

Mechanism of Lateral Self-Association of Von Willebrand Factor.

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Von Willebrand Factor (VWF) is a multimeric plasma protein that chaperones coagulation Factor VIII and binds platelets to an injured vascular wall. VWF molecules are secreted from vascular endothelial cells as variable numbers of disulphide-linked homodimers. When exposed to the shear forces found in the flowing blood, VWF molecules undergo lateral self-association that results in a meshwork of VWF fibres that bind platelets. Plasma VWF contains unpaired cysteine thiols and lateral association is inhibited by thiol-alkylating agents, implying that this process involves thio/disulphide exchange between VWF molecules. A recombinant C-terminal fragment of VWF was exposed in mammalian cells and examined for unpaired cysteine thiols using tandem mass spectroscopy. The Cys2431-Cys2453 disulphide bond in the VWF C2 domain was shown to be reduced in approximately 75% of the molecules. Fragments containing all three C domains or just the C2 domain formed monomers, dimers and higher-order oligomers when expressed in mammalian cells. From mutagenesis studies, both the Cys2431-Cys2453 and nearby Cys2451-Cys2468 disulphide bonds were found to be involved in oligomer formation. The findings imply that lateral VWF dimers form when a Cys2431 thiolate anion attacks the Cys2431 sulphur atom of the Cys2431-Cys2453 disulphide bond of another VWF molecule. Trimers and higher-order oligomers can also form when the Cys2451 thiolate anion of one of the VWF molecules in the dimer attacks the Cys2431-Cys2453 disulphide bond of another VWF molecule. These observations provide the basis for exploring defects in lateral VWF association in patients with unexplained hemorrhage or thrombosis.

No conflict of interest to disclose