Cleavage of von Willebrand Factor Requires the Spacer Domain of the Metalloprotease ADAMTS13*

Xinglong Zheng‡§, Kenji Nishio‰, Elaine M. Majerus¶¶ and J. Evan Sadler†¶¶¶

From the ¶Department of Pathology and Immunology, ‡Howard Hughes Medical Institute, and †¶¶¶Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

Received for publication, May 21, 2003
Published, JBC Papers in Press, June 5, 2003, DOI 10.1074/jbc.M305331200

ADAMTS13 consists of a repolysin-type metalloprotease domain followed by a disintegrin domain, a thrombospondin type 1 motif (TSP1), Cys-rich and spacer domains, seven more TSP1 motifs, and two CUB domains. ADAMTS13 limits platelet accumulation in microvascular thrombi by cleaving the Tyr1605-Met1606 bond in von Willebrand factor, and ADAMTS13 deficiency causes a lethal syndrome, thrombotic thrombocytopenic purpura. ADAMTS13 domains required for substrate recognition were localized by the characterization of recombinant deletion mutants. Constructs with C-terminal His6 and V5 epitopes were expressed by transient transfection of COS-7 cells or in a baculovirus system. No association with extracellular matrix or cell surface was detected for any ADAMTS13 variant by immunofluorescence microscopy or chemical modification. Both plasma and recombinant full-length ADAMTS13 cleaved von Willebrand factor subunits into two fragments of 176 kDa and 140 kDa. Recombinant ADAMTS13 was divalent metal ion-dependent and was inhibited by IgG from a patient with idiopathic thrombotic thrombocytopenic purpura. ADAMTS13 that was truncated after the metalloprotease domain, the disintegrin domain, the first TSP1 repeat, or the Cys-rich domain was not able to cleave von Willebrand factor, whereas addition of the spacer region restored protease activity. Therefore, the spacer region is necessary for normal ADAMTS13 activity toward von Willebrand factor, and the more C-terminal TSP1 and CUB domains are dispensable in vitro.

Thrombotic thrombocytopenic purpura (TTP)† is a syndrome characterized by microangiopathic hemolytic anemia and thrombocytopenia, and it may be accompanied by neurological dysfunction, renal failure, and fever (1–3). If untreated, the mortality can exceed 90%, but plasma-exchange therapy has reduced the mortality to less than 20% (4). Although the pathophysiology of TTP is not fully understood, a plausible model has been proposed in which the proteolysis of von Willebrand factor (VWF) plays a central role (5). VWF is a multimeric protein that binds receptors on the surface of platelets and in connective tissue, thereby mediating the adhesion of platelets to sites of vascular injury. If unchecked, the process can lead to microvascular thrombosis. A plasma VWF-cleaving protease has been described that acts on the Tyr1606–Met1606 bond in the central A2 domain of the VWF subunit, and cleavage is stimulated by shear forces like those occurring at sites of thrombosis or by low concentrations of urea or guanidine (6, 7). This proteolytic reaction limits VWF-dependent platelet adhesion, and most adults with idiopathic TTP have an acquired autoantibody that inhibits the VWF-cleaving protease (8, 9). Therefore, therapeutic plasma exchange may ameliorate TTP by replacing the missing protease and removing the inhibitory antibody.

The VWF-cleaving protease was recently purified and identified as a new member of the ADAMTS family of metalloproteases (10, 11), so named for the combination of a disintegrin-like and metalloprotease (reprolysin type), with thrombospondin type 1 motifs (12). The primary structure of the ADAMTS13 precursor was determined by cDNA cloning (13, 14) and by positional cloning in families with inherited ADAMTS13 deficiency (15). It consists of 1427 amino acid residues comprising a signal peptide, a short propeptide ending in the sequence QQRR, a reprolysin-like metalloprotease domain, a disintegrin domain, a thrombospondin-1 repeat (TSP1), a cysteine-rich domain and spacer characteristic of the ADAMTS family, seven additional TSP1 repeats, and two CUB domains (13–15). Several alternatively spliced mRNA species have been identified that could encode truncated forms of ADAMTS13 lacking various C-terminal domains (13–16).

The complex multidomain structure of ADAMTS13 is conserved across vertebrates as diverse as mammals, birds, and fish (17),‡ suggesting that motifs outside the metalloprotease domain are required for its biological function. This concept is supported by the finding that missense mutations in domains far from the metalloprotease domain cause inherited ADAMTS13 deficiency and thrombotic microangiopathy (15, 18, 19). The specificity of ADAMTS13 for a single bond in VWF and the remarkable regulation of cleavage by tensile stress on the substrate suggest that accessory domains are critical for substrate recognition. However, the structural requirements for ADAMTS13 activity have not been determined. To address this question, ADAMTS13 constructs with nested C-terminal deletions were characterized. The results suggest that the spacer domain is required to recognize and cleave VWF. More distal domains may contribute to activity but do not appear to be necessary in vitro.

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§ Present address: Dept. of Pathology and Laboratory Medicine, The Children’s Hospital of Philadelphia, University of Pennsylvania School of Medicine, 34th St. and Civic Center Blvd., Philadelphia, PA 19104.
¶ To whom correspondence should be addressed: Howard Hughes Medical Inst., Washington University School of Medicine, 660 S. Euclid Ave., Box 8022, St. Louis, MO 63110. Tel.: 314-362-9029; Fax: 314-454-3012; E-mail: esadler@im.wustl.edu.
¶¶ The abbreviations used are: TTP, thrombotic thrombocytopenic purpura; PBS, phosphate-buffered saline; TSP1, thrombospondin type 1; VWF, von Willebrand factor.

† J. E. Sadler, unpublished observations.
EXPERIMENTAL PROCEDURES

**Plasmid Constructs**—A cDNA construct covering the ADAMTS13 coding sequence was assembled from partial cDNA clones (13) using a PCR-based strategy. The open reading frame of ADAMTS13 for constructs with C-terminally cleaved and cloned into pcDNA3.1/V5-His-TOPO (Intronvit) to generate pcADAMTS13-V5-His. Constructs with C-terminally truncated cleavages were created similarly. This cloning strategy appends the following vector-encoded amino acid sequence onto the ADAMTS13 sequence: a linker region (RKNSADQHRSGSGLSSLEGR, the V5 epitope (GR-PR), a tripeptide (RTG), and a poly(His) tag (HHHHHH)). All PCR amplifications were performed with high fidelity PfuTurbo Hotstart DNA polymerase (Strategene, La Jolla, CA) in the presence of 7.5% Me2SO. The accuracy of all constructions was confirmed by DNA sequencing.

Using ADAMTS13 constructs in vector pcDNA3.1/V5-His-TOPO at the template, recombination sites attB1 and attB2 were introduced at the 5′- and 3′-ends of the insert by PCR with oligonucleotides 5′-agg gac aag tgt cta gaa agg att ctt act gCA gca gca gCA tca ccc gCC gGC AAG-3′ (attB1 site in lowercase) and 5′-agg gac cac ttt gta cga aag aic gtc gta ATG ATG ATG ATG ACC G-3′ (attB2 site in lowercase). PCR products were recombined with plasmid pDONR201 and BP Clonase to prepare entry clones according to the manufacturer’s instructions (Intronvit). Entry clones were sequenced to verify the accuracy of the PCR and recombination reactions. Bacmid expression vectors were prepared by recombination of each entry clone with vector pDEST5s and LR Clonase (Intronvit). Baculovirus stocks were prepared for each bacmid by transfection of Spodoptera frugiperda Sf9 cells and repeated amplification of the recombinant baculovirus.

**EXPRESSION—COOH Versus—NCOOH**—Cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, penicillin, and streptomycin. Cells were seeded on 6-well tissue culture plates at 45% confluency 12 h before seedings. The 5′-ggg gac /H11032- and 3′-agc tgg gtc TCA ATG GTG ATG ATG ATG ATG ACC G-3′ (attB2 site in lowercase) were amplified and cloned into pcDNA3.1/V5-His-TOPO (Invitrogen) to generate pcADAMTS13-V5-His, 12 μl of LipofectAMINE, and 8 μl of Pluses reagent (Intronvit) in 2 ml of Opti-MEM I. After 48 h, medium was collected, and cells were washed extensively with ice-cold PBS. The cells were lysed on ice for 30 min in 2 ml of water containing 0.1% protease inhibitor mixture (Sigma) and 1% phenylmethylsulfonyl fluoride. The lysate was removed, and remaining cell membranes and matrix were washed extensively with ice-cold water. The extracellular matrix was solubilized by scraping into 100 μl of PBS-PAGE sample buffer (15 mM Tris-HCl, pH 6.8, 2.5% glycerol, 0.5% SDS, 178 mM β-mercaptoethanol, and 0.25% bromophenol blue). Equal percentages of lysate, medium, and matrix solutions were solubilized at 100 °C for 5 min, then diluted 2.5-fold in 50 mM sodium citrate, pH 5.5, with or without 500 units of recombinant Streptomyces plicatus endoglycosidase H (New England Biolabs, Inc., Beverly, MA) and incubated at 37 °C for 2 h. Products were analyzed by SDS-PAGE and Western blotting with anti-V5 antibody or monoclonal anti-human fibronectin antibody (clone IST-3, catalog no. F0974, Sigma).

**Cell Surface Biotinylation**—Transfected cells in 6-well plates were washed three times with ice-cold PBS and incubated at 4 °C for 30 min with 1 ml of PBS containing 1.5 mg/ml sulforoscinimidyl-2-biotinimi-
doethyl-I,3-dithiopropionate (NHS-SB-Biotin, Pierce) (24). Reaction was stopped by washing with ice-cold PBS, and cells were lysed in 1.5 ml of PBS containing 1% Triton X-100 and 1% protease inhibitor mixture (Sigma). The lysate was clarified by centrifugation and split into two equal samples. ADAMTS13 proteins in one sample were concentrated by adsorption onto 100 μl of TALON metal affinity beads (BD Biosciences Clontech). Biotin-labeled proteins from the second sample were adsorbed onto 100 μl of streptavidin-agarose (Pierce) at room temperature for 2 h, and unbound ADAMTS13 in the flow-through fraction was concentrated by adsorption onto 100 μl of TALON beads. After washing four times with PBS, proteins were eluted from TALON or streptavidin beads with 40 μl of 0.4% SDS containing 5% β-mercaptoethanol. Samples (30 μl) were diluted with 30 μl of water and incubated at 100 °C for 1 min in a total volume of 50 μl containing 100 mM sodium citrate phosphate, pH 5.5, 2% Triton X-100, 4% β-mercaptoethanol, and 0.02% sodium azide without (control) or with 2 milligrams of recombinant S. plicatus endoglycosidase H (Oxford Glycosciences, Abingdon, UK) (24). Products were analyzed by SDS-PAGE and Western blotting with anti-V5 antibody.

**RESULTS**

**Expression and Localization of Recombinant ADAMTS13 Variants**—Plasma ADAMTS13 consists of an N-terminal metalloprotease domain followed by several conserved structural motifs of unknown function. To assess the role of these various domains in substrate recognition, nested C-terminal deletions were constructed with a V5 epitope, and poly(His) tag was appended to facilitate detection of the recombinant proteins (Fig. 1). In a preliminary comparison of expression systems by Western blotting, stably transfected COS-7, CHO, or BHK cell lines expressed much less ADAMTS13 than did transiently transfected COS-7 cells, suggesting that high levels of ADAMTS13 confer a selective disadvantage. Therefore, ADAMTS13 variants were expressed in transiently transfected COS-7 cells. All constructs were synthesized with similar efficiency varying only twice in the amount of secreted protein detected by Western blotting with anti-V5 antibody.



\[X. Zheng, K. Nishio, E. M. Majerus, and J. E. Sadler, unpublished observations.\]
The cellular localization of ADAMTS13 proteins was assessed by immunofluorescence microscopy (Fig. 2). The variant containing only the metalloprotease domain (del6) exhibited a perinuclear distribution consistent with localization in the Golgi apparatus. Larger constructs exhibited a more extensively diffuse plus granular pattern consistent with transient localization in the endoplasmic reticulum during biosynthesis and secretion. Staining of nonpermeabilized cells, either in culture at 4 °C or after fixation with paraformaldehyde, did not identify any ADAMTS13 variant on the cell surface. The absence of cell-surface ADAMTS13 was confirmed for full-length ADAMTS13 by a chemical modification approach (Fig. 3). Cell surface proteins were biotinylated and recovered from cell lysates by affinity chromatography on avidin-agarose. Contamination of the biotinylated fraction by intracellular proteins was assessed by digestion with endoglycosidase H, which does not act on complex oligosaccharides of secreted ADAMTS13 in conditioned medium. Almost all of the ADAMTS13 was not biotinylated and had endoglycosidase H-sensitive oligosaccharides consistent with localization in the endoplasmic reticulum. Trace amounts of biotinylated ADAMTS13 had endoglycosidase H-sensitive oligosaccharides and probably represent intracellular protein derived from broken cells. Therefore, secreted ADAMTS13 does not bind detectably to COS-7 cells.

Because some ADAMTS proteases interact with extracellular matrix components, the association of ADAMTS13 with extracellular matrix was examined. Recombinant full-length ADAMTS13 was not detected in the extracellular matrix of transfected COS-1 cells by immunofluorescence or by Western blotting (data not shown), but COS-1 cells produce a relatively sparse matrix in culture. Therefore, similar experiments were performed in primary rat lung fibroblasts, which produce a more substantial matrix and have been used extensively in studies of extracellular matrix localization (25). In RFL-6 cells, the oligosaccharides of intracellular ADAMTS13 were endoglycosidase H-sensitive, whereas oligosaccharides of secreted ADAMTS13 were endoglycosidase H-resistant (Fig. 4). Trace amounts of endoglycosidase H-sensitive ADAMTS13 were recovered in solubilized extracellular matrix (Fig. 4), although endoglycosidase H-resistant fibronectin was abundant (data not shown). Overexposure did not demonstrate any ADAMTS13 in the matrix fraction that was resistant to endoglycosidase H. Similar results were obtained by immunofluorescence: fibronectin was detected readily in RFL-6 extracellular matrix, but ADAMTS13 was not. Thus, secreted ADAMTS13 does not appear to bind to the extracellular matrix of cultured RFL-6 or COS-1 cells.

**Proteolytic Activity of ADAMTS13 Variants**—Recombinant ADAMTS13 proteins were assayed under conditions previously

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**FIG. 1.** ADAMTS13 constructs. Secreted ADAMTS13 consists of a metalloprotease domain, a disintegrin domain, 8 thrombospondin-1 (TSP1) repeats, a Cys-rich and spacer domain, and two CUB domains. The signal peptide and propeptide that occur in the primary translation product (residues 1–74) are not part of mature ADAMTS13 and are not shown. Truncation mutants were constructed by inserting a V5 epitope, His6 tag, and termination codon after the following amino acid residues: full-length (FL) after Thr1427; del1 after Ala1191; del2 after Ala685; del3 after Cys555; del4 after Glu439; del5 after Gly385; and del6 after Gln289. The domains present in each construct are shown schematically.

**FIG. 2.** Immunofluorescence localization of ADAMTS13. COS-7 cells expressing full-length ADAMTS13 (FL) or the indicated deletion mutants described in Fig. 1 were fixed with ethanol:acetic acid and incubated with anti-V5 IgG and Cy3-conjugated anti-mouse IgG (in red). Cell nuclei were stained with 4',6-diamidino-2-phenylindole (in blue).

**FIG. 3.** Surface biotinylation does not detect secreted cell-surface ADAMTS13. Full-length ADAMTS13 was expressed in COS-7 cells. Cell surface proteins were biotinylated and collected by adsorption on streptavidin-agarose. Samples of the initial cell lysate, the streptavidin flow-through fraction (Unbound), the streptavidin eluate (Bound), and conditioned medium (Medium) were digested with endoglycosidase H (EndoH). Products were separated by SDS-PAGE and visualized by Western blotting with anti-V5 antibody. The position of molecular mass standards is indicated at the left.

**FIG. 4.** Secreted ADAMTS13 is undetectable in extracellular matrix. Full-length ADAMTS13 was expressed in RFL-6 cells. Cell lysate, conditioned medium, and extracellular matrix were prepared, and samples were digested with endoglycosidase H (EndoH) as described under “Experimental Procedures.” Products were separated by SDS-PAGE and visualized by Western blotting with anti-V5 antibody. The position of molecular mass standards is indicated at the left.
Established for plasma ADAMTS13, ADAMTS13 does not cleave VWF at a significant rate under conditions of physiological ionic strength and in the absence of fluid shear stress. However, reaction occurs readily in buffers of low ionic strength that are supplemented with low concentrations of urea or guanidine (6, 7). The relative concentration of recombinant ADAMTS13 variants was determined by Western blotting with anti-V5 antibody and densitometry. Samples containing equivalent amounts of recombinant ADAMTS13 (~5 μl of concentrated conditioned medium) were incubated for 16 h with VWF substrate in buffer containing 5 mM Tris-HCl, pH 8.0, 3 mM BaCl2, and 1.5 M urea, and the integrity of the remaining VWF multimers was assessed in a collagen binding assay. The results were normalized to the values obtained with full-length ADAMTS13 (FL). The bars represent the S.E. of four independent assays.

Fig. 5. ADAMTS13 variants expressed in COS-7 cells. A, COS-7 cells were transiently transfected with plasmids encoding the indicated construct that is described under Fig. 1 or with vector only (Control). Conditioned medium was concentrated 5-fold, samples (50 μl) were analyzed by SDS-PAGE on a 5–15% gradient gel, and Western blotting was done with anti-V5 antibody. B, the ability of recombinant ADAMTS13 proteins to cleave plasma VWF was measured in a two-stage assay as described under “Experimental Procedures.” The relative concentration of recombinant ADAMTS13 proteins was determined by Western blotting with anti-V5 antibody and densitometry. Samples containing equivalent amounts of recombinant ADAMTS13 (~5 μl of concentrated conditioned medium) were incubated for 16 h with VWF substrate in buffer containing 5 mM Tris-HCl, pH 8.0, 3 mM BaCl2, and 1.5 M urea, and the integrity of the remaining VWF multimers was assessed in a collagen binding assay. The results were normalized to the values obtained with full-length ADAMTS13 (FL). The bars represent the S.E. of four independent assays.

Fig. 6. Cleavage of VWF subunits by ADAMTS13 variants. VWF (10 μg/ml) was incubated with the indicated recombinant ADAMTS13 protein expressed in COS-7 cells or with normal human plasma (Plasma) in buffer containing 5 mM Tris-HCl, pH 8.0, 3 mM BaCl2, and 1.5 M urea as described under “Experimental Procedures.” Control reactions containing full-length recombinant ADAMTS13 (FL) were performed in the presence of IgG (2.4 mg/ml) from a patient with autoimmune TTP (lane 1) or 10 mM EDTA (lane 2). Products were analyzed by SDS-PAGE under reducing conditions on a 5% gel and Western blotting with polyclonal anti-VWF antibody. The mass of the intact VWF subunit and the cleavage products generated by plasma ADAMTS13 are indicated at the left.

Metal ions or by autoantibodies to ADAMTS13 from a patient with idiopathic TTP (22) confirming the specificity of the reaction. Construct del1, which lacks both CUB domains, had normal activity toward VWF. Further deletion of TSP1 domains 2–8 had a variable effect. Among four experiments, the average activity of construct del2 was ~70% relative to the full-length protein, but the standard error was large, and the activity of del2 was not significantly different from that of del1 or full-length ADAMTS13. Deletion of the spacer domain in construct del3 abolished activity, and the shorter constructs del4, del5, and del6 also were inactive. The results obtained in the collagen-binding assay were confirmed by direct visualization of VWF multimers. Full-length ADAMTS13 and truncation mutants del1 and del2 digested VWF rapidly into small multimers, whereas constructs del3, del4, del5, and del6 had no discernable effect on the VWF multimer distribution (data not shown).

Plasma ADAMTS13, VWF (10 μg/ml) was incubated with the indicated recombinant ADAMTS13 protein expressed in COS-7 cells or with normal human plasma (Plasma) in buffer containing 5 mM Tris-HCl, pH 8.0, 3 mM BaCl2, and 1.5 M urea as described under “Experimental Procedures.” Control reactions containing full-length recombinant ADAMTS13 (FL) were performed in the presence of IgG (2.4 mg/ml) from a patient with autoimmune TTP (lane 1) or 10 mM EDTA (lane 2). Products were analyzed by SDS-PAGE under reducing conditions on a 5% gel and Western blotting with polyclonal anti-VWF antibody. The mass of the intact VWF subunit and the cleavage products generated by plasma ADAMTS13 are indicated at the left.
The indicated ADAMTS13 constructs were expressed by transient transfection of COS-7 cells or by baculovirus infection of Sf9 cells. Samples of concentrated conditioned medium were assayed for VWF cleaving activity in a collagen-binding assay. COS-7 cells: full-length (FL), 1.4 units/ml; del2, 0.73 units/ml; del6, 0 units/ml. Sf9 cells: full-length, 0.67 units/ml; del2, 1.42 units/ml; del6, 0 units/ml. The amount of recombinant protein was determined by SDS-PAGE, Western blotting with anti-V5 antibody and densitometry as described under “Experimental Procedures.” COS-7 cells: full-length, 12.9 nmol/liter; del2, 9.0 nmol/liter; del6, 12.9 nmol/liter. Sf9 cells: full-length, 6.4 nmol/liter; del2, 13.1 nmol/liter; del6, 13.4 nmol/liter. The signals obtained for the standard protein (Positope, Invitrogen) are shown at the right. Its apparent mass is 53 kDa, and its mass calculated from amino acid sequence is 47.7 kDa.

Whether expressed in COS-7 cells or Sf9 cells, full-length ADAMTS13 and the variant truncated after the spacer domain (del2) had similar specific activities of ~80–110 units/nmol (Fig. 7), confirming that domains C-terminal to the spacer domain are not required for proteolytic activity in vitro. If full-length recombinant and plasma ADAMTS13 (~170 kDa) have similar specific activity, the results suggest that the plasma concentration of active ADAMTS13 is ~1.6 μg/ml, which is similar to the value of 1 μg/ml based upon the recovery of ADAMTS13 during purification from plasma (11).

**DISCUSSION**

The complex modular structure of many ADAMTS proteases is highly conserved among vertebrates, suggesting that their noncatalytic domains perform specific selectable functions. The properties of several ADAMTS proteases supports this conclusion. For example, the Cys-rich and spacer domains and three TSP1 repeats of ADAMTS1 are necessary to bind heparin or extracellular matrix (28, 29). The C-terminal TSP1 domains of ADAMTS1 are reported to bind endothelial cells (29) and to have anti-angiogenic activity, which is attributed to binding and sequestration of vascular endothelial growth factor (30). The TSP1 motif of ADAMTS4 is required for aggrecan cleavage (31), and its Cys-rich and spacer domains bind sulfated glycosaminoglycans (32). ADAMTS12 also employs C-terminal TSP1 domains to bind extracellular matrix (33). Therefore, ADAMTS structures remote from the metalloprotease domain can participate in tissue localization, substrate recognition, and possibly other activities unrelated to proteolysis.

Although several ADAMTS proteases associate with extracellular matrix or cell surfaces, ADAMTS13 does not appear to fit this pattern. Full-length ADAMTS13 did not bind detectably to the surface of COS-7 cells (Fig. 3) and was not incorporated into the extracellular matrix of COS-1 or RFL-6 cells (Fig. 4). If ADAMTS13 binds cell surfaces or matrix in vivo, such interactions could depend on binding partners that are not expressed in these cultured cell lines.

The properties of truncated ADAMTS13 proteins suggest that substrate recognition requires the participation of several domains. Full-length recombinant ADAMTS13 was able to cleave VWF with apparently normal specificity, but truncation mutants lacking the spacer domain could not (Figs. 5–7). Therefore, TSP1 repeats 2–8 and the CUB domains are not required for VWF cleavage in vitro. Whether they are important under fluid shear stress or under more physiological conditions in vivo requires further study. If these C-terminal domains were necessary in vivo, one might predict the occurrence of patients with inherited TTP caused by mutations in the ADAMTS13 gene but with normal plasma ADAMTS13 activity upon laboratory testing. To date, however, all reported mutations distal to the spacer domain have been associated with severe ADAMTS13 deficiency (15, 19, 34).

Deletion of the ADAMTS13 spacer domain abolished the activity of construct del3 toward VWF (Figs. 5–7), suggesting that the spacer domain participates in substrate recognition. This conclusion would be strengthened if construct del3 and smaller variants were shown to have catalytic activity toward another substrate, to react with an active site inhibitor, or to have native conformation by another method. Unfortunately, no ADAMTS13 substrates other than VWF and certain fragments thereof have been reported. A variety of other proteins and small synthetic substrates have been tested, but none were cleaved detectably; also, with the exception of metal-ion chelators (6, 7), no synthetic or natural active site inhibitor including α2-macroglobulin has been shown to react with ADAMTS13.

The efficient synthesis and secretion of small inactive ADAMTS13 constructs (Figs. 2 and 7) suggest they are folded properly, but direct evidence for structural integrity will require a different experimental approach.

Although not addressed in this study, the more proximal disintegrin and Cys-rich domains and the first TSP1 repeat may also participate in substrate recognition. For example, the mutation P475S in the ADAMTS13 Cys-rich domain was identified in a Japanese patient with inherited TTP; the corresponding recombinant mutant ADAMTS13 was secreted efficiently but had extremely low activity toward VWF (18). Other missense mutations that cause inherited ADAMTS13 deficiency have been found in the disintegrin, Cys-rich, and first TSP1 domains (15, 19), but protein expression studies have not yet been reported for them.

Proteolytic processing can generate distinct forms of several ADAMTS proteases, and this variation may control tissue localization or substrate specificity. For example, ADAMTS4 is autoproteolytically cleaved into products that lack variable portions of the spacer and Cys-rich domains, and the truncated forms have reduced affinity for sulfated glycosaminoglycans (32). Some cells remove the C-terminal TSP1 repeat from ADAMTS1, which reduces its affinity for heparin and endothelial cells (29). At least one site within the C-terminal TSP1 repeat of recombinant full-length ADAMTS13 appears to be sensitive to cleavage (Fig. 7), suggesting that it could be subject to proteolytic processing in vivo. Such processing could occur intracellularly as indicated by the sensitivity of the C-terminal fragment to digestion with endoglycosidase H (Fig. 3). Additional structural heterogeneity for several ADAMTS proteases may be produced by alternative mRNA splicing (16, 35, 36), although the biological significance of the predicted products has not been established. In the case of ADAMTS13, alternatively spliced forms have been cloned that encode variants truncated after the metalloprotease domain, the spacer domain, the second TSP1 repeat, or the first CUB domain (13–15). Similar deletion mutants (Fig. 1) were secreted efficiently by COS-7 cells and Sf9 cells, and ADAMTS13 purified from

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Specificity of ADAMTS13

plasma was shown to contain species of 110, 130, 140, and 150 kDa that had the same N-terminal amino acid sequence (11). Thus, truncated forms of ADAMTS13 may circulate in vivo and could potentially be derived from alternatively spliced transcripts. Based on the properties of recombinant ADAMTS13 could potentially be derived from alternatively spliced transcripts. Based on the properties of recombinant ADAMTS13 mutants (Figs. 5–7), such variation in the complement of C-terminal domains would dramatically affect ADAMTS13 sub-

Acknowledgments—We thank Claudine Mazurier (CRTS, Lille, France) for providing purified human plasma VWF and Robert Mecham (Washington University, St. Louis, MO) for providing RFL-6 cells and for advice on assessing the extracellular matrix localization of proteins.

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