Distinct Isoforms of Chicken Decorin Contain Either One or Two Dermatan Sulfate Chains*

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Decorin, a member of a family of proteins with leucine-rich repeat motifs, is a widely distributed extracellular matrix proteoglycan that is thought to be responsible for the structure, tissue organization, and surface properties of fibrils. In mammals, decorin carries one chondroitin/dermatan sulfate chain as a distinction from its homologue, biglycan, which contains two glycosaminoglycan chains. With the aim to study decorin-collagen interactions in chicken, where the fibrillar organization of cartilage collagens is best understood, we have isolated decorin-related proteoglycans from sternal cartilage of 40-day-old broiler chickens. Small chondroitin/dermatan sulfate proteoglycans were resolved by hydrophobic interaction chromatography into two fractions, DCN I and DCN II. Both forms contained dermanatan sulfate and, in addition, keratan sulfate chains. Tryptic fingerprinting revealed that the core proteins of DCN I and DCN II were identical. The protein was identified as decorin by amino-terminal sequencing. DCN II was found to contain two dermatan sulfate chains, whereas DCN I had a single dermatan sulfate chain. The dermatan sulfate attachment sites are located near the NH₂ terminus of the core protein, i.e. at Ser-4 and Ser-16 in DCN II and at Ser-4 in DCN I. The keratan sulfate attachment sites are located in the central portion of the core protein, at Asn-179 and Asn-230. The presence of two dermatan sulfate chains renders the chicken proteoglycan DCN II structurally similar to mammalian biglycan. Interestingly, biglycan has not been detected in chicken. Therefore, in birds, DCN II may function as a biglycan substitute.

The mechanical properties of a connective tissue are largely determined by the composition of its extracellular matrix and the interactions between the matrix macromolecules. Tensile strength is generated by a framework of insoluble fibrils that are rich in collagen. The fibrils are embedded in a hydrated gel formed by proteoglycans and other glycoproteins. In cartilage fibrils, collagens II, IX, and XI form a heterotypic aggregate which, presumably, is essential for the control of fibril dimensions and surface properties (1, 2). Other participants in the fibril assemblages are collagen-binding glycoproteins, such as decorin and fibromodulin (3), but their functions are not well understood.

Decorin (4) is a small chondroitin sulfate (CS)/dermatan sulfate (DS) proteoglycan present in most extracellular matrices (reviewed in Refs. 3 and 5). It belongs to a family of secreted glycoproteins also including the CS/DS proteoglycans biglycan (6) and PG-Lb (7) and the keratan sulfate (KS) proteoglycans fibromodulin (8), lumican (9), and keratocan (10), as well as the proteins chondroadherin (11) and PRELP (12). Within this group of macromolecules, the protein structures are composed mainly of repeated motifs of 20–25 amino acid residues with abundant leucine residues in conserved positions (3). Leucine-rich repeats are known to occur in more than 60 different proteins of eucaryotic or prokaryotic origin, and in many cases they appear to be involved in protein-protein interactions (13, 14). In the members of the decorin family, the leucine-rich repeat region is located in the center of the protein and is flanked by structurally less conserved NH₂ and COOH-terminal regions. The NH₂-terminal region harbors four conserved cysteine residues, with a disulfide bond connecting the first with the fourth (15). Similarly, there is an intrachain disulfide bond between two conserved cysteines near the COOH terminus. There are significant structural differences between these molecules in the segment NH₂-terminal to the first cysteine. Mammalian decorin contains a single CS/DS chain that is attached to a serine residue at position 4 (16). Biglycan usually has two CS/DS chains attached to serine residues at positions 5 and 11 (15). Fibromodulin and lumican do not have glycosaminoglycans in the NH₂-terminal regions, but tyrosine sulfate residues may be present instead (17).

Ultrastructural studies suggest that decorin is associated with collagen-containing fibrils in a number of connective tissues (18–20). Decorin binds to collagens I and II and inhibits collagen fibril formation in vitro (21, 22). The effects on collagen I fibrillogenesis in vitro include a delayed initial assembly of collagen molecules and a decreased final fibril diameter (23). The proteoglycan shows a high affinity for native collagen I molecules, with a dissociation constant in the order of 10⁻⁸ M (22, 24), or even 10⁻⁹ M when the decorin has been isolated under strictly nonnondenaturing conditions (25, 26). High affinity binding depends on the core protein, but the glycosaminoglycan chain can provide additional collagen binding sites (27). The latter interactions presumably are electrostatic since they are disrupted in the presence of phosphate or sulfate ions at >20 mM (28). Analogously to decorin, fibromodulin binds to collagens I and II and delays fibrillogenesis in vitro (22). However, the two proteoglycans bind to separate distinct sites on collagen fibrils (27). The core protein of biglycan, which is 50–60% homologous to that of decorin, does not show a similar interaction with collagens (24, 27, 28) although a weaker affinity for

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1 The abbreviations used are: CS, chondroitin sulfate; DS, dermatan sulfate; KS, keratan sulfate; PAGE, polyacrylamide gel electrophoresis; DCN, decorin; HPLC, high performance liquid chromatography.
collagen (Kc ~ 10^{-7} m) has been reported (25). Recently, it was shown that a recombinant proteoglycan chimera having the structure of biglycan except for leucine-rich repeats 4–5, which were from decorin, was able to bind to collagen I with almost the same affinity as recombinant decorin (26). This suggests that the affinity for collagen is determined by the detail structure of a stretch of some 40 amino acid residues within the central portion of the core protein.

With a view to investigating the properties of cartilage fibrils, our attention has focused on small interstitial proteoglycans of chicken, because collagen structure in chicken cartilage is already well studied. In this article, we report on the isolation of decorin from chicken sternal cartilage. A novel variant with two DS chains is described. Since the avian counterpart to biglycan has not yet been identified, we speculate that this decorin isoform might partially substitute for biglycan functions.

**EXPERIMENTAL PROCEDURES**

**Materials**—Chondroitinase ABC was purchased from Seikagaku Kogyo (Tokyo, Japan). Endo-B-galactosidase, endoproteinase Asp-N, N-glycosidase F were from Boehringer Mannheim. Trypsin (1-tosylamide-2-phenylthyl chloromethyl ketone-treated), papain, chondroitin sulfate A, and dermatan sulfate were from Sigma. Octyl-Sepharose CL-4B and Sephadex G-25M were from Pharmacia Biotech Inc., and DEAE-cellulose was DE-52 from Whatman Ltd. Peroxidase-conjugated goat anti-(mouse IgG) antibodies were from Kirkegaard and Perry Laboratories (Gaithersburg, MD). Coomassie Brilliant Blue R-250 and dimethylmethylene blue were from Serva Feinbiochemica (Heidelberg, Germany). Guanidine HCl solutions were prepared from an 8 M stock solution, which had been treated overnight with activated charcoal and then filtered. Urea solutions were prepared from an 8 M stock solution, which was deionized with Servol MB-3 (Serva) prior to use.

**Isolation of DCN I and DCN II**—Sterna from 40-day-old broiler chickens were taken within 1 h after slaughter. Cartilage was freed from adherent tissues and homogenized in a 15-fold excess of 4 M guanidine HCl, 50 mM sodium acetate, pH 5.8, containing fresh protease inhibitors (100 mM 6-aminohexanoic acid, 10 mM N-ethylmaleimide, 10 mM benzamidine HCl, 50 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride). The suspension was stirred for 24 h at 4°C and then centrifuged at 17,000 x g. The supernatant was collected, and its density was adjusted to 1.40 g/ml by addition of solid CsCl. A density gradient was formed using a 2.5-fold mixture of N-ethylmaleimide for 2 h at 20°C. The proteoglycans were recovered by ethanol precipitation and digested with trypsin (10 μg/mg of proteoglycan) for 12 h at 37°C in 1% NH4HCO3, pH 8. After lyophilization, the peptides were dissolved in 0.1% (v/v) trifluoroacetic acid and injected onto an ODS Ultrasphere reversed phase HPLC column, 0.15 μM NaCl, 50 mM Tris-HCl, pH 7.4, containing 1 mM o-vanillic acid. These samples were incubated for 4 h at 37°C. Digestion with endo-B-galactosidase (0.2 milliunit/μg of proteoglycan) was for 2 h at 37°C in 0.15 μM NaCl, 50 mM sodium acetate, pH 5.8. Treatment with N-glycosidase F was carried out with samples in 0.1% (v/v) trifluoroacetic acid for 10 min at 37°C, 50 mM Tris-HCl, pH 7.4, that had been preheated to 60°C for 10 min. After cooling to 37°C, enzyme (0.05 unit/μg of proteoglycan) was added and digestion was allowed to proceed for 12 h.

**Uronic Acid Assay**—Uronic acids were identified according to the procedure of Formato et al. (31) with modifications. 1,2,3,4,6-Penta-O-benzoyl-D-glucopyranose and 2-benzamido-1,3,4,6-tetra-O-benzoyl-D-xylose were standards for uronic acid analysis. The uronic acid residues of glycosaminoglycan chains were reduced in the system of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide-NH2 in aqueous solution. The product was hydrolyzed with 2 M trifluoroacetic acid. Monosaccharides, i.e. D-glucose, 1,6-anhydro-D-idose, and D-galactosamine, were perbenzoylated with benzoic anhydride in pyridine. Samples were then lyophilized and dissolved in acetonitrile. Aliquots were injected onto an C-18 ODS Ultrasphere reversed phase HPLC column (0.15 x 15 cm; same as above) and eluted with a linear gradient of 0–10% 0.1% (v/v) trifluoroacetic acid containing acetonitrile in stepwise increased proportions: 0–10 min, 0%; 10–20 min, 25%; 20–35 min, 25–60%; 35–45 min, 60%; 45–50 min, 60–75%; 50–70 min, 75%. The flow rate was 1 ml/min and the effluent was monitored for absorbance at 235 nm.

**Disaccharide Analysis**—The proportion of chondroitin 4-sulfate to chondroitin 6-sulfate was determined after digestion with chondroitinase ABC according to the method of Zebrower et al. (32). Chondroitin sulfate A, a mixture of 70% chondroitin 4-sulfate and 30% chondroitin 6-sulfate, was used as standard.

**Size Exclusion Chromatography of Glycosaminoglycans**—O-Linked glycaminoglycans were liberated from the proteoglycans by treatment with 0.5 M NaBH4, 0.1 M NaOH, at 48°C for 48 h (33). The samples were neutralized with acetic acid and chromatographed on a column of Sephacryl S-300 HR (100 x 6.0 cm) eluted with 0.1% (v/v) trifluoroacetic acid containing acetonitrile in stepwise increased proportions: 0–10 min, 0%; 10–20 min, 25%; 20–35 min, 25–60%; 35–45 min, 60%; 45–50 min, 60–75%; 50–70 min, 75%. The flow rate was 1 ml/min and the effluent was monitored for absorbance at 235 nm.

**Isoforms of Chicken Decorin**

Samples of proteoglycans were digested with papain (200 μg/mg of proteoglycan) in 50 mM KH2PO4, 10 mM EDTA, 2 mM dithioerythritol, pH 6.8, at 40°C for 24 h, and chromatographed on the Sephacryl S-300 column. Disaccharide Analysis—A sample of 2 mg of DCN II was reduced, alkylated, and digested with trypsin as described above. The peptides were dissolved in 300 μl of 2 M guanidine HCl, 0.25 mM sodium acetate, pH 6.3. In a separate experiment, a sample of proteoglycan was digested with papain (200 μg/mg of proteoglycan) in 50 mM KH2PO4, 10 mM EDTA, 2 mM dithioerythritol, pH 6.8, at 40°C for 24 h, and chromatographed on the Sephacryl S-300 column. Disaccharide Analysis—A sample of 2 mg of DCN II was reduced, alkylated, and digested with trypsin as described above. The peptides were dissolved in 300 μl of 2 M guanidine HCl, 0.25 mM sodium acetate, pH 6.3, and separated by size-exclusion chromatography on a column of Sephacryl S-300 HR (100 x 1.0 cm) in the 2 M guanidine HCl buffer. Fractions of 1 ml were collected. These were analyzed for protein by monitoring the absorbance at 280 nm and for glycosaminoglycans as described (34). Fractions containing Ks were pooled and lyophilized, followed by desalting on a column of Sephadex G-25M (11.5 x 15 cm) in 1% NH4HCO3, A separate pool, which contained the CS/DS, was dialyzed against water and lyophilized. This material was digested with 2 μg of endoproteinase Asp-N in 10 mM Tris-HCl, 50 mM sodium phosphate, pH 8.0, for 14 h at 37°C. It was then re-chromatographed on Sepharose S-300 HR. Fractions containing CS/DS were pooled, treated with 10 milliliters of chondroitinase ABC, and subjected to reversed phase HPLC as described under “Tryptic Peptide Mapping.” Peptide fractions were collected and vacuum-dried.

**Polyacrylamide Gel Electrophoresis**—Samples to be analyzed by SDS-PAGE were precipitated by adding nine volumes of ice-cold ethanol and centrifuged at 4°C for 30 min at 17,000 x g. The precipitates were resuspended in sample buffer containing 2% β-mercaptoethanol, heated to 95°C for 3 min, and then electrophoresed in 3.5–12% polyacrylamide gradient gels using the buffer system of Laemmli (30). After completion of electrophoresis, gels were stained with Coomassie Brilliant Blue.
Peptides eluted with the breakthrough were purified further by gel filtration on Sephadex G-25M (100 x 0.6 cm) in 1% NH₄HCO₃ 2-5 M guanidine HCl. Detection of glycosaminoglycans—Concentrations of sulfated glycosaminoglycans were determined by using the reagent 1,9-dimethylmethane blue as described by Fairdale et al. (34).

KS was detected using an immunooassay with the monoclonal antibody 5D4 (35). Aliquots of column fractions, diluted in 0.15 M NaCl, 50 mM Tris-HCl, pH 7.4, were kept in well plates for 6 h at room temperature to allow adsorption of molecules. The plates were then rinsed with buffer containing 0.05% (v/v) Tween 20. The wells were incubated for 3 h with peroxidase-conjugated goat anti-mouse IgG antibodies at a dilution of 1:10,000, followed by incubation for 2 h with peroxidase-conjugated goat anti-mouse IgG antibodies at a dilution of 1:5000. Enzyme activity was measured after addition of 200 µl/well of a substrate solution prepared by combining o-phenylenediamine (0.4 mg/ml) and 30% H₂O₂ (0.4 µl/ml) in 0.05 M sodium citrate, 0.1 M sodium phosphate. The reaction was stopped by addition of 50 µl of 2.5 M H₂SO₄. Absorbance was read at 490 nm.

KS was also detected by immunoblotting (36) using the antibody 5D4 at a 1/5000 dilution and the peroxidase-conjugated goat anti-mouse IgG antibodies at a 1/5000 dilution.

RESULTS

Isolation of Proteoglycans—Extracts of sternal cartilage of 40-day-old chickens were subjected to CsCl-density gradient centrifugation in 4 M guanidine hydrochloride, and fractions containing low buoyant density proteoglycans (1.30–1.45 g/ml) were pooled. The material was chromatographed on a column containing low buoyant density proteoglycans (1.30–1.45 g/ml) of Octyl-Sepharose CL-4B eluted with a linear gradient of 2–8 M guanidine HCl. Two CS/DS proteoglycans were recovered within a pool collected at guanidine HCl concentrations between 2.2 and 3.8 M. Contaminating glycoproteins were removed from this material by chromatography on DEAE-cellulose. Finally, the two proteoglycans were separated by hydrophobic interaction chromatography on Octyl-Sepharose (Fig. 1). Material that was eluted at the beginning of the gradient, between 2.2 and 2.7 M guanidine HCl, migrated as a single diffuse band upon SDS-PAGE. The position of the band indicated a molecular mass of more than 200 kDa. Fractions eluted toward the end of the gradient contained a small proteoglycan, with an apparent molecular mass of 80–150 kDa. The latter proteoglycan was tentatively designated as DCN I and the >200-kDa form as DCN II.

Identification of DCN I and DCN II—DCN I and DCN II were identified as isoforms of chicken decorin by sequence analysis of 20 NH₂-terminal residues (Fig. 2). Blank cycles were observed in positions 4 and 10 of DCN I and in positions 4, 10, and 16 of DCN II. Comparison with an amino acid sequence derived from the published cDNA sequence of chicken decorin (37) revealed that these blank positions were occupied by Ser, Thr, and Ser, respectively. Moreover, the identity between the core proteins of DCN I and DCN II was confirmed by tryptic peptide mapping. DCN I and DCN II gave rise to identical patterns (Fig. 3).

Treatment of either DCN I or DCN II with chondroitinase ABC yielded a more compact band in a subsequent SDS-PAGE, corresponding to a molecular mass of 45–55 kDa (Fig. 4, lanes 1 and 4). Digestion of the chondroitinase-treated proteoglycans with endo-β-galactosidase did not substantially increase the electrophoretic mobility but resulted in a further sharpening of the bands (Fig. 4, lanes 2 and 5). Separately, chondroitinase-treated samples were digested with N-glycosidase F, an enzyme removing N-linked oligosaccharides including N-linked keratan sulfate. For DCN I as well as DCN II, a single core protein with an apparent molecular mass of 40 kDa was observed (Fig. 4, lanes 3 and 6). The susceptibility to endo-β-
suggests that the glycosaminoglycan chains of DCN II and bovine decorin are of similar size, i.e. 37 kDa (39).

Attachment Sites of Dermatan Sulfate Chains in DCN II—
The apparent molecular weight of DCN II was unusually high, although neither the core protein nor the DS chains were larger than in other decorin molecules. Therefore, it was suspected that DCN II harbors more than one DS chain. The most likely attachment sites are serine residues in positions 4 and 16, both of which gave blank cycles in the amino acid sequencing. A dash represents a residue of DCN I being identical with that of DCN II. •, DS attachment site in DCN I and DCN II; ◦, DS attachment site in DCN II only. Panel c, peptides obtained by digestion of the NH₂-terminal tryptic fragment of DCN II with endoproteinase Asp-N. ■, peptide-(1–5); □□□, peptide-(6–25); ○○○, peptide-(13–25).

Fig. 2. Comparison of the NH₂-terminal amino acid sequences of DCN I and DCN II with the sequence deduced from chick decorin cDNA. Panel a, amino acid sequence of the NH₂-terminal tryptic fragment of decorin as predicted from a cDNA nucleotide sequence (37). Arrows indicate the endoproteinase Asp-N cleavage sites. Panel b, NH₂-terminal amino acid sequences of DCN II and DCN I as determined from 20 cycles of Edman degradation. Xaa indicates a residue giving a blank in the amino acid sequencing. A dash represents a residue of DCN I being identical with that of DCN II. •, DS attachment site in DCN I and DCN II; ◦, DS attachment site in DCN II only. Panel c, peptides obtained by digestion of the NH₂-terminal tryptic fragment of DCN II with endoproteinase Asp-N. ■, peptide-(1–5); □□□, peptide-(6–25); ○○○, peptide-(13–25).

Fig. 3. Tryptic peptide patterns of DCN I and DCN II. Reduced and alkylated DCN I (top) or DCN II (bottom) were digested with trypsin. The resulting peptides were chromatographed on a C-18 reversed phase HPLC column eluted with a gradient of acetonitrile (-----) in 0.1% trifluoroacetic acid. Absorbance at 220 nm was monitored (----).

Fig. 4. SDS-PAGE of DCN I and DCN II core proteins. Samples of the proteoglycans were digested with chondroitinase ABC (CSase ABC), endo-β-galactosidase (KSase), and N-glycosidase F (PNGase F). The samples were electrophoresed on a 3.5–12% gradient SDS-polyacrylamide gel, which was then stained with Coomassie Blue. The deglycosylated core proteins of DCN I and DCN II show identical migration upon SDS-PAGE, corresponding to a size of 43–45 kDa. A sharp band at the position of 66 kDa represents bovine serum albumin, which was present in the chondroitinase ABC preparation.

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The isolated tryptic fragment (Fig. 5b, pool I) was digested with endoproteinase Asp-N. This enzyme is known to cleave peptide–alysolate bonds, but not between two adjacent aspartate residues (40). The amino-terminal tryptic peptide of chicken decorin that comprises residues 1–25 harbors two cleavage sites for endoproteinase Asp-N, i.e. between residues 5 and 6 and between residues 10 and 11. Both sites are located between the presumptive DS attachment sites at residues 4 and 16 (Fig. 2). Peptides generated by cleavage with endoproteinase Asp-N were re-chromatographed on Sephacryl S-300 HR (Fig. 5c). The glycosaminoglycan carrying peptides eluted in fractions corresponding to $K_{av} = 0.16$, similarly as after cleavage with papain. This material was further resolved into three fractions by reversed phase HPLC after removal of the DS chains by chondroitinase ABC (Fig. 6). One peptide was recovered from the breakthrough fraction, together with the digestion products of the glycosaminoglycan (Fig. 6, pool I). This peptide was separated from disaccharides by gel filtration on Sephadex G-25M (Fig. 7). The peptide was recovered from fractions 20–25 and brought to sequence analysis. It was identified as a pentapeptide consisting of the five NH₂-terminal amino acids of decorin (Fig. 2, solid line). Again, Ser-4 was not detected by automated Edman degradation because of the glycosylation of this amino acid residue.

A second pool of material eluted as a broad peak from the reversed phase HPLC-column at about 20–35% CH₃CN (Fig. 6,
pool II). Sequence analysis by Edman degradation revealed that this material consisted of two peptides generated by a nearly quantitative cleavage by endoproteinase Asp-N between Ala-5 and Asp-6 (>95%) and an incomplete fragmentation between Thr-10 and Asp-11 (50%; see also Fig. 2). However, the observed amino acid sequences corresponded to peptide-(6–25) (Fig. 2, dashed line) and 13–25 (Fig. 2, dotted line), respectively. Hence, the two expected amino-terminal aspartyl residues were not observed in peptide-(13–25). It is likely that cleavage between Asp-12 and Pro-13 occurred spontaneously in the enzymatically generated peptide-(11–25), since Asp-Pro peptide bonds are characteristically labile in acid solutions as those used here for HPLC (41). However, both peptides produced a blank cycle at position 16, indicating the presence of a second DS stub at this serine residue.

The identified DS-carrying peptides were recovered from the fractions corresponding to the two major peaks in the reversed phase chromatogram (Fig. 6, pools I and II). The third peak was much smaller (Fig. 6, pool III), and no peptide material was found in the corresponding fractions. Therefore, the described sequence analyses confirmed that O-linked glycosaminoglycans were attached to Ser-4 and Ser-16 only.

**Attachment Sites of Keratan Sulfate Chains—** Peptides containing KS eluted in fractions with \( K_{AV} \) values between 0.12 and 0.67 (Fig. 5b). Amino acid sequencing of this material showed two peptides in similar amounts, representing tryptic peptide-(175–185) and peptide-(218–240). Each peptide contained one of the two Asn-Xaa-Ser/Thr sequences, which are
potential N-glycosylation sites. Hence, the KS attachment sites were Asn-179 and Asn-230.

**DISCUSSION**

In this study, we find that the predominant isoform of decorin in chicken cartilage differs from its mammalian counterpart in that it contains two CS/DS chain substituents. The two CS/DS attachment sites are located close to each other near the NH2 terminus of the core protein, i.e., at the residues Ser-4 and Ser-16. In addition to the biglycanated form, DCN II, we also find a monoglycanated form, DCN I, with the characteristics published previously for chick corneal decorin (37, 38, 42).

The functions of the small interstitial proteoglycans are incompletely understood. However, the broad tissue distribution of decorin and the high degree of structural conservation of the decorin protein sequence indicate that the function of this proteoglycan is of general importance. It is assumed that decorin influences matrix assembly and supramolecular organization through interactions with other macromolecules. A number of studies have concerned decorin interactions in vitro. In most cases the binding capacity is provided by the core protein, as for example with collagens I, II, and VI, fibronectin, and transforming growth factor-β (see Ref. 3 for references). There also exist some glycosaminoglycan-mediated decorin interactions, for example with collagen XIV (43) and collagen I (28). Interactions of the latter type could be strongly influenced by the number of CS/DS chains.

A major function of collagen-associated decorin might be to confer negative charges to the fibril surface. In tissues like cartilage, interactions between fibrils and perifibrillar components are necessary to prevent fusion of the fibrils and segregation of the extracellular substance. It is reasonable to assume that these interactions show variation between and within tissues, to meet the need of biomechanical diversity. Since the fibril surfaces are rich in glycosaminoglycans, provided by decorin as well as collagen IX, it is likely that electrostatic forces modulate the interactions at the fibril surface. Therefore, the variable glycosylation of decorin may have a role in tuning of the mechanical properties of cartilage tissue.

The biosynthesis of O-linked glycosaminoglycans follows upon the transfer of xyloside to serine residues in a polypeptide. It has been suggested that the responsible enzyme, xylosyltransferase, recognizes the tetrapeptide Ser-Gly-Xaa-Gly preceded by a few acidic residues (44). In chicken, however, the glycosaminoglycan attachment sites are Gly-Ser in DCN I, in collagen IX (45), and probably in PG-Lb (7). Hence, it has been supposed that avian xylosyltransferase activity differs from that in mammals (37). Now we find that the second CS/DS substitution site in chicken decorin corresponds to a classical acceptor sequence. None of the five other Ser-Gly or Gly-Ser sequences of the core protein receives a glycosaminoglycan substituent. These observations support the notion that xylosyltransferase recognition is not specific for a certain peptide sequence, but may involve conformational determinants (46).

The finding of both mono- and biglycanated forms of decorin raises the question as to whether the substitution with glycosaminoglycan chains is regulated in a tissue-specific manner. If so, biglycanated decorin could be rather restricted in its tissue distribution. It is not specific for cartilage, however, as it is found in chicken tendon as well. In addition, close inspection of the published SDS-PAGE of intact corneal decorin (Fig. 6 in Ref. 38) reveals that both DCN II and DCN I may be present in cornea. However, in an immunoblot of chick embryonic skeletal muscle proteoglycans, only monoglycanated decorin was detected (47).
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