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Identification of a point mutation in type IIB von Willebrand disease illustrating the regulation of von Willebrand factor affinity for the platelet membrane glycoprotein Ib–IX receptor

(thrombosis/bleeding disorder/platelet adhesion/platelet aggregation)

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ABSTRACT **von Willebrand factor (vWF) supports platelet adhesion on thrombogenic surfaces by binding to platelet membrane glycoprotein (GP) Ib in the GP Ib–IX receptor complex. This interaction is physiologically regulated so that it does not occur between circulating vWF and platelets but, rather, only at a site of vascular injury. The abnormal vWF found in type IIB von Willebrand disease, however, has a characteristically increased affinity for GP Ib and binds to circulating platelets. We have analyzed the molecular basis of this abnormality by sequence analysis of a type IIB vWF cDNA and have identified a single amino acid change, Trp^{550} to Cys^{550}, located in the GP Ib-binding domain of the molecule comprising residues 449–728. Bacterial expression of recombinant fragments corresponding to this vWF domain yielded molecules that, whether containing a normal Trp^{550} or a mutant Cys^{550} residue, bound directly to GP Ib in the absence of modulators and with similar affinity. In contrast, mammalian cell expression of the same segment of sequence yielded molecules that, when containing the normal Trp^{550}, did not bind to GP Ib directly but, like native vWF, bound in the presence of ristocetin. However, molecules containing the point mutation (Cys^{550}) behaved like type IIB vWF—namely, bound to GP Ib even without ristocetin modulation and, in the presence of ristocetin, had 10-fold higher affinity than molecules with normal sequence. These results identify a region of vWF that, although not thought to be directly involved in binding to GP Ib, may modulate the interaction through conformational changes.**

von Willebrand factor (vWF) exhibits a central role in the process of hemostasis and, in pathological conditions, may be involved in the development of acute vascular occlusion (1–3). Under flow conditions characterized by high shear stress, platelet adhesion and thrombus formation onto altered vascular surfaces depend on the concurrent interaction of vWF with subendothelial components like collagen (4, 5) and heparin-like molecules (6), on one side, and platelet membrane glycoprotein (GP) Ib in the GP Ib–IX receptor complex (7) on the other. In contrast, soluble vWF and platelets circulate in blood without interacting with each other. The regulation of vWF affinity for GP Ib may depend on a specific functional conformation, different from that of circulating vWF, acquired by binding to subendothelial components, and/or on changes induced in the ligand and/or receptor by molecules acting as modulators of the interaction at the site of ongoing thrombogenesis.

Among the many subtypes of von Willebrand disease described to date, type IIB (8, 9) is of particular interest because purified IIB vWF in solution has an increased affinity for platelets and, unlike normal vWF, binds to GP Ib in the absence of any modulating substance (10). Further evidence that circulating IIB vWF interacts with platelets derives from the observation that patients with type IIB von Willebrand disease may exhibit thrombocytopenia, thought to be the consequence of intravascular platelet clumping mediated by the aberrant vWF (11). In this report we demonstrate that the vWF-encoding DNA of a type IIB von Willebrand disease patient contains a nucleotide transversion changing the Trp^{550} codon to a Cys^{550} codon. This mutation is located in the vWF domain that contains the interaction sites for GP Ib, collagen, and heparin, between residues 449 and 728 (6, 12, 13). In particular, the substituted amino acid is within a 186-residue loop created by the intrachain disulfide bond between Cys^{509} and Cys^{695} (13–15). Expression and functional characterization of this type IIB point mutation suggest that changes in vWF conformation may regulate the *in vivo* interaction of vWF with platelet GPIb, thus providing an important clue to the mechanisms that regulate platelet responses to vascular injury.

MATERIALS AND METHODS

Genetic Characterization of the Propositus. The relevant laboratory parameters of this propositus, identified as case no. 7 in a previous publication, have been reported (16); they are all consistent with the diagnosis of type IIB von Willebrand disease (8). Blood was drawn from the patient after obtaining informed consent according to the Declaration of Helsinki and following institutional guidelines of the University of Vienna. For genetic analysis, platelets were obtained from 50 ml of blood drawn into 1/10 volume of 3.2% trisodium citrate as anticoagulant, and residual platelet RNA was isolated by ultracentrifugation through a cesium chloride cushion, as described (17). Total platelet RNA was subjected to a first-strand cDNA reaction primed with a vWF-specific oligonucleotide (complementary to the mRNA that encodes residues 899–908 of the mature vWF subunit) and catalyzed by Moloney murine leukemia virus reverse transcriptase (GIBCO/BRL) (18). Aliquots of the first-strand reaction were subjected to a polymerase chain reaction (19) using one oligonucleotide identical to that used in the first-strand reaction and a second one identical to the coding sequence for amino acid residues 428–436. A vWF pseudogene with an

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Abbreviations: vWF, von Willebrand factor; GP, platelet membrane glycoprotein.

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intron–exon arrangement similar to that of the functional gene within the region targeted for amplification was selectively excluded from the reaction by choosing oligonucleotides complementary to exons 23 and 24, which are separated by a 2000-base-pair intron (20). The amplified fragments were subcloned into M13-based vectors for DNA sequence analysis using either the M13 universal or vWF-specific primers (21).

Expression of Normal and Mutant vWF Peptides. The expression of *Escherichia coli*-derived vWF peptides utilized the T7 bacterial system developed by Studier *et al.* (22, 23). The expression plasmids were prepared by amplifying the coding sequence for vWF residues 441–733 in a polymerase chain reaction and cloning the amplified product into M13mp18 for DNA sequence analysis. After verifying that the amplified coding sequence was intact, one M13 template was mutated to replace the vWF Trp^{550} codon with a Cys^{550} codon (24). vWF cDNA inserts from both M13 constructs were cloned into the expression plasmid pET3a and directed the synthesis of molecules differing only for the single amino acid substitution at residue 550. As a consequence of the cloning strategy employed, the vWF fragments expressed by both *E. coli* constructs contained a 17-residue amino-terminal sequence from the gene 10 capsid protein of the pET3a vector (23).

The expression of vWF fragments in Chinese hamster ovary cells (CHO-K1; ATCC 61) utilized a chimeric vWF cDNA sequence containing the following coding elements in a contiguous open reading frame: (*i*) the coding sequence for the vWF signal peptide; (*ii*) the first three codons of the vWF propeptide; (*iii*) the coding sequence for mature vWF residues Arg^{441} to Asn^{730}; and (*iv*) a translation stop codon. This modified vWF cDNA sequence was constructed in a series of cloning experiments utilizing the polymerase chain reaction and site-directed mutagenesis, and the final chimeric cDNA fragment was verified by sequence analysis. Modified inserts containing the native vWF sequence and a mutated sequence in which the vWF codon for Trp^{550} was changed to a Cys^{550} codon were each cloned into a eukaryotic expression plasmid identical to pcDM8 (25) except for the presence of a neomycin resistance gene. Transfection of CHO-K1 cells with the expression plasmids was performed by a standard procedure (26). Cells resistant to Geneticin (Sigma) and secreting vWF were identified using the anti-vWF monoclonal antibodies NMC-4 and LJ-RG46. Production (6, 12, 27) and characterization (13, 15) of both antibodies have been reported; NMC-4 recognizes a disulfide bond-dependent epitope present on a 116-kDa tryptic fragment of vWF comprising residues 449–728, whereas LJ-RG46 recognizes a linear sequence within residues 474–488. Transformed cells were grown in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum, 0.5 mM nonessential amino acids, and 2 mM L-glutamine (Whittaker Bioproducts).

Purification of Recombinant Peptides. *E. coli*-synthesized peptides were isolated from inclusion body preparations (18) and solubilized in 6 M guanidine hydrochloride containing 10 mM dithiothreitol. After a 60-min incubation at 37°C, the extracts were *S*-carboxyamidomethylated with iodoacetamide as described (6, 12). The *E. coli*-derived vWF peptides were purified by high-performance liquid chromatography (Perkin–Elmer) employing the anion-exchanger Q Sepharose Fast Flow (Pharmacia) followed by the cation-exchanger Protein-Pack SP 8HR (Waters). vWF molecules expressed by CHO-K1 cells were isolated using immunoaffinity column chromatography with monoclonal antibody NMC-4 (15). Transformed CHO-K1 cells expressing vWF residues 441–730, either normal or with the Cys^{550} substitution, synthesized vWF antigen at similar levels. Culture medium from both cell lines yielded ≈200 μg of immunopurified protein for each 500 ml. All purified fragments were dialyzed extensively against Hepes-buffered saline (20 mM Hepes, pH 7.4, with 0.15 M NaCl) and their concentration was measured with the Micro bicinchoninic acid assay kit (Pierce); they were stored at −70°C until used.

Western Blot Analysis. Purified peptides were subjected to SDS/polyacrylamide gel electrophoresis under nonreducing or reducing conditions (65 mM dithiothreitol, 55°C, for 20 min). Following transfer onto nitrocellulose, immunoreactive fragments were visualized by incubation with antibody NMC-4 or LJ-RG46 followed by ^{125}I-labeled rabbit anti-mouse antibody and subsequent autoradiography, as described (13, 15).

Inhibition of Anti-GP Ib Monoclonal Antibody Binding to Platelets. This assay was based on the demonstrated ability of native vWF to inhibit the platelet binding of an anti-GP Ib monoclonal antibody, LJ-Ib1 (28). Since the antibody in turn is a potent inhibitor of vWF binding to platelets, the corresponding epitope must overlap with the vWF-binding site in GP Ibα, thus explaining the cross-inhibition. LJ-Ib1 was iodinated by the procedure of Fraker and Speck (29) using Iodo-Gen (Pierce). Platelets were washed by the albumin density gradient technique (30) and used at a final count of 1×10^8 per ml. Half-maximal binding of antibody to platelets was observed at 10 μg/ml, as determined in preliminary experiments, and this concentration of ^{125}I-labeled LJ-Ib1 was used to evaluate the inhibiting effect exerted by vWF and related molecules on antibody binding. Initial studies demonstrated that native purified vWF in the absence of the modulator ristocetin (31) had no effect on LJ-Ib1 binding to platelets; however, in the presence of ristocetin at 1 mg/ml, vWF inhibited binding of the antibody in a dose-dependent manner. Ristocetin alone had no effect on antibody binding. To measure inhibition of binding, platelets were incubated for 30 min at 22–25°C with various concentrations of recombinant peptides and a constant amount of ^{125}I-labeled LJ-Ib1. Ristocetin (Sigma) was included at a final concentration of 1 mg/ml in the indicated experiments. At the end of the incubation, platelets with bound radioactivity were separated from free antibody by centrifugation at 12,000 × *g* through a 20% sucrose layer in a microcentrifuge tube. Nonspecific binding was determined in the presence of a 100-fold molar excess of unlabeled ligand and was subtracted from all data points. Binding of the antibody was expressed as percentage of that measured in a control mixture lacking recombinant peptides.

RESULTS AND DISCUSSION

In the attempt to identify the molecular basis of type IIB von Willebrand disease, we hypothesized that mutations affecting vWF affinity for GP Ib would occur within the domain containing the binding site for this platelet receptor, between residues 449 and 728 (12, 15). Indeed, sequence analysis of recombinant cDNA clones derived from the platelet vWF mRNA of a patient with type IIB von Willebrand disease identified, in some clones, a single guanine to cytosine transversion changing the Trp^{550} codon (TGG) of the mature vWF subunit to a Cys^{550} codon (TGC). Other clones from the same patient contained the normal Trp^{550} codon, indicating that the patient was heterozygous for the observed amino acid change, a finding consistent with the autosomal dominant mode of inheritance seen in this as well as most kindreds with type IIB von Willebrand disease (8). The observed nucleotide transversion destroys an *Ava* II restriction site within the vWF gene and this was used to confirm that the base change exists in the patient's genomic DNA.

Although an amino acid substitution in the GP Ib-binding domain represents a possible cause for the functional abnormality typical of type IIB vWF—namely, increased affinity for GP Ib (10, 32, 33)—this finding alone does not prove the

molecular pathogenesis of type IIB von Willebrand disease. Therefore, normal and mutant vWF molecules were expressed to obtain experimental evidence that the observed mutation can affect vWF interaction with platelets. Rather than expressing whole vWF, which would have resulted in a heterogeneous population of oligomers of varying molecular mass (34, 35), we elected to proceed with the expression of a specific vWF domain on the premise that this would yield a homogeneous population of molecules. This approach, in addition to giving higher expression levels, also facilitated the interpretation of the possible functional consequences of a single amino acid substitution, eliminating the considerable influence that variations in the size distribution of multimers have on the affinity of vWF for platelet receptors (10, 36).

The choice of expressing an isolated vWF domain was also supported by previous results defining a tryptic fragment that recapitulates crucial functions of native vWF by retaining the ability to interact with GP Ib, collagen, and heparin and to mediate ristocetin-dependent platelet agglutination (13). This 116-kDa fragment, a homodimer of the segment of sequence comprising residues 449–728 (Fig. 1), contains two intrachain disulfide bonds between Cys^{471} and Cys^{474} and Cys^{509} and Cys^{695}, and at least one interchain bond involving one of the Cys residues at positions 459, 462, and 464 (Fig. 1). The isolated 116-kDa domain with intact disulfide bonds expresses the epitope of a conformation-dependent monoclonal antibody, NMC-4, suggesting that it retains the same conformation of the corresponding region of the native vWF molecule (13, 15). After disulfide bond reduction, the constituent monomeric 52/48-kDa fragment still expresses binding to GP Ib (12) and heparin (6) but fails to bind to native fibrillar collagen (5) and, as expected, fails to react with the conformation-dependent antibody, NMC-4 (15). Based on these considerations, we expressed the GP Ib-binding site of vWF as a single-chain polypeptide without native conformation, using a bacterial expression system, and also as a molecule with native folding by using a mammalian cell expression system.

We constructed two *E. coli* recombinant plasmids that express vWF residues 441–733, essentially corresponding to the primary sequence (residues 449–728) of the reduced and alkylated 52/48-kDa tryptic fragment (6, 12) and differing only at residue 550. As expected, both *E. coli*-expressed peptides were contained in insoluble intracellular inclusion bodies that, after cell lysis and solubilization, yielded random aggregates of disulfide-linked vWF fragment molecules with high molecular mass. After reduction and irreversible alkylation of Cys residues, a solution of stable monomeric molecules was obtained, with an apparent molecular mass of 36 kDa, appropriate for the GP Ib-binding domain of vWF without carbohydrate chains (Fig. 2). Thus, with regard to primary sequence, the *E. coli*-expressed fragments are equivalent to the 52/48-kDa tryptic fragment of vWF with reduced and alkylated Cys residues (6, 12). The recombinant *E. coli*-expressed fragment with normal sequence was identified as $r36/Trp^{550}$, whereas the mutant fragment was identified as $r36/Cys^{550}$.

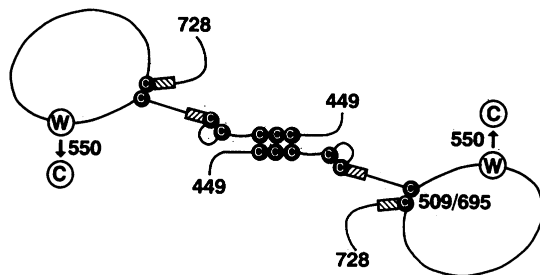

FIG. 1. Schematic model of the dimeric 116-kDa domain of vWF comprising residues 449–728. Seven Cys residues within each monomeric subunit of the normal 116-kDa domain of vWF are shown with their appropriate inter- or intramolecular disulfide bridges. A triplet of Cys residues at positions 459, 462, and 464 contributes to the intermolecular bridge that links monomeric subunits together, but the exact arrangement of these disulfide bonds is not yet known. Two intramolecular disulfide loops, between residues 471 and 474 and residues 509 and 695, are present within each monomeric subunit. The 509–695 disulfide loop (identified within the monomeric subunit on the right) contains structural elements that allow vWF to interact with collagen and heparin. The position of two noncontiguous sequences necessary for binding to GP Ib is indicated by hatched boxes. The single amino acid change identified in the type IIB vWF molecule is shown at residue 550.

For expression of the GP Ib-binding domain of vWF with native conformation, we constructed two mammalian cell expression plasmids with coding sequence (residues 441–730) corresponding closely to the 116-kDa tryptic fragment. One plasmid contained normal vWF sequence, whereas the other coded for Cys^{550} instead of Trp^{550}, as found in the cDNA of the propositus. Both plasmids contained the coding sequence for the vWF signal peptide and three residues of the vWF propeptide to facilitate secretion of the expressed vWF molecules from mammalian cells. Both expression plasmids were transfected into CHO-K1 cells, and stable transformants constitutively secreting $r116/Trp^{550}$ or $r116/Cys^{550}$ were selected. In both cases, the protein secreted in culture medium had the molecular mass of 116 kDa expected of the dimeric, glycosylated GP Ib-binding domain from native vWF (13, 15) and reacted with the conformation-dependent monoclonal antibody NMC-4 (Fig. 2). Following disulfide bond reduction, the constituent subunit fragment had the expected molecular mass of 52/48 kDa and did not react with NMC-4 but only with antibody LJ-RG46 (Fig. 2), which recognizes an epitope not dependent on native conformation (13, 15).

Purified recombinant fragments representing the GP Ib-binding domain of vWF, either expressed in bacteria or mammalian cells, were tested for their ability to interact with

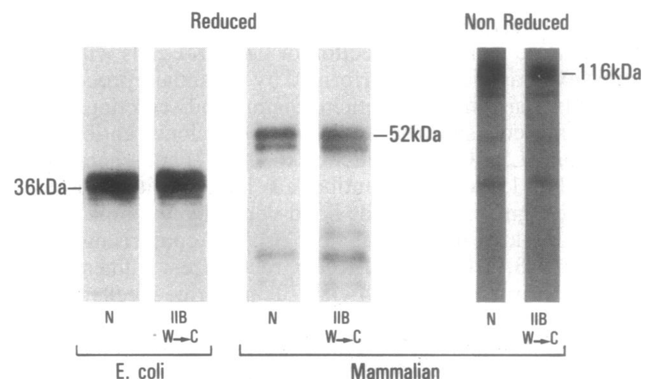

FIG. 2. Electrophoretic characterization of recombinant vWF fragments. Purified recombinant vWF fragments were subjected to SDS/polyacrylamide gel electrophoresis after reduction of disulfide bonds (Reduced) or in the absence of reducing agent (Non Reduced). Fragments were transferred onto nitrocellulose by electroblotting and allowed to react with anti-vWF monoclonal antibody LJ-RG46 (reduced samples) or NMC-4 (nonreduced samples). *E. coli*-derived peptides $r36/Trp^{550}$ (N) and $r36/Cys^{550}$ (IIB, W → C) are shown on the left. Mammalian cell-derived vWF fragments $r116/Trp^{550}$ (N) and $r116/Cys^{550}$ (IIB, W → C) migrate as dimeric molecules of ≈116 kDa before reduction and as monomeric 52/48-kDa fragments after reduction. The heterogeneity in apparent molecular mass seen after reduction is also seen in fragments obtained by proteolysis of purified vWF and is due to differences in glycosylation (6, 12). Minor immunoreactive bands seen in the gels were not present before purification and are assumed to be proteolytic fragments. Indicated molecules masses were calculated from the relative mobility of marker proteins.

GP Ib on platelets as measured by inhibition of anti-GP Ib monoclonal antibody binding. Both *E. coli*-expressed fragments, either with normal or mutant IIB sequence, inhibited platelet binding of the antibody in the absence of ristocetin and with the same IC_{50} (the concentration of peptide necessary to inhibit 50% of antibody binding) (Fig. 3). The precipitation that invariably occurred upon addition of ristocetin to solutions containing the *E. coli*-expressed fragments precluded the use of this modulator in the binding assay. The results in the absence of ristocetin are compatible with the concept that an unfolded molecule corresponding to the GP Ib-binding site of vWF can interact directly with the receptor, as demonstrated previously with the reduced and alkylated 52/48-kDa tryptic fragment (12) and with synthetic peptides representing the GP Ib-binding sequences in vWF (15). In the context of a molecule lacking native conformation and with the inherent ability to interact with GP Ib, the Cys for Trp substitution at position 550 has no functional consequence.

The inhibitory effects of mammalian-expressed vWF fragments on anti-GP Ib antibody binding to platelets were different. First, the recombinant 116-kDa fragment with native sequence failed to inhibit binding when tested alone in the absence of a modulator, even at molar concentrations equivalent to those of *E. coli*-expressed fragments showing pronounced inhibition of antibody binding (compare Figs. 3 and 4). The same fragment, however, displayed considerable inhibitory activity in the presence of ristocetin (Fig. 4), thus reproducing the results observed with native multimeric vWF. Therefore, the affinity for GP Ib seems to decrease when a fragment containing the GP Ib-binding domain of vWF acquires a conformation in solution corresponding to that of the native molecule but can be up-regulated by appropriate modulators. This finding suggests that the two segments of sequence known to contain structural elements necessary for interaction with GP Ib, between residues 474 and 488 and residues 694 and 708 (15), may be prevented from interacting with the receptor when vWF acquires its native conformation in solution. However, in contrast to the fragment with native sequence, $r116/Cys^{550}$ clearly inhibited anti-GP Ib antibody binding to platelets even in the absence

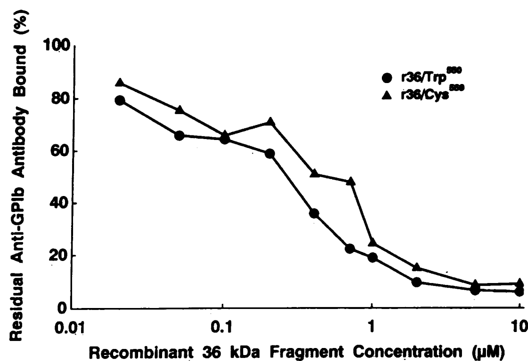

FIG. 3. Interaction of *E. coli*-derived vWF fragments with GP Ib–IX on platelets. The ability of *E. coli*-expressed peptides to bind the platelet GP Ib–IX complex was evaluated from the inhibition of binding of anti-GP Ib monoclonal antibody LJ-Ib1. Washed platelets (1×10^8 per ml) were incubated with ^{125}I-labeled IgG of LJ-Ib1 (10 μg/ml) and the indicated concentrations of purified *E. coli* peptides $r36/Trp^{550}$ (closed circles) or $r36/Cys^{550}$ (closed triangles) for 30 min at room temperature. Following the incubation, platelet-bound antibody was separated from free antibody by centrifugation through 20% sucrose at $12,000 \times g$ in a microcentrifuge and platelet-bound radioactivity was measured in a γ scintillation spectrometer. The inhibiting effect of *E. coli*-derived peptides was demonstrated in the absence of modulator. Residual LJ-Ib1 binding is expressed as a percentage of a control mixture lacking recombinant peptides. Both recombinant peptides have a similar inhibiting effect with 50% inhibition at ≈ 0.5 μM.

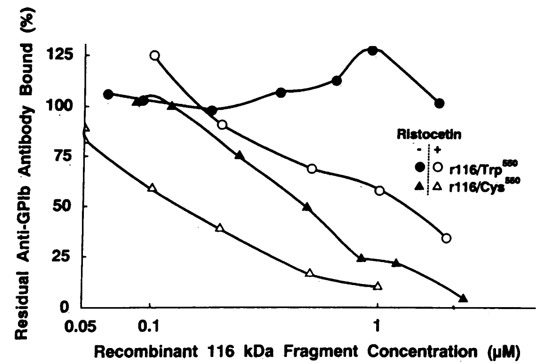

FIG. 4. Interaction of mammalian cell-derived vWF fragments with GP Ib–IX on platelets. The ability of mammalian-expressed vWF fragments to inhibit LJ-Ib1 binding to platelets was assayed as described in the legend to Fig. 3. In contrast to the *E. coli*-derived peptides, recombinant 116-kDa domain containing the normal vWF primary sequence ($r116/Trp^{550}$; closed circles) fails to compete for LJ-Ib1 binding to GP Ib–IX in the absence of modulator. The same molecule in the presence of the modulator ristocetin (open circles) interacts with GP Ib–IX and inhibits antibody binding. In contrast, the expressed recombinant 116-kDa domain containing the amino acid substitution identified in the type IIB vWF molecule ($r116/Cys^{550}$) has a measurable interaction with GP Ib–IX even in the absence of modulator (closed triangles). The same molecule in the presence of ristocetin (open triangles) inhibits LJ-Ib1 binding at a 4-fold lower concentration than in the absence of ristocetin and is 10-fold more effective than the normal molecule in the presence of ristocetin.

of ristocetin (Fig. 4), thus reproducing the typical functional abnormality of type IIB vWF—namely, that of interacting with GP Ib in the absence of modulators in solution (10). Accordingly, when ristocetin was present, $r116/Cys^{550}$ was $\approx$10-fold more effective than $r116/Trp^{550}$ with native sequence in inhibiting anti-GP Ib antibody binding. On the basis of the present results, expression of the GP Ib-binding domain of vWF containing the Cys for Trp substitution at position 550 appears to recreate the crucial phenotypic abnormality of type IIB vWF—that is, increased affinity for GP Ib (10, 32, 33). Since the bleeding disorder in these patients follows an autosomal dominant mode of inheritance, the presence of normal subunits along with mutant ones is not sufficient to prevent expression of the abnormal phenotype in heterozygous individuals. Normal mRNA and mutant mRNA were identified in the platelets of the propositus, indicating that the vWF synthesized in these patients is indeed heterogeneous with regard to subunit composition. Most likely, all vWF molecules in this patient are composed of a mixture of normal and abnormal subunits, as opposed to the alternative possibility of two populations of molecules, some composed of normal and some of abnormal subunits. Indeed, all vWF molecules in patients with IIB von Willebrand disease seem to express the abnormal phenotype, as shown by binding to platelets at low ristocetin concentration (9).

These results provide insights into the mechanisms that regulate vWF affinity for GP Ib. The native conformation of vWF in solution prevents binding to GP Ib, presumably because the two discontinuous segments of sequence directly involved in the interaction, residues 474–488 and 694–708 (Fig. 1), are not favorably oriented in the folded molecule. Exposure of the binding site seems to require conformational changes that may be experimentally mimicked by disruption of disulfide bonds but, in pathophysiological situations, must be the consequence of more subtle transitions. The mutation observed at position 550 in the type IIB patient studied here does not seem to cause directly an increased affinity for GP Ib since unfolded recombinant molecules comprising residues 441–733 interact with platelets in a similar manner

regardless of the presence or absence of the mutation. This is consistent with the observation that residue 550 is located in a region of the GP Ib-binding domain of vWF, between residues 512 and 673, that is not directly necessary for vWF binding to this receptor (13). In the context of a vWF fragment with intrinsic folding properties, however, the observed mutation greatly increases the affinity for GP Ib, suggesting that subtle alterations of the conformational state of this domain are paramount in regulating the GP Ib-binding function of vWF.

Because the mutation at position 550 is within a disulfide-dependent loop comprising the residues between Cys^{509} and Cys^{695} that are involved in mediating vWF binding to subendothelial components (4, 13), the present results suggest that a physiological equivalent of the conformational change caused by the Cys^{550} for Trp^{550} substitution may be represented by vWF binding to collagen or heparin-like proteoglycans. These interactions may regulate vWF function through the involvement of the loop sequence between residues 509 and 695, since changes in its conformation appear to affect the functional orientation of the two GP Ib-binding sequences located on the two sides of the loop (Fig. 1). In the case of the patient described here, the new cysteine residue may alter the distribution of inter- or intrachain bonds within the GP Ib-binding domain, thus creating a conformation not present in circulating normal vWF. If this hypothesis is correct, distinct mutations located between residues 509 and 695 of vWF may have similar structural and functional consequences and may represent the common molecular basis of type IIB von Willebrand disease.

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