A Disintegrin and Metalloproteinase with Thrombospondin Motifs-5 (ADAMTS-5) Forms Catalytically Active Oligomers*

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The metalloproteinase ADAMTS-5 (A disintegrin and metalloproteinase with thrombospondin motifs) degrades aggrecan, a proteoglycan essential for cartilage structure and function. ADAMTS-5 is the major aggrecanase in mouse cartilage, and is also likely to be the major aggrecanase in humans. ADAMTS-5 is a multidomain enzyme, but the function of the C-terminal ancillary domains is poorly understood. We show that mutant ADAMTS-5 lacking the catalytic domain, but with a full suite of ancillary domains inhibits wild type ADAMTS activity, in vitro and in vivo, in a dominant-negative manner. The data suggest that mutant ADAMTS-5 binds to wild type ADAMTS-5; thus we tested the hypothesis that ADAMTS-5 associates to form oligomers. Co-elution, competition, and in situ PLA experiments using full-length and truncated recombinant ADAMTS-5 confirmed that ADAMTS-5 molecules interact, and showed that the catalytic and disintegrin-like domains support these intermolecular interactions. Cross-linking experiments revealed that recombinant ADAMTS-5 formed large, reduction-sensitive oligomers with a nominal molecular mass of ~400 kDa. The oligomers were unimolecular and proteolytically active. ADAMTS-5 truncates comprising the disintegrin and/or catalytic domains were able to competitively block full-length ADAMTS-5-mediated aggrecan cleavage, measured by production of the G1-EGE373 neoepitope. These results show that ADAMTS-5 oligomerization is required for full aggrecanase activity, and they provide evidence that blocking oligomerization inhibits ADAMTS-5 activity. The data identify the surface provided by the catalytic and disintegrin-like domains of ADAMTS-5 as a legitimate target for the design of aggrecanase inhibitors.

ADAMTS-5 (TS5) is one member of a family of secreted, multidomain, zinc-dependent proteinases known as A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS).2 TS5 is a major aggrecan-degrading enzyme (aggrecanase) (1) mediating proteolysis and loss of aggrecan from articular cartilage in arthritic diseases. TS5 inhibitors are currently in development for arthritis therapies (2, 3), although ADAMTS-4 (TS4) might also have a role in human joint disease (4–6). The most important aggrecanase activity is cleavage at the EGE373→374 ALG (numbering is from1EEVP of the mouse mature protein [NCBI accession number Q61282]) bond in the aggrecan interglobular domain. Aggrecanases also cleave at conserved sites in the chondroitin sulfate-rich region of aggrecan, including SELE1L279↓1280GRGT, REE1467↓1468GLGS, TAQE1572↓1573AGEG, and VSQE1672↓1673LGHG (7, 8). Fragments arising from proteolysis at these sites have been detected in cartilage and synovial fluids from patients with arthritic diseases including rheumatoid arthritis, osteoarthritis, and juvenile idiopathic arthritis (9–12).

TS5 is a multidomain enzyme comprising an N-terminal pro (Pro)-domain, a catalytic (Cat) domain, a disintegrin (Dis)-like domain, two thrombospondin (TS)-type 1 motifs, a cysteine-rich (CysR) region, and a spacer (Sp) domain as reviewed previously (13–15). It is expressed as a zymogen that requires cleavage by pro-protein convertases (16, 17) at a polyarginine consensus sequence to remove the pro-domain. Further processing at the C terminus by autocatalysis gives rise to multiple forms of TS5, and recombinant C-terminal truncates have altered enzyme activity and substrate specificity (18, 19). The active and C terminally truncated forms of TS5 are readily detected in tissue extracts and experimental systems (20–22).

Previous studies have shown that the C-terminal ancillary domains of TS4 and TS5 govern the specificity of the enzymes by modulating substrate binding (18, 19, 23) and internalization via lipoprotein receptor-related protein receptors (24, 25). The results of studies in HTB-94 chondrosarcoma cells suggest that the CysR domain helps localize TS5 in the extracellular matrix (19), whereas in TS4, the spacer domain is thought to mediate matrix binding (26–29). Hyaluron binding to the Dis domain (20) and binding of heparan sulfate proteoglycans to the CysR domain, het, heterozygous; mBSA, methylated BSA; MMP, matrix metalloproteinase; MS, mass spectrometry; Pro, N-terminal prodomain; Sp, spacer domain; TS, thrombospondin-type 1 domains; TS5ΔCat, catalytically inactive ADAMTS5 with an in-frame deletion of exon 3; TS5 null, Adams5 deficiency created by substitution of exon 2 for an IRES-laZ-pgk neomycin (Neo) resistance cassette; TS5-FLAG, ADAMTS5 with a C-terminal FLAG tag; TS5-Myc, ADAMTS5 with a C-terminal Myc tag.

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2 The abbreviations used are: ADAMTS, A disintegrin and metalloproteinase with thrombospondin motifs; Cat, catalytic; CysR, cysteine-rich; Dis, disintegrin; het, heterozygous; mBSA, methylated BSA; MMP, matrix metalloproteinase; MS, mass spectrometry; Pro, N-terminal prodomain; Sp, spacer domain; TS, thrombospondin-type 1 domains; TS5ΔCat, catalytically inactive ADAMTS5 with an in-frame deletion of exon 3; TS5 null, Adams5 deficiency created by substitution of exon 2 for an IRES-laZ-pgk neomycin (Neo) resistance cassette; TS5-FLAG, ADAMTS5 with a C-terminal FLAG tag; TS5-Myc, ADAMTS5 with a C-terminal Myc tag.

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and Sp domains, and possibly TSS1 domain (19, 20) might also help to facilitate matrix localization.

To better understand the role of the TSS5 C-terminal ancillary domains and their influence on TSS5 activity, we compared aggrecanolysis in cartilage explanted from mice that are heterozygous for the TSS5cat mutation (TSS5+/−/cat) or the TSS5 null mutation (TSS5+/−). TSS5cat mice express the full suite of ancillary domains but lack TSS5 activity due to an in-frame deletion of exon 3 within the catalytic domain (30, 31). In contrast, TSS5 null mice express neither catalytic nor ancillary domains due to substitution of exon 2 in the catalytic domain, with an IRES-lacZ-pkg neomycin (Neo) resistance cassette (32). Our preliminary studies revealed that TSS5+/− mice had the same level of aggrecanase activity as wild type mice, however, TSS5+/−/cat mice had significantly reduced aggrecanase activity. This unexpected result suggested the possibility of an interaction between wild type and mutant TSS5 molecules in TSS5 cat cartilage, in which binding of the mutant TSS5cat protein, with its full set of ancillary domains, to wild type TSS5 inhibits enzyme activity in a dominant-negative manner. Accordingly, we hypothesized that native, functional TSS5 is a dimer or higher order oligomer. In this study we describe biochemical, we describe biochemical, experimental procedures and in vivo experiments designed to test this hypothesis and to reveal the underlying mechanism of oligomerization. Collectively, our results show that TSS5 forms catalytically active, oligomeric complexes via disintegrin and/or catalytic domain interactions.

Experimental Procedures

TSS5-deficient Mice—Mouse lines containing deletions of either exon 2 or exon 3 within the catalytic domain were used for this study. The TSS5cat mice containing an in-frame deletion of exon 3, produced by Lexicon and provided by Johnson and Johnson Pharmaceutical Research and Development LLC, have been published previously (30, 31, 33). The TSS5 null mice containing an IRES-lacZ-pkg neomycin (Neo) resistance cassette, substituted for exon 2 (Adams5m12gen), produced by Deltagen and purchased from Jackson Laboratories (Bar Harbor, ME) have also been published previously (32). Both mutant strains were back-crossed to the C57BL/6 background for more than six generations. The procedures for mouse work were approved by the Murdoch Childrens Research Institute Animal Ethics Committee, Melbourne, Australia.

TSS Expression Constructs—C-terminal FLAG-tagged human TSS5 in the pCEP4 vector (TSS5-FLAG-pCEP4) provided by Professor Hideaki Nagase and Dr. Kazuhiro Yamamoto has been described previously (19). A construct encoding C-terminal Myc-tagged human TSS5 in the pBudCE4.1 vector (TSS5-Myc-pCEP4) provided by Professor Hideaki Nagase and Dr. Kazuhiro Yamamoto. The modification was made using a forward primer 5′-ACTGTTACCCACCATGCTGCTCGGGTGCCC3′ containing a KpnI restriction site (underlined), Kozak sequence (bold), and TSS5 sequence (italics) and a reverse primer, 5′-AGCAGATCTC-TACAGATCCTCCTCCTCGATGACGCTTCTGTC-3′ containing a BglII restriction site (underlined), stop codon (bold), and a Myc sequence (italics). The construct was ligated into the EF-1α multiple cloning site of the pBudCE4.1 vector (Invitrogen), following predigestion with KpnI and BglII. Two constructs encoding TSS5 terminating after the Dis domain (TSS5–5–V5) or the catalytic domain (TSS5–6–V5) were prepared with both V5 epitope and histidine tags at the C terminus. The nomenclature used for the production of these truncated fragments is that published by Gendron et al. (19). Briefly, cDNA prepared from HEK293 cells using standard protocols was used as a template for PCR using the forward primer 5′-AGGTAACCACTATGCTGCTGCTCGGGTGCCCC-3′ containing a KpnI restriction site (underlined), Kozak sequence (bold), and TSS5 sequence (italics) and either the Dis domain reverse primer, 5′-GACGATCTCCTCCTCGATGACGCTTCTGTC3′ containing a BglII restriction site (underlined) or the catalytic domain reverse primer, 5′-AGCAGATCTCCTCCTCGATGACGCTTCTGTC-3′ containing a BglII restriction site (underlined). The PCR products were TA cloned, sequence verified, and subcloned into the EF-1α multiple cloning site of the pBudCE4.1 vector (Invitrogen) using the KpnI and BglII sites, maintaining the frame with the C-terminal V5 and histidine tags encoded by the vector.

Stable Expression and Purification of Recombinant Human TSS—After transfecting the TSS5-FLAG construct into HEK293-EBNA cells using LipofectamineTM LTX (Invitrogen), cells resistant to 250 μg/ml of hygromycin B were used to produce a stable cell line expressing FLAG-tagged TSS5 (TSS5-FLAG). These cells were subsequently transfected with the Myc-TSS5-pBud construct and cells resistant to 75 μg/ml of G418 (Sigma) were used to produce double, stable transfectants expressing both TSS5-FLAG and TSS5-Myc. For protein expression, transfected cells were cultured for 72 h in serum-free DMEM containing 0.2% lactalbumin hydrolysate (Sigma), then heparin (100 μg/ml; Sigma), was added for 3 days to displace TSS5 from the cell surface (19) and to prevent its internalization by LR1 (24). The conditioned media were collected and centrifuged to remove cell debris. Conditioned medium containing TSS5-FLAG was applied to a 2-ml column of anti-FLAG M2 Affinity Gel (Sigma) equilibrated in 150 mM NaCl, 50 mM Tris-HCl, pH 7.5 (Tris-buffered saline; TBS), washed with TBS containing 500 mM NaCl to remove heparin that may have bound to TSS5, then eluted in 0.5-ml fractions with 100 μg/ml of FLAG peptide (Sigma) in TBS for experiments to analyze enzyme activity, or into tubes containing 20 μl of 0.5 M EDTA (to prevent autolysis) for experiments to analyze the size of TSS5 fragments. Conditioned medium containing TSS5–5–V5 and TSS5–6–V5 double-tagged with V5 epitope and a His tag was applied to a 1-ml column of His-Select Nickel Affinity Gel (Sigma) in TBS for experiments to analyze enzyme activity, or into 1-ml columns of Zeta Spin desalting columns (Thermo Fisher Scientific). Briefly, the Zeta Spin columns were washed and equilibrated with 50 mM Tris, pH 7.5, 150 mM NaCl, 5 μM ZnCl2, 5 mM CaCl2 according to the man-
Antibodies—Commercial antibodies used in this study include α-FLAG M2 mouse monoclonal (Sigma), α-cMyc rabbit polyclonal (Sigma-Aldrich), α-cMyc 9E10 mouse monoclonal (Santa Cruz Biotechnology, Santa Cruz, CA), α-V5 mouse monoclonal (AbD Serotec), and HRP-conjugated secondary antibodies (Dako Cytomation, Denmark). The rabbit polyclonal α-EGE antibody against the ADAMTS-generated aggrecan EGE neoepitope was home-made and has been described previously (34). A rabbit polyclonal antibody was raised against a synthetic peptide, SISRAGVGGC, conjugated to ovalbumin using a method described previously (35). This antibody is specific for the SISRA neoepitope created at the new N terminus following pro-protein convertase-mediated loss of the prodomain. The immunogen used to raise the TS5 antibody was full-length recombinant mouse TS5 solubilized from bacterial inclusion bodies. After analysis of the immunogen by SDS-PAGE and Coomassie stain, the five most strongly stained bands ranging from 30 to 120 kDa in molecular size were excised for mass spectrometry analyses, which confirmed the presence of the TS5 Prodomain (5/5 bands), the Cat domain (3/5 bands), the Sp domain (1/5 bands), and the CysR domain (1/5 bands). This immunogen was used to immunize a sheep at the South Australian Health and Medical Research Institute (SAHMRI), South Australia.

Gel Electrophoresis, Western Blotting, Gel Zymography, and Cross-linking Experiments—Laemmli SDS-PAGE gels were prepared in-house or 7.5% Mini-PORTEAN® TGXTM precast gels were purchased from Bio-Rad. Reagents used for Western blotting included Immobilon-P transfer membrane (Millipore), animal-free blocker (Vector Laboratories), Amersham Biosciences™ ECL™ Prime, and Amersham Biosciences™ ECL™. Select Western blotting reagent (GE Healthcare Life Sciences, UK). For gelatin zymography, 7.5% SDS gels were prepared with 0.5 mg/ml of gelatin, but without urea. After electrophoresis, the gel was incubated in 2.5% (v/v) Triton X-100 to allow TS5 refolding. Thereafter, the gel was incubated in zymography buffer containing 50 mM Tris, pH 7.5, 200 mM NaCl, 5 mM ZnCl₂, 5 mM CaCl₂, 0.02% Brij35, and 0.02% NaN₃ at 37 °C. For cross-linking experiments, stocks of disuccinimidyl sulhide (spacer arm 11.4 Å) and disuccinimidyl glutarate (spacer arm 7.7 Å) (Thermo Fisher Scientific) were prepared in dimethyl sulphoxide and used at a final concentration of 1 mM for 1 h at 4 °C with gentle mixing, then the 80-μl reactions were quenched with 4 μl of 50 mM Tris, pH 7.5. The samples were boiled in SDS and resolved by SDS-PAGE under reducing and non-reducing (±dithiothreitol, respectively) conditions.

Competition Assay for Measuring Aggrecanase Activity—Fixed volumes of purified TS5-FLAG and increasing volumes of purified TS5–5-V5 or TS5–5-6-V5 competitor were preincubated for 1 h at 37 °C. Thereafter, the mixtures were incubated with 160 μg of purified aggrecan for 24 h at 37 °C. The samples were treated with chondroitinase ABC (Seikagaku, Japan) and keratanase (Seikagaku, Japan) to remove glycosaminoglycans from the aggrecan substrate. The G1-EGE neoepitope cleavage products were detected by Western blot using an α-EGE antibody.

Aggrecanase Activity in Mouse Femoral Head Cartilage Explant Cultures—For cartilage explant cultures, 3-week-old mouse femoral head cartilage (hips) harvested from TS5+/Δcat, TS5+/-, and wild type mice were placed in serum-free DMEM, with or without 10 ng/ml of human recombinant IL-1α (Peprotech) and cultured for 72 h at 37 °C. The concentration of sulfated glycosaminoglycans released from the cartilage explants into the conditioned media, and present in cartilage extracts, was analyzed by the 1,9-dimethylmethylen blue dye binding assay (36).

Mouse Model of Inflammatory Arthritis—Acute inflammatory arthritis was induced in knee joints of 8–9-week-old mice using the antigen-induced arthritis model described (31, 37, 38). Briefly, animals received an intradermal injection of 100 μg of mBSA in 100 μl of Freund’s Complete adjuvant at two sites near the base of the tail on days 0 and 14 to elicit an immune response. Seven days later, the mice received an intra-articular injection of 10 μl of 20 mg/ml of mBSA in 0.9% sterile saline or vehicle alone, into the left and right knees, respectively. The intra-articular mBSA produced a localized inflammatory response that drove cartilage erosion and aggrecan loss. Animals were culled 4 days later and their knees were harvested and processed for histology. Sagittal 6-μm sections through the central weight-bearing region of the medial femorotibial joint were stained with toluidine blue/fast green and scored for aggrecan loss (0–3) as described (39). The mean scores from two investigators blinded to the treatment and genotype were analyzed using the Mann-Whitney U test for unpaired non-parametric data.

In Situ Proximity Ligation Assay (PLA)—We used the PLA to detect TS5 molecules located within 40 nm of each other at the cell surface. Stably transfected HEK293-EBNA cells expressing TS5-FLAG and TS5-Myc were seeded overnight in 8-well LabTek II Nunc chamber slides (Thermo Fisher Scientific), fixed, permeabilized, and visualized with reagents from the Duolink® In Situ Detection Reagents kit (Sigma), which uses PLA® Technology. Specifically, 5 μg/ml of α-FLAG M2 mouse monoclonal antibody and 5 μg/ml of α-c-Myc rabbit polyclonal antibody were added to the cells for 2 h at room temperature, followed by anti-rabbit PLUS and anti-mouse MINUS PLA probes for 1 h at 37 °C. For interaction experiments using truncated constructs, stably transfected HEK293-EBNA cells expressing TS5-FLAG and TS5-Myc were transfected with either the TS5–5–V5 or TS5–6–V5 constructs and the proximity of expressed proteins was detected by interactions between α-V5 mouse monoclonal and α-c-Myc rabbit polyclonal antibody antibodies and the MINUS and PLUS probes, respectively. After ligation and amplification of the Duolink probes the slides were dried and mounted for fluorescence detection with Duolink In Situ Mounting Medium.

NanoLC Tandem Mass Spectrometry—To identify high M₁ proteins present in TS5 oligomeric aggregates, silver-stained bands excised from reduced and non-reduced gels were dried, then rehydrated in digest buffer (40 mm ammonium bicarbonate and 10% (v/v) acetonitrile) and digested with 20 ng/μl of proteomics grade trypsin (Sigma) as described previously (40). Peptides were reconstituted in 20 μl of HPLC buffer A (5% acetonitrile in 0.2% formic acid) and analyzed by nanoLC-
MS/MS using an LTQ-Orbitrap XL (Thermo Fisher Scientific) using a Top8 method when survey scans were acquired in the Orbitrap (FT) and MS/MS spectra were concurrently acquired in the LTQ mass analyzer on the eight most intense ions (43). The acquired MS/MS data were extracted from Xcalibur Raw files and searched against the *Homo sapiens* complete proteome set database. Semi-tryptic searches using parent ion and fragment ion mass tolerances of 10 ppm and 0.5 Da, respectively, were done using X!Tandem using the Computational Proteomics Analysis System (41). Variable oxidation of methionine was specified as the only modification. The Peptide Prophet and Protein Prophet algorithms were applied to the X!Tandem search results to assign probabilities to peptide and protein matches, respectively (42). Peptide-spectrum matches were accepted if the peptide was assigned a probability greater than 0.95 as specified by the Peptide Prophet algorithm.

**Results**

Before analyzing the role of the TS5 ancillary domains in enzyme activity, we first confirmed that these domains were present in TS5Δcat, and absent in TS5 null cartilage (30, 31, 33). Fig. 1A shows the structure of the TS5 gene and the genetic modifications made to the TS5 null and TS5Δcat genomes. The results in Fig. 1, B and C, show that oligonucleotide primers 22F and 254R amplified sequences from the TS5 Pro domain (Fig. 1B), generating amplicons of 232 bp from both TS5Δcat and TS5 null cDNA (Fig. 1C), as expected. In contrast, primers amplifying the region upstream and including part of exon 2 (1007F-1233R) produced amplicons of 226 bp from wild type and TS5Δcat cDNA, but not from TS5 null cDNA. The presence of the 468-bp amplicon from the TS5Δcat cDNA confirms that the mutant RNA arising from the in-frame deletion of exon 3 is stable and not degraded by nonsense-mediated decay. The primer pair 1007F-1643R spanning exons 2, 3, and part of exon 4 produced amplicons of 636 bp from wild type and 468 bp from the TS5Δcat cDNA, respectively, but there were no amplicons produced from TS5 null cDNA. Primers specific for the neomycin-resistance gene present in the IRES/LacZ/Neo cassette, which was substituted for exon 2 in the TS5 null mouse, were included as a positive control for the integrity of the TS5 null
Active ADAMTS-5 Is an Oligomer

To analyze interactions between TS5 molecules we expressed recombinant TS5 with C-terminal FLAG or Myc tags (Fig. 1E) and analyzed the TS5 species that were co-purified from immunoaffinity columns. TS5 species in lysates of HEK293-EBNA cells expressing TS5-FLAG were first identified by Western blotting with antibodies that recognize total TS5 (polyclonal α-TS5 antibody) or active TS5 without its prodomain (α-SISRA antibody) (Fig. 3A). The α-SISRA Western blot revealed four major species of active enzyme (Fig. 3A, lane 2), in addition to a large aggregated species that was retained in wells at the top of the gel (Fig. 3A, lane 2, arrowhead). The approximate sizes of the SISRA-positive TS5 species were 80, 60, 47, and 43 kDa. The band at ~80 kDa is consistent with the size of active TS5 with its full suite of C-terminal ancillary domains. Bands at ~60, 47, and 43 kDa, represent recombinant truncates of TS5, progressively shortened from the C terminus, as reported previously for recombinant, engineered (19) and naturally occurring TS5 fragments. Analysis of the same sample with an antibody raised against the full-length TS5 molecule revealed additional distinct bands (Fig. 3A, lane 1). The 120-kDa band detected by the TS5 antibody is not detected by the α-SISRA antibody and most likely represents the full-lengthzymogen form of TS5 (Fig. 3A, lane 1, arrow). A larger SISRA-negative band and two smaller bands migrating at ~50 and 55 kDa were detected with the polyclonal α-TS5 antibody. TS5 that failed to enter the resolving gel was detected by both α-SISRA and α-TS5 antibodies (Fig. 3A, arrowhead).

When conditioned medium from HEK293-EBNA cells expressing TS5-FLAG was analyzed by Western blotting, six SISRA-positive TS5 species were detected prior to affinity purification on a FLAG affinity column (Fig. 3B, lane 1), and four TS5 species were recovered after purification (Fig. 3B, lane 2). SISRA-positive molecules including species lacking the C-terminal TS and Sp domains, with or without the CysR and internal TS domains, were consistently eluted from the FLAG column, despite the absence of FLAG tags on these fragments (Fig. 3B, lane 2). A fourth band of ~43 kDa, consistent with a protein comprising both the Cat and Dis domains was also weakly detected (Fig. 3B, lane 2). These results suggest that a spectrum of TS5 fragments that lack FLAG tags interact with full-length TS5-FLAG and co-elute from the FLAG-affinity column. In contrast, 37-kDa SISRA-positive fragments lacking all or part of the Dis domain (Fig. 3B, lane 1, asterisk) were not co-eluted from the column (Fig. 3B, lane 2). These results showing that TS5 species lacking the Dis domain might not be able to interact with TS5-FLAG suggest that the Dis domain is required for interactions between TS5 monomers.

In separate co-elution experiments, TS5 species secreted into conditioned medium from double transfectants expressing Myc-tagged and FLAG-tagged TS5 were eluted from a FLAG affinity column and detected with α-TS5, α-Myc, or α-SISRA antibodies (Fig. 3C). Full-length TS5-Myc co-eluted with TS5-FLAG from the FLAG affinity column (Fig. 3C, lane 2), as did truncated fragments of TS5 that were detected by the α-SISRA antibody (Fig. 3C, lane 1). These results show that intact and fragmented TS5 species without FLAG tags co-elute from FLAG-affinity columns by binding to TS5-FLAG. In a recipro-

cDNA (Fig. 1C). These results confirm that TS5 C-terminal domains are expressed in the TS5Δcat but not the TS5 null mice (Fig. 1D).

The TS5Δcat Protein Has a Dominant-negative Effect on Aggrecanase Activity in Vitro—We and others have shown that aggrecanase activity in cartilage explants from homozygous TS5Δcat mice is barely detectable (30, 31) and that the homozygous TS5 null mice are full genetic nulls (32); thus, homozygous animals were not analyzed further in this study. To compare the contribution of the TS5 ancillary domains to in vitro aggrecanolyis, cartilage explants from wild type, TS5+/Δcat, and TS5+/- cartilage explants and B the corresponding statistical analysis of the differences in aggrecan release from independent samples for wild type (n = 6), TS5+/Δcat (n = 9), and TS5+/- mice (n = 7).

**TABLE 2**

| Genotype | Treatment | Mean aggrecan loss (µg/mL CS) | Fold increase treated vs untreated |
|----------|-----------|------------------------------|-----------------------------------|
|          | IL-1α     | 6                            | 25.81                             |
|          | IL-1α     | 6                            | 19.5                              |
|          | IL-1α     | 6                            | 7                                |
|          | IL-1α     | 6                            | 2.2                              |
|          | IL-1α     | 6                            | 3.1                              |

**FIGURE 2.** Dominant-negative effect of TS5 ancillary domains on catalytic activity in vitro. Levels of IL-1α-induced aggrecan release into the medium from wild type, TS5+/Δcat, and TS5+/− cartilage explants and B the corresponding statistical analysis of the differences in aggrecan release from independent samples for any genotype (Fig. 2, A–D). The IL-1α-induced aggrecan loss from untreated explants (Fig. 2, A and B). The IL-1α-induced aggrecan loss from wild type and TS5 null explants was 3.3- and 3.1-fold higher than from untreated explants, respectively (Fig. 2, A and B). However, IL-1α-induced aggrecan loss from the TS5Δcat explants was only 2.2-fold higher than untreated explants (Fig. 2, A and B). When the level of induced aggrecan loss was compared between genotypes, the results showed that there was no significant difference between aggrecan loss from wild type and het TS5 null explants (p = 0.0856), however, aggrecan loss from the het TS5Δcat explants was significantly less than from wild type (p = 0.0041) and also significantly less than from the het TS5 null mice (p = 0.0347) (Fig. 2A). This unexpected finding suggests that in cartilage from het mice, the mutant TS5Δcat protein expressed from the mutant allele inhibits the aggrecanase activity expressed from the wild type allele. Accordingly, we propose that wild type TS5 activity requires the interaction of two or more TS5 monomers, and that the mutant TS5Δcat proteins have a dominant-negative effect on TS5 activity.
Active ADAMTS-5 Is an Oligomer

FIGURE 3. Interactions between recombinant TS5 monomers and TS5 fragments in vitro. A, Western blot analysis of cell lysates from TS5-FLAG cells resolved under reducing conditions and detected with α-TS5 (lane 1) or α-SISRA (lane 2) antibodies. Predicted molecular mass, mass by SDS-PAGE, and schematics of individual molecular species of TS5 are shown. B, α-SISRA Western blot of TS5-FLAG and C terminally truncated (FLAG-free) TS5 fragments under reducing conditions, before (lane 1) and after (lane 2) elution from a FLAG-affinity column. C, conditioned medium from double transfectants expressing TS5-FLAG and TS5-Myc was applied to a FLAG-affinity column and elutes analyzed on reducing gels by α-SISRA (lane 1), α-Myc (lane 2), and α-FLAG (lane 3) Western blotting. D, visualization of TS5-FLAG and TS5-Myc proximity by PLA with two negative controls including (E) untransfected HEK293-EBNA cells and (F) double transfectants with the addition of the α-FLAG antibody only, to control for non-specific probe ligation. G, PLA of double transfectants visualized by confocal microscopy.

To further explore our hypothesis that TS5 monomers interact to form oligomers, we analyzed TS5-FLAG/TS5-Myc double transfectants by in situ PLA to detect an interaction between the Myc- and FLAG-tagged species. Strong PLA signals were detected (Fig. 3D), indicating that TS5-Myc and TS5-FLAG are often found within 40 nm (or less) of each other. The controls for this experiment included untransfected HEK293-EBNA cells (Fig. 3E), as well as double transfectants with the addition of only α-FLAG antibody, to exclude the possibility of non-specific ligation of the PLUS and MINUS probes (Fig. 3F). No PLA signal was detected in the negative controls. In addition, heparitinase treatment of the cultured cells did not disrupt the PLA signals suggesting that the PLA signal is not mediated by heparan sulfate bridging of adjacent TS5 molecules (data not shown). Imaging the PLA-positive TS5 interactants by confocal microscopy revealed that they had a perinuclear localization within the cell (Fig. 3G), suggesting that these interactions are likely to occur within the secretory pathway.

TS5-TS5 Interactions Are Mediated via the Catalytic and Disintegrin Domains—Because the shortest species that co-purified on the α-FLAG affinity column comprised both the Cat and Dis domains, (Fig. 3B, lane 1, asterisk), we hypothesized that the Dis and/or Cat domain(s) were required for TS5 interactions. We therefore transfected the TS5–5–V5 construct encoding the Cat and Dis domains (Figs. 1E and 4A), the TS5–6–V5 construct encoding the Cat domain (Figs. 1E and 4B), or the empty vector (Fig. 4C), into the TS5-FLAG/TS5-Myc double stable transfectants and analyzed the cells by PLA. The TS5–5–V5 and TS5–6–V5 proteins each carry a C-terminal His tag and a V5 tag. An α-rabbit IgG was used to detect the c-Myc epitope on full-length TS5, and α-mouse IgG was used to detect the V5 epitope, prior to amplification with the species-specific PLA probes. The results show that the TS5–5–V5 and TS5–6–V5 proteins were sufficiently close to TS5-Myc molecules to ligate and amplify the PLA signal. Within this experimental setting, which reports on proximity rather than direct interactions, we conclude that the Dis and/or Cat domains of neighboring TS5 molecules are regularly in close enough proximity to enable molecular interactions.

In a more definitive experiment, TS5-FLAG cells were transiently transfected with vectors expressing TS5–5–V5, TS5–6–V5, or an empty vector control. Conditioned medium from the triple transfectants was passed over (i) a His-Select affinity column comprised both the Cat and Dis domains, (Fig. 4D, lanes 1–3), or (ii) an α-FLAG affinity column eluted with FLAG peptide (Fig. 4D, lanes 4–6). Western blotting showed that the 43-kDa TS5–5–V5 protein eluted from the His-Select column as expected (Fig. 4D, lane 1), and also from the FLAG affinity column (Fig. 4D, lane 4) presumably via its interaction with TS5-FLAG, and was strongly detected by the α-SISRA and the α-V5 antibodies. Similarly, the 31-kDa TS5–6–V5 protein eluted from both the His-Select column (Fig. 4D, lane 2), and the α-FLAG column (Fig. 4D, lane 5). No epitopes were detected in eluates from cell lines co-transfected with the empty vector controls (Fig. 4D, lanes 3 and 6). Collectively these results show that the Dis and Cat domains can mediate interactions between TS5 molecules.

To determine whether these TS5 interactions are required for aggrecanase activity, purified aggrecan (substrate) was incubated with TS5-FLAG, in the presence and absence of TS5–5–V5 or TS5–6–V5, which were included as competitors to...
block TS5-FLAG catalytic activity that produces G1-EGE373 neoepitope fragments. Western blotting clearly showed that in the absence of competitor (Fig. 4E, lanes 2 and 7), TS5-FLAG cleaved aggrecan to produce the G1-EGE373 neoepitope, confirming that TS5-FLAG was catalytically active. However, when increasing amounts of purified TS5–5-V5 competitor (Fig. 4E, lanes 3–5) or TS5–6-V5 competitor (Fig. 4E, lanes 8–11) were incubated with TS5-FLAG, levels of the EGE373 neoepitope were markedly reduced. These results suggest that TS5 oligomerization is required for aggrecanase activity, and that oligomerization is mediated via the Dis and/or Cat domains.

**Analysis of TS5 Oligomers**—To characterize the TS5 oligomers, conditioned medium from TS5-FLAG transfectants was analyzed by Western blotting after SDS-PAGE under reducing and non-reducing conditions (Fig. 5A). As expected, TS5 monomers migrated faster at ~75 kDa under non-reducing conditions compared with their reduced counterparts, which migrated at ~80 kDa (Fig. 5A). In addition, larger TS5 species were detected in the non-reduced samples, migrating above the 250-kDa marker, but within the resolving gel, at nominally ~400 kDa (Fig. 5A, asterisk). These data provide evidence for TS5 oligomers stabilized by inter-molecular disulfide bonding.

To further investigate whether purified TS5 assembled into oligomers via additional interactions that are sensitive to dissociation during SDS-PAGE, we used chemical cross-linkers to stabilize TS5 associations via non-covalent interactions. Strikingly, the majority of the SISRA-positive TS5 and all of the FLAG-positive TS5 was stabilized by the non-reducible cross-linking reagents, disuccinimidyl suberate and disuccinimidyl glutarate. After cross-linking disuccinimidyl suberate and disuccinimidyl glutarate, TS5 migrated as the ~400 kDa oligomer under reducing conditions (Fig. 5B, gels 1 and 2), consistent with the TS5 species previously detected in conditioned medium containing competitor.

**FIGURE 4.** TS5-TS5 interactions are mediated via the disintegrin and/or catalytic domain. TS5-FLAG and TS5-Myc double transfectants transiently transfected with (A) TS5–5-V5, (B) TS5–6-V5, or (C) empty vector were visualized by PLA. D, TS5 proteins expressed in cells stably expressing TS5-FLAG and TS5-Myc and transiently expressing TS5–5-V5, TS5–6-V5, or empty vector control, were eluted from a His-Select affinity column with imidazole (lanes 1–3) or from an α-FLAG affinity column with FLAG peptide (lanes 4–6). Eluted proteins were detected by α-SISRA (lanes 1–3) and α-V5 (lanes 4–6) by Western blot analysis. E, production of the EGE373 aggrecan fragment by purified TS5-FLAG was inhibited in the presence of excess, purified TS5–5-V5 or TS5–6-V5 competitors.

**FIGURE 5.** TS5 forms catalytically active, SDS-stable, disulfide bonded oligomers. Western blot analysis of TS5 under reducing and non-reducing conditions (A), in the presence and absence of the chemical cross-linkers disuccinimidyl suberate (DSS) and disuccinimidyl glutarate (DSG) (B). Analysis of purified TS5 by non-reducing gel electrophoresis and α-SISRA Western blot showing a strong monomer band, compared with gelatin zymography showing strong oligomer bands (arrows) but a weak monomer band (asterisk) (C).
Active ADAMTS-5 Is an Oligomer

observed under non-reducing conditions (Fig. 5A). Under non-reducing conditions, cross-linked TS5 migrated not only at the position of the ~400 kDa oligomer but also as a larger multimeric species that failed to enter the gel (Fig. 5B, gels 3 and 4, arrows).

We also used gelatin zymography to detect catalytic activity in affinity-purified TS5 monomers and oligomers. The results in Fig. 5C show that although the α-SISRA antibody detected a strong band of TS5 monomer and only a weak band of TS5 oligomer, gelatin zymography of the same samples revealed abundant catalytic activity in two high molecular mass bands at ~400 kDa (Fig. 5C, arrows), but only weak gelatinolytic activity in the 75-kDa monomer band (Fig. 5C, asterisk). The gelatinase activity of species at ~400 and 75 kDa was inhibited by EDTA (Fig. 5C), confirming that these species are metalloproteinases. Collectively these data indicate that TS5 associates into oligomeric complexes via disulfide bonds that can be stabilized using chemical cross-linking, and that these oligomeric species are catalytically active.

Other investigators working with native TS5 in human osteoarthritic cartilage have reported interactions between TS5 and hyaluronan (24). To determine whether the large molecular complexes comprised TS5 oligomers complexed with hyaluronan, we treated purified TS5-FLAG, or conditioned media from TS5-FLAG transfected cells, with hyaluronidase and analyzed the samples under non-reducing conditions. The hyaluronidase treatment did not alter the amount of TS5 entering the gel, nor did it alter the electrophoretic mobility of the ~400 or 75 kDa species of TS5-FLAG (data not shown), suggesting that hyaluronan was not complexed with TS5 in this in vitro system.

We then used mass spectrometry (MS) to further characterize the nature of the SISRA-positive TS5 isoforms detected by non-reducing SDS-PAGE, zymography, and chemical cross-linking. Affinity-purified TS5-FLAG was resolved under reducing and non-reducing conditions and protein bands were detected by Western blotting using the SISRA antibody and silver staining (data not shown). The SISRA-positive protein bands excised from the silver-stained gel for MS analysis were the ~400 kDa band and larger oligomers shown in Fig. 5B (asterisks and arrows, respectively) and the band at ~80 kDa corresponding to the mass of full-length monomeric ADAMTS-5 as shown in Fig. 3B. The strongest SISRA-positive bands under both reducing and non-reducing conditions corresponded to the TS5 monomer and yielded the highest number of distinct TS5 peptides and MS/MS spectra (MS runs 5–8 in Table 1). Consistent with Western blot and zymography data, TS5 peptides were also detected in the ~400-kDa band and larger oligomers that were detected under non-reducing conditions (MS runs 2 and 4, respectively). In contrast, no TS5 peptides were detected in control gel slices at the equivalent positions in samples resolved under reducing conditions, providing further evidence for disulfide-bonded TS5 oligomers of ~400 kDa and larger multimeric species.

Whereas TS5 was the major protein detected in the high-molecular mass protein bands, intracellular structural proteins (the cytoskeletal keratins KRT10, KRT9, and KRT1, tubulin chains TUBA1C and TUBB8, and actin), heat shock proteins HSP90AB1 and HSPA1A, and the secreted lectin, galectin-3 binding protein, were also detected. However, because these proteins were mostly detected in the MS analysis of the ~80 kDa TS5 monomers, and not in the ~400 kDa band, they are likely to be contaminants rather than authentic TS5-binding proteins. Importantly, no peptides corresponding to the MMP-9 gelatinase were detected in any analyses. Based on these results we conclude that the TS5 isoforms described in Fig. 5 are predominantly unimolecular oligomers of catalytically active TS5.

In Vivo Assessment of the Dominant-negative Effect of TS5Δcat Protein on Aggrecanolysis in Heterozygous Mice—The results in Fig. 2 raised the possibility of an interaction between wild type and mutant TS5 molecules in TS5+/Δcat cartilage, in which binding of the mutant TS5Δcat protein to the wild type TS5 protein inhibits its activity in a dominant-negative manner. The results in Figs. 3–5 suggest that this is indeed the case, and show that the Dis and/or Cat domains are required for oligomerization and maximum catalytic activity. In our final experiment we tested whether the mutant TS5Δcat protein had a dominant-negative effect in vivo, by assessing whether the same pattern of suppressed aggrecanase activity observed in TS5+/Δcat cartilage in vitro also occurred in the TS5+/Δcat mice in vivo, using an antigen-induced model of inflammatory arthritis (39) (Fig. 6). Following an inflammatory insult, the extent of aggrecan loss from mouse knee joints was compared histologically between wild type, TS5+/Δcat, and TS5+/− mice. The results showed that the mean score for femoral aggrecan loss from wild type mice was almost maximal, reaching 2.5 of a possible maximum score or 3.0, as we have showed previously (39). The results also showed that aggrecan loss from TS5+/− mice was the same as from wild type mice. However, femoral aggrecan loss from TS5+/Δcat mice was significantly less than the loss from both wild type (p = 0.0063) and TS5+/− (p = 0.0014) mice, and the mean score was 2.0. The results in Fig. 6 support our hypothesis that the mutant TS5Δcat proteins have a dominant-negative effect on TS5 activity in vivo.

Discussion

In this study we show that TS5 forms oligomers that are proteolytically active. Analysis of recombinant FLAG-tagged TS5 under non-reducing conditions and after treatment with chemical cross-linkers revealed the formation of ~400 kDa and larger multimeric species stabilized by both inter-molecular disulfide bonds and non-covalent interactions. Both multimeric isoforms were apparently more active against gelatin than monomeric TS5 and the results of mass spectrometry analysis indicated that these complexes were predominantly unimolecular. Co-elution, competition, and in situ PLA experiments using full-length and truncated recombinant TS-5 indicated that the Cat and Dis domains support TS-5 intermolecular interaction. Truncates comprising the Dis and/or Cat domains competitively inhibited production of the G1-EGE373 neoepitope by full-length TS-5 in aggrecanase assays, further supporting the data showing that TS5 oligomers are required for full aggrecanase activity, and that the Dis and/or Cat domains mediate oligomerization. The finding that TS5 forms catalytically active oligomers is con-
sistent with our observations in vitro and in vivo that mutant TS5 protein lacking the C-terminal ancillary domains inhibited wild type TS5 aggrecan degradation, in a dominant-negative manner.

The formation of dimers and higher-order oligomers is not uncommon within the metalloproteinase family, although the mechanism and consequences of these associations are diverse, and not readily predicted. MMP2, MMP9, MT1-MMP, MT4-MMP, MT6-MMP, ADAM-10, ADAM-17, and meprins form homo- or hetero-oligomers (43–50). The major advantage of oligomerization is the formation of an energetically favorable structure. Other advantages include enhanced enzyme activation, improved stability, and regulation of substrate accessibility. For example, MT1-MMP dimerizes via its hemopexin domain to enable collagenolytic activity (44), and also dimerizes via the transmembrane domain to facilitate MT1-MMP-mediated activation of MMP-2 (45). MMP-2 dimerizes via disulfide bonding between two neighboring cysteine residues in the pro-domain (43). The cysteine residue is part of the conserved cysteine-switch motif, hence dimerization disrupts cysteine-zinc ion pairings and mediates activation of MMP-2 by thrombin. MMP-9 forms homodimers, or heterodimers in combination with TIMP-1 (47, 51–53). Dimerization is mediated via the hemopexin domain (51, 53) and monomers are more efficiently activated than dimers (52), suggesting that dimer formation promotes the stability of the zymogen.

Early purifications of procollagen N-proteinase (ADAMTS-2) and von Willebrand factor cleaving factor (ADAMTS-13) indicated that these enzymes formed oligomers (54–56), however, the constituents proved difficult to characterize (57, 58). In the intervening years since these studies, there has been no further characterization of these oligomers, nor publication of oligomer formation by other ADAMTS enzymes, highlighting the novelty of our finding that TS5 forms catalytically active homo-oligomers.

Many proteolytic enzymes form oligomers to localize and concentrate proteolytic activity, particularly if their substrates are large; indeed mature glycosylated aggrecan substrate has a molecular size of ~2,500 kDa. The C-terminal domains of TS5

| MS Run # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|----------|---|---|---|---|---|---|---|---|
| Gel      | Red | Non-Red | Red | Non-Red | Red | Non-Red | Red | Non-Red |
| Band excised | High MW (~400 kDa) | Non-reducing SDS-PAGE, detection by silver staining and in-gel trypsin digestion. | Monomer (upper) | Monomer (lower) |

**TABLE 1**

Mass spectrometry analysis of TS5-FLAG protein bands

Proteins identified by tandem MS analysis of SISRA-positive TS5-FLAG protein bands after separation by reducing and non-reducing SDS-PAGE, detection by silver staining and in-gel trypsin digestion.

| Description | Gene Name | Found In Runs | MS/MS spectra | Unique peptides | MS/MS spectra | Unique peptides | MS/MS spectra | Unique peptides | MS/MS spectra | Unique peptides | MS/MS spectra | Unique peptides | MS/MS spectra | Unique peptides |
|-------------|-----------|---------------|---------------|----------------|---------------|----------------|---------------|----------------|---------------|----------------|---------------|----------------|---------------|----------------|
| Keratin, type I cytoskeletal 10 | KRT10 | 6 | 12 | 2 | 2 | 36 | 26 | 54 | 26 | 14 | 10 | 5 | 9 | 11 | 10 | 3 | 2 |
| Keratin, type II cytoskeletal 1 | KRT1 | 8 | 13 | 10 | 9 | 63 | 20 | 76 | 23 | 14 | 13 | 36 | 23 | 26 | 20 | 5 | 6 |
| ADAM metalloproteinase with thrombospondin type 1 motif, 5 preprotein | ADAMTS5 | 6 | 16 | 11 | 8 1 | 23 | 100 | 22 | 83 | 21 | 184 | 37 | 120 | 25 | |
| ADAMTS-1 precursor | ADAMTS1 | 6 | 2 | 2 | 4 | 4 | 5 | 4 | 2 | 5 | 4 | 3 | 2 |
| Galectin-3-binding protein precursor | LGALS3BP | 6 | 3 | 3 | 14 | 10 | 37 | 13 | 10 | 9 | 21 | 10 | 5 | 4 |
| Heat shock protein HSP 90-beta | HSP90AB1 | 6 | 3 | 3 | 15 | 18 | 14 | 15 | 2 | 2 | 6 | 6 | 8 | 8 |
| Heat shock 70 kDa protein 1 | HSPA1A | 5 | 10 | 9 | 26 | 19 | 10 | 9 | 2 | 2 | 24 | 16 |
| Keratin, type I cytoskeletal 9 | KRT9 | 5 | 8 | 7 | 28 | 16 | 44 | 23 | 11 | 9 | 12 | 10 |
| Q4 Vas 2 homo sapiens keratin 2a | KRT2 | 5 | 10 | 10 | 34 | 25 | 23 | 20 | 7 | 9 | 9 | 15 |
| Actin, alpha skeletal muscle | ACTA1 | 4 | 4 | 3 | 5 | 5 | 21 | 12 | |
| Keratin, type I cytoskeletal 20 | KRT20 | 4 | 8 | 11 | 8 | 11 | 23 | 22 | 4 | 5 |
| Keratin, type II cytoskeletal 6C | KRT6C | 4 | 9 | 13 | 9 | 13 | 9 | 25 | 2 | 8 |
| Tubulin alpha-1C chain | TUBA1C | 3 | 7 | 6 | 3 | 8 | 5 | 10 | 8 |
| 6-phosphofructokinase | PFKL | 2 | 2 | 2 | 2 | 4 |
| 6-phosphofructokinase type C | PFKP | 3 | 7 | 6 |
| DNA replication licensing factor MCM4 | MCM4 | 2 | 2 | 2 | 8 | 7 |
| DNA replication licensing factor MCM7 | MCM7 | 2 | 6 | 5 | 9 | 8 |
| Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 precursor | PLOD1 | 2 | 3 | 3 | 7 | 7 |
| Putative pre-mRNA-spooling factor ATP-dependent RNA helicase DHX15 | DHX15 | 2 | 15 | 12 | 10 | 9 |
| Vesicle-fusing ATPase | NSF | 1 | 4 | 4 |
| Vacuolar protein sorting-associated protein 35 | VPS35 | 1 | 6 | 6 |
| Cullin-4A | CUL4B | 1 | 3 | 2 |
Active ADAMTS-5 Is an Oligomer

Oligomer formation often results in a conformational change of the enzyme, which might expose the active site or conceal it. Using gelatin zymography we found that TS5 oligomers were able to cleave gelatin, suggesting the oligomerization did not conceal the active site of TS5. Furthermore, by comparing TS5 signal intensity by Western blot, and TS5 enzyme activity by gelatin zymography, we showed that TS5 oligomers were substantially more active than the monomeric form of TS5. The crystal structures of TS-4 and TS5 (59) showed that their catalytic sites have two conformations: an open, ligand accessible conformation and a closed, autoinhibited form. Such flexibility at the catalytic site is unique within the metalloproteinase family. Given that TS5 oligomers were active against gelatin, which is generally a poor substrate for TS5, we propose that oligomer formation is energetically favorable for the open conformation of the catalytic site, and therefore promotes ligand binding and proteolysis.

Co-elution experiments and aggrecanase assays showed that the TS5–5-V5 and TS5–6-V5 truncates inhibited aggrecanase activity, by competing with full-length TS5 for either oligomerization or substrate binding. Furthermore, TS5Δcat protein inhibited aggrecanolysis, in vitro and in vivo. Taken together, the data emphasize that the Cat and Dis domains are important determinants of TS5 activity. The crystal structures of TS-4 and TS5 show that the Cat and Dis domains form a single folding unit; this could provide an uninterrupted surface for ligand binding (59). We propose that this surface is important for oligomerization.

TS5 is a target for the development of a disease-modifying osteoarthritis drug. A recent study has described monoclonal antibodies (mAbs) to TS-4 and −5 that blocked aggrecan loss from human osteoarthritic cartilage ex vivo (3). Intriguingly, domain mapping of the mAbs predicted that they recognize similar epitopes spanning the Cat and Dis domains, further supporting our conclusion that the Cat and Dis domains are, together, important for TS5 activity. Of particular importance to disease-modifying osteoarthritis drug development is the fact that the TS5 mAb inhibited structural damage in mouse models of osteoarthritis, and a humanized version of the mAb-reduced aggrecan loss from cartilage in a non-human primate model. The authors propose an "allosteric lock" model to describe the action of the TS-4 and −5 mAbs, wherein the antibodies bind to the Cat and Dis domains simultaneously, limiting flexibility of the catalytic site, and consequently impairing ligand binding and proteolysis. From our observations of TS5 oligomerization, we consider that there is an alternative interpretation, wherein antibody binding to the Cat and Dis domains blocks TS5 oligomerization, which is essential for full catalytic activity. Further studies are required to determine whether the TS5 mAb blocks TS5 oligomerization.

The significance of our study is that in vivo, in heterozygous TS5+/Δcat mice, disrupted oligomer formation by the TS5Δcat mutant protein blocks TS5 activity and protects against aggrecan loss in a mouse model of joint disease. This is proof-of-principle that blocking oligomerization can inhibit the activity of ADAMTS-5. Together with the study by Larkin et al. (3) the new mechanistic insights described herein identify
the surface provided by the Cat and Dis domains as a legitimate target for the design of TS5 inhibitors.

Author Contributions—A. J. F. conceived the study and coordinated the work. F. M. R. helped coordinate part of the study. Experimental design was by A. J. F., H. J. K., and F. M. R. Experimental work was done by H. J. K. (Figs. 1–5), S. B. G. (Fig. 2), K. L. (Figs. 4 and 5), V. C. R. (Fig. 5), F. M. R. and S. J. G. (Fig. 6), and R. W. (Table 1). R. W. did the mass spectrometry experiments. P. H. designed and constructed vectors for expression of TS5–5–V5 and TS5–6–V5. S. L. provided technical advice. The paper was written by A. J. F., H. J. K., and H. S. All authors reviewed

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