Investigating self-inhibition of ADAMTS13 by massive protein-protein interaction sampling

Unveiling the structural role of distal domains in global latency of ADAMTS13

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In Short

- Thrombotic thrombocytopenic purpura (TTP)
- ADAMTS13 global latency mechanism
- TIGER2h replica-exchange MD simulations
- Large scale protein-protein interaction sampling

Thrombotic thrombocytopenic purpura (TTP) is a rare, life-threatening blood disorder. Severe deficiency of ADAMTS13 (A Disintegrin and Metalloprotease with Thrombospondin type 1 repeats-13) can be caused by mutations in the ADAMTS13 gene (hereditary TTP) or by anti-ADAMTS13 antibodies (immune-mediated TTP). This leads to ultra-large von Willebrand factor (VWF) multimers to accumulate and the formation of microthrombi, resulting in microangiopathic hemolytic anemia and severe thrombocytopenia. VWF is present in the form of disulfide-linked multimers (ranging from dimers to 100-mers) and is critical for platelet recruitment to damaged blood vessels. While too little VWF leads to a bleeding disorder called von Willebrand disease, high plasma VWF concentrations and large multimers are risk factors for thrombosis. Hence, strict and precise regulation of both VWF concentration and VWF multimer size is physiologically important. The size of VWF is proteolytically regulated by ADAMTS13. Upon shear stress, the A2 domain of VWF unfolds to reveal a specific binding and cleavage site for ADAMTS13. Multiple VWF-binding exosites connecting to specific ADAMTS13 domains are known to determine the specificity and participate in the two separate allosteric mechanisms of ADAMTS13 activation by VWF (molecular zipper). A "global latency" state is defined by the CUB1-2 domains being bound to the Spacer module, and an additional "local latency" mode is characterized by the central gatekeeper residues blocking the entry of the substrate into the active site cleft at the MP domain. The crystal structures of the ADAMTS13-MDTCS domains and the CUB1-2 domains have been solved recently, and molecular docking experiments between the CUB1-2 domains and the ADAMTS13 Spacer domain have provided insights into the Spacer-CUB1-2 interface. Current treatment options for TTP are plasma exchange, immunosuppressants, or plasma infusion, reducing overall mortality from 85-95% to 10-20%. The delivery of recombinant ADAMTS13 has been suggested as an alternative therapeutic approach. Consequently, it is crucial to understand the underlying molecular principles responsible for VWF recognition and engagement with ADAMTS13, as well as the inherent latency mechanisms[1–3].

In multiple previous projects, we developed and refined an optimized derivative of the replica exchange molecular dynamics simulation method (REMD) termed TIGER2h(s) and successfully applied it for the extensive structural sampling of protein structures, and protein-protein complexes[4].

In a recent project [5], we applied the TIGER2h method to investigate the large configurational space of the isolated ADAMTS13-MDTCS domains and its binding to the unfolded VWF-A2 domain, which acts as both substrate and activating cofactor to ADAMTS13. There, the sampling of binding states was guided in accordance with the available experimental data and we were able to extensively refine the exosite information for each ADAMTS13 domain and provide, for the first time, direct pairs of interacting amino acids for the entire interaction surface. We were able to create the first molecular interaction model that puts previous experimental results in perspective to better understand disease-related mutations in view of improved therapies. A number of experimental targets are proposed to verify the stated binding pathways, refine the general picture of MP binding pockets, the involvement of Dis-binding in MP activation, and the passage of the Cys-rich domain by VWF-A2, thereby deepening the understanding of a highly dynamic interaction. Our model can be used as a structural platform for the design of novel therapeutics not only for TTP, but also for other diseases associated with a perturbed ADAMTS13/VWF axis, such as myocardial infarction, stroke, and malaria.

In the present project, we aim to address the "global latency" mechanism of ADAMTS13, which, together with our previous work on "local latency" and substrate binding, will add to complete the molecular picture of the proteolytic regulation of ADAMTS13 and how this is perturbed in life-threatening disorders such as TTP.

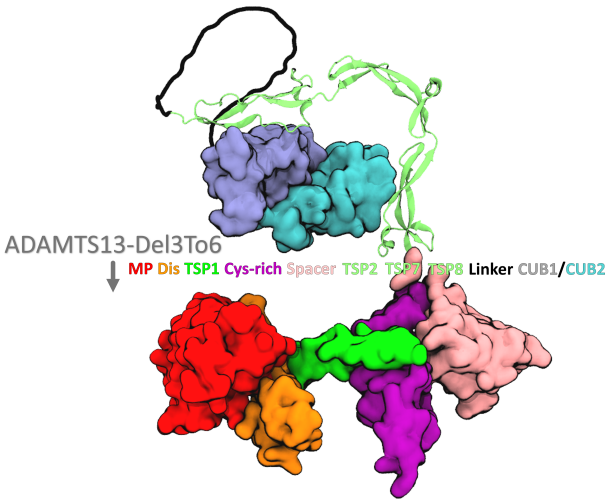

Figure 1: *AlphaFold2 model of minimal functional ADAMTS13-Del3To6 construct.*

The "global latency" mode of ADAMTS13 is controlled by the binding of the distal CUB1 and CUB2 domains to the Spacer domain. This binding is physiologically disrupted by VWF-D4CK, but can also be induced by anti-Spacer or anti-CUB monoclonal antibodies (mAb). This activation results in a two-fold increase in the activity of ADAMTS13. However, it has been shown that this gain in activity is not caused by the exposure of the Spacer or Cys-rich domain exosites for VWF-A2. A VWF-A2 substrate construct lacking the corresponding reciprocal region for binding either the Spacer or Cys-rich domain showed lower proteolytic efficiency but still a two-fold increase when the Spacer-CUB binding was disrupted via VWF-D4CK or corresponding mAbs. Therefore, the rupture of the Spacer-CUB binding somehow influences the activity of the remote MP domain. To date, there's no satisfying explanation for this long-range allosteric effect.

Multiple works have shown that not only the two C-terminal CUB domains are required for this process, but also the TSP8 repeat and the flexible Linker region between TSP8 and the CUB1-2 domains are needed. The shortest ADAMTS13 construct that still allows this two-fold auto-inhibition can delete TSP3 through TSP6 and is similar to ADAMTS13 from pigeons (Figure 1). Under physiological conditions, global ADAMTS13 activation occurs via VWF-D4CK, which shows the strongest binding to CUB1, but also binds TSP8. Hence, TSP8 is one major factor required for the "global latency" mechanism that has not gained much attention previously.

A cryptic epitope in the MP domain was revealed by binding studies with anti-MP domain mAb's 6A6 and 3H9. While 3H9 can bind similarly well to full-length ADAMTS13 or the MDTCS part truncated by the distal domains, 6A6 displayed a strong difference, as it could bind MTDCS much better than full-length constructs. The same behavior could be shown with full lengths ADAMTS13 that was conformationally activated by anti-Spacer or anti-CUB mAb's. This was formerly explained by allosterically induced conformational changes to the MP domain, but this long-range effect could not be described in detail.

We hypothesize, that TSP8 binds near the MP domain and thereby exerts the two-fold reduced activity. We, therefore, aim to investigate the binding of TSP8 to the area around the MP domain and how the distal domains will wrap around the MDTCS surface when the CUB1-2 domains are bound to Spacer in the globally latent form.

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More Information

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DFG Subject Area

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