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ADAMTS13 conformations and mechanism of inhibition in immune thrombotic thrombocytopenic purpura

Konstantine Halkidis[1], **X. Long Zheng**[2,3]

[1]Division of Hematologic Malignancies and Cellular Therapeutics, Department of Internal Medicine, The University of Kansas Medical Center, Kansas City, Kansas, USA

[2]Department of Pathology and Laboratory Medicine, The University of Kansas Medical Center, Kansas City, Kansas, USA

[3]Institute of Reproductive and Developmental Sciences, The University of Kansas Medical Center, Kansas City, Kansas, USA

Abstract

ADAMTS13, a plasma metalloprotease that cleaves von Willebrand factor, is crucial for normal hemostasis. Acquired autoantibody-mediated deficiency of plasma ADAMTS13 results in a potentially fatal blood disorder, immune thrombotic thrombocytopenic purpura (iTTP). Plasma ADAMTS13 protease appears to exist in multiple conformations. Under physiological conditions, plasma ADAMTS13 exists predominantly in its “closed” conformation (or latent form), which may be activated by lowering pH, ligand binding, and binding of an antibody against the distal domains of ADAMTS13. In patients with iTTP, polyclonal antibodies target at various domains of ADAMTS13. However, nearly all inhibitory antibodies bind the spacer domain, whereas antibodies that bind the distal C-terminal domains may activate ADAMTS13 through removing its allosteric inhibition. Additionally, the anti-C-terminal antibodies may alter the potency of inhibitory antibodies towards ADAMTS13 activity. This review summarizes some of the most recent knowledge about the ADAMTS13 conformation and its mechanism of inhibition by its autoantibodies.

Keywords

activation; ADAMTS13; autoantibody; inhibition; TTP/HUS

Correspondence Konstantine Halkidis, Division of Hematologic, Malignancy and Cellular Therapeutics, Department of Internal Medicine, The University of Kansas Medical Center, 3901 Rainbow Blvd, Kansas City, Kansas 66160, USA. khalkidis@kumc.edu, X. Long Zheng, Department of Pathology and Laboratory Medicine, The University of Kansas Medical Center 3901 Rainbow Blvd, Kansas City, Kansas 66160 USA. xzheng2@kumc.edu.

AUTHOR CONTRIBUTIONS

Konstantine Halkidis and X. Long Zheng designed the study, analyzed the results, and wrote the manuscript. Both authors approved the final version of the manuscript for submission.

CONFLICT OF INTEREST

X.L.Z. was a speaker for Alexion and Sanofi, but continues serving a consultant for Alexion, Sanofi, and Takeda. X.L.Z. is also the co-founder of Clotsolution. K.H. has declared no relevant conflict.

1 | INTRODUCTION

Immune thrombotic thrombocytopenic purpura (iTTP) is an autoimmune disease, caused by autoantibody-mediated inhibition of a plasma metalloprotease ADAMTS13.[1–5] The only known function of ADAMTS13 to date is to cleave the glycoprotein von Willebrand factor (VWF), which recruits platelets to sites of vascular injury for hemostasis.[6,7] In the absence of functional ADAMTS13, ultra-large VWF multimers accumulate and cause a thrombotic microangiopathy that is potentially fatal unless being recognized early in the disease course and treated accordingly.[8–10] Though significant progresses have been made over the past decades regarding the understanding of pathogenesis of iTTP, treatment options remain crude and nonspecific. Therapeutic plasma exchange has been part of the standard of care for iTTP, and it must be initiated early in the disease course to prevent high mortality associated with disseminated microvascular thrombosis.[11–13] The monoclonal antibodies against CD20 on B-cells, rituximab and anti-VWF nanoboy, caplacizumab are the adjunct treatments that work indirectly to block the formation of autoantibodies and the recruitment of platelets to ultra-large VWF, respectively.[14–23] To improve on our current therapeutic standards, there is still a need to explore the mechanism(s) by which autoantibodies cause iTTP. To do so, we must also learn more about how ADAMTS13 works. Recent work by our group and others suggests a complex landscape of conformations and functional states of ADAMTS13, both in the native context and when bound to antibodies.[24–37] Both structural and functional studies have been used to gain insight into the function and antibody-mediated inhibition of ADAMTS13. We will breifly review the current state of knowledge of ADAMTS13 function and inhibition.

2 | ADAMTS13 STRUCTURE

The partial and then completed primary structures of ADAMTS13 were first reported by several groups in 2001.[1,38–41] Secreted ADAMTS13 protein consists of a metalloprotease (M) domain, a disintegrin (D) domain, the first thrombospondin type 1 (TSP1–1) repeat, the Cys-rich (C) and spacer (S) domains, followed by 7 more TSP1 repeats and two CUB domains, associated with complement components C1r/C1s, sea Urchin epidermal growth factor, Bone morphogenetic protein (Figure 1A). While the tertiary structure of a full-length ADAMTS13 protein has yet to be determined, the truncated versions of recombinant ADAMTS13 protein (e.g., MDTCS or its variants) have recently been described using a variety of techniques, including X-ray crystallography, small angle X-ray scattering, and mass spectrometry plus hydrogen/deuterium exchange, and cross-linking mass spectrometry.[27,28,42,43] The MDTCS fragment contains all the necessary and sufficient domains of ADAMTS13 protein for substrate specificity and efficient cleavage.[28,43,44,45] From these studies, coupled with functional assays, the spacer domain of ADAMTS13 has been identified to be crucial for substrate cleavage and specificity.[45–48]

The crystal structure of the DCTS fragment of ADAMTS13 was reported in 2009,[44] which demonstrates that the spacer domain is highly structured, with 10 beta sheets forming a “sandwich” that exposes various loops. These loops are important epitopes for antibody binding, as we will review later. However, it was not until 2019 that the structure of MDTCS domains was determined,[27] using a variant with the catalytic glutamate in

the metalloprotease domain substituted for a glutamine (E225Q), bound with a mouse anti-human ADAMTS13 monoclonal antibody against the metalloprotease domain, which stabilizes the protein. The structure revealed that the active site is conformationally hidden, or buried, in this context. The extent to which MDTCS must be manipulated to obtain a crystal structure suggests that the wild-type native protein, as well as full-length recombinant ADAMTS13, may be somewhat disordered. This may also suggest that the metalloprotease domain may be flexible and plastic. This poses a significant challenge to structural biologists since ADAMTS13 was first discovered and cloned in 2001.[1,38–41] Recently, cross-linking mass spectrometry was used to demonstrate that, when bound to a surrogate substrate (VWF73), the metalloprotease, disintegrin, cysteine-rich, and spacer domains undergo a significant rearrangement, with the first TSP1 repeat functioning as a "hinge" region, placing the spacer domain, which is the primary determinant of substrate specificity, in close proximity to the metalloprotease domain.[43] This likely occurs via a series of low-affinity interactions with VWF at various exosites that causes dozens, if not hundreds, of small refolding events. The authors describe this as a "fuzzy complex" with a "dynamic zipper" mechanism, offering evidence that ADAMTS13 is likely an intrinsically disordered protein.[43]

3 | ADAMTS13 CONFORMATIONS

The role of the other distal C-terminal domains of ADAMTS13 has started to become clearer in recent years. The CUB domains may associate with the spacer domain when the protein is in a less active state (e.g., before it is bound by a substrate).[36,49] This likely prevents ADAMTS13 from indiscriminately cleaving VWF and possibly other proteins, suggesting an autoregulatory role of the distal domains. The crystal structure of the two CUB domains of human recombinant ADAMTS13 has been published recently, yielding important insights into the function of the CUB domains, which will be further discussed later.[50]

Though we begin to get a handle on what the CUB domains are doing, we have largely limited ourselves to the consideration of an intramolecular interaction between the distal C-terminal domains of ADAMTS13 and the proximal N-terminal domains. Yang et al. have performed molecular modeling and demonstrated that there may be three potential sites in the spacer domain—exosite 3 (R568, F592, R660, and Y661), G607-S610, and exosite 4 (E634, D635, and R636)—that may interact with the first CUB domain (Figure 1B).[51] Mutations in the exosite 3 appear to disrupt the interaction between spacer and CUB, leading to an increase of ADAMTS13 activity.[34,36,52]

Recent data published by Rottensteiner et al. have demonstrated that there may be a role for the intermolecular ADAMTS13 interactions as well, with evidence that recombinant ADAMTS13 may form dimers or oligomers in nonreducing conditions.[53] These oligomers may conceivably have interactions between the CUB domains on one monomer and the spacer domain on another monomer, for example. The implications of this oligomerization, as well as whether it is relevant in physiologic conditions, is an important area of ongoing investigation with tremendous implications regarding the function and inhibition of ADAMTS13.

Despite these significant advances, we still do not have any structural studies that are available for a full-length ADAMTS13. This is important because, though allosteric inhibition is implied by structural studies to some degree, functional studies suggest that allosteric changes can be communicated along the entire length of the protein.[24,25,32,54] Our understanding of the mechanisms by which this allosteric regulation works is still evolving. More importantly, the study of anti-ADAMTS13 antibodies might have revealed surprising insights into the function and inhibition of ADAMTS13, as we will be discussing later.

4 | ADAMTS13 INHIBITION: POTENTIAL NOVEL MECHANISM OF AUTOANTIBODY-MEDIATED INHIBITION

Most patients with iTTP have immunoglobulin G antibodies that bind the spacer domain to mediate their inhibition, although multiple other domains of ADAMTS13 may be targeted as well.[55–64] To date, there is no published study to elucidate precisely how the anti-spacer (inhibitory) IgG work to prevent the VWF cleavage, with a widely held assumption that they somehow sterically interfere with substrate binding being unchallenged.[33] Furthermore, a significant subset of patients may harbor antibodies that bind the C-terminal domains of ADAMTS13.[26,56,65] Mouse monoclonal antibodies against the C-terminal domains have been developed, some of which are capable of stimulating ADAMTS13 activity instead of inhibiting it.[24,25,66,67] This led to the hypothesis that these anti-C-terminal antibodies may allosterically create an “open” conformation of ADAMTS13 by relieving the autoinhibition of the CUB domains that are presumably binding to the spacer domain. This binding interaction may induce conformational changes that at the very least move the CUB domains away from spacer domain[30] (Figure 2A).

To explore this hypothesis, Kim et al. used *in silico* docking simulations to identify the residues in the CUB domains that likely interact with the spacer domain. They then generated full-length variants of recombinant ADAMTS13 with mutations in putative interaction sites to test enzymatic activity in the presence of a truncated surrogate VWF substrate.[50] They also assessed the activity of these variants in the presence and absence of an activating mouse monoclonal antibody (17G2). The hypothesis was that the “open” conformation would be favored in variants with decreased affinity between spacer and the CUB domains, and thus 17G2 should not increase ADAMTS13-mediated substrate cleavage. This was based on the assumption that the action of the stimulatory antibody is to decrease the CUB-mediated steric barrier to ADAMTS13-VWF binding. The authors then used a second round of docking simulations to test the hypothesis that the residues identified as abrogating the effects of 17G2 (W1245, W1250, K1252, R1326, E1387, and E1389) were important in the spacer-CUB interaction. The highest scoring pose using two different crystal structures of the spacer domain (PDB codes: 3GHM and 6QIG) and two different programs (ClusPro and HADDOCK) paired these CUB residues with spacer domain exosite residues previously shown to be important both in the function of ADAMTS13 and its inhibition by anti-ADAMTS13 antibodies. However, no *in vitro* binding data have yet been published to corroborate this hypothesis, and the mechanistic consequences are still not clear. For instance, it is not known if the spacer-CUB interaction sterically prevents VWF

binding to ADAMTS13 or if other mechanism(s) are involved. The mechanism(s) by which 17G2 and other similar antibodies stimulate ADAMTS13 activity is still not known.

Our group recently used a human monoclonal antibody that binds to the C-terminal ADAMTS13 domains identified via phage display from a patient with iTTP,[56] which showed strong activation of ADAMTS13.[30] This is the first evidence of human monoclonal antibody-mediated stimulation of ADAMTS13 activity.[30,56] We also showed that at physiologic pH, a condition in which ADAMTS13 activity is extremely low *in vitro*, our stimulatory antibody was capable of not only recovering activity equivalent to standard assay conditions, but in fact increased reaction velocity by threefold at saturating antibody concentrations.[32,68,69] Presumably this may be mediated by promoting the "open" conformation of ADAMTS13. However, the physiological relevance of antibody-mediated activation of ADAMTS13 in patients with iTTP remains to be determined. Further in this review, we will revisit this data and discuss the potential implications.

Using a cryptic epitope of ADAMTS13 that is only recognized in the "open" conformation, it has recently been shown that the vast majority of patients with acute iTTP are in the "open" conformation, a seeming contradiction because these patients have little to no detectable ADAMTS13 activity.[25,71] The group also showed that patients in remission who had ADAMTS13 in the "open" conformation were found to be more likely to develop a relapsed disease.

Combined with the structural insights reviewed previously, this paints a very complicated picture of ADAMTS13 biology in patients with iTTP, in which conformational accessibility of ADAMTS13 to bind VWF probably cannot explain exactly what is happening in these patients. For this reason, it is critical to identify whether anti-C-terminal and/or other "opening" antibodies are central to the pathogenesis of iTTP. If their mechanism of action can be elucidated, we can explore ways to rescue ADAMTS13 activity by similar mechanisms, or at the very least to prevent inhibitory anti-spacer domain antibodies from forming because of prolonged epitope exposure in their presence. Next, we will discuss recent mechanistic insights into ADAMTS13 using enzymology-based approaches.

5 | KINETIC STUDIES: A STORY OF BINDING VS. ACTIVITY

The epitopes that inhibitory (anti-spacer domain) antibodies bind are also required for specific recognition of VWF.[45,46,52,71] It was hypothesized that inhibitory anti-spacer antibodies may physically block the binding of ADAMTS13 to VWF, based on hydrogen exchange plus mass spectrometric analysis.[33] As such, if this hypothesis is correct, the functional assays should reveal that the presence of inhibitory anti-spacer domain antibodies should lead to an increase in the substrate concentration that leads to the half-maximal reaction velocity. To test this hypothesis, most groups have relied on Michaelis–Menten kinetics-based experiments to determine the parameters k_{cat}, K_M, and k_{cat}/K_M of ADAMTS13.[24,72] To wit, if the inhibitory antibodies cause problems with substrate binding, the K_M should increase out of proportion to any change in k_{cat}. If k_{cat} is more affected than K_M, it suggests that antibodies may affect the protein in a way that is independent of alterations in substrate binding.

One group has used a stimulatory mouse monoclonal antibody against the C-terminal domains of ADAMTS13 to determine the k_{cat}, K_M, and k_{cat}/K_M of recombinant ADAMTS13 in the presence of various concentrations of a surrogate substrate.[24] Notably, they found that the k_{cat} was more affected than the K_M. This is consistent with an alternative hypothesis that the stimulatory antibody may not simply make ADAMTS13 easier to bind VWF by swinging the CUB domains away from the spacer domain as shown (Figure 2B), but somehow increases the catalytic turnover rate of ADAMTS13 by other unknown mechanisms. Clearly, this is potentially paradigm-shifting in the field, and it is a major focus of our group's ongoing studies. This may help better elucidate ADAMTS13 function and regulation towards developing better diagnostic and therapeutic tools for management of iTTP in the future.

6 | COOPERATIVITY: IMPLICATIONS FOR FUTURE RESEARCH IN iTTP

Cooperativity in protein chemistry is classically described by the example of the hetero-tetramer hemoglobin, in which binding of a ligand to one subunit of hemoglobin affects the ligand-binding affinity of adjacent subunits.[73–75] It can also be seen in monomeric proteins, as in the other classic example of hexokinase, where stable intermediate conformations with different ligand binding affinities are more or less probable at different ligand concentrations.[76]

Our recent data using a human monoclonal anti-C-terminal antibody, isolated from a patient with iTTP, have shown that the antibody is not only capable of stimulating ADAMTS13 activity, but also exhibits a remarkable positive cooperativity.[30] This is particularly evident when normal human plasma containing a wild-type ADAMTS13 is titrated with a stimulatory antibody under physiologic pH conditions. Even more strikingly, the simultaneous presence of stimulatory and inhibitory antibodies in physiologic conditions showed strong positive cooperativity. Our findings imply that ADAMTS13 may function as a multimeric protein in which the binding of one antibody to one monomer increases the probability of another antibody binding to an adjacent monomer. Alternatively, a monomeric ADAMTS13 may adapt multiple stable intermediate forms, only becoming more capable of binding to other antibodies when the ligand concentrations increase above a certain threshold.

Whether the cooperativity we observed occurs in the context of monomeric or multimeric ADAMTS13 is of critical importance because we do not know how ADAMTS13 would function optimally, poorly, or not at all as a monomer or a member of a complex. Identifying the factors that affect the multimer formation or the stability of multiple functional conformations of ADAMTS13, if they exist, would also enhance our understanding of the pathophysiology of iTTP. It may be also possible to exploit these properties to increase ADAMTS13 activity.

Despite the recent and historical elucidation of the role of ADAMTS13 in iTTP outlined here, which represents an impressive body of scientific achievement in a relatively short time frame, much work still needs to be done to elucidate the structure and function, as well as regulation of ADAMTS13 activity, the mechanism of antibody-mediated inhibition, and

the pathophysiology of iTTP to improve our clinical management of this potentially fatal blood disease, and other inflammatory and thrombotic disorders that might be associated with imbalance of ADAMTS13/VWF axis.

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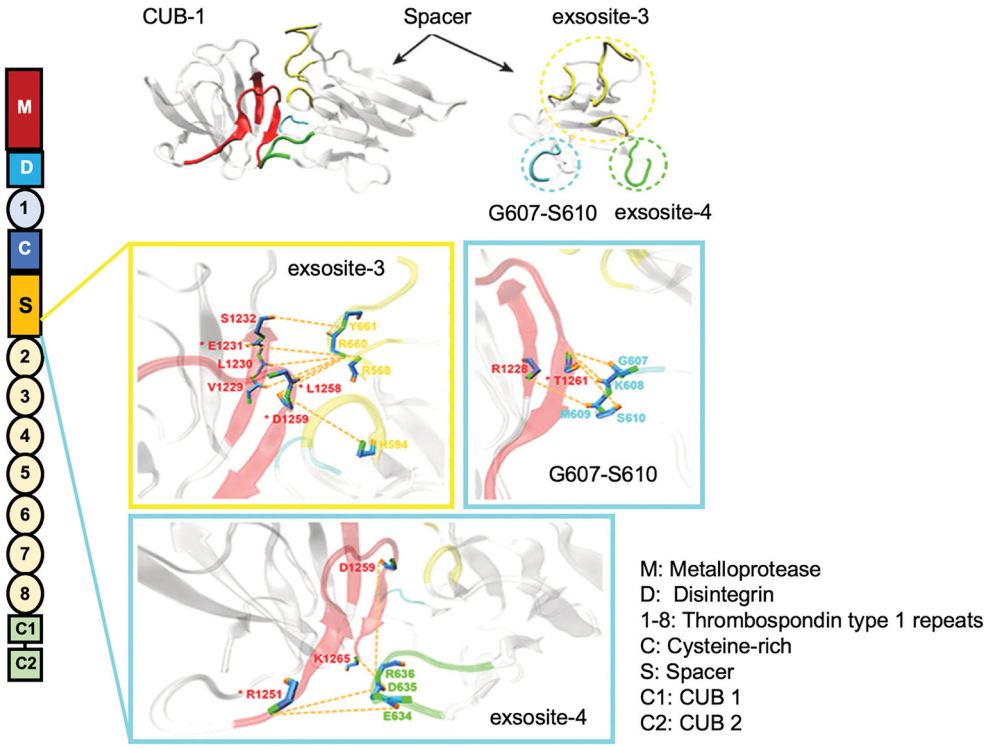

FIGURE 1.

The primary domain structure of ADAMTS13 and proposed binding interaction between CUB and spacer domain. (A) ADAMTS13 consists of a metalloprotease, a disintegrin, the first TSP1 repeat, Cys-rich and spacer domain, followed by seven more TSP1 repeats, and two CUB domains. (B) Multiple binding regions in the Spacer interface including exosite 3 (R568, F592, R660, Y661, Y665), G607-S610, and exosite 4 (E634, D635, and R636) appear to interact with the CUB1 domain (E1231, R1251, L1258, D1259, and T1261) to mediate autoinhibition (Adapted from Yang et al.[51]).

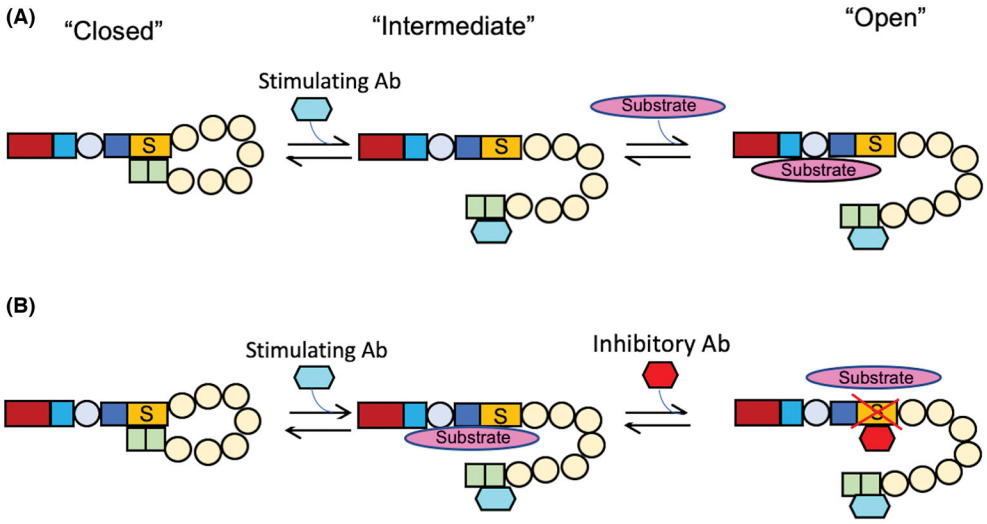

FIGURE 2.

Conformational changes of ADAMTS13 induced by antibodies. (A) Transition from closed to open conformation of ADAMTS13 upon its binding with a human monoclonal anti-CUB IgG (scFv3–3) and its subsequent cleavage of VWF73 peptide. (B) The possible mechanism underlying the synergistic effect of a stimulating anti-CUB (scFv3–3) and an inhibitory anti-Spacer (i.e., scFv4–20) on proteolytic cleavage of VWF substrate (i.e., VWF73) by ADAMTS13 (Adapted from Halkidis and Zheng[30])