Recombinant Decorin Glycoforms

PURIFICATION AND STRUCTURE*

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The vaccinia virus/T7 bacteriophage expression system was used to express human decorin in HT-1080 cells by co-infection with vTF7-3, encoding T7 RNA polymerase, and vDCN1, encoding the decorin core protein fused to a polyhistidine-insulin signal sequence fusion-protein cassette. Overexpression using the vaccinia virus/T7 phage system resulted in secretion of approximately 30 mg of decorin/10⁹ cells per 24 h which enabled purification and separation of multiple glycoforms under native conditions. Cells were cultured in the presence of [³⁵S]methionine or a mixture of [³H]glucosamine and [³⁵S]sulfate, and recombinant glycoprotein purified by metal affinity chromatography which resolved the secreted decorin into two classes, a proteoglycan form and a core protein form. About 25% of the recombinant protein was secreted into the culture medium as core protein devoid of glycosaminoglycan chains. The decorin core protein was resolved into two forms (49 and ~53 kDa) that differed in the extent of N-linked oligosaccharide substitution (2 and 3 N-linked oligosaccharides, respectively). Deglycosylation of the recombinant proteoglycans and core proteins resulted in a single band migrating with an apparent molecular mass ~43 kDa when analyzed by SDS-polyacrylamide gel electrophoresis. Far-UV circular dichroism spectra of native decorin proteoglycan showed a minima at 218 nm, consistent with a secondary structure that is predominantly β-sheet. Circular dichroism spectra of bovine decorin extracted from articular cartilage and recombinant decorin similarly treated revealed a minima of 205 nm indicating a loss of secondary structure. The affinity of decorin proteoglycan and core protein for collagen-like molecules was demonstrated, with the complement component C1q exhibiting the most striking affinity for decorin, although adherence to collagen types I and V was also observed. The extensive secondary structure maintained in the purified recombinant protein is likely to be important for the biological function of decorin.

Decorin, a small chondroitin sulfate proteoglycan is a ubiquitous component of the extracellular matrix of many tissues (1, 2). The decorin mRNA encodes a prepro-core protein of 360 amino acids (3, 4). This core protein can be divided into several distinct structural domains comprising: 1) a short signal sequence of 16 amino acids that targets the protein to the rough endoplasmic reticulum; 2) a propeptide of 14 amino acids of unknown function; 3) the glycosaminoglycan (GAG)³ acceptor region with the chondroitin/Dermapan sulfate chain substituted at the Ser-4 residue of the mature core protein; 4) a hyper-variable cysteine globular domain; 5) a leucine-rich domain comprising a major portion of the core protein and containing three N-linked oligosaccharide attachment sites; and 6) a carboxy-terminal globular domain. The mature decorin molecule that is recovered from tissues lack the propeptide domain. Decorin belongs to a growing family of proteoglycans with one or more GAG chains, that are characterized by core proteins of about 40 kDa containing 8–12 homologous leucine-rich repeats (LRR) of 20–29 residues with leucines in conserved positions (3, 6). Similar LRR motifs are found in other proteoglycans like biglycan (7), fibromodulin (8), and lumican (9), and numerous other apparently unrelated molecules such as the porcine ribonuclease inhibitor (10), (11). In the ribonuclease inhibitor, each LRR contains an alternating β sheet-α helix structural unit, with the short β-strand and the α-helix approximately parallel to each other (12). The leucine-rich sequences of some of these proteins have been postulated to mediate protein-protein (13) and protein-membrane (14) interactions. The LRR repeats in the extracellular matrix proteoglycans are flanked by disulfide loops located at conserved positions near the NH₂ and COOH termini and thus may comprise a specialized subgroup within the LRR superfamily (3, 6). Although biglycan, fibromodulin, lumican, and decorin are similar in their general structural features they differ markedly in their gene regulation, pattern of expression, and functional interactions.

Most of the biological functions ascribed to decorin are believed to relate to complex formation with other molecules via the GAG or core protein domains. Thus decorin has been demonstrated in vitro to bind to several proteins including fibronectin (15), transforming growth factor-β (16), and membrane receptors required for its endocytosis (17). In addition, decorin binds to certain types of fibrillar collagens including type I (18), type II (19), and type VI (20) and retards fibrillogenesis (19). Decorin also interacts with the collagen-like molecule C1q, a subcomponent of the C1 complex, the first component of the classical pathway of complement activation (21, 22). However, without exception these studies have utilized decorin extracted from tissues under harsh denaturing conditions which are likely to alter the secondary structure of this molecule. In fact, we demonstrate in this study that decorin purified under native conditions has an extensive secondary structure that is severely and irreversibly disrupted when treated by standard extraction techniques (23, 24). Binding of denatured decorin with other matrix molecules may not reflect biologically rele-

* This work was supported by a grant from the National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health Grant RO1 AR42826. 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.

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³ The abbreviations used are: GAG, glycosaminoglycan; PAGE, polyacrylamide gel electrophoresis; GdnHCl, guanidine hydrochloride; CD, circular dichroism; LRR, leucine-rich repeat; PCR, polymerase chain reaction; BSA, bovine serum albumin.
vant interactions. In the present study we have successfully utilized the vaccinia virus/T7 phage expression system to produce chemical amounts of differentially glycosylated forms of decorin that encompass the range of species described to date for tissue extracts and cell culture systems. We have purified the decorin glycoforms under native conditions and analyzed their glycosylation patterns. Preliminary investigation of the secondary structure of the decorin and biglycan extracted under denaturing and non-denaturing conditions is presented. The affinity of these recombinant native forms of decorin for collagenous extracellular matrix molecules is likely to better represent interactions that may occur in vivo.

**EXPERIMENTAL PROCEDURES**

All experimental procedures are as described in the accompanying paper (34), except for the following.

First-strand cDNA Synthesis and PCR Amplification—First-strand cDNA synthesis was done (Gene Amp kit, Perkin Elmer) with specific downstream oligonucleotide primers (0.5 μg/reaction) and 1–2 μg of RNA in a final reaction volume of 20 μl. First-strand cDNA synthesis was done (using the upstream primer, DCN-M1 5′-GCCAGGTATAAAAATGAGGGGATCCTGAGA-3′; and downstream primer, P4 5′-GGCTTATTGCAGCTGTAAAGATGAGGC-3′) for 60 min, and the reaction terminated by incubation at 100 °C for 5 min. PCR cDNA amplification was done using the entire 20-μl first-strand cDNA reaction as a template in a final reaction volume of 100 μl containing upstream oligonucleotide primers (0.5 μg) and 2.5 units of Pfu DNA polymerase.

**RESULTS**

Human Decorin Expression Vector—A recombinant expression vector, pDCN1 (Fig. 1a), was developed for optimum production and secretion of decorin in eukaryotic cells using the vaccinia virus/bacteriophage T7 polymerase expression system, essentially as described in the accompanying paper (34). The vector, pDCN1, contains a cDNA that encodes the mature decorin core protein and does not contain sequence encoding the endogenous signal sequence or the putative propeptide domain. pDCN1 was transcribed and translated in vitro in the presence of Trans35S-label (Fig. 1b). The radiolabeled products migrated as a single predominant band with an apparent molecular mass of 46 kDa (lane 1) and were co-infected with PR2 decorin-specific antibody (lane 2) showing that the translation product (Fig. 1a) was immunoreactive decorin. The material eluted from the column with 0.3 mg/ml nitroblue tetrazolium and 0.15 mg/ml 5-bromo-4-chloro-3-indolyl phosphate in 100 mM Tris-HCl, 100 mM NaCl, 5 mM MgCl2, pH 9.5 was used for SDS-PAGE and visualized by fluorography.

**FIG. 1. Fusion protein expression vector and in vitro transcription and translation of decorin core protein. a, the expression construct pDCN1 comprising an insulin signal sequence (INS), six consecutive histidine residues (His × 6), a factor Xa protease recognition sequence (Xa), and sequence encoding the mature decorin core protein under the control of the bacteriophage T7 promoter (T7). b, pDCN1 was transcribed in vitro with T7 RNA polymerase and the resultant RNA translated with rabbit reticulocyte lysate. Total in vitro translation product (lane 1) and immunoprecipitated translation product with PR2 decorin-specific antibody (lane 2) were resolved by 7.5% SDS-PAGE and visualized by fluorography.
Fig. 2. Purification of recombinant decorin. HT-1080 cells were co-infected with vDCN1 and vTF7-3 at a multiplicity of infection of 10 plaque-forming units/cell. Cells were incubated for 24 h in serum-free medium containing Trans35S-label and the medium was harvested. a, nickel chelating chromatography. Fractions eluted from the nickel column were monitored for radioactivity (•), and imidazole concentration (○). b, SDS-PAGE. Aliquots from selected fractions (*, panel a) were concentrated and resolved by 7.5% SDS-PAGE and visualized by fluorography. Square bracket indicates the position of the intact decorin proteoglycan, double lines indicate the doublet core protein bands.

Fig. 3. Synthesis and purification of chemical amounts of recombinant decorin. HT-1080 cells (~2.5 x 10^9 cells) were co-infected with vDCN1 and vTF7-3 and the conditioned media harvested at 30 h and applied to a nickel column. Aliquots from the 60 mM imidazole elution (lanes 1–3) and 80 mM imidazole elution (lane 4) were resolved on a 7.5% SDS-PAGE and stained with Coomassie Blue R-250. Ten μg (lane 1), 5 μg (lane 2), and 1 μg (lane 3) of decorin proteoglycan (square bracket) and 5 μg (lane 4) of decorin core protein (indicated by the double lines) were loaded onto the gel.

About 75% of the total recombinant protein purified from the culture supernatant was the proteoglycan form of decorin based on incorporation of 35S-radioactivity (Cys and Met) before and after chondroitinase ABC digestion. In addition to the apparent high level of purity attained by the nickel column, we also noted that there was some resolution into subpopulations: the early eluting decorin proteoglycan was of larger molecular size compared to the later eluting proteoglycan (Fig. 2b, fractions 30 and 46, square brackets). The resolution of two sub-populations of proteoglycan was clearer when the proteins were labeled with [35S]sulfate and [3H]glucosamine (see below). Although separation into subpopulations was more evident using a step elution procedure, it was still apparent during gradient elution procedures (data not shown). The core protein form of decorin also migrated on SDS-PAGE as a doublet, molecular mass ~ 53 and ~ 49 kDa, with the larger core protein eluting slightly ahead of the smaller form on the nickel column (Fig. 2b, fractions 46–52).

Recombinant decorin was isolated from a large number of co-infected HT-1080 cells (2.5 x 10^9) by a preparative scale nickel column. The concentration of purified samples were estimated by theoretical molar extinction coefficient and analyzed by 7.5% SDS-PAGE stained with Coomassie Blue (Fig. 3). Decorin proteoglycan was predominantly eluted in the 60 mM imidazole fraction, and 10, 5, and 1 μg were applied to the gel (Fig. 3, lanes 1–3, respectively). Decorin core protein was enriched in the 80 mM imidazole elution and an estimated 5 μg was loaded (Fig. 3, lane 4) which migrated as a doublet, with the larger form (molecular mass ~ 53 kDa) more abundant. No other contaminating protein bands were visualized on the gel, indicating purity of the sample. Based on these data, we are able to estimate that expression and purification of recombinant decorin under native conditions (i.e. in the absence of chaotropic solvents or detergents) yields about 10 mg of proteoglycan and 3.3 mg of core protein/10^9 cells per 24 h. Recombinant decorin proteoglycan was also isolated and purified under denaturing conditions from HT-1080 culture supernatant in the presence of the chaotropic solvent 4 M GdnHCl and the detergent 0.5% Triton X-100. The use of this solvent throughout the extraction and purification protocol increased the final yield of proteoglycan 2–3-fold compared with the yield under
native conditions. The significant increase in yield of recombinant protein in the presence of detergent was most likely a consequence of the hydrophobicity of native material.

Differential Glycosylation of Recombinant Decorin—Samples of Trans35S-labeled decorin proteoglycan and core protein were subjected to differential digestion with glycosidases to assess the nature of the carbohydrate substitutions. Undigested sample (Fig. 4, lane 1) shows the presence of the proteoglycan smear and a doublet core protein. Following digestion with chondroitinase ABC (Fig. 4, lane 2), the proteoglycan smear is no longer visible and all the radiolabel is coincident with the core protein doublet. Separate experiments showed that the proteoglycan was equally susceptible to chondroitinase ACII digestion indicating that the proteoglycan contained predominantly chondroitin sulfate. Chondroitinase ABC-treated sample was further incubated with N-glycosidase F (Fig. 4, lane 3) which resulted in the doublet core protein reducing to a single band with an apparent molecular mass ~ 43 kDa. Chondroitinase ABC digestion of proteoglycan generated a doublet core protein, indicating that both core protein forms contain GAG.

Proteoglycan Characterization—HT-1080 cells co-infected with vDCN1 and vTF7–3 were incubated in the presence of [35S]sulfate and [3H]glucosamine. Recombinant decorin proteoglycan was purified by nickel chelating affinity chromatography in physiological solvents (50 mM Tris-HCl, pH 6.5) and was resolved into two distinct peaks: one eluting at 60 mM imidazole (pool 1) and the second eluting at 70–80 mM imidazole (pool 2). Aliquots of each pool were subjected to Superose 6 gel filtration chromatography. Panels a, pool 1, untreated; b, pool 2, untreated; c, pool 1, incubated with chondroitinase ABC; d, pool 2, incubated with chondroitinase ABC.

\[ K_d = 0.40 \text{ (mass } - 94 \text{ kDa, compared to globular protein standards)} \]

\[ K_d = 0.60 \text{ (mass } - 80 \text{ kDa)} \]

\[ 35S : 3H \text{ ratio reflected the composition of the two pools. The early eluting peak (pool 1) and the later eluting peak (pool 2) were collected and aliquots subjected to gel filtration chromatography on Superose 6. Pool 1 eluted predominantly as a single broad peak (Fig. 5a) centered at } K_d \text{ 0.40 (mass } - 94 \text{ kDa, compared to globular protein standards), with the } 35S \text{ and } 3H \text{ coincident. Pool 2 resolved into a single broad } 35S \text{-labeled peak centered at } K_d \text{ 0.45 (mass } - 80 \text{ kDa); and two distinct } 3H \text{-labeled peaks, one coincident with the } 35S \text{ label, and a second, sharp peak at } K_d \text{ 0.60 (mass } - 50 \text{ kDa)} \]

which consisted exclusively of } 3H \text{ label (Fig. 5b). Based on the
predicted composition of the two pools (see Fig. 2b), pool 1 represents decorin proteoglycan alone and pool 2 comprises a mixture of proteoglycan and core protein. The core protein from pool 2 is radiolabeled via 3H incorporation into N-linked oligosaccharides. The broad, high molecular weight peaks in both pools represent proteoglycan containing 35S- and 3H-labeled chondroitin sulfate chains. This was confirmed by chondroitinase ABC digestion, the Superose 6 column resolves a core protein pool of molecular mass 54% Kd 0.60 (Fig. 5, asterisk). After chondroitinase ABC digestion, the Superose 6 column resolves a core protein pool of molecular mass -50 kDa, consistent with the core protein size determined on SDS-PAGE (49 and -53 kDa).

Molecular weight
Disaccharide Analysis

| Pool | Pool 1 | Pool 2 |
|------|-------|-------|
| Mass | 94 | 80 |
| 3H | 30 | 20 |
| 3S | 19 | 30 |
| 4S | 7 | 15 |
| 6S | 74 | 55 |

The molecular size difference between the decorin proteoglycan in pools 1 and 2 is illustrated by their different migration positions on SDS-PAGE (Fig. 6a). This size difference is largely due to differences in chondroitin sulfate chain size as determined from Superose 6 chromatography after alkaline hydrolysis. However, anomalous migration of proteoglycans on SDS-PAGE may be influenced by microheterogeneity in disaccharide composition which could affect the total charge density of the molecule. Disaccharide analysis of the chondroitin sulfate chains (summarized in Fig. 6b) show ratios of Δdi-0S:Δdi-4S:Δdi-6S in the larger chondroitin sulfate chains (pool 1) of 0.7:1.0:1.0, markedly different for that observed for the shorter chains (pool 2) of 2.0:1.0:3.7. These two subpopulations partially resolve by nickel chelating chromatography and this may relate to both the size and different disaccharide composition of the chondroitin sulfate chains.

Circular Dichroism—Recombinant decorin proteoglycan purified under native conditions was analyzed by CD spectroscopy. The far-UV spectra of the native proteoglycan showed greater than 70% secondary structure which was predominantly β-sheet as indicated by a sharp minima at 218 nm (Fig. 7a, closed circles). Analysis of the CD spectra based on computer modeling (27) predicted the native proteoglycan to comprise 54% β-sheet, 14% β-turn, 12% α-helix, and 20% random coil. Recombinant decorin proteoglycan was also isolated and purified under denaturing conditions from HT-1080 culture supernatant in the presence of the chaotropic solvent 4 M GdnHCl and 0.5% Triton X-100. After exchange back into physiological solvent, the CD spectra obtained for this material (Fig. 7a, open circles) revealed a sharp minima around 205 nm indicating an unordered conformation with no detectable secondary structure. Decorin extracted from bovine articular cartilage, representative of material utilized in numerous binding studies (29, 30), was also devoid of any secondary structure (Fig. 7a, open squares), and was similar to the spectra obtained for native decorin after thermal denaturation. The CD spectra for similarly produced recombinant biglycan (34) was identical to that for recombinant decorin; and exposure to denaturants resulted in disruption of the secondary structure. Consistent with these observations, significant secondary structure in the recombinant decorin proteoglycan, the PR2 antiserum selectively immunoprecipitated guanidine HCl-extracted decorin core protein (Fig. 7b, lane 1) but not the native recombinant decorin (Fig. 7b, lane 2), however, both denatured and native preparations of recombinant decorin were recognized by Western blot analysis (Fig. 7b, lanes 3 and 4). This is consistent with the epitope being inaccessible in the native conformation (i.e. conditions for immunoprecipitation), but available upon unfolding of the molecule (i.e. SDS treatment and transfer to nitrocellulose membrane).

The binding of 3H-labeled proteoglycan and core protein to C1q was subjected to competition by the addition of unlabeled decorin proteoglycan and core protein to collagen types I, II, III, V, VI, C1q or BSA was examined, and showed an identical pattern to that of biglycan (34). The binding of decorin to the fibrillar collagens was overshadowed by the much greater binding observed with C1q. Interaction with C1q appears to be mediated by the core protein of decorin, with binding up to 18-fold over the BSA control compared to 9-fold binding of intact proteoglycan. Krumdieck et al. (21) have shown that decorin proteoglycan and chondroitinase ABC-generated core protein from bovine articular cartilage also bind C1q.

The binding of 125I-labeled proteoglycan and core protein to C1q was subjected to competition by the addition of unlabeled decorin proteoglycan and core protein (Fig. 8a, open and closed circles, respectively). Both decorin proteoglycan and decorin core protein inhibited binding of 125I-labeled decorin to C1q. The IC50 (concentration of competitor required for half-maximal inhibition) for the core protein was 28 μg/ml (0.62 μM) and for intact proteoglycan the IC50 value was 18 μg/ml (0.2 μM; Fig. 8a). Saturability of the decorin-C1q interaction was...
Recombinant Decorin Expression

Fig. 8. Decorin-C1q interaction. a. $^{125}$I-labeled decorin proteoglycan (○) and $^{125}$I-labeled core protein (●) binding to C1q-coated microtiter wells was inhibited with increasing amounts of unlabeled recombinant intact proteoglycan (■) and core protein (●). All determinations were done in triplicate (mean ± S.D.). b and c. Scatchard analysis. Microtiter wells coated with 2 µg of C1q or 1% BSA were incubated with increasing concentrations of $^{125}$I-labeled decorin proteoglycan or $^{125}$I-labeled core protein (specific activity 200,000 cpm/µg). The amount of bound and free ligand was quantitated for decorin proteoglycan (○) and core protein (●). The insets show that binding was saturable.

![Graph showing Scatchard analysis](image)

In this study, differentially glycosylated forms of decorin have been overexpressed in a eukaryotic cell line with a connective tissue origin (HT-1080). These decorin glycoforms were separated from other secreted molecules to greater than 95% purity, and further partially resolved by metal chelating affinity chromatography into two distinct populations: proteoglycan forms and core protein forms. The proteoglycan population was further partially resolved into two species distinguished by different sized chondroitin sulfate chains with distinct disaccharide composition; the core protein subpopulation was also represented by two distinct forms, distinguished by the extent of N-linked oligosaccharide substitution. The core protein preparations generated by chondroitinase ABC digestion of the proteoglycan pool comprised both the 2 and 3 N-linked oligosaccharide forms; we cannot exclude the possibility that the different sized GAG chains are on different core proteins (i.e., the 2 and 3 N-linked oligosaccharide forms, respectively), however, this would seem unlikely given a similar bi-modal population of GAG chains was observed for recombinant biglycan (34) where there is no evidence for expression of two core proteins.

The two non-proteoglycan forms of recombinant decorin had an apparent mobility of ~49 and ~53 kDa on SDS-PAGE. Differential N-glycosylation of decorin synthesized by human skin fibroblasts in vitro has been demonstrated previously (32), whereby chondroitinase ABC-digested decorin migrated as a doublet core protein preparation which further reduced to a single band when synthesized in the presence of tunicamycin. Although these investigators were able to induce N-glycan-free decorin proteoglycan synthesis through exogenous addition of tunicamycin, we saw no evidence for expression of similarly N-glycan-free decorin core protein expression, nor is there any evidence that this is a biosynthetic product in vivo. In fact, it is likely that core protein devoid of both GAG and N-linked oligosaccharides may be insoluble, as evidenced by the difficulties associated with bacterial expression of these molecules (33).

The different glycoforms of decorin expressed in this system are similar to the variety of decorin glycanated and non-glycanated species already identified in tissues. The proteoglycan form of the recombinant decorin is characteristic of material extracted from tissues in terms of chondroitin sulfate chain length and pattern of sulfation, as well as differential N-glycosylation. The recombinant decorin secreted devoid of GAG chains is likely a product of overexpression of core protein exceeding the capacity of one or more glycosyltransferases. Purification and quantitation of the recombinant decorin indicates that the HT-1080 cells secreted up to 30–40 mg of decorin/10⁹ cells per 24 h, about 75% as a proteoglycan form and the remainder as core glycoprotein. Under native conditions we experienced significant losses of material, but are nevertheless able to purify chemical amounts that are correctly folded and biologically active.

Circular dichroism spectra and secondary structure prediction clearly demonstrate that about 70% of the native recombinant intact decorin assumes a secondary structure with a high proportion of β-sheet content. This is not surprising given that about 70% of the core protein is comprised of the LRR domain which is predicted to assume a complex structure in solution. However, both recombinant decorin subjected to denaturing conditions and decorin extracted from cartilage by standard techniques (29, 30) demonstrated a loss of secondary structure in their CD spectra. This strongly argues that techniques commonly employed for isolation of decorin and biglycan from tissues (23, 24, 29) have a permanent effect on the conformation of the core protein. These changes in secondary structure may have implications on the interpretation of data reported by others related to interactions between decorin and biglycan with other extracellular matrix molecules. A recent
study (23) reported that decorin extracted from cartilage with GdnHCl was not an effective competitor of native decorin binding to type I collagen. Given many previous reports of GdnHCl-extracted decorin interacting with type I collagen in a variety of assays, it is possible that there are both secondary structure-dependent and-independent interactions. It is likely that interactions requiring correctly folded decorin are important in vivo so it is important to investigate these interactions using native preparations.

Decorin bound very effectively to the complement component C1q, showing an 18-fold increase in binding with core protein compared to BSA, and a 9-fold increase in binding with the proteoglycan form. Since the presence of a GAG chain appears to have attenuated the extent of binding it is likely that this interaction is mediated via the core protein, and the GAG chain may interfere with the ability of C1q to access the core protein domain. C1q is a subcomponent of the C1 complex, the first component of the classical pathway of complement activation (21, 22). The C1q molecule consists of two domains, a collagen-like triple helical domain at the N terminus and a globular configuration at the C terminus and circulates in the plasma as a Ca\(^{2+}\)-dependent complex with C1r and C1s (22). Although the biological significance of C1q-decorin interaction is not known, decorin may modulate complement-mediated inflammation at sites where extracellular matrices containing decorin are exposed to complement components (21). The C1q-decorin interaction was competitively inhibited by both core protein and proteoglycan preparations, however, an intact proteoglycan (i.e., substituted with a GAG chain) is required for inhibition of the complement cascade (21). Although both native and denatured decorin bind to C1q, it remains to be determined if the two forms interact via the same domain or whether the interaction with denatured decorin is simply nonspecific binding. The specificity and affinities of native decorin glycoforms for collagen-like molecules is the subject of further study.

This study, and the accompanying paper (34), have demonstrated the high level expression and purification under native conditions of the small interstitial proteoglycans, decorin and biglycan. These glycoconjugates possess a high degree of secondary structure not apparent in similar molecules extracted from hard tissues by standard techniques, in addition to maintaining biological activity in several binding assays. Furthermore, several glycoforms have been isolated that are similar to subpopulations present in a variety of tissues in vivo. These recombinant decorin proteoglycan and core protein forms isolated in chemical amounts will allow further study on the biological role and molecular interactions of this class of LRR-containing molecules.

Acknowledgments—We thank Drs. Karen House-Pompeo and Steve LaBrenz for help with the CD analysis, and Dr. Gurusiddappa for making the synthetic peptide. Bovine decorin extracted from articular cartilage was kindly provided by Dr. Larry Rosenberg (Montefiore Medical Center, New York). We also thank Dr. Magnus Höök for critical review of the manuscript.

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