. SDS/PAGE and immunodetection of the C1 Q-Sepharose pool (refer to Figure 3, panel C, fractions 8–12) from old cartilage resolved three G1 bands with molecular masses of 35, 42 and 55 kDa (Figure 8, arrows) and these were not substituted with keratan sulphate and did not stain with the 5-D-4 antibody. The 55 kDa band was the predominant G1 fragment in extracts of old cartilage. Some 55 kDa fragments that were eluted at higher salt concentrations in pool C2 (Figures 3 and 8) were larger and stained more intensely with 5-D-4 antibody suggesting that these fragments were substituted with gradually increasing amounts of keratan sulphate.

Essentially, the same core protein fragments that were identified in young cartilage extracts (Figure 7) were also present in extracts of old cartilage (results not shown). The only significant qualitative difference in the small aggrecan fragments found in old and young tissue was the absence of the very small (35 and 42 kDa) keratan sulphate-free G1 fragments in young cartilage.

These results indicate that there are at least eight naturally occurring species of G1 and G1–G2 fragments that accumulate in cartilage with age. The two smallest species carry no keratan sulphate chains. The 55 and 70 kDa fragments may or may not be substituted with keratan sulphate, while the larger fragments always contain variable amounts of keratan sulphate. Substitution with keratan sulphate accounts for the polydispersity of the fragments and causes their overlapping: only fragments that carry no keratan sulphate chains run on SDS/PAGE as separated bands. The three largest species contain both the G1 and G2 domains. Clearly this accounts for the retention of G2 fragments in the tissue, as G2 fragments lacking the hyaluronan-binding G1 domain are rapidly lost.

DISCUSSION

Our results provide new information concerning the size of core proteins of populations and fragments of aggrecan present in human articular cartilage. Two large populations of aggrecan were shown, in fact, to consist of proteoglycans comprising four different core proteins. The 180 kDa core protein corresponds to the largest core protein that has been recently identified in human synovial fluids [25], all other protein cores identified here were larger. In addition to these large populations of aggrecan, that are always present in human cartilage, we demonstrated the presence of eight different small G1-containing products that accumulate in the tissue with age. These results are intriguing for two reasons. First, they suggest that there are multiple sites of proteolytic attack within the G1 and connected interglobular domain. It is possible to speculate, based on the apparent

molecular mass of the G1 fragments, that the 55 and 70 kDa fragments may correspond to cleavage at the predominant metalloproteinase cleavage sites [5–7] and the aggrecanase site [8,10,11] respectively. Other strategies, however, are required to confirm this. Secondly it is of interest that very small G1 fragments are produced, and also retained in the tissue. While high concentrations of plasmin have been shown to cleave within the proteoglycan tandem repeat loop (loop B) of G1 [26], G1 is relatively proteinase-resistant and indeed classical procedures for its purification involve extensive trypsin digestion of proteoglycan aggregates to release the G1 domain intact.

Our findings on the distribution of proteoglycan species and fragments between different fractions separated from the extracts are summarized in Figure 1(d). Extracts of young and old cartilage have not differed significantly in the nature of the proteoglycans present, however there are marked differences in the relative abundance of several components. The data clearly document an age-related shift from proteoglycans of high buoyant density in young tissue, to proteoglycans of medium buoyant density in older tissue, and show that this is due to an accumulation of truncated aggrecan molecules, many of which have lost their entire chondroitin-sulphate-bearing domains.

Fragments of aggrecan were found not only in old cartilage, but also in cartilage of young individuals. With the exception of two small G1 fragments that were found exclusively in old cartilage, the proteoglycans present in samples of old and young tissue were identical. Hence, the repertoire of aggrecan fragments found in normal tissue does not appear to change significantly with age. This finding raises the question of whether the same repertoire of fragments is found in degenerating (osteoarthritic) cartilage or in cartilage from a joint with acute inflammation, where conditions that affect the expression and activity of proteolytic enzymes may vary.

Decorin, biglycan and fibromodulin were present in extracts of old and young tissue, albeit in very low yield in old tissue. Proportions of all three small proteoglycans sedimented during associative centrifugation to the high-density fractions and subsequently were recovered from the low-density (i.e. D₅) dissociative fractions. This distribution presents the interesting possibility that a proportion of these proteoglycans may be associated with high-buoyant-density aggregates. It has recently been shown that decorin, biglycan and fibromodulin can each bind to collagen type VI via their core proteins [27], and probably via a site located within the leucine-rich repeats. A similar interaction of small proteoglycans with aggregates might also be mediated through this common structural motif.

We thank Dr. Bruce Caterson, Dr. Larry W. Fisher, Dr. Timothy E. Hardingham and Dr. Anna H. K. Plaas, who generously donated antibodies used in this study, and Dr. P. Valouch who dissected tissue specimens. The technical assistance of Mrs. Jana Hamakova is much appreciated. This project was supported by a grant 0227-3 from the Internal Grant Agency of the Ministry of Health, Czech Republic.

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