Binding of the NG2 Proteoglycan to Type VI Collagen and Other Extracellular Matrix Molecules*

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Previous studies have suggested that the NG2 proteoglycan interacts with type VI collagen. We have further characterized this interaction using a solid phase binding assay in which purified NG2 was shown to bind to pepsin-solubilized type VI collagen. In addition, NG2 bound a recombinant a2 (VI) collagen chain but did not appreciably bind to the recombinant a1 (VI) chain or the N-terminal domain of a3 (VI) (N9–N2). Binding of NG2 to type VI collagen was shown to be concentration-dependent and saturable and to depend mainly on the NG2 core protein, since chondroitinase-treated NG2 bound the collagen as well as undigested samples. In addition, the binding studies revealed several other possible ligands for NG2, including type II collagen, type V collagen, tenascin, and laminin. Binding of the proteoglycan to these molecules was also shown to be mediated by domains contained within the NG2 core protein. The ability of NG2 to bind to these extracellular matrix molecules was compared with that of the chondroitin sulfate proteoglycan decorin, revealing an almost identical binding pattern of the two proteoglycans to the different collagen types. In addition, decorin was found to effectively inhibit the ability of NG2 to bind to collagen, thus suggesting that the two proteoglycans may bind to some of the same regions on the collagen substrates. In contrast, decorin did not bind tenascin and was ineffective in inhibiting the binding of NG2 to tenascin or laminin, indicating that NG2 may bind these two molecules using a separate domain that is distinct from its collagen binding region.

Proteoglycans represent a diverse class of macromolecules the defining feature of which is the addition of one or more glycosaminoglycan sugar chains to a core glycoprotein. Characterization of this group of molecules has expanded in recent years, revealing an enormous diversity in number, structure, and function (for review, see Refs. 1–4). Although early work emphasized the importance of the glycosaminoglycan chains in mediating proteoglycan interactions with other ligands, sequence information obtained from the growing list of cloned proteoglycan core proteins has revealed the presence of binding motifs similar to those found in other proteins (3). This suggests that proteoglycan core proteins may, in fact, be responsible for many of the interactions attributed to proteoglycans. Proteoglycans contained within the extracellular matrix (ECM), 1 which include the large aggregating proteoglycans aggrecan (5) and versican (6), the smaller leucine-rich family of proteoglycans typified by decorin, and the basement membrane proteoglycan perlecan, all have been shown to interact with several other matrix components, including laminin (7), fibronectin (8, 9), tenasin (10), hyaluronic acid (5, 6, 11), and collagen (12–14). These interactions are believed to be important for the proper assembly, maintenance, and function of the ECM. In addition, cell surface proteoglycans such as syndecan and CD44 have been shown to bind matrix molecules, including fibronectin (15, 16), hyaluronic acid (17), thrombospondin (18), and the collagen types I, III, and V (19), and therefore may serve to modulate cell-ECM interactions.

NG2 is a 500-kDa integral membrane chondroitin sulfate proteoglycan that is widely expressed on numerous cell types, including chondroblasts (20, 21), myoblasts, fibroblasts, 2 and O2A glial progenitor cells (22, 23). The rat NG2 molecule has been cloned and shown to have very limited homology to any other proteoglycan or protein (24). Although the function of NG2 remains speculative, several studies have suggested that NG2 may interact with type VI collagen (21, 25). In addition, several cell lines that produce type VI collagen have been shown to bind type VI collagen to the cell surface. Transfection of these cells with full-length NG2 cDNA resulted in colocalized expression of NG2 and type VI collagen on the cell surface. Transfection of these cells with full-length NG2 cDNA resulted in colocalized expression of NG2 and type VI collagen on the cell surface. Retention of type VI collagen on the cells could be inhibited using specific antisera against the NG2 proteoglycan. Furthermore, both monoclonal and polyclonal antibodies against NG2 coprecipitate type VI collagen from detergent extracts of cells (21, 25). Together, these studies strongly suggest that NG2 may represent a cellular receptor for type VI collagen.

Structurally, type VI collagen consists of three polypeptide chains (for review, see Ref. 26). The a1 (VI) and a2 (VI) chains are approximately 140 kDa, whereas the a3 (VI) chain is a larger 210-kDa protein. These chains form heterotrimeric monomers, which further associate into larger oligomers (dimers and tetramers) that assemble into distinct microfibrils (27, 28). Type VI collagen is a rather unusual collagen, in that its chains contain relatively short triple helical segments and unusually large N- and C-terminal globular domains (26–29). Interactions between type VI collagen and other ECM components, including collagen types I (14, 31), II (12), and XIV (30),

1 The abbreviations used are: ECM, extracellular matrix; PBS, phosphate-buffered saline; BSA, bovine serum albumin.
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hyaluronic acid (31, 32), von Willebrand factor (33), and the leucine-rich proteoglycans decorin (13), fibromodulin (14), and lumican (34), have previously been demonstrated. Although it is thought that some of these interactions may be mediated by domains contained within the large globular domains of the type VI collagen molecule, several studies have suggested instead that the triple helical domains are responsible for most of the functional interactions of type VI collagen examined to date (35, 36). Several studies have demonstrated cell attachment to type VI collagen molecules, which is presumably mediated by α1β1- and α2β1-integrins (37, 38).

To further characterize the interaction between NG2 and type VI collagen, the present study examines the ability of purified NG2 molecules to bind directly to type VI collagen using a solid phase binding assay. We also examined the ability of NG2 to interact with the recombinant α1 (VI), α2 (VI), and N9–N2 α9 (VI) polypeptides. In addition, the binding of NG2 to other collagens and several other ECM components was tested. The binding of the proteoglycan decorin to these substrates was also tested and compared with the binding pattern found for NG2.

EXPERIMENTAL PROCEDURES

Antibodies—Production and characterization of polyclonal and monoclonal antibodies against NG2 have been previously described (21, 24). Protein G-purified immunoglobulins from ascites preparations of the monoclonal antibodies were used for some of the inhibition studies reported here. Polyclonal antiserum against human type VI collagen was kindly provided by Dr. Eva Engvall (La Jolla Cancer Research Center). Decorin polyclonal antiserum was obtained from Life Technologies, Inc.

Proteins—Laminin and pepsin-solubilized collagen types I–V were purchased from Sigma. These collagens were isolated from pepsin extracts of human placenta as described previously (39). Additional experiments were performed using pepsin-solubilized type V collagen purified from bovine bone, which was provided by Dr. F. Ruggiero (Institut de Biologic et Chimie des Proteines). Human tenascin and mouse laminin were obtained from Chemicon. Purified decorin was kindly provided by Dr. Yu Yamaguchi (La Jolla Cancer Research Center).

Purification of NG2 and Collagen Type VI Recombinant Proteins—NG2 was purified from B49 cells in a manner similar to that previously described (21). Briefly, NG2 was extracted from B49 cells using PBS (0.137 M NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, 1.47 mM KH2PO4), pH 7.4, and 50 mM octyl glucoside in the presence of phenylmethylsulfonyl fluoride and soybean trypsin inhibitor. The detergent extract was subjected to DEAE chromatography followed by size exclusion chromatography using a Sepharose CL-4B column. The NG2 sample was then dialyzed against 10 mM ammonium acetate and concentrated by lyophilization to a small volume. The purity and integrity of the NG2 was visually determined by examination of samples that had been electrophoresed on a SDS-polyacrylamide gel and Coomassie Blue stained. A Western blot was also performed on the purified NG2 preparation as described previously (20). The protein concentration of the purified NG2 was determined using the Bio-Rad protein assay. The presence of any contaminating type VI collagen in the purified NG2 preparation was determined by Western blotting using a specific polyclonal antiserum against human placental type VI collagen.

Recombinant α1 (VI) and α2 (VI) and the C-terminal fragment, N9–N2, of α3 (VI) collagen were purified as described previously (35, 36).

Binding Assay—Microtiter wells were coated with 2 μg of collagen, laminin, or tenascin or 2% BSA diluted into 0.05 ml of 10 mM ammonium acetate, pH 7.2, at 4 °C, overnight. Nonspecific binding sites in the wells were blocked by incubation with 2% BSA in PBS for 1 h at room temperature. Wells were washed three times with PBS and incubated with purified NG2 or decorin in 0.05 ml of PBS containing 2% BSA for 2 h at room temperature. After several washes with PBS, a 1:1000 dilution of either NG2 or decorin polyclonal antiserum in 0.05 ml of PBS was added to the wells, and they were incubated an additional 1 h. The wells were washed, and 1.5 × 105 cpm of 125I-goat anti-rabbit IgG in 0.05 ml of PBS was added to the wells and incubated 1 h. After final washing, the wells were separated, and the bound radioactivity was determined using a γ counter. All binding assays were repeated at least three times using duplicate wells in each case. Each figure presents the data as mean values ± S.D. from the pooled results of three trials.

To determine the amount of collagen, laminin, or tenascin adsorbed to the wells, samples of these proteins were 125I-labeled, diluted with unlabeled protein, and allowed to adsorb to the microtiter wells overnight. The amount of protein bound to the wells was then calculated. Approximately 1 μg of each collagen, laminin, fibronectin, and tenascin was found to adsorb to the microtiter wells under the conditions of this study.

To determine whether any type VI collagen was present in the NG2, laminin, tenascin, and other collagen samples, microtiter wells were coated overnight with the various proteins. The presence of type VI collagen was detected by incubation of the wells with a collagen type VI polyclonal antibody followed by incubation with 125I-goat anti-rabbit IgG.

Iodinations—The various collagens and the goat anti-rabbit IgG were iodinated using the chloramine T method followed by purification using G50-Sepharose chromatography.

Chondroitinase Treatment of Proteoglycans—NG2 and decorin core proteins were prepared by digestion with chondroitinase-ABC (Seikagaku). Proteoglycans were diluted into 0.1 ml of PBS containing protease inhibitors and incubated with 0.1 unit of chondroitinase-ABC for 2 h at 37 °C.

RESULTS

Binding of NG2 to Immobilized Collagens—NG2 was purified from rat glia cells using sequential DEAE and Sephacryl CL-4B chromatography. To confirm the purity and integrity of the NG2, an aliquot was analyzed by SDS-polyacrylamide gel electrophoresis. Coomassie Blue staining illustrates the purity of the NG2 preparation used in the binding studies (Fig. 1A). A Western blot of the NG2 preparation (Fig. 1B) also indicates that the NG2 used in the present studies was the proper size and that chondroitinase digestion of the sample resulted in the conversion of the proteoglycan to the intact 300-kDa NG2 protein core. The ability of purified NG2 to bind to various collagens was tested using a solid phase binding assay. Purified NG2 proteoglycan was added to microtiter wells coated with either pepsin-extracted collagens or BSA, and the

![Fig. 1. Evaluation of NG2 purity and integrity.](http://www.jbc.org/)

1. Binding to immobilized collagen types I, II, and III. Lane 1, BSA control; lane 2, NG2 control; lane 3, chondroitinase-treated NG2.
binding assay was performed as described under “Experimental Procedures.” The results demonstrate that NG2 binds to type VI collagen. Binding of NG2 was also observed to collagen types II and V but not to collagen types I, III, and IV or BSA-coated wells (Fig. 2A). Controls in which either the NG2 or the anti-NG2 antiserum was not added showed no binding above background (data not shown).

We were interested in comparing the specificity of the binding of NG2 to various collagens with that of other proteoglycans known to interact with collagen. Decorin has previously been shown to interact specifically with collagen type VI and, to a lesser extent, with collagen type V in a similar binding assay (12). We therefore examined the binding of decorin with collagen types I–VI. The binding assay was performed in parallel to the NG2 binding assays using purified decorin in conjunction with decorin polyclonal antisera. The results show that decorin binds type VI collagen and also binds collagen types II and V (Fig. 2B). This binding pattern is similar to that seen for NG2 but differs from a previous study in which decorin did not bind to collagen type II (12). The difference in these results may be due to differences in the preparation of the type II collagen or decorin samples. To determine whether any contaminating type VI collagen was present in the other collagen samples, we tested these preparations using a type VI collagen polyclonal antiserum. Minor levels of type VI collagen were detected in the other collagen substrates tested. When tested in this same manner, the NG2 preparation was found to contain no detectable amounts of type VI collagen.

Binding of NG2 to Collagen Type VI Recombinant Chains—To further examine the interaction of NG2 and type VI collagen, we tested the ability of NG2 to bind to recombinant forms of the α1 (VI) and α2 (VI) chains as well as to the N-terminal N9–N2 globular domain of the α3 (VI) chain. Production and initial characterization of these collagen type VI recombinant products have been previously described (37, 38). The results indicate that NG2 binds to the recombinant α2 (VI) chain but does not bind to the α1 (VI) chain or the N9–N2 domain of the α3 (VI) chain (Fig. 3A). Decorin also specifically bound the α2 (VI) chain but did not appreciably bind to the other type VI collagen recombinant proteins (Fig. 3B).

Since these results seem to suggest some similarity between the interaction of NG2 and decorin with collagens, we further characterized the interaction of the proteoglycans with collagen type VI. We first examined whether the binding of the proteoglycans to type VI collagen was a saturable process. Increasing concentrations of purified NG2 were added to collagen type VI-coated wells, and the amount of NG2 bound was determined. The results (Fig. 4A) indicate a concentration-dependent, saturable binding of NG2 to type VI collagen, with half-maximal binding attained with 2 μg of NG2 (130 nM). The binding of decorin to type VI collagen was also shown to be a concentration-dependent, saturable process, with half-maximal binding occurring at approximately 0.5 μg (70 nM) of purified decorin (Fig. 4B).

Specificity of NG2 Binding to Type VI Collagen—Since the
collageneffectivelyinhibitthebindingofNG2toconcentration-dependentmanner,withmaximalbindingseenwith1mone another in binding to collagen type VI. NG2 (5
next examined the ability of NG2 and decorin to compete with
whereas control immunoglobulins had little effect.
clonal antibodies was also effective in inhibiting the binding,
slightlyenhancethisinteraction.Amixtureofanti-NG2mono-
represent means
the highest concentration of NG2 or decorin was set at 100%. Values
performedatleastthreetimes.ThecpmboundtotypeVIcollagenusing
experiments.
NG2 is found
tobindtothecollageninaconcentration-dependent,saturablemanner,
with maximal binding occurring with 5 μg and half-maximal binding
with 2 μg (~130 nM). B, decorin also binds type VI collagen in a concentration-
dependentmanner, with maximal binding seen with 1 μg and half-maximal binding with 0.15 μg (~70 nM). Experiments were
performed at least three times. The cpm bound to type VI collagen using
the highest concentration of NG2 or decorin was set at 100%. Values
represent means ± S.D. (bars) obtained from three separate experiments.
main focus of these studies was to further characterize the interaction of NG2 with type VI collagen, we next examined the ability of soluble type VI collagen and anti-NG2 monoclonal antibodies to specifically inhibit the interaction of NG2 with bound type VI collagen. The results (Table I) show that when preincubated with NG2, soluble type VI collagen and a mixture of anti-NG2 monoclonal antibodies both quite effectively inhibit the ability of soluble NG2 to bind to type VI collagen-coated wells. In contrast, BSA, fibronectin, and control immunoglobulins do not appreciably affect binding.
We performed a similar analysis to specifically test the binding of NG2 to the α2 (VI) recombinant fragment. The results (Table I) indicate that the soluble α2 (VI) chain and type VI collagen effectively inhibit the binding of NG2 to α2 (VI)-coated wells, whereas the α1 (VI) chain and the N9–N2 (VI) chain slightly enhance this interaction. A mixture of anti-NG2 monoclonal antibodies was also effective in inhibiting the binding, whereas control immunoglobulins had little effect.
Decorin Effectively Inhibits NG2-Collagen Interactions—We next examined the ability of NG2 and decorin to compete with one another in binding to collagen type VI. NG2 (5 μg) was added to collagen type VI-coated wells in the presence of increasing amounts of decorin, and the subsequent binding of NG2 to the collagen was determined using the NG2 antisera.
The results demonstrate that decorin is an effective inhibitor of NG2 binding to type VI collagen (Fig. 5A). NG2 binding to type VI collagen is inhibited by 50% with the addition of 0.5 μg of decorin and inhibited by 66% with 2.0 μg of added decorin. Conversely, NG2 appears to be a much less potent inhibitor of

![Figure 4](image4.png)

**Fig. 4. Concentration dependence of NG2 and decorin binding to type VI collagen.** Microtiter wells coated with type VI collagen were incubated with varying concentrations of NG2 (A) or decorin (B), and binding assays were performed as described in Fig. 1. A, NG2 is found to bind to the collagen in a concentration-dependent, saturable manner, with maximal binding occurring with 5 μg and half-maximal binding with 2 μg (~130 nM). B, decorin also binds type VI collagen in a concentration-dependent manner, with maximal binding seen with 1 μg and half-maximal binding with 0.15 μg (~70 nM). Experiments were performed at least three times. The cpm bound to type VI collagen using the highest concentration of NG2 or decorin was set at 100%. Values represent means ± S.D. (bars) obtained from three separate experiments.

![Figure 5](image5.png)

**Fig. 5. Competition between decorin and NG2 for binding to type VI collagen.** A, microtiter wells coated with type VI collagen were incubated with a mixture of NG2 (5 μg) and varying amounts of decorin (0–2 μg) for 3 h at room temperature. After washing, wells were incubated with NG2-specific antisera, followed by incubation with 125I-anti-rabbit IgG. The bound radioactivity was then determined. B, microtiter wells coated with type VI collagen were incubated with a mixture of decorin (1 μg) and varying amounts of NG2 (0–5 μg) for 3 h at room temperature. Wells were then incubated with decorin-specific antisera, followed by incubation with 125I-anti-rabbit IgG. The bound radioactivity was then determined. The cpm bound to type VI collagen with the addition of no soluble inhibitor was set at 100%. Data shown are expressed as means ± S.D. (bars) from three separate experiments.

**Table I**

| Soluble competitor | Type VI collagen | α2 (VI) |
|--------------------|-----------------|---------|
| None               | 100             | 100     |
| BSA                | 93.5 ± 3.4      | 95.2 ± 3.2 |
| Fibronectin        | 120.1 ± 4.2     | 89.9 ± 5.1 |
| α1 (VI)            | ND*             | 110.4 ± 3.6 |
| α2 (VI)            | ND              | 18.7 ± 8.1 |
| NG2–N2             | ND              | 115.3 ± 3.4 |
| Collagen VI        | 16.0 ± 8.9      | 33.9 ± 4.3 |
| NG2 monoclonal antibody | 46.8 ± 7.1 | 39.9 ± 7.2 |
| IgG control        | 89.9 ± 5.2      | 84.7 ± 5.1 |

*ND, not determined.*
NG2 Binding to Other ECM Ligands—We next tested the binding of NG2 and decorin to various other ECM molecules. Wells were coated with either laminin or tenasin, and the ability of NG2 and decorin to bind to these ligands was determined. NG2 was found to bind both laminin and tenasin (Fig. 8A). Decorin also bound laminin but did not bind to tenasin (Fig. 8B). The distinction between the ability of NG2 and decorin to bind to tenasin is the first example in the present study in which the two proteoglycans failed to bind to the same substrates. This suggests that the interaction of NG2 with tenasin is mediated by a domain(s) separate from its collagen binding domains. To further characterize the interaction between NG2 and these other ECM ligands, we tested the binding of chondroitinase-treated NG2 to laminin and tenasin. Chondroitinase treatment did not affect the ability of NG2 to bind to either laminin or tenasin, establishing that these interactions were also mediated via domains contained within the protein core of NG2 (Fig. 9). We were interested in determining whether decorin could effectively inhibit NG2 binding to these ligands, as was the case for the NG2-collagen interactions. We therefore examined the ability of NG2 to bind to the ligands in the presence of decorin (1.0 μg). The results (Fig. 9) indicate that decorin does not inhibit the ability of NG2 to bind to either laminin or tenasin, again suggesting that the binding of NG2 to these ECM molecules is mediated by domains separate from its collagen binding region. The results also suggest that NG2 and decorin may bind to different domains of laminin.

NG2 Binds Type VI Collagen via Its Protein Core—Previous studies have determined that the interaction of decorin with collagen is largely mediated by domains contained in its protein core. To establish whether NG2 binds to the different collagen types in a similar fashion, we tested the ability of chondroitinase-treated NG2 samples to bind to collagen type VI as well as the α2 (VI) chain. The results demonstrate that chondroitinase-digested NG2 binds type VI collagen nearly as efficiently as untreated NG2, indicating that the interaction is mediated through the NG2 protein core rather than the chondroitin sulfate chains (Fig. 7A). Similarly, chondroitinase-treated decorin effectively bound to type VI collagen, thus confirming that this binding was also due to protein-protein interaction (Fig. 7B).

DISCUSSION

In the present study we have extended our earlier observations regarding the interaction of NG2 and type VI collagen, establishing that purified NG2 is capable of directly binding type VI collagen in a solid phase binding assay. NG2 was shown to bind to both a pepsin-solubilized type VI collagen preparation and the recombinant α2 (VI) collagen chain. These interactions could be specifically inhibited with a mixture of anti-NG2 monoclonal antibodies, which had previously been shown to inhibit the anchoring of type VI collagen to NG2 on B28 glioma cells in culture (25). The NG2-type VI collagen interaction was also shown to be mediated by domains contained within the NG2 core protein, as chondroitinase treatment of the NG2 proteoglycan did not diminish binding to type VI collagen substrates. Similarly, the decorin core protein was found to bind to the pepsin-solubilized type VI collagen as well as to the α2 (VI) collagen chain. Moreover, it was found that decorin effectively competes with the ability of NG2 to bind to the type VI collagen substrates. Although NG2 and decorin share no sequence homology, the central extracellular domain of NG2 contains a leucine-rich region, which may function in a manner similar to the classic leucine-rich motif found in decorin and other related proteins (3). Previous studies have suggested that the type VI collagen binding domain of NG2 is localized to this central one-third of the NG2 extracellular domain (21). The similarity in binding patterns of NG2 and
Fig. 8. Binding of NG2 and decorin to other ECM molecules. Microtiter wells, coated with the indicated ECM molecule, were incubated with NG2 (A) or decorin (B), and binding assays were performed as described above. The cpm bound to type VI collagen was set at 100%. Data shown are expressed as means ± S.D. (bars) from three separate experiments. CVI, collagen type VI; TN, tenascin; LN, laminin.

Fig. 9. Binding of chondroitinase-treated NG2 to tenascin and laminin. Microtiter wells coated with either tenascin (TN), laminin (LN), or BSA were incubated with either intact NG2 (■), NG2 plus decorin (1 µg) (△), NG2 plus core protein (□). Wells were subsequently incubated with NG2 polyclonal antisera, followed by incubation with 125I-anti-rabbit IgG, and bound radioactivity was determined. The cpm bound to tenascin in the presence of no soluble inhibitor was set at 100%. Data shown are expressed as means ± S.D. (bars) from three separate experiments.

decorin for the collagens and the ability of decorin to effectively inhibit the interaction of NG2 with the collagen substrates suggest that NG2 and decorin could bind to at least some of the same or closely associated domains on collagen substrates. The relative inefficiency of NG2 in blocking the binding of decorin to the collagens suggests that either decorin binds with a much greater affinity to the collagen, that decorin interacts with many sites on the collagen, whereas NG2 interacts with only a limited number of these domains, or that decorin blocks NG2 binding by binding to a noncompetitive site. Although this study demonstrates that the half-maximal binding of decorin to type VI collagen (70 nM) is not appreciably greater than the half-maximal binding of NG2 to type VI collagen (130 nM), the use of 125I-NG2 and 125I-decorin will be needed to directly determine the binding affinities of the proteoglycans for the various collagen species. The functions of type VI collagen remain relatively unknown. Immunohistological studies reveal that type VI collagen is concentrated around basement membranes and cell surfaces, suggesting that type VI collagen may function to anchor tissues and cells to connective tissue ECM (40, 41). Indeed, the fact that type VI collagen is capable of interacting with numerous ECM components, including type I collagen (13) and hyaluronic acid (31, 32), as well as with cell surface receptors, which include NG2 (21, 25) and integrins (37, 38), supports this idea.

Recently, recombinant forms of the α1 (VI) and α2 (VI) chains as well as a large portion of the N-terminal region of the α3 (VI) chain have been purified and characterized (35, 36). The α2 (VI) chain exhibited binding to fibronectin and the heparin-sulfate proteoglycan perlecan, whereas the α1 (VI) recombinant protein failed to bind to any of the substrates tested (35). Surprisingly, the N- and C-terminal globular domains of the recombinant α2 (VI) chain were unable to bind these ligands, suggesting that the triple helical region of the α2 (VI) chain was largely responsible for the observed binding. The present study demonstrates that both NG2 and decorin also bind specifically to the α2 (VI) chain, and preliminary experiments suggest that these two proteoglycans are also interacting with the triple helical portion of the collagen chain. On the other hand, the N-terminal N9–N2 globular domain of the α3 (VI) chain has been shown to bind heparin and hyaluronic acid, therefore suggesting that this globular region of type VI collagen may indeed be important for the association of collagen with other proteoglycans and glycosaminoglycans (36).

The observation that decorin and NG2 may compete with one another for type VI collagen binding raises several possibilities. Decorin, NG2, and type VI collagen display an overlapping pattern of expression in many developing tissues, including developing blood vessels (21, 25, 42, 43), skin (44, 45), cartilage (20, 21, 46), and peripheral nerves (47–49). An increase in decorin synthesis may result in a decreased association of type VI collagen with NG2 and the cell surface. The result may be a decrease in cell-ECM interactions, which may lead to changes in cell shape, proliferation, migration, or differentiation. In support of this idea, decorin has previously been shown to inhibit fibroblast attachment to fibronectin (50) or thrombospondin (51) substrates. The interaction of decorin with these ECM molecules is thought to prevent their association with cellular receptors. The possibility that decorin may interfere with cell attachment to type VI collagen via NG2 awaits experimental confirmation.

Decorin has also been shown to bind transforming growth factor β and is thought to modulate the activity of this growth factor (52, 53). Overexpression of decorin in Chinese hamster ovary cells was found to decrease cell proliferation, in part due to the inability of decorin-sequestered transforming growth factor β to bind to its cell surface receptors. The similar binding pattern of NG2 and decorin to collagens revealed in the present study raises the possibility that the two proteoglycans may also show similar growth factor binding capabilities. Competition between the two proteoglycans may therefore regulate the availability of various growth factors on NG2-expressing cells. The present study also suggests several other possible ligands for NG2. NG2 appears to bind to both type II and type V collagen. The binding of NG2 to these collagens was also shown to be mediated by the NG2 protein core. The fact that decorin displayed the same specificity of binding to these collagens and was also capable of inhibiting NG2 binding to these molecules suggests that decorin and NG2 might bind to some of the same sites on these different collagen types. Further studies need to be done to determine whether an interaction of NG2 with these collagens can be demonstrated either in vivo or in tissue.
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culture.

One particularly intriguing result of these studies is that tenascin also appears to bind to NG2 via its core protein. Decorin was not found to associate with tenascin and was incapable of blocking NG2 binding to tenascin, suggesting that the interaction of NG2 interaction with this molecule is being mediated by domains separate from its collagen binding domain. Tenascin has previously been shown to interact with neurocan, suggesting that NG2 interaction with this molecule is being mediated by domains separate from its collagen binding domain. Tenascin also appears to bind to NG2 via its core protein.

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