Decorin Is a Zn$^{2+}$ Metalloprotein*

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Vivian W-C Yang‡‡, Steven R. LaBrenz‡, Lawrence C. Rosenberg¶, David McQuillan§, and Magnus Höök§**

From the ‡Graduate School of Biomedical Sciences, University of Texas, Houston Health Science Center, Houston, Texas 77030, the §Center for Extracellular Matrix Biology, Albert B. Alkek Institute of Biosciences and Technology, the ¶Department of Biochemistry and Biophysics, Texas A&M University, Houston, Texas 77030, and the §Orthopaedic Research Laboratory, Montefiore Medical Center, Bronx, New York 10467

Decorin is ubiquitously distributed in the extracellular matrix of mammals and a member of the proteoglycan family characterized by a core protein dominated by leucine-rich repeat motifs. We show here that decorin extracted from bovine tissues under denaturing conditions or produced in recombinant “native” form by cultured mammalian cells has a high affinity for Zn$^{2+}$ as demonstrated by equilibrium dialysis. The Zn$^{2+}$-binding sites are localized to the N-terminal domain of the core protein that contains 4 Cys residues in a spacing reminiscent of a zinc finger. A recombinant 41-amino acid long peptide representing the N-terminal domain of decorin has full Zn$^{2+}$ binding activity and binds two Zn$^{2+}$ ions with an average $K_D$ of $3 \times 10^{-7}$ M. Binding of Zn$^{2+}$ to this peptide results in a change in secondary structure as shown by circular dichroism spectroscopy. Biglycan, a proteoglycan that is structurally closely related to decorin contains a similar high affinity Zn$^{2+}$-binding segment, whereas the structurally more distantly related proteoglycans, epiphycan and osteoglycin, do not bind Zn$^{2+}$ with high affinity.

Decorin, a small chondroitin/dermatan sulfate proteoglycan, is found in the extracellular matrix of a variety of tissues such as skin (1–5), cartilage (6, 7), and bone (6, 7). This proteoglycan is composed of a 40-kDa core protein and one glycosaminoglycan chain attached to a serine residue in the N-terminal part of the protein. The decorin core protein is dominated by a central region composed of 10 leucine-rich repeat units. Each unit contains 21–26 amino acid residues and is proposed to adopt a characteristic $\alpha$-helix/$\beta$-sheet folding pattern (8, 9). The C- and N-terminal regions of the core protein are believed to form globular structures stabilized by disulfide bonds between sets of cysteine residues. Several proteoglycans have core proteins of similar size and structural organization. These related molecules are considered to form a family called small leucine-rich proteoglycans (SLRPs)† (9, 10). The family of SLRPs include decorin, biglycan, and epiphycan, all of which contain chondroitin/dermatan sulfate chains attached to the N-terminal domain of the core protein and fibromodulin, lumican, keratocan, PRELP, and osteoglycin which often have keratan sulfate linked to asparagine residues in the central region of the core protein. The extracellular matrix glycoprotein chondroadherin has a structural organization similar to the core proteins of the SLRPs but has not been shown to be substituted with glycosaminoglycan chains (11). The biological importance of the different SLRPs is unclear. In vitro binding studies have shown that decorin, biglycan, and fibromodulin can interact with several types of collagen (12–16) and different SLRPs are believed to be important regulators of collagen fibrillogenesis. In support of this hypothesis, a decorin-deficient mouse was found to have fragile skin with an abnormal organization of collagen fibers (17). The phenotype appears to be largely restricted to the skin, perhaps suggesting that other SLRPs have similar functions and may fulfill this role in other collagenous tissues. In fact, a lumican-deficient mouse also exhibited abnormal collagen fibers both in the skin and cornea (18).

Decorin may also affect the production of other extracellular matrix components by regulating the activity of transforming growth factor-β (19, 20). Additionally, decorin can modulate the interactions of matrix molecules such as fibronectin with cells (21–23). These observations suggest that decorin and perhaps other SLRPs regulate at several levels the production and assembly of the extracellular matrix and hence the remodeling of connective tissue.

Zinc, a divalent cation, is one of the essential trace elements for eukaryotic organisms. Zinc ions play a key role in biological processes by being directly involved in enzyme catalysis or by binding to specific sites in a protein to stabilize the conformations which are of importance to the function of the protein (24, 25). In the extracellular matrix, zinc is required for the activity of matrix metalloproteinases which are responsible for the degradation of structural extracellular matrix components (26).

Breakdown and remodeling of the ECM occur in normal embryonic development, wound healing, and many pathological processes such as cancer, arthritis, and osteoporosis (26, 27). Structural extracellular matrix molecules such as laminin (29), link protein (30), nidogen (31), and COMP (32) have been shown to bind zinc ions. The Zn$^{2+}$-binding sites have been located to subdomains of some of these proteins (31, 33).

In a previous study, biglycan was shown to self-associate in the presence of zinc ions and shown to bind Zn$^{2+}$, Ni$^{2+}$, and Cu$^{2+}$ in metal chelate affinity chromatography studies (34). The binding affinity and binding domain, however, have not been reported. In this study, we demonstrate that decorin is a metalloprotein and binds two zinc ions per core protein with an average $K_D$ of $\sim 1 \mu$M. The Zn$^{2+}$-binding domain is mapped to the N-terminal region in the decorin core protein and a short
A mouse embryo cDNA library (CLONTECH, Palo Alto, CA) was used as a template along with appropriate primers (Table I) to PCR amplify the decorin cDNA segments. Conditions for PCR were 94 °C for 7 min, followed by 35 cycles involving 94 °C for 1 min, 50 °C for 2 min, and 72 °C for 3 min. The individual segments were purified, cleaved with appropriate restriction enzymes, and ligated into the pMAL-p2 expression vector. To produce MBP as a control protein without any segment of decorin fused to it, a modified pMAL-p2 vector containing a stop codon and a short polylinker was constructed.

### EXPERIMENTAL PROCEDURES

#### Preparation of Decorin Proteoglycans—Decorin was extracted and purified from bovine skin under denaturing conditions as described previously (3). Intact recombinant decorin was produced in HT1080 cells using a vaccinia virus-based expression system. The recombinant virus construct contains a segment encoding a N-terminal polyhistidine tag, a Factor Xa cleavage site, and the mature human decorin core protein starting with an Asp residue found at position 31 of the full-length human sequence (35). The polyhistidine tag in the recombinant protein allows for its efficient purification by Ni²⁺ binding activity. Furthermore, the Zn²⁺ binding activity of the corresponding segments in the structurally related core proteins of biglycan, epiphycan, and osteoglycin are analyzed.

#### Recombinant Decorin Core Protein Fragments—A series of recombinant core protein fragments were produced in *Escherichia coli*. A mouse embryo cDNA library (CLONTECH, Palo Alto, CA) was used as a template along with appropriate primers (Table I) to PCR amplify the decorin cDNA segments. Conditions for PCR were 94 °C for 7 min, followed by 35 cycles involving 94 °C for 1 min, 50 °C for 2 min, and 72 °C for 3 min. The individual segments were purified, cleaved with appropriate restriction enzymes, and ligated into the pMAL-p2 expression vector (New England Biolabs, Beverly, MA) which contain a segment encoding the maltose-binding protein (MBP) at the 5′ end of the expression cassette. The cDNA fragments (MD and MD3) generated with the DCNR2 primer which contains an EcoRI site at the 3′ end were first subcloned into the pBluescript SK- vector (Stratagene, La Jolla, CA) through BamHI and EcoRI sites. Subsequently, the MD/pBluescript and MD3/pBluescript constructs were digested with BamHI and HindIII and then ligated into the pMAL-p2 expression vector. To produce MBP as a

#### Expression and Purification of Recombinant Decorin Core Protein Subdomains—Previously, a method developed for the production of full-length bovine decorin core protein using a pMAL expression system has been reported (37). This procedure involved solubilization and refolding of protein recovered from inclusion bodies. In our case, we obtained significant amounts of soluble recombinant protein from all constructs and we did not attempt to solubilize protein from inclusion bodies.

The expression vector constructs were used to transform *E. coli* strain TB1. The procedure adopted for the expression and purification of the MBF-decorin fusion protein was based on the manual provided by New England Biolabs. In brief, a flask containing 950 ml of Lennox LB (Sigma) was inoculated with 50 ml of a TB1/MBP-decorin overnight culture and grown on a platform shaker for 3–4 h at 37 °C when the culture reached an OD₆₀₀ of 0.6–0.8. Expression of fusion protein was induced by adding isopropyl-1-thio-β-D-galactopyranoside (Life Technologies, Inc., Gaithersburg, MD) to the culture at a final concentration of 0.2 mM and incubation was continued for another 3 h. The bacterial cells were harvested by centrifugation at 4000 rpm for 20 min. The cell pellet was resuspended in Buffer A (20 mM Tris, 200 mM NaCl, pH 8.0) to a final volume of 10 ml/liter of culture and frozen at −80 °C for a minimum of 18 h. To purify the fusion proteins, the cells were thawed and lysed using a French press. The lysate was cleared by centrifugation at 40,000 rpm for 20 min and the supernatant was applied to a 5-ml amylose affinity column (New England Biolabs, Beverly, MA) equilibrated with 25 ml of Buffer A. The column was washed with 50 ml of
Buffer A and the MBP-decorin fusion protein was eluted by Buffer A containing 10 mM maltose. The purity of the fusion protein was >70% as judged by SDS-PAGE and the yield was 5–10 mg/liter of culture. To improve the purity of fusion proteins, an additional purification step was required. Ion exchange chromatography was used for further purification. MBP-MD3 protein was applied to a NaCl gradient (25–500 mM) in 20 mM Tris, pH 8.0, and applied to a column of Q-Sepharose (Pharmacia Biotech, Piscataway, NJ) equilibrated with the same buffer. The protein was subsequently eluted with a NaCl gradient (25–500 mM) in 20 mM Tris, pH 8.0. Metal ion chelating chromatography on iminodiacetic acid immobilized Sepharose 6B resin (Sigma) charged with Zn2+ was used to further purify MBP-MD, MBP-MD1, and MBP-MD4. The procedure used for charging the column with Zn2+ ions is described in the following section. The purified recombinant proteins were analyzed on SDS-PAGE and Western blot using two polyclonal antibodies that were raised against synthetic peptides corresponding to amino acid residues 31–47 and 308–326 of the mouse decorin core proteins, respectively. The protein concentrations were determined based on the absorbance at A280 and the calculated molar extinction coefficient of the different protein constructs (38).

Production and Purification of a N-terminal Decorin Peptide (MD4)—To produce a large quantity of MD4 peptide, we used a pGEX-2T expression vector (Pharmacia Biotech Inc., Piscataway, NJ). This MD4/pGEX-2T construct encodes glutathione S-transferase (GST) followed by aHis-tag, an N-terminal and the MBP-decorin fusion protein. The eluted GST-MD4 protein was eluted from the column into a buffer composed of 25 mM NaCl, 20 mM Tris, pH 8.0, and applied to a column of Q-Sepharose (Pharmacia Biotech, Piscataway, NJ) equilibrated with the same buffer. The protein was subsequently eluted with a NaCl gradient (25–500 mM) in 20 mM Tris, pH 8.0. Metal ion chelating chromatography on iminodiacetic acid immobilized Sepharose 6B resin (Sigma) charged with Zn2+ was used to further purify MBP-MD, MBP-MD1, and MBP-MD4. The procedure used for charging the column with Zn2+ ions is described in the following section. The purified recombinant proteins were analyzed on SDS-PAGE and Western blot using two polyclonal antibodies that were raised against synthetic peptides corresponding to amino acid residues 31–47 and 308–326 of the mouse decorin core proteins, respectively. The protein concentrations were determined based on the absorbance at A280 and the calculated molar extinction coefficient of the different protein constructs (38).

Dialysis of the Protein—In preparation for Zn2+ binding experiments, all proteins were dialyzed against Buffer D (20 mM Tris-HCl, 150 mM NaCl, pH 7.0) supplemented with 5 mM EDTA (Sigma) to remove possibly contaminating ions and subsequently dialyzed against Buffer D (without EDTA) with three changes. Buffer D was shown to contain less than 5 × 10−5 M Zn2+ ions as analyzed by 4-(2-pyridyl)diazoresorcinol (PAR) (Sigma) complex formation (40, 41). Zn2+/chelating Affinity Column Chromatography—A resin composed of iminodiacetic acid-immobilized Sepharose 6B (Sigma) was charged with 10 volumes of 2 mg/ml ZnCl2 (Sigma) in deionized water. The resin was then washed with 5 volumes of Buffer E (20 mM Tris-HCl, 150 mM NaCl, pH 8). The charged Sepharose 6B was packed on top of an equal amount of uncharged iminodiacetic acid-Sepharose 6B that has been pre-equilibrated in Buffer E. The purpose of having the uncharged resin on the bottom of the column is to trap zinc ions released from the charged resin during the experiment. The dialyzed test sample was loaded into thecolumn, and the column was washed with 5 volumes of Buffer E. After overnight incubation of Buffer E and the bound protein was eluted by Buffer F in which the pH of Buffer E was titrated to 4.0 by addition of glacial acetic acid. Zn2+/Equilibrium Dialysis—The equilibrium dialysis experiments were carried out in a double acrylic microdialysis module (Hoffer, San Francisco, CA). A dialysis membrane was assembled between the two modules to separate each of the eight chambers into two compartments. The column was preincubated at 4°C for at least 18 h. The cell was thawed and lyzed using a French press. One ml of 10% Trition X-100 (Sigma) was added to the cell lysate and mixed until homogenous. The cell homogenate was centrifuged and filtered through a 0.45-μm membrane to remove cellular debris.

The GST-MD4 fusion protein present in the filtered supernatant was purified by affinity chromatography on a 10-ml column of glutathione-agarose (Sigma) equilibrated with 50 ml of Buffer B (phosphate-buffered saline with 1 mM EDTA, pH 7.5) supplemented with 5 mM EDTA and adjusting the pH 7.5 to a final volume of 10 ml/liter of culture and incubated at 4 °C for 3–4 h. The column was then washed with 5 volumes of Buffer E (20 mM Tris-HCl, 150 mM NaCl, pH 8). The charged Sepharose 6B was packed on top of an equal amount of uncharged iminodiacetic acid-Sepharose 6B that has been pre-equilibrated in Buffer E. The purpose of having the uncharged resin on the bottom of the column is to trap zinc ions released from the charged resin during the experiment. The dialyzed test sample was loaded into the column and the column was washed with 5 volumes of Buffer E. After 18 h, the column was washed with Buffer F in which the pH of Buffer E was titrated to 4.0 by addition of glacial acetic acid. Zn2+/Equilibrium Dialysis—The equilibrium dialysis experiments were carried out in a double acrylic microdialysis module (Hoffer, San Francisco, CA). A dialysis membrane was assembled between the two modules to separate each of the eight chambers into two compartments. The column was preincubated at 4°C for at least 18 h. The cell was thawed and lyzed using a French press. One ml of 10% Trition X-100 (Sigma) was added to the cell lysate and mixed until homogenous. The cell homogenate was centrifuged and filtered through a 0.45-μm membrane to remove cellular debris.

To isolate the MD4 peptide, the fusion protein was cleaved with thrombin. The pH and the concentration of NaCl of the eluate containing thrombin-MD4 was adjusted to pH 8.3, 0.15 M NaCl using 1 M NaOH and 3 M NaCl stock solutions. Bovine thrombin (Sigma) was added to give a 1/40 (w/w) enzyme-substrate ratio. The digestion was allowed to proceed overnight at room temperature. Subsequently, β-mercaptoethanol was added to the incubation mixture to a final concentration of 1% (v/v) and the solution was adjusted to pH 9.5 by addition of 1 M NaOH. The solution was incubated at 37 °C for 30 min and filtered through a 0.45-μm membrane to remove any particulate material. The MD4 peptide was purified on a Waters 25 × 200-mm RCM semi-preparative C18 HPLC column and eluted with a gradient of 28 to 36% solvent B (95% acetonitrile, 5% H2O, 0.1% trifluoroacetic acid) in solvent A (95% H2O, 5% acetonitrile, 0.1% trifluoroacetic acid) over 10 min at a flow rate of 25 ml min−1.

The purity of the peptide was monitored by running a three-layer Tricine-SDS-PAGE gel consisting of a 15% acrylamide slab, 10% acrylamide spacer, and a 3.5% acrylamide stacker (39). The identity of the peptide was confirmed by matrix-assisted laser desorption ionization-mass spectrometry (The Center for Analytical Chemistry, University of Texas in Houston, Houston, TX).

Metalloprotein

Biglycan Preparation—Biglycan can be isolated from bovine articular cartilage by extraction and purified under denaturing conditions as described previously (3). Recombinant biglycan containing a N-terminal polyhistidine tag was produced in HT1080 cells using the vaccinia virus expression system as described (36). The polyhistidine tag was removed and the recombinant proteoglycan was re-isolated as described above. The preparation of recombinant biglycan contains a mixture of proteoglycan and core protein forms (36). The N-terminal segment of the biglycan core protein was produced as a recombinant MBP fusion protein in E. coli. The encoded fusion protein MBP-MB-N is composed of the malese-binding protein followed by a biglycan peptide, corresponding to amino acid residues 38 to 77. The primers BGNF11 and BGNR13 (Table I) and the mouse embryo cDNA library were used to PCR amplify an appropriate cDNA fragment. The resulting PCR product was cleaved, purified, and ligated into the pMAL-p2 vector. Protein expression and purification protocols were the same as those described above in the preparation of MBP-decorin fusion proteins.

Diazoacidic acid-immobilized Sepharose 6B (Sigma) was charged with 10 volumes of 2 mg/ml ZnCl2 (Sigma) in deionized water. The resin was then washed with 5 volumes of Buffer E (20 mM Tris-HCl, 150 mM NaCl, pH 8). The charged Sepharose 6B was packed on top of an equal amount of uncharged iminodiacetic acid-Sepharose 6B that has been pre-equilibrated in Buffer E. The purpose of having the uncharged resin on the bottom of the column is to trap zinc ions released from the charged resin during the experiment. The dialyzed test sample was loaded into the column and the column was washed with 5 volumes of Buffer E. The concentration of Zn2+ in the dialysis chamber at the beginning of the experiment. The [Zn2+]free term is multiplied by a factor of 2 to take into account the dilution of [Zn2+]total at equilibrium in the absence of protein. An equation derived for multiple, independent binding sites (Equa-
**RESULTS**

*The Zn$^{2+}$ Binding Activity of Decorin Is Located to the N-terminal Domain of the Decorin Core Protein*—We recently reported that a Zn$^{2+}$ charged column of iminodiacetate-Sepharose could be used to separate decorin and epiphycan isolated from fetal bovine epiphysyal cartilage (43). Decorin was retained on the column suggesting that the proteoglycan can bind Zn$^{2+}$. Equilibrium dialysis has now been used to demonstrate a saturable binding of Zn$^{2+}$ to decorin (Fig. 1). The proteoglycan isolated from bovine skin (BDCN) and recombinant human decorin produced in mammalian cells (vvHDCN) bound a maximum approaching two Zn$^{2+}$ ions per decorin molecule with an average $K_D$ of 1.0 $\pm$ 0.3 $\mu$M and 3.9 $\pm$ 1.8 $\mu$M, respectively (Fig. 1, A and B).

The recombinant mammalian decorin appears as a mixture of molecular species including proteoglycans and core proteins without glycosaminoglycan chains. The observation that the recombinant proteoglycan/core protein mixture could bind similar amounts of Zn$^{2+}$ suggests that the primary Zn$^{2+}$-binding sites in decorin are located in the core protein. This hypothesis was further examined by analyzing the Zn$^{2+}$ binding properties of prokaryotic recombinants. Recombinant mouse decorin (MD) core protein produced in *E. coli* as a fusion protein where the core protein is linked at its N terminus to the MBP also bound Zn$^{2+}$ when examined by equilibrium dialyses whereas no saturable binding of Zn$^{2+}$ to MBP alone could be detected (Fig. 1, C and D). A maximum of 1.8 Zn$^{2+}$ ions could bind per molecule of MBP-MD fusion protein with a $K_D$ of 3 $\mu$M under these conditions (Fig. 1C). Isolated dermatan sulfate or chondroitin sulfate polysaccharides were not retained on a Zn$^{2+}$ charged iminodiacetate-Sepharose (data not shown), suggesting that the core protein alone is responsible for Zn$^{2+}$ binding activity of decorin.

*The Zn$^{2+}$ Binding Activity Is Located to the N-terminal Domain of the Decorin Core Protein*—To further locate the domain responsible for the Zn$^{2+}$ binding activity in the core protein, we analyzed recombinant segments of the mouse decorin core protein produced in *E. coli* as fusion proteins linked to the C terminus of MBP. The different constructs made are shown in Fig. 2. All the fusion proteins which were at least partially soluble when produced as described under “Experimental Procedures,” were purified by a combination of affinity chromatography on amylose-Sepharose and ion exchange chromatography. In the presence of reducing agent, a major component in the different preparations of core protein fragments migrated as expected when analyzed by SDS-PAGE (data not shown).

Chromatography of the different fusion proteins on a Zn$^{2+}$ charged matrix demonstrated that MBP-MD, MBP-MD1, and MBP-MD4 bound Zn$^{2+}$ whereas MBP-MD2 and MBP-MD3 did not bind the metal ions (summarized in Fig. 2). The Zn$^{2+}$-binding recombinant proteins all contained the N-terminal segment of the decorin core protein whereas this segment was not...
present in the non-binders. Equilibrium dialyses showed that MBP-MD4 could bind Zn\(^{2+}\) in a process that exhibits saturation kinetics approaching a maximum of two Zn\(^{2+}\) ions bound per protein molecule (Fig. 3A). The average \(K_D\) for these interactions was 2.4 ± 0.6 \(\mu\)M. Binding of Zn\(^{2+}\) to MBP-MD2 and MBP-MD3, respectively, could not be demonstrated using equilibrium dialyses (data not shown). These results suggest that the 41-amino acid long N terminus of decorin retain full zinc binding activity.

**Characterization of Zn\(^{2+}\) Binding to the MD4 Peptide**—We decided to examine the Zn\(^{2+}\) binding behavior of the MD4 peptide in the absence of a fusion protein partner. A new MD4 expressing plasmid using the pGEX-2T vector was constructed. The resulting recombinant fusion protein is composed of GST followed by a linker containing a thrombin cleavage site and MD4. This fusion protein was expressed, purified, and cleaved. The released MD4 peptide, which contains 4 extra amino acid residues (Gly-Ser-Asn-Gly) at the N terminus followed by residue 31–71 of mouse decorin, was purified by RP-HPLC. The expected size of the peptide was 4878.56 Da as calculated from the amino acid sequence and the molecular mass of the isolated peptide was 4878.37 Da as determined by matrix-assisted laser desorption ionization-mass spectroscopy.

The purified MD4 peptide bound to a Zn\(^{2+}\) charged iminodiacetate-Sepharose column (data not shown) and equilibrium dialyses experiments showed that the MD4 peptide bound Zn\(^{2+}\) ions in a concentration dependent manner that approached a maximum of two Zn\(^{2+}\) ions bound per peptide molecule (Fig. 3B). Analyses of the binding data assuming the presence of two independent Zn\(^{2+}\)-binding sites in the MD4 peptide suggest an average \(K_D\) of 0.28 ± 0.03 \(\mu\)M for these sites. Analyses of the binding data in a Hill plot did not reveal any pronounced cooperativity between the two binding sites.

**Zn\(^{2+}\) Binding Induces a Conformational Change in the MD4 Peptide**—Analyses by circular dichroism spectroscopy (Fig. 4) showed that the MD4 peptide has a different conformation in the presence of Zn\(^{2+}\) ions. In the presence of EDTA (but absence of Zn\(^{2+}\) ions), the spectra exhibit a minimum of 200 nm. When Zn\(^{2+}\) ions are added either in the form of ZnSO\(_4\) or ZnCl\(_2\), the minima has a reduced amplitude and is shifted to a slightly lower wavelength. In addition, a shoulder appears at 215–220 nm. These observations demonstrate a change in secondary structure in the peptide on Zn\(^{2+}\) binding.

**The Zn\(^{2+}\) Binding Activity of Biglycan**—The amino acid sequence of the Zn\(^{2+}\) binding MD4 decorin peptide is partially conserved in biglycan but less conserved in epiphycan and osteoglycin. In previous studies, tissue extracted biglycan from bovine articular cartilage have been shown to form multimers in the presence of Zn\(^{2+}\) (34). We therefore examined the Zn\(^{2+}\) binding activity of different forms of biglycan. Binding to a Zn\(^{2+}\) charged column was demonstrated for biglycan isolated under denaturing conditions from bovine articular cartilage (BBGN) or produced as a recombinant proteoglycan in HT1080.
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**DISCUSSION**

In this article, we report that decorin and biglycan are Zn\(^{2+}\) metalloproteins. These macromolecules are capable of binding Zn\(^{2+}\) regardless of whether they are purified under denaturing conditions from tissues or are produced and isolated under non-denaturing conditions from cultured cells infected by recombinant viruses. Furthermore, the ability to bind Zn\(^{2+}\) is not restricted to decorin from one particular species since in this study, we demonstrate a binding of Zn\(^{2+}\) to decorin derived from human, mouse, or bovine tissue. Decorin and biglycan are members of a growing family of ECM proteins that exhibit high affinity for Zn\(^{2+}\). In addition to matrix metalloproteases (28), this family includes laminin (29), link protein (30), nidogen (31), and COMP (32).

The Zn\(^{2+}\) binding activity of decorin is localized to a segment in the N-terminal part of the core protein. A GAG attachment site is also located in this region and involves a Ser residue at position 34 in the mouse decorin sequence. The carbohydrate components of the proteoglycans, however, are not directly involved in Zn\(^{2+}\) binding. In fact, a recombinant peptide corresponding to the decorin N-terminal domain and produced in *E. coli* has full Zn\(^{2+}\) binding activity. The amino acid sequence of this peptide which contains four Cys residues is reminiscent of a zinc finger but the spacing of the Cys residues is different from the previously reported Zn\(^{2+}\)-binding motifs (24, 25). This apparently novel Zn\(^{2+}\) binding sequence must adopt a conformation that allows the binding of two Zn\(^{2+}\) ions per peptide.

Cys residues appear to be involved in coordinating the Zn\(^{2+}\) ions since in preliminary experiments we have shown that reduction and alkylation of the MBP-MD4 protein resulted in loss of Zn\(^{2+}\) binding activity. Earlier analyses of tryptic peptides obtained from bovine biglycan showed that the first and the fourth Cys residues in the N-terminal domain are linked through a disulfide bond (44). This observation leaves the second and third Cys residues as potential Zn\(^{2+}\) coordinators. If in fact the Cys residues are involved in Zn\(^{2+}\) binding, this could help explain the different affinities we see for Zn\(^{2+}\) among the different forms of decorin tested. The isolated MD4 peptide which was isolated as a homogenous form by RP-HPLC has the highest apparent affinity for Zn\(^{2+}\).

The Zn\(^{2+}\) binding MBP fusion proteins all occurred as mixtures of several molecular forms where some Cys residues appear to be engaged in forming disulfide linked multimers. The measured *K*\(D\) values for Zn\(^{2+}\) binding to these proteins were higher compared with that recorded for the isolated MD4 peptide. Perhaps Cys residues engaged in coordinating Zn\(^{2+}\) in the MD4 peptides in the multimers are involved in disulfide...
linkages. Alternatively, this observation may suggest that structures outside the actual binding site may influence the Zn\(^{2+}\) binding activity of this domain. Thus the isolated sequence present in the peptide appears to have a higher affinity for Zn\(^{2+}\) than when the sequence is part of a larger structure as in an intact core protein or a proteoglycan. Decorin contains in addition to a traditional N-terminal signal, a 13-amino acid long propeptide, which also has been removed from the proteoglycan isolated from tissues. The decorin MD4 peptide and the proteoglycan forms here shown to bind Zn\(^{2+}\) with a high affinity do not include the propeptide sequence. It is unclear if the presence of a propeptide may affect Zn\(^{2+}\) binding to decorin or biglycan.

Recombinant N-terminal segments of epiphycan and osteoglycin core protein made as MBP fusions, which contain sequences similar to the Zn\(^{2+}\) binding sequences present in decorin and biglycan, are also retained on a Zn\(^{2+}\)-charged iminodiacetate-Sepharose. However, the affinity of the MBP-fusion proteins for Zn\(^{2+}\) is too low to measure reliable binding constants by equilibrium dialyses. In an earlier study, we found that Zn\(^{2+}\) chelating chromatography could be used to fractionate tissue-extracted epiphycan (which did not bind to the column) from decorin (which bound to the column). This result is in contrast to the chromatography data obtained in the current study and could be explained if the full-length epiphycan proteoglycan has a lower affinity for Zn\(^{2+}\) compared with the recombinant N-terminal peptide. Such a pattern was established for decorin. In the N-terminal segments of epiphycan and osteoglycin, the Cys residues are spaced differently than in the Zn\(^{2+}\)-binding peptides of decorin and biglycan; there are two compared with 3 amino acid residues between the first and second Cys in epiphycan osteoglycin, and decorin/biglycan, respectively. It is possible but not yet demonstrated that the spacing of Cys residues determines the Zn\(^{2+}\) binding activity of these segments.

The concentration of Zn\(^{2+}\) in body fluids such as blood plasma is approximately 15 μM, which suggests that both decorin and biglycan in vivo occur in complex with Zn\(^{2+}\). The significance of this complex is unclear. Decorin and biglycan which are abundant molecules in the tissues could serve as Zn\(^{2+}\) storage pools where the metal ions could be released to proteins which have a higher affinity for and need Zn\(^{2+}\) ions for their activity. In addition, Zn\(^{2+}\) may stabilize a conformation in the proteoglycan core proteins which are important for their functions. In fact, analyses of the MD4 peptides using circular dichroism spectroscopy demonstrate that the conformation of the peptide is altered in the presence of Zn\(^{2+}\). This observation is consistent with the observation of Liu et al. (34) who found that biglycan had a tendency to aggregate in the presence of Zn\(^{2+}\), which could be caused by a conformational change in the protein induced by Zn\(^{2+}\) binding. Future studies of the biology of decorin and biglycan must take into account the fact that these molecules are Zn\(^{2+}\) metalloproteins.

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