Shear-dependent Interactions of von Willebrand Factor With Factor VIII and Protease ADAMTS 13 Demonstrated at a Single Molecule Level by Atomic Force Microscopy

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INTRODUCTION

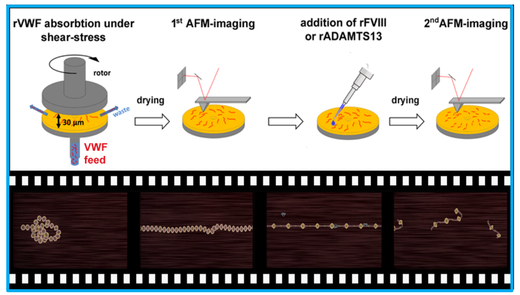
- Von Willebrand Factor (VWF) is a large glycoprotein which is essential in blood coagulation and has also been found to play multiple roles in inflammation, apoptosis, cancer propagation, and other physiological and pathological processes.
- Regarding haemostasis, VWF mediates adhesion of platelets to injured endothelial cells on the one hand, facilitating platelet recruitment especially at high wall shear stress, and acts as a carrier for Factor VIII (FVIII) on the other, thereby essentially prolonging its half-life in the circulation.
- VWF circulates as long, loosely coiled multimer chain (also termed concatemer) which abruptly forms large fibers when shear rates exceed a certain threshold value of yet unclear order of magnitude.
- These conformational changes increase its binding activity towards components of the subendothelium matrix of a disrupted vessel wall, and renders VWF susceptible to cleavage by the protease ADAMTS13. Cleavage occurs between residues Tyr-1605 and Met-1606 which are buried inside the VWF A2-domain and become accessible only upon stretching of this domain.
- In the context of these conformation-dependent functions, a further key function of VWF merits elucidation, namely, the ability to bind FVIII, which has not been investigated in terms of shear rate dependence at a macro-molecular level.
- The effect of VWF elongation on its ability to bind FVIII is not yet completely elucidated.

OBJECTIVE

- To study the elongation of VWF at a single molecular scale by atomic force microscopy (AFM)
- To explore critical shear forces required for molecular stretching by combining a microfuidic device with AFM imaging
- To study the interaction of fluid-mechanically stretched VWF molecules and FVIII at single molecule level

METHODS

Schematic Diagram of Experimental Procedure



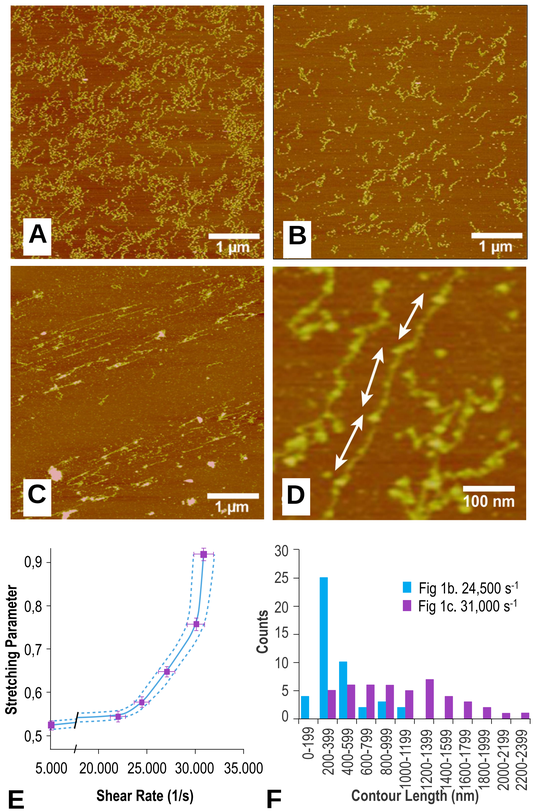
- Full-length recombinant FVIII (ADVATE, Baxalta) was used as FVIII. Recombinant VWF (Baxalta) served as a source for VWF, recombinant ADAMTS13 (Baxalta) as a source for ADAMTS13.
- Adsorption was carried out on a mica substrate from a solution containing 0.86 µg/mL VWF in Tris buffered saline (TBS, 50 mM Tris, 150 mM NaCl, 0.4 µM $MgCl_2$, pH 7.4) for 5 min.
- VWF molecules were stretched in a custom-made, rheometer-type microfluidic device.
- Molecules were rinsed with Milli-Q water before AFM imaging.
- Tapping mode AFM imaging was done with a NanoScope V (Bruker, Santa Barbara, CA).
- NanoScope Software version v7.1.30 (Bruker) was used for Image Processing and Data Analysis.
- Stretching parameters were determined with Datalab version 3.5.30 (Epina, Austria)

RESULTS

Critical Shear Rate for Stretching of VWF

- **Figure 1** shows AFM images of VWF molecules at increasing shear rates (Fig. 1A: 22.000 s^{-1}, Fig. 1B: 24.500 s^{-1}, Fig. 1C: 31.500 s^{-1}). The number of molecules affected by shearing (disentangling and elongation) increased with the shear rate.
- **Figure 1D** shows molecules stretched at 30.000 s^{-1}. The linear parts of chains show dimer lengths of 80−100 nm as indicated by two-headed arrows.
- Between 27.000 and 31.000 s^{-1}, an apparently sharp transition into a strongly elongated state with chain lengths of several micrometers was observed (**Figure 1E**).
- The stretching parameter used in **Figure 1E** was calculated as the quotient of the maximum one-dimensional length of the (coiled) molecules and the actual full contour length of the whole molecule.
- Whereas disentangling of the molecules already starts at shear rates above 20.000 s^{-1}, stretching of individual molecules also occured at higher shear rates (around 30.000 s^{-1}), as shown by the full contour lengths in **Figure 1F**.

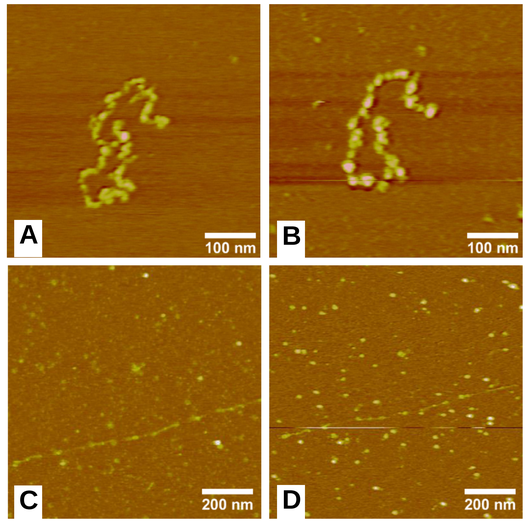
Figure 1: VWF Stretched at Increasing Shear Rates



FVIII Binding Activity of Shear-Stretched VWF

- **Figure 2** shows AFM images of non-stretched (**Figure 2A and B**) and stretched (**Figure 2C and D**) VWF molecules before (**Figure 2A and C**) and after (**Figure 2B and D**) treatment with FVIII.
- Identical molecules before and after FVIII interaction were detected using a nanoscopic scratch on the mica substrate surface as a marker.
- Adsorption on the surface occurred under static conditions (no flow).

Figure 2: Shear-dependent Interaction Between VWF and FVIII



- For non-stretched VWF, binding of FVIII was shown by large bright knots along the VWF chain (**Figure 2B**).
- For fully stretched VWF, the molecule chain appeared more or less identical before (**Figure 2C**) and after (**Figure 2D**) treatment with FVIII, indicating that FVIII does not attach to stretched VWF chains.

Binding of ADAMTS13 to VWF and Proteolysis of Shear-Stretched VWF

- **Figure 3** shows stretched VWF molecules before (**Figure 3A**) and after (**Figure 3B, C and D**) treatment with ADAMTS13.
- For fully stretched VWF (**Figure 3A and B**), complete proteolytic cleavage of the VWF chain was demonstrated after 22 s.
- At a lower degree of stretching (**Figure 3C and D**), after 22 s (**Figure 3C**) cleavage was not yet be observed, and after 60 s (**Figure 3D**) only a partly cleaved VWF chain was observed.
- **Figure 4** shows non-stretched VWF molecules before (Figure 4A) and after (Figure 4B) treatment with ADAMTS13. Large bright dots indicate binding of ADAMTS13 to VWF. Under these conditions, proteolytic cleavage of the VWF chain was not observed.

Figure 3: Proteolysis of Shear-Stretched VWF by ADAMTS13.

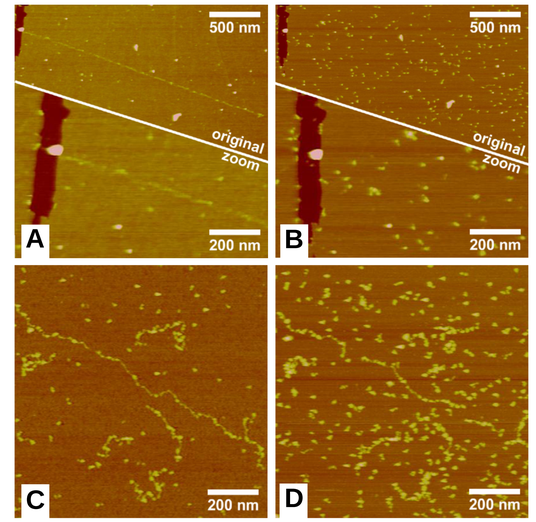
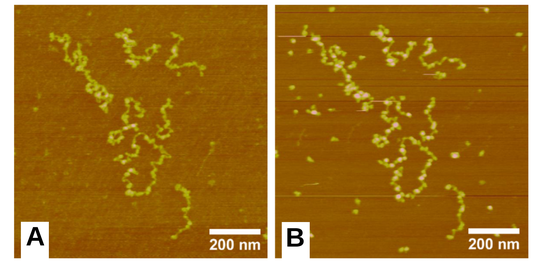


Figure 4: Interaction between Non-Stretched VWF and ADAMTS13



CONCLUSION

- Using a single molecule approach, stretching of individual VWF molecules and the effect of stretching on the interaction of VWF with FVIII and ADAMTS13 was visualized.
- The results indicate that VWF serves as a molecular bus for FVIII as long as the molecule is coiled and releases FVIII upon shear stress.
- Binding of ADAMTS13 to VWF was also visualized. Proteolytic cleavage of VWF by ADAMTS13 is strongly enhanced when VWF molecules are elongated by mechanical shear stress.

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