Investigating Inter-Domain Regulation of von Willebrand Factor Interactions with Platelets

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Abstract/Introduction

Von Willebrand factor (vWF), a large multimeric blood plasma protein, is integral to *in vivo* platelet aggregation and clot formation (Figure1) [1]. Of particular interest are the shear dependent interactions between the A_1-domain of vWF and platelet glycoprotein (GP) Ib_α [2]. Weak, transient bonding between these two partners anchors platelets and vWF long enough for other glycoprotein and integrin mediated bonds to form [3]. Disruption of these transient bonds, such as mutations that abolish the shear dependence of the interaction, lead directly to clinical illnesses such as von Willebrand disease, the most common hereditary blood clotting disorder [1]. To detect the presence of inter-domain regulation within vWF, we investigated the interaction of platelets with the isolated A_1, $A_1A_2A_3$, $D'D_3A_1A_2A_3$ and $\Delta D'D_3$ ($A_1A_2A_3D_4B_1B_2B_3C_1C_2C_k$) domains of vWF under flow conditions. In addition, we conducted ristocetin induced platelet aggregation (RIPA) assays to verify construct functionality, and enzyme linked immunosorbent assays (ELISA) to quantitate construct surface absorption.

Results

ELISA, which were visualized at A_{650} with a horseradish peroxidase (HRP)/HRP antibody system developed by Invitrogen, revealed nonlinear concentration dependence as well as variable coat concentration for the different constructs tested — multimeric vWF, A_1, $A_1A_2A_3$, $D'D_3A_1A_2A_3$. Construct size dependent adsorption was observed as expected — A_1 coated at ~24%, $A_1A_2A_3$ at ~58% and $D'D_3A_1A_2A_3$ at ~67% of multimeric vWF coating (data not shown).

RIPA assays verified the functionality of the dimeric ($D'D_3A_1A_2A_3$ and $\Delta D'D_3$), but not monomeric (A_1 and $A_1A_2A_3$) constructs. RIPA assays rely on the fact that platelets, in combination with ristocetin (antagonist) and a cross linker should aggregate in solution. In Figure 2, our negative control (platelets + vWF) shows no aggregation, while our positive control (platelets + vWF + ristocetin) and dimeric constructs (platelets + construct + ristocetin) aggregate in solution. This reveals that both vWF and dimeric constructs are functionally active in solution; that is, they bind platelets with high affinity in the presence of a ristocetin antagonist. For monomeric, the paradigm is the opposite— platelets + vWF + ristocetin + monomer should actually inhibit platelet aggregation versus platelets + vWF + ristocetin. No platelet aggregation inhibition was consistently observed, suggesting that our A_1 and $A_1A_2A_3$ were functionally inactive. This is curious, considering that A_1 is widely known to inhibit platelet aggregation in the presence of ristocetin (or botrocetin, a close analog) [2,4].

Flow chamber experiments were conducted for plates coated with multimeric vWF, A_1, $A_1A_2A_3$, $D'D_3A_1A_2A_3$ and $\Delta D'D_3$ in the range of 0.0025 Pa to 16 Pa wall shear stress. Platelet interactions occurred for all constructs other than A_1 and $A_1A_2A_3$, where the interaction is quantified as the number of platelets bound to the surface in two minutes under flow conditions. Bovine serum albumin (BSA)-coated plates were used as a negative control and a baseline threshold for platelet interactions; that is, constructs

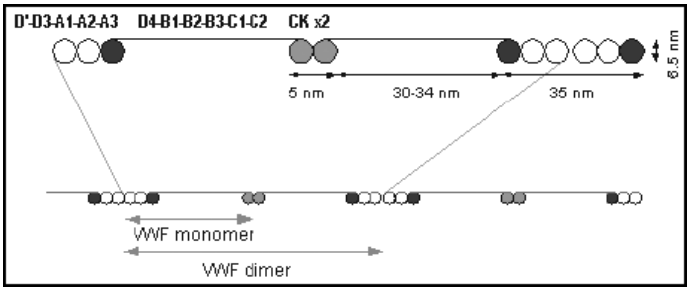

Figure 1: Domain-level structure of von Willebrand factor.

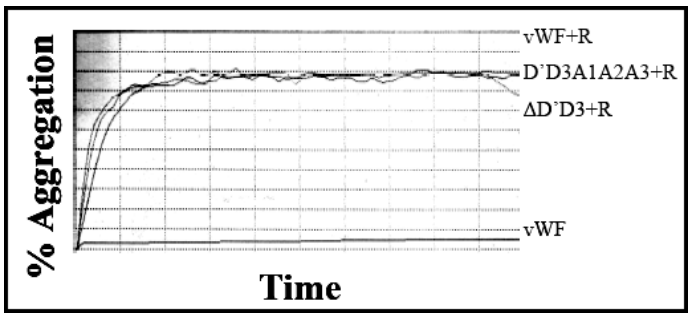

Figure 2: RIPA assay for dimeric vWF constructs.

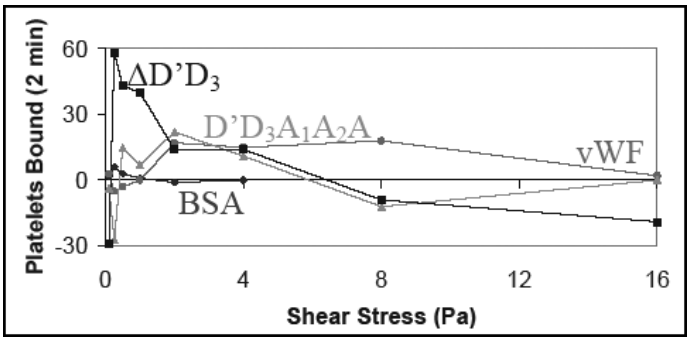

Figure 3: Flow chamber experiment comparing the shear-dependent platelet binding of vWF constructs.

that bound less platelets than BSA, such as A_1 and $A_1A_2A_3$, were considered nonspecific and discounted.

Four experiments were performed in the same conditions on separate days and Figure 3 shows the results from one representative/typical experiment. Note that the $\Delta D'D_3$ construct experiences peak platelet binding at a much lower shear than both multimeric vWF and $D'D_3A_1A_2A_3$. This demonstrates that the $\Delta D'D_3$ domain has a role in regulating the shear dependence of vWF platelet interactions.

Furthermore, at high shear (> 2 Pa), platelets tend to detach from the dimeric constructs while platelet binding occurs throughout the entire high shear range for multimeric vWF.

Methods

Flow chamber experiments were conducted with circular parallel plate flow chambers (Glycotech) following the protocol of Dogget, et. al. 2003 [5]. Variable concentrations of multimeric vWF (Haematologic Technologies Human von Willebrand factor VIII Free, Cat#: HCVWF0191, Stock 210 μg/mL), A_1, $A_1A_2A_3$, $D'D_3A_1A_2A_3$ and/or $\Delta D'D_3$ were coated onto 35 mm round polystyrene petri dishes (Corning) via a 90 minute incubation at 37°C. Recombinant vWF-A_1 [6] and $A_1A_2A_3$, $D'D_3A_1A_2A_3$ and $\Delta D'D_3$ were prepared and isolated as previously described [7]. vWF construct coated dishes were mounted to the flow chamber, suffused with a platelet rich suspension isolated from healthy donor whole blood and visualized under a 10X light microscope (Nikon) [5]. RIPA assays were conducted following the protocol of Cruz, et. al. 2000 [2].

Conclusion

This study reveals that inter-domain regulation does indeed occur in vWF platelet interactions. Flow chamber experiments demonstrate that the D'D3 domain regulates shear dependent platelet binding by inhibiting low shear vWF platelet interactions by an, as of yet, undetermined mechanism. One possible mechanism is that the $D'D_3$ domain inhibits the A_1 domain at low shear, preventing GP Ib_α binding. However, at high shear, vWF and $D'D_3$ are elongated by shear forces, exposing the A_1 domain.

While in general agreement with our findings, the elongation theory does not explain the discrepancy between the dimeric constructs and vWF platelet binding at high shear. Repeating flow chamber experiments with GP Ib_α coated polystyrene beads instead of intact platelets and characterization under an atomic force microscope are needed to cement our findings as well as to test mechanistic hypothesizes.

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References

[1] Wagner DD. (1990) An. Rev. Cell. Bio. 6:217242.
[2] Cruz MA, et. al. (2000) J. Bio. Chem. 275.25:1909819105.
[3] Doggett TA et. al. (2002) Biophys. J. 83:194–205.
[4] Fukuda K et. al. (2005) Nature Struct. Mol. Bio. 12:2:152159.
[5] Doggett TA et. al. (2003) Blood 102:1:152160.
[6] Cruz MA et. al. (1993)) J. Bio. Chem. 268.28:2123821245.
[7] Ulrichts H et. al. (2006). J. Biol. Chem., 281.8:46994707.