The Dermatan Sulfate Proteoglycans of Bovine Sclera and Their Relationship to Those of Articular Cartilage

AN IMMUNOLOGICAL AND BIOCHEMICAL STUDY*

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Dermatan sulfate proteoglycans were isolated from adult bovine sclera and adult bovine articular cartilage. Their immunological relationships were studied by enzyme-linked immunosorbent assays using polyclonal antibodies raised against the large and small dermatan sulfate proteoglycans from sclera and a polyclonal and monoclonal antibody directed against the small dermatan sulfate proteoglycan from cartilage. The small dermatan sulfate proteoglycans from sclera and cartilage displayed immunological cross-reactivity while there was no convincing evidence of shared epitope(s) with the larger dermatan sulfate proteoglycans, nor did these larger proteoglycans share any common epitopes with each other. A hyaluronic acid binding region was detected immunologically on the larger scleral dermatan sulfate proteoglycan but was absent from the larger dermatan sulfate proteoglycan of cartilage and both the small dermatan sulfate proteoglycans.

These antibodies were used in immunofluorescence microscopy to localize the scleral proteoglycans and molecules containing these epitopes in the eye. The large scleral dermatan sulfate proteoglycan was restricted to sclera while molecules related to the small scleral cartilage proteoglycans were found in the sclera, anterior uveal tract, iris, and cornea. Amino acid sequencing of the amino-terminal regions of the core proteins of the small dermatan sulfate proteoglycans from sclera and articular cartilage showed that all the first 14 amino acids analyzed were identical and the same as reported earlier for the small bovine skin and tendon dermatan sulfate proteoglycans.

These studies demonstrate that the larger dermatan sulfate proteoglycans of sclera and cartilage are chemically unrelated to each other and to the smaller dermatan sulfate proteoglycans isolated from these tissues. The latter have closely related core proteins and probably represent a molecule with a widespread distribution in which the degree of epimerization of glucuronic acid and iduronic acid varies between tissues.

Dermatan sulfate proteoglycans (DS-PG) have been isolated from various connective tissues such as articular cartilage, blood vessel walls, cornea, sclera, skin, tendon, and a variety of other sources such as follicular fluid and yolk sac tumor (1). Although they may often be of similar size, the iduronic acid contents of these dermatan sulfate chains vary (1).

Dermatan sulfate proteoglycans have been isolated from adult bovine articular cartilage and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis into two species with different mobilities at high ionic strength (2). The molecular mass of the smaller proteoglycan (DS-PGII) was from 87,000 to 120,000 kDa and that of the larger proteoglycan (DS-PGI) from 165,000 to 285,000 kDa. They both had a core protein with an M, of 45,000. A monoclonal antibody raised against the small DS-PGII did not react with the large DS-PGI, indicating that they have different core proteins and represent distinct species of dermatan sulfate proteoglycans. Dermatan sulfate proteoglycans were earlier isolated from bovine sclera (3, 4). The larger proteoglycan (PGI) has an M, of about 200,000 and its side chains have a low content of iduronic acid (4, 5). The smaller proteoglycan (PGII) has an M, of about 90,000, a core protein with an M, of 46,000 and its side chains are rich in iduronic acid (3, 5). They constitute two separate species of dermatan sulfate proteoglycans having different core proteins and sharing no common immunological epitopes (6).

This investigation was undertaken to elucidate further the chemical and immunochemical relationships between the dermatan sulfate proteoglycans of sclera and articular cartilage. We also wanted to study the localizations within the eye of those proteoglycans isolated from sclera.

MATERIALS AND METHODS

Tissue Preparations—Adult bovine eyes were removed from the animal immediately after death. For immunochemistry, eyes were cut into strips along the median axis, frozen in OCT compound (Tissue Tek), cut into 4-μm sections in a cryostat, and fixed for 5 min in 4% formaldehyde (7, 8).

†††† The abbreviations used are: DS-PG, dermatan sulfate proteoglycan; ELISA, enzyme-linked immunosorbent assay.
**Proteoglycan Preparations**—Dermatan sulfate proteoglycans PGI and PGII from adult bovine sclera (3, 4) and DS-PGI and DS-PGII from adult articular cartilage (2) were isolated as previously described. The large aggregating cartilage proteoglycan (AID1) containing chondroitin sulfate and keratan sulfate side chains was isolated from bovine adult articular cartilage (2).

**Antibodies to Proteoglycan**—R59 and R57 are polyclonal rabbit antisera raised against the large (PGI) and small (PGII) scleral dermatan sulfate proteoglycans, respectively (6). R103 is a rabbit antiserum to the hyaluronic acid binding region of high buoyant density aggregating cartilage proteoglycan isolated from the Swarm rat chondrosarcoma (9). R164 is a rabbit antisera directed against the small cartilage DS-PGII (2). R110 recognizes the bovine native articular proteoglycan (AID1) and the antibody was prepared as described for R114 (9). DS1 is a mouse monoclonal antibody against the small dermatan sulfate proteoglycan (DS-PGII) from cartilage (2); AN9P1 is a mouse monoclonal antibody against the keratan sulfate binding region of the large aggregating adult human cartilage proteoglycans (9). IgG Fab' antibody subunits were prepared as described (8).

**Immunoassay**—ELISA assays were performed essentially as reported earlier (2) with the following modifications. Proteoglycans were bound at 4°C overnight at 100 ng/50 μl unless otherwise indicated. Alkaline phosphatase-conjugated pig IgG anti-mouse or anti-rabbit IgG were used for the color reaction.

**Immunolocalization**—After fixation, sections were washed in phosphate-buffered saline. The sections were then treated with chondroitinase ABC (12.5 milliunits) at 37°C for 90 min (7). IgG Fab' (1), was derived from antisera R59 (3.8 mg/ml), R57 (3.8 mg/ml), and nonimmune rabbit serum (3.5 mg/ml). Ascites fluid DS1 (0.16 mg/ml) and control ascites fluid were used at the concentrations indicated as described earlier (10). Each section was treated for 30 min with 50 μl of one of the mouse or rabbit immunoreagents followed by a wash in phosphate-buffered saline (7). All sections were then treated for 30 min with fluorescein isothiocyanate-conjugated pig Fab' anti-mouse IgG (10) or Fab' anti-rabbit IgG (8) and then washed. The preparation of sections and microscopy were performed as described earlier (8). Photographs were taken with green only fluorescence emission.

**Amino Acid Sequencing**—Dermatan sulfate proteoglycans DS-PGII from cartilage (0.3 nmol) and PGI from sclera (1.5 nmol) were applied to a Biobind™-coated glass filter of an Applied Biosystem model 470 gas phase sequenator. The released anilothiazoleamine amino acids were converted to phenylthiohydantoin amino acids using the trifluoroacetic acid conversion program. The phenylthiohydantoin amino acids were analyzed on a Beckman 346 HPLC chromatograph using the procedure described by Lazare et al. (11).

**RESULTS**

The dermatan sulfate proteoglycans from articular cartilage (2) and sclera (3, 4) used in this study have been characterized extensively regarding their molecular mass, size of core protein, glycosaminoglycan chains, and carbohydrate compositions. The small DS-PGII from cartilage and the small PGII from sclera are very alike in molecular mass, in size of the core protein, and in iduronic acid content of the glycosaminoglycan side chains.

**ELISA Assays**

The immunological relationships of the different species of DS-PGs from sclera and cartilage were studied by ELISA assays. The results for intact proteoglycans are shown in Table 1. Chondroitinase ABC treatment of the proteoglycans increased the reactivity where immunological reactivity was found for the intact proteoglycans (not shown). It has earlier been shown that the large and small DS-PG from the same tissue do not cross-react (2, 6) but some of these results are included for comparison. Evidence for the presence of hyaluronic acid binding region (detected with antisera R103) and cross-reactivity with the large cartilage chondroitin sulfate PG (AID1) (detected with antisera R110 and R59) was only found for the large PGI from sclera. No cross-reactivity was found between the two large DS-PG from sclera and cartilage using antisera R59. There was no cross-reactivity between the large and small proteoglycans of sclera. The large DS-PGI from cartilage did not react with any of the antibodies used except for a limited reaction with antisera R166 to the small DS-PGII of cartilage. None of these dermatan sulfate proteoglycans reacted with the antibody (AN9P1) which recognizes keratan sulfate. The two small DS proteoglycans displayed complete immunological cross-reactivity with each other as shown by studies with antisera R57, R166, and monoclonal antibody DS1.

**Immunolocalization**

Tissue sections treated with either nonimmune rabbit IgG Fab' (Fig. 1H) or with control ascites fluid (Fig. 1L) exhibited a complete lack of green fluorescein fluorescence.

**Antiserum R59 to Scleral PGI**—In sections treated with antibody R59 a strong nonuniform fluorescence could be seen extracellularly distributed throughout the sclera (Fig. 1B). No fluorescence could be detected in the cornea (Fig. 1, A and D), iris (Fig. 1C), or uveal tract (not shown).

**Antiserum R57 to Sceral PGI**—In the sclera there was strong uniform staining in extracellular sites (Fig. 1F). Strong staining was also detected in the iris (Fig. 1G) and uveal tract (not shown). The cornea was also stained (Fig. 1E).

**Antibody AN9P1 to Cartilage DS-PGII**—The results for antibody DS1 were virtually identical to those for R57. Strong staining was seen in the extracellular matrix of the cornea (Fig. 1J), sclera (Fig. 1J), iris (Fig. 1K), and uveal tract (not shown).

**Amino Acid Sequencing**

A comparison of the results obtained for the cartilage DS-PGII and scleral PGII and the published data for the skin dermatan sulfate proteoglycan (12) is shown in Table II. They are identical for the first 14 residues in all cases and for the first 20 (sclera and skin) except for residue 4 which could not be resolved in the case of the small cartilage proteoglycan. With the scleral molecule, serine was detected at position 4 but the signal was reduced. In skin, position 4 has been shown to be a serine residue to which a single dermatan sulfate chain is attached (12). Hence, we conclude that the dermatan sulfate chain may be similarly attached to the core protein isolated from cartilage and sclera. The molecular masses of these molecules (2, 5, 12) suggest that 1–2 dermatan sulfate chains are bound to core protein. The amino-terminal sequence analysis of the small dermatan sulfate proteoglycan isolated from tendon (13) has revealed an identical sequence to that reported here for cartilage and sclera (14). As yet, we have been unable to obtain any continuous sequence data for the larger DS-PGI from cartilage and the scleral PGII; that which has been derived demonstrates the unrelatedness of these proteoglycans to each other and to the smaller molecules (data not shown).

**GENERAL DISCUSSION**

It has recently been proposed that the galactosaminoglycan containing proteoglycans, isolated from various tissues including sclera and cartilage, can, according to size, be initially divided into two groups consisting of large and small proteoglycans. Within each group, they are immunologically related and have similar peptide patterns (6). Thus, our data and earlier results together indicate that the large dermatan sulfate proteoglycan from sclera (PGI) would belong to the group of larger proteoglycans having a core protein of Mr = 70,000 or greater and a hyaluronic acid binding region. This group
TABLE I
Immunoreactivities of proteoglycans from sclera (PGI and PGII) and cartilage (A1D1, DS-PGI, and DS-PGII) in ELISA assay

<table>
<thead>
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<th>Antibody</th>
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<th>Antibody binding measured at $A_{450nm}$</th>
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<tr>
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<td></td>
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<td>R57 (1:4)</td>
<td>PGI from bovine sclera</td>
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<td>R59 (1:8)</td>
<td>PGI from bovine sclera</td>
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<td>R166 (1:20)</td>
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<td>NM</td>
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<td>R110 (1:8)</td>
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<td>R103 (1:16)</td>
<td>Hyaluronic acid binding region of rat cartilage PG</td>
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<td>NRS (1:4)$^e$</td>
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<td>AN9P1 (1:16)</td>
<td>Adult human A1D1</td>
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<td>DS1 (1:8)</td>
<td>Mixture of DS-PGI and DS-PGII from bovine articular cartilage</td>
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<tr>
<td>Ascites fluid</td>
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* Proteoglycans bound at 225 ng/well.
* Data from Ref. 2.
* NM, not measured.
* NRS, nonimmune rabbit serum.

Fig. 1. Localizations of proteoglycans in fixed sections of cornea, sclera, and iris treated with chondroitinase ABC. Longitudinal sections of sclera (B) show intense staining with antiserum R59 to large dermatan sulfate proteoglycan (PGI) from sclera and at the sclera-cornea junction (D); there is no staining in the iris (C) and cornea (A). Using antiserum R57 to the small DS-PG (PGII) from sclera, staining can be seen in the cornea (E), sclera (F), and iris (G); with monoclonal antibody (DS1) to the small DS-PG from articular cartilage (PGII) there is staining in cornea (I), sclera (J), and iris (K). Control sections of sclera showed absence of staining when treated with nonimmune rabbit serum (H) and the ascites fluid control (L). The control section for cornea was identical to A (results not shown). Scales = 50 μm.
The widespread distribution of these molecules, therefore would appear to be related to this interaction with type I collagen through the core protein (24), (25), the structure of which appears to be common to all of these molecules.

The related characteristics of the other dermatan sulfate cartilage proteoglycan DS-PGI would indicate that this is a molecule with a distinct core protein and function which remains to be elucidated.

Acknowledgments—We would like to thank Isabelle Pidoux, Agnes Reiner, and Elisa de Miguel for their technical assistance and Freda Rowbotham for processing the manuscript.

REFERENCES


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<tr>
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* Results taken from Ref. 12. PDS, proteodermatan sulfate.
* Unidentified residue.

includes the large chondroitin sulfate-rich cartilage proteoglycan, the large tendon proteoglycan, and the large aorta proteoglycan (1). A major difference from the large cartilage molecule is that sclera PGI has no keratan sulfate, as defined immunologically, nor is there any published evidence for the presence of keratan sulfate in these other large aggregating molecules.

The ability of the large PGI from sclera to aggregate with hyaluronic acid suggests that this interaction is probably stabilized by the link protein we previously detected in sclera (7), in the manner observed for cartilage link protein (15-17). Thus, the aggregated scleral proteoglycan PGI probably plays an important role, as in cartilage (18), in endowing sclera with compressive stiffness and the ability to sustain loading resulting from intraocular pressure.

The small dermatan sulfate proteoglycan from sclera (PGII) would belong to the group of small proteoglycans. Adult articular cartilage contains two different dermatan sulfate proteoglycans, both having a size of 45 kDa after removal of glycosaminoglycans by chondroitinase ABC. Our immunological and sequencing data indicate that the small DS-PGI from articular cartilage is unrelated to the other molecule, DS-PGI, but can be assigned to the group of small proteoglycans which would appear to have similar or the same core proteins, which include the small scleral proteoglycan PGI, proteodermatan sulfate from skin, and the small tendon proteoglycan.

The physicochemical properties of these proteoglycans are probably largely influenced by the galactosaminoglycan chains on the proteoglycan. The degree of epimerization of glucuronic acid to iduronic in the galactosaminoglycan chain may vary considerably, depending upon the tissue and this can in turn influence the properties of the molecule (1). Also, the type and number of side chains varies within (2, 3, 13) and between (1, 3) tissues. Thus, the corneal staining detected immunologically with antibodies to scleral PGIII would appear to represent the reaction of the small proteoglycan that has been isolated from cornea and which contains relatively little iduronic acid (19) and which has already been shown to be immunologically related to the small proteoglycan PGIII from sclera (6).

Immunological (20) and histochemical (21, 22) studies have revealed that these small proteoglycans are bound to type I collagens. They are also probably involved in fibrillogenesis (23, 24). The widespread distribution of these molecules, therefore would appear to be related to this interaction with type I collagen through the core protein (24, 25), the structure of which appears to be common to all of these molecules.