Heparan Sulfate Proteoglycans from Mouse Mammary Epithelial Cells

CELL SURFACE PROTEOGLYCAN AS A RECEPTOR FOR INTERSTITIAL COLLAGENS

(Received for publication, December 3, 1984)

Joy E. Koda, Alan Rapraeger, and Merton Bernfield

From the Department of Pediatrics, Stanford University School of Medicine, Stanford, California 94305

A heparan sulfate-rich proteoglycan is on the surface of NMuMG mouse mammary epithelial cells apparently intercalated into their plasma membranes. Mild treatment of the cells with trypsin releases the GAG-bearing region (ectodomain) of this molecule as a discrete proteoglycan which is readily purified. At physiological pH and ionic strength, the ectodomain binds collagen types I, III, and V but not types II, IV, or denatured type I. The proteoglycan binds to a single class of high affinity saturable sites on type I collagen fibrils, sites which are selective for heparin-like glycosaminoglycans. The binding of NMuMG cells to type I collagen duplicates that of their cell surface proteoglycan; cells bind to native but not denatured collagen, and binding is inhibited by heparin but not by other glycosaminoglycans. These binding properties suggest that cell surface heparan sulfate proteoglycans could act as receptors for interstitial collagens and mediate changes in cell behavior induced by collagen matrices.

MATERIALS AND METHODS

RESULTS

Preparation of Assessment of Purity of the Cell Surface Proteoglycan

Binding of the Ectodomain to Type I Collagen

Affinity of the Ectodomain for Other Collagen Types—Binding of 35S-PG to collagen immobilized on polystyrene chloride wells was used to compare binding to different collagen types as well as to native and heat-denatured type I collagen. Native collagen types I, II, III, IV, and V (including two different preparations each of types IV and V), the 7S domain of type IV collagen, and heat-denatured type I were immobilized on separate microtiter wells. After washing, the amount of each collagen type immobilized was assayed (Bailey, 1962) and was found to range from 1 to 10 µg/well. To test for binding, 35S-PG in PBS containing 1% BSA was added to collagen-treated wells, and the reactions were incubated for varying periods at room temperature. Binding to types I and III collagen was initially rapid and then slowed after 30 min of incubation while binding to type V progressed linearly during the 90-min incubation (Fig. 1). In contrast to these interstitial collagens, no appreciable binding was observed with types II and IV collagen or with heat-denatured type I collagen under these conditions (Fig. 1). These differences were not due to variation in the amount of collagen on the well. Therefore, differences

* This work was supported by National Institutes of Health Grant CA 05753, New Investigator Research Award HD17146, and Fellowship DE 05275, and by a fellowship from the Cystic Fibrosis Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† The abbreviations used are: PG, proteoglycan; PAGE, polyacrylamide gel electrophoresis; BSA, bovine serum albumin; PVC, polyvinyl chloride; PBS, phosphate-buffered saline.

ECTODOMAIN is the GAG-bearing region of the cell surface proteoglycan released by mild treatment with trypsin (Rapraeger and Bernfield, 1985).

* Portions of this paper (including "Materials and Methods," part of "Results," and Figs. 3-6) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-3636, cite the authors, and include a check or money order for $5.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
in the ability of the PG to bind must be due to differences in the structure of the collagens.

The quaternary structure of the collagens immobilized on the well is not known. However, it is clear that a native, possibly fibrillar, structure is required since the PG binds to native but not to denatured type I collagen. Binding may not occur to type IV collagen because only the 7 S domain of this molecule resembles a fibril, and this structure, which is maintained by covalent cross-links, is probably destroyed when the type IV collagen is extracted under reducing conditions, as was done here. To determine if the native cross-linked 7 S region of type IV collagen interacts with the proteoglycan, the isolated unreduced domain was immobilized on wells. However, no binding is detected (Fig. 1). Therefore, under these conditions, the PG binds to interstitial collagen types I, III, and V, but not to type II collagen, type IV collagen, or to the 7 S aggregate derived from type IV.

**Binding of Cells to Collagen**—The affinity of the ectodomain for interstitial collagens suggests that the cell surface PG could function as a collagen receptor. Therefore, we examined the ability of intact cells to duplicate the selectivity of the PG binding to type I collagen. Microtiter wells coated with BSA, native, or denatured type I collagen were incubated with NMuMG cells suspended by scraping after EDTA treatment. The binding of the cells exhibited the same specificity as the binding of the ectodomain. The cells bound to native but not to denatured collagen or BSA, and the binding was unaltered by including 100 μg/ml chondroitin 6-sulfate, chondroitin 4-sulfate, dermatan sulfate, or hyaluronic acid in the incubation mixture (Fig. 2). In contrast, binding was prevented by including 5–10 μg/ml heparin or 100 μg/ml dextran sulfate (M, 500,000), polyanions which interfere with the binding of cell surface PG to collagen (Fig. 2). The inhibition of binding by heparin is due to blocking of binding sites on the collagen because identical results are obtained when the wells are pretreated with heparin and the assay is run in the absence of heparin (not shown).

**DISCUSSION**

A heparan sulfate-rich proteoglycan is on the surface of NMuMG mouse mammary epithelial cells and behaves as an integral membrane protein (Rapraeger and Bernfield, 1983, 1985). The ectodomain of this PG, the region containing the glycosaminoglycan chains, is cleaved from its putative membrane-associated domain by mild trypsin treatment, producing a PG which is no longer lipophilic and can be readily handled in the absence of detergent. This ectodomain binds specifically to interstitial collagen types I, III, and V at physiological pH and ionic strength and saturates a single class of high affinity sites on type I collagen fibrils (see Miniprint Section). The binding of the ectodomain is inhibited by heparin and dextran sulfates but not by other glycosaminoglycans at the concentrations tested (see Miniprint Section). NMuMG cells also bind to type I collagen and mimic, in several respects, the binding of their cell surface PG; the cells bind to native but not denatured type I collagen, and this binding is inhibited by heparin and dextran sulfate, but not by other glycosaminoglycans. Because the behavior of mammary epithelial cells is markedly altered by culture on and within type I collagen gels, they apparently have a mechanism for recognizing collagen fibrils. The cell surface PG could function as a receptor for the interstitial collagens, potentially mediating the collagen-induced changes in cell behavior.

**Binding of the Ectodomain Is Similar to the Binding of a Basal Extracellular Proteoglycan**—The ectodomain of the cell surface PG bears primarily heparan sulfate chains (M, of 36,000) but also 15–24% chondroitin sulfate chains (M, of 17,000) attached to the same core protein (Rapraeger et al.*). Although prepared by proteolytic treatment, the ectodomain behaves as a single PG species on DEAE-cellulose, gel filtration, and collagen affinity chromatography (Rapraeger et al.*). Based on gradient PAGE, it shows a broad molecular weight distribution, possibly, as in other PGs, due to variation in the number of glycosaminoglycan or N-linked oligosaccharide chains (Hascall and Hascall, 1981).

The ectodomain is slightly smaller in molecular size range but identical in other respects to an extracellular heparan sulfate-rich PG deposited beneath the basal surfaces of the NMuMG cells when they are cultured on plastic (Koda and Bernfield, 1984). The ectodomain binds to type I collagen with properties that are nearly identical to those previously reported for this extracellular PG (Koda and Bernfield, 1984). Both PGs (i) bind to type I collagen at physiological pH and...
The structural features of the inhibitory polyanions suggest a selective binding site on collagen fibrils. Heparan sulfate contains highly sulfated regions that are also present in heparin and potentially occur in dextran sulfate, polyanions which compete with PG binding to collagen. These highly sulfated regions are lacking in the polyanions which are ineffective competitors of the binding; for example, the sulfates occur uniformly along the chondroitin sulfate polymer rather than in "block" regions. Extensive sulfation alone is insufficient for inhibitory potential. Dextran sulfate, which has 3.3 sulfate residues/disaccharide (Windholz et al., 1976), on the average, is 10-fold less potent than similarly sized heparin, which bears 2 to 3 sulfate residues/disaccharide. Interestingly, increasing the sulfate concentration by using a larger-sized dextran sulfate increases its inhibitory potency. However, the binding is not simply a function of sulfated region because the sulfate concentrations provided by the noninhibitory glycosaminoglycans were 10- to 500-fold greater than the effective sulfate concentration required of any of the inhibitory polyanions. However, the chondroitin sulfates do bind to collagen (Lindahl and Hook, 1978), although apparently at sites different than heparan sulfate, suggesting that the heparan sulfate-rich PG binds to specific sites on the collagen which are selective for polysaccharides containing regions of extensive sulfation interspersed with regions of low or no sulfation.

The Ectodomain Binds Selectively to Interstitial Collagens—At physiological pH and ionic strength, the ectodomain binds to collagen types I, III, and V, but not to collagen types II and IV. Thus, the ectodomain shows selectivity for the interstitial collagens with the exception of type II. The reason for this is not known but may be because the type II collagen fibril formed in vitro under physiological ionic conditions differs from that of the other interstitial collagens.

Our failure to show binding to type IV collagen under physiologic conditions is also consistent with the requirement for collagen fibrils. Type IV collagen does not form fibrils but does form a multimeric complex of amino termini, the 7 S domain (Timpl et al., 1979; Risteli et al., 1980) which may resemble a fibril. However, the failure of PG binding to this isolated domain indicates that it does not satisfy this structural requirement.

The fibrils formed by collagen types I, III, and V under physiological conditions have a similar distribution of clustered polar residues (Kuhn, 1982). Thus, these collagens may have a similar heparan sulfate PG binding site. Collagen types I, III, and V also have a similar tissue localization, appearing together in extracellular matrices of many tissues (Kuhn, 1982). The tissue distribution of type IV collagen differs, being localized to the basal lamina (Kefaldes, 1973). It is puzzling that the ectodomain does not bind to type IV collagen because mammary epithelial cells do lie on a basal lamina in situ. Interactions of other basal laminar components may be required to facilitate complex formation of the cell surface PG with type IV collagen.

The Cell Surface Proteoglycan May Be a Receptor for Interstitial Collagens—In vitro, fibrillar type I collagen causes mammary epithelial cells to accumulate matrix materials, organize into distinct structures, and respond to physiological stimuli. For example, when cultured on top of collagen gels, mouse mammary epithelial cells form a basal lamina-like layer (Emerman and Pitelka, 1977; David and Bernfield, 1979). When embedded in collagen gels, branched ductlike structures form (Yang et al., 1979; Bennett, 1980) and, in such cultures, the cells respond to lactogenic hormones and secrete α-lactalbumin and casein (Haeuptle et al., 1983). These effects may involve a receptor on the mammary epithelial cell surface that recognizes the collagen.

Receptors are defined by their (i) high affinity, (ii) saturability, (iii) reversibility, (iv) ligand specificity, and (v) biological response produced by the receptor-ligand interaction (Hollenberg and Cuatrecasas, 1979). The cell surface PG satisfies certain of these criteria; the ectodomain reversibly binds type I collagen fibrils with high affinity ($K_d \sim 10^{-9} M$), saturates a finite number of highly specific sites on the fibrils, and binds to only interstitial collagens. The cell surface PG may be a cellular receptor for collagen fibrils because its binding specificity is duplicated by the NMuMG cells. The cells bind to native but not denatured type I collagen and, based upon inhibition studies, bind at an identical site as their isolated cell surface PG.

Cells appear to have multiple mechanisms for attachment to or recognition of collagens. Indeed, the number and type of mechanisms may vary with the type of cell. Other cell types may also use heparan sulfate PGs, suggested by the finding that heparan sulfate or heparin inhibits the attachment of mouse myeloma cells to type I collagen fibrils (Stamatoglou and Keller, 1983). Other types of membrane-associated molecules interact with collagens but differ from the cell surface PG in their binding and chemical properties. Some bind to denatured collagen (gelatin) (Koehler et al., 1980) or to isolated α chains (Chiang and Kang, 1982) or show a different specificity for the collagen types (Mollenhauer and von der Mark, 1983; Kurkinen et al., 1984). Where described, these molecules are glycoproteins of molecular weights ranging from 31,000 to 95,000 and apparently do not contain glycosaminoglycan chains. The function of these cell surface collagen-binding molecules is not clear. Their presence on the cell surface may be to bind cells to collagens or to assist in the secretion and assembly of collagens at the cell surface. Alternatively, they may appear on the cell surface only transiently. For example, they may have a role in secretory vesicles and appear on the cell surface upon fusion of these vesicles with the plasma membrane and then be reinternalized.

If the ectodomain is, as proposed, a receptor which binds cells to interstitial collagens, then some mechanism is required to release cells from this association. Release of the cells could be accomplished by cleavage of the ectodomain from the cell surface. In the presence of a collagen substratum, such release would result in a stable collagen-proteoglycan complex. In the absence of a collagen substratum, the release mechanism could result in a soluble PG, perhaps the basal extracellular PG described previously.

In addition to binding interstitial collagens, apparently via its heparan sulfate chains as shown here, heparan sulfate PG or heparan sulfate or heparin chains are also thought to bind to fibronectin (Yamada et al., 1980), laminin (Ruoslahti and Engvall, 1980; Woodley et al., 1983), and a complex of fibronectin with collagen (Ruoslahti and Engvall, 1980). Thus, the cell surface proteoglycan may interact, as a receptor, with various matrix components.

Acknowledgments—We thank Drs. Saryu Dixit, Daniel Herbage, and Robert Treistad for collagens and Margareta Svensson-Rosenberg for technical skill.
Cell Surface Proteoglycan as Collagen Receptor

Neurog9 mouse mesencephalon epithelial cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM-G1, as described previously) and plated on collagen (0.1 mg/ml) or on the extracellular matrix of rat tail tendon (laminin, bovine type II collagen, fibronectin, and vitronectin) in vitro. The cells were washed with ice-cold Tris-buffered saline (TBS, 150 mM NaCl, 2.7 mM KCl, 10 mM Tris HCl, pH 7.4), and the supernatant was aspirated. The adhered cells and the collagen were fixed with 4% paraformaldehyde in PBS for 1 h at room temperature. After fixation, the samples were washed thoroughly with PBS and processed for immunocytochemistry. The cells were incubated with monoclonal antibodies to laminin (1:100), type II collagen (1:100), fibronectin (1:100), and vitronectin (1:100) and FITC-conjugated goat anti-mouse or goat anti-rabbit IgG (1:100) for 1 h at room temperature. The cells were then washed with PBS and mounted on glass slides with glycerol containing 0.5% Triton X-100. The slides were observed with a fluorescent microscope (Olympus, Japan) equipped with a fluorescence filter set and a cooled charge-coupled device camera (Hamamatsu, Japan). The images were captured and analyzed using ImageJ software (National Institutes of Health, USA). The images were then processed for contrast enhancement and color balance adjustment. The results were analyzed using a computer program written in MATLAB (MathWorks, USA). The data were presented as mean ± standard deviation. The significance of the differences was determined using Student's t-test. A p-value of <0.05 was considered statistically significant.

Cell Surface Proteoglycan as Collagen Receptor

Polymerizable Gel Electrophoresis

Polyacrylamide gel electrophoresis was performed using 10% acrylamide (37:1) and 0.8 M Tris-boric acid (pH 8.8). The gels were stained with Coomassie blue R250 and destained with 10% acetic acid. The stained bands were scanned and analyzed using ImageJ software. The data were presented as mean ± standard deviation. The significance of the differences was determined using Student's t-test. A p-value of <0.05 was considered statistically significant.

Cell Surface Proteoglycan as Collagen Receptor

REFERENCES

Bailey, J. L. (1962) Techniques in Protein Chemistry, p. 73, Elsevier, New York.

Bennett, D. C. (1980) Nature 285, 657–659.

Chandrasana, G., Torchia, D. A., and Piez, K. A. (1983) J. Biol. Chem. 258, 6062–6067.

Chang, T. M., and Kang, A. H. (1983) J. Biol. Chem. 258, 7581–7588.

Cooper, S. A., and Bernfield, M. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 798–790.

Dixit, S. N., Mainardi, C. L., Beachey, E. H., and Kang, A. H. (1983) Collagen Relat. Res. 31, 263–270.

Eisenbarth, G. S., Rankin, R. B., III, Haynes, B. F., and Fauci, A. S. (1980) J. Immunol. Methods 39, 387–392.

Emerson, J. T., and Pikelis, D. R. (1977) In Vitro 13, 316–318.

Freytag, J. W., Noellen, M. E., and Hudson, B. G. (1979) Biochemistry 18, 4761–4768.

Goldberg, B. (1979) Cell 16, 265–275.

Haseulte, M.-T., Suard, Y. L. M., Bogenmann, E., Reggio, H., Racine, L., and Kraehnebuil, J.-P. (1983) J. Cell Biol. 96, 1425–1434.

Hascall, V. C., and Hascall, G. (1981) in Cell Biology of the Extracellular Matrix (Hay, E., ed) pp. 39–63, Plenum Publishing Corp., New York.

Hollenberg, M. D., and Cuatrecasas, P. (1979) in The Receptors, A Comprehensive Treatise (O’Brien, R. D., ed) Vol. 1, pp. 193–214, Plenum Publishing Corp., New York.

Koehler, J. K., Nudelman, E. D., and Hakomori, J. T. (1962) J. Biol. Chem. 237, 259, 5915–5922.

Laemmli, U. K. (1970) Nature 227, 680–685.

Liotta, L. A., and Hook, M. (1970) Annu. Rev. Biochem. 47, 385–417.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.

Mauer, A. Demarches, M., Herbage, D., Grimard, J.-A., Druguet, M., Hartmann, D., and Sengel, P. (1982) Dev. Biol. 94, 93–105.

Mollenhauer, J., and von der Mark, K. (1983) J. Cell Biol. 95, 247–257.

Oakley, B. R., Kirsch, D. R., and Morris, N. R. (1980) Anal. Biochem. 105, 361–363.

Rapraeger, A., and Bernfield, M. (1983) J. Cell Biol. 95, 3632–3638.

Rapraeger, A., and Bernfield, M. (1985) J. Biol. Chem. 260, 4093–4109.

Risteli, J., Bachinger, H. P., Engel, J., Furthmayr, H., and Timpl, R. (1980) Eur. J. Biochem. 108, 233–250.

Roshol, E., and Engvall, E. (1980) Biochem. Biophys. Acta 631, 350–358.

Stamatoglou, S. C., and Keller, J. M. (1983) J. Cell Biol. 96, 1820–1823.

Timpl, R., Risteli, J., and Bachinger, H. P. (1979) FEBS Lett. 101, 21–25.

Trelstad, R. L., Catanese, V. M., and Rubin, D. F. (1976) Anal. Biochem. 71, 114–118.

Trelstad, R. L., Lawley, K. R., Hayashi, E., Ehrlich, H. P., and Silver, F. H. (1981) Collagen Relat. Res. 1, 39–52.

Windholz, M., Budavari, S., Gilman, L., and Fertig, M. (eds), The Merck Index, Ninth Ed., p. 387, Merck, Rahway, NJ.

Woodley, D., Rau, C., Hassell, J., Liotta, L., Martin, G., and Kleinman, H. (1985) Biochem. Biophys. Acta 761, 278–283.

Yang, J., Richards, J., Bowman, P., Guzman, R., Enami, J., McComb, K., Hamamoto, S., Pelletta, D., and Nandi, S. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 3401–3405.

Polymerizable Gel Electrophoresis

Polyacrylamide gel electrophoresis was performed using 10% acrylamide (37:1) and 0.8 M Tris-boric acid (pH 8.8). The gel was stained with Coomassie blue R250 and destained with 10% acetic acid. The stained bands were scanned and analyzed using ImageJ software. The data were presented as mean ± standard deviation. The significance of the differences was determined using Student’s t-test. A p-value of <0.05 was considered statistically significant.

Collagen Preparations

Type I collagen was purified from rat tail tendons by the method of Chandrasana et al. (1976) which yields a pure collagen preparation enriched in alpha chains as judged by PAGE (Coomassie, 1901) and staining with Coomassie brilliant blue. Staining with Coomassie blue gel electrophoresis (Advantec Biotechniques, Type I collagen purified all protein bands). Chicken collagen type V, 111 (T叻et al., 1991), and V (Trastel et al., 1991), were from Dr. Daniel Herbage (Maugen, 1982). Type V collagen was also purified by quantitative chloride extraction of the CVS tendon, reducing conditions (Kleinman et al., 1982). The tissue included, isolated homogenate of human placenta type IV collagen was from Dr. Sergey Stark (Joint et al., 1993). Type I collagen fibrils were prepared by dialysis of a 5 mg/ml solution in 0.1 M acetic acid against PBS for 12 hours at 4°C. A uniform suspension to collagen fibrils was generated by brief, sonication on ice for 1 minute, using a Heat Systems-Ultrasonic Model 405C at setting 5 with the special ultrasonic probe.

Collagen Binding Assays

Binding was assayed in three distinct assays:

1. Fibril Suspension Assay: Binding to suspended fibrils of type I collagen was assayed by centrifugation or filtration. For the centrifugation assay, the fibrils were added to a sample containing a 10-fold excess of collagen in PBS. The samples were centrifuged at 500 × g for 10 min. The supernatant was aspirated, and the precipitate was resuspended in PBS. The amount of collagen in the supernatant was determined by measuring the absorbance at 280 nm.

2. Fibril Suspension Assay: Binding to suspended fibrils of type I collagen was assayed by centrifugation or filtration. For the centrifugation assay, the fibrils were added to a sample containing a 10-fold excess of collagen in PBS. The samples were centrifuged at 500 × g for 10 min. The supernatant was aspirated, and the precipitate was resuspended in PBS. The amount of collagen in the supernatant was determined by measuring the absorbance at 280 nm.
Cell Surface Proteoglycan as Collagen Receptor

The stoichiometry of binding to collagen fibrils was analyzed by examining binding in the fibril suspension assay as a function of the amount of PG added. Varying amounts of the PG were incubated with 8 pg of collagen fibrils for 60 min at 22°C (Figure 5A). Fibrils were collected by centrifugation, and the amounts of bound and unbound PG were determined. At low concentrations, the amount of PG bound is a linear function of the amount added. Thus, the binding of cell surface PG to collagen fibrils is saturable, suggesting that the fibrils contain a finite number of PG-binding sites.

To analyze these findings further, the data were redrawn as a Scatchard plot (Figure 5B). The data yield a highly significant straight line (y = 0.905, suggesting that the fibrils contain a single class of equal affinity binding sites for the PG. Assuming 95% confidence intervals for this line and that the PG has an Mr of 250,000, the apparent dissociation constant for these sites is 1.0 x 10^(-7) M. The Mr of the PG estimated by PAGE may vary by as much as 40% and using this variation in molecular weight, the calculated dissociation constant ranges from 0.7 to 2.8 x 10^(-7) M.

To assess the nature of the collagen binding site, the ability of various anions to elute the eotuchonectin from collagen fibrils was tested using a TCA-agarose column. Anions such as Na+, K+, and Cl- were found to be effective in eluting the fibrils, indicating that the interaction between the fibrils and the PG is strongly affected by anion concentration. However, it should be noted that TCA-agarose is a very effective eluting agent for plasminogen activator (PA), and the effect of anions on the fibrils may be due to their inhibitory effects on PA.

Different ECD values were observed for various anions. The ECD for chloride is 460, which for phosphatase is 31, and that for sulfate is 36, these are several-fold greater than physiological concentrations. These data suggest that the collagen binding site shows anion selectivity.

The ECD for various sulfated glycosaminoglycans provides even more evidence for this selectivity. The ECD for heparin is ca. 10^5 M, similar to that for a large dextran sulfate (Mr 5000) but 10-50 fold lower than that for a small dextran sulfate (Mr 8000). An ECD for chondroitin-4-sulfate, chondroitin-6-sulfate, and dextran sulfate could not be established; these GAGs were not effective at eluting PG from collagen at concentrations up to 1000-fold that of the ECD. These results suggest that the binding site is not selective for polysaccharides containing highly sulfated regions (Koda and Beneski, 1984).

RESULTS

Preparation and Assay of Purity of the Eotuchonectin of the Cell Surface Proteoglycan

Preparation of the Eotuchonectin of the Cell Surface Proteoglycan

The Eotuchonectin was released from the cell surface by mild trypsin treatment and applied to the column in buffer A and eluted with a 100 ml linear gradient from 0.05 M to 0.2 M NaCl. The peak of radioactivity was pooled and dialyzed against buffer B against the appropriate buffer (4 M NaCl and then buffer A).

Binding Studies with Cell Surface Proteoglycan

Studies with soluble, extracellular heparan sulfate-rich PG from mononuclear cells revealed that binding to type I collagen requires heparin at physiological pH and ionic strength.
Cell Surface Proteoglycan as Collagen Receptor

Fig. 5: Saturation of collagen fibrils with the chondroitin of the cell surface PG. Increasing amounts of 35S-PG were incubated for 60 min at 22°C with 2 mg of collagen fibrils suspended in 100 μL PBS, 1% BSA. Bound PG is plotted vs. the calculated total amount of PG added per assay (A). Triplicate points from a representative experiment are plotted in the figure. Data from the same experiment are replotted as a Scatchard plot in (B). The correlation coefficient = 0.95 and the P value <0.001.