Enhanced Local Disorder in a Clinically Elusive von Willebrand Factor Provokes High-Affinity Platelet Clumping

Alexander Tischer[1], Venkata R. Machha[1], Juan P. Frontroth[2], Maria A. Brehm[3], Tobias Obser[3], Reinhard Schneppenheim[3], Leland Mayne[4], S. Walter Englander[4] and Matthew Auton[1]

*1 - **Division of Hematology,** Departments of Internal Medicine and Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN 55905, USA*
*2 - **Laboratorio de Hemostasia y Trombosis,** Servicio de Hematologia y Oncologia, Hospital de Pediatria, "Prof. Dr. Juan P. Garrahan", Buenos Aires, Argentina*
*3 - **Department of Paediatric Haematology and Oncology,** University Medical Centre Hamburg-Eppendorf, Hamburg, Germany*
*4 - **Johnson Research Foundation,** Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA*

Correspondence to Juan P. Frontroth, Reinhard Schneppenheim, S. Walter Englander and Matthew Auton:.
jfrontroth@garrahan.gov.ar; schneppenheim@uke.de; engl@mail.med.upenn.edu; auton.matthew@mayo.edu
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Abstract

Mutation of the cysteines forming the disulfide loop of the platelet GPIbα adhesive A1 domain of von Willebrand factor (VWF) causes quantitative VWF deficiencies in the blood and von Willebrand disease. We report two cases of transient severe thrombocytopenia induced by DDAVP treatment. Cys1272Trp and Cys1458Tyr mutations identified by genetic sequencing implicate an abnormal gain-of-function phenotype, evidenced by thrombocytopenia, which quickly relapses back to normal platelet counts and deficient plasma VWF. Using surface plasmon resonance, analytical rheology, and hydrogen–deuterium exchange mass spectrometry (HXMS), we decipher mechanisms of A1-GPIbα-mediated platelet adhesion and resolve dynamic secondary structure elements that regulate the binding pathway. Constrained by the disulfide, conformational selection between weak and tight binding states of A1 takes precedence and drives normal platelet adhesion to VWF. Less restrained through mutation, loss of the disulfide preferentially diverts binding through an induced-fit disease pathway enabling high-affinity GPIbα binding and firm platelet adhesion to a partially disordered A1 domain. HXMS reveals a dynamic asymmetry of flexible and ordered regions common to both variants, indicating that the partially disordered A1 lacking the disulfide retains native-like structural dynamics. Both binding mechanisms share common structural and thermodynamic properties, but the enhanced local disorder in the disease state perpetuates high-affinity platelet agglutination, characteristic of type 2B VWD, upon DDAVP-stimulated secretion of VWF leading to transient thrombocytopenia and a subsequent deficiency of plasma VWF, characteristic of type 2A VWD.

Introduction

The primary hemostatic function of the human von Willebrand factor (VWF) is to sequester platelets from blood flow in response to vascular injury so that secondary hemostasis and blood coagulation can proceed to stop the bleeding [1]. Like a fishing line with multiple hooks, VWF is a multidomain multimeric plasma glycoprotein with multiple sites (A1 domains) to which platelets adhere. Efficient formation of a hemostatic platelet plug is dependent on the high-molecular-weight multimeric forms of VWF in plasma. Similar to the quantitative VWF deficiency phenotypes of von Willebrand disease (VWD), lack of the mid- to high-molecular-weight multimers of VWF multimers in type 2A VWD significantly impairs primary hemostasis causing hemorrhage.

Type 2A VWD refers to qualitative functional variants in which VWF-dependent platelet adhesion is diminished or absent because the proportion of large VWF multimers is decreased [2]. This limits the detection of VWF in blood and its ristocetin cofactor activity in clinical assays [3]. Ristocetin is a standard macroglycopeptide antibiotic used to induce *in vitro* VWF-platelet agglutination in patient plasma in order to assess gain or loss of function. Subtypes of 2A result from mutations that impair intracellular multimerization [4] and from mutations that enhance the degradation of high-molecular-weight multimers by the blood metalloprotease, ADAMTS13 [5]. A few mutations classified in the 2A phenotype, however, also occur in the platelet GPIbα binding A1 domain of VWF [6], indicating that the VWF multimer/function disproportionality characteristic of the type 2A phenotype is also a result of gain-of-function even when thrombocytopenia is not immediately apparent.

Chief among type 2A mutations are cysteine substitutions that break the disulfide bond in the A1 domain. Alkylation of these cysteines has been demonstrated to enhance GPIbα affinity and rheological platelet adhesion to recombinant A1 domains [7–10]. Recombinant VWF studies of how cysteine mutations in the A1 domain alter the ristocetin-induced platelet binding activity of VWF have been ambiguous, indicating a gain or loss of function depending on which cysteine is mutated [11,12]. However, reduced and alkylated recombinant VWF shows an enhanced affinity for platelets in the absence of ristocetin [13]. Evidence for high affinity in VWD patients due to loss of the disulfide bond has not been previously observed [14–16]. Here, evidence is presented from two patients, which confirms that cysteine amino acid substitutions that break the disulfide bond induce a gain-of-function phenotype that manifests in a 1-desamino-8-D-arginine vasopressin (DDAVP)-treatment-induced transient thrombocytopenia, which promptly restores to a homeostasis of low VWF and normal platelet counts within hours following treatment [17,18]. DDAVP is a vasopressin analog that stimulates the release of VWF from Weibel–Palade bodies of vascular endothelial cells into the blood.

Despite 2 decades of research, a structure-based mechanism of high-affinity binding has not been fully elucidated. The dynamic character of the A1 domain has limited many crystallographic attempts to identify a specific high-affinity conformation with type 2B gain-of-function VWD variants of the A1 domain [19–22]. Recent experimental evidence for mutation-induced A1 domain misfolding in both type 2B gain-of-function and type 2M loss-of-function VWD phenotypes has further complexed the issue [23]. These phenotypes contain many mutations that collectively enhance (2B) or diminish (2M) VWF-dependent platelet interactions; however, the extent of gain or loss of function is variably broad in range [23]. Here, a structural thermodynamic mechanism for high-affinity platelet adhesion to the A1 domain lacking a disulfide bond is deciphered using a combination of CD, surface plasmon resonance (SPR), analytical rheology, and hydrogen–deuterium exchange mass spectrometry (HXMS). In the normal condition, platelet GPIbα affinity is regulated by a conformational selection [24] between weak and tight binding states of A1 within the native ensemble. In disease, loss of the disulfide partially disorders the A1 domain and restricts binding through an induced-fit pathway [24] involving a conformational change from the weakly bound complex to a tightly bound complex that perpetuates abnormally high-affinity platelet adhesion. HXMS reveals that both binding mechanisms share common dynamic structural regions that regulate the thermodynamic path to high affinity. GPIbα is able to adapt to local disorder in the A1 domain through flexibility of the leucine-rich repeats (LRRs) and complementary disorder in a loop previously referred to as the β-switch. The interaction between platelets and VWF is driven by local flexibility within the A1 domain that, left unrestrained, leads to localized conformational disorder, thereby intensifying GPIbα affinity and aggravating thrombocytopenia when patients are challenged with DDAVP.

Results and Discussion

Patients

Two unrelated girls (6.3y and 9.6y) with a historical diagnosis of type 2A VWD presented with low VWF antigen (VWF:Ag; 22 and 27%) and undetectable VWF ristocetin cofactor activity (VWF:RCo) and VWF collagen binding (VWF:CB) without prior evidence of thrombocytopenia or *in vitro* hyper responsiveness to low dose ristocetin (0.5 mg/mL). These presentations are typical of a type 2A diagnosis, which is characterized by reduced VWF-dependent platelet adhesion due to a quantitative loss of the largest VWF multimers in plasma [16]. Desmopressin, DDAVP treatment, is frequently used to induce the release of normal VWF from cellular compartments when plasma VWF:Ag is quantitatively deficient [17], but it is contraindicated in gain-of-function type 2B VWD [25]. DDAVP treatment of these patients induced transient thrombocytopenia in which platelet counts decreased 30 min post-DDAVP infusion from normal counts (Patient 1 = $194*10^9$ L^{-1}, Patient 2 = $274*10^9$ L^{-1}) to $27*10^9$ L^{-1} and $52*10^9$ L^{-1}, respectively, with complete restoration at 120 min (Fig. 1a**)**. Thrombocytopenia was paralleled by an increase in median platelet volume (Table 1), and platelet clumps were observed in blood smears (Fig. 1b**)**. Spontaneous platelet aggregation and enhanced response to low-dose ristocetin-induced platelet agglutination (RIPA; 0.5 mg/mL)