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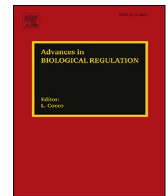

Biophysical approaches promote advances in the understanding of von Willebrand factor processing and function

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ABSTRACT

The large multimeric plasma glycoprotein von Willebrand factor (VWF) is essential for primary hemostasis by recruiting platelets to sites of vascular injury. VWF multimers respond to elevated hydrodynamic forces by elongation, thereby increasing their adhesiveness to platelets. Thus, the activation of VWF is force-induced, as is its inactivation. Due to these attributes, VWF is a highly interesting system from a biophysical point of view, and is well suited for investigation using biophysical approaches. Here, we give an overview on recent studies that predominantly employed biophysical methods to gain novel insights into multiple aspects of VWF: Electron microscopy was used to shed light on the domain structure of VWF and the mechanism of VWF secretion. High-resolution stochastic optical reconstruction microscopy, atomic force microscopy (AFM), microscale thermophoresis and fluorescence correlation spectroscopy allowed identification of protein disulfide isomerase isoform A1 as the VWF dimerizing enzyme and, together with molecular dynamics simulations, postulation of the dimerization mechanism. Advanced mass spectrometry led to detailed identification of the glycan structures carried by VWF. Microfluidics was used to illustrate the interplay of force and VWF function. Results from optical tweezers measurements explained mechanisms of the force-dependent functions of VWF's domains A1 and A2 and, together with thermodynamic approaches, increased our understanding of mutation-induced dysfunctions of platelet-binding. AFM-based force measurements and AFM imaging enabled exploration of intermonomer interactions and their dependence on pH and divalent cations.

These advances would not have been possible by the use of biochemical methods alone and show the benefit of interdisciplinary research approaches.

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1. Introduction

The best-described function of the multimeric glycoprotein von Willebrand factor (VWF) is its role in primary hemostasis. At sites of vascular injury, where conditions of elevated hydrodynamic forces are present, VWF recruits platelets to the damaged endothelium. Efficient binding of VWF to platelets correlates with the transition of VWF molecules from a rather globular to an elongated "string"-like conformation (Ruggeri et al., 2006; Schneider et al., 2007). The string-like structure of VWF results from its linear multimeric nature. VWF multimers are formed exclusively in endothelial cells (ECs) and megakaryocytes – the precursor cells of platelets. First, in the endoplasmic reticulum (ER), two single VWF molecules (= monomers; subdomains are schematically shown in Fig. 1A) are connected to dimers via C-terminal disulfide bonds (Fig. 1B). Only dimers proceed into the Golgi apparatus where they are N-terminally linked by interdimer disulfide bonds between the D'D3 domains (Wagner, 1990), resulting in multimers that consist of two to more than 40 dimeric subunits. Using a multilateral approach involving quantitative gel analysis, fluorescence correlation spectroscopy, and total internal reflection fluorescence microscopy it was recently shown that VWF multimer size follows an exponential size distribution (Lippok et al., 2013). The multimers become compacted and are stored in storage organelles called Weibel-Palade bodies (WPB's) in ECs (Wagner et al., 1982) and α-granules in platelets (Jeanneau et al., 1984; Zucker et al., 1979). VWF can be secreted from these organelles upon extracellular stimuli (Fernandez et al., 1982; Loesberg et al., 1983; Miyata and Ruggeri, 1999; Sporn et al., 1989), and, while still attached to the cell, can elongate in the bloodstream (Ruggeri et al., 2006; Schneider et al., 2007). The transition from a compact into a stretched conformation leads to the activation of VWF's A1 domain to bind platelets. Remarkably, also the down-regulation of VWF's hemostatic activity – achieved by the cleavage of long VWF multimers into shorter, hemostatically less active ones – is force-induced, as the specific cleavage site is buried within VWF's A2 domain and exposed by unfolding of this domain (Zhang et al., 2009). Constitutively secreted, soluble VWF travels the circulation in a rather globular conformation. In addition to the abovementioned roles of VWF, this soluble VWF also fulfills a force-independent function as a transporter of coagulation factor VIII, which is thereby protected from degradation (Bennett et al., 1972).

The importance of VWF for primary hemostasis is illustrated by von Willebrand Disease (VWD), the most common hereditary bleeding disorder, which arises from a variety of mutations in the VWF gene (reviewed in Sadler, 1998). Three types of VWD have been defined: While type 1 is characterized by low levels of functional VWF, patients with type 3 have virtually no VWF in their plasma and platelets. In VWD type 2, VWF exhibits structural and functional defects (Sadler et al., 2006). Type 2 is further divided into subtypes 2A, 2B, 2M and 2N. VWD 2A is associated with a significant reduction of VWF high molecular weight multimers (HMWM) resulting in deficits in platelet-dependent function of VWF. 2M shows a similar phenotype, although HMWM are present at almost normal levels. 2B exhibits lack of HMWM due to enhanced VWF proteolysis and increased platelet binding, which often leads to strongly reduced platelets counts (thrombocytopenia). In subtype 2N, mutations diminish factor VIII binding, leading to a phenotype similar to hemophilia A (Schneppenheim and Budde, 2011).

Further studies have revealed that VWF is also involved in arterial (Spiel et al., 2008) and venous thrombosis (Takahashi et al., 2009) as well as stroke (Kleinschnitz et al., 2009; Nieswandt and Stoll, 2010; Zhao et al., 2009). VWF has been described as a negative regulator of angiogenesis (Starke et al., 2011), and is able to stimulate smooth muscle cell proliferation (Bosmans et al., 1997; Qin et al., 2003). Moreover, VWF contributes to platelet and tumor cell apoptosis (Baud'huin et al., 2009) as well as to inflammatory processes (Bernardo et al., 2005; Denis et al., 2001; Petri et al., 2010), and it influences physiological bone remodeling via its interaction with osteoprotegerin (Shahbazi et al., 2007).

Key to VWF's functional diversity are its highly complex multi-domain structure and its extraordinary responsiveness to external forces, which in the vasculature result from the interplay of VWF's length with hydrodynamic flow (Springer, 2014). Both VWF's size (multimers can reach more than 20,000 kDa) and the importance of force-induced conformational changes for its function make this protein a highly interesting system from a biophysical point of view and an ideal research object for biophysical techniques. Recently, a variety of biophysical methods have been employed to investigate diverse aspects of VWF.

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