observe a $64^\circ$ bend between the 2 spectrin repeats, and hypothesize that the pocket formed by this bend provides many of the high-affinity contacts involved in ankyrin binding. Ipsaro et al, in contrast, see no such bend, but they do observe crystallographic evidence for considerable flexibility within this region, suggesting that the linker could be flexible and that the spectrin conformer immobilized in their crystal structure assumes a nonbent conformation. Possible resolution of this discrepancy can be found in a recent online article by Davis et al\(^5\) that describes the crystal structure of the ankyrin binding domain (repeats 14–16) of $\beta_2$-spectrin as having a bent structure. As observed by Stabach et al, Davis et al also find that differences in interrepeat contacts, involving an homologous loop in $\beta_2$-spectrin, may be responsible for the unusual bend seen in the 2-repeat structure that binds ankyrin.

In summary, a possible unifying interpretation that emerges from all of the data are that acidic amino acids near the C-terminus of helix C in repeat 14, together with the linker connecting repeat 14 to repeat 15 and the loop linking helices B and C of repeat 15, all contribute to formation of a flexible pocket on spectrin that associates with ankyrin. Because defects in the spectrin-ankyrin function are associated with such human pathologies as hereditary spherocytosis, cardiac arrhythmia, premature aging, deafness, and ataxia,\(^6\) further examination of this very important membrane complex is clearly warranted.

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REFERENCES


A team player: the disintegrin domain of ADAMTS13

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In this issue of Blood, de Groot and colleagues identify novel interactions between the disintegrin domain of ADAMTS13 and the A2 domain of VWF. This provides another piece in the puzzle of how this domain of ADAMTS13 works in concert with other domains to bind and process VWF.

ADAMTS13, a member of A disintegrin and metalloprotease with thrombospondin type 1 repeats (ADAMTS) family,\(^1,2\) is a multidomain glycoprotein found in plasma.\(^3\) It consists of numerous domains including a metalloprotease domain, a disintegrin domain, first thrombospondin type 1 (TSP1) repeat, a cysteine-rich domain, and a spacer domain (see panel A in the figure). These are followed by 7 more TSP1 repeats and 2 CUB (complement C1r/c1s, sea urchin epidermal growth factor, and bone morphogenetic protein) domains (figure panel A). ADAMTS13 makes a precise cut on the von Willebrand factor (VWF) molecule after the amino acid residue Tyr$^{1605}$ at the central A2 domain. This proteolytic cleavage is essential to reduce the size of VWF polymers so that they remain functional enough to stop bleeding, but not so sticky as to cause unwanted thromboses in the small arterioles.\(^4,5\) However, it is still unclear how each of the ADAMTS13

A (A) Schematic domain structure of ADAMTS13. Human ADAMTS13 is composed of a catalytic region (ie, metalloprotease domain [MP]) and noncatalytic region that is divided into 3 parts: the proximal, middle, and distal. The proximal noncatalytic part has a disintegrin domain (Dis), first thrombospondin type 1 (TSP1) repeat, a cysteine-rich domain (Cys), and a spacer domain. The middle and distal noncatalytic regions contain 7 more TSP1 repeats and CUB domain, respectively. (B) Homolog model of the metalloprotease and disintegrin domains of ADAMTS13. The metalloprotease domain is shown in light blue. Three active sites His and catalytic zinc ion (pink). The disintegrin domain is depicted in light green, light pink, and red. The hypervariable region (HVR) is highlighted in light pink with Arg349 and Leu350 highlighted in red. These 3 amino acids lie adjacent to the active site cleft. Arg349 is located approximately 26 Å from the active site Zn$^{2+}$. See the complete figure in the article beginning on page 5609.
domains contributes to the binding and proteolytic processing of VWF under physiologic conditions.

Previous studies have shown that the metalloprotease domain of ADAMTS13 alone is ineffective in cleaving VWF, 6,7 but if the various noncatalytic domains are incrementally added back, proteolytic activity is gradually restored. 6,7 These results support a linear relationship between the domains of ADAMTS13 and VWF proteolysis. Gao et al 8 have identified several potential sites on the VWF-A2 domain that may make direct contacts with various proximal noncatalytic domains of ADAMTS13 under static conditions. This result is in agreement with that reported previously by Ait et al, 6 in which ADAMTS13 variants truncated after the spacer domain with an additional internal deletion of either disintegrin domain or disintegrin plus TSP1-1 repeat have markedly reduced proteolytic activity toward VWF fragment and exhibit no proteolytic activity toward full-length VWF. Collectively, these data support a hypothesis that all the proximal noncatalytic domains of ADAMTS13 are required for productive engagement with VWF-A2 domain at least under static/denaturing conditions.

In this issue of Blood, de Groot et al 9 focus on the involvement of the disintegrin domain of ADAMTS13 in VWF processing in more detail. They use molecular modeling (panel B in the figure) and site-directed mutagenesis to identify the involvement of the disintegrin domain of ADAMTS13 and site-directed mutagenesis to identify the involvement of the disintegrin domain of ADAMTS13 in VWF processing in more detail. They use molecular modeling (panel B in the figure) and site-directed mutagenesis to identify the involvement of the disintegrin domain of ADAMTS13 in VWF processing in more detail. They use molecular modeling (panel B in the figure) and site-directed mutagenesis to identify the involvement of the disintegrin domain of ADAMTS13 in VWF processing in more detail. They use molecular modeling (panel B in the figure) and site-directed mutagenesis to identify the involvement of the disintegrin domain of ADAMTS13 in VWF processing in more detail.

...suggest that binding of all the proximal noncatalytic domains of ADAMTS13 to VWF is necessary to position the active site of ADAMTS13 to the scissile bond (Tyr1605-Met1606) on VWF, resulting in productive cleavage.

...It remains to be seen how this domain functions in concert with the other domains of ADAMTS13 in the presence of shear stress that alters VWF conformation in a more physiologic way. Could it be that the other domains of ADAMTS13 are more important than the disintegrin domain in binding VWF in order to align it with the scissile bond for cleavage in vivo? For instance, the recent report by Zhang et al 10 suggests a role of the middle and distal parts of the noncatalytic region in participating in binding and proteolytic processing of VWF under fluid shear stress. Therefore, further investigation of the precise interactions between each of ADAMTS13 domains and VWF may shed light on understanding the pathogenesis of thrombotic thrombocytopenic purpura, a potentially fatal illness caused primarily by the absence of plasma ADAMTS13 proteolytic activity, as a result of ADAMTS13 mutations or acquired autoantibodies against ADAMTS13 enzyme.

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REFERENCES


...RTEs: lazy T-cell teenagers

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In this issue of Blood, Opiela and colleagues analyze the phenotype and function of the lymphoid periphery’s youngest T cells, RTEs.

Recent thymic emigrants (RTEs) are T cells that have just exited from the thymus, having completed an approximately 2-week journey that takes them from stem cell to committed T cell. Only 1% to 5% of thymocytes survive this complex maturation process that begins with T–cell receptor gene rearrangement and ends with a select population of lineage committed T cells that are both self-major histocompatibility complex (MHC)–restricted and self-tolerant. 1 Throughout the lifetime of the individual, RTEs are essential for the maintenance of a diverse population of naive peripheral T cells, ready to further differentiate into appropriate effector T cells upon encounter with foreign antigen.

...It has long been of interest to identify and analyze RTEs as a population distinct from the bulk of peripheral T cells, in order to quantify thymic output and to assess whether T-cell maturation continues after thymic egress. Understanding RTE biology is of particular importance for predicting recovery of the immune system following lymphoablative therapy or viral infection, and for the study of immunity in neonates (in which the bulk of the lymphoid periphery consists of RTEs) and in aged individuals (in which RTEs represent a small minority of peripheral T cells). Previous methods for tagging RTEs have included intrathymic injection of fluorochromes, transplantation of congenically marked thymus...
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