Opticin is a class III member of the extracellular matrix small leucine-rich repeat protein (SLRP) family that was initially identified in the eye in association with the collagen fibrils of the vitreous humor. Recombinant and tissue-extracted forms of bovine opticin were subjected to biochemical and biophysical characterization. Following SDS-PAGE the predominant component produced by both forms was a broad band between 45–52 kDa. There was evidence for two-stage processing and, additionally, a proteolytic cleavage product of ~25 kDa. Deconvolution of circular dichroism spectra revealed β-sheet (41%), β-turn (21%), and α-helix (10%), and thermal denaturation experiments showed a transition with a midpoint of 47 °C. Weight-averaged molecular mass measurements using both light scattering and analytical ultracentrifugation demonstrated that opticin exists in solution as a stable dimer of ~90 kDa, which can be dissociated into a monomer by denaturation with 2.5 M guanidine hydrochloride or during SDS-polyacrylamide electrophoresis. Opticin remains a dimer after removal of the amino-terminal region by O-sialoglycoprotein endopeptidase digestion, suggesting that dimer formation is mediated by the leucine-rich repeats. Dimerization could have a number of functional consequences, including divergent ligand interactions.

The extracellular matrix small leucine-rich repeat proteins (SLRPs) are a family of molecules to which various functions have been ascribed, including regulation of matrix assembly, binding to growth factors, and suppression of cell growth (for review, see Ref. 1). They contain a varying number of leucine-rich repeats (LRRs) that contain the consensus sequence LNNXXL, where X is any amino acid, L is leucine, isoleucine or valine, and N can be asparagine, cysteine or threonine. The LRRs are generally flanked by 4 cysteine residues at the amino-terminal end and two at the carboxyl-terminal end. The SLRPs have been subdivided into three classes depending upon their secondary structure and thermal denaturation profile to class I and II SLRPs, little is known about the properties of the class III members. Here we show that opticin has a similar LRR domain structure with 73% of amino acids showing identity or similarity between the three protein sequences. However, the regions amino-terminal to the 4 cysteine clusters are more conserved among class III members, including regulation of matrix assembly, binding to growth factors and suppression of cell growth.

Opticin was first identified in a 4 M guanidine hydrochloride (GdnHCl) extract of collagen fibrils from the vitreous humor of the eye (3). Opticin expression is largely confined to the eye and, in the mouse, was localized specifically to the non-pigmented epithelium of the ciliary body (8). However, expressed sequence tag analyses of adult human iris and retinal pigment epithelium/choroid (available at neibank.nei.nih.gov/index.shtml) suggest that opticin is a very common transcript in these tissues (9–11). Furthermore, recently published immunolocalization data suggests the presence of opticin in a number of ocular tissues, including the cornea, iris, ciliary body, vitreous, choroid, and retina (12). Friedman et al. (12) also suggest that mutations in the opticin gene may be associated with age-related macular degeneration, the commonest cause of blindness in the Western World.

Opticin, epiphycan, and osteoglycin/mimecan possess very similar LRR domains with 73% of amino acids showing identity or similarity between the three protein sequences. However, the regions amino-terminal to the 4 cysteine clusters are more diverse. The amino-terminal region of opticin contains a cluster of sialylated O-linked oligosaccharides (3), epiphycan/PG-Lb has an amino-terminal dermatan sulfate side chain and two O-linked oligosaccharides (5), whereas osteoglycin/mimecan bears N-linked keratan sulfate side chains that are attached in the LRR domains (7).

Although there is a body of data concerning the physical properties of the class I and II SLRPs, little is known about the class III members. Here we show that opticin has a similar secondary structure and thermal denaturation profile to class I and II SLRPs and that it exists as a stable dimer in solution.

**EXPERIMENTAL PROCEDURES**

Cloning and Sequencing of Bovine Opticin cDNA—A custom oligo(dT) primed Uni-ZAP™ cDNA library was prepared by Stratagene from RNA extracted from whole fetal bovine eyes. Part of the coding region of bovine opticin was amplified from the library by PCR using the primers 5′-atgagagctctgctgtctgtc-3′ and 5′-tcagggcaggttcag-3′, which were designed by aligning the human and mouse opticin cDNA sequences. The 3′ end of the cDNA sequence was obtained by 3′ rapid amplification of cDNA ends using oligo(dT)™-primed total RNA extracted from fetal bovine eyes. The sense primer 5′-agtctctcaggccca-3′ was designed in the coding region of the bovine opticin cDNA. This primer was used in conjunction with primers complementary to the 3′

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adaptor and added during the 3′ rapid amplification (Invitrogen). PCR products were cloned using the TA Cloning® method (Invitrogen) and sequenced using M13R and M13(-20) primers with ABI Prism™ dye terminator cycle sequencing reagents (PerkinElmer Life Sciences). The products were analyzed on an ABI 377 machine. The 5′ end of the open reading frame was completed after identifying a bovine opticin expressed sequence tag sequence (AW430351). The translation of this sequence was interrogated with various proteomics tools at the ExPaSy web site (ca.expasy.org).

Construction of Expression Vector—Bovine ciliary body RNA was used as a template for an oligo(dT)–primed reverse transcription–polymerase chain reaction using Superscript II reverse transcriptase (Invitrogen). The cDNA was generated using a polymerase chain reaction with the primers 5′-tagtagcatctctcagagagaaag-3′ and 5′-ctgtctgaagtctggcctgtc-3′ corresponding to nucleotides 85–1120–1135 of the opticin cDNA sequence (Fig. 1) with the addition of NheI and BamHI restriction sites, respectively. The amplified cDNA was digested with the restriction enzymes NheI and BamHI and cloned into the pCEP4 vector (Invitrogen) incorporating the BM40 signal peptide, which was used to replace the opticin signal peptide. The resultant fusion protein, after removal of the signal peptide, was predicted to contain three amino-terminal vector-derived amino acids (i.e. APL).

Protein Expression and Purification—The expression vector was transfected into 293-EBeta cells (human embryonic kidney cells, Invitrogen) using Lipofectamine2000 reagent (Invitrogen). The cells were cultured in Dulbecco’s modified Eagle medium containing 2% fetal calf serum and, after 24 h, the transfected cells were selected by the addition of puromycin (5 μg/ml). Upon confluence, the medium was replaced with Dulbecco’s modified Eagle medium without fetal calf serum. The cells were incubated in this medium for 48 h, the conditioned medium was collected, centrifuged at 3000 × g for 5 min, and the protease inhibitors EDTA (5 μM, final concentration) and phenylmethylsulfonyl fluoride (0.5 mM, final concentration) were added.

Purification of Recombinant Bovine Opticin—Conditioned medium (2–4 liters) was applied at a flow rate of 5 ml/min to a 30 ml Fast Flow DEAE-Sepharose (Sigma) column equilibrated in 50 mM Tris-HCl, pH 7.4 (Tis buffer). The column was washed with 0.2 mM NaCl in Tris buffer (TBS) containing 0.2% CHAPS and then with TBS alone. The column was then eluted with 0.7 mM NaCl in Tis buffer. The fraction containing opticin was diluted to a final NaCl concentration of 0.1 M and applied to a 3 ml Jacalin lectin-Sepharose (Sigma) affinity column equilibrated with 0.1 M NaCl in Tis buffer. The column was washed with the same buffer prior to elution with 1 mM melibiose in 0.1 M NaCl, Tris buffer. The effluent from this column was applied directly to a 1.5 ml Fast Flow DEAE-Sepharose column equilibrated in TBS. The column was washed with TBS and eluted with a linear gradient of 0.2–0.7 M NaCl in Tris buffer. Fractions (0.5 ml) were collected, and those containing opticin were identified by SDS-PAGE with silver staining and Western blotting. The opticin was stored in frozen (−70 °C), concentrated aliquots and not subjected to lyophilization during any of the experiments.

Purification of Opticin from Bovine Vitreous Humor—Frozen bovine vitreous humor from 100 bovine eyes was thawed, and the collagen fibrils were pelleted by centrifugation. The pellet was initially washed with Tris buffer containing 0.15 M NaCl, 2 mM EDTA, 5 mM N-ethylmaleimide, 5 mM benzamidine, and 2 mM phenylmethylsulfonyl fluoride. The pellet was then washed three times with 5-liters volumes of 0.1 M NaCl in Tris buffer (TBS) containing 0.2% CHAPS and then with TBS alone. The column was then eluted with 0.7 M NaCl in TBS buffer. The fraction containing opticin was diluted to a final NaCl concentration of 0.1 M and applied to a 3 ml Jacalin lectin-Sepharose column equilibrated in TBS and then with TBS alone. The column was then eluted with 0.7 M NaCl in Tis buffer. The fraction containing opticin was diluted to a final NaCl concentration of 0.1 M and applied to a 3 ml Jacalin lectin-Sepharose (Sigma) affinity column equilibrated with 0.1 M NaCl in Tis buffer. The column was washed with the same buffer prior to elution with 1 mM melibiose in 0.1 M NaCl, Tris buffer. The effluent from this column was applied directly to a 1.5 ml Fast Flow DEAE-Sepharose column equilibrated in TBS. The column was washed with TBS and eluted with a linear gradient of 0.2–0.7 M NaCl in Tris buffer. Fractions (0.5 ml) were collected, and those containing opticin were identified by SDS-PAGE with silver staining and Western blotting. The opticin was stored in frozen (−70 °C), concentrated aliquots and not subjected to lyophilization during any of the experiments.
Thr residues differ between species, this region is present in all of them (Fig. 2) and, at least in human and mouse genomic sequences, is contained within a single exon (i.e., exon 3). The Sulfinator program (21) predicts that Tyr61 is sulfated and that the equivalent Tyr residue is sulfated in all four species.

Purification and Comparison between Recombinant and Extracted Bovine Opticin

The sialylated O-linked oligosaccharides of opticin were utilized to purify both recombinant and extracted opticin with sialylation contributing anionic charge for ion-exchange chromatography and Jacalin lectin binding to the core Gal(β1→3)GalNAc- of the O-linked oligosaccharides. During the second DEAE ion-exchange step the opticin eluted from the column between 0.25 and 0.7 M NaCl, and fractions eluting between 0.35 and 0.7 M NaCl were deemed pure. The recovery of purified recombinant opticin was between 0.5 and 0.75 mg/ml of conditioned culture medium.

Analysis of the tissue-extracted and recombinant opticin by SDS-PAGE and silver staining in both cases revealed a rather broad band of reactivity in the 45- to 52-kDa region, with the extracted protein(s) migrating somewhat faster than the recombinant protein(s) (Fig. 3). The OPT-C antiserum appeared to detect the same components as the silver stain. However, the OPT-N antiserum appeared to detect only the slower migrating protein in both the extracted and recombinant preparations. Because the OPT-N antiserum recognizes the amino-terminal region of the mature protein, we surmise that the faster migrating components in the 45- to 52-kDa regions are proteolytically cleaved forms lacking the amino-terminal region. In ad-

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**Fig. 1.** cDNA sequence and deduced protein sequence of bovine opticin. The positions of the primers used to determine the central portion of the cDNA sequence by amplification of a fetal bovine eye cDNA library are underlined (single line). The 3' end of the expressed sequence tag sequence (GenBank™ accession number AW303551) used to complete the 5' end of the opticin cDNA sequence is indicated (Δ). The primer sequence used for the 3' rapid amplification of cDNA ends is underlined (dotted line). The translation initiation codon and termination codon are boxed, and the predicted polyadenylation sequence is double-underlined. In the protein sequence, the signal peptide is double-underlined. The cysteine residues are highlighted with a gray background, and the LRR consensus sequences are in boldface.

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dition, a minor 25-kDa component was observed in both the tissue-extracted and recombinant opticin that was recognized by OPT-C, but not OPT-N, suggesting that it represents the carboxyl-terminal half of the molecule. Peptide mass mapping demonstrated that this 25-kDa component contains the opticin LRR domain (data not shown), but the sequence of the amino-terminal end of this component could not be identified. Mass mapping of peptides produced by trypsin, with and without reduction of disulphide bonds, confirmed that the two carboxyl-terminal cysteines form a disulfide bridge. A minor 90-kDa component was observed in the recombinant opticin that probably represents a small amount of opticin dimer that is resistant to cleavage during SDS-PAGE (including after analysis under reducing conditions; data not shown), possibly due to non-disulfide cross-link formation (see "Discussion").

Circular Dichroism and Melting Temperature Determination—The circular dichroism spectra for tissue-extracted and recombinant opticin were very similar (Fig. 4A). They both demonstrated three Cotton effects, including minima at 217 and 195 nm, and a weak maximum at 204 nm. Three deconvolution programs, including CONTIN/LL, SELCON3, and CDSSTR, were used to estimate secondary structure. All three methods gave generally similar results, but CDSSTR gave the best fit to the data. The data for extracted and recombinant opticin were virtually identical, and average values for secondary structure composition from CDSSTR analysis were 41% β-sheet, 21% β-turn, and 10% α-helix. The melting temperature of both extracted and recombinant opticin were determined by monitoring ellipticity at 205 nm (Fig. 4B). The thermal denaturation curves were similar, and both showed a single, cooperative transition with a mid-point (T_m) of about 47 °C. Interestingly, there appeared to be a slight decrease in ellipticity at 38–40 °C when compared with 35 °C, suggesting that subtle changes in secondary structure occur around physiological temperatures.

MALLS of Recombinant Opticin—Recombinant opticin (between 0.5 and 1 mg) was subjected to Superose 12 size exclusion chromatography in TBS and the column effluent passed in-line through a differential refractometer and multi-angle laser light scattering detector. A single major refractive index peak was observed eluting at -11 ml, and SDS-PAGE analysis revealed that this peak contained the opticin (data not shown).
However, a large light scattering signal was associated with a small amount of material in the void volume of the column that spilled over into the opticin-derived light scattering peak and interfered with the $M_w$ estimations. Therefore, a 1-ml fraction that straddled the opticin-derived refractive index peak (at 11 ml) was collected and rechromatographed on the Superose 12 column (Fig. 5A). This step minimized the spillover of light scattering from the void volume and allowing a more accurate $M_w$ estimation. A refractive index peak was observed at 10.4 ml, and the $M_w$ of the material that eluted between 10 and 12 ml (i.e. the opticin) was calculated using the ASTRA™ software. This experiment was repeated four times (using two different preparations of recombinant opticin), and the opticin concentration within the analyzed peaks (calculated from the refractive index) was between 0.2 and 1 M. The average $M_w$ obtained for four different experiments was found to be 88.4 ± 3.7 kDa (Table I). Because the monomer molecular mass is ~45 kDa (from SDS-PAGE or analysis of compositional analysis), opticin appears to behave as a dimer under these conditions. Only a single refractive index peak (at 10.4 ml) was observed, so there was no evidence of dissociation of the 88.4-kDa material into monomer-sized material at this concentration.

Experiments were then undertaken to determine the $M_w$ of opticin in chaotrophic concentrations of guanidine hydrochloride. The initial opticin-containing peak after the first round of Superose 12 chromatography was dialyzed into Tris-GdnHCl and reanalyzed on the same column equilibrated in Tris-GdnHCl (Fig. 5B). This time a single peak was observed at 11.75 ml (Fig. 5B). The $M_w$ was calculated of the material between 11.3 and 12.2 ml and found to be 43 kDa, i.e. similar to that of an opticin monomer.

In a further experiment, the opticin-containing peak following the first round of chromatography was digested with O-sialoglycoprotein endopeptidase prior to re-analysis on the Superose 12 column in TBS. This enzyme cleaves peptide bonds near Ser or Thr residues that are substituted with sialylated O-linked oligosaccharides (22). A peak was observed at 12.9 ml, and the $M_w$ of the material that eluted between 12.3 and 13.7 ml was calculated to be 63 kDa, i.e. larger than that of the opticin monomer (Fig. 5C). This material was analyzed by SDS-PAGE along with intact opticin for comparison, and the resultant polyacrylamide gel was either silver stained or Western blotted and probed with OPT-A, OPT-C, and Jacalin lectin (Fig. 5D). Following SDS-PAGE the pooled fractions between 12 and 14 ml migrated to ~32 kDa, and peptide mass mapping of the silver-stained band (data not shown) demonstrated that this component contained the LRR domain of opticin. Both the full-length and the enzyme-digested fragment of opticin were recognized by OPT-C and the Jacalin lectin. However, only the undigested opticin was recognized by OPT-A. Taken together these results confirmed that the O-sialoglycoprotein endopeptidase digestion had cleaved opticin within the O-glycosylated domain thus removing the amino-terminal part of the protein. The rest of the molecule, which was composed mainly of LRRs, remained dimerized.
Analysis of the sedimentation equilibrium data from the three chromatography in TBS the fraction that straddled a single refractive index peak at ~11 ml (data not shown) was rechromatographed for $M_w$ analysis. In each graph the thin, solid line represents the signal from the refractometer thus showing the mass distribution of the material eluting from the column. A, sample rechromatographed in TBS. Thick line (composed of data points) shows the light scattering signal at 90 degrees, and the bar represents the material (opticin) used for $M_w$ determination; $V_0$ = void volume. Material eluting between 10 and 12 ml was analyzed by SDS-PAGE and silver staining (inset) demonstrating that it contained opticin. B, sample rechromatographed in Tris-GdnHCl; the thick line shows the molecular weight profile across the peak. C, sample digested with O-sialoglycoprotein endopeptidase and rechromatographed in TBS. The individual data points show the molecular weight distribution across the peak. The material that eluted between 12 and 14 ml was pooled and analyzed by SDS-PAGE (with intact opticin for comparison). D, following SDS-PAGE, the intact opticin (lanes 1, 3, 5, and 7) and pooled fraction between 12 and 14 ml (lanes 2, 4, 6, and 8) were stained with silver (lanes 1 and 2) or Western blotted and probed OPT-C (lanes 3 and 4), Jacalin lectin (lanes 5 and 6), and OPT-A (lanes 7 and 8).

TABLE I

Summary of $M_w$ and shape data for opticin

| Physical property                  | Value ± S.D. |
|-----------------------------------|--------------|
| Weight-averaged molecular mass    | 88.4 ± 3.7   |
| from MALLS, $M_w$ (kDa)            |              |
| Weight-averaged molecular mass    | 90.6 ± 7.8   |
| from sedimentation equilibrium, $M_w$ (kDa) |        |
| Sedimentation coefficient, $s_{20,w}$ (s) | 3.41 ± 0.05 |
| Partial specific volume, $v$ (ml/g) | 0.721        |
| Translational frictional ratio, $f_f$ | 2.05 ± 0.03 |
| Hydrodynamic radius from gel filtration, $R_H$ (nm) | 6.9 ± 0.13 |
| Hydrodynamic radius from sedimentation velocity, $R_V$ (nm) | 6.0 ± 0.10 |

where $N_A$ is Avogadro’s number and $\rho$ and $\eta$ are the density and viscosity of water at 20 °C (17). When the values for $M$ (90.6 ± 7.8 kDa), $s_{20,w}$ (3.41 ± 0.05), and $\eta$ (0.721) for recombinant opticin are used, then the $f_f$ value obtained is 2.01 ± 0.03. This shows that the protein in solution is behaving as an elongated particle rather than as a sphere. The behavior on gel filtration chromatography is characteristic of a particle having a hydrodynamic radius, $R_H$ of 6.9 ± 0.13 nm, which is comparable to the estimated 6.0 ± 0.1 nm from sedimentation velocity analysis. Although the $f_f$ function does not take into consideration the effects of molecular hydration, the hydrodynamic radius estimates also suggest that the molecule has an elongated rather than a compact globular conformation.

On gel filtration chromatography the O-sialoglycoprotein endopeptidase-digested opticin eluted at 12.9 ml, and the $R_V$ was estimated to be decreased to 3.5 nm, i.e. half that of the undigested opticin ($R_H$ of 6.9 ± 0.13). These data show that, after removal of the amino-terminal regions of the opticin dimer the dimerized LRR domains adopt a compact configuration.

DISCUSSION

Here we provide the first biophysical characterization of a class III SLRP. In several of the experiments two forms of opticin were used; i.e. a recombinant form and a tissue form that had been extracted in 4 M GdnHCl and refolded. The recombinant and tissue-extracted forms showed similar profiles following SDS-PAGE with the main opticin component being a broad band of 45–52 kDa containing multiple forms. However, the broad band derived from the recombinant form migrated slightly more slowly than that derived from the tissue-extracted form. The presence of multiple forms and the slower migration on SDS-PAGE of the recombinant form may be due, in part, to variations in glycosylation. In addition,
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proteolysis contributed to the generation of multiple forms as the OPT-N antiserum only labeled the slower migrating components of the broad bands produced by both the recombinant and tissue-extracted opticin. This antiserum recognizes an epitope close to the amino terminus of the mature protein, so we conclude that a proportion of the mature opticin had undergone proteolytic cleavage to remove the OPT-N epitope (Figs. 2 and 6). Two-step processing (i.e. removal of the signal peptide and further removal of a propeptide) has been observed with other SLRPs, including decorin, biglycan, and epiphycan, although the functional significance of the second cleavage step remains undetermined (5, 23).

The susceptibility of opticin to digestion with O-sialoglycoprotein endopeptidase confirms that the Ser/Thr-rich region of the core protein has a molecular weight of 33,578 implying that the opticin monomer both by SDS-PAGE and MALSS in Tris-GdnHCl provide values of ~45 kDa. The core protein has a molecular weight of 33,578 implying that the O-linked oligosaccharides account for ~10 kDa. This “mucin-like” region is unique among SLRP family members.

The tissue-extracted opticin was exposed to 4 M GdnHCl and refolded, whereas the recombinant form had not been exposed to chaotropes. Concern has been expressed about the ability of the SLRP decorin to refold after exposure to GdnHCl (24). However, we have recently demonstrated that it is possible to refold decorin following exposure to GdnHCl (25) and the refolded, tissue-extracted opticin gave a CD spectrum that was indistinguishable from that of the recombinant form. The secondary structure estimated from the CD spectrum of opticin comprises β-sheet and β-turn as the main components, which is in keeping with previously published analyses of class I and II SLRPs (25–27). The lower proportion of ordered secondary structure in opticin, compared with that reported for decorin (26), is consistent with the smaller number of LRRs (7 as opposed to 12) and the probable random coil structure of the glycosylated amino-terminal region, which may be inferred from its susceptibility to proteolysis. The melting temperature of opticin was determined and the Tm found to be 47 °C; this relatively low value is similar to the Tm of 43.5 °C previously reported for decorin (28). Therefore, the differences in the number of LRRs between the class III and class I SLRPs do not greatly affect their thermal stability.

Two strategies were used to show that opticin exists as a stable dimer in solution. MALSS analysis demonstrated that virtually all the opticin eluted as a single peak with an Mr of 88.4 ± 3.7 kDa. Sedimentation equilibrium confirmed the Mr of opticin and therefore that under these experimental conditions it is a dimer. Furthermore, the MALSS analysis demonstrated that the dimer is stable down to a concentration of 0.2 μM. However, denaturation of the opticin in GdnHCl or during SDS-PAGE causes the dimers to dissociate into monomers, so that dimer formation may be dependent upon secondary structure. A small proportion of the opticin remained dimerized after SDS-PAGE, possibly due to cross-link formation. Similar dimer forms of chondroadherin have been observed following SDS-PAGE (29, 30).

We have recently shown that the Class I SLRP family member decorin exists as a stable dimer in solution (25). Furthermore, we have provided evidence that the decorin dimer is formed by association of the LRR domains, which interact through the inner β-sheets resulting in a conformation resembling intertwining Cs (25). Here we have demonstrated that opticin forms a dimer and remains dimerized after removal of most of the amino-terminal region with O-sialoglycoprotein endopeptidase, implying that opticin also dimerizes through its LRR domains. O-Sialoglycoprotein endopeptidase-digested opticin has a compact structure with an RH of 3.5 nm, which is compatible with the “intertwining Cs” model of dimerization. Although Class III SLRPs differ from Class I and II SLRPs in possessing approximately seven as opposed to twelve LRRs, we have now provided evidence that a Class III SLRP (opticin) and a Class I SLRP (decorin) dimerize through their LRRs. Furthermore, the Class I SLRP biglycan has been shown to form dimers reversibly (31). Taken together, these data raise the possibility that all SLRPs are capable of forming dimers through a similar mechanism.

Based upon our decorin model (25) and the data obtained in this study we propose a tentative model for opticin (Fig. 6). We suggest that opticin dimerizes in a similar manner to decorin and that the relatively long, glycosylated amino-terminal regions of opticin (i.e. amino-terminal to the four cysteine cluster) extend away from opposite sides of the compact LRR dimer, thus approximately doubling the RH of the molecule when compared with the LRR dimer alone.

Opticin and decorin form stable dimers in vitro under our experimental conditions and so far, the only way we have found to dissociate these dimers is to denature the protein. Therefore, it is possible that they remain dimerized when interacting with other molecules such as collagen in vitro. This would mean that such interactions could not involve a large part of the β-sheet as this would be buried within the dimer, so other parts of the molecules may be involved. As dimers, these molecules would be capable of bivalent interactions with consequences for calculations of the stoichiometry and the affinity of ligand binding.

Acknowledgments—We thank Dr. Tim Dafforn for assistance with CD spectroscopy and Professor Tim Hardingham for critical reading of the manuscript.

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Fig. 6. Tentative model of the opticin dimer. The LRR domains link together to form an intertwining Cs shape with the inner β sheets opposed to one another. The amino-terminal regions with “mucin-like” domains (oligosaccharides represented by fine lines) extend away from the compact LRRs. Regions containing intrachain disulfide bonds are indicated with vertical hatching. Black arrows indicate where recombinant and tissue-extracted forms appear to be susceptible to proteolytic cleavage during tissue processing or degradation, and the gray block arrows indicate where the opticin is apparently cleaved by O-sialoglycoprotein endopeptidase digestion.
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