Allosteric activation of ADAMTS13 by von Willebrand factor


The metalloprotease ADAMTS13 cleaves von Willebrand factor (VWF) within endovascular platelet aggregates, and ADAMTS13 deficiency causes fatal microvascular thrombosis. The proximal metalloprotease (M), disintegrin-like (D), thrombospondin-1 (T), Cys-rich (C), and spacer (S) domains of ADAMTS13 recognize a cryptic site in VWF that is exposed by tensile force. Another seven T and two complement C1r/C1s, sea urchin epidermal growth factor, and bone morphogenetic protein (CUB) domains of uncertain function are C-terminal to the MDTCS domains. We find that the distal T8–CUB2 domains markedly inhibit substrate cleavage, and binding of VWF or monoclonal antibodies to distal ADAMTS13 domains relieves this autoinhibition. Small angle X-ray scattering data indicate that distal T-CUB domains interact with proximal MDTCS domains. Thus, ADAMTS13 is regulated by substrate-induced allosteric activation, which may optimize VWF cleavage under fluid shear stress in vivo. Distal domains of other ADAMTS proteases may have similar allosteric properties.

hemostasis | metalloproteases | allosteric regulation

After vascular injury, platelets adhere to von Willebrand factor (VWF) multimers bound to endothelial cell surfaces and connective tissue. The force of flowing blood on a growing platelet-rich thrombus stretches the central A2 domain of VWF and exposes a Tyr1605–Met1606 cleavage site for ADAMTS13 (Fig. 1A) (1–5), a metalloprotease that severs VWF and releases adherent platelets. Deficiency of ADAMTS13 disrupts this feedback regulatory mechanism and causes thrombotic thrombocytopenic purpura (TTP), which is characterized by life-threatening microvascular thrombosis (3, 6, 7).

The recognition and cleavage of VWF is a formidable challenge. VWF and ADAMTS13 occur at ∼10 μg/mL and ∼1 μg/mL, respectively, compared with total plasma protein of ∼80,000 μg/mL. ADAMTS13 is constitutively active and has no known inhibitors in vivo. Nevertheless, VWF is the only identified ADAMTS13 substrate, and VWF is resistant to cleavage until subjected to fluid shear stress (8), adsorbed on a surface (9), or treated with denaturants (8, 10). This specificity depends on structural features of both ADAMTS13 and VWF that have not been characterized fully. The proximal metalloprotease (M), disintegrin-like (D), thrombospondin-1 (T), Cys-rich (C), and spacer (S) domains of ADAMTS13 bind to cryptic sites that are uncovered by unfolding VWF domain A2 (11–15) (Fig. 1B), and these interactions are required for efficient cleavage of VWF or peptide substrates. More distal ADAMTS13 domains bind to sites in or near VWF domain D4 that are always available (16–18). Deletion of distal ADAMTS13 domains impairs the cleavage of VWF multimers in vitro (16, 19) and increases VWF-dependent microvascular thrombosis in vivo (20) but accelerates the cleavage of peptide substrates (12, 13). In addition, ADAMTS13 cleaves guanidine hydrochloride-treated VWF multimers with an apparent Km of ∼15 nM (21), which is 100-fold lower than the Km of ∼1.6–1.7 μM for peptide substrates that are based on the sequence of VWF domain A2 (12, 14). These striking differences suggest that distal T or complement C1r/C1s, sea urchin epidermal growth factor, and bone morphogenetic protein (CUB) domains regulate ADAMTS13 activity. We have now shown that these distal domains inhibit ADAMTS13, and binding to VWF relieves this autoinhibition.

Results

Activation of ADAMTS13 by Antibodies and Low pH. Evidence for allosteric regulation was obtained unexpectedly in the course of analyzing plasma samples from patients with TTP. The majority of adult patients with acquired TTP have autoantibodies that inhibit ADAMTS13 and reduce its activity in plasma to <5% of normal, but one patient proved to be a remarkable exception. When assayed with a fluorogenic ADAMTS13 substrate, VWF71 (Fig. 1B) (22), patient BCW49 had high-titer autoantibodies that paradoxically activated exogenous ADAMTS13 threefold (Fig. 1C). Activation occurred at pH 7.4, which is characteristic of blood, but not at pH 6, which is used routinely for clinical ADAMTS13 assays (23). Furthermore, BCW49 plasma had no effect on the activity of MDTCS at either pH 7.4 or pH 6.

The loss of pH dependence for MDTCS suggested a regulatory function for the distal domains that are missing from this truncated ADAMTS13 construct. We (22) and others (23, 24) have observed that full-length ADAMTS13 is most active at pH 6, with markedly decreased activity at pH 7.4 (Fig. 1D and Fig. S1). This phenomenon has been attributed to ionization of a Zn2+-bound...
ADAMTS13 up to T8, and CUB domains had additive effects and activated those of BCW49 plasma and identified several that increased the ADAMTS13 monoclonal antibodies for properties similar to cles) and MDTCS (blue circles). Error bars indicate 95% confidence intervals cleavage were determined as a function of pH for ADAMTS13 (orange cir-

Fig. 1. Activation of ADAMTS13 by autoantibodies from a patient with TTP or by low pH. (A) Structure of ADAMTS13. (B) Fluorogenic substrates ter-
minate at VWF residues indicated by arrows. Each substrate has Lys1617 replaced with Arg, N-terminal Gly modified with IRDye QC-1 (QC1), and Asn1616 replaced by Cys and modified with DyLight 633 (DyL) (22). The arrowhead indicates the cleaved Tyr-Met bond. Secondary structure elements of the VWF A2 domain (11) are indicated below and segments that interact with specific ADAMTS13 domains (13) are indicated above the sequence. (C) BCW49 plasma activated ADAMTS13 with a titer of 9.6 U at pH 7.4 (orange squares), but not at pH 6.0 (orange circle). BCW49 plasma did not activate MDTCS at pH 6 (blue circle) or pH 7.4 (blue circle); (D) Rates of VWF71 cleavage were determined as a function of pH for ADAMTS13 (orange circles) and MDTCS (blue circles). Error bars indicate 95% confidence intervals and if not shown are smaller than the symbols.

Allosteric Activation of ADAMTS13 by VWF. We screened anti-
ADAMTS13 monoclonal antibodies for properties that similar to those of BCW49 plasma and identified several that increased the rate of VWF71 cleavage (Fig. 2A). When combined at saturating concentrations (Fig. S2), certain antibodies that recognized T67, T8, and CUB domains had additive effects and activated ADAMTS13 up to ∼4.2-fold at pH 7.4 (Fig. 2A), which is comparable to the magnitude of ADAMTS13 activation achieved by changing to pH 6 (Fig. 1D). These antibodies had markedly decreased effects on ADAMTS13 activity at pH 6 (Fig. S2). Auto-
antibodies in BCW49 plasma also bound to the CUB domains (Fig. 2B). These data suggest that specific distal T and/or CUB domains contribute to autoinhibition of ADAMTS13 at physiological pH.

Only some monoclonal antibodies that recognize a particular distal domain can activate ADAMTS13 (Fig. 2A), and these differences correlate with epitope specificity. For example, anti-T67 antibody 7C4 activates ADAMTS13 but 8C10 does not, and these antibodies bind distinct sites on ADAMTS13. Activating anti-T8 antibodies 14D2 and 19H4 bind competitively to ADAMTS13 at a site distinct from that recognized by nonactivating anti-T8 antibodies 11E2 and 20A5. Finally, anti-CUB antibodies 12D4 and 12H6 recognize different epitopes, but 12D4 activates ADAMTS13, whereas 12H6 does not (Fig. 2A). These results suggest that activ-
ating antibodies bind and stabilize an activated ADAMTS con-
formation, whereas nonactivating antibodies show no preference for such a conformation.

Several proximal ADAMTS13 domains participate in recogni-
tion of the extended VWF A2 domain that contains the scissile Tyr1605-Met1606 bond (12–15) (Fig. 1B). We prepared a series of fluorogenic substrates to determine whether activation involved specific proximal ADAMTS13 domains that engage distinct segments of VWF (Fig. 1B). As expected (13), the cleavage rate decreased as the substrate length decreased. However, the magnitude of activation by low pH (Fig. 2C) increased to ∼ninefold even for the shortest substrate VWF25, even though VWF25 interacts only with the MD domains of ADAMTS13 (13). The activation ratios, which correspond to the change in free energy of transition state stabilization (∆GTS) (25), do not depend strongly on substrate length. In addition, antibodies that increased the cleavage of fluorogenic substrates by ADAMTS13 had no effect on MDTCS (Fig. 2C).

More importantly, adding VWF or recombinant VWF D4 markedly increased fluorogenic substrate cleavage (Fig. 2D). Staphylococcal V8 protease cleaves VWF after Glu2129 between the VWD4 and C8–4 subdomains of D4 and more slowly after Glu1672 between the A2 and A3 domains (26) (Fig. S3). These proteolytic fragments—SPI, SPII, and SPIII—had little effect on ADAMTS13 (Fig. 2D), suggesting that a functional ADAMTS13 binding site requires cooperation between motifs on both sides of the V8 cleavage site in the D4 domain.

These results indicate that the VWF D4 domain binds distal ADAMTS13 domains and allosterically activates the N-terminal M domain. The interaction of these distal domains with proximal MDTCS domains might be expected to reduce the affinity of ADAMTS13 binding to VWF A2 domain sequences that contain the scissile Tyr-Met bond, and this prediction was confirmed by using ADAMTS13(E225Q) variants that can bind substrates normally but cannot cleave them (17) (Fig. S4). MDTCS bound to a chimeric GST-VWF73 construct with a Kd of 210 ± 30 nM (SE), whereas ADAMTS13 bound slightly less tightly with a Kd of 440 ± 50 nM (SE).

The mutation G1505E, which was identified in patients with von Willebrand disease type 2A, prevents complete folding of VWF domain A2, exposes the scissile bond, and allows ADAMTS13 to cleave it rapidly and completely in the absence of shear stress (27). MDTCS bound to VWF construct A1-CK(G1505E) but did not bind to wild-type A1-CK (with a folded A2 domain) or to construct D4-CK (with no A2 domain). In contrast, ADAMTS13 did bind to VWF constructs D4-CK and A1-CK, presumably at the D4 site. ADAMTS13 also bound to A1-CK(G1505E) with increased affinity, which is consistent with binding to both A2 and D4 sites (Fig. S4).

ADAMTS13 Distal Domains Facilitate VWF Cleavage Under Shear Stress. The natural substrate of ADAMTS13, multimeric plasma VWF, is resistant to cleavage until subjected to fluid shear stress that exposes the cleavage site in the A2 domain. The time course of this reaction can be analyzed by vortexing plasma VWF with ADAMTS13 variants and detecting specific cleavage products by Western blotting (16, 17). Under these conditions, full-length ADAMTS13 cleaved VWF ~1.7-fold faster than MDTCS (Fig. 3 A and C), even though ADAMTS13 is ~fourfold less active toward small peptide substrates (Fig. 1). The preferential cleavage of VWF by ADAMTS13 is not explained by an effect of shear stress on the enzyme, because vortexing ADAMTS13 with VWF71 for 10 min increased product generation only 1.15 ± 0.07-fold
This small effect of vortexing may be due to frictional heating of the reaction.

Anti-CUB antibody 12D4 increased the rate of VWF cleavage \( \sim \) twofold (Fig. 3B and C). Thus, distal ADAMTS13 domains that bind VWF domain D4 confer an advantage for cleaving VWF that can be increased further by allosteric activation. Although anti-T8 antibody 19H4 also accelerates the cleavage of peptide substrates (Fig. 2A), it had no effect on the cleavage of VWF (Fig. 3D).

**ADAMTS13 Distal Domains Are Adjacent to Its Proximal Domains.** The regulation of ADAMTS13 metalloprotease activity by its distal T-CUB domains implies that proximal and distal domains are close enough to make contact, and small angle X-ray scattering (SAXS) data (Fig. 4A) indicate that ADAMTS13 does have such a structure. Pair distance distributions (Fig. 4B) show that MDTCS, MT8, and ADAMTS13 have maximum dimensions of \( \sim 140 \) Å, \( \sim 200 \) Å, and \( \sim 230 \) Å, respectively. For comparison, a molecular model of ADAMTS13 has a maximum extended length of \( \sim 500 \) Å (Fig. 4C). Agreement was excellent between an atomic model for MDTCS and experimental scattering profiles \( (\chi = 1.86) \) (28) (Fig. 4A) or ab initio envelopes (Fig. 4D). The envelopes for full-length ADAMTS13 (Fig. 4E) are too compact for fully extended ADAMTS13 but readily accommodate a folded or condensed conformation. Specific flexible segments have not been identified, but the extended linkers after T4 and T8 are susceptible to proteolysis by thrombin, plasmin, or elastase (29, 30) and may act as hinges. Comparisons of SAXS envelopes for ADAMTS13 and construct MT8, which lacks both CUB domains, indicate that the CUB domains are adjacent to proximal ADAMTS13 domains and occupy a bulge in the envelope for ADAMTS13 (Fig. 4E) that is missing from the envelope for MT8, which cannot accommodate the CUB domains in this location (Fig. 4F). This folded conformation would enable distal T7-CUB domains to allosterically regulate ADAMTS13 by variably occluding the active site and substrate binding exosites. The occurrence of condensed ADAMTS13 is supported by quick-freeze deep-etch EM, which shows both elongated and folded conformations for ADAMTS13 (Fig. 4G).

**Discussion**

Our results indicate that VWF promotes its own destruction by allosterically activating ADAMTS13, and this mechanism could have evolved to balance the competing needs to promote VWF-dependent hemostasis and prevent VWF-dependent thrombosis. In addition, the finding that VWF activates ADAMTS13 raises...
cardial infarction (32)

activity seem to increase the risk of ischemic stroke and myo-
clinical relevance. Relatively modest decreases in ADAMTS13

etermine the relative importance of substrate binding and allosteric
domains other than T7-CUB2. Further study is required to de-
activate mouse Adamts13, which suggests that allosteric activation
ADAMTS13, deletion of the distal T7-CUB2 domains may not
that are similar to VWF71 (19, 31). Therefore, in contrast to human
Adamts13, which is consistent with the role we propose for binding
distal domains to VWF D4 (19). However, long and short
Adams13 may rely mainly on substrate binding rather than allostery.

Some mouse strains express a “long” Adamts13 that is similar to
full-length human ADAMTS13. Other strains have an intracisternal
A-particle (IAP) retrotransposon inserted in the
ADAMTS13 gene and express a “short” Adamts13 that is truncated after the T6 (31).
Long Adams13 cleaves VWF multimers ninefold faster than short
Adams13, which is consistent with the role we propose for binding
distal domains to VWF D4 (19). However, long and short
Adams13 reportedly have similar activity toward small substrates
that are similar to VWF71 (19, 31). Therefore, in contrast to human
ADAMTS13, deletion of the distal T7-CUB2 domains may not
activate mouse Adamts13, which suggests that allosteric activation
is less significant than for human ADAMTS13 or depends on
domains other than T7-CUB2. Further study is required to de-
terminate the relative importance of substrate binding and allosteric
activation for ADAMTS13 in humans, mice, and other vertebrates.

The allosteric properties of ADAMTS13 are likely to have
clinical relevance. Relatively modest decreases in ADAMTS13
activity seem to increase the risk of ischemic stroke and myo-
cardial infarction (32–34). In animal models, ADAMTS13 de-
ciciency promotes vascular inflammation and atherosclerosis (35).
Therefore, inherited or acquired defects in the allosteric regulation
of ADAMTS13 could inhibit VWF cleavage and contribute to
thrombosis in these conditions.

Conversely, agents that block allosteric activation could be
useful to treat bleeding caused by excessive proteolysis of VWF, which
occurs mainly in two settings. Von Willebrand disease type
2A frequently is due to mutations like G1505E (27) that impair
the folding of the VWF A2 domain and allow cleavage by
ADAMTS13 even in the absence of fluid shear stress. As a result,
the VWF multimers that circulate in the blood are too small for
effective hemostasis (36, 37). Acquired von Willebrand syndrome
also is characterized by deficiency of the largest VWF multimers,
often as a result of pathologically increased fluid shear stress that
promotes cleavage by ADAMTS13. For example, this type of
acquired von Willebrand syndrome is very common in patients
with implanted left ventricular assist devices, who experience re-
current major gastrointestinal bleeding as a result (38). Because
ADAMTS13 can be allosteric activated by ∼fourfold to ∼10-fold,
an inhibitor of allosteric activation could reduce ADAMTS13 activity to
no lower than ∼10%, which is not low enough to cause TTP (6, 7)
but may be useful to treat bleeding. The feasibility of this ap-
proach is supported by a study demonstrating that a mono-
oclonal antibody against the VWF D4 domain inhibits ADAMTS13
binding and markedly reduces the excessive degradation of VWF multimers in an ex vivo model using a HeartMate II left ventricular assist device (39).

Like ADAMTS13, other ADAMTS proteases and related ADAMTSL proteins also have complex C-terminal structures with up to 15 T repeats and variable numbers of proteoglycan, a pro tease and lacunin (PLAC) domain, and Gon-1 modules that are involved in mediated regulatory protein–protein interactions (40, 41). For example, N-terminal procollagen processing depends on ADAMTS2, and defects in ADAMTS2 cause a variant of Ehlers-Danlos syndrome (41). Microfibrol fibrils regulate the activation of TGF-β, which is force-dependent (42), as is the processing of VWF by ADAMTS13 (8, 11). The biogenesis of microfibrols involves several ADAMTS and ADAMTSL proteins, and mutations in them cause developmental disorders such as Weill-Marchesani syndrome (ADAMTS10, ADAMTS17), geleophysic dysplasia (ADAMTSL2), and ectopia lentis (ADAMTS17, ADAMTS14) (41, 43). The distal domains of these homologous proteins share many structural features with ADAMTS13 and may have similar allosteric properties that are essential for their biological functions.

**Materials and Methods**

**Antibodies.** Mouse monoclonal antibodies to ADAMTS13 were made by DNA immunization with a plasmid encoding human ADAMTS13, i.p. injection of recombinant human ADAMTS13, and isolation of hybridomas by standard methods (44). Epitopes were localized and binding affinities determined by ELISAs (44) and immunoprecipitation (45) using a panel of ADAMTS13 constructs with C-terminal truncations or internal domain deletions.

Plasma samples from patients with TTP or healthy donors were obtained with informed consent under protocols approved by the institutional review board of Washington University or BloodCenter of Wisconsin.

**ADAMTS13 Assays.** Fluorogenic peptide substrate FRETs-rVWF71 (VWF71) has an N-terminal Gly followed by VWF Gly1959 Arg1668 with mutations N1610C and K1617R. The N terminus is modified with IRDye Q-1 N-hydroxysuccinimide ester (Li-COR), and Cys1610 is modified with DiLight 633 maleimide (Thermo Scientific) (22). FRETs-rVWF72, FRETs-rVWF74, and FRETs-rVWF62 were prepared similarly except with C-terminal residues Asn1622 Arg1641, and Arg1659, respectively.

ADAMTS13 was assayed with fluorogenic substrates as previously described (22). Standard assays included 50 mM Bis-Tris (pH 6) or Hepes (pH 7.4), 150 mM NaCl, 10 mM CaCl2, 0.05% Tween-20, 1 mg/mL BSA, 1 μM fluorogenic substrate. For recombinant ADAMTS13 variants in a total volume of 200 μL. Reaction was initiated by adding buffer containing substrate to enzyme in 96-well white microplates (Optiplate-96, PerkinElmer) at 30 °C. Product generation was monitored as an increase in fluorescence using a VictorV Multilabel Counter (PerkinElmer) or Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek) with 635 ± 10-nm excitation and 660 ± 10-nm emission filters. Assays by this method have an interassay coefficient of variation of <2% (22).

ADAMTS13 activity as a function of pH was assayed similarly, except the buffer included 20 mM Bis-Tris, 20 mM Hepes, and 20 mM Tris-HCl at pH values between pH 6 and pH 7.4 in increments of 0.2 pH unit.

Shear-induced cleavage of VWF was assayed as described previously (16, 17), with modifications. Reactions (30 μL total volume) were performed in 50 mM Hepes (pH 7.4), 150 mM NaCl, 5 mM CaCl2, and 1 mg/mL BSA at room temperature with plasma VWF (40 nM) and ADAMTS13 variants (50 nM) without or with 10 mM EDTA, without or with fluid shear stress, and with or without antibody 12D4 or 19H4. To be sure that ADAMTS13 was saturated with antibodies, the final concentrations of 19H4 (100 μg/mL) and 12D4 (40 μg/mL) were −20-fold higher than the EC50 for activating ADAMTS13 toward VWF71 (Fig. S2). Reactions were stopped by adding EDTA to 10 mM. Values between pH 6 and pH 7.4 in increments of 0.2 pH unit.

**Binding Assays.** Dissociation constants (Kd) were determined by biolayer interferometry (BLI) using an Octet RED96 (Fortebio). GST-VWF73 (12) was captured by biotinylated anti-GST antibody bound to streptavidin-biosensors. Binding to ADAMTS13 proteins was performed in 20 mM Hepes (pH 7.4), 150 mM NaCl, 2 mg/mL BSA, and 0.02% Tween 20. A buffer-only reference was subtracted from all curves. Affinities were determined from global kinetic analysis for a 1:1 binding model using Octet RED software, Version 5.2.

**Molecular Modeling.** An atomic model of MDTCS was constructed from the crystallographic structure of DTCs domains (15) and a homology model of MD domains (13) that was based on ADAMTS4 (46).

Full-length ADAMTS13 was built using Hhpred (47) to model distal T domains, CUB domains, and linkers after T4 and T8. Templates for T domains included 414-CK, A1-CK, and A1-CK(G1505E) were captured on Ni-NTA biosensors. Binding to ADAMTS13 proteins was performed in 20 mM Hepes (pH 7.4), 150 mM NaCl, 2 mg/mL BSA, and 0.02% Tween 20. A buffer-only reference was subtracted from all curves. Affinities were determined from global kinetic analysis for a 1:1 binding model using Octet RED software, Version 5.2.

**Activation Assays.** Pooled normal Li+-heparin plasma (PNP) was prepared from >35 healthy donors. Plasma samples from patients with TTP were screened for activation of ADAMTS13 in assays (200 μL) with FRETs-rVWF71, 50 μL PNP, and 50 μL of plasma. Monoclonal antibodies were screened with 25 μL PNP and ~2 μg of antibody per reaction. VWF and VWF fragments were added at varying concentrations. EC50 values for activators were determined from assays of serial dilutions and nonlinear regression (22). Differences between mean values were assessed with the unpaired Student t test.

**Small Angle X-ray Scattering.** SAXS data were collected for ADAMTS13 variants on the SIBYLS beamline 12.3.1.2 at the Advanced Light Source (ALS), a national user facility operated by the Lawrence Berkeley National Laboratory and supported by the Director, Office of Science, Office of Basic Energy Sciences, of the US Department of Energy under Contract DE-AC02-05CH11231 (51). All proteins were monodisperse and yielded similar scattering profiles at three or four concentrations between 0.5 mg/mL and 3.0 mg/mL. Pair distance distribution functions were obtained using DATGONM (52).

The quality of the SAXS data was evaluated by comparison with an atomic model of MDTCS. The M domain was modeled (13) on ADAMTS4 (46), DTCs domains were from the corresponding crystal structure (15), and N-linked oligosaccharides were added with GLYPROT (53). This structure was compared with the experimental scattering profiles using CRYSTOL (28), and agreement was excellent with χ < 1.86 (28). Models (envelopes) were generated from scattering profiles using DAMMIN (54), averaged (n = 15) using DAMAVER (55), and superimposed on the atomic structure of MDTCS using SUPCOMB (56).

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Supporting Information

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SI Materials and Methods

ADAMTS13 and VWF Proteins. Recombinant ADAMTS13 (1) was obtained from Baxter Innovations. Purified human plasma VWF (Laboratoire Français du Fractionnement et des Biotechnologies, Lille, France) was chromatographed on a Superdex 200 equilibrated with 50 mM Hepes (pH 7.4), 150 mM NaCl, 5 mM CaCl₂, and 1 μM ZnCl₂ to remove traces of human serum albumin. Trace amounts of residual ADAMTS13 were removed by adsorption on monoclonal antibody 3H9-agarose.

VWF fragments SPI, SPII, and SPIII were prepared by digestion of plasma VWF with Staphylococcal V8 protease (Thermo Scientific Pierce) and purified by ion exchange chromatography (2).

Human cDNA constructs encoding VWF domain D4 (Ser₁⁸⁷₃-Thr²²⁵₃) (3), full-length mature ADAMTS13 (4), and ADAMTS13 truncated after domain S (Ala₉₈₅-MDTCS), T7 (Arg¹⁰⁷₅-M-T7), or T8 (Ala¹¹⁹₁-M-T8), with or without the mutation E₂₂₅Ｑ, were cloned into pTriEx-7 Ek/LIC (EMD Millipore). These constructs encode the following (underlined) mouse IgM signal peptide, StrepTag II, and enteropeptidase recognition pore. These constructs encode the following (underlined) mouse IgM signal peptide, StrepTag II, and enteropeptidase recognition pore. These constructs encode the following (underlined) mouse IgM signal peptide, StrepTag II, and enteropeptidase recognition pore. These constructs encode the following (underlined) mouse IgM signal peptide, StrepTag II, and enteropeptidase recognition pore.

Constructs D4-CK in vector pcDNA3.1 (Invitrogen) encodes VWF residues Met¹-Cys²² and Gly¹⁰⁷₄-Lys¹⁰⁷₅ followed by a 6xHis tag. Constructs A1-CK in vector pSV7D (6) and A1-CK(G1₅₀₅E) in vector pcDNA3.1 encode VWF residues Met¹-Cys²² and Glu¹₆₈₀-Lys²₀₁₃ followed by a 6xHis tag. These three VWF constructs contain the mutation C₂₇₇₃ₐ to prevent C-terminal dimerization (7). A1-CK(G1₅₀₅E) and D4-CK were expressed in stably transfected HEK293 cells. A1-CK was expressed in transiently transfected BHK cells. Conditioned Freestyle serum-free medium was adsorbed on Q-Sepharose, and proteins were eluted with 20 mM Hepes (pH 7.4) and 1 M NaCl. Appropriate pooled fractions were dialyzed against 20 mM Hepes (pH 7.4) and 150 mM NaCl. Imidazole (20 mM) was added, and proteins were adsorbed on HisPur Cobalt agarose (Thermo Scientific Pierce). After washing with 20 mM Hepes (pH 7.4), 150 mM NaCl, and 20 mM imidazole, proteins were eluted with buffer containing 300 mM imidazole.

All proteins were further purified by chromatography on a TSK-G2000SW or Superdex 200 and stored at −80 °C. The concentration of proteins was determined in BCA protein assays (Thermo Scientific Pierce) standardized with BSA.

For epitope localization, ADAMTS13 constructs were cloned in pcDNA4/TO with C-terminal V5 and (His)₉ tags and expressed transiently in T-Rex 293 cells (8). C-terminal deletions were truncated after domain M (Gln¹²⁸⁶), D (Gly¹⁶₈₅), T1 (Glu¹⁴⁹₃), C (Cys¹₆₅₅), S (Ala₉₈₅), T2 (Tyr¹₃₈), T3 (Arg¹⁰⁷₅), T7 (Arg¹⁰⁷₅), T8 (Ala¹¹⁹₁), or CUB1 (Ala¹₂⁹₉). Internal deletions lacked domain T2 (Trp⁶₆₆⁵, Tyr⁷₄₅), T3 (Trp⁷₄⁶, Arg¹⁰⁷₅), T4 (Trp⁸₀₈⁵-Ala⁸⁹₄), T5 (His⁹⁵⁹-Pro⁹₅₂), T₆ (Ala⁹₅₃, Arg¹₀₁₅), T₇ (Trp¹₀₁₆, Arg¹⁰⁷₅), T₈ (Trp¹₀₇₆-Ala¹₁₉₈), or CUB1 (Cys¹₁₉₂-Glu¹₂₉₈).


Fig. S1. Reaction time course for MDTCS and ADAMTS13 as a function of pH. Enzymes MDTCS and ADAMTS13 were assayed with substrate VWF71 at the indicated pH values.
Fig. S2. Dependence of ADAMTS13 activation on monoclonal antibody concentration and pH. Concentration dependence of ADAMTS13 activation at pH 7.4 (open circles) by monoclonal antibody 12D4 (Left) or 19H4 (Right). At pH 6.0 (open squares), ADAMTS13 activity was less affected by either antibody. Antibody 12D4 recognizes ADAMTS13 CUB domains, and antibody 19H4 recognizes ADAMTS13 T8 domain. ADAMTS13 activity values are expressed as the ratio of the VWF71 cleavage rate in the presence/absence of antibody. Error bars indicate 95% confidence intervals.

Fig. S3. Structure of VWF, V8 protease digestion products, and recombinant VWF fragments. (A) Dimensions of the VWF subunit are to scale based on EM data, and the total length is ~65 nm (1). Reannotated D3 and D4 assemblies contain VWD (blue), C8 (green), TIL (yellow), and E or D4N domains (violet); D’ consists of TIL and E domains. Other domains are VWA (pink), VWC (violet), and CK (chartreuse). E, D4N, and C domains are homologous. Whiskered lines are O-glycosylated segments. Staphylococcal V8 protease cleaves after Glu^{2129} (2) at the boundary between domains VWD4 and C8-4 to generate fragments SPII and SPIII as indicated. Additional cleavage after Glu^{1675} between domains A2 and A3 produces fragment SPI. Recombinant A1-CK and D4-CK have the CK domain mutation C2773A and are monomeric. (B) Gel electrophoresis of purified VWF proteolytic fragments and recombinant VWF proteins.

Fig. S4. Binding of MDTCS and ADAMTS13 to VWF constructs. BLI sensorgrams, steady-state analyses, and $K_d$ values ± SE are shown for MDTCS(E225Q) (Left) and full-length ADAMTS13(E225Q) (Right) binding at pH 7.4 to the indicated VWF derivatives.