von Willebrand factor folds into a bouquet

Volker Gerke

Centre for Molecular Biology of Inflammation (ZMBE), Institute of Medical Biochemistry, University of Muenster, Muenster, Germany.
Correspondence to: gerke@uni-muenster.de

The EMBO Journal (2011) 30, 3880–3881. doi:10.1038/emboj.2011.321

The large glycoprotein von Willebrand factor (vWF) serves an important role in orchestrating the blood vessel's response to injury. It is released from activated endothelial cells and forms long multimeric strings that bind exposed extracellular matrix and circulating platelets and thereby initiate the formation of a platelet plug sealing the wound. Central to this process is the rapid transition of multimeric vWF from a tightly packed storage form present in acidic intraendothelial granules, to the elongated string that is active at neutral vascular pH. In this issue of *The EMBO Journal*, Zhou and coworkers now provide compelling structural evidence for internal conformational changes that accompany this transition and thereby serve important regulatory functions. They show that the C-terminal part of the protein zips up into a dimeric bouquet at the acidic internal pH of the storage granules but opens up at the neutral pH of plasma, thereby assisting the establishment of elongated vWF strings capable of efficiently capturing platelets.

Blood vessel wounds have to be repaired rapidly and in a tightly controlled manner to prevent excessive leakage and maintain vascular function. An immediate response to vessel injury is the formation of a platelet plug that is initiated by the acute release of vWF from activated endothelial cells. vWF is a multimeric glycoprotein that binds to platelet glycoprotein Ib and also other receptors on activated platelets. To efficiently capture platelets at sites of vessel injury vWF has a unique property, it forms elongated strings of covalently linked concatamers that can consist of > 100 vWF molecules and can span a length of more than 100 µm (making it the largest soluble protein in vertebrates). Importantly, string formation of vWF is tightly regulated and only occurs once the protein is released into the vasculature. Inside endothelial cells vWF multimers are stored as a condensed tubular form in acidic organelles, the Weibel-Palade bodies (WPB; Sadler, 1998, 2009; Wagner, 1990; Metcalf *et al*, 2008; Valentijn *et al*, 2011). How is the switch from condensed tubules to elongated strings brought about and how is the tight packing of vWF concatamers in the WPB achieved?

To answer these questions, we have to consider the complex structure and maturation of vWF. It is synthesized as a preproprotein at the ER with the signal peptide cleaved off following synthesis. In the ER, vWF dimerizes via interchain disulphide bond formation in the C-terminal cysteine-knot (CK) region (Figure 1A). Further maturation occurring in the

Golgi apparatus includes furin cleavage between domains D2 and D' and another interchain disulphide linkage between C3 domains of adjacent dimers. Thereby vWF forms long concatamers linked by tail–tail (C-terminal) and head–head (N-terminal) disulphides. To allow efficient and space-saving package into WPB, the vWF multimers are condensed by the formation of helical tubules, in which the D'D3 domains of neighbouring dimers are non-covalently joined via a dimer of the D1D2 propeptide that remains associated with the rest

Figure 1 Schematic representation of the vWF domains and their folding into tubular concatamers. (A) Linear sequence of the pro-vWF domains. Furin cleavage liberates domains D1 and D2 that remain associated with the mature vWF peptide at the acidic pH of the Golgi and WPB. (B) Helical arrangement of the multimeric vWF tubules found in WPB. The N-terminal domains (D1D2 and D'D3) form a helix whereas A2-CK fold into a dimeric bouquet (see Zhou *et al*, 2011). The sequences neighbouring A1 are likely to function as a flexible hinge allowing the C-terminal part (A2-CK) to pack between adjacent vWF tubules. Colours of the domains as in (A). See text for details.

of the molecule following furin cleavage (Huang *et al*, 2008; Berriman *et al*, 2009). This process proceeds in maturing WPB and results in WPB that contain densely packed, paracrystalline vWF tubules enwrapped in a tight-fitting membrane. The tightly packed vWF tubules thereby define the unique structure of WPB, long cigar-like organelles 1–5 µm in length and ~200 nm in diameter. Critical for the propeptide-assisted assembly of vWF into helical tubules are elevated Ca^{2+} concentrations and a low pH met in the Golgi and in mature WPB, which have a luminal pH of ~5.5 (Erent *et al*, 2007; Huang *et al*, 2008). The pH dependence of propeptide-assisted vWF packing allows vWF concatamers to rapidly unfurl once exocytic fusion of WPB with the plasma membrane leads to alkalization (Michaux *et al*, 2006). Thus as in many other processes, a pH switch regulates the transition from an inactive (storage) form, here the paracrystalline vWF tubules, to the physiologically active variant, the long vWF strings present in the vasculature.

While the propeptide-chaperoned helical tubularization of vWF that involves the N-terminal domains of the molecule provides the basis for tight packing, the structural organization of the C-terminal part of the protein (domains A1 to CK) had remained elusive. Zhou *et al* (2011) now provide evidence for the folding of these C-terminal domains that is also pH regulated and contributes to both the tight packing in WPB and the efficient unfurling at exocytotic sites. Using truncated derivatives of vWF and analysing their hydrodynamic (gel filtration) and structural properties (negative stain EM) they show that the C-terminal two thirds of mature vWF adopt an elongated dimeric conformation at the low luminal pH of WPB. In this fold, the B1-C2 domains of the two subunits align with each other and form a stem-like structure covalently linked by the disulphide bridge between the two CK domains located at the base of this stem. The preceding domains, A2, A3 and D4, could be identified as three pairs on top of this stem assuming a flower-like structure that the authors refer to as raceme. Finally, Zhou *et al* (2011) also obtained evidence to position the A1 domain that is flanked by two flexible regions rich in O-glycosylations above the raceme (Figure 1B). Importantly, this structural arrangement

of A2-CK dimers is only seen at acidic pH, at neutral pH the entire structure opens up, most likely due to a loss of contact between the B1-C2 domains. Thus, the C-terminal part of vWF acts in concert with the helical part of the concatamers to execute a global pH-regulated switch from a compact to an extended conformation.

As discussed by Zhou *et al* (2011) this dynamic structural behaviour of the C/C3-CK dimer has important physiological consequences for the assembly, secretion and vascular function of vWF. During formation of the vWF multimers in the Golgi/WPB, the arrangement of dimeric stem/raceme structures helps to ensure that the D'D3 domains are positioned in a way supporting helical assembly and that the N-terminal disulphide bond formation occurs between adjacent D'D3 domains of two vWF dimers and not within a dimer or with a neighbouring vWF tubule. Furthermore, although the stem/raceme structure appears rather rigid at low pH, the highly glycosylated, flexible linkers adjacent to A1 (thin lines in Figure 1B) allow a bending of the stem that enables this C-terminal part to pack properly between adjacent vWF tubules in the paracrystalline arrangement in mature WPB. Alkalization that occurs upon WPB exocytosis and proceeds from the fused end of the WPB to the more distal one (Erent *et al*, 2007) rapidly triggers an opening of the helical conformation since contacts to the D1D2 propeptide are lost. As shown by Zhou *et al* (2011) this opening of the structure also propagates into the C-terminal two thirds of the molecule, resulting in a loss of the stem-like conformation. Thus, the N- and C-terminal parts of vWF unfurl in a concerted manner at sites of exocytosis, most likely from one end of the tubule to the other, thereby ensuring a maximal length of vWF strings most potent in binding platelets.

Acknowledgements

I thank Anja Biesemann for preparing the figure

Conflict of interest

The author declares that he has no conflict of interest.

References

- Berriman JA, Li S, Hewlett LJ, Wasilewski S, Kiskin FN, Carter T, Hannah MJ, Rosenthal PB (2009) Structural organization of Weibel-Palade bodies revealed by cryo-EM of vitrified endothelial cells. *Proc Natl Acad Sci USA* **106**: 17407–17412
- Erent M, Meli A, Moiso N, Babich V, Hannah MJ, Skehel P, Knipe L, Zupancic G, Ogden D, Carter T (2007) Rate, extent and concentration dependence of histamine-evoked Weibel-Palade body exocytosis determined from individual fusion events in human endothelial cells. *J Physiol* **583**: 195–212
- Huang RH, Wang Y, Roth R, Yu X, Purvis AR, Heuser JE, Egelman EH, Sadler JE (2008) Assembly of Weibel-Palade body-like tubules from N-terminal domains of von Willebrand factor. *Proc Natl Acad Sci USA* **105**: 482–487
- Metcalfe DJ, Nightingale TD, Zenner HL, Lui-Roberts WW, Cutler DF (2008) Formation and function of Weibel-Palade bodies. *J Cell Sci* **121**: 19–27
- Michaux G, Abbitt KB, Collinson LM, Haberichter SL, Norman KE, Cutler DF (2006) The physiological function of von Willebrand's factor depends on its tubular storage in endothelial Weibel-Palade bodies. *Dev Cell* **10**: 223–232
- Sadler JE (1998) Biochemistry and genetics of von Willebrand factor. *Annu Rev Biochem* **67**: 395–424
- Sadler JE (2009) von Willebrand factor assembly and secretion. *J Thromb Haemost* **7**: 24–27
- Valentijn KM, Sadler JE, Valentijn JA, Voorberg J, Elkenboom J (2011) Functional architecture of Weibel-Palade bodies. *Blood* **117**: 5033–5043
- Wagner DD (1990) Cell biology of von Willebrand factor. *Annu Rev Cell Biol* **6**: 217–246
- Zhou Y-F, Eng ET, Nishida N, Lu C, Walz T, Springer TA (2011) A pH-regulated dimeric bouquet in the structure of von Willebrand factor. *EMBO J* **30**: 4098–4111