Binding of Fibromodulin and Decorin to Separate Sites on Fibrillar Collagens*

(Received for publication, June 1, 1993, and in revised form, August 10, 1993)

Erik Hedbom and Dick Heinegård
From the Department of Physiological Chemistry, University of Lund, P. O. Box 94, S-221 00 Lund, Sweden

The small proteoglycans, decorin, fibromodulin, biglycan, and lumican, represent a family of structurally related but genetically distinct molecules present in many types of connective tissues. Fibromodulin and decorin interact with collagen I and II (Hedbom, E., and Heinegård, D. (1989) J. Biol. Chem. 264, 6898–6905). These interactions have been characterized further by using native radiolabeled components from fibroblast cultures and nonlabeled proteoglycans purified from guanidine hydrochloride extracts of bovine tendon. Binding of metabolically labeled macromolecules to collagen I was measured in an assay based on precipitation of collagen fibrils formed in vitro. Among a large number of secreted fibroblast products, decorin and fibromodulin represented the vast majority of the collagen binding components. These molecules showed poor binding to denatured collagen, in contrast to fibronectin, which was also present in the medium. Decorin and fibromodulin bind to different sites on collagen I fibrils, since the binding of either radiolabeled component could be competed for only by the corresponding nonlabeled proteoglycan. Similarly, these proteoglycans showed binding to separate sites on collagen II. Binding of isolated fibromodulin and decorin to collagens in solution was measured in a solid-phase inhibition assay. Each of the proteoglycans interacted with triple helical molecules, but not with denatured collagen chain constituents or fragments. For fibromodulin, the data indicated an average of one binding site per collagen I molecule (Kd = 9.9 nM). The data on decorin indicated additional interactions, some apparently mediated by the dermatan sulfate side chain. The results suggest that the small proteoglycans bind to distinct triple helical sites, apparently differing from several other similar structures within each collagen molecule.

The collagens are proteins that contain three polypeptides forming at least one extended domain with a characteristic triple-stranded helix. As yet, some 15–16 different collagen types have been identified (van der Rest and Garrone, 1991; Pan et al., 1992). These can be classified on the basis of molecular shape and properties (Miller and Gay, 1987; Kiely et al., 1990). The major class, i.e. the fibrillar collagens, comprises molecules having a single helical domain that makes up more than 95% of the molecule. The rodlike shape and regular structure of these molecules offer exceptional capacity for lateral association and aggregation into fibers. The fibrillar collagens are type I, which is present in most tissues, type II in cartilage, type III in distensible connective tissues, and types V and XI, which appear associated with types I and II, respectively. Another distinct class of collagens, including types IX, XII, and XIV, contains multiple short helical domains interrupted by nonhelical domains. These do not form fibrils by themselves, but they are associated with the major collagen fibrils. Collagen IX, for example, can be located to the surface of collagen II fibers in cartilage, periodically arranged, with globular domains protruding at regular intervals (Vaughan et al., 1988).

The proteoglycans are proteins that have a variable number of sulfated carbohydrate chains, glycosaminoglycans, covalently attached (reviewed in Kjellan and Lindahl (1991)). These molecules, together with other glycoproteins and the glycosaminoglycan hyaluronan, form the gel in which the collagen fibrils are embedded. There are two major classes of proteoglycans present interstitially in mesenchymal connective tissues. One is represented by large molecules (>10^6 Da) having the capacity to form aggregates with hyaluronan (Heinegård et al., 1985; Mörgelin et al., 1988) (for review see Wight et al. (1991)). The other major class of interstitial proteoglycans has a low molecular mass (<10^5 Da). They are present in many tissues and predominate in fibrous connective tissues. Three members of this group have been studied more extensively, i.e. decorin (PG-S2, PGII, PG40), biglycan (PG-S1, PG1), and fibromodulin (reviewed in Heinegård and Oldberg (1989, 1993)). A fourth member is a keratan sulfate proteoglycan (Axelsson and Heinegård, 1978) that is referred to as lumican (Blochberger et al., 1992). These molecules display core proteins of M, of about 40,000 that are structurally related but genetically distinct. They contain some 10 homologous repeats of about 25 amino acids in their central domains and cysteine residues located at conserved positions. Decorin contains a single CS/DS chain, attached to the N-terminal part of the protein. Biglycan carries two CS/DS chains similarly located. Fibromodulin contains keratan sulfate (Oldberg et al., 1989) with usually one or two such chains distributed.

Connective tissues contain collagen and proteoglycans as predominant components. Each of these is now recognized to represent large families of distinct extracellular matrix molecules. Appropriate assembly of these and other components into a well organized matrix is of crucial importance to the tissue function. Most likely, specific interactions between the individual macromolecules determine the organization at the supramolecular level.

*This study was supported by grants from the Swedish Medical Research Council, Axel och Margaret Ax:son Johnsons Stiftelse, Folksams Stiftelse, Kock’s Stiftelse, Konung Gustaf V:s 80-årsfond, Österlunds Stiftelse, and the Medical Faculty, University of Lund.

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.

1 The abbreviations used are: CS, chondroitin sulfate; DS, dermatan sulfate; PBS, phosphate-buffered saline; BSA, bovine serum albumin; TES, 2-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)amino)ethanesulfonic acid.

27307
among four sites in the central domain (Plaa et al., 1990). There are several sulfated tyrosine residues in the N-terminal part of fibromodulin (Antonsson et al., 1991).

Proteoglycans and fibrillar collagens appear to interact, which is of potential importance in extracellular matrix assembly (for references see Scott (1988), Hedbom and Heinegärd (1989), and Bidanset et al. (1992)). Collagen IX, which occurs associated with fibers of collagen II, is actually also a proteoglycan containing a glycosaminoglycan side chain (Bruckner et al., 1985). Ultrastructural studies on several tissues suggest that the small CS/DS proteoglycans can be regularly associated with collagen fibers (Scott and Orford, 1981; Pringle and Dodd, 1990). Both decorin (Vogel et al., 1984) and fibromodulin (Hedbom and Heinegärd, 1989) inhibit collagen fibril growth in vitro. These two proteoglycans show similar affinity for collagen I, with apparent dissociation constants of approximately 10^{-8} M (Hedbom and Heinegärd, 1989). Biglycan, although very similar in structure to decorin and fibromodulin, does not appear to bind to fibril-forming collagen (Brown and Vogel, 1989).

The present study was undertaken to characterize the collagen structures recognized as binding sites by decorin and fibromodulin. The primary aim was to reveal whether these sites are different for the individual proteoglycans.

**EXPERIMENTAL PROCEDURES**

**Preparation of Collagen**—Collagen I was prepared by pepsin digestion or by acid extraction from the fibrous proximal part of bovine flexor tendon (Vogel et al., 1984; Hedbom and Heinegärd, 1989). To avoid proteolysis, acid extraction was done with 0.5 M acetic acid containing the proteinase inhibitors phenylmethylsulfonyl fluoride (0.01 M) and pepstatin (5 μM), N-ethylmaleimide (2 mM), and EDTA (5 mM). Collagen II was prepared from pepsin extracts of bovine nasal cartilage using the method of Miller (1972) with previously described modifications (Vogel et al., 1984). The preparations were stored freeze-dried.

Samples were dissolved at 4 or 5 mg/ml in 0.5 M acetic acid, then dialyzed into 0.1 M acetic acid and kept at +4 °C until used in the binding experiments. The collagen II preparation was further processed to eliminate molecules having the capacity to form fibrils. The acidic solution was diluted to 1 mg/ml in PBS (0.14 M NaCl, 30 mM sodium phosphate, pH 7.3) and incubated at 37 °C for 24 h. Precipitated proteins were removed by centrifugation at 4000 × g for 30 min. This material was again dissolved in 0.5 M acetic acid at +4 °C and dialyzed into 0.1 M acetic acid. Finally, the solution was centrifuged at 10,000 × g for 30 min to remove a minor insoluble component. The collagen concentrations were determined by quantitation of hydroxyproline in samples after hydrolysis in 6 M HCl for 24 h at 100 °C (Steigeman and Stalder, 1967).

Denaatured collagen chain constituents were obtained by heating a sample, diluted into 10 mM acetic acid, at 60 °C for 10 min. Peptides derived from the collagen chains were prepared by cleavage at methionyl residues with 2% (w/v) CNBr in 70% (v/v) formic acid for 20 h at 20 °C (Brownstein and Piek, 1969).

**Isolation of Proteoglycans**—Decorin and fibromodulin were isolated from a 4 mM guanidine hydrochloride extract of adult bovine tendon and purified essentially as described elsewhere (Vogel and Heinegärd, 1986; Heinegärd et al., 1986). The proteoglycans were pooled and stored in 4 M guanidine HCl, 0.05 M sodium acetate, pH 5.8, frozen as aliquots. Before use in the collagen binding experiments, proteoglycans in thawed samples were precipitated by adding 10 volumes of ethanol, pelleted by centrifugation, and briefly vacuum-dried. These samples were dissolved in the buffer used for the binding assay and added to collagen within 10 min.

**Preparation of Radiolabeled Components**— Fibroblasts from the deep flexor tendon of a 2-year-old calf were allowed to colonize 145 cm² plastic dishes (Nunclon, A/S Nunc). The cells were cultured in Ham's F-12 supplemented with 10% fetal calf serum and utilized at passages 5-15. Confluent cultures were radiolabeled for 20 h in serum-free medium containing 20 μCi/ml [3H]leucine. To the harvested culture medium was added phenylmethylsulfonyl fluoride (0.5 mM), N-ethylmaleimide (2 mM), EDTA (2.5 mM), and BSA (50 μg/ml). This medium, containing a mixture of secreted fibroblast products, was prepared for binding studies by desalting on a 10-ml column of Sephadex G-50 (Pharmacia LKB Biotechnology Inc.) eluted with PBS diluted with 3 volumes of water. Samples containing the macromolecules were pooled, freeze-dried, and then dissolved in the appropriate volume of matrix buffer.

Proteoglycans from radiolabeled culture medium were partially purified by ion-exchange chromatography. The medium, obtained as described above, was dialyzed with an equal volume of water and chromatographed on a column (1.0 × 6.4 cm) of DEAE-Sepharose (Pharmacia). The column was eluted with 0.1 M LiCl, 25 mM Tris/ HCl, pH 7.3, followed by a linear gradient of 0.1-1.1 M LiCl in 100 ml of the Tria buffer. Fractions of 1 ml were collected and analyzed by β-scintillation counting and SDS-polyacrylamide gel electrophoresis (see below). Fractions containing fibromodulin and decorin were pooled separately, dialyzed against water, and freeze-dried.

**Samples of Fibroblasts**—Samples containing radiolabeled molecules, and sometimes nonlabeled proteoglycans, were combined with double-strength PBS, water, BSA, and finally 20 μg of acid-extracted collagen I or 100 μg of pepsin-extracted collagen II. When ready, each sample was in 200 μl of 140 mM NaCl, 30 mM sodium phosphate, pH 7.5, and containing ~100 μg of BSA. The samples were incubated at 37 °C for 20 h to permit the formation of insoluble collagen fibrils. These fibrils were pelleted by centrifugation at 10,000 × g for 10 min at 37 °C, and the supernatant was removed. The precipitate was washed once with 0.2 ml of PBS at 37 °C and then dissolved in 0.1 M HCl. In the initial experiments, with crude mixtures of radiolabeled components, these samples were directly electrophoresed on SDS-gel electrophoresis. However, large amounts of collagen affected the appearance of the radiolabeled molecules. In the following experiments, only one-tenth of each sample was electrophoresed as such. The remaining nine-tenths was digested for 6 h at 37 °C, with 0.4 units of high purity collagenase (type VII, Sigma) in 0.1 M NaCl, 5 mM CaCl₂, 50 mM Tris/HCl, pH 7.4, containing 10 μg/ml ovomucoid trypsin inhibitor (type II-0, Sigma) and electrophoresed separately. The relative amounts of collagen in the nondigested samples were determined by densitometric scanning of the Coomassie Blue-stained gels.

**Collagen-Agarose Binding Assay**—Pepsin-extracted collagen I at 2.5 mg/ml in PBS was coupled to CNBr-activated Sepharose 4B (Pharmacia) at 4 °C overnight. Similarly, gel beads were incubated in PBS containing heat-denaturated collagen I or no additives. Remaining reactive groups were blocked with 0.2 M ethanolamine, followed by several rinses with 0.1 M acetic acid, PBS, and finally PBS containing 0.2% (v/v) of Tween 20. Samples of the collagen-containing solutions, taken before and after the coupling, were used to determine the degree of coupling. Typically, there was 3.4-4.0 mg of collagen/ml of gel.

**Microtiter Plate Assay**—The wells of microtiter plates were coated overnight with pepsin-extracted collagen I at 10 μg/ml in 0.15 M NaCl, 10 mM Tris/HCl, pH 7.4 (Hedbom and Heinegärd, 1989). Control wells were coated with BSA at 10 μg/ml. The adsorbed protein was cross-linked by treatment with 0.25% glutaraldehyde in 0.15 M NaCl, 0.5 mM NaCNBH₃, 20 mM TES, pH 7.4, for 2.5 h at 37 °C, followed by blocking of remaining reactive groups with 0.2 M ethanolamine in the same buffer for 0.5 h at 37 °C. To prevent nonspecific binding of components, all wells were incubated with 10 μg/ml of BSA in 0.15 M NaCl, 10 mM Tris/HCl, pH 7.4, for 4 h at 20 °C. In some cases, the wells were additionally incubated for 1 h with 0.2 mg/ml of CS, prepared as described in Antonopoulos et al. (1967). Isolated proteoglycans, dissolved in 0.15 M NaCl, 10 mM Tris/HCl, pH 7.4, containing 0.05% (v/v) of Tween 20 were combined with soluble collagen at various concentrations and preincubated for 5 h at 20 °C. These samples were transferred without washing to the wells at the same time at 20 °C. Proteoglycans bound to the wells were detected by using an enzyme-linked immunosorbent assay procedure (Hedbom and Heinegärd, 1989). In some experiments, the dissolved decorin was first digested with 0.05 units of chondroitinase ABC (Sigma) per mg of protein for 30 min at 37 °C. These samples were diluted with Tris-buffer containing 0.05% (v/v) of Tween 20 and immediately used for the binding assay.

**SDS-Polyacrylamide Gel Electrophoresis**—Electrophoresed samples of fractions from the described chromatography steps, pre-
cipitates from the collagen fibril binding assay, and gel beads from the collagen-agarose binding assay were incubated in electrophoresis sample buffer containing SDS and β-mercaptoethanol (Laemmli, 1970). The samples were applied on polyacrylamide gradient gels. After electrophoresis, gels were stained with 0.1% Coomassie Brilliant Blue R-250. Radiolabeled components were detected by fluorography with sodium salicylate (Chamberlain, 1979).

RESULTS

Binding of Metabolically Radiolabeled Components to Collagen—Bovine tendon fibroblasts in confluent cultures were labeled with [3H]leucine. The macromolecules in the culture medium were separated from low molecular weight components and transferred into PBS by gel filtration. The proteins were analyzed by SDS-polyacrylamide gel electrophoresis. As shown in Fig. 1, a sample representing the total fibroblast medium contained several radioactive components. Among these, fibronectin and procollagen I were predominant.

The mixture of components was tested for binding to collagen I, using two different methods. In one of these, the sample was added to a solution of collagen at the onset of in vitro fibrillogenesis. The two small proteoglycans, decorin and fibromodulin, were the only radiolabeled molecules that showed pronounced binding to the precipitated collagen fibrils (Fig. 1). Some fibronectin and trace amounts of procollagen were also associated with the collagen. The other detectable components remained entirely in the supernatant. Before fluorography, the gels were stained with Coomassie Blue in order to check that appropriate amounts of collagen I (>95%) had been precipitated. A typical result is shown in Fig. 1, lane 4. Due to the presence of large amounts of collagen quenching the radioactivity, pale zones were seen on the fluorography plates at the positions of the collagen bands affecting the appearance of decorin and fibronectin.

Binding of radiolabeled components to collagen was also tested by using Sepharose beads to which collagen molecules had been covalently linked. If the coupled collagen was in the form of denatured α-chains, large amounts of fibronectin were bound (Fig. 2, lane 2). The small proteoglycans, however, were not associated with the beads. If instead the collagen was coupled under conditions promoting the retention of mainly triple helical monomers, binding of decorin and fibromodulin was observed in addition to that of fibronectin (Fig. 2, lane 3). Thus, the binding of decorin and fibromodulin is depend-
were combined with isolated nonlabeled proteoglycan in PBS. Soluble to collagen fibrils in fibromodulin.

bacterial collagenase and analyzed by SDS-polyacrylamide 4-12% gradient gel electrophoresis. Radiolabeled proteoglycans were de-
tected by fluorography. It was found to be essential to prevent
interactions between the soluble and the coated collagen molecules. For this reason, the assay was performed in Tris buffer instead of the previously used PBS. Furthermore, primary amino groups in the coated protein were modified by cross-linking with 0.25% glutaraldehyde. This treatment
apparently blocks the aggregation of collagen molecules, whereas proteoglycan binding still occurs. The binding of isolated fibromodulin to the collagen-coated wells displayed an apparent linear relationship to the fibromodulin concentration over 0-1 µg/ml and reached saturation above 40 µg/ml (data not shown).

Preincubation of the proteoglycan with triple helical collagen I in solution resulted in inhibition of the solid-phase binding (Fig. 4, a and b). Triple helical collagen II also showed inhibition, but at slightly higher concentrations (Fig. 4b). Collagen I in the form of denatured α-chains showed no such activity and neither did the peptides obtained after cleavage with CNBr (Fig. 4b). At higher concentrations of these (>32 µg/ml), there was a dramatic increase in binding of fibromodulin to the wells. This was probably due to nonspecific interactions. Large amounts of proteoglycan were also bound to the control wells not coated with collagen. Likewise, the inhibitory effect of collagen II was abolished if the conformation was altered by cleavage or denaturation (not shown). Thus, the binding of fibromodulin requires triple helicity but not fibrillar assembly.

The data on fibromodulin acting as a ligand to triple helical
collagen I were used for Scatchard (1949) analysis. It was assumed that the measured solid-phase binding was a linear function of the concentration of free ligand. The proportion of ligand molecules that were bound would then be equal to the percentage of inhibition. Even though the scatter of data points was a limiting factor, the analysis gave an indication that the major, if not the only, class of binding sites had an apparent $K_d$ of 9.9 nM and was represented by one site per collagen molecule on the average.

The data on fibromodulin binding to collagen II did not display a similar linearity (not shown). Clearly, there were fewer and/or weaker binding sites in the collagen molecules of this preparation, as compared with collagen I.

Binding of Isolated Decorin to Collagen Molecules in Solution—The interaction of decorin with collagen, when measured as inhibition in a solid-phase assay, appeared different from that of fibromodulin. Triple helical collagen I, at concentrations of 2–8 µg/ml in the solution, produced increased binding of decorin to the coated collagen (Fig. 5a). At higher concentrations, the effect of the collagen was inhibitory. If the DS chain of decorin was first removed by digestion with chondroitinase ABC, the binding enhancement was less pronounced (Fig. 5a). Alternatively, the enhancing effect could be suppressed by addition of CS to the coated wells before these were used in the assay (Fig. 5b). These observations indicate that not only the core protein of decorin but also the glycosaminoglycan chain is involved in collagen interactions.

The core protein-mediated solid-phase binding was inhibited by triple helical collagens I and II (Fig. 5b). Neither was inhibition shown by denatured or CNBr-cleaved collagen I (Fig. 5b) nor with collagen II (not shown). At concentrations above 8 µg/ml of such competitors, there was a nonspecific increase in the binding of decorin to the coated surfaces.

DISCUSSION

The binding sites for decorin and fibromodulin on collagens I and II have been examined. The interactions between these proteoglycans and collagen in the form of fibrils, monomers, denatured chain constituents, and fragments have been evaluated. The proteoglycans used were either native as taken directly from cell culture medium or pure as isolated from tissue extracts.

Binding of metabolically radiolabeled components was tested in a collagen fibrillogenesis assay. Among the secreted fibroblast macromolecules, decorin and fibromodulin were those predominantly bound to the precipitated collagen fibrils. The two proteoglycans bound to separate sites on the fibrils, as revealed by competition experiments. Saturation of the binding sites occurred at relatively low concentrations of the added proteoglycan, representing 1–3 times the molar concentration of the collagen. Binding saturation of one of the proteoglycans apparently did not affect the binding of the other or the amount of collagen that was precipitated as fibrils. These observations taken together indicate that the binding sites for the two proteoglycans are distinct, specific, and limited in number.

One advantage of the fibril binding assay was that the radiolabeled proteoglycans never had been exposed to denaturing conditions or even been incorporated in a matrix. This contrasts with previous studies where proteoglycans have been extracted and purified in solutions of guanidine hydrochloride and urea. It is possible that such preparations only partially renature upon removal of the denaturing solvent and, therefore, show protein conformation heterogeneity and binding artifacts. Self-interaction of the molecules, whether artificial or genuine, may complicate the binding studies. The isolated small proteoglycans are indeed known to self-interact (Ward et al., 1987; Morgelin et al., 1989). This may at least partially explain some apparently conflicting data on the binding of decorin to fibrillar collagens (Bidanset et al., 1992) and its effect on collagen fibrillogenesis (Uldbjerg and Danielsen, 1988). When the isolated proteoglycans were used in our study, care was taken to perform the experiments immediately after the proteoglycans had been redissolved. Then, the isolated and renatured fibromodulin was able to bind to all of the sites that were available for the radiolabeled native molecules, shown as complete inhibition in the competition experiments. Thus the binding sites of the proteoglycans appear properly renatured. In the competition between decorin molecules for binding to collagen, a slightly larger excess of the isolated proteoglycan was required.

There was also some binding of radiolabeled fibronectin to the collagen fibrils, but this glycoprotein preferentially interacts with denatured collagen (Engvall et al., 1978). Interestingly, fibromodulin and decorin showed no affinity for denatured collagen I linked to Sepharose beads. Obviously, the proteoglycans bind to collagen by a different mechanism than fibronectin. The lack of binding of decorin and fibromodulin to the fibronectin-denatured collagen-Sepharose complex also indicates that these proteoglycans do not occur as complexes with fibronectin under the conditions described.

The interaction sites within the collagens were further examined in a solid-phase binding inhibition assay using the
isolated and renatured proteoglycans. It was found that each of the proteoglycans could interact with native collagen molecules in solution but not with denatured collagen chain constituents or fragments. The collagen II preparation was less effective than collagen I with regard to binding inhibition, albeit the fact that this preparation contained collagen II molecules that had been selected on the basis of their ability to form fibrils. From the fibromodulin binding inhibition data on collagen I, a dissociation constant of 9.9 nM was calculated. This value agrees well with the relative $K_d$ value of 35 nM previously obtained in a direct binding assay (Hedbron and Heinegård, 1989). The analysis clearly indicated the presence of one fibromodulin binding site per collagen molecule. This result does not exclude the possible existence of more than one proteoglycan binding site within a collagen molecule, but it brings further support to the view that the sites are limited in number and highly specific.

The interaction of decorin with collagen, as observed in the solid-phase assay, was more complex than that of fibromodulin. Higher concentrations of collagen in the solution were required for inhibition of the binding to coated molecules. At low concentrations the soluble collagen instead increased the binding. Since this effect was reduced after removal of the DS chains, it appears that either DS-collagen interactions (Obirink, 1973) or DS-DS interactions (Fransson et al., 1982) were involved. In support, the addition of isolated CS chains reduced the binding. A possible explanation of the increased binding is that decorin binds to collagen in solution by protein-protein interaction, and then the dermatan sulfate side chain provides interaction with the coated collagen. This may then indicate that the glycosaminoglycan chain preferentially binds to a collagen molecule other than the one to which the core is bound, consistent with previous data showing little alterations of binding upon removal of the DS chain (Hedbron and Heinegård, 1989). It is thus possible that one function of decorin is to connect neighboring collagen fibrils.

Decorin has recently been shown to bind collagen VI by a core protein-mediated interaction (Bidanset et al., 1992). In that study, binding to collagen I–IV was poor, perhaps suggesting that the interaction with collagen VI is differentially mediated. An interaction between decorin and fibronectin has been studied (Lewandowska et al., 1987; Schmidt et al., 1987, 1991) suggesting a role for the small proteoglycans in the regulation of cell attachment and modulation of the extracellular matrix. The binding of native decorin or fibromodulin to fibrillar collagens does not depend on any mediating molecules according to the present data. However, the exact function of these matrix components in vivo may require additional interactions. In continued studies, the combined effects of the multiple interactions and the possible interlinking of matrix constituents should be considered. Since each of the small proteoglycans shows distinct collagen binding characteristics, these molecules should be studied individually and not regarded as functionally identical.

The present study shows that the small proteoglycans bind to distinct triple helical sites, presumably selected among a large number of very similar structures in each collagen molecule. The limited structural differences between these proteoglycans determine the exact binding specificity and probably the function of the molecule.

Acknowledgment—We are grateful to Malin Molander for skillful experimental work.

REFERENCES

Antonopoulos, C. A., Fransson, L.-A., Heinegård, D., and Gardell, S. (1967) Biochim. Biophys. Acta 148, 158–163
Antonsson, P., Heinegård, D., and Oldberg, Å. (1991) J. Biol. Chem. 266, 16859–16861
Axelson, I., and Heinegård, D. (1978) Biochem. J. 169, 517–530
Bidanset, D. J., Guidry, C., Rosenberg, L. C., Choi, H. U., Timpl, R., and Hook, M. E. (1982) J. Biol. Chem. 257, 6333–6338
Bidder, T., Vigeois, J.-P., Hempel, J., and Hasel, J. (1992) J. Biol. Chem. 267, 347–352
Bornstein, P., and Piez, K. A. (1965) Science 148, 1353–1355
Brown, D. C., and Vogel, K. G. (1989) Matrix 9, 468–478
Brown, D. C., Vaughan, L. and Winterhalter, K. H. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 2906–2912
Chamberlain, J. P. (1979) Anal. Biochem. 98, 132–135
Engvall, E., Russel, E., and Miller, E. J. (1978) J. Exp. Med. 147, 1584–1595
Fransson, L.-A., Coster, L., Malmström, A., and Sheehan, J. (1982) J. Biol. Chem. 257, 6333–6338
Hedbron, E., and Heinegård, D. (1989) J. Biol. Chem. 264, 8888–8905
Heinegård, D., and Oldberg, A. (1989) FASEB J. 3, 2542–2551
Heinegård, D., and Oldberg, A. (1993) in Connective Tissue and Its Heritable Disorders (Royce, P. M., and Steinmann, B., eds) pp. 189–209, Wiley-Liss Inc., New York
Heinegård, D., Björne-Persson, A., Coster, L., Franzén, A., Gardell, S., Malmström, A., Paulsson, M., Sandalkal, R., and Vogel, K. (1986) Biochem. J. 230, 181–194
Heinegård, D., Larson, T., Sommarin, Y., Franzén, A., Paulsson, M., and Hedbron, E. (1986) J. Biol. Chem. 261, 13866–13872
Kjety, C. M., Hopkinson, L., and Grant, M. E. (1993) in Connective Tissue and Its Heritable Disorders (Royce, P. M., and Steinmann, B., eds) pp. 103–147, Wiley-Liss Inc., New York
Kjellén, L., and Lindahl, U. (1991) Annu. Rev. Biochem. 60, 443–475
Laemmli, U. K. (1970) Nature 227, 680–685
Lewandowska, K., Choi, H. U., Rosenberg, L. C., Zardi, L., and Culp, L. A. (1987) J. Cell Biol. 105, 1443–1454
Miller, E. J. (1972) Biochemistry 11, 4900–4909
Miller, E. J., and Gay, B. (1987) Methods Enzymol. 144, 3–41
Mörgelin, M., Paulsson, M., Hardingham, T. E., Heinegård, D., and Engel, J. (1988) Biochem. J. 253, 179–185
Mörgelin, M., Paulsson, M., Malmström, A., and Heinegård, D. (1989) J. Biol. Chem. 264, 12080–12086
Paavilainen, T., Zhang, R.-Z., Mattei, M.-G., Timpl, R. and Chu, M.-L. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6656–6659
Plaa, A. H. K., Neave, P. J., Nivens, C. M., and Reiss, L. (1990) J. Biol. Chem. 265, 2644–2646
Pringle, G. A., and Dodd, C. M. (1993) J. Histochem. Cytochem. 38, 1404–1411
Scatchard, G. (1949) Ann. N. Y. Acad. Sci. 51, 660–672
Schmidt, A., Nieminen, T., and Hook, M. E. (1990) Biochem. J. 199, 267–273
Scatchard, G., Robeneck, H., Harrach, B., Gösel, J., Nolte, V., Hörman, H., Richter, H. and Kresse, H. (1987) J. Cell Biol. 104, 1683–1691
Schaubmuller, J. M., and Heatley, R. W. (1989) J. Biol. Chem. 264, 411–414
Scott, J. E. (1986) Biochem. J. 252, 313–323
Scott, J. E., and Oxford, C. R. (1981) Biochem. J. 197, 213–216
Segel, H. and Stadler, K. (1967) Clin. Chim. Acta 18, 267–273
Sheehan, J., and Robeneck, H. (1991) Biochem. J. 270, 841–845
Uldenberg, N., and Danielsson, C. C. (1988) Biochem. J. 251, 643–648
van der Rest, M., and Gannone, R. (1991) FASEB J. 5, 2814–2823
Vogel, K. G., and Heinegård, D. (1985) J. Biol. Chem. 260, 9298–9306
Wahl, N. P., Scott, J. E., and Coster, L. (1987) Biochem. J. 242, 761–766
Wight, T. N., Heinegård, D. K., and Hasell, V. C. (1991) in Cell Biology of Extracellular Matrix (Hay, D., ed) 2nd Ed., pp. 45–78, Plenum Press, New York

27312 Binding of Fibromodulin and Decorin to Fibrillar Collagens