Isolation of a Chondroitin Sulfate Proteoglycan from a Rat Yolk Sac Tumor and Immunochromatographic Demonstration of Its Cell Surface Localization*

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A proteoglycan was isolated from ascites fluid produced by a rat yolk sac tumor. The glycosaminoglycan chains of the proteoglycan are all sensitive to digestion with chondroitinase ABC and about 90% are sensitive to chondroitinase AC. The proteoglycan contains 5% protein. Amino acid analysis revealed a high content of serine and glycine which together constitute 37% of the amino acids. Glutamic acid (glutamine) and aspartic acid (asparagine) are also abundant. Galactosamine accounts for 97% of the hexosamine and the remainder is glucosamine. These characteristics indicate that the glycosaminoglycan side chains of this proteoglycan are predominantly chondroitin sulfate with a smaller amount of dermatan sulfate.

Antibodies to the proteoglycan were prepared by immunization of a rabbit with purified alcalki-treated proteoglycan. Affinity-purified antibodies from the antisera immunoprecipitated (35S)Sulfate-labeled radioactivity from culture media of the yolk sac tumor cells known to contain chondroitin sulfate proteoglycan. This binding was inhibited by the intact purified proteoglycan but not by proteoglycan treated with papain, suggesting dependence of the reactivity of the antibodies on integrity of the protein part of the proteoglycan. Immunofluorescence of the cultured yolk sac tumor cells revealed localization of immune reactive proteoglycans at the cell surface.

Proteoglycans are ubiquitous compounds composed of a core protein and glycosaminoglycan side chains (1). Proteoglycans and glycosaminoglycans are implicated in a number of important cell surface phenomena. They are present in extracellular matrices, in basement membranes, and at cell surfaces. In addition to their structural role in tissues such as cartilage, proteoglycans are thought to be involved in adhesion of normal cells, and changes in the expression of cell surface proteoglycans may contribute to the altered adhesive properties of malignant cells (9-11). Proteoglycans may also function as mediators of specific cell surface interactions directing cellular differentiation and movement during development (12, 13).

While extensive studies have been conducted on cartilage proteoglycan (reviewed in Ref. 14), relatively little is known about the molecular properties and distribution of the proteoglycans associated with nonchondrocytic cells. It has been proposed that the proteoglycans present in cartilage and synthesized by chondrocytes are structurally different from chondroitin sulfate proteoglycans synthesized by fibroblast-like cells (15, 16). Because of the known or suspected involvement of proteoglycans in important biological phenomena, their further characterization would clearly be desirable. We describe here the isolation and partial chemical and immunochromatographic characterization of a chondroitin sulfate proteoglycan from a rat yolk sac tumor.

EXPERIMENTAL PROCEDURES

Materials

A rat yolk sac tumor, L2, established by R Albrechtsen and U. Wewer, University of Copenhagen, Denmark, was used as a source of proteoglycan. The tumor was maintained in ascites form in Lewis rats (17) and was serially transplanted every 2-3 weeks. Ascites fluid from tumor-bearing rats was centrifuged (1000 x g, 5 min) to remove cellular material, phenylmethanesulfonfyl fluoride was added to a final concentration of 0.2 mM and the fluid was stored at -20 °C. (35S)Sulfate-labeled ascitic fluid was prepared by injecting tumor-bearing rats with 0.5 mc of (35S)Sulfate intraperitoneally. After 2 h the rats were killed and the tumor and ascitic fluid were collected as described above. The tumor cells were cultured as described previously (17).

Papain (twice crystallized) was obtained from Sigma. Chondroitinase ABC and chondroitinase AC II were from Miles. Hyluronidase (Streptomyces hyaluronicus) was purchased from Seikagaku Kogyo Co. (Tokyo, Japan). Chondroitin sulfate and hyaluronic acid standards were generous gifts from Dr. A. Wasteson, University of Uppsala, Sweden. The heparin preparation used was kindly provided by Dr. U. Lindahl, Swedish University of Agricultural Sciences (Uppsala, Sweden). These glycosaminoglycans have been previously described (18). Sephadex and Sepharose were from Pharmacia, and DE52 cellulose was from Whatman. (35S)Sulfate (688.1/mCi/mmol, carrier-free) and [3H]methionine (1314.5 Ci/mmol) were from New England Nuclear.

Methods

Uronic acid and protein were determined according to Bitter and Murz (19) and Lowry et al. (20), respectively. 35S radioactivity was measured in a Beckman LS 250 liquid scintillation counter using Aquasol-2 (New England Nuclear).

Identification of Glycosaminoglycans in Ascites Fluid—Ascites fluid (2 ml) was digested with papain and the digest was applied to a column (1.5 x 30 cm) of Sephadex G-50, eluted with 1 M NaCl. The material that eluted in the void volume of the column was pooled and diluted with 0.05 M sodium acetate buffer, pH 4.0, to a concentration of 0.2 M NaCl. The sample was chromatographed on a DE52 cellulose column (0.5 x 8 cm) eluted with a linear gradient (100 ml) of 0.2 to 1 M NaCl. The column was calibrated with hyaluronic acid and chondroitin sulfate standards. Two-mll fractions were collected and analyzed for uronic acid. Fractions containing uronic acid were pooled and digested with chondroitinase ABC or hyaluronidase. The degree of degradation was investigated by separating the digests on a Sephadex G25 column (0.5 x 50 cm) equilibrated with 1 M NaCl.

Purification of Proteoglycan—Ascites fluid was mixed with an
equal volume of saturated ammonium sulfate and held for 2 h at 6°C. The precipitate was removed by centrifugation (20,000 × g, 15 min) and the supernatant was dialyzed against several changes of 0.3 M NaCl at 6°C. Subsequently, 200 ml of the dialyzed ammonium sulfate supernatant (corresponding to 80 ml of acetic acid) were added to an equal volume of 2% CPC in 0.3 M NaCl. A flocculent precipitate formed and after 30 min at room temperature, the precipitate was collected by centrifugation (2,000 × g, 10 min). The CPC precipitate was dissolved in 10 ml of 2 NaCl and mixed with 90 ml of 95% ethanol. After 20 h at 6°C the mixture was centrifuged (2,000 × g, 10 min). The pellet was dissolved in 5 ml of 1 M NaCl and applied to a Sepharose CL-4B column eluted with 1 M NaCl (see Fig. 1). The fractions from the Sepharose CL-4B column were pooled and diluted with 0.05 M sodium acetate, pH 4.0, to a concentration of 0.2 M NaCl. The sample was applied to a DE52-cellulose column. After washing with 250 ml of 0.2 M NaCl, 0.05 M sodium acetate, pH 4.0, the column was eluted with a 300-ml linear gradient of 0.2 to 1.0 M NaCl in 0.05 M sodium acetate, pH 4.0 (see Fig. 2). Uronic acid-containing fractions from the ion-exchange chromatography were pooled, extensively dialyzed against deionized water, and lyophylized. The same procedures were used to isolate and characterize proteoglycan extracted homogenate from rat yolk sac tumor from about 8 pregnant rats.

Enzyme Digestions—Incubations of proteoglycan with chondroitinase ABC and ABC were carried out as previously described (8). When the papain-digested proteoglycan was used in immunological studies, the digestion was terminated by adding iodoacetamide to a final concentration of 50 mM. Hyaluronidase digestion was carried out in 0.05 M acetate, pH 5.0, containing 2 turbidity-reducing units of Streptomyces hyaluronidase. Polysaccharide samples (1 mg/ml) were digested for 16-18 h at 37°C.

Alkali treatment of the proteoglycan was performed in 0.5 M NaOH, 0.1 M N-ethylmaleimide, and pH 7.0, containing 15 mM N-ethylmaleimide, 10 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride. The proteoglycan was extracted from the homogenate with 3.5 M NaCl.

Enzyme Digestions—Incubations of proteoglycan with chondroitinase ABC and ABC were carried out as previously described (8). When the papain-digested proteoglycan was used in immunological studies, the digestion was terminated by adding iodoacetamide to a final concentration of 50 mM. Hyaluronidase digestion was carried out in 0.05 M acetate, pH 5.0, containing 2 turbidity-reducing units of Streptomyces hyaluronidase. Polysaccharide samples (1 mg/ml) were digested for 16-18 h at 37°C.

Amino acid analyses were performed in a Beckman 119 automatic analyzer. The analyses were kindly performed by Dr. E. Miller, University of Alabama, and by the amino acid analysis facility of Scripps Clinic and Research Foundation (Grant GM27452 from The National Institute of General Medical Sciences, Department of Health and Human Services). Proteoglycan samples were hydrolyzed in 6 M HCl at 110°C for 24 h. The correction factors for labile amino acids were the same as in previous work (22).

Preparation of Antiserum to Proteoglycan—A rabbit antiserum was prepared against alkali-treated proteoglycan. For this purpose, the alkali treatment was performed with 0.05 M NaOH at 20°C, and after 6 h the reaction was terminated by neutralization with 1 M acetic acid. A fraction, representing 2 mg (0.1 mg of protein) of the β-eliminated proteoglycan in 0.5 ml of NaCl/Pi, mixed with 0.25 ml of Freund’s complete adjuvant. Similar injections were given every second week and the rabbit was bled 8-10 days after the third and each subsequent injection. The antiserum was absorbed by passage through a column of rat serum proteins coupled to Sepharose 4B. Typically, 10 ml of antiserum were absorbed with 10 ml of rat serum Sepharose (about 10 mg of protein/ml of Sepharose 4B). The antibodies specific for the proteoglycan were isolated by affinity chromatography on proteoglycan-Sepharose. The proteoglycan was coupled to AH-Sepharose 4B using carbodimide (23) to give about 1 mg of proteoglycan/ml of gel. Antibodies from 10 ml of antiserum were bound to 1 ml of proteoglycan-Sepharose, the column was washed with 0.5 M NaCl/Pi, and the bound antibodies were eluted with 0.1 M glycine-HCl, pH 2.6. Finally, the affinity-purified antibodies were dialyzed against NaCl/Pi, and concentrated to 1 ml.

&sup2;S-labeled Antigens—Yolk sac tumor cells (L2 cells) were maintained in culture as previously described (17). The cell cultures were labeled for 24 h with &sup2;S-sulfate (20 μCi/ml of media) or &sup2;S-methionine (5 μCi/ml of media). Labeled macromolecules in the media were precipitated by addition of 5 volumes of 95% ethanol. After incubation at 8°C for 24 h, the precipitates were collected by centrifugation, redissolved in NaCl/Pi, (1/5 × original volume) and dialyzed against NaCl/Pi.

Immunoprecipitation and Enzyme Immunoassay—Antiserum dilutions were incubated with &sup2;S-sulfate-labeled antigen in a volume of 525 μl. After incubation at 6°C for 16-18 h, 1 ml of protein A-Sepharose (50% suspension in NaCl/Pi) was added and the incubation continued for 1 h at 20°C. The gel was washed 5 times with 2 ml of NaCl/Pi, and after that radioactivity was extracted by two successive additions of 1 ml of glycine-HCl, pH 2.6. Binding of antibodies to various antigens was studied in enzyme immunoassay (ELISA) by coating microtiter wells with the antigens followed by detection of binding of antibodies to such wells by enzyme-conjugated anti-rabbit IgG as described (24).

Immunofluorescence—Proteoglycan was detected in cell layers by immunofluorescence as described (25), after fixation with 3% paraformaldehyde or acetone.

RESULTS

Identification of Glycosaminoglycans in Ascites Fluid—Uronic acid-containing macromolecules, obtained after papain digestion of ascites fluid were chromatographed on a column of DEAE-cellulose. The polysaccharides emerged as two peaks with elution positions identical with standard hyaluronic acid and chondroitin sulfate.

The glycosaminoglycan with an elution position coinciding with that of the hyaluronic acid standard, represented 18% of the total uronate content in the ascitic fluid. The polysaccharide was degraded by treatment with Streptomyces hyaluronidase, an enzyme that specifically degrades hyaluronic acid (26). Based on this susceptibility to Streptomyces hyaluronidase and the elution position in DEAE-cellulose chromatography, the glycosaminoglycan was identified as hyaluronic acid.

Most of the uronic acid-containing material had the same elution position in DEAE-cellulose chromatography as the chondroitin sulfate standard and was degraded to disaccharides by treatment with chondroitinase ABC. This glycosaminoglycan represented 82% of the total uronic acid content in ascitic fluid and was identified as chondroitin/dermatan sulfate. No heparin or heparan sulfate was detected.

Purification of Proteoglycan—The steps in the isolation of proteoglycan from clarified ascitic fluid are summarized in Table I. Most of the uronic acid was recovered in the supernatant after precipitation with 50% saturated ammonium sulfate. CPC precipitation of the dialyzed supernatant from the ammonium sulfate precipitation step yielded only 1 mg of proteoglycan/ml of NaCl. This was presumably due to a loss of low molecular weight oligosaccharides and hyaluronic acid, which are soluble in CPC at this ionic strength. After ethanol precipitation, the uronic acid-containing material was applied to a column of Sepharose CL-4B (Fig. 1). The uronic acid eluted as a broad peak immediately after the void volume of the column. As indicated by absorbance at 260 nm, this purification step resulted in the removal of most of the nucleic acids co-precipitated with the proteoglycan in the preceding steps. Effluent fractions from the Sepharose CL-4B fractionation were pooled as shown and further purified by DEAE-cellulose chromatography (Fig. 2). In this final purification step, the proteoglycan eluted as a single peak at about 0.6 M NaCl. Based on the absorbance at 260 nm, nucleic acids

| Recovery of uronic acid | mg | % |
|-------------------------|----|---|
| Starting material (80 ml of ascitic fluid) | 26.9 | 100 |
| Ammonium sulfate precipitation | 17.9 | 72 |
| CPC and ethanol precipitation | 10.9 | 48 |
| Sepharose CL-4B gel chromatography | 9.3 | 40 |
| DEAE-cellulose chromatography | 7.2 | 32 |

| Isolation of proteoglycan from rat yolk sac tumor ascites fluid | Purification step |
|-----------------------|------------------|
| Alfalfa extract | 80 ml of ascites fluid |
| Protein A-Sepharose (50% suspension in NaCl/Pi) | 525 μl |
| Uronic acid content | 525 μl |
| Enzyme Immunoassay—Antiserum dilutions | 525 μl |
| Immunoprecipitation of Proteoglycan | 525 μl |
| Immunofluorescence | 525 μl |

*The abbreviations used are: CPC, cetylpyridinium chloride; ELISA, enzyme-linked immunosorbent assay; NaCl/Pi, phosphate-buffered saline (157 mM NaCl, 1.7 mM KCl, 8.1 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.3).
Yolk Sac Tumor Proteoglycan

Fraction Number

FIG. 1. Gel filtration on Sepharose CL-4B of material obtained from ascites fluid after ethanol precipitation. The sample (5 ml) was applied to a column (0.5 x 110 cm) of Sepharose CL-4B eluted with 1 M NaCl at 20 °C and 9-ml fractions were collected. These were analyzed for uronic acid (O) and A280 nm (O) and pooled as indicated. V0 and Vt mark the void volume and total volume of the column, respectively.

FIG. 2. DEAE-cellulose chromatography of uronic acid-containing material from Sepharose CL-4B chromatography. Pooled fractions from the Sepharose CL-4B chromatography were adjusted to 0.2 M NaCl with 0.05 M acetate buffer, pH 4.0, and then applied to a DE52 cellulose column (20 ml). After washing with 250 ml of the same buffer, the column was eluted at 20 °C with a 300-ml linear gradient of 0.2 to 1.0 M NaCl in the acetate buffer. Fractions of 5 ml were collected and analyzed for uronic acid (O) and A280 (O). Fractions were pooled as indicated. Constituted less than 1% (w/w) of the material in this peak. The yield was 7.2 mg of uronic acid from 80 ml of ascitic fluid. This represented a 32% recovery of uronic acid. Identical procedures when applied to ascitic fluid or extracts of tumor from 35SO4-injected rats yielded labeled material which eluted in gel filtration and DEAE-cellulose chromatography at the same position as the ascites proteoglycan (result not shown).

Characterization of the Proteoglycan—The proteoglycan-containing fractions from the DEAE-chromatography were pooled as shown in Fig. 2 and after lyophilization the properties of the proteoglycan were studied. The preparation contained 23% (w/w) hexuronic acid and 5% (w/w) protein. These values are similar to those obtained for proteoglycans isolated from hyaline cartilage (27). Chondroitin sulfate and dermatan sulfate proteoglycans from aortic tissue (28) and lung tissue (29) contain more protein, 18 and 22%, respectively.

The amino acid composition of the proteoglycan is shown in Table II. In general, the composition is similar to that found in other proteoglycans (27–29) with large amounts of serine, glycine, glutamic acid, and aspartic acid. The serine and glycine residues were present in equimolar amounts and constituted 37% of the total amino acids. No cysteine was detected. Alkaline treatment of the proteoglycan resulted in a 57% reduction in the serine content, indicating that these serine residues were substituted with glycosaminoglycan via serine-xylose bonds (30). Both galactosamine and glucosamine were present in the proteoglycan preparation, but galactosamine constituted 97% of the hexosamines.

The purified proteoglycan was chromatographed on a column of Sepharose CL-2B (Fig. 3). The chromatogram showed a heterogeneous size distribution and a major peak with a Kav of 0.46. Reduction and alkylation of the proteoglycan did not change the elution position on Sepharose CL-2B, suggesting

| Amino acid composition of the proteoglycan Residues/1000 | Aspartic acid | Thrreonine | Serine | Glutamic acid | Proline | Glycine | Alanine | Cysteine | Valine | Methionine | Isoleucine | Leucine | Tyrosine | Phenylalanine | Histidine | Lysine | Arginine | GalNH2 | GlcNH2 |
|--------------------------------------------------------|--------------|------------|--------|---------------|---------|---------|---------|----------|--------|------------|-----------|---------|----------|----------------|----------|--------|-----------|---------|---------|
| Aspartic acid                                          | 88           | 34         | 183    | 116           | 61      | 185     | 68      | 10       | 72     | 18         | 20        | 20      | 43       | 61              | 22       | 15     | 15         | 2287    | 82      |

a Value for tyrosine was not obtained because it was obscured by the large galactosamine peak.

b The numbers of hexosamine residues are expressed per 1000 amino acid residues, without correction for destruction during hydrolysis.
that the proteoglycan was not aggregated with hyaluronic acid (31).

The size of the polysaccharide chains was investigated by Sepharose CL-6B chromatography (Fig. 4). After alkali treatment, the glycosaminoglycans eluted from the column with a \( K_v \) of 0.35. A similar result was obtained when the proteoglycan was chromatographed after papain digestion. This \( K_v \) is the same as was obtained by Oegema et al. (28) for a papain-digested proteoglycan isolated from bovine aorta and suggests a \( M_w \) \( \approx \) 40,000 for the polysaccharide chains.

Chondroitinase ABC digestion of the polysaccharide chains obtained by alkali treatment of the proteoglycan resulted in an almost quantitative conversion to disaccharides as shown by gel filtration on Sephadex G-25 (Fig. 5). A similar treatment with chondroitinase AC digestion demonstrated that 9% of the glycosaminoglycans were resistant to this enzyme. As chondroitinase AC is more active as an exoglycosidase than as an endoglycosidase (32), the results suggest that the proteoglycan preparation contains at most 9% dermatan sulfate. The colorimetric method of Kresse et al. (33) gave an iduronic acid content of 5% for the proteoglycan. As iduronic acid is the hexuronic constituent in dermatan sulfate, this result suggests a dermatan sulfate content of 10%. The remaining 90-95% of the glycosaminoglycans in the proteoglycan preparation appears to be chondroitin sulfate.

We also studied the proteoglycans of the L2 tumor tissue. As the amount of proteoglycan obtained from the tumor was smaller than what was recovered from ascites, radiolabeled material was used. The \( \text{[35S]} \) sulfate-labeled material isolated from extracts of tumors from such animals showed similar size on gel filtration and similar sensitivity to digestion with chondroitinase ABC and AC as the ascites proteoglycan (results not shown).

**Immunological Studies**—An antiserum against alkali-treated proteoglycan was induced in a rabbit. Antibodies to the proteoglycan were detected by immunoprecipitation of \( \text{[35S]} \) sulfate-labeled culture media from yolk sac tumor cells. Judged from the susceptibility to chondroitinase ABC, 75-80% of the \( \text{[35S]} \) labeled macromolecules in the L2 culture media were chondroitin/dermatan sulfate. A 5-fold dilution of the antiserum precipitated about 30% of the \( \text{[35S]} \) radioactivity (Fig. 6) while normal rabbit serum gave a background precipitation of 2%. All of the \( \text{[35S]} \) sulfate-labeled macromolecules precipitated by the antiserum were susceptible to digestion with chondroitinase ABC and were identified as chondroitin/dermatan sulfate. When \( \text{[35S]} \) methionine-labeled culture medium was precipitated in the same way, no radioactivity above the level given by normal rabbit serum could be detected in the precipitate.

Competitive binding studies were performed to determine the ability of the proteoglycan and proteoglycan-derived preparations to inhibit the interaction between the antiserum and \( \text{[35S]} \) labeled proteoglycans from L2 medium (Fig. 7). The intact...
proteoglycan was the most efficient inhibitor, with proteoglycan corresponding to about 0.2 μg of uronic acid displacing 50% of the labeled antigen. Alkali-treated proteoglycan, which was used to induce the antiserum, was about half as active as the intact proteoglycan. Papain-digested proteoglycan did not inhibit the interaction between the antibodies and the antigen.

The antiserum did not react with fibronectin or laminin as a control gives no staining (c). The undiluted inhibitors were present at a concentration corresponding to 86 μg of uronic acid/ml. Antibody-bound radioactivity was determined as described under "Experimental Procedures."

The antiserum was used at a 1:10 dilution and the inhibitors were diluted as indicated. The undiluted inhibitors were isolated at a concentration corresponding to 86 μg of uronic acid/ml. Antibody-bound radioactivity was determined as described under "Experimental Procedures."

**DISCUSSION**

The rat yolk sac tumor we used to isolate the proteoglycan described here produces large amounts of extracellular material. Laminin, a newly described basement membrane protein (34, 35), is a major component of this extracellular material (17). Laminin is also found in the ascites fluid produced by the tumor as well as in culture media of these tumor cells grown in vitro (17). The proteoglycan we have described shows a similar distribution. It was isolated from ascites fluid and, judging from the presence of a similar material that could be metabolically labeled in tumor tissues and in cultured tumor cells, it seems to be a product of the tumor cells. Some transformed cell lines have been found to have elevated amounts of cell surface chondroitin sulfate (10, 36). The synthesis of large amounts of chondroitin sulfate proteoglycan by our yolk sac tumor cells may relate to the fact that these are also malignant cells. It could also be that this proteoglycan is a characteristic of the extraembryonic endoderm.

The extracellular matrix produced by yolk sac tumors is thought to correspond to Reichert's membrane of the normal embryo and this basement membrane is rich in laminin, one of the products of the yolk sac tumor (37, 38). Basement membranes have been shown to contain heparan sulfate proteoglycan (39). The proteoglycan we have isolated contains predominantly chondroitin sulfate as demonstrated by its sensitivity to chondroitinase and the presence of galactosamine as the main amino sugar. Studies to establish the in vivo localization of the L2 chondroitin sulfate proteoglycans are presently underway.

Chondroitin sulfate proteoglycans have been isolated from a number of tissues. The L2 yolk sac tumor proteoglycan appears to be different from the extensively studied cartilage proteoglycan in that the latter has a higher molecular weight. The $K_w$ value of the L2 proteoglycan on Sepharose CL-2B was 0.46, while that of the main component in cartilage proteoglycan was 0.15 to 0.23 (28, 29). The chondroitin sulfate proteoglycans isolated from aorta (28) and lung tissue (29) also have higher $K_w$ values than the yolk sac tumor proteoglycan. However, proteoglycans isolated previously have shown polydispersity, and components with $K_w$ values similar to that of our proteoglycan have been observed (40-42).

**Fig. 7.** Radioimmunoassay inhibition curves. Inhibition of the binding of (35S)sulfate-labeled proteoglycans from L2 cell culture media to antiserum to chondroitin sulfate proteoglycan by the intact proteoglycan (C), alkaline-treated proteoglycan (O), and papain-digested proteoglycan (A). The antiserum was used at a 1:10 dilution and the inhibitors were diluted as indicated. The undiluted inhibitors were present at a concentration corresponding to 86 μg of uronic acid/ml. Antibody-bound radioactivity was determined as described under "Experimental Procedures."

**Fig. 8.** Immunofluorescent staining of cultured yolk sac tumor cells with affinity-purified antibodies to chondroitin sulfate proteoglycan. Comparison of fluorescent staining (a) and phase contrast micrograph (b) of the same field shows that the staining follows cellular outlines. Substitution of the antibodies with normal rabbit serum (diluted 1:10) as a control gives no staining (c and d).
the fact that the successful immunization was with the alkali-treated proteoglycan has any significance. The rationale for the latter approach was that the covalently bound glycosaminoglycan chains could cover the protein part so as to render it nonimmunogenic. We did not want to use chondroitinase digestion to release the core protein because immunization with proteoglycan treated this way has resulted in antibodies directed against the unsaturated uronic acid residues that remain attached to the protein after chondroitinase digestion (47).

We found that papain treatment abolished the reactivity of the proteoglycan with the antibodies. The possibility that the oligosaccharide chains would react with the antibody when coupled by the protein into a multivalent unit but not as single chains was excluded by the fact that alkali-treated proteoglycan reacted with the antibodies nearly as well as the intact proteoglycan. Based on these results, it appears that our antibodies are directed against the core protein of the proteoglycan. The antiserum precipitated only about one-half of the chondroitin sulfate proteoglycan radioactivity in L2 culture media. The reason for the incomplete reactivity of the material identified as chondroitin sulfate proteoglycan is not clear, but considering the observed polydispersity of the proteoglycan with regard to molecular weight, antigenic heterogeneity is not unexpected.

Our antibodies stained cultured yolk sac tumor cells in immunofluorescence. The appearance of the staining suggested a cell surface localization of the proteoglycan antigen. The fact that cells fixed with paraformaldehyde were stained but not after this fixation unless additional steps are taken to ensure penetration of the antibody (48). The antibodies to this proteoglycan could be useful in studies on the expression and function of proteoglycans at the cell surface.

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REFERENCES

1. Lindahl, U., and Högk, M. (1978) Annu. Rev. Biochem. 47, 381-417
2. Hascall, V. C., and Sajdera, S. W. (1970) J. Biol. Chem. 245, 4920-4930
3. Obrink, B., and Ocklind, C. (1978) Biochim. Biophys. Acta 85, 837-843
4. Hay, E. D., and Meir, S. (1974) J. Cell Biol. 52, 889-898
5. Kanwar, Y. S., and Farquhar, M. G. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 1303-1307
6. Parthasarathy, N., and Spiro, R. G. (1979) J. Biol. Chem. 254, 507-513
7. Kraemer, P. M. (1971) Biochemistry 10, 1437-1445
8. Oldberg, A., Hook, M., Obrink, B., Peterfi, E., and Rubin, K. (1977) Biochem. J. 164, 75-81
9. Culp, L. A., Rollins, B. J., Baniel, J., and Hitri, S. (1978) J. Cell Biol. 79, 788-801
10. Dietrich, C. P., and Armean, H. A. (1978) Biochem. Biophys. Res. Commun. 84, 794-801
11. Schubert, D., and LaCorbiere, M. (1980) J. Biol. Chem. 255, 11564-11569
12. Toole, B. P. (1976) in Neuronal Recognition (Baronde, S. H., ed) pp. 275-329, Plenum Publishing Corp., New York
13. Gordon, J. R., and Bernfield, M. R. (1980) Dev. Biol. 74, 118-135
14. Muir, H. (1977) in Cell and Tissue Interactions (Lash, J. W., and Burger, M. M., eds) pp. 87-99, Raven Press, New York
15. Okayasu, M., Pacifici, M., and Holtzer, H. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 3224-3228
16. Stevens, R. L., and Hascall, V. C. (1981) J. Biol. Chem. 256, 2053-2068
17. Wewer, U., Albrechtsen, R., and Ruoslahti, E. (1981) Cancer Res. 41, 1518-1524
18. Kjellen, L., Oldberg, A., and Höök, M. (1980) J. Biol. Chem. 255, 10407-10413
19. Bitter, T., and Muir, H. M. (1962) Anal. Biochem. 4, 330-334
20. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 285-275
21. Norgaard, B., Gilmeus, B., Westermarck, B., and Wasteson, A. (1978) Biochem. Biophys. Res. Commun. 84, 914-921
22. Miller, E. J. (1972) Biochemistry 11, 4903-4909
23. Tengblad, A. (1979) Biochim. Biophys. Acta 578, 281-289
24. Engvall, E. (1980) Methods Enzymol. 70, 419-439
25. Hayman, E. G., Engvall, E., and Ruoslahti, E. (1981) J. Cell Biol. 88, 352-357
26. Oyha, T., and Kaneko, Y. (1970) Biochim. Biophys. Acta 198, 607-609
27. Sajdera, S. W., and Hascall, V. C. (1969) J. Biol. Chem. 244, 77-87
28. Oegema, T. R., Jr., Hascall, V. C., and Eisenstein, R. (1979) J. Biol. Chem. 254, 1312-1318
29. Radhakrishnamurty, B., Smart, F., Dalpare, E. R., Jr., and Benson, G. S. (1980) J. Biol. Chem. 255, 7675-7682
30. Andersson, B., Hoffman, P., and Meyer, K. (1965) J. Biol. Chem. 240, 156-167
31. Hascall, V. C., and Riolo, R. L. (1972) J. Biol. Chem. 247, 4529-4538
32. Hyama, K., and Okada, S. (1978) J. Biochem. (Tokyo) 80, 1201-1207
33. Kresse, H., Heidel, H., and Buddecke, E. (1971) Eur. J. Biochem. 23, 557-562
34. Chung, A. E., Jaffe, R., Freeman, I. L., Vergnes, J.-P., Braginski, J. E., and Martin, G. R. (1979) Cell 16, 277-287
35. Timpl, R., Rhode, H., Robey, P. G., Rennard, S. I., Foidart, J.-M., and Martin, G. R. (1979) J. Biol. Chem. 254, 9933-9937
36. Chiarugi, V., and Dietrich, C. P. (1979) J. Cell Physiol. 99, 201-206
37. Hogan, B. (1980) Dev. Biol. 76, 275-285
38. Sakashita, S., and Ruoslahti, E. (1980) Arch. Biochem. Biophys. 205, 283-290
39. Hassell, J. R., Robey, P. M., Burrach, H.-J., Wilczek, J., Rennard, I. R., and Martin, G. R. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 4494-4498
40. Mayes, R. W., Mason, R. M., and Griffin, D. C. (1973) Biochem. J. 131, 541-553
41. Harding, T. E., and Muir, H. (1974) Biochem. J. 139, 565-581
42. Heinegard, D. (1977) J. Biol. Chem. 252, 1980-1989
43. Vertel, B. M., and Dorfman, A. (1978) Dev. Biol. 62, 1-12
44. Weislander, A., and Heinegard, D. (1979) Biochem. J. 178, 35-45
45. Sparks, K. J., Lever, P. L., and Goetinck, P. F. (1980) Arch. Biochem. Biophys. 199, 579-586
46. Poole, A. R., Heiner, A., Tang, L.-H., and Rosenberg, L. (1980) J. Biol. Chem. 255, 9255-9305
47. Christner, J. E., Caterson, B., and Baker, J. R. (1980) J. Biol. Chem. 255, 7102-7105
48. Biberfeld, P., Biberfeld, G., Molnar, Z., and Fagraeus, A. (1974) J. Immunol. Methods 4, 135-148