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A chondroitin sulphate proteoglycan from human cultured glial and glioma cells

Structural and functional properties

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A chondroitin sulphate proteoglycan capable of forming large aggregates with hyaluronic acid was identified in cultures of human glial and glioma cells. The glial-cell- and glioma-cell-derived products were mutually indistinguishable and had some basic properties in common with the analogous chondroitin sulphate proteoglycan of cartilage: hydrodynamic size, dependence on a minimal size of hyaluronic acid for recognition, stabilization of aggregates by link protein, and precipitability with antibodies raised against bovine cartilage chondroitin sulphate proteoglycan. However, they differed in some aspects: lower buoyant density, larger, but fewer, chondroitin sulphate side chains, presence of iduronic acid-containing repeating units, and absence (<1%) of keratan sulphate. Apparently the major difference between glial/glioma and cartilage chondroitin sulphate proteoglycans relates to the glycan rather than to the protein moiety of the molecule.

Chondroitin sulphate proteoglycan (CSPG) from cartilage is capable of reacting specifically with hyaluronic acid (HA) with the formation of large aggregates (Hardingham, 1981). These aggregates are deposited in the extracellular matrix of cartilage and are held to be of importance for the structure and function of this compartment; they were long considered to be unique to cartilage (Wiebkin *et al.*, 1975). The demonstration that cultures of human glial cells manufacture aggregating CSPG became an indicator that CSPG–HA interaction may be a more generalized phenomenon, not restricted to the extracellular matrix of cartilage (Norling *et al.*, 1978). Support for this idea has since accumulated, as new tissues and cell types have been found to be producers of aggregating CSPG (Wight & Hascall, 1983).

Whereas the structural features of cartilage CSPG have been studied in detail (Hardingham, 1981), those of the analogous non-cartilage product are still relatively unknown (Oegema *et al.*, 1979; McMurtrey *et al.*, 1979; Cöster *et al.*, 1979; Poole *et al.*, 1982; Wight & Hascall, 1983). Cartilage CSPG has a large hydrodynamic size ($K_{av.} = 0.3$ on Sepharose CL-2B) and a high buoyant density (>1.70g/ml) due to a high degree of substitution with CS chains (M_r about 20000). It interacts with HA via a globular CS-poor portion of the core protein; the binding is stabilized by link proteins. Only HA fragments consisting of five or more repeating disaccharide units are recognized by the binding site. These properties were utilized in the present work for a comparative analysis of CSPG derived from cultures of glial and glioma cells; they both were similar to, but not identical with, cartilage CSPG.

Abbreviations used: CS, chondroitin sulphate; CSPG, chondroitin sulphate proteoglycan; HA, hyaluronic acid; GuHCl, guanidinium chloride.

Experimental

Chemicals

Carrier-free [^{35}S]sulphate and [^{125}I]iodide were obtained from The Radiochemical Centre, Amersham, U.K. Sepharose and Sephadex gels and DEAE-Sephacel were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Whatman DEAE-cellulose (DE-52) was a product of Whatman Biochemicals, Maidstone, Kent, U.K. Bacte-

rial chondroitinase ABC (EC 4.2.2.4) and chondroitinase AC (EC 4.2.2.5), and the unsaturated reference disaccharides from chondroitin sulphate (CS), 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-4-*O*-sulpho-D-galactose (Δ-di-4S) and 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid-6-*O*-sulpho-D-galactose (Δ-di-6S), were provided by Miles Laboratories, Elkhart, IN, U.S.A. Testicular hyaluronidase (EC 3.2.1.35) was provided by AB Leo, Helsingborg, Sweden, Practical-grade guanidinium chloride (GuHCl) was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.; it was further purified as described by Norling *et al.* (1978).

Preparation of standards

High-molecular-mass HA was prepared by chromatographing HA on Sepharose CL-2B in 4M-GuHCl; the excluded portion (HA_I) was pooled.

HA oligosaccharides of specific size classes were prepared by partial digestion of HA with testicular hyaluronidase, followed by chromatography on a Sephadex G-50 (superfine grade) column (2cm × 200cm) in 1M-NaCl (Wasteson *et al.*, 1973). Fractions corresponding to the even-numbered oligosaccharides tetrasaccharides to dodecasaccharides (HA_4 to HA_{12}) were pooled, desalted and freeze-dried. Standards of HA_8, HA_{10} and HA_{12} were generously given by Dr. T. C. Laurent of this Department.

Cartilage proteoglycan was prepared from bovine nasal septum, essentially as described by Hascall & Sajdera (1969). A number of proteinase inhibitors were included during the preparation, as suggested by Oegema *et al.* (1975). The procedure included centrifugation first under associative conditions (fraction A1), then under dissociative conditions (fraction A1–D1) (Heinegård, 1972). The product was dialysed against 0.15M-NaCl and kept at −20°C until used. Cartilage CSPG was labelled with ^{125}I by using the chloramine-T method (Hunter & Greenwood, 1962).

Link proteins (Hardingham, 1979) were recovered from the lightest fractions of the dissociative step mentioned above. It contained two proteins with M_r values about 41000 and 44000, as shown by sodium dodecyl sulphate/polyacrylamide-electrophoresis under reducing conditions (Laemmli, 1970). The material was dialysed against 4M-GuHCl and stored at −20°C; a portion of the preparation was labelled with ^{125}I (Hunter & Greenwood, 1962). Fibronectin was kindly given by Dr. S. Johansson and collagen types I and III by Dr. K. Rubin of this Department; laminin was purchased from Bethesda Research Laboratories, Rockville, MD, U.S.A. The preparations were labelled with ^{125}I as described above.

CS fractions of known M_r values were available in our laboratory (Wasteson, 1971). ^{14}C-labelled hexa- or tetra-saccharides were obtained after hyaluronidase treatment of ^{14}C-labelled CS, followed by Sephadex G-25 chromatography (Amadò *et al.*, 1974; Ingmar & Wasteson, 1979). The analogous penta- and tri-saccharides were obtained from the respective higher oligosaccharides by digestion with β-glucuronidase. Treatment of ^{14}C-labelled CS with chondroitinase ABC yielded the unsaturated disaccharides, Δ-di-4S and Δ-di-6S.

Cell culture and labelling conditions

The normal human glial-cell lines U-787 CG and U-1508 CG and the human established malignant glioma-cell line U-251 MG were kept under conditions previously described (Glimelius *et al.*, 1978*a*). The experimental details for incubation of cultures with [^{35}S]sulphate have been described previously (Glimelius *et al.*, 1978*a*).

AH-Sepharose chromatography

^{35}S-labelled samples were applied to a 1.0cm × 2.0cm column of AH-Sepharose 4B, equilibrated with 10mM-Tris/HCl buffer, pH8.0, containing 0.15M-NaCl, 1mM-Na_2SO_4 and 0.1% bovine serum albumin and operated at 4°C at a flow rate of 18ml/h. The column was then washed with the same buffer, supplemented with 0.4M-GuHCl, to decrease the radioactivity in the eluate to below 100c.p.m./ml. Elution was then made with 10mM-Tris/HCl buffer, pH8.0, containing 4M-GuHCl, 0.15M-NaCl, 1mM-Na_2SO_4 and 0.1% bovine serum albumin (buffer A). Under these conditions both intact [^{35}S]proteoglycans and free ^{35}S-labelled glycosaminoglycan chains were eluted from the column; gradient elution showed that the critical concentration of GuHCl was about 1.0M, indicating a stronger binding of CSPG to AH-Sepharose 4B than to DEAE derivatives (DE-52 DEAE-cellulose and DEAE-Sephacel); in the latter case desorption occurred at about 0.4M-GuHCl. The strong binding of CSPG conferred selectivity on to the AH-Sepharose step; e.g. ^{125}I-fibronectin or ^{125}I-laminin adsorbed on the column were desorbed at 0.35M-GuHCl, i.e. at a markedly lower concentration of GuHCl than required for the elution of glycan components (results not shown). Analogous experiments with ^{125}I-labelled (Hunter & Greenwood, 1962) calf serum proteins showed that about 50% of the applied radioactivity was retained on the column; virtually all of this material was desorbed at 0.20M-GuHCl.

Gel chromatography

Gel chromatography on Sephadex G-25 (1cm × 190cm column) and Sephadex G-50

(2cm × 200cm or 1cm × 190cm column) was carried out in 1M-NaCl. The Sephadex G-50 columns were calibrated with known standards of HA oligosaccharides (HA_8, HA_{10} and HA_{12}). Sepharose CL-2B chromatography was either on a 1cm × 90cm column, equilibrated with 10mM-Tris/HCl buffer, pH8.0, containing 0.15M-NaCl, 1mM-Na_2SO_4 and 0.1% bovine serum albumin, or a 1cm × 190cm column, equilibrated with buffer A. Sepharose CL-4B (1cm × 190cm column) and Sepharose CL-6B (1cm × 140cm or 1cm × 190cm column) chromatography was performed with buffer A. The former Sepharose CL-6B column was calibrated with CS fractions of known M_r values; a calibration curve was constructed by plotting log M_r versus $K_{av.}$ (Wasteson, 1971). [Desalting of oligosaccharides was carried out by gel chromatography on a Sephadex G-15 column (2cm × 90cm) equilibrated with 10% (v/v) ethanol.] All columns were operated at 4°C.

Density-gradient centrifugation in CsCl

Centrifugation was carried out in an MSE SS65 ultracentrifuge with an 8 × 14ml angle rotor. Samples were prepared for centrifugation by mixing with weighed amounts of CsCl (final concentration 0.28, 0.45 or 0.70g/ml). Runs were performed at 100000*g* for 60–72h at 18°C. After the run the tubes were either punctured and evacuated via a steel needle through the bottom of the tubes or emptied in the reverse direction by introducing a dense solution (Fluorinert FC-77; 3M, St. Louis, MO, U.S.A.) at the bottom of the tubes, driving the lighter fractions through a tight-fitting cap at the top. Fractions of volume 0.5–0.8ml were collected; the density of individual fractions was determined by weighing 200μl samples in a glass pipette.

Isolation and identification of CSPG from cell culture medium

In order to minimize the influence of possible preparation artifacts, three different methods were used to isolate CSPG from glial-cell or glioma-cell culture medium; the resulting products turned out to be similar in all respects examined. The harvested medium was used either after 10-fold concentration under reduced pressure (methods I and II) or used directly (method III). The recovery of CSPG in individual steps was 90% or better.

In method I the sample of concentrated medium was first acidified by the addition of 0.3ml of 0.1M-sodium acetate buffer (pH5.5)/ml of concentrated medium and then digested with platelet heparitinase (Oldberg *et al.*, 1980) (10μg of platelet protein/2ml of concentrated medium). After addition of an equal volume of 8M-GuHCl the sample was chromatographed on a Sepharose CL-4B column (1cm × 190cm) eluted with buffer A. ^{35}S-labelled material eluted with the void volume of the column was pooled. It was identified as CSPG by the following criteria. It was susceptible to papain digestion (Glimelius *et al.*, 1978*a*) or treatment with alkali (Norling *et al.*, 1978), yielding components with $K_{av.}$ 0.83 on Sepharose CL-2B and $K_{av.}$ 0.30 on Sepharose CL-6B. Further, on DEAE-cellulose ion-exchange chromatography, the products migrated like a standard of free CS chains, carrying, on an average, one sulphate residue per disaccharide unit. Digestion with chondroitinase AC converted 90% of the material into products chromatographing in the included-volume fraction of a Sephadex G-25 column eluted with 1M-NaCl.

In method II, HA_I was first added to the sample (10μg/2ml of concentrated medium). After incubation at room temperature for 6h or more, the sample was chromatographed on a Sepharose CL-2B column (1cm × 90cm) eluted with 10mM-Tris/HCl buffer, pH8.0, containing 0.15M-NaCl, 1mM-Na_2SO_4 and 0.1% bovine serum albumin. ^{35}S-labelled material eluted in the void volume was pooled and concentrated to 2ml in a Minicon cell; then an equal volume of 8M-GuHCl was added and the sample chromatographed on a Sepharose CL-2B column (1cm × 190cm) in buffer A. The ^{35}S-labelled material was now shifted to an included position, whereas HA_I remained in the void-volume fraction. The pooled ^{35}S-labelled material was shown to be CSPG by the same criteria as given above for method I.

In method III the unprocessed culture medium was passed through a column of AH-Sepharose 4B (see above). ^{35}S-labelled material eluted with buffer A was pooled and purified by density-gradient centrifugation in CsCl (final concentration 0.28g/ml); the material recovered in the bottom 5/20 of the gradient was pooled. The pool was rechromatographed on AH-Sepharose 4B; the radioactivity eluted from the column with buffer A was collected and rechromatographed on Sepharose CL-2B. Fractions corresponding to $K_{av.}$ 0.2–0.5 were pooled, mixed with 2mg of fraction A1–D1 carrier and re-centrifuged in a CsCl density gradient (final concentration 0.70g/ml); material having $\rho = 1.48$–1.59g/ml was collected. It was subjected to another cycle of AH-Sepharose 4B chromatography and finally chromatographed on Sepharose CL-2B, producing a peak with $K_{av.}$ 0.3. This material was pooled and identified as CSPG by the criteria described above.

Aggregation assay

The capability of glial-cell or glioma-cell CSPG to form large aggregates in the presence of HA_I under different conditions was tested by gel

chromatography. HA_I (about 2 μg in 10 μl of 4M-GuHCl) was added to ^{35}S-labelled CSPG (40000c.p.m. in 1.0ml of 10mM-Tris/HCl buffer, pH8.0, containing 0.15M-NaCl) and the mixture was left at room temperature for 6h. The sample was then chromatographed on a column of Sepharose CL-2B operated under associative conditions. The radioactivity eluted with the void volume was ascribed to [^{35}S]CSPG–HA_I aggregates. HA oligosaccharides of different size classes were tested for their effect on HA-induced aggregate formation. A 150-fold excess (about 300 μg) of oligosaccharides was then added at the same time as HA_I.

The stabilizing effect of link protein was tested either with a preparation of purified link protein (15 μg per incubation) or with CSPG A1 fraction (Heinegård, 1972; 15 μg per incubation), added together with HA_I. The mixtures were made to 4M-GuHCl and then dialysed against an associative buffer (10M-Tris/HCl buffer, pH8.0, containing 0.15M-NaCl). At that time HA oligosaccharides were added, and their effect on HA_I–CSPG aggregates was examined as described above.

Immunological techniques

Antibodies against cartilage proteoglycan were raised in a rabbit by injecting 130 μg portions of the antigen (isolated as described above) into a lymph node on the back of each thigh. The first dose was mixed with an equal volume of Freund's complete adjuvant; booster doses given 2 and 4 weeks later were mixed with Freund's incomplete adjuvant. At 2 weeks after the last injection the rabbit was bled to death; the recovered serum was stored at −20°C until used.

Antiserum reacting only with cartilage link proteins but not with cartilage CSPG was prepared by passing antiserum against bovine link proteins (kindly provided by Dr. A. Tengblad of this Department) over a column of Sepharose 4B with bound chondroitinase ABC-digested cartilage CSPG.

Immunoprecipitation was performed by mixing the sample (5 μl, containing ^{35}S-labelled CSPG or ^{125}I-labelled cartilage CSPG in 4M-GuHCl containing 0.1% bovine serum albumin) with 50 μl of antiserum or pre-immune serum. Incubation was at room temperature for 1h and at 4°C overnight; then 100 μl of suspended fixed *Staphylococcus* A bacteria (Arvidsson *et al.*, 1970; Kessler, 1976) [15% (v/v) in 10mM-sodium phosphate buffer, pH7.4, containing 0.15M-NaCl, 0.1% Tween 80 and 0.02% NaN_3] was added and incubation prolonged with agitation at room temperature for 1h. The suspension was finally diluted with 2ml of 10mM-Tris/HCl buffer, pH8.0, containing 0.15M-NaCl, 0.1% Tween 80, 0.1% bovine serum albumin, 1mM-Na_2SO_4 and 0.02% NaN_3, and centrifuged. The resulting supernatant and pellet were analysed for radioactivity.

Degradative methods

Treatment with alkali (β-elimination), digestion with chondroitinase AC or chondroitinase ABC and reductive alkylation were carried out as previously described (Glimelius *et al.*, 1978*a*; Norling *et al.*, 1978). The preparation of chondroitinase ABC, used to produce CS-free CSPG protein core, was first shown to have no degrading activity on ^{35}S-labelled heparan sulphate proteoglycan, a markedly proteinase-susceptible molecule. Degradation with testicular hyaluronidase or β-glucuronidase was as described by Ingmar & Wasteson (1979). Periodate oxidation and subsequent alkaline elimination was carried out as described by Fransson & Carlstedt (1974); non-labelled dermatan sulphate (kindly given by Dr. U. Lindahl of this Department) was added as a carrier.

High-voltage paper electrophoresis

High-voltage paper electrophoresis was performed as described by Glimelius *et al.* (1978*b*). Disaccharides migrating half-way between the standards of monosulphated disaccharide (Δdi-4/6-S) and inorganic sulphate were assigned two sulphate residues per repeating disaccharide unit.

Paper chromatography

The distribution of Δdi-4-S and Δdi-6-S in disaccharides obtained after chondroitinase treatment was determined by descending paper chromatography as described by Ingmar & Wasteson (1979) with solvent C (butan-1-ol/acetic acid/1M-NH_3, 2:3:1, by vol.); standards of Δdi-4-S or Δdi-6-S were located by a silver-dip technique (Smith, 1960).

Radioactivity

^{35}S radioactivity was measured in a Nuclear–Chicago Isocap/300 liquid-scintillation counter with Scintillator 299 as the scintillation medium; ^{125}I was measured in a Packard model 5166 Autogamma spectrometer.

Results

Analysis of intact CSPG

Hydrodynamic size and buoyant density. Gel chromatography on Sepharose CL-2B in the presence of 4M-GuHCl indicated that CSPG isolated from glial-cell or glioma-cell culture medium had similar hydrodynamic size; they both produced symmetrical peaks with a $K_{av.}$ of 0.30 (Fig. 1). A standard of ^{125}I-labelled CSPG from

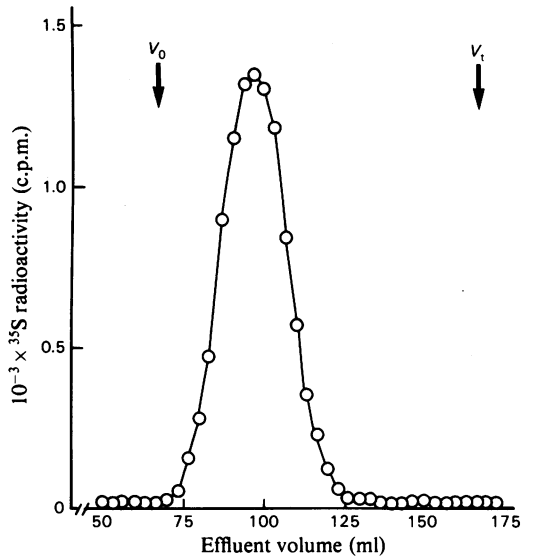

Fig. 1. *Gel chromatography on a Sepharose CL-2B column (1 cm × 190 cm) of CSPG isolated from the medium of ^{35}S-labelled glial-cell (U-787CG) cultures*
For full experimental details see the text. In this and subsequent Figures V_0 and V_t indicate void volume and total volume respectively.

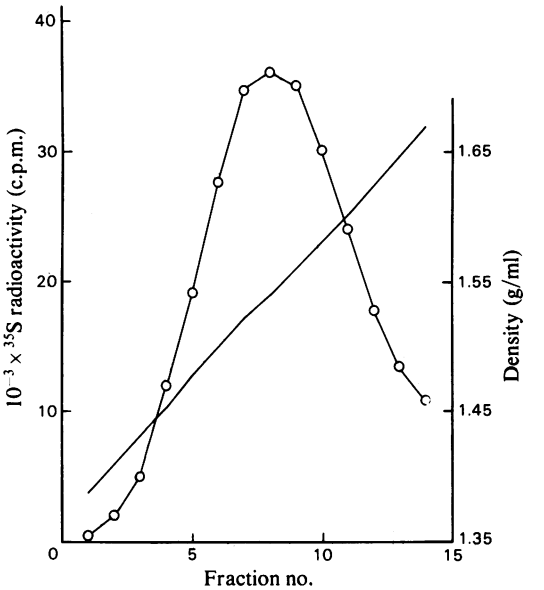

Fig. 2. *Density gradient centrifugation in CsCl (0.70 g/ml) of CSPG isolated from the medium of ^{35}S-labelled glioma-cell (U-251 MG) cultures*
For full experimental details see the text.

bovine nasal cartilage migrated with a similar $K_{av.}$, 0.29.

Isopycnic centrifugation in CsCl in the presence of 4M-GuHCl showed buoyant densities of about 1.55 g/ml for both glial-cell and glioma-cell derived CSPG (Fig. 2). In contrast, the standard of ^{125}I-labelled cartilage CSPG under the same conditions did not form a band in the gradient but appeared in the high-density bottom fractions (> 1.67 g/ml).

Aggregation with HA. Titration of different size classes of HA oligosaccharides (HA_4–HA_{12}) for their capacity to inhibit complex-formation between glial-cell or glioma-cell CSPG and HA showed that the smallest active oligosaccharide was the decasaccharide, HA_{10} (Fig. 3). Although the octasaccharide fraction also had some effect, this was ascribed to decasaccharide, known to contaminate the octasaccharide preparation. This view was supported by the findings of control experiments, in which the HA-induced aggregation of cartilage CSPG was similarly affected by the respective oligosaccharide preparations. It was concluded that the HA-binding region of glial-cell or glioma-cell and cartilage CSPG had the same requirement for minimal size of the HA molecule.

In contrast, the aggregate formed by HA and glial-cell or glioma-cell CSPG in the presence of link proteins derived from bovine nasal cartilage was not affected by HA oligosaccharides (results not shown). This would indicate that the cartilage-derived link proteins stabilized the complex in which glial-cell or glioma-cell CSPG was involved. Similar conclusions were obtained in analogous experiments in which solubilized cartilage CSPG–HA aggregate (fraction A1; Heinegård, 1972) was reconstituted in the presence of small amounts of glial-cell CSPG. The latter was apparently integrated in the newly formed link-stabilized complex, since HA oligosaccharides failed to displace any glial-cell CSPG from the high-molecular-mass material (results not shown).

Reduction and alkylation of glial-cell or glioma-cell CSPG completely abolished the formation of aggregates with HA (results not shown). Thus these CSPGs, in this respect, were similar to cartilage CSPG.

Immunoprecipitation. Rabbit antiserum raised against bovine nasal-cartilage CSPG precipitated 54% (over the control) of the ^{35}S-labelled CSPG isolated from glial-cell culture medium. In an analogous experiment 67% of cartilage CSPG was precipitated by the same antiserum. The antiserum did not react with a number of isolated extracellular-matrix components, such as human or bovine ^{125}I-labelled fibronectin, laminin or collagen type I, III or V, but showed some activity against CSPG link proteins of bovine origin. The latter finding raised the possibility that link

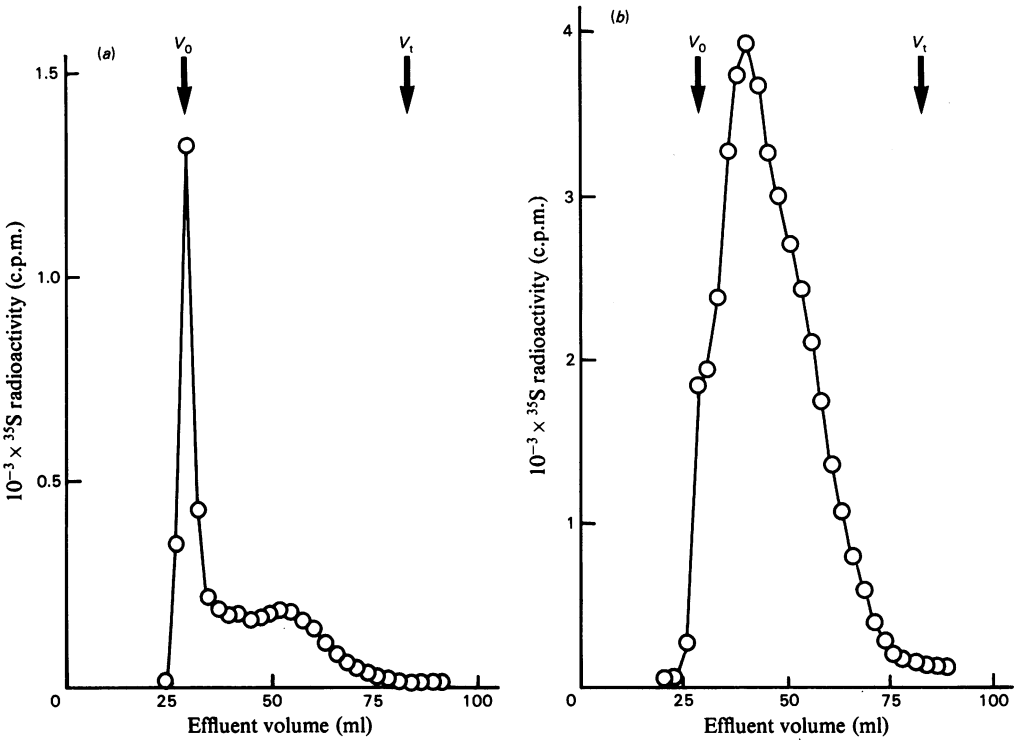

Fig. 3. *Effect of HA oligosaccharides on HA-induced aggregation of CSPG isolated from glial cells (U-787 CG)* Under associative conditions addition of HA to glial-cell CSPG led to the formation of aggregates as revealed by the excluded-volume material on Sepharose CL-2B chromatography (1 cm × 90 cm column), eluted with 10 mM-Tris/HCl buffer, pH 8.0, containing 0.15 M-NaCl, 1 mM-Na_2SO_4 and 0.1% bovine serum albumin (*a*). This phenomenon was inhibited in the presence of HA_{10}, as shown by the marked increase in included material (*b*). For full experimental details see the text.

proteins caused the effect on [^{35}S]CSPG, i.e. that the [^{35}S]CSPG were co-precipitated with link proteins without being specifically recognized by the antiserum. Therefore an antiserum against link protein was tested for its ability to precipitate the CSPG; it had no activity in this respect. It was concluded that the glial-cell-derived CSPG had antigenic determinants in common with the cartilage-derived analogue.

Analysis of chondroitinase ABC digested CSPG

Chondroitinase ABC digestion of ^{35}S-labelled glial-cell CSPG left 2% of the radioactivity in a high-M_r form, representing protein core with remnants of CS chains still attached (Hascall *et al.*, 1972). Chromatography of this material on Sepharose CL-6B produced one major peak, as shown in Fig. 4(*a*). In contrast, cartilage CSPG subjected to the same treatment (Fig. 4*b*) showed a more heterogeneous distribution, presumably as a result of the presence of keratan sulphate. However, its major included component was similar in size to that of glial-cell CSPG. After alkali treatment of the chondroitinase ABC-resistant glial-cell material half of it was eluted as a homogeneous peak slightly ahead of a disaccharide on chromatography on Sephadex G-50. The other half of the material showed M_r values in the range 5000–20000, as determined by chromatography on Sepharose CL-6B. It may represent incompletely degraded CS chains, or possibly keratan sulphate. Therefore, if present, keratan sulphate could only amount to a maximum of 1% of the total ^{35}S radioactivity in glial-cell CSPG.

Analysis of CS chains obtained by β-elimination of CSPG

Hydrodynamic size and charge properties. Ion-exchange chromatography of [^{35}S]CS chains derived from the CSPG of the medium of ^{35}S-labelled glial-cell or glioma-cell cultures yielded indistinguishable, sharp and symmetrical peaks, eluted only slightly after the standard of CS, carrying one sulphate group per disaccharide unit (results not shown). Gel chromatography of the same components on Sepharose CL-6B similarly

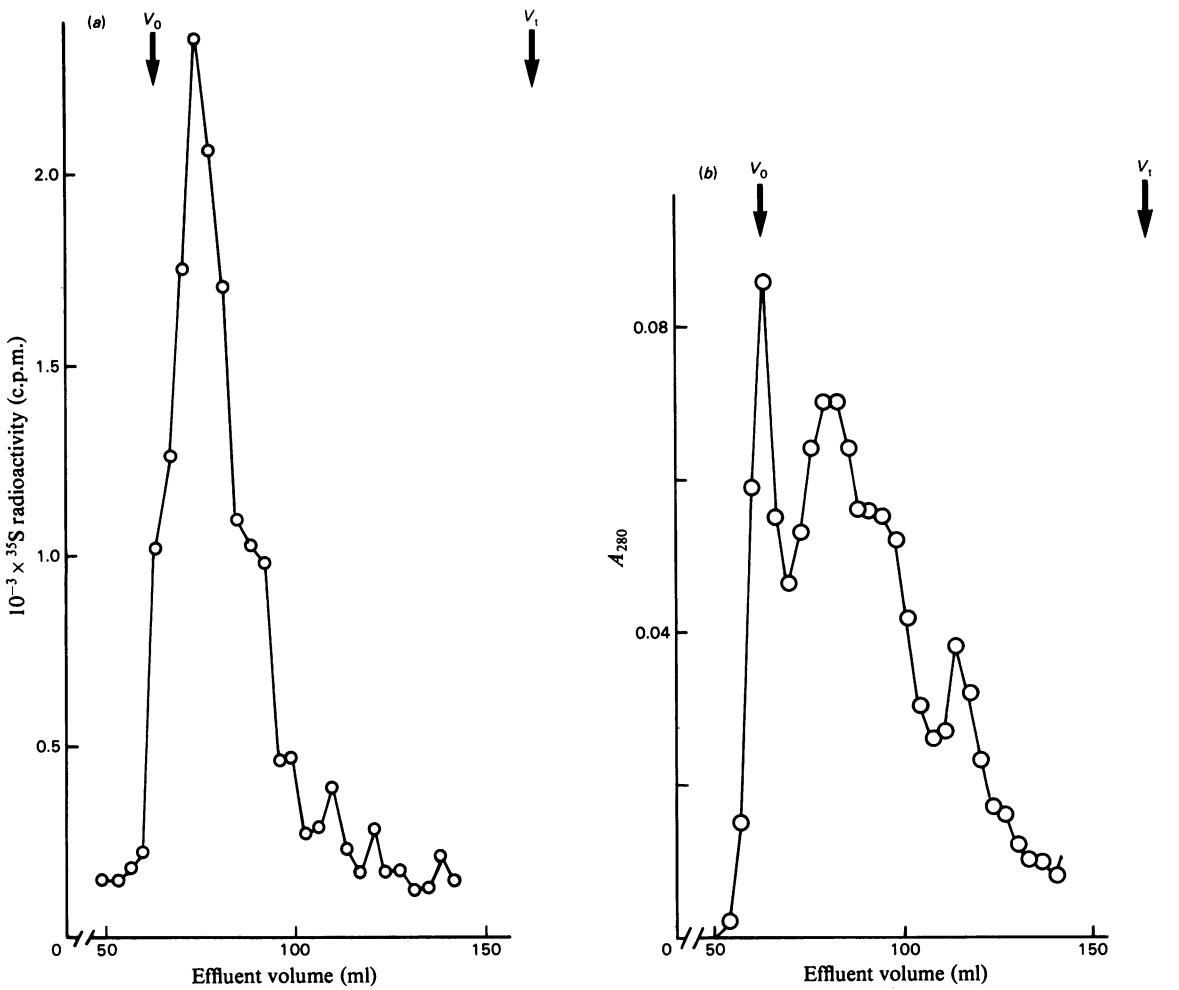

Fig. 4. *Gel chromatography on a Sepharose CL-6B column (1 cm × 190 cm) of chondroitinase ABC-digested glial-cell (^{35}S) or cartilage CSPG (A_{280})*

Only the macromolecular chondroitinase products were applied; the low-M_r products had been removed by Sephadex G-50 chromatography. For full experimental details see the text.

produced identical elution profiles (results not shown); the $K_{av.}$ values of the peak fraction were 0.30 and 0.31 for the glial-cell- and glioma-cell-derived CS chains respectively. Judged from the $K_{av.}$ values of calibration standards (Wasteson, 1971), these values correspond to M_r values of 44000 and 42000 respectively

Susceptibility to chondroitinase. Chondroitinase ABC digestion of the glial-cell- and glioma-cell-derived ^{35}S-labelled CS chains converted about 98% of the glial-cell material as well as the glioma-cell material into disaccharides, as demonstrated by Sephadex G-25 chromatography (results not shown). The remaining non-disaccharide portion (2%) was eluted slightly ahead of the standard tetrasaccharide. Since most of it was degraded to disaccharides by chondroitinase AC, it probably represented material located close to the carbohydrate–protein linkage region; the analogous position in cartilage-derived CS is partially resistant to chondroitinase ABC (Hascall *et al.*, 1972).

Treatment of the glial-cell or glioma-cell CS chains with chondroitinase AC resulted in identical product patterns, as indicated by Sephadex G-25 chromatography (Fig. 5). A major part (about 80%) was converted into disaccharides. A minor portion (9%) appeared at a position slightly after that of a standard tetrasaccharide. Periodate/alkali treatment (known to degrade unsulphated but not 2-sulphated iduronic acid; Fransson & Carlstedt 1974) of this material caused later elution on the Sephadex G-25 column, half of the products migrating slightly ahead of the disaccharide fraction, and half being eluted at the position of

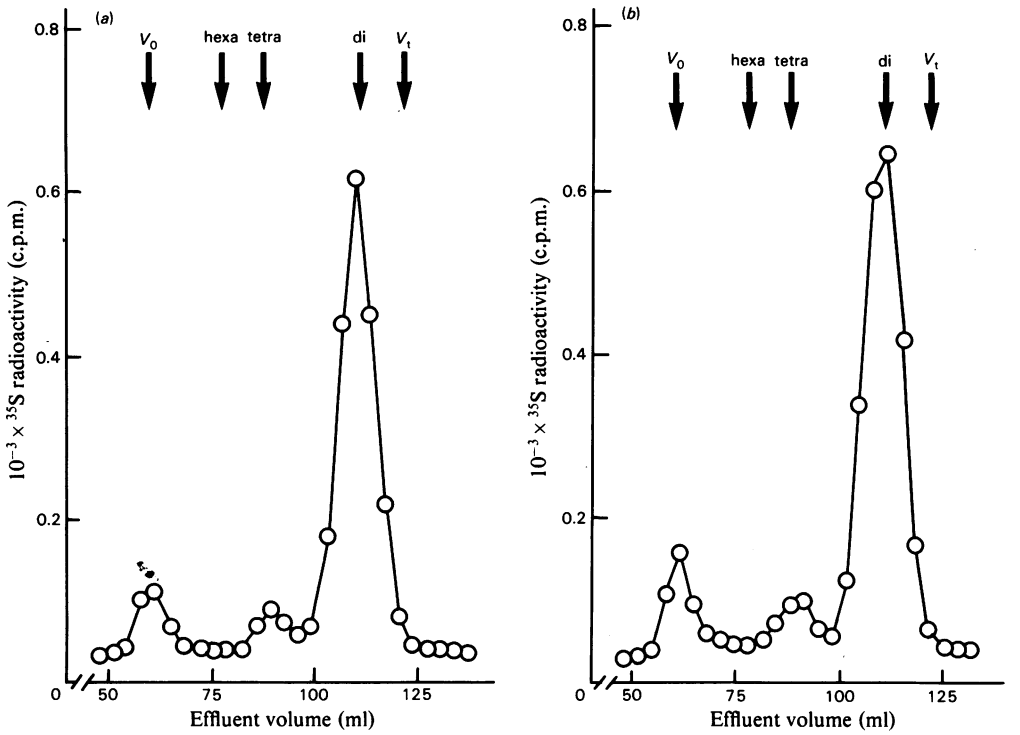

Fig. 5. *Gel chromatography on Sephadex G-25 of glial-cell (a) or glioma-cell (b) CS digested with chondroitinase AC*
For full experimental details see the text. The position of oligosaccharides are indicated.

inorganic sulphate (results not shown). These products should be derived from a tetrasaccharide containing a non-sulphated iduronic acid residue at its penultimate position. The remaining portion of the chondroitinase AC products (11%) was in the excluded-volume fraction on Sephadex G-25; it stayed in that position after the periodate/alkali step (results not shown) and therefore should be composed of contiguous blocks of disaccharides containing 2-sulphated iduronic acid residues.

Constituent disaccharides. High-voltage paper electrophoresis of the disaccharide portions of the chondroitinase ABC or chondroitinase AC digests revealed no differences between the glial-cell- and glioma-cell-derived products. Whereas essentially only monosulphated disaccharides were found in chondroitinase AC digests, significant amounts (5%) of disulphated disaccharide were demonstrated in the material obtained after digestion with chondroitinase ABC. Paper chromatography of the disaccharide fraction showed approximately equal amounts of mono-4- and mono-6-sulphated species. The average disaccharide composition of an individual CS chain that could be deduced from the present analyses was the same for the glial-cell product as for the glioma-cell product (Table 1). As

Table 1. *Calculated average distribution of constituent disaccharides in glial-cell or glioma-cell CS*
CSPG was isolated from the culture medium of glial-cell or glioma-cell cultures; CS chains were then obtained by alkali-induced β-elimination. The results are based on degradative analysis of the CS chains, assuming a uniform chain length (92 disaccharides) and a uniform composition in the respective populations. Abbreviations: IdUA, iduronic acid; GlcUA, glucuronic acid.

	Number per CS chain	
Disacharide type	Glial-cell CS	Glioma-cell CS
IdUA(2-S)-GalNAc(4-S)	5	5
IdUA-GalNAc(4-S)	3	3
GlcUA-GalNAc(4-S)	42	42
GlcUA-GalNAc(6-S)	42	42

can be seen from the Table the observed pattern of iduronic acid-containing to glucuronic acid-containing disaccharides (10:90) was different from that held to govern the process of self-aggregation among CS/dermatan sulphate chains (50:50) (Fransson, 1976).

Discussion

The present results demonstrate that the CSPGs produced by human normal glial or malignant glioma cells in culture have structural and functional properties in common with cartilage-derived CSPG. The hydrodynamic sizes of the monomeric molecules were the same, as judged by Sepharose CL-2B chromatography under dissociative conditions, and large-size aggregates were similarly formed with HA under associative conditions. The same principles were apparently operating in the formation of these aggregates. Thus, for the recognition of HA, the glial-cell- or glioma-cell-derived CSPG showed the same minimal requirement for HA chain length as the analogous cartilage product. Further, their binding to HA was similarly abolished by reducing agents, and stabilized by link proteins. This suggests that the different CSPGs may have similar HA-binding structures. The common antigenic determinants indicated by the immunoprecipitation experiments may therefore reside in this region of the CSPG molecule; the CSPG antibodies were probably directed against the globular portion of the molecule, since intact rather than chondroitinase-treated CSPG had been used for immunization.

However, significant structural differences were noted between glial-cell/glioma-cell CSPG and cartilage CSPG. Whereas they all had the same hydrodynamic size, they differed with respect to buoyant density ($\rho = 1.55$ g/ml versus > 1.67 g/ml) and size of the chondroitin sulphate side chains (M_r 40000 versus 20000). Since the size of the polypeptide core was similar, it can be inferred that glial-cell/glioma-cell CSPG contains fewer CS side chains than does cartilage CSPG. Additional differences relate to the composition of the substituent glycosaminoglycan chains in the respective types of CSPG. In contrast with the analogous cartilage CSPG, glial-cell/glioma-cell CSPG showed significant amounts of iduronic acid-containing repeating units (about 10% of the total) and virtually lacked keratan sulphate.

It is concluded that the glial-cell CSPG is indistinguishable from the glioma-cell CSPG, and thus much more related to the latter than to cartilage CSPG, i.e. a product of a cell with a different origin. The major difference between glial-cell/glioma-cell CSPG and cartilage CSPG relate to the glycan part of the molecule; the protein cores may well be identical. If so, it would appear as if the particular biosynthetic machinery of each cell type would determine the subsequent substitution with carbohydrate.

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