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# Adhesive properties of the isolated amino-terminal domain of platelet glycoprotein Ibα in a flow field

PATRIZIA MARCHESE, ENRIQUE SALDÍVAR, JERRY WARE, AND ZAVERIO M. RUGGERI*

Roon Research Center for Arteriosclerosis and Thrombosis, Division of Experimental Hemostasis and Thrombosis, Departments of Molecular and Experimental Medicine, and Vascular Biology, The Scripps Research Institute, La Jolla, CA 92037

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**ABSTRACT** **We have examined the interaction between the amino-terminal domain of platelet glycoprotein (GP) Ibα and immobilized von Willebrand Factor (vWF) under flow conditions in the absence of other components of the GP Ib–IX–V complex. Latex beads were coated with a recombinant fragment containing GP Ibα residues 1–302, either with normal sequence or with the single G233V substitution that causes enhanced affinity for plasma vWF in platelet-type pseudo-von-Willebrand disease. Beads coated with native fragment adhered to vWF in a manner comparable to platelets, showing surface translocation that reflected the transient nature of the bonds formed. Thus, the GP Ibα extracellular domain is necessary and sufficient for interacting with vWF under high shear stress. Beads coated with the mutated fragment became tethered to vWF in greater number and had lower velocity of translocation than beads coated with the normal counterpart, suggesting that the G233V mutation lowers the rate of bond dissociation. Our findings define an approach for studying the biomechanical properties of the GP Ibα–vWF bond and suggest that this interaction is tightly regulated to allow rapid binding at sites of vascular injury, while permitting the concurrent presence of receptor and ligand in the circulation.**

Platelet adhesion to von Willebrand factor (vWF) immobilized at sites of vascular injury initiates thrombus formation in areas of rapid blood flow (1) and represents a critical mechanism in hemostasis and thrombosis. The process is mediated by the glycoprotein (GP) Ib–IX–V complex, in which the amino-terminal domain of the GP Ibα-chain (2, 3) contains the binding site for the vWF A1 domain (4, 5). This interaction supports platelet tethering to surfaces even at extremely high shear rates but without irreversible attachment. If no other bonds are formed, tethered platelets translocate in the direction of flow, albeit at a markedly lower velocity than freely flowing blood cells (6). On reactive substrates, however, initial contact allows the rapid establishment of additional bonds typically mediated by receptors of the integrin superfamily and results in essentially instantaneous irreversible adhesion, followed by events leading to subsequent thrombus development (1). At present, it is unknown whether any single domain of the GP Ib–IX–V complex can exhibit the vWF binding function of the intact receptor expressed on the platelet surface. Information in this regard could indicate whether linkage to the cytoskeleton (7, 8) and/or interactions between components of the complex (9, 10) contribute to vWF binding.

We have addressed these questions by using a recombinant fragment comprising GP Ibα residues 1–302 (11). The isolated domain has been shown to undergo tyrosine sulfation and support modulator-dependent vWF binding as the native receptor (12). In the present studies, plastic beads were coated with the fragment, and its activity was tested in a flow field to mimic the function of a surface-expressed cell membrane receptor during vascular injury. In addition to the GP Ibα fragment with normal sequence, we also tested a mutant molecule with a single Gly → Val substitution at position 233 (G233V; ref. 13). The latter is associated with platelet-type pseudo-von-Willebrand disease (14, 15) and may be responsible for the enhanced interaction with plasma vWF typical of this congenital bleeding disorder (16). We found that beads coated with the 45-kDa GP Ibα domain behave like platelets in the interaction with immobilized vWF under flow conditions. Moreover, the mutant fragment had altered function, causing beads to translocate on immobilized vWF with markedly decreased velocity. Thus, the G233V substitution may alter the equilibrium of ligand binding to GP Ibα, prolonging the lifetime of single bonds. These results show that we have developed an effective tool for studying adhesive interactions in a fluid dynamic environment and provide evidence that the GP Ibα amino-terminal domain may be the only structure of the GP Ib–IX–V complex directly involved in the regulation of vWF binding.

## MATERIALS AND METHODS

**Expression and Purification of Recombinant GP Ibα Amino-Terminal Domain Fragments.** Two recombinant cDNA constructs encoding the GP Ibα amino-terminal domain residues 1–302, pMW2-WT (native/wild type) and pMW2-G233V (mutant for the substitution G233V), were prepared as described (11, 17). Chinese hamster ovary K1 cells were transfected with pMW2-WT or pMW2-G233V and grown in DMEM (BioWhittaker) containing 10% (vol/vol) FCS. After reaching confluence, the cells were washed twice with DMEM and supplemented with serum-free culture medium for 24–28 h. The recombinant fragment secreted into the culture medium was precipitated by using 75% (vol/vol) saturated ammonium sulfate at 4°C with overnight stirring. After centrifugation at 8,000 × *g* for 1 h at 4°C, the protein pellet was resuspended in 1/200 of the original volume, dialyzed extensively against Tris buffer (10 mM Tris, pH 7.4/100 mM NaCl/0.02% $NaN_3$), and applied to a Superdex 200 size-exclusion column (Amersham Pharmacia). The collected fractions were analyzed by 10% PAGE in the presence of SDS (18), followed by immunoblotting with specific antibodies (19). Protein was transferred onto a nitrocellulose strip and incubated first for 1 h in a solution containing 20 mM Hepes and 150 mM NaCl (pH 7.4) with the addition of 50 mg/ml nonfat powdered milk and 0.05% Tween 20 (Blotto solution; ref. 20). The nitrocellulose strip was then incubated for 2 h in a solution containing the monoclonal antibody LJ-Ibα1, which recognizes an epitope that does not depend on native conformation and is located within the GP Ibα sequence

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Abbreviations: vWF, von Willebrand factor; GP, glycoprotein; WT, wild type.

*To whom reprint requests should be addressed. e-mail: ruggeri@scripps.edu.

1–181 (2, 21). The solution was prepared by diluting ascitic fluid to 1:1,000 in Blotto solution. At the end of the incubations, the nitrocellulose was washed three times for 10 min each in Blotto solution and then incubated for 30 min with $^{125}$I-labeled rabbit IgG directed against mouse IgG. The labeled antibody was diluted in Blotto solution to give ≈0.05 mCi of total radioactivity. After this incubation, the nitrocellulose was washed three times, dried, and exposed overnight to a Kodak X-Omat RPXRP-1 film with a Dupont Cronex Quanta III intensifying screen. The fractions containing bands corresponding to a molecular mass of 45 kDa and reacting with the antibody were evaluated for protein concentration and used in subsequent experiments.

**Coating of Latex Beads with Recombinant GP Ibα Fragment.** The protein concentration of the solution containing GP Ibα fragment was adjusted to the desired value by using PBS (pH 7.4; GIBCO/BRL), and latex beads (Fluoresbrite plain; 2-μm diameter; Polysciences) were added at a final count of 500,000 per μl. This mixture was incubated overnight at 4°C with gentle rotation. After centrifugation at 13,000 × *g*, the supernatant was removed and replaced by PBS containing BSA fraction V (Calbiochem) at a final concentration of 50 mg/ml. Incubation was continued for 1 h at room temperature, after which the beads were centrifuged, resuspended in modified Tyrode's buffer containing 50 mg/ml BSA, and counted. Control beads were prepared in the same manner, but BSA substituted for the partially purified GP Ibα fragment. Coated beads were stored at 4°C until used and were found to retain activity for up to 4 weeks.

**Binding of $^{125}$I-LJ-P3 Fab to Beads Coated with Recombinant GP Ibα Fragment.** The conformation-dependent epitope recognized by the monoclonal antibody LJ-P3 is located within the GP Ibα sequence 1–237 (2, 21). IgG was purified by affinity chromatography on protein A (22). The purified antibody was digested with papain (23), and the Fc portion was separated from the Fab by binding to protein A. The monovalent Fab was labeled with $^{125}$I by using Iodo-Gen (24). For the binding assay, beads coated with GP Ibα fragment or control beads coated with BSA were suspended at a final concentration of 100,000 per μl in modified Tyrode's buffer (25), and a saturating concentration (6 μg/ml) of $^{125}$I-LJ-P3 was added together with 10% (vol/vol) BSA. After 15 min, the beads where layered on top of an 8% (vol/vol) sucrose gradient and centrifuged at 13,000 × *g* for 10 min to separate them from unbound antibody. The tip of the tube containing the sedimented beads was removed by cutting, and bound $^{125}$I-LJ-P3 was measured by using a γ-scintillation counter (Packard). The number of antibody molecules bound per bead was calculated by using the known specific activity.

**Evaluation of Recombinant GP Ibα Fragment Function Under Flow Conditions.** These experiments were performed by using the method for studying platelet adhesion in flowing blood that has been described (6) with minor modifications. Glass coverslips were coated with purified vWF (25) at a concentration of 50 μg/ml in PBS (6). Blood was collected from the antecubital vein of healthy donors through a 19-gauge needle into syringes containing 1:6 acid-citrate-dextrose (75 mM trisodium citrate/38 mM citric acid/138 mM dextrose, pH 4.5). The blood was centrifuged at 600 × *g* for 20 min at room temperature (22–25°C), and the platelet-rich plasma on top of the erythrocytes was replaced with an equal volume of modified Tyrode's buffer. Red cells were resuspended and centrifuged at 1,800 × *g*. This procedure was repeated four times, always removing the supernatant-fluid-containing platelets and other cells on top of the red cells and replacing them with modified Tyrode's buffer. After the final wash, the red cells were reconstituted to the original hematocrit with modified Tyrode's buffer (pH 7.4) containing 50 mg/ml BSA. Beads were added to the washed blood cell suspension at a final count between 1,000 and 7,000 per μl, and the interaction with vWF immobilized onto a surface was analyzed in a chamber reproducing the design described by Usami *et al.* (26) with slight modifications. The tapered shape of the silicone gasket used in the chamber causes a progressive change in flow velocity as a linear function of the distance from the inlet, thus varying the wall shear rate. Blood was aspirated through the flow chamber by a syringe pump (Harvard Bioscience, South Natick, MA), typically at 2.65 ml/min. Visualization of events on the surface was obtained by epifluorescence microscopy with an Axiovert 135 inverted microscope (Zeiss). Interactions between the fluorescent beads and immobilized vWF were evaluated in real time, and all experiments were recorded on videotape (1). The function of GP Ibα was blocked by using 150 μg/ml LJ-Ib1, a monoclonal antibody that recognizes the GP Ibα sequence 1–237 (2, 21) and is a competitive inhibitor of vWF binding. The antibody was used as purified IgG prepared by chromatography of ascitic fluid on a protein A column (22). Bead motion and velocity of translocation were evaluated as reported for studies with platelets (1, 6).

## RESULTS

**Characterization of Latex Beads Coated with Recombinant GP Ibα Amino-Terminal Domain.** The recombinant GP Ibα domain used in these experiments was a single species with an apparent molecular mass of 45 kDa as judged by specific immunoblotting (Fig. 1). Staining with Coomassie blue after SDS/PAGE allowed visualization of unidentified bands eluting with the GP Ibα fragment in the fractions obtained by size-exclusion chromatography of culture medium proteins. By visual judgment, it was determined that these contaminants were similar in both normal and mutant fragment preparations (not shown) and were deemed not likely to interfere with the specific GP Ibα–vWF interaction (see below). Latex beads coated with the GP Ibα fragment acquired the ability to bind an anti-GP Ibα antibody, and the number of bound IgG

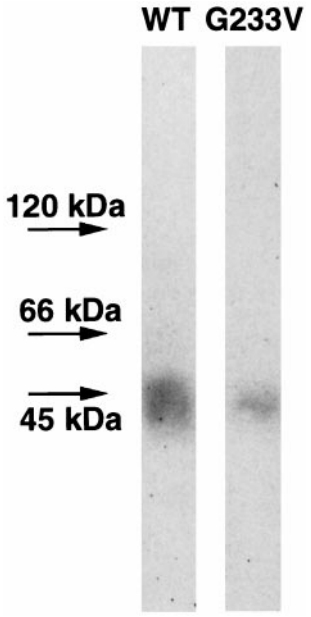


FIG. 1. Immunoblotting analysis of native and mutant GP Ibα amino-terminal domain fragments. Recombinant fragments with either normal sequence (WT) or the G233V mutation were analyzed after ammonium sulfate precipitation from culture medium and partial purification by size-exclusion column chromatography. Each fraction obtained from the chromatography separation was evaluated for reactivity with the anti-GP Ibα monoclonal antibody LJ-Ibα1 after SDS/PAGE and protein transfer to nitrocellulose. Positive bands were visualized with rabbit anti-mouse IgG labeled with $^{125}$I and autoradiography.

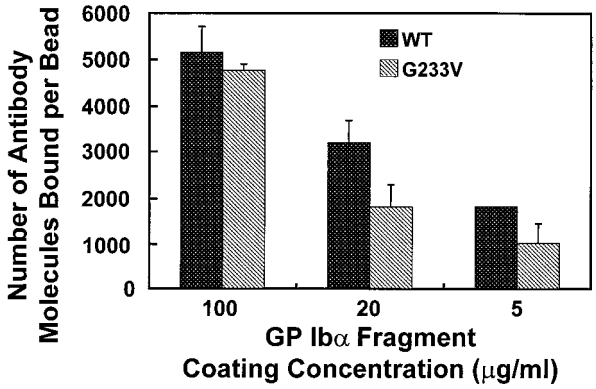


FIG. 2. Binding of $^{125}$I-LJ-P3 Fab to latex beads coated with GP Ibα amino-terminal domain fragments. These experiments were performed with beads coated by using solutions at three different concentrations of either normal (WT) or G233V mutant fragment. Each experiment was performed in duplicate, and the results are shown as means ± SEM. The counts of $^{125}$I-LJ-P3 Fab bound to BSA-coated beads were considered as background and were subtracted from the counts bound to beads coated with GP Ibα fragments to calculate specific binding. Such background counts did not exceed 20% of total bound counts. Each molecule of monovalent $^{125}$I-LJ-P3 Fab binds to one molecule of GP Ibα fragment, thus the numbers shown correspond to the number of GP Ibα fragment molecules exposed on the surface of each bead. The apparent difference in coating efficiency observed with the mutant fragment may be the consequence of conformational changes caused by the G233V substitution.

molecules was directly related to the recombinant fragment concentration in the coating solution (Fig. 2). Coating of the beads with comparable concentrations of BSA resulted in antibody binding that was never >20% of that observed with GP Ibα-coated beads; such binding was considered to be nonspecific (not shown). Antibody binding to beads was similar whether the coating solution contained recombinant fragment with native sequence or with the G233V mutation (Fig. 2).

**Adhesion of GP Ibα-Coated Beads to Immobilized vWF in a Flow Field: Effect of the G233V Mutation.** Latex beads coated with the native GP Ibα fragment adhered to immobilized vWF in a manner similar to platelets expressing the intact GP Ib–IX–V complex (6). The beads became tethered to the surface even at the relatively high wall shear rate of 1,300 $s^{-1}$ (Fig. 3*A*), but they did not adhere irreversibly; rather, they translocated in the direction of flow with a rolling motion (Fig. 3*B*). Two lines of evidence indicated that the interaction was specific: first, beads coated with BSA showed essentially no adhesion to a vWF-coated surface (Fig. 3*A*), and, second, the adhesion of beads coated with GP Ibα fragment was inhibited >90% by a specific anti-GP Ibα monoclonal antibody (Fig. 3*C*). As previously observed with platelets, adhesion was greatly enhanced when the wall shear rate increased from the lower value of 300 $s^{-1}$ to 1,300 $s^{-1}$ and then decreased only slightly at the highest value (2,000 $s^{-1}$) tested in these experiments (Fig. 4).

Presence of the mutation G233V enhanced the adhesive properties of the GP Ibα amino-terminal domain. As determined by visual judgment, more beads coated with mutant fragment than those coated with native fragment adhered to immobilized vWF (Fig. 3*A*), and the velocity of translocation on the surface decreased considerably. The latter phenomenon could be easily appreciated by analyzing successive steps of the rolling motion at 1-s intervals and superimposing the corresponding images, thus showing the closer distance between consecutive positions in the case of beads coated with the mutant fragment (Fig. 3*B*). Of note, even the more adhesive beads coated with the G233V mutant GP Ibα fragment could not attach irreversibly to the vWF-coated surface.

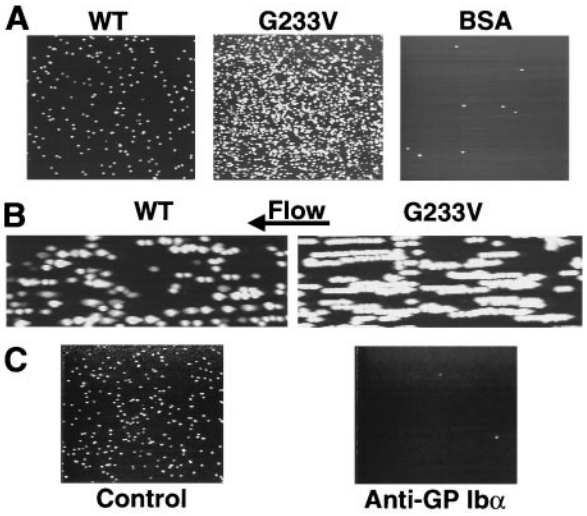


FIG. 3. (*A*) Images of latex beads coated with GP Ibα amino-terminal domain fragment adhering to immobilized vWF. Fluorescent beads were coated with a 100-μg/ml solution of GP Ibα fragment, which was either normal (WT) or mutant (G233V). Control beads were coated with the same concentration of BSA. Each image, representing a surface area of 1.28 $mm^2$, is a single frame taken from a recording of beads adherent to vWF in a flow field with a wall shear rate of 1,300 $s^{-1}$. The beads (7,000 per μl) were flowing in a suspension of washed human red cells with hematocrit of 45%. Interactions on the surface were visualized by epifluorescence video microscopy and recorded in real time (30 frames per s). (*B*) Visual rendition of the translocation (rolling) of beads interacting with immobilized vWF. The beads used in these experiments were coated with solutions of normal or mutant GP Ibα fragment at a concentration of 20 μg/ml. Such a coating concentration resulted in a number of adherent beads that facilitated evaluation of individual interactions. Each image represents a surface area of 0.088 $mm^2$, and 15 consecutive frames, sampled at 1 frame per s from a real-time videotape recording, were digitized and superimposed to show the progressive change in position of the beads on the surface. Each track formed by closely spaced fluorescent dots represents a single bead moving along the direction of flow. (*C*) Inhibitory effect of a specific monoclonal antibody on the interaction between beads coated with native GP Ibα fragment and immobilized vWF. Experimental conditions as described for *A* except that, when indicated, the anti-GP Ibα antibody LJ-Ib1 (150 μg/ml) was added to the suspension containing the beads and incubated for 15 min at 22–25°C before perfusion.

**Quantitative Analysis of the Interaction Between GP Ibα Amino-Terminal Domain and Immobilized vWF Under Flow Conditions.** The number of GP Ibα amino-terminal domain molecules exposed on the surface of latex beads (Fig. 2) was directly proportional to the number of beads interacting with surface-bound vWF (Fig. 5). The G233V substitution had a considerable effect on the latter parameter. Even when its surface density was ≈25% of that of the native counterpart, as achieved with coating at 5 μg/ml as opposed to 100 μg/ml (Fig. 2), the mutant fragment supported adhesion to vWF of twice as many beads at a relatively high wall shear rate (Fig. 5). Whether coated with normal or mutant GP Ibα amino-terminal domain, the number of surface interacting beads tended to remain constant in time (Fig. 5), and essentially all were rolling (Fig. 3*B*).

The velocity of translocation on immobilized vWF was evaluated with beads coated at two concentrations of native or mutant GP Ibα fragment, differing by 5-fold. Unlike the observed effect on the number of beads adhering to immobilized vWF (Fig. 5), decreasing the surface density of either fragment did not considerably alter the corresponding rolling motion, which showed comparable velocity peaks at 10–15 or 1–5 μm/s, respectively (Fig. 6). Approximately 60% of the beads coated with native GP Ibα fragment adhered to immo-

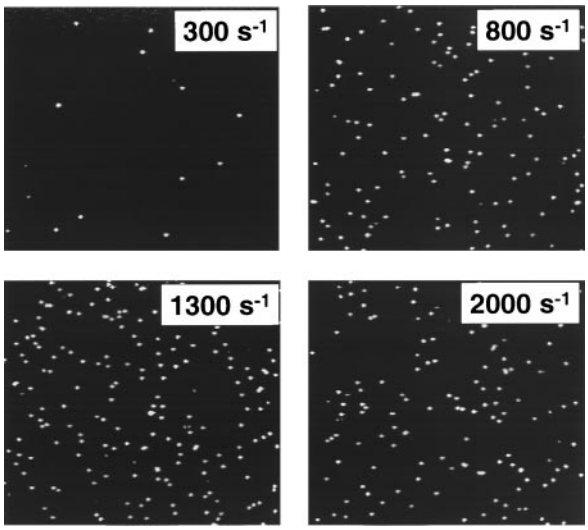


FIG. 4. Effect of wall shear rate on the adhesion to immobilized vWF of latex beads coated with native GP Ibα amino-terminal domain fragment. The experimental conditions were similar to those described for Fig. 3*A*, except that perfusion of the beads in suspension with red cells was performed at flow rates resulting in the indicated wall shear rates.

bilized vWF with surface contact time limited to 1–2 s; <10% could be tracked for >5–6 s, and essentially none could be tracked for >11–12 s (Fig. 7). In contrast, >60% of the beads interacting with vWF through the mutant fragment remained in contact with the surface for >1–2 s, and >25% could be tracked for >5–6 s. Some of these beads adhered with rolling motion for the entire observation period of 14 s. The results were similar at the two different coating concentrations for both the normal and mutant fragment (Fig. 7).

## DISCUSSION

These studies show that the isolated amino-terminal domain of GP Ibα is comparable to the entire GP Ib–IX–V complex in its ability to interact with vWF. In fact, coating with a recombinant GP Ibα fragment comprising residues 1–302 confers, to beads similar in size to platelets, the ability to resist high shear stress and roll at low velocity onto immobilized vWF. Such a distinctive function has been identified as the hallmark of GP Ib–IX–V participation in thrombogenesis (6), allowing platelet contact with reactive surfaces for the time sufficient to promote activation and irreversible adhesion through integrin receptors (1). Our findings, therefore, indicate that the GP Ibα amino-terminal domain may represent the only cellular function required for the initiation of platelet tethering to vascular lesions under flow conditions that are relevant for hemostasis (27) and arterial thrombosis (28). Furthermore, the considerable enhancement of GP Ibα adhesive function caused by the single amino acid substitution G233V indicates the possibility that the receptor may undergo conformational regulation of its activity with effects on the stability of binding to vWF. If a comparable functional transition occurs on platelets at wound sites, the reduced rolling velocity in a flow field and prolonged duration of contact with reactive surfaces may enhance activation, release, and the rate of thrombus growth.

Because the GP Ibα–vWF pairing is intrinsically short-lived (6), the number of effective bonds established at any given time between a particle expressing the receptor and vWF on a surface determines the overall efficiency of the adhesive process. Under flow conditions, bonds must form rapidly to initiate tethering at high shear rates (29). In this respect, native and mutant GP Ibα fragment seem to have similar efficiency, as both could promote deposition of coated beads onto

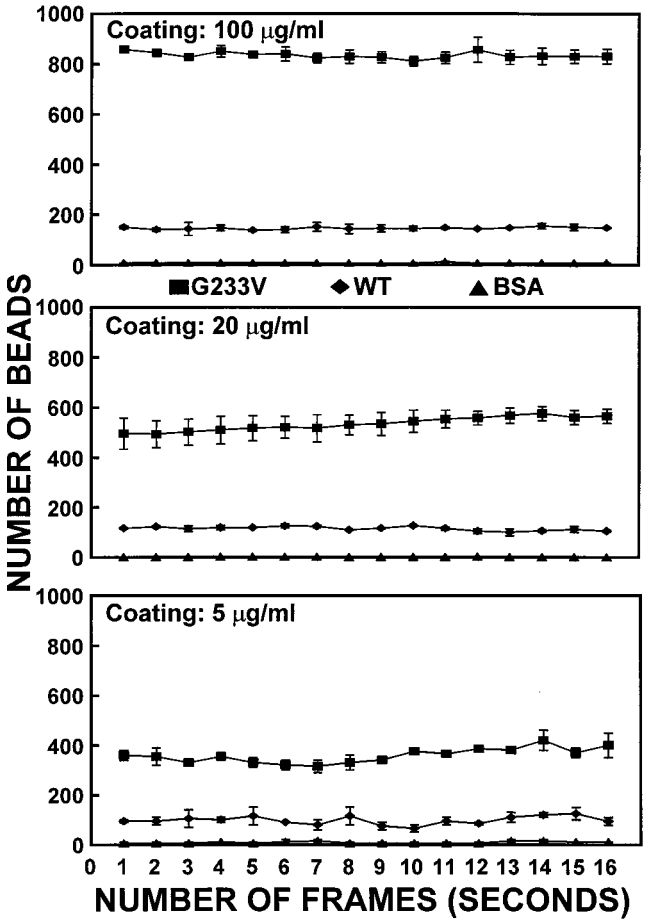


FIG. 5. Quantitative evaluation of the interaction between latex beads coated with GP Ibα amino-terminal domain fragments and immobilized vWF. The beads used in these experiments were coated with solutions at three different concentrations of normal (WT) or mutant (G233V) GP Ibα fragment. Control beads were prepared with equivalent concentrations of BSA. The beads (7,000 per μl) were perfused in suspension with washed human red cells (hematocrit of 45%) at a wall shear rate of 1,300 $s^{-1}$. The number of beads interacting with immobilized vWF on a surface area of 1.28 $mm^2$ was evaluated in 16 consecutive individual frames from a videotape recording sampled at 1-s intervals. Thus, each frame provided information on surface coverage every second. Two nonoverlapping series of frames obtained between 2 and 5 min after the beginning of perfusion were evaluated for each experimental condition, and the corresponding average values are shown here (±SEM).

immobilized vWF at the highest shear rate tested (2,000 $s^{-1}$). After attachment to the surface, bonds at the back of a particle in relation to the direction of flow are subjected to tensile stress and tend to dissociate, while new bonds form at the front (30). The net result is a rolling forward of the particle in contact with the surface (31, 32). In this respect, the bonds formed by the mutant G233V fragment proved to be remarkably more efficient than those supported by the native counterpart, as the rolling velocity of the corresponding beads was significantly lower. Taken together, our observations suggest but do not unequivocally prove that the main consequence of the specific G233V substitution in GP Ibα is a lower dissociation rate of bound vWF. This observation can explain why particles coated with the mutant GP Ibα fragment establish contacts of longer duration (Fig. 7) and, consequently, accumulate in greater number on the immobilized ligand (Fig. 5). Such findings are in agreement with the concept that adhesion mediated by GP Ibα binding to vWF is regulated by a tight equilibrium between bond formation and dissociation. Modulation of the latter and variations in receptor density inde-

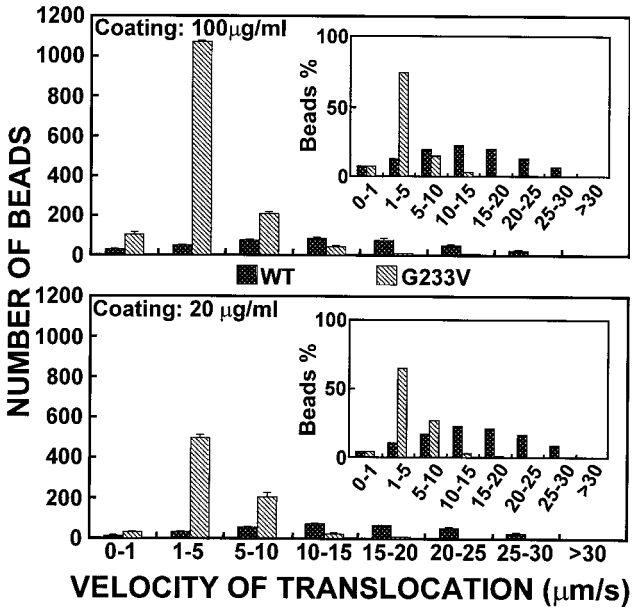


FIG. 6. Velocity of translocation of latex beads coated with GP Ibα amino-terminal domain fragments rolling on surface-immobilized vWF. The beads used in these experiments, coated with normal (WT) or mutant (G233V) GP Ibα fragment, were prepared and perfused at a 1,300-$s^{-1}$ wall shear rate as described in the legend of Fig. 5. The results represent the average of two separate measurements performed on nonoverlapping series of 16 frames sampled at a rate of 1 frame per s from a real-time videotape recording and are shown here with the corresponding SEM. Velocities are grouped arbitrarily in eight discrete categories. Because of the notable difference in reactivity of beads coated with normal or mutant fragment, the velocity distribution is also shown in the *Insets* as percentage of beads in each category relative to the total number present on the surface.

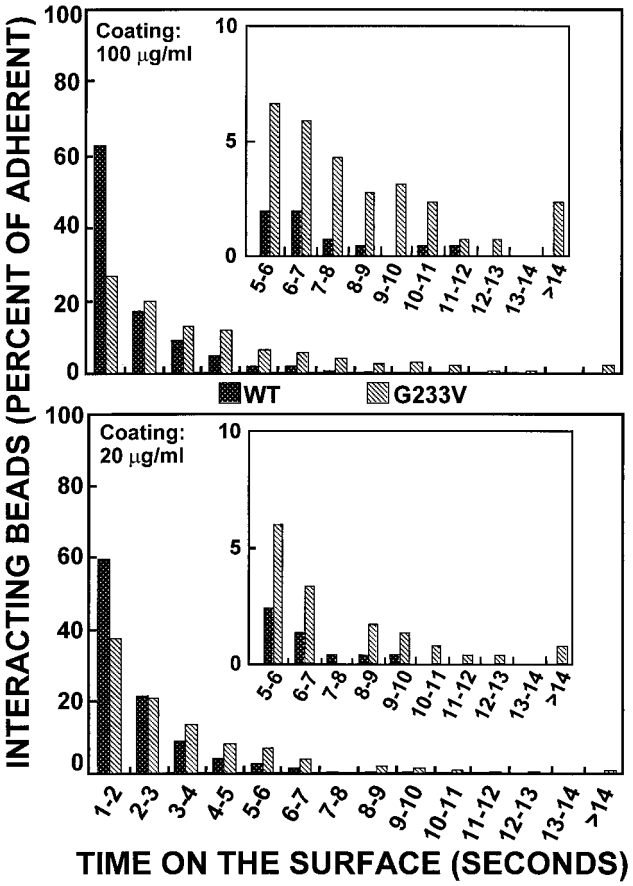


FIG. 7. Time course of individual interactions between latex beads coated with GP Ibα amino-terminal domain fragments and immobilized vWF. The beads used in these experiments, coated with normal (WT) or mutant (G233V) GP Ibα fragment, were prepared and perfused at a 1,300-$s^{-1}$ wall shear rate as described in the legend of Fig. 5. The duration of contact of each individual bead rolling on immobilized vWF is presented here as a distribution in arbitrary categories. Only beads that could be followed from the beginning of interaction with the surface were considered. The percentage of beads in each category was calculated relative to the total number of beads interacting with the surface. The *Insets* show selected results with a reduced scale on the ordinate to provide a better representation of the lower values in the categories reflecting longer duration of contact with the surface.

pendently influence the number of effective bonds and, consequently, shift the equilibrium in the number of rolling particles that may instantaneously adhere to immobilized vWF.

The enhanced stability conferred by the G233V substitution to the vWF–GP Ibα interaction may explain the pathogenesis of platelet-type pseudo-von-Willebrand disease, the congenital bleeding disorder in which the mutation was identified originally (13). It may seem contradictory to propose that the cause of a phenotype characterized by defective hemostatic function is a mutation directly enhancing the adhesive potential of GP Ibα. Indeed, a functional alteration such as the latter would be expected to result in more rapid thrombus formation rather than inefficient primary hemostasis. This paradox may be resolved by considering that prolongation of the effective lifetime of the GP Ibα–vWF bond caused by the G233V substitution may alter the association–dissociation equilibrium that normally permits soluble vWF and platelets to coexist in blood. Indeed, both clinical and experimental evidence suggest that circulating plasma vWF binds with enhanced affinity to platelets in patients with platelet-type pseudo-von-Willebrand disease (14–16). Thus, it is conceivable that the mutant receptor on circulating platelets, because of its heightened ligand-binding capacity, becomes blocked by plasma vWF and is consequently unable to interact with vascular surfaces when required for initiating hemostasis at sites of tissue injury (33). Of note, the same phenotype of platelet-type pseudo-von-Willebrand disease and a similar pathogenetic mechanism characterize type 2B von Willebrand disease (34–36), except that, in the latter case, the alteration in GP Ibα–vWF binding is caused by mutations in the ligand (37) rather than in the receptor (38, 39).

The present data support the concept that the coexistence of vWF and GP Ibα in circulating blood depends on the intrinsically high dissociation rate of the corresponding bond. Indeed, as shown here for GP Ibα and elsewhere for vWF (40), it seems that both ligand and receptor in their native conformation and without the need for induction by exogenous modulators are endowed with the ability to bind rapidly to one another. From a functional point of view, such an event may favor a prompt response to vascular injury but requires a mechanism controlling the lifetime of formed bonds to limit the consequences of the interaction in the circulation. Thus, showing that the vWF-binding function of GP Ibα may be regulated by conformational changes in the amino-terminal domain of the receptor provides information relevant to understanding the molecular bases of platelet function and devising strategies for the inhibition of pathological thrombosis.

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