Shear-Induced Platelet Membrane Glycoproteins Ib and IIb-IIIa

By D.M. Peterson, N.A. Statopoulos, T.D. Giorgio, J.D. Hellums, and J.L. Moake

Different types of platelets in various types of plasma were subjected to levels of shear stress that produce irreversible platelet aggregation in normal platelet-rich plasma (PRP). At shear stresses of 90 or 180 dyne/cm² applied for 30 seconds or five minutes, aggregation was either absent or only transient and reversible using severe von Willebrand factor (vWF) Bernard-Soulier syndrome (BSS) PRP (≤1% von Willebrand factor, vWF): Bernard-Soulier syndrome (BSS) PRP (platelets deficient in the membrane glycoprotein Ib, GPIb); normal PRP plus monoclonal antibody (MoAb) to GPIb; thrombasthenic PRP (platelets deficient in membrane glycoprotein IIb-IIIa complex, GPIb-IIla); and normal PRP plus MoAb to GPIb-IIa. Shear-induced aggregation was inhibited under the above conditions, even though the platelets were activated to release their granular contents. Sheared normal platelets in vWD plasma aggregated in response to added vWF. These studies demonstrate that the formation of stable platelet aggregates under conditions of high shear requires vWF and the availability of both GPIb and GPIb-IIIa on platelet membranes. The experiments demonstrate that vWF-platelet interactions can occur in the absence of artificial agonists or chemical modification of vWF. They suggest a possible mechanism for platelet aggregation in stenosed or partially obstructed arterial vessels in which the platelets are subjected to relatively high levels of shear stress.

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Platelet aggregation induced by various agonists in plasma is dependent on the binding of plasma proteins to specific lectoglycoprotein receptors on platelet surface membranes. Ristocetin, a cationic glycopeptide antibiotic, artificially promotes platelet clumping in vitro by inducing the binding of large factor VIII-related von Willebrand factor (vWF) multimeric forms to or near to platelet membrane glycoprotein Ib (GPIb) molecules. Adenosine diphosphate (ADP), which is released (along with serotonin) from the dense granules of stimulated platelets, promotes platelet aggregation by inducing the attachment of fibrinogen to platelet membrane glycoprotein IIb-IIIa complexes (GPIb-IIla). Thrombospondin, a protein released from the α-granules of stimulated platelets, stabilizes fibrinogen binding to platelet surface glycoprotein IIb-IIIa complexes. Thrombin-stimulated platelets are altered in a manner that enables their glycoprotein IIb-IIIa complexes to bind fibrinogen, thrombospondin, fibronectin, and, with low affinity, vWF multimers. Fluid shear stress applied in vitro to normal PRP can itself induce platelets to aggregate and secrete their granular contents in the absence of added agonists. Shear stress may reach levels as high as 200 to 400 dyne/cm² when arterial vessels become stenosed or partially occluded, and as by arteriosclerosis or during vascular spasm. Thus platelet aggregation induced by shear stress may be of considerable importance both in normal hemostasis and in the pathophysiology of thrombus formation in small arteries, arterioles, and capillaries of the microcirculation.

The present study was undertaken to evaluate the dependence of shear-induced platelet aggregation on plasma vWF and on platelet GPIb and the IIb-IIIa complex and to study their involvement in the mechanism of shear-induced platelet aggregation.

MATERIALS AND METHODS

Whole blood samples were collected from 12 healthy adults and three patients into siliconized test tubes containing 0.38% sodium citrate as anticoagulant (one volume of anticoagulant to nine volumes of blood). The patients had severe vWD (one patient; plasma and platelet vWF levels <1% of normal); BSS (one patient); and thrombasthenia (one patient). All have had life-long mucosal and posttraumatic bleeding and prolonged template bleeding times. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared by centrifugation as described elsewhere. The PRP...
platelet count was adjusted to 300,000/μL with PPP. BSS PRP had a platelet count of 50,000/μL; however, the BSS platelets were four times the mean volume of normal platelets.

The PRP samples were subjected to controlled shear stress levels in a stainless steel cone-plate viscometer (Ferranti Electric Model 781) at 24°C. Shear stress-induced platelet aggregation in this instrument has been demonstrated to be independent of any minimal platelet-surface interactions. There was also no measurable attachment of vWF antigen or particular multimeric forms to the surface of the viscometer, with or without the application of shear stress, as studied by methods described elsewhere. The number of shear experiments with normal and patient PRP were normal PRP, 25 experiments; severe vWD PRP, four experiments; BSS PRP, four experiments; and thrombasthenic PRP, two experiments.

Some normal PRP samples were incubated for five minutes with monoclonal antibodies (MoAbs) prior to shearing in concentrations of 6 μg/mL (6D1 antibody against GPIb; ten experiments with five normal PRP samples); 10 μg/mL (10E5 antibody against the GPIIb-IIIa complex; six experiments with five normal PRP samples); or 20 μg/mL (control MAB3I antibody that binds to an undefined platelet membrane antigen and produces no functional defect; four experiments with two normal PRP samples). Monoclonal antibodies to GPIb (6D1) and GPIIb-IIIa complex (10E5) were generously provided by Dr Barry S. Coller of the State University of New York at Stony Brook, and we are grateful for his important contribution. The control MAB3 MoAb was produced in our laboratory by methods described by Coller et al.

After shearing, 10 μL of PRP samples were dispensed in 20 mL of Coulter Koton II diluent containing 0.5% gluteraldehyde and were counted. Particle count and size distribution were done with an electronic particle counter (Coulter Electronics Model ZBI with Channelyzer) using a 50 μm diameter aperture. Particles with sizes ± 20% of the mean platelet distribution of the unsheared PRP were considered as "single platelets." It is known, of course, that this size distribution range includes some small aggregates consisting of doublets and triplets. However, studies in which EDTA was used to disaggregate control samples suggest that the "single platelets," as reported here, agree with the actual particle numbers (EDTA-treated) to within about 30%. The disappearance of "single platelets" was accounted for by the formation of platelet aggregates; thus the percent decrease in "single platelets" is directly related to the percent increase in platelet aggregates. Lactic dehydrogenase activity (LDH) and 14C-serotonin release from platelets were measured by methods described previously.

Large vWF multimeric forms were purified from normal human cryoprecipitate by the method of Thorell and Blombäck.

RESULTS

The dependence of shear-induced platelet aggregation on plasma vWF and on platelet GPIb and the GPIIb-IIIa complex is shown in Figs 1 and 2. Normal platelets sheared in normal plasma at 90 or 180 dyne/cm² for varying times were aggregated extensively. Exposure to shear stress for 30 seconds or more resulted in platelet aggregation that could be observed directly and microscopically and was quantified as a decrease in the number of single platelets by nearly 80%. After five minutes of shearing some platelets disaggregated, as shown by the increase in particle count; however, about 50% remained in stable aggregates. In contrast, stable platelet aggregates failed to form in the shear fields using PRP from a patient with severe vWD; BSS (deficient in the ristocetin-induced binding component of GPIb), thrombasthenic (deficient in the GPIIb-IIIa complex), and normal individuals in the presence of MoAbs either to GPIb or to the GPIIb-IIIa complex.

The shear-induced increases in particle counts observed in the BSS, thrombasthenic, and anti-GPIIb-IIIa-treated PRP was presumably due to dissolution of some microaggregates that were initially present before application of the shear field.

The control MoAb (MAB31) that binds to human platelets at a site other than GPIb or the IIb-IIIa complex did not impair shear-induced platelet aggregation in PRP, as shown in Fig 2.

PRP consisting of normal platelets in severe vWD plasma prepared by methods described elsewhere, sheared at 180 dyne/cm² for 30 seconds or five minutes, failed to produce aggregates (data not shown). Immediately following the shearing, PRP samples placed in an aggregometer aggregated in response to added vWF (100 U/DL). This extent of aggregation was significantly higher than in nonsheared PRP samples. Sheared PRP samples, which were incubated for five minutes after the shear, aggregated in response to added vWF to a degree intermediate between the unsheared control and the sheared but not incubated specimen. Other results (not shown) revealed that vWF did not attach to the viscometer and that vWF multimeric forms were not altered in the shear field.

![Fig 1](https://www.bloodjournal.org/fig1.png)

**Fig 1.** Effects of shear stress and shearing time on normal (○), Bernard-Soulier (□), severe von Willebrand’s disease (vWD) (▲), and anti-GPIb antibody-treated (△) platelet-rich plasma. In (A) shear stress was 180 dyne/cm²; in (B) shearing time was five minutes. Symbols represent mean values and bars one SD.

![Fig 2](https://www.bloodjournal.org/fig2.png)

**Fig 2.** Effect of time in the shear field on normal (○), thrombasthenic (□), anti-GPIIb-IIIa antibody-treated (△) control monoclonal antiplatelet antibody (MAB31)-treated (▲) platelet-rich plasma sheared at 90 dyne/cm² (A), and 180 dyne/cm² (B). The bars denote one SD.
Platelets in the different types of samples released ^14^C-serotonin in response to shear stress (Fig 3). These results indicate that activation of the platelets occurred in the shear fields, even when there was little or no aggregation.

**DISCUSSION**

The present study has demonstrated that vWF and the availability of both GPIb and the GPIIb-IIIa complex on platelet membranes are essential for aggregation at relatively high shear stresses, as may be attained in stenosed or partially occluded arterial vessels.\(^{10-12}\) No externally added agonist (eg, ristocetin) or chemical modification of vWF (eg, desialation) is required for this effect. Our findings are compatible with other observations\(^ {13} \) that implied the involvement of vWF in platelet thrombus formation in a flowing system and with a preliminary report\(^ {14} \) of defective shear-induced platelet aggregation in vWD PRP. Our results may help to explain the disappearance of large vWF multimeric forms during acute episodes of systemic platelet aggregation in thrombotic thrombocytopenic purpura\(^ {15} \) and during extensive intrarenal platelet aggregation in the hemolytic-uremic syndrome.\(^ {16} \)

Our results reported elsewhere\(^ {17} \) show that the large multimers are especially active in platelet-platelet binding in the shear field.

It is possible that vWF attachment to platelets is necessary but not sufficient to cause aggregation during the application of relatively high shear stresses. Additional plasma proteins may be involved. Our results confirm the possibility expressed by others\(^ {23-24} \) that shear induces a direct alteration of platelet membrane GPIb and GPIIb-IIIa complexes. According to this hypothesis, vWF attaches predominantly to GPIb, and this binding further perturbs the conformation of GPIIb-IIIa; then additional vWF, along with fibrinogen (and, possibly, fibronectin), attach to the altered GPIIb-IIIa complexes and potentiate aggregation.

**ACKNOWLEDGMENT**

This work has been supported by NIH Grants ROI H1 18584, PO1 NS 23327, and PO1 HL 13262. We appreciate the excellent technical assistance provided by Nancy Turner.

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Shear-induced platelet aggregation requires von Willebrand factor and platelet membrane glycoproteins Ib and IIb-IIIa

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