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# Fine Structure of Heparan Sulfate Regulates Syndecan-1 Function and Cell Behavior*

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**Two myeloma cell lines, MPC-11 and P3X63Ag8.653 (P3), have almost identical amounts of syndecan-1 at their cell surface. The syndecan-1 molecules from both lines are similar in size, have indistinguishable core proteins, and have similarly sized heparan sulfate chains. Nevertheless, syndecan-1 on MPC-11 mediates cell adhesion to type I collagen, whereas P3 cells do not bind collagen. Affinity co-electrophoresis reveals that intact syndecan-1 isolated from P3 cells binds collagen poorly and that syndecan-1 heparan sulfate isolated from MPC-11 has a 20-fold higher affinity for collagen than syndecan-1 heparan sulfate from P3. Analysis of disaccharide composition and oligosaccharide mapping also reveals differences between MPC-11 and P3 heparan sulfate. Most notably, the level of *N*-sulfation and 2-*O*-sulfation is higher, and 6-*O*-sulfation lower, in syndecan-1 heparan sulfate from MPC-11 than from P3. Interestingly, levels of total sulfation of syndecan-1 heparan sulfate from MPC-11 and P3 are similar (75.6 and 72.6 sulfates/100 disaccharides, respectively), indicating that the difference in their affinity for collagen is not due to a difference in net charge. These data indicate that the fine structure of heparan sulfate can differ on identical proteoglycan core proteins, and these differences can control fundamental cellular properties such as cell-matrix adhesion.**

Heparan sulfate proteoglycans are present on most cell surfaces where they participate in the regulation of cell behavior by binding structural proteins, enzymes, and growth factors (1, 2). Considerable diversity exists in the numbers and lengths of heparan sulfate chains that are found on cell surface proteoglycans, and considerable heterogeneity exists in the composition and fine structure of these chains.

The extent to which the molecular heterogeneity of heparan sulfate proteoglycans contributes to specificity in their biological activities is poorly understood. At least one heparan sulfate-binding protein, antithrombin III, binds specifically to a pentasaccharide sequence that is only found in some heparan sulfate chains (3, 4). Several other proteins bind with moderate preferences to subsets of the heparan sulfate-related glycosaminoglycan heparin, although the structural basis for this binding is not yet known (5). Studies indicate that bFGF[1] is capable of interacting preferentially with certain heparan sulfate and heparin oligosaccharides of defined sequence (6–10). However, information is lacking on whether full-length naturally occurring heparan sulfates differ in their interactions with, and ability to mediate the biological activity of, bFGF, although one recent study suggests that this may be the case (11).

Indeed, the large size and complexity of intact proteoglycans has made it difficult to obtain clear correlations between proteoglycan structure and function. The most progress to date has been made with syndecan-1, a member of the syndecan family of proteoglycans (12). Syndecan-1 exhibits broad structural heterogeneity between cell and tissue types due to differences in the number and length of glycosaminoglycans, types of glycosaminoglycans (heparan sulfate *versus* chondroitin sulfate), and heparan sulfate fine structure (*e.g.* sulfate content, disaccharide composition, and distribution of heparinase cleavage sites) (13–16).

Syndecan-1 from various cell types has been shown to bind to interstitial collagens (17), fibronectin (18), thrombospondin (19), tenascin (16), and bFGF (20, 21). In some cases, cell type-specific differences in binding properties were seen. For example, solid phase assays indicate that a 250–300-kDa form of syndecan-1 isolated from tooth mesenchyme and having only heparan sulfate chains binds strongly to tenascin, whereas a 100–250-kDa form of syndecan-1 from normal murine mammary epithelial (NMuMG) cells and having both heparan sulfate and chondroitin sulfate chains binds weakly to tenascin (16). In contrast, syndecan-1 from NMuMG epithelial cells binds with 4-fold higher affinity to type I collagen than does syndecan-1 from a murine myeloma cell line that expresses a form of syndecan-1 that is 70–150 kDa and has shorter and fewer heparan sulfate chains than does NMuMG syndecan-1 (22).

These data suggest that cell type-specific forms of syndecan-1 can have distinct ligand binding properties. However, two critical questions have yet to be resolved. First, what differences in syndecan-1 structure cause these differences in binding behavior? Conceivably, such differences could depend on

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[1] The abbreviations used are: bFGF, basic fibroblast growth factor; P3, P3X63Ag8.653; NMuMG, normal murine mammary gland epithelial; BSA, bovine serum albumin; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; CHAPS, 3-(3-cholamidopropyl)dimethylammonio-1-propanesulfonate; MOPSO, (3-(*N*-morpholino)-2-hydroxypropanesulfonic acid; SAX, strong anion exchange; HPLC, high performance liquid chromatography; GlcA, glucuronic acid; IdoA, iduronic acid; IdoA(2S), iduronic acid 2-sulfate; GlcNac, *N*-acetyl glucosamine; $GlcNSO_3$, *N*-sulfated glccosamine; $GlcNSO_3$(6S), *N*-sulfated glucosamine 6-sulfate; GlcA(2S), glucuronic acid 2-sulfate; ΔUA, unsaturated uronic acid residue.

aspects of large scale structure (*e.g.* number, length, or net charge of glycosaminoglycan chains), fine structure (specific oligosaccharide sequences with defined patterns of sulfation), or both. Second, are the differences that have been observed in syndecan-1 binding behavior sufficiently large to have a determining influence on cell behavior (*i.e.* does the structure of a cell's syndecan-1 control its behavior)?

To address these questions, we have been studying the role of syndecan-1 as the type I collagen receptor of myeloma cells. Previous work has demonstrated that syndecan-1 mediates the binding of murine and human myeloma cells to type I collagen (22, 23). In the present paper, we identify a murine myeloma cell line, P3, that possesses syndecan-1 that fails to mediate cell binding to collagen. These P3 cells contain the same amount of cell surface syndecan-1 as MPC-11 mouse myeloma cells (which do bind to collagen), but syndecan-1 from P3 cells possesses heparan sulfate chains that bind 20-fold less tightly to type I collagen than the heparan sulfate chains of syndecan-1 from MPC-11 cells. Furthermore, evidence is provided to show that P3 and MPC-11 syndecan-1 are similar in their gross properties, including core protein size and net charge, but substantially different in the fine structure of their heparan sulfate chains. Specifically, the heparan sulfate from MPC-11 syndecan-1 (which binds with high affinity to type I collagen) has higher levels of *N*-sulfation and 2-*O*-sulfation, lower levels of 6-*O*-sulfation, and a different arrangement of heparitinase cleavage sites than the heparan sulfate of P3 syndecan-1. These data suggest that a causal relationship may exist between heparan sulfate fine structure, proteoglycan binding properties, and at least some aspects of cell behavior.

## EXPERIMENTAL PROCEDURES

*Cell Culture*—MPC-11 and P3 mouse myeloma cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.05 mM 2-mercaptoethanol, 100 units/ml penicillin, and 100 μg/ml streptomycin sulfate.

*Cell Binding Assays*—MPC-11 and P3 cell binding to type I collagen was assayed as described previously (17, 22). Polyvinyl 96-well U-bottom plates (Dynatech, Chantilly, VA) were incubated with type I collagen (rat tail, 1 mg/ml; Collaborative Biomedical Products, Bedford, MA) or BSA (Fraction V, 1 mg/ml, Sigma) overnight at 4 °C. Wells were washed with PBS, and remaining protein binding sites within the wells were saturated by incubation with 10 mg/ml BSA for 30 min. Plates were then washed three times with PBS prior to addition of cells. Cell cultures were established at a concentration of $2 \times 10^5$ cells/ml 24 h prior to performing cell binding assays. Cells were harvested by centrifugation at 4 °C, washed with ice-cold PBS, and resuspended in cold PBS at a concentration of $4 \times 10^5$ cells/ml. Cells were added to microtiter wells (200 μl/well), incubated for 30 min at room temperature, and the plate was centrifuged at $120 \times g$ for 10 min in a Beckman TJ-6 centrifuge. Following centrifugation, 50 μl of 4% glutaraldehyde in PBS was added to each well and the plate placed at 4 °C overnight. After removal of the buffer, cells were stained with 4% trypan blue in PBS. In this assay, if cells do not bind to the protein-coated wells, a clearly visible pellet is formed in the bottom of the well following centrifugation. If cells bind to wells, they remain as a uniform coating over the well surface.

*Quantification of Syndecan-1*—Syndecan-1 on the surface of cells was quantified using $^{125}$I-labeled antibody 281.2, an antibody specific for an epitope within the extracellular domain of syndecan-1 (24). Cells growing at a concentration of $4 \times 10^5$ cells/ml were harvested by centrifugation and washed two times in ice cold complete cell culture media. Cells were then added to wells of a 96-well plate ($5 \times 10^5$ cells/well) that had been preincubated with PBS containing 10 mg/ml BSA to prevent cells from binding to the wells. Cells in the wells were incubated with normal rat serum to prevent nonspecific binding of labeled antibody, followed by incubation for 30 min on ice with excess $^{125}$I-labeled monoclonal antibody 281.2, or with $^{125}$I-labeled antibody L3T4 (Pharmingin, San Diego, CA), an isotype-matched control monoclonal antibody. Cells were then centrifuged, washed four times with ice-cold complete media, resuspended, and bound antibody detected by γ counting.

*Purification and Analysis of Syndecan-1 and Syndecan-1 Heparan Sulfate*—MPC-11 and P3 cells were harvested by centrifugation and washed three times in PBS containing 0.5 mM disodium EDTA. Cells were resuspended, and syndecan-1 was released from the cell surface by the addition of 20 μg/ml trypsin for 10 min on ice (25). Soybean trypsin inhibitor (Calbiochem) was added to a concentration of 100 μg/ml and cells pelleted by centrifugation at $735 \times g$ for 3 min. Supernatants containing the released syndecan-1 were then brought to 6 M urea, 50 mM sodium acetate, pH 4.5, 1 mM phenylmethylsulfonyl fluoride and boiled for 10 min. Tubes were cooled to room temperature, and DEAE-Sepharose beads (100 μl of beads/$10^7$ cell equivalents) were added and the mixture placed on a rocker for 1 h at room temperature. DEAE beads were pelleted by gentle centrifugation ($240 \times g$ for 2 min), placed in a clean 0.5-ml microcentrifuge tube, and washed four times with PBS. The beads were then eluted with PBS containing a final NaCl concentration of 1 M. Eluates were then diluted with 10 mM Tris, pH 7.4, to a final concentration of 0.15 M NaCl and incubated with monoclonal antibody 281-2 that was covalently bound to Sepharose CL-4B beads (281-2 beads). Following incubation overnight at 4 °C with gentle rocking, beads were washed extensively in PBS then distilled water and bound syndecan-1 was eluted as described below.

Western blotting of core proteins was as described previously (26). Briefly, syndecan-1 bound to 281.2 beads was treated with heparitinase and chondroitinase ABC (Seikagaku, Rockville, MD) and core proteins released from beads by boiling in SDS-PAGE sample buffer. Samples were run on 3.8–20% gels, transferred to a cationic nylon filter (Genetrans; Plasco, Woburn, MA), and probed with $^{125}$I-labeled antibody 281.2.

For Sepharose CL-4B (Pharmacia LKB Biotechnology Inc.) chromatography, cells were grown for 24 h in medium containing 100 μCi/ml $H_2{}^{35}SO_4$. The $^{35}SO_4$-labeled syndecan-1 was purified as described above, and the syndecan-1 bound to 281.2 beads was eluted by boiling beads in buffer containing 4 M guanidine hydrochloride, 0.1% Triton X-100, and 50 mM sodium acetate, pH 5.8. The samples (50 μl) were applied to Sepharose CL-4B columns (0.5 × 50 cm) and eluted at a flow rate of 6 ml/h. Fractions were collected and assayed by liquid scintillation counting.

For analysis of syndecan-1 heparan sulfate chains, $^{35}SO_4$-labeled or [$^3$H]glucosamine-labeled syndecan-1 bound to 281.2 beads was treated with ABCase (0.05 units/ml) for 1 h at 37 °C to remove chondroitin sulfate chains. After washing beads to remove released chondroitin sulfate, the syndecan-1 was eluted with 50 mM triethylamine, pH 11.0, and immediately neutralized by addition of a 1/5 volume of 1 M Tris, pH 7.4. The core protein was then degraded by addition of 1 mg/ml Pronase for 2 h at 37 °C. The remaining heparan sulfate chains were then concentrated on DEAE beads, eluted with 1 M NaCl, and desalted by passing over an Excellulose G-5 column equilibrated with $dH_2O$. Samples were lyophilized and stored until use. For analysis of heparan sulfate chain size, samples were loaded on a Sepharose CL-6B (Pharmacia) column (0.5 × 50 cm) and eluted in buffer containing 1% SDS, 0.15 M NaCl, 50 mM sodium acetate, pH 5.0, and 0.02% sodium azide.

*Affinity Co-electrophoresis*—Samples of $^{35}SO_4$-labeled syndecan-1 and heparan sulfate derived from syndecan-1 were subjected to electrophoresis through 1% agarose gels containing lanes in which rat tail type I collagen had been incorporated at different concentrations. Electrophoresis buffer consisted of 0.1 M sodium acetate, 50 mM MOPSO, 0.5% CHAPS, pH 7.0. Collagen dilutions were prepared at 10 times final concentration in 20 mM acetic acid (at 4 °C), then mixed with 9 volumes of molten low gelling temperature agarose (1.11% w/v) in electrophoresis buffer plus 0.5% CHAPS and 2.22 mM NaOH and immediately introduced into preformed lanes (27). Following electrophoresis, gels were dried with forced warm air and labeled material imaged using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). For analysis of heparan sulfate chains, syndecan-1 was digested with chondroitinase ABC (0.1 unit/ml, 1 h, 37 °C), followed by Pronase (0.2 mg/ml, 1 h, 37 °C). The reaction was terminated by adding SDS to 0.1% and heating to 100 °C for 10 min. Samples were brought to 1% in Triton X-100 and electrophoresed.

*SAX-HPLC of Disaccharides and Oligosaccharides*—Disaccharide composition was analyzed by complete depolymerization of heparan sulfate to disaccharide products with a mixture of heparitinase, heparitinase II, and heparinase (Seikagaku) as described previously (28, 29). Briefly, these lyases were used at concentrations of 20–50 milliunits/ml in 100 mM sodium acetate, pH 7.0, containing 0.2 mM calcium acetate and porcine mucosal heparan sulfate (0.5–1 mg/ml) as carrier. Heparan sulfate from syndecan-1 was prepared as described above and then initially incubated at 37 °C with heparinase for 2 h, followed by addition of heparitinase and a further 2-h incubation. Finally, heparitinase II was added and the incubation stopped after 16 h,

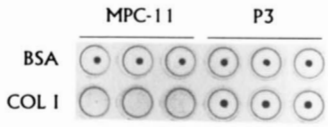


FIG. 1. **P3 cells do not bind to type I collagen.** Microtiter wells were coated with BSA or type I collagen. Following incubation of cells within wells for 30 min, the plate was gently centrifuged. Bound cells form a uniform coating on the well surface, and unbound cells form a pellet in the center of the well. Triplicate wells of each are shown.

by heating the sample at 100 °C for 2 min. Disaccharides were recovered by chromatography on a Bio-Gel P-2 column (1 × 120 cm) eluted with 500 mM $NH_4HCO_3$ at a flow rate of 4 ml/h. The disaccharide products (yields ≥ 90%) were lyophilized and separated by SAX-HPLC on a ProPac PA1 analytical column (4 × 250 mm; Dionex, Surrey, United Kingdom). After equilibration in mobile phase (double-distilled water adjusted to pH 3.5 with HCl) at 1 ml/min, samples were injected and disaccharides eluted with a linear gradient of sodium chloride (0–1 M over 45 min) in the same mobile phase. The eluant was monitored in-line for UV absorbance ($A_{232}$ for unlabeled disaccharide standards) and $^3H$ and $^{35}S$ radioactivity (Radiomatic Flo-one/Beta A-200 detector, Canberra Packard, Pangbourne, United Kingdom).

*Oligosaccharide Mapping by Gradient PAGE*—Oligosaccharide mapping by gradient PAGE was carried out using methods described in detail previously (28, 30). Briefly, $^{35}S$-labeled heparan sulfate samples depolymerized with heparitinase were electrophoresed (400 V for 4 h) on 25–30% gradient gels (24 cm × 16 cm × 0.75 mm) and electrotransferred to cationic nylon membranes (Biodyne B; Pall BioSupport Division, Portsmouth, United Kingdom). The fractionated oligosaccharides were detected by fluorography using EN$^3$HANCE (DuPont NEN).

## RESULTS AND DISCUSSION

*Identification of a Myeloma Cell Line That Expresses Syndecan-1 but Does Not Bind Collagen*— Previous studies have provided evidence that syndecan-1 mediates the binding of murine and human myeloma cells to type I collagen. For example, MPC-11 murine myeloma cells, which express syndecan-1 as their only major cell surface heparan sulfate proteoglycan, are inhibited from binding collagen by heparin, by pretreatment of cells with heparitinase or by growth of cells in an inhibitor of glycosaminoglycan sulfation (22). Moreover, isolated sydnecan-1 from MPC-11 cells (which is virtually indistinguishable from the form of syndecan-1 found on normal plasma cells) exhibits demonstrable binding to collagen (22). Finally, when cells from several human myelomas were tested for binding to type I collagen, all of those that bound collagen expressed syndecan-1, whereas all of those that did not bind collagen lacked syndecan-1 (23).

In the course of these studies, however, one murine myeloma line, P3, was identified that did not attach to type I collagen (Fig. 1), yet P3 cells did express syndecan-1. This observation suggested that the syndecan-1 on P3 cells was either present in insufficient quantity or structurally different from the syndecan-1 on other myeloma cells. To address this question, experiments were undertaken to compare the abundance, structure, and binding properties of syndecan-1 isolated from P3 cells with syndecan-1 isolated from MPC-11 cells.

*MPC-11 and P3 Cells Express Syndecan-1 in Similar Amounts and of Similar Molecular Size*—To assess the basis for the difference in ability of these two cell lines to adhere to collagen, we first analyzed the amount and size of syndecan-1 on MPC-11 and P3 cell surfaces. Quantification of syndecan-1 using $^{125}I$-labeled monoclonal antibody 281.2 indicates that nearly identical amounts of the proteoglycan are present on the surfaces of the two cell types (Table I).

To compare the size of syndecan-1 from the two cell types, purified syndecan-1 was analyzed by Sepharose CL-4B chromatography and its core proteins by Western blotting. The intact syndecan-1 from MPC-11 and P3 cells are similar in size (Fig. 2*A*), with the P3 being only slightly smaller, and the core proteins are identical in size (Fig. 2*B*). Because the core proteins are the same size, and there is no evidence for alternative splicing of syndecan-1 mRNA (31, 32), it is likely that the core proteins from the two cell lines are identical in structure. Analysis of isolated heparan sulfate chains from the syndecans by Sepharose CL-6B chromatography shows that a large proportion of the MPC-11 and P3 heparan sulfate peaks overlap, with the overall average size of the MPC-11 heparan sulfate chains being slightly larger than the P3 chains (Fig. 2*C*). These results

TABLE I
*MPC-11 and P3 cells have nearly identical amounts of syndecan-1 at their cell surface*

Cells were harvested by centrifugation, washed, and placed in wells of a microtiter plate. Cells were incubated at 4 °C for 30 min with excess $^{125}I$-labeled antibody 281.2 or as a control, an isotype-matched monoclonal antibody against L3T4 (Pharmingen), a surface antigen not expressed on myeloma cells. Bound radioactivity was determined by γ counting. Values represent mean of quadruplicate samples ± S.D.

| | $^{125}I$-Labeled | |
|---|---|---|
| | 281.2 | L3T4 |
| | *cpm/5 × 10$^5$ cells* | |
| MPC-11 | 99,014 ± 5,606 | 496 ± 224 |
| P3 | 99,815 ± 4,970 | 609 ± 284 |

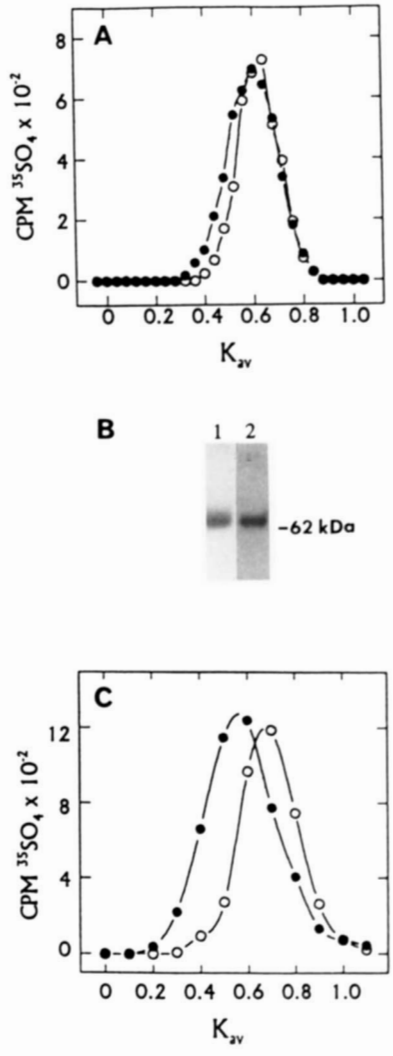


FIG. 2. **Syndecan-1 from MPC-11 (●) and P3 (○) cells are similar in molecular size.** Syndecan-1 from the two cell lines was purified by DEAE and antibody 281.2 affinity chromatography and analyzed for intact proteoglycan size by CL-4B chromatography (*A*), for core protein size by Western blotting (*lane 1*, MPC-11; *lane 2*, P3) (*B*), and for heparan sulfate chain size by CL-6B chromatography (*C*).

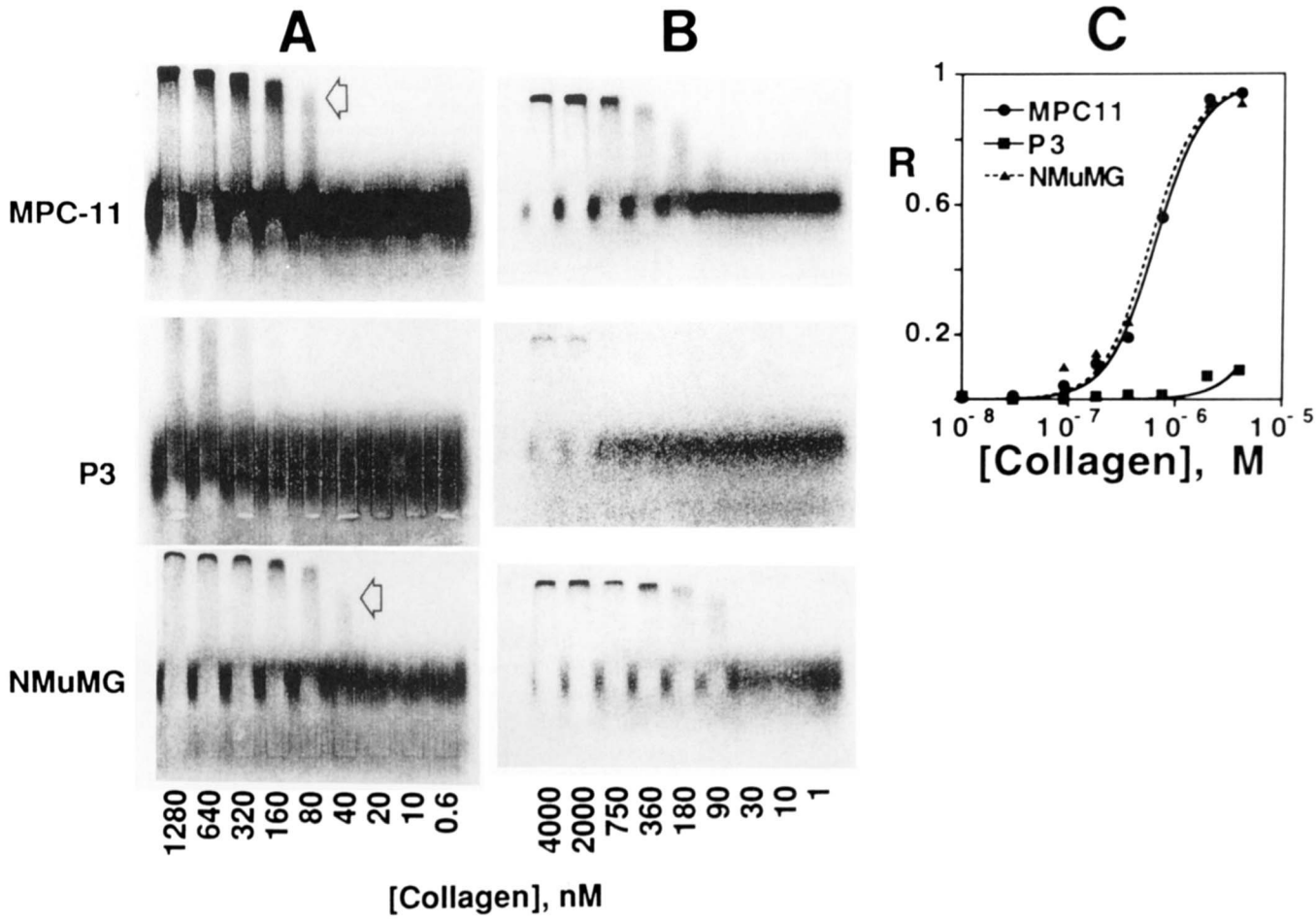


FIG. 3. **Analysis of binding of syndecan-1 and syndecan-1 heparan sulfate to type I collagen by affinity co-electrophoresis.** *A*, labeled syndecan-1 was prepared from NMuMG mouse mammary epithelial cells and MPC-11 and P3 cells by trypsinization of cells cultured in [$^{35}$S]sulfate and purified by DEAE chromatography and immunoaffinity chromatography using antibody 281.2. Collagen concentrations are shown at the *bottom*. Progressive retardation of syndecan-1 mobility from low to high collagen concentration demonstrates binding (collagen-free zones between each lane permit the mobility at each collagen concentration to be compared with the mobility of free syndecan-1 molecules). The presence of smears in some lanes, rather than tight bands, is indicative of binding heterogeneity within each sample, *i.e.* some syndecan-1 molecules bind collagen more tightly than others. *Arrows* illustrate the presence of high-affinity subpopulations in the MPC-11 and NMuMG samples that are not seen in the P3 sample. *B*, heparan sulfate chains were prepared from the samples used in *A* and subjected to affinity co-electrophoresis. Collagen concentrations are shown at the *bottom*. As in *A*, the MPC-11 and NMuMG samples exhibit high-affinity fractions not seen in the P3 sample. *C*, binding isotherms derived from the data in *B*. For each lane of the autoradiograms, an average retardation coefficient ($R$) was determined after numerically determining (by integrating pixel intensities) the median mobility (5, 27). $R$ values vary from 0 (no retardation) to 1 (complete arrest of mobility) and are plotted as a function of collagen concentration. Curves were fit to the equation $R = R_{max}/[1 + (K^d_{,app}/[C_{tot}])^2]$, where $R_{max} = 0.97$ (22) and yield apparent values of $K_d$ of $5.8 \times 10^{-7}$ M (NMuMG), $6.4 \times 10^{-7}$ M (MPC-11), and $1.1 \times 10^{-5}$ M (P3).

demonstrate that the amount of syndecan-1 and the molecular size of syndecan-1 and its heparan sulfate chains are similar on MPC-11 and P3 cells and therefore are unlikely to account for the difference in the ability of these cells to bind to type I collagen.

*Heparan Sulfate Chains of Syndecan-1 from P3 Cells Have a Low Affinity for Type I Collagen*—To more closely analyze the molecular basis for the difference in cell binding to collagen, we purified syndecan-1 from MPC-11 and P3 cells and, using the technique of affinity co-electrophoresis (27), compared their affinities for type I collagen (Fig. 3). As a control, we also analyzed intact syndecan-1 from normal murine mammary gland epithelial (NMuMG) cells which is known to have a high affinity for type I collagen (17, 22). All of the samples tested show broad heterogeneity in binding affinities; however, syndecan-1 from both MPC-11 and NMuMG cells has a distinct high affinity fraction that is absent from the syndecan-1 from P3 cells (Fig. 3*A*, *arrows*). Analysis of heparan sulfate chains isolated from purified syndecan-1 gave similar results although these showed less heterogeneity than did the intact syndecan-1 (Fig. 3*B*). Calculated values of $K_d$ of the heparan sulfate chains for type I collagen were $5.8 \times 10^{-7}$ M, $6.4 \times 10^{-7}$ M, and $1.1 \times 10^{-5}$ M, for NMuMG, MPC-11, and P3, respectively (Fig. 3*C*). Thus, the heparan sulfate chains of syndecan-1 from MPC-11 cells have an approximately 20-fold higher affinity for type I collagen than do the heparan sulfate chains of syndecan-1 from P3 cells. This difference provides a likely explanation for the observed inability of P3 cells to attach to collagen (Fig. 1).

It is noteworthy that although the heparan sulfate chains of MPC-11 syndecan-1 are significantly smaller than those of NMuMG syndecan-1 (17 *versus* 36 kDa, respectively) (22), they have very similar affinities for collagen. This indicates that at least in this instance, heparan sulfate fine structure may be more important than chain length in determining affinity of heparan sulfate for collagen. This notion is further supported by the observation that P3 heparan sulfate, which is only slightly smaller than MPC-11 heparan sulfate (Fig. 2), has a much lower affinity for collagen than does MPC-11 heparan sulfate.

*Heparan Sulfate of Syndecan-1 from MPC-11 and P3 Cells Differ in Their Fine Structure*—Because the heparan sulfate chains from MPC-11 and P3 cells are similar in size (Fig. 2*C*),

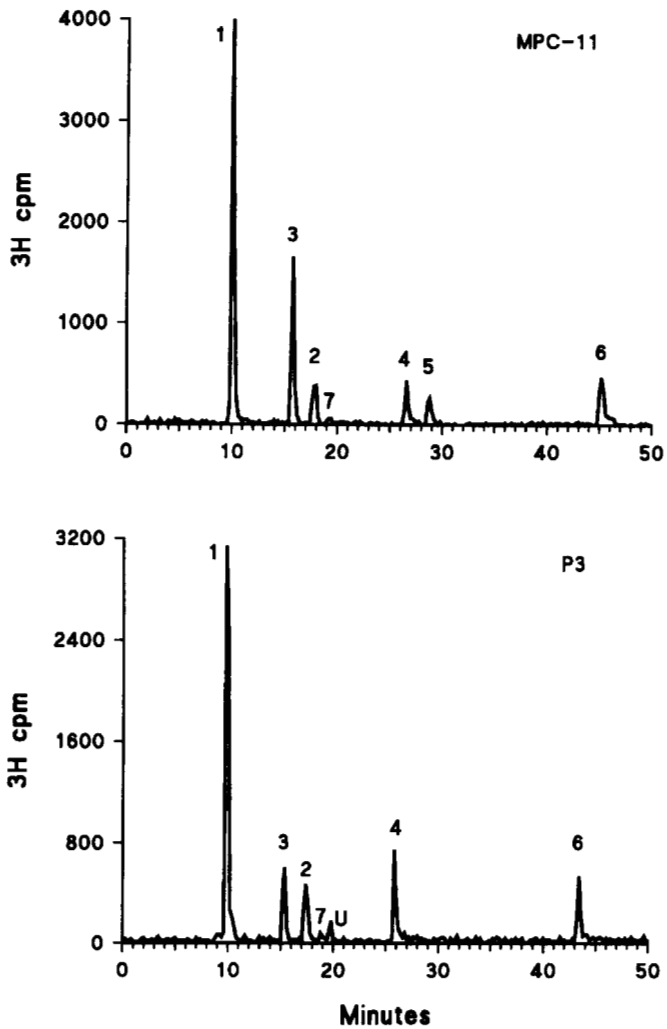


FIG. 4. **SAX-HPLC analysis of disaccharide composition of syndecan heparan sulfate from MPC-11 and P3 cells.** Disaccharide composition of syndecan-1 heparan sulfate (biosynthetically radiolabeled with [$^3$H]glucosamine) from P3 and MPC-11 cell lines was analyzed by SAX-HPLC as described under "Experimental Procedures." For the identity of the numbered peaks, see Table II.

their difference in affinity for collagen is likely due to differences in their fine structure. Consistent with this, data on disaccharide composition (Fig. 4 and Table II) and oligosaccharide mapping profiles (Figs. 5 and 6) demonstrate that syndecan-1 heparan sulfate from MPC-11 and P3 cells are structurally distinct. In the disaccharide composition it is particularly noticeable that the P3 heparan sulfate has reduced levels of both *N*-sulfate and 2-*O*-sulfate groups relative to MPC-11 heparan sulfate (Table II). Indeed, the disaccharide ΔUA(2S)-GlcNS$O_3$ is entirely absent from the P3 heparan sulfate. However, the level of 6-*O*-sulfate is higher in P3 than in MPC-11, and thus the overall level of sulfation of the heparan sulfate species from the two cell lines is very similar (72.6 and 75.6% respectively, Table II). Therefore, the difference in the affinity of P3 and MPC-11 syndecan-1 heparan sulfate for type I collagen are not simply due to differences in net charge. Although the overall level of sulfation of syndecan-1 from normal B cells is unknown, the levels seen here for P3 and MPC-11 cells are broadly within the same range as several normal cell types (33), despite the relatively low *N*-sulfate content. This indicates that these transformed cells may not differ significantly from their normal counterparts in regard to their average level of sulfation.

TABLE II
*Disaccharide composition of syndecan-1 heparan sulfate from MPC-11 and P3 cells*

Syndecan-1 was isolated from MPC-11 and P3 cells, and heparan sulfate oligosaccharides were prepared and their disaccharide composition analyzed by strong anion exchange HPLC as described under "Experimental Procedures." Peak numbers on the left side of the table are from Fig. 4.

| | MPC-11 | P3 |
|---|---|---|
| 1 UA-GlcNAc | 50.6 | 56.2 |
| 2 UA-GlcNAc(6S) | 7.4 | 10.1 |
| 7 UA(2S)-GlcNAc | 0.6 | 1.0 |
| U Unknown mono S | —[a] | 2.5 |
| 3 UA-GlcNS$O_3$ | 21.2 | 10.2 |
| 4 UA-GlcNS$O_3$(6S) | 5.9 | 11.2 |
| 5 UA(2S)-GlcNS$O_3$ | 4.3 | —[a] |
| 6 UA(2S)-GlcNS$O_3$(6S) | 10.0 | 8.8 |
| Polymer sulfation | 75.6 | 72.6 |
| *N*-Sulfates/100 disaccharides | 37.4 | 30.2 |
| *O*-Sulfates/100 disaccharides | 38.2 | 42.4 |
| N:O ratio | 0.98 | 0.71 |
| 2-*O*-Sulfates/100 disaccharides | 14.9 | 9.8 |
| 6-*O*-Sulfates/100 disaccharides | 23.3 | 30.1 |

[a] Not detected.

The data in Table II suggest that heparan sulfate affinity for collagen correlates strongly with the presence of *N*- and 2-*O*-sulfate groups. However, compositional data *per se* are not necessarily predictive of the nature of the collagen binding sequence.

Gradient PAGE oligosaccharide mapping using the enzyme heparitinase which acts specifically on the hexosamine-glucuronic acid linkage (34) demonstrates qualitatively that the MPC-11 and P3 $^{35}$S-labeled heparan sulfates give rise to distinct banding patterns (Fig. 5). Differences in the presence and relative content of specific oligosaccharide bands were evident, clearly indicating that the fine structures of these two heparan sulfate species are markedly different. This was confirmed by quantitative oligosaccharide mapping by SAX-HPLC using heparitinase which also revealed distinct elution profiles (Fig. 6). The greater susceptibility of the P3-derived heparan sulfate (note the high content of small $^{35}$S fragments eluting between 15 and 20 min) suggests a relative enrichment of *N*- and *O*-sulfate groups in the vicinity of the heparitinase cleavage sites. In contrast, the MPC-11 heparan sulfate contains an increased proportion of larger more highly sulfated saccharides composed of heparitinase-resistant GlcNS$O_3$-IdoA repeats, with variable *O*-sulfation (28, 29), which elute between 30 and 80 min. Because the overall level of sulfation of syndecan-1 heparan sulfate is similar between MPC-11 and P3 cells (Table II), the mapping data suggest that in the MPC-11 polysaccharide the sulfated residues are tightly clustered in relatively large blocks or domains, whereas in the P3 counterpart, the blocks of sulfated disaccharides are more commonly interspersed with GlcA-containing disaccharides that can be cleaved by heparitinase.

It seems likely that critical differences in the distribution of sulfated disaccharides such as those described above are responsible for the observed differences in collagen binding properties of syndecan-1 heparan sulfate. It is becoming increasingly clear that heparan sulfate binds biologically relevant protein ligands through specific sugar sequences. For example, high affinity binding of heparan sulfate to bFGF is mediated by a heparitinase-resistant fragment seven disaccharides in length with an internal repeat of five IdoA(2S)-GlcNS$O_3$ units (6–8), although it is not clear whether all the sulfate groups within sequences of this type are required for the bFGF interaction (9). Sequences of the same basic size and structure have been shown to mediate activation of bFGF in a mitogenic assay

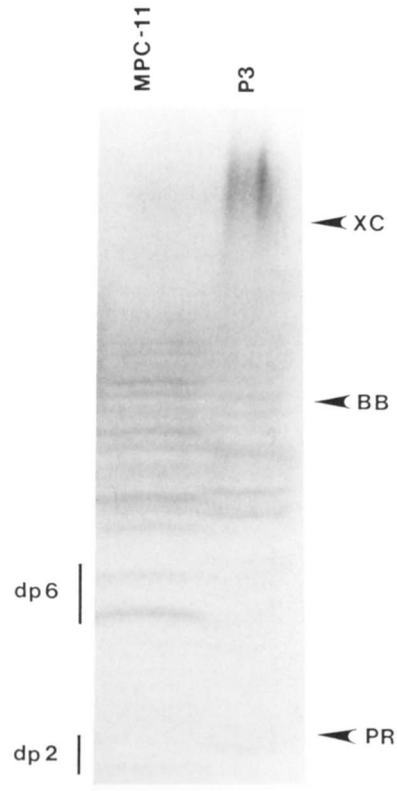


FIG. 5. **Oligosaccharide mapping of syndecan-1 heparan sulfate from MPC-11 and P3 cells by gradient PAGE.** $^{35}$S-Labeled heparan sulfate from MPC-11 and P3 syndecan-1 was treated with heparitinase and the resulting oligosaccharides separated by gradient PAGE, transferred to a nylon membrane, and detected by fluorography. The migration positions of disaccharides (*dp2*), hexasaccharides (*dp6*), bromphenol blue (*BB*), phenol red (*PR*), and xylene cyanol (*XC*) were as indicated. Oligosaccharides of dp approximately 12 migrate to a similar position to that of bromphenol blue.

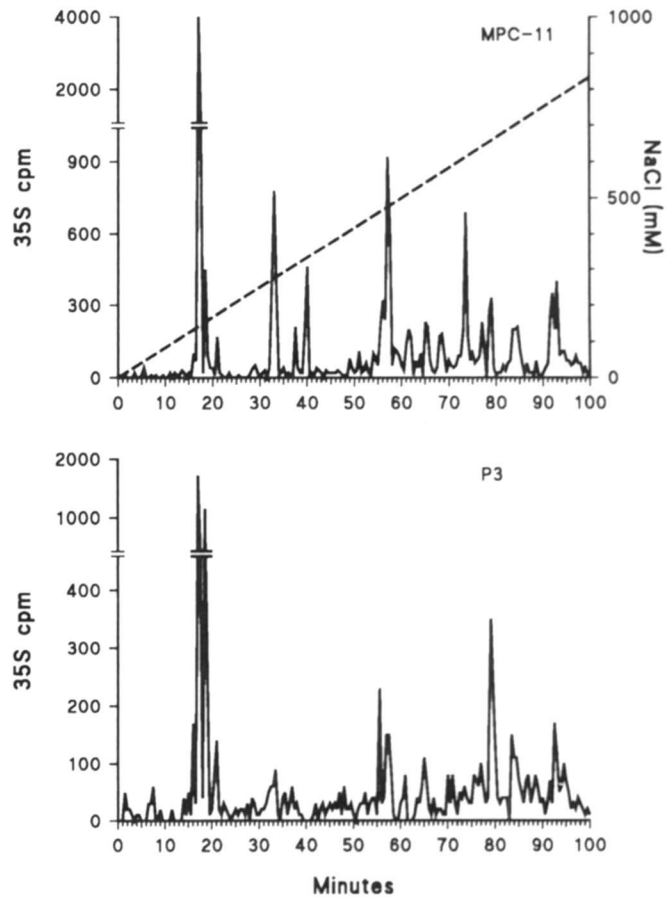


FIG. 6. **Oligosaccharide mapping of syndecan-1 heparan sulfate from MPC-11 and P3 cells by SAX-HPLC.** $^{35}$S-Labeled heparan sulfate from MPC-11 and P3 syndecan-1 was treated with heparitinase and the resulting oligosaccharides mapped by SAX-HPLC as described under "Experimental Procedures" except the salt elution gradient was 0–1.5 M over 180 min. The *dashed line* indicates the salt elution gradient. Additional radiolabel (representing only a minor proportion of total radiolabel) eluting in the range of 100–160 min is not shown. Note that the $^{35}$S cpm axis of the two plots is on different scales.

employing 3T3 cells rendered heparan sulfate-deficient by chlorate treatment (35). By contrast, the anti-thrombin III binding site in heparan sulfate (and heparin) is characterized by 3-*O*- and 6-*O*-sulfated glucosamines and a glucuronic acid residue (3, 4). Within the context of the present study, it will be essential to identify the structure of the collagen binding site in the syndecan-1 heparan sulfate as this sequence will have a major role to play in regulating cell adhesion and migration in the interstitial matrix.

*Syndecan-1 Heparan Sulfate and Cell Behavior*—Overall from these data we conclude that: (i) the syndecan-1 core protein can bear structurally different heparan sulfate chains in different, yet related, cells, and (ii) such structural variation in syndecan-1 heparan sulfate can give rise to substantial differences in the proteoglycan's binding properties. Furthermore, the data strongly suggest that such differences in proteoglycan binding properties can play a determining role in an important cell behavior, cell-matrix adhesion.

These results have several important implications regarding the regulation of proteoglycan function. First, they support the view that, at least for syndecan-1, the fine structure of heparan sulfate is not determined by information encoded in the core protein. Second, they argue that structural variations in heparan sulfates serve to encode functionally important information and do not merely reflect redundant biosynthetic variability. This is in accord with the demonstration of specific binding sequences for protein ligands such as anti-thrombin III (3, 4) and bFGF (6–8). Third, they demonstrate that an extracellular matrix molecule, type I collagen, can recognize information encoded in heparan sulfate, by a means other than simple detection of net polymer charge.

Taken together, these findings indicate that heparan sulfate fine structure is a critical determinant of proteoglycan function. It is clear that more needs to be learned about the range of heparan sulfate structural variation that occurs *in vivo*, as well as the range of heparan sulfate-binding proteins that are sensitive to such variation. Until then, caution should be exercised in drawing conclusions about the *in vivo* functions of any heparan sulfate proteoglycan based solely on the distribution of its core protein.

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