The synthesis of dermatan sulphate proteoglycans by fetal and adult human articular cartilage

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Non-aggregating dermatan sulphate proteoglycans can be extracted from both fetal and adult human articular cartilage. The dermatan sulphate proteoglycans appear to be smaller in the adult, this presumably being due to shorter glycosaminoglycan chains, and these chains contain a greater proportion of their uronic acid residues as iduronate. Both the adult and fetal dermatan sulphate proteoglycans contain a greater amount of 4-sulphation than 6-sulphation of the *N*-acetylgalactosamine residues, in contrast with the aggregating proteoglycans, which always show more 6-sulphation on their chondroitin sulphate chains. In the fetus the major dermatan sulphate proteoglycan to be synthesized is DS-PGI, though DS-PGII is synthesized in reasonable amounts. In the adult, however, DS-PGI synthesis is barely detectable relative to DS-PGII, which is still synthesized in substantial amounts. Purification of the dermatan sulphate proteoglycans from adult cartilage is hampered by the presence of degradation products derived from the large aggregating proteoglycans, which possess similar charge, size and density properties, but which can be distinguished by their ability to interact with hyaluronic acid.

INTRODUCTION

Hyaline cartilage has been shown to contain both aggregating and non-aggregating proteoglycans (Heinegård *et al.*, 1982). In mature bovine articular cartilage two small non-aggregating proteoglycans have been characterized, namely DS-PGI and DS-PGII (Rosenberg *et al.*, 1985). Both contain about 45% of their uronic acid as iduronate, but differ with respect to their core-protein structure and their ability to self-associate. DS-PGs similar to those reported for bovine articular cartilage have been described in a number of other mature connective tissues, such as bovine periodontal ligament and skin (Pearson & Gibson, 1982), pig skin (Damle *et al.*, 1982), bovine bone (Sato *et al.*, 1985) and bovine tendon (Vogel & Heinegård, 1985). With the exception of the bone molecules, where only chondroitin sulphate was reported, the other proteoglycans were characterized by their high content of iduronic acid. This class of proteoglycans is not unique to adult tissues, but has also been shown to be prevalent in immature tissues, such as fetal bovine tendon (Honda *et al.*, 1987; Vogel & Evanko, 1987), fetal bovine cartilage (Rosenberg *et al.*, 1982) and developing human bone (Fisher *et al.*, 1987). No major differences were reported for iduronate contents of the dermatan sulphate chains between immature and mature tissues, though there were indications that the proportion of DS-PGI and DS-PGII could vary with age. In bovine tendon both DS-PGI and DS-PGII were extracted from the fetus, but only DS-PGII was extracted from the adult (Vogel & Evanko, 1987). In contrast, both DS-PGI and DS-PGII were extracted from adult bovine cartilage (Rosenberg *et al.*, 1985). In addition to DS-PG type

variation with age, it has also been demonstrated that at least for DS-PGII there may also be variation in its distribution throughout the tissue with age (Poole *et al.*, 1986).

It has now been established that molecules of the DS-PGII class are immunologically related (Vogel & Fisher, 1986; Cöster *et al.*, 1987), and probably represent the product of a single gene that is ubiquitous in its expression among connective tissues (Day *et al.*, 1986), and whose translation product is represented by a cDNA clone derived from human fibroblasts (Krusius & Ruoslahti, 1986). The identity of DS-PGI between different tissues is not so clear. The molecule described as DS-PGI in bovine sclera differs from that described as DS-PGI in bovine cartilage in both core-protein size and immunological cross-reactivity (Rosenberg *et al.*, 1985; Cöster *et al.*, 1987; Cöster & Fransson, 1981), though both do self-associate (Rosenberg *et al.*, 1985; Cöster *et al.*, 1981). The scleral DS-PGI may be related to the aggregating class of proteoglycan, as it does appear to possess a hyaluronate-binding region (Cöster *et al.*, 1987), albeit a non-functional one. It remains to be established whether all other molecules identified as DS-PGI represent the same gene product, as, to date, protein sequence data have only been described for the species from bone (Fisher *et al.*, 1987), and no cDNA clone representing this gene has been reported.

DS-PGs have recently been characterized in human articular cartilage (Stanescu *et al.*, 1988; Sampaio *et al.*, 1988). However, it is unclear from previous work whether DS-PGI and DS-PGII are expressed to similar or different degrees throughout life, and the work reported in the present paper was designed to address this point.

Abbreviation used: DS-PG, dermatan sulphate proteoglycan.

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EXPERIMENTAL

Preparation of non-aggregating proteoglycans

Human articular cartilage was obtained from the distal femur of both a fetus (28 weeks gestation) and a mature adult (58 years old) at the time of autopsy, and within 20 h of death. Proteoglycan was extracted from the tissue with 4 M-guanidinium chloride in the presence of proteinase inhibitors, and non-aggregating proteoglycans were separated from large aggregating proteoglycans and matrix proteins by a combination of CsCl-density-gradient centrifugation, ion-exchange chromatography and gel-filtration chromatography, in a manner similar to that described by Rosenberg *et al.* (1985). The major difference was that centrifugation was performed directly under dissociative conditions, with a starting density of 1.4 g/ml. At each stage of the purification the position of the non-aggregating proteoglycan was monitored by agarose/polyacrylamide-gel electrophoresis.

Cartilage culture and [35 S]sulphate-labelling

Adult or fetal cartilage was cut into small pieces and cultured in Dulbecco's modified Eagle's medium (GIBCO), containing 4.5 g of glucose/l, and supplemented with 2.0 g of NaHCO_3 /l, 0.8 g of sodium glutamate/l, 1.2 g of sodium pyruvate/l, 20 mM-Hepes, and penicillin and streptomycin, each at 50 units/ml. The pH of the medium was adjusted to 7.3. Culture was performed in 25 ml flasks, with each flask containing 2 g of tissue in 10 ml of medium, and the tissue was preincubated at 37 °C for 24 h under normal atmospheric conditions. After preincubation the spent medium was removed, and replaced with fresh medium containing $\text{H}_2^{35}\text{SO}_4$ at 150 $\mu\text{Ci}/\text{ml}$ of medium. Incubation was then continued at 37 °C for a further 24 h. After labelling, the tissue was washed three times with phosphate-buffered saline (145 mM-NaCl/6 mM- Na_2HPO_4 /4 mM- KH_2PO_4 buffer, pH 7.2), then extracted as described above.

Electrophoretic techniques

Proteoglycans were analysed by agarose/polyacrylamide-gel electrophoresis in slab gels as described by Heinegård *et al.* (1985). Interaction with hyaluronic acid was assessed by adding 20% (w/w) hyaluronic acid to the sample before electrophoresis. Authentic samples of DS-PGI and DS-PGII from bovine articular cartilage were used as reference markers, and were kindly supplied by Dr. L. C. Rosenberg (Montefiore Hospital, New York, NY, U.S.A.). For analysis of fractions from chromatography or density gradients, samples were first dialysed into water. Gels were stained with Toluidine Blue.

SDS/polyacrylamide-gel electrophoresis was performed in 4–20% gradient slab gels as described by Rosenberg *et al.* (1985). Gels were stained successively with Coomassie Brilliant Blue R-250, then Alcian Blue, as described by Fisher *et al.* (1983). For analysis of samples from chromatography or density gradients, samples were first dialysed into 0.125 M-Tris/HCl buffer, pH 6.8, containing 0.1% SDS. Estimation of core protein size was performed after digestion of samples with chondroitin ABC lyase, with 0.05 unit of enzyme/mg of proteoglycan in 0.1 M-sodium acetate/0.1 M-Tris/HCl buffer, pH 7.3, for 20 h at 40 °C.

Fluorography

After electrophoresis, staining and destaining, gels were immersed in En^3Hance (New England Nuclear) with gentle agitation for 2 h with agarose/polyacrylamide gels or 1 h with the thinner SDS/polyacrylamide gels. The En^3Hance was then replaced by water, and agitation was continued for 30 min to precipitate the fluors. The gel was then placed on filter paper and dried on a slab-gel dryer, with the use of a minimum of heating for the agarose/polyacrylamide gels or 60 °C for the SDS/polyacrylamide gels. After being dried, the gel was placed against Kodak X-Omat AR film and exposed at –70 °C until the desired intensity was achieved (usually 3 days).

Alternatively, the proteoglycans in the agarose/polyacrylamide gels were transferred to Nylon 66 membranes (Nytran) as described by Heimer & Sampson (1987). After blocking of the transfer by incubation with 10% (w/v) bovine serum albumin in 200 mM-NaCl/15 mM-Tris/HCl buffer, pH 7.4, it was stained with Alcian Blue, then destained. The stained transfer was then

Fig. 1. CsCl-density-gradient centrifugation of cartilage extracts

Centrifugation was performed in the presence of 4 M-guanidinium chloride with a starting density of 1.4 g/ml. Gradients were divided into 12 fractions, which were analysed by agarose/polyacrylamide-gel electrophoresis, and newly synthesized proteoglycan was detected by fluorography. Fluorographs are shown for fractions from (a) fetal and (b) adult cartilage. Fractions from the centre of the gradient, enriched in the component of fastest electrophoretic mobility, were pooled ($\leftrightarrow$) for further purification.

Fig. 2. DEAE-cellulose ion-exchange chromatography of pooled material from the CsCl gradients

Chromatography was performed in the presence of 6 M-urea, and, after application of the sample, elution was performed with a linear gradient of NaCl (—). Fractions were analysed by measurement of A_{280} (■—■) and by scintillation counting of ^{35}S radioactivity (-----). Chromatographic profiles are depicted for (a) fetal and (b) adult material. Fractions that were retained by the resin and contained proteoglycan were pooled ($\leftrightarrow$) for further purification.

sprayed with En^3Hance , allowed to dry, then exposed to X-ray film as described above.

Collagenase digestion

The collagenous nature of samples was assessed by digestion with bacterial collagenase before electrophoresis as described by Broek *et al.* (1985). Samples were dissolved or dialysed into 50 mM-Tris/HCl buffer, pH 7.6, containing 0.2 M-NaCl, 5 mM- CaCl_2 and 10 mM-*N*-ethylmaleimide, then heated for 3 min at 100 °C. After cooling, collagenase (Advanced Biofactures) was added at 250 units/mg of protein, and the samples were incubated at 37 °C for 5 h. The reaction was terminated by adding SDS to a final concentration of 2% (w/v) and boiling for 3 min.

Composition of dermatan sulphate

The position of sulphation along the dermatan sulphate chains was determined by h.p.l.c. (Hjerpe *et al.*, 1979) following release of component disaccharides by digestion of the proteoglycan with chondroitin ABC lyase (Lee & Tieckelmann, 1979). The same chromatographic technique was used to assess the proportion of glucuronic acid and iduronic acid in the dermatan sulphate chains following digestion with chondroitin AC lyase. This method will tend to overestimate the iduronate content of the molecule, as not all glucuronic acid residues

will be released as disaccharides following digestion with chondroitin AC lyase. All oligosaccharides generated containing iduronic acid will have one modified glucuronic acid residue at their non-reducing terminus. The disaccharides produced from the resident dermatan sulphate chains were monitored by absorption at 232 nm. Those from the newly synthesized proteoglycans were monitored by scintillation counting of 200 μl fractions of the column eluate, to each of which 5 ml of Ready-Solv EP (Beckman) had been added.

RESULTS

DS-PGs were initially purified from fetal and adult cartilage extracts by using dissociative CsCl-density-gradient centrifugation at a starting density of 1.4 g/ml. Under these conditions components with analogous properties to authentic DS-PGs can be detected in the lower central portion of the gradient by agarose/polyacrylamide-gel electrophoresis (Fig. 1). In both cases the bulk of the aggregating proteoglycan was in the bottom two fractions, and those fractions of lower density containing predominantly the DS-PG were pooled for further purification.

Anion-exchange chromatography in 6 M-urea separated the bulk of the proteoglycan from remaining protein, which was eluted at lower ionic strength (Fig. 2).

Fig. 3. Sepharose CL-4B chromatography of pooled material from the ion-exchange chromatography

Chromatography was performed in the presence of 4 M-guanidinium chloride. Fractions were analysed by measurement of A_{280} (■—■) and by scintillation counting of ^{35}S radioactivity (-----). Chromatographic profiles are depicted for (a) fetal and (b) adult material. Fractions that were retarded by the column and contained proteoglycan were subjected to further analysis.

More protein contamination was evident in the fetal preparations than in the adult ones, which appear to be relatively homogeneous by this technique. In the fetus, the peak of uronic acid-containing material was eluted with 0.28 M-NaCl, whereas the ^{35}S sulphate-labelled material was eluted with 0.31 M-NaCl. The elution position of the ^{35}S sulphate-labelled adult proteoglycan was at 0.20 M-NaCl.

The fractions containing ^{35}S sulphate and uronic acid were pooled and subjected to dissociative Sepharose CL-4B chromatography. The fetal preparation showed a minor peak of proteoglycan eluted at the void volume, presumably representing contaminating aggregating proteoglycan, and a major included peak, compatible with the DS-PGs (Fig. 3a). The ^{35}S sulphate-labelled proteoglycans in the fetal preparation were less retarded than the unlabelled proteoglycan (K_{av} values of 0.33 and 0.41 respectively), indicating that the newly synthesized proteoglycans are of larger size. In the adult preparation, only a small proportion of material was eluted at the void volume, and the included newly synthesized proteoglycans were eluted with a K_{av} value of 0.41, indicating that they were of smaller size than their fetal counterparts (Fig. 3b). In contrast with the unimodal peak obtained for the ^{35}S sulphate, measurement of protein content for the adult preparation showed a bimodal distribution of components (with peak K_{av} values of 0.29

Fig. 4. SDS/polyacrylamide-gel electrophoresis of fractions from Sepharose CL-4B chromatography

Fractions containing proteoglycan (Fig. 3) from both the fetal and adult cartilage preparations were analysed by electrophoresis under reducing conditions. The resulting gels were subjected to (a) direct staining by consecutive treatment with Coomassie Brilliant Blue, then Alcian Blue, or (b) detection of ^{35}S sulphate-labelled material by fluorography. M_r values of the detected proteoglycans were estimated relative to the migration of protein standards, and the migration positions of authentic DS-PGI and DS-PGII from bovine articular cartilage are indicated.

and 0.51), suggesting substantial heterogeneity within the resident adult proteoglycan preparation.

Fractions from the Sepharose CL-4B columns were analysed by SDS/polyacrylamide-gel electrophoresis. The fetal preparation showed the occurrence of both DS-PGI and DS-PGII in the resident proteoglycans, with average M_r values of 200 000 and 100 000 respectively (Fig. 4a). Both proteoglycan populations were also apparent in the newly synthesized material, though they were of larger average size (Fig. 4b), with average M_r values of 220 000 and 120 000 respectively. The molecules representing DS-PGI were on average less retarded on Sepharose CL-4B than were those representing DS-PGII, for both the resident and newly synthesized populations, with those representing DS-PGI appearing to predomi-

Fig. 5. Agarose/polyacrylamide-gel electrophoresis of aggregating and non-aggregating proteoglycans

Aggregating proteoglycans (Agg. PG) were obtained from the bottom fractions of the CsCl gradients used in the preparation of the non-aggregating proteoglycans (Fig. 1). The non-aggregating proteoglycans (DS-PG) represent peak fractions from the Sepharose CL-4B chromatography (Fig. 3). The resulting gels, which contain samples from both fetal (F) and adult (A) preparations, were subjected to (a) direct staining with Toluidine Blue, or (b) detection of [35 S]sulphate-labelled material by fluorography.

nate in abundance. In contrast, the resident proteoglycans in the adult preparation did not show distinct species analogous to those of the fetus. A smear of Alcian Blue-staining material was observed encompassing the region where DS-PGI and DS-PGII migration was to be expected (Fig. 4a), making it impossible to confirm the presence of either population, and suggesting the presence of additional proteoglycan species. The newly synthesized proteoglycans in the adult did, however, reveal the presence of DS-PGI and DS-PGII, though the latter was by far the most abundant species (Fig. 4b). The M_r value of the DS-PGII was about 100 000, being smaller than its newly synthesized counterpart in the fetus. The resolution of distinct proteoglycan species by fluorography, but a smear on Alcian Blue staining, would suggest that much of the material present in the resident proteoglycan of the adult preparation represents degradation products derived from larger proteoglycan species.

Analysis by agarose/polyacrylamide-gel electrophoresis shows that the adult aggregating proteoglycans are more heterogeneous and more mobile than the fetal molecules (Fig. 5a), this presumably being due to the smaller size and greater degree of structural variation of the former. In contrast, fluorography does not reveal such a large difference, with the newly synthesized adult

Fig. 6. Agarose/polyacrylamide-gel electrophoresis of fractions from Sepharose CL-4B chromatography

Fractions containing proteoglycan (Fig. 3) from both fetal and adult cartilage preparations were analysed. The resulting gels were subjected to (a) direct staining with Toluidine Blue, or (b) detection of [35 S]sulphate-labelled material by fluorography. The migration position of a mixture of DS-PGI and DS-PGII from bovine articular cartilage is indicated.

molecules being only slightly more mobile than the fetal molecules (Fig. 5b). This lends credence to the idea that much of the heterogeneity in composition observed in the adult aggregating proteoglycans may be due to proteolytic processing. The newly synthesized DS-PGs also show distinct migration differences between the fetus and the adult (Fig. 5b), with the adult molecules being less mobile, indicative of their lower charge density, as also illustrated by their elution on ion-exchange chromatography. Unlike SDS/polyacrylamide-gel electrophoresis, the agarose/polyacrylamide-gel system does not reveal a clear difference between the migration of DS-PGI and DS-PGII.

Further analysis of the Sepharose CL-4B fractions by agarose/polyacrylamide-gel electrophoresis reveals that in this system both newly synthesized and resident fetal DS-PGs co-migrate (Fig. 6). Comparison of migration positions in the adult fractions shows that some fractions also contain co-migrating Alcian Blue-stained and [35 S]sulphate-labelled material. Such resident material is under the more retarded of the two peaks observed in the protein elution profile (Fig. 3b). This suggests that DS-PGs are indeed present in the adult extract, and, as with the fetus, they are of smaller size than the newly synthesized material. The Alcian Blue-staining material

Fig. 7. Agarose/polyacrylamide-gel electrophoresis of fetal proteoglycans in the presence of hyaluronic acid

Aggregating proteoglycans (Agg. PG) were obtained from the bottom fractions of the CsCl gradients (Fig. 1a), and non-aggregating proteoglycans represent selected fractions from the Sepharose CL-4B chromatography (Fig. 3a). Samples were electrophoresed either directly (—) or after (+) equilibration with 20% (w/w) hyaluronic acid (HA). The resulting gels were subjected to (a) direct staining with Toluidine Blue, or (b) detection of [³⁵S]sulphate-labelled material by fluorography.

of lower mobility in the adult does not have any newly synthesized counterpart. As this material is of a similar low buoyant density and charge density to the DS-PGs on CsCl-density-gradient centrifugation and ion-exchange chromatography respectively, yet of larger size on Sepharose CL-4B, the most likely explanation for its origin is from the hyaluronate-binding terminus of the large aggregating proteoglycans, with several glycosaminoglycan chains still being attached.

Some evidence in favour of this hypothesis was obtained when agarose/polyacrylamide-gel electrophoresis was repeated, with hyaluronic acid being added to the fractions before analysis. As expected, the fetal DS-PGs show no change in mobility under these conditions, indicative of their inability to interact with hyaluronic acid (Fig. 7). In contrast, both the intact aggregating proteoglycans and the less-mobile material from the adult column fractions do show evidence of material being retarded in the presence of hyaluronic acid (Fig. 8). Thus at least some of the material that copurifies with the adult DS-PGs does contain functional hyaluronate-binding regions.

Fig. 8. Agarose/polyacrylamide-gel electrophoresis of adult proteoglycans in the presence of hyaluronic acid

Aggregating proteoglycans (Agg. PG) were obtained from the bottom fractions of the CsCl gradients (Fig. 1b), and non-aggregating proteoglycans represent selected fractions from the Sepharose CL-4B chromatography (Fig. 3b). Samples were electrophoresed either directly (—) or after (+) equilibration with 20% (w/w) hyaluronic acid (HA). The resulting gels were subjected to (a) direct staining with Toluidine Blue, or (b) detection of [³⁵S]sulphate-labelled material by fluorography.

The resident fetal DS-PGs have about 3 times as much 4-sulphation as 6-sulphation along their dermatan sulphate chains. This was not the situation in the aggregating proteoglycans, where a similar degree of sulphation at the two positions is observed (Table 1). In the adult the aggregating proteoglycans bear over 10 times as much sulphation at the 6-position, yet the proteoglycans present in the DS-PG preparation are still predominantly sulphated in the 4-position. Differences between the DS-PG preparations and the aggregating proteoglycans are also apparent in the newly synthesized material. For both the fetal and adult preparations the DS-PGs have over twice as much 4-sulphation as 6-sulphation, whereas the situation is reversed in the aggregating proteoglycans. Perhaps surprisingly, no differences were observed, however, between equivalent preparations from the fetus and adult.

Both newly synthesized and resident aggregating proteoglycans for the fetus or the adult contained little, if any, iduronic acid. However, iduronic acid was apparent in both resident and newly synthesized DS-PGs (Table 1). In the fetus the resident proteoglycans may contain up to 40% iduronate, whereas in the adult the equivalent preparation may have over 70% iduronate. Comparable degrees of epimerization also appeared to be present in the newly synthesized DS-PGs. In the fetus,

Table 1. Analysis of disaccharides produced after incubation of proteoglycans with chondroitin ABC lyase and chondroitin AC lyase

DS-PG is the peak fraction of non-aggregating proteoglycans from Sepharose CL-4B chromatography (Fig. 3). Aggregating proteoglycan (Agg. PG) was obtained from bottom fractions of CsCl gradients used in preparation of non-aggregating proteoglycans (Fig. 1). Resident proteoglycans were measured by absorbance at 232 nm. Newly synthesized proteoglycans were measured by scintillation counting of ^{35}S radioactivity.

	Fetal		Adult	
	DS-PG	Agg. PG	DS-PG	Agg. PG
(a) Ratio of 6-/4-sulphated disaccharides released by chondroitin ABC lyase				
Resident	0.31	1.28	0.58	10.35
Newly synthesized	0.38	2.14	0.34	2.28
(b) % of total disaccharides released by chondroitin AC lyase				
Resident	58	100	28	100
Newly synthesized	48	98	31	100
(c) % of 4-sulphated disaccharides released by chondroitin AC lyase				
Resident	49	100	6	100
Newly synthesized	40	97	16	98
(d) % of 6-sulphated disaccharides released by chondroitin AC lyase				
Resident	86	100	67	100
Newly synthesized	69	98	71	100

the disaccharides released by chondroitin AC lyase contain the bulk of the 6-sulphated residues and about half the 4-sulphated residues. This would imply that the glucuronate-rich blocks within the dermatan sulphate chains contain both 6-sulphation and 4-sulphation, whereas the iduronate-rich blocks, by inference, are predominantly 4-sulphated. In the adult, the disaccharides released by chondroitin AC lyase still contain the majority of the 6-sulphated residues but only little of the 4-sulphated residues. Hence the glucuronate-rich blocks are now predominantly 6-sulphated, whereas the iduronate-rich blocks are still rich in 4-sulphation. Similar changes were apparent in both the resident and the newly synthesized proteoglycans.

Finally, the identification of type IX collagen as proteoglycan (Vaughan *et al.*, 1985) opened up the possibility that some of the material present as low-density non-aggregating proteoglycans could represent this component. However, digestion of either fetal or adult DS-PG preparations with bacterial collagenase gave no change in the electrophoretic mobility of either the resident or the newly synthesized components (results not shown). Thus in this work no evidence for the presence of a type IX collagen proteoglycan in the cartilage extracts was obtained.

DISCUSSION

DS-PGs were extracted from both fetal and adult human articular cartilage. Both DS-PGI and DS-PGII were clearly identified in the fetal extracts and could be readily purified from the larger, more-glycosylated,

aggregating proteoglycans. In the adult it was not clear whether both types of DS-PG were present, as analysis of the preparations was obscured by unrelated material that co-purified with the DS-PG. This material appears to be derived from the aggregating proteoglycans by proteolysis, and there is evidence that such degradation products, rich in the hyaluronate-binding region, do accumulate in adult cartilage (Roughley *et al.*, 1984, 1985; Stanescu *et al.*, 1988). Although the data presented here do not allow the distinction between DS-PGI and DS-PGII in the adult extracts, preliminary results on several specimens indicate that DS-PGII is by far the predominant species in the adult extracts. One cannot, however, exclude the possibility that, in the adult, DS-PGI remains within the cartilage residue after extraction. If DS-PGI is present in significant amounts, this would be in contrast with the newly synthesized DS-PGs, where both DS-PGI and DS-PGII are readily detected in the fetus, with DS-PGI predominating, whereas in the adult the synthesis of DS-PGII is clearly much more prevalent. The presence of both DS-PGI and DS-PGII was previously reported in adult bovine articular cartilage (Rosenberg *et al.*, 1985), whereas adult bovine tendon contains only DS-PGII (Vogel & Heinegård, 1985). The chromatographic and electrophoretic behaviour of the DS-PGs suggests that the dermatan sulphate chains are shorter in the adult than the newborn, a situation previously reported for the chondroitin sulphate chains of the aggregating proteoglycans (Roughley & White, 1980). This difference is maintained in the newly synthesized dermatan sulphate chains, though in culture the chains are longer than their resident counterparts. However, changes in glycosaminoglycan chain length have been shown to correlate with the rate of proteoglycan synthesis and hence vary under different culture conditions (Mitchell & Hardingham, 1981).

With increasing age, the amount of iduronic acid in the DS-PGs appears to increase. This apparent increase with age is in contrast with the bovine system, where in both adult and fetal tendon (Vogel & Heinegård, 1985; Vogel & Evanko, 1987) and articular cartilage (Rosenberg *et al.*, 1982, 1985) DS-PGs the iduronate content shows little change. In the aggregating proteoglycans of human articular cartilage little, if any, iduronic acid was detected in either the fetal or the adult preparations. Comparison of the position of sulphation of the glycosaminoglycan chains between the DS-PGs and the aggregating proteoglycans is also instructive. In the aggregating proteoglycans 6-sulphation is always predominant, increasing from degrees just in excess of 4-sulphation to over 10 times 4-sulphation with age. On the other hand, the DS-PGs always show more 4-sulphation, and though the degree of 6-sulphation increases in the adult, it does not rise much above half that of 4-sulphation. This increase in 6-sulphation of the dermatan sulphate chains appears to occur mainly in glucuronate-rich regions of the molecule. These regions, which decrease in abundance in the adult, are also predominantly 6-sulphated in the adult. These differences in polymer modification of the chondroitin sulphate chains, initially made in the two types of proteoglycans, show that all proteoglycans are not recognized equally by the epimerase and sulphotransferase systems, and suggest that, if the same cellular compartments are responsible for the synthesis of the aggregating and non-aggregating proteoglycans, then the nature of the core protein may play a role in

modulating the activity of some synthetic enzymes. Such modulation is still apparent in the newly synthesized proteoglycans produced in tissue culture, though in this case differences in sulphation position between fetal and adult counterparts is no longer produced. This suggests that, although the activity of the epimerase may be inherent to the age of the cartilage, the activity of the sulphotransferases can be modified by exogenous agents that differ at different ages.

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