Biglycan and Decorin Bind Close to the N-terminal Region of the Collagen VI Triple Helix*

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The binding of native biglycan and decorin to pepsin-extracted collagen VI from human placenta was examined by solid phase assay and by measurement of surface plasmon resonance in the BIAcore™2000 system. Both proteoglycans exhibited a strong affinity for collagen VI with dissociation constants (K_D) of ~30 nM. Removal of the glycosaminoglycan chains by chondroitinase ABC digestion did not significantly affect binding. In coprecipitation experiments, biglycan and decorin bound to collagen VI and equally competed with the other, suggesting that biglycan and decorin bind to the same binding site on collagen VI. This was confirmed by electron microscopy after negative staining of complexes between gold-labeled proteoglycans and collagen VI, demonstrating that both biglycan and decorin bound exclusively to a domain close to the interface between the N terminus of the triple helical region and the following globular domain. In solid phase assay using recombinant collagen VI fragments, it was shown that the α2(VI) chain probably plays a role in the interaction.

Collagens are a large family of extracellular structural proteins made up of three α chains that are intracellularly associated and folded into specific structures including characteristic triple helical domains (1). The major class, recognized as the fibril-forming collagens, contains molecules with one large uninterrupted triple helical domain (for review, see Refs. 1 and 2). Other members of the collagen family have one or more non-triple helical domains, which may constitute the major part of the protein. Most of these collagens do not form prominent lateral aggregates in a manner similar to that of the fibril-forming collagens. Instead, they form complex aggregates together with other matrix macromolecules. Collagen VI is one example, forming multimolecular filamentous beaded structures after secretion from the cell (for review, see Ref. 3). This collagen is composed of three different peptide chains (α1(VI), α2(VI), and α3(VI)), which form the basic unit consisting of a relatively short triple helical domain flanked by two large multidomain globular regions (4). These are composed primarily of repeating units of von Willebrand type A domains (5).

Collagen VI assembles intracellularly into antiparallel, overlapping dimers that then align and form tetramers (6). These structures are stabilized by disulfide bonds. Secreted tetramers assemble extracellularly in a characteristic end-to-end fashion into thin (3–10 nm) beaded filaments with a periodicity of about 100 nm (7–9). Further supramolecular assembly includes lateral associations of the beaded filaments into microfibrils (8, 9).

Collagen VI is ubiquitous. It can be found intermingled with fibril-forming collagens and is often enriched in the pericellular matrix (for review, see Refs. 3 and 10). Decreased amounts of secreted collagen VI resulting from mutations in COL6A1 have been shown in Bethlem myopathy (11, 12), a dominantly inherited disorder characterized by progressive muscle weakness and wasting. This suggests an important role for collagen VI in tissue integrity.

Collagen VI has been shown to interact with several different matrix constituents. It may have a role in the development of the matrix supramolecular structure as well as in tissue homeostasis by mediating interactions of cells with the extracellular matrix. More specifically, interactions of collagen VI with collagen XIV, collagen IV, the fibrillar collagens type I and II, decorin, microfibril-associated glycoprotein MAGP-1, and hyaluronan as well as the α1β1 and α2β1 integrins and the cell surface proteoglycan NG2 have been demonstrated (13–20). Collagen VI interacts via its triple helical domain with perlecan and fibronectin (21). Furthermore, a recombinant α3(VI) N-terminal fragment containing domains N9–N2 (5) interacts with both heparin and hyaluronan (22).

A family of extracellular matrix proteins with characteristic leucine-rich repeats has several members that show tight binding to collagens. They are present in collagen networks and modulate their functional properties. One extensively studied example is the small proteoglycan decorin. Biglycan and decorin represent two distinct but closely related members of a subgroup within the family of leucine-rich repeat proteins in the extracellular matrix. They contain 10 leucine-rich repeats of each some 25 amino acids. Decorin and biglycan are proteoglycans with one and two chondroitin/dermatan sulfate chains, respectively (for review, see Refs. 23 and 24).

Decorin interacts with fibrillar collagens (25, 26) and intervenes in collagen fibrillogenesis in vitro (27, 28). Decorin has been shown also to interact with collagen VI (16) through the α2(VI) chain (20) and to colocalize with collagen VI in the cornea (29). Inactivation of the decorin gene leads to alterations of the collagen fibril network, primarily in skin (30).

Decorin is found primarily at a distance from cells, but biglycan is distributed mainly close to the cells and even pericel-
lularly (31, 32). Mice deficient in biglycan show major alterations in bone (33). Biglycan can be extracted from, e.g., cartilage and purified under denaturing conditions. Such preparations have been used in studies of functional properties indicating that biglycan can inhibit binding of decorin to collagen VI (16).

In the present study, native biglycan and decorin were shown to interact tightly via their core protein with the same binding site close to the N-terminal region of the collagen VI helical domain, possibly via the α2(VI). Native decorin was shown to bind to collagen VI with higher (10×) affinity than has been shown previously for the molecule isolated under denaturing conditions (16).

**EXPERIMENTAL PROCEDURES**

**Purification of Collagens**—Pepsinized collagen VI was prepared from human placenta (34). Briefly, the tissue was homogenized in formic acid and incubated with pepsin for 24 h at room temperature. After a series of salt precipitations, the collagen VI was purified by gel filtration, dialyzed into dilute acetic acid, and freeze dried (34).

Recombinant α1(VI) and α2(VI) chains and the N-terminal fragment N9–N2 of the α3(VI) chain were purified as described previously (21, 22). Medium containing recombinant α3(VI) chain was produced by excising the Nci-I BamHI fragment from the pCI-neo α3(VI) N9–C5 cDNA construct (12) and cloning it into pCEP4-BM40-hisEK (35). The expression construct was transfected into 293-EBNA cells using FuGENE™6 (Roche Molecular Biochemicals, Mannheim, Germany), and transfected cells were selected in growth medium containing 250 μg/ml hygromycin. The recombinant α3(VI) chain containing an N-terminal His tag, the first six N-terminal domains (N6–N1), the tripeptide helical domain, and the complete C-terminal domain (C1–C5) was isolated from serum-free conditioned medium under native conditions by nickel affinity chromatography on a chelating Sepharose Fast Flow column (Amersham Pharmacia Biotech) and purified further by gel filtration chromatography on a Superose 6 HR 10/30 column (Amersham Pharmacia Biotech).

Collagen I was prepared by acid extraction from the fibrous proximal part of bovine flexor tendon (28, 36).

**Purification of Recombinant Proteoglycans**—Recombinant human biglycan (37) and bovine decorin (38) were produced in human HeLa cells and Chinese hamster ovary cells, respectively (26). Cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. Medium was changed every 2nd day, and the collected medium was stored frozen at −20 °C until purification. For purification of proteoglycans, thawed medium (900–1,400 ml) was applied onto a 20-ml Q-Sepharose (Amersham Pharmacia Biotech) column, equilibrated in 10 mM Tris/HCl, 100 mM NaCl, pH 7.4. The column was washed with 4 volumes of equilibration buffer, and bound material was eluted with a gradient of 0.1–1 M NaCl in equilibration buffer. Fractions containing decorin/biglycan were identified by SDS-polyacrylamide gel electrophoresis, pooled, and dialyzed with 5 mM phosphate buffer, pH 7.4, containing 150 mM NaCl. They were subsequently applied onto a 1.7-ml column of DEAE-Sepharose (Amersham Pharmacia Biotech). To block nonspecific binding of glycosaminoglycans to the DEAE column two different solutions, containing 6-sulfated chondroitin sulfate (see below) and bovine serum albumin (Serva, Heidelberg, Germany) at 1 mg/ml, had been chromatographed under similar conditions prior to the sample. Dermatan sulfate was eluted with a linear gradient of 0–1 M NaCl in 20 mM Tris/HCl, pH 7.4. Fractions containing glycosaminoglycans were identified by the dimethylmethylene blue assay (41), pooled, dialyzed against water, and lyophilized.

Biglycan isolated from nucleus pulposus as described previously (42). Hyaluronic acid (Healon) was obtained from Amersham Pharmacia Biotech.

**Coprojection of Collagen and Proteoglycan**—Native radiolabeled proteoglycans were tested for binding to precipitated collagens. Samples were combined with collagen VI at 20 μg/ml and bovine serum albumin at 50 μg/ml in 10 mM Tris/HCl, 150 mM NaCl, pH 7.4 (TBS), and subsequently incubated for 18 h at 20 °C. Alternatively, samples were combined with acid-extracted collagen I at 100 μg/ml and bovine serum albumin at 500 μg/ml in 30 mM sodium phosphate, 140 mM NaCl, pH 7.4 (25), followed by incubation for 18 h at 37 °C. Precipitated material was collected by centrifugation at 10,000 × g for 10 min at the respective temperatures. The pellets and the supernatants were electrophoresed in 4–12% SDS-PAGE and detected by fluorography with sodium salicylate (44). For quantification of the radiolabeled proteoglycans, fluorographs were scanned using a digital scanner and evaluated using the Gel-Pro Analyzer™ software (Media Cybernetics, Silver Spring, MD). For inhibition experiments, native radiolabeled proteoglycans were combined with collagen VI as described above but in the presence of 4 μg of nonlabeled decorin or 5 μg of biglycan, respectively. The quotient of decorin and biglycan found in the precipitate was calculated after quantification as described above.

**Chondroitinase ABC Digestion**—To remove glycosaminoglycan chains from recombinant proteoglycans, digestion with chondroitinase ABC (Seikagaku Corporation, Tokyo, Japan) (1.0 milliunit/mg of proteoglycan) at a final proteoglycan concentration of 16–48 μg/ml in TBS was performed. The digestion of the sample was monitored at 232 nm, showing formation of unsaturated disaccharides. Samples were then further analyzed immediately after complete digestion, typically after 0.5–2 h at 37 °C to minimize the risk of core protein self-aggregation after removal of glycosaminoglycan chains. For chondroitinase ABC digestion, 0.5–5 μg/ml collagen VI in TBS was adsorbed overnight onto microtiter plates (Maxisorb plates, Nunc, Roskilde, Denmark). This and all of the following steps were done at room temperature.

To avoid nonspecific interactions, wells were blocked for 1 h with 0.03 mg/ml α-casein (Sigma) in TBS. Coated wells were incubated overnight with recombinant biglycan at different concentrations (0–1.2 μg/ml) in TBS containing 0.05% s-casein and 0.05% Tween. In control experiments, only α-casein was coated onto the plate, or the incubation step with biglycan was omitted. The amount of bound biglycan was determined by incubation with affinity-purified, polyclonal anti-biglycan antibodies. Bound IgG was detected with alkaline-phosphatase-conjugated anti-rabbit IgG antibody. Enzyme activity was measured with p-nitrophenyl phosphate as the substrate at 405 nm. To check the specificity of the interaction, biglycan was preincubated in inhibition experiments with collagen VI at different concentrations before being added to wells coated with the collagen.

**Radiolabelling of Small Proteoglycans**—Proteoglycans were prepared from confluent bovine fibroblast monolayer cultures (25), which were labeled for 20 h with either 20 μCi/ml [3H]leucine or 50 μCi/ml [35S]sulfate. The medium was passed through a 2-ml column of DE32 cellulose (Whatman), eluted with 10 mM Tris/HCl, 100 mM NaCl, pH 7.4. Bound proteoglycans were eluted in 100 mM Tris/HCl, 1.5 mM NaCl, pH 7.4, followed by desalting on a 10 ml column of Sephade G-50 (25) (Amersham Pharmacia Biotech).

Recombinant proteoglycans were purified as described above and radiolabeled using IODO-BEADS (Pierce) according to the recommendations of the manufacturer. Briefly, 10 μg of protein was added to 0.5 ml Na2HPO4, and in a trial containing 10 ml of 10 mM sodium phosphate buffer, pH 7.4, and 1 ml IODO-BEAD. The reaction was terminated after 15 min. Free isotope was removed by desalting on a PD-10 column (Amersham Pharmacia Biotech).

**Isolation of Glycosaminoglycans**—Decorin from bovine articular cartilage was purified as described previously (39, 40) (a kind gift from Dr. Mark Hickery). The decorin was digested with 0.2 unit of crystalline papain (papaya latex, Sigma) of proteoglycan in 0.1 M sodium phosphate, 0.1 mM NaCl, 5 mM cysteine/HCl, 10 mM EDTA, pH 7.4, at 60 °C for 16 h. The digest was then dialyzed against 20 mM Tris/HCl, pH 7.4, and applied onto a 1.7-ml column of DEAE-Sepharose (Amersham Pharmacia Biotech). To block nonspecific binding of glycosaminoglycans to the DEAE column two different solutions, containing 6-sulfated chondroitin sulfate (see below) and bovine serum albumin (Serva, Heidelberg, Germany) at 1 mg/ml, had been chromatographed under similar conditions prior to the sample. Dermatan sulfate was eluted with a linear gradient of 0–1 M NaCl in 20 mM Tris/HCl, pH 7.4. Fractions containing glycosaminoglycans were identified by the dimethylmethylene blue assay (41), pooled, dialyzed against water, and lyophilized.

**Fractionation of biglycan 6-sulfate was isolated from nucleus pulposus as described previously (42). Hyaluronic acid (Healon) was obtained from Amersham Pharmacia Biotech.**

1 The abbreviations used are: TBS, Tris-buffered saline; EDAC, 1-ethyl-3(3-dimethylaminopropyl)carbodiimide.
bination of radiolabeled proteoglycans to aggregates of collagen VI. Medium was collected from fibroblast monolayer cultures labeled with [35S]sulfate. After purification of labeled proteoglycans, mixtures of radiolabeled samples and collagen were incubated for 18 h under conditions that were chosen to promote precipitation of the collagen. The precipitates were recovered by centrifugation. Pellets and supernatants were analyzed by fluorography after SDS-polyacrylamide gel electrophoresis. In α, the proteoglycans (approximately 50,000 cpm) were incubated with 10 μg of collagen VI in 500 μl of TBS at 20 °C. The lanes show the supernatants (S) and the precipitates (P) recovered in the absence of nonlabeled proteoglycans (panel I), in the presence of 4 μg of nonlabeled decorin (panel II), and in the presence of 5 μg of nonlabeled biglycan (panel III). b, radiolabeled proteoglycans (approximately 10,000 cpm) were incubated with 20 μg of collagen I in 200 μl of phosphate/saline buffer at 37 °C. The lanes show total sample (T), supernatant (S), and precipitate (P). c, the proportion (percent) of total radiolabeled proteoglycan found in precipitate after incubation with collagen VI in the absence (I) or presence of nonlabeled decorin (II) and biglycan (III) is shown. The biglycan and decorin labels in a and b indicate the positions of reference proteoglycans.

FIG. 1. Binding of metabolically labeled proteoglycans to aggregating collagens. Medium was collected from fibroblast monolayer cultures labeled with [35S]sulfate. After purification of labeled proteoglycans, mixtures of radiolabeled samples and collagen were incubated for 18 h under conditions that were chosen to promote precipitation of the collagen. The precipitates were recovered by centrifugation. Pellets and supernatants were analyzed by fluorography after SDS-polyacrylamide gel electrophoresis. In α, the proteoglycans (approximately 50,000 cpm) were incubated with 10 μg of collagen VI in 500 μl of TBS at 20 °C. The lanes show the supernatants (S) and the precipitates (P) recovered in the absence of nonlabeled proteoglycans (panel I), in the presence of 4 μg of nonlabeled decorin (panel II), and in the presence of 5 μg of nonlabeled biglycan (panel III). In b, radiolabeled proteoglycans (approximately 10,000 cpm) were incubated with 20 μg of collagen I in 200 μl of phosphate/saline buffer at 37 °C. The lanes show total sample (T), supernatant (S), and precipitate (P). c, the proportion (percent) of total radiolabeled proteoglycan found in precipitate after incubation with collagen VI in the absence (I) or presence of nonlabeled decorin (II) and biglycan (III) is shown. The biglycan and decorin labels in a and b indicate the positions of reference proteoglycans.

RESULTS
Binding of Metabolically Labeled Proteoglycans to Aggregating Collagens—Purified, native [35S]sulfate-labeled proteoglycans were tested for binding to precipitating collagen VI. An assay for pepsin-extracted collagen VI was developed in which 50–70% of collagen VI was precipitated. Under these experimental conditions, 16% of the total biglycan was recovered bound to collagen VI, whereas the proportion of decorin bound was 18% (Fig. 1, a and c). The binding of radiolabeled proteoglycans was competed for when nonlabeled, purified proteoglycans were added (Fig. 1a). The addition of either decorin (panel II) or biglycan (panel III) resulted in a markedly decreased binding of radiolabeled proteoglycans of both types (Fig. 1, a and c). In a corresponding assay with collagen I, more than 95% of the decorin was coprecipitated with the collagen fibrils. Biglycan was almost totally recovered in the supernatant, and less than 5% was found precipitated with the collagen (Fig. 1b).

The capacity of biglycan, decorin, and glycosaminoglycans to inhibit binding of proteoglycans in the precipitation assay was examined further. Almost complete inhibition of decorin binding was achieved when the molar concentration of nonlabeled proteoglycan exceeded that of collagen VI (Fig. 2), indicating that the proteoglycan binding site(s) on collagen VI are saturable and limited in number. Isolated biglycan was equal or even slightly more efficient in inhibiting decorin binding to collagen VI than decorin (Fig. 2). The amounts of collagen VI in the precipitates were not different in the presence or absence of proteoglycans or glycosaminoglycans, as revealed by Coomassie staining of the gels prior to fluorography (not shown). In conclusion, the inhibition experiments show that the native biglycan and decorin interact close to or at the same defined site(s) on collagen VI.

The isolated biglycan core protein, prepared by treatment of the intact proteoglycan with chondroitinase ABC, competed for collagen VI binding to the same extent as the intact proteoglycan (Fig. 2). Neither free chondroitin sulfate nor hyaluronic acid inhibited the binding of radiolabeled decorin (Fig. 2).

Interaction of Intact Biglycan with Collagen VI (Microtiter Plate Assay)—Incubation of pepsin-extracted collagen VI-coated wells with biglycan at different concentrations showed
Interactions Studied by Surface Plasmon Resonance—Im mobilization of collagen VI resulted in surface concentrations of 3 ng/mm² (3,000 relative units) for sensorchip B1 and about 6 ng/mm² (6,000 relative units) for sensorchip CM-5, reflecting the lower degree of carboxylation of the B1 chip. The kinetic data presented in Table I refer to evaluation with a 1:1 binding model of interaction studies on sensorchip B1 (intact proteoglycans) and CM-5 (core proteins). With sensorchip CM-5, reliable data for intact proteoglycans could not be obtained, probably because of repulsion effects from remaining, not activated, dermatan sulfate chains. By using sensorchip B1, having a lower degree of carboxylation, and by increasing the time of activation and deactivation of this surface, we could minimize the effects of repulsion of molecules with high negative charge density which caused problems in initial experiments with intact proteoglycans. The interaction kinetics of collagen VI with proteoglycan core proteins were consistent on both chips, although the kinetics of core protein binding to collagen VI showed a higher variability when sensorchip B1 was used. Kinetic evaluation of intact biglycan and decorin interacting with collagen VI (Fig. 5, a and b) according to a 1:1 binding model gave a dissociation constants (Kd) close to 30 nM (Table I). The kinetics of the proteoglycan core protein binding (Fig. 5, c and d) did not differ significantly from those of the intact proteoglycans (Table I).

Injections of chondroitinase ABC gave no signal, verifying a lack of binding to immobilized collagen VI.

Electron Microscopy—Biglycan and decorin were labeled with 5 nm colloidal gold. The proteoglycans were subsequently used to characterize the interaction with collagen VI. The molecules and their complexes were visualized by negative staining and electron microscopy.

Both labeled biglycan and decorin were found to bind exclusively at the small N-terminal globular domain remaining on pepsin-extracted collagen VI (Fig. 6 a, arrowheads). These domains appear as small globules (asterisks) located at the end of collagen VI dimers and tetramers. Biglycan and decorin treated with chondroitinase ABC and subsequently tagged by gold labeling showed binding at the N-terminal part of collagen.

The interaction kinetics of biglycan with collagen VI showed binding to α2(VI), but it was significantly lower compared with collagen VI. In solution, as visualized by electron microscopy after negative staining (inset), biglycan (arrow) could be seen to interact moderately only with α2(VI), identified with gold labeling (arrowhead).

Table I

| Analyte | Kd (nM) | k_d (× 10^-3) | k_a (× 10^-3) |
|---------|---------|---------------|---------------|
| Biglycan |         |               |               |
| Intact   | 32 (±19) | 2.9 (±1.0)    | 127 (±104)    |
| Core     | 18 (±4)  | 1.5 (±0.5)    | 85 (±18)      |
| Decorin  |         |               |               |
| Intact   | 27 (±11) | 4.0 (±1.3)    | 156 (±36)     |
| Core     | 14 (±4)  | 1.5 (±0.6)    | 104 (±18)     |

Fig. 3. Binding of intact, recombinant biglycan to collagen VI in solid phase assay. Pepsin-extracted collagen VI (○) and casein (□) were adsorbed onto microtiter plates. Biglycan at different concentrations was allowed to interact, and bound biglycan was quantified using antibodies as described under “Experimental Procedures.” The absorbance reflecting the amount of bound biglycan is plotted against the concentration added.

Fig. 4. Binding of radiolabeled, recombinant biglycan to collagen VI and recombinant collagen VI fragments in solid phase assay and in solution. Pepsin-extracted collagen VI, α1(VI), α2(VI), N9–N2 and N6–C5 fragment of α3(VI), or casein (control) was adsorbed onto breakable microtiter strips followed by incubation with radiolabeled recombinant biglycan. Biglycan showed binding to α2(VI), but it was significantly lower compared with collagen VI. In solution, as visualized by electron microscopy after negative staining (inset), biglycan (arrow) could be seen to interact moderately only with α2(VI), identified with gold labeling (arrowhead).

Fig. 5. Interaction of biglycan and decorin with collagen VI in the BIAcore™2000 system. Intact recombinant biglycan (α), intact recombinant decorin (β), chondroitinase ABC-treated, recombinant biglycan (γ), and chondroitinase ABC-treated recombinant decorin (δ) at different concentrations (20–200 nM) in TBS containing 0.05% surfactant P20 were injected over immobilized pepsin-extracted collagen VI at flow rates of 40–50 μl/min. The arrows indicate the beginning and the end of injections.
Interaction of Biglycan and Decorin with Collagen VI

Recombinant biglycan and decorin showed moderate binding to both nonlabeled and gold-labeled α2(VI) (Fig. 4, inset). The recombinant fragments of collagen VI appeared as globular rather than linear structures when analyzed with electron microscopy after negative staining.

**DISCUSSION**

Interactions of the leucine-rich repeat proteoglycans biglycan and decorin with collagen VI were characterized. It was considered important that preparations of extracellular matrix macromolecules, e.g. decorin, classically obtained via a number of denaturing steps, sometimes show a weaker binding than recombinant, native protein expressed in eukaryotic cells, as shown in the case of decorin binding to collagen I (26). Because denatured proteins may have an altered binding to their ligands, we used the native forms of biglycan and decorin, purified under mild conditions, for our binding studies. It was shown that decorin binds 10-fold stronger to collagen VI than in a previous study (16), where decorin that had been exposed to denaturing conditions was used. In that study, collagen VI from the same source as ours was used in solid phase assay. Even if we had used a different method, the BIAcore system, it is unlikely that this accounts for the differences in the reports because we do not see any major differences between the values of $K_D$ measured in the enzyme-linked immunosorbent assay system compared with values measured with the BIAcore system. Instead, the weaker binding of decorin to collagen VI reported by Bidanset et al. (16) is likely to depend on exposure of decorin to denaturing agents. This is consistent with data on decorin binding to collagen I (26) and suggests that these interactions depend on an optimal secondary structure of decorin.

The core protein plays the major role in the interaction between these two small proteoglycans and collagen VI. Treatment of biglycan and decorin with chondroitinase ABC did not alter the binding kinetics to collagen VI, showing that the glycosaminoglycan side chains do not have a significant role in the interaction. This was supported by experiments demonstrating that the isolated chains were not able to inhibit the interaction in vitro (data not shown). This is also consistent with the lack of apparent effects of the glycosaminoglycan chain observed in studies of decorin binding to collagen VI (16). In that study, direct binding of biglycan core protein was not explored. Some uncertainties exist in the literature as to whether glycosaminoglycan substitutions are necessary for the interaction. In a recent study (48) with radiolabeled biglycan, no interaction could be shown in the solid phase assay for biglycan core protein. In our experimental setup the biglycan core protein interacts with collagen VI. The difference from previous data can be the result of blocking of binding sites when collagen VI is adsorbed to a plastic surface. Indeed, in the enzyme-linked immunosorbent assay we also observed an abolished interaction after chondroitinase ABC digestion. Further, in the same system with radiolabeled proteoglycans we observed a significantly diminished binding. However, presence of glycosaminoglycan chains appears not to modulate binding strength of the intact biglycan to collagen VI. Whether the glycosaminoglycan chains in vivo contribute by interacting with matrix constituents other than the collagen to which the core protein is bound remains to be answered.

Our studies of the biglycan-collagen VI interaction show a binding of equal strength ($K_D$ 32 nM) to that of decorin
Biglycan and decorin apparently share the same binding structure on collagen VI as is indicated by the coprecipitation experiments, showing that either proteoglycan inhibited the binding of the other.

In previous studies decorin has been shown to bind to the recombinant α2 chain of collagen VI and to collagen I within the C-terminal CNBr peptide CB6 of α1(I) (49), whereas the binding site in collagen XIV is located in the fibronectin type III-repeat in the non-collagenous N-terminal domain (50). Here, both biglycan and decorin bound to the N-terminal part that remained on the collagen VI preparation after pepsin digestion as revealed by electron microscopy, i.e. either to the N-terminal part of the triple helical domain or to the first von Willebrand type A-like domains of the N-terminal globular domain (4). We can also show a weak binding to the recombinant α2(VI) chain but not to the N6–C5 fragment of α3(VI) containing the fibronectin type III-repeat like domain C4 (5). Given the weak binding to the α2(VI) chain in solid phase, the interaction might require a binding site created by all three chains in combination. A less than appropriate folding in the absence of the triple helix may also affect the binding.

In developing murine and adult human cartilage, collagen VI is localized mainly in the pericellular compartment (51, 52). The same localization is found for biglycan, whereas decorin primarily colocalizes with collagen fibrils in the interterritorial matrix (31, 53). Thus, in cartilage, based on its localization with collagen VI to the same compartment, biglycan appears as a more likely ligand for the collagen VI than decorin.

In skin and tendon, collagen VI seems to form mesh-like structures adjacent to or in contact with fibrillar collagen (54, 55), indicating a closer interrelation of the fibrillar collagen and microfibrillar collagen VI networks. However, studies of the decorin-deficient mouse showed no apparent disruption of the collagen VI network (54) but suggested that another member of the family of small proteoglycans was present. Based on our studies, it is possible that this proteoglycan is biglycan. Even if biglycan is not up-regulated at the transcriptional level in the skin in collagen-deficient mice (30), it should be kept in mind that the protein level may be altered by a slower catabolism. By analogy, lumican protein is increased in the fibrovascular tissue and microfibrillar collagen VI networks. However, studies of the decorin-deficient mouse showed no apparent disruption of the collagen VI network (54) but suggested that another member of the family of small proteoglycans was present. Based on our studies, it is possible that this proteoglycan is biglycan. Even if biglycan is not up-regulated at the transcriptional level in the skin in collagen-deficient mice (30), it should be kept in mind that the protein level may be altered by a slower catabolism. By analogy, lumican protein is increased in the fibrovascular tissue and microfibrillar collagen VI networks. However, studies of the decorin-deficient mouse showed no apparent disruption of the collagen VI network (54) but suggested that another member of the family of small proteoglycans was present. Based on our studies, it is possible that this proteoglycan is biglycan. Even if biglycan is not up-regulated at the transcriptional level in the skin in collagen-deficient mice (30), it should be kept in mind that the protein level may be altered by a slower catabolism. By analogy, lumican protein is increased in the fibrovascular tissue and microfibrillar collagen VI networks. However, studies of the decorin-deficient mouse showed no apparent disruption of the collagen VI network (54) but suggested that another member of the family of small proteoglycans was present. Based on our studies, it is possible that this proteoglycan is biglycan. Even if biglycan is not up-regulated at the transcriptional level in the skin in collagen-deficient mice (30), it should be kept in mind that the protein level may be altered by a slower catabolism. By analogy, lumican protein is increased in the fibrovascular tissue and microfibrillar collagen VI networks. However, studies of the decorin-deficient mouse showed no apparent disruption of the collagen VI network (54) but suggested that another member of the family of small proteoglycans was present. Based on our studies, it is possible that this proteoglycan is biglycan.
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