The cytoplasmic domain of the α-subunit of glycoprotein (GP) Ib mediates attachment of the entire GP Ib-IX complex to the cytoskeleton and regulates von Willebrand factor-induced changes in cell morphology.

(Received for publication, November 26, 1995, and in revised form, January 26, 1996)

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The glycoprotein (GP) Ib-IX complex is one of the major platelet membrane glycoproteins. Its extracellular domain binds von Willebrand factor at a site of injury, an interaction that leads to activation of intracellular pathways. Its intracellular domain associates tightly with the platelet membrane glycoproteins. Its extracellular domain binds von Willebrand factor at a site of injury, leading to activation of intracellular pathways, release of platelet granule contents, and aggregation of platelets.

The cytoplasmic domain of the GP Ib-IX complex associates with the platelet cytoskeleton (13–15). Based on the commounoprecipitation of GP Ib-IX with actin-binding protein from detergent lysates, binding of purified GP Ib-IX to purified actin-binding protein, and association of expressed GP Ib-IX with actin-binding protein in transfected cells, it has been concluded that the association of GP Ib-IX with the cytoskeleton is mediated by actin-binding protein (16–21), a homodimer with rod-like 280-kDa subunits that cross-links actin filaments. Studies with synthetic peptides identified sequences in the cytoplasmic domain of the GP Ib, subunit that can bind to actin-binding protein in vitro binding assays (22) but the importance of these sequences in mediating the interaction of the GP Ib-IX complex with the cytoskeleton in the intact cell is not known.

The function of several types of adhesion receptors including integrins, selectins, and members of the immunoglobulin-like family of receptors, appears to be affected by the cytoplasmic domain of the receptor (23–28). In several cases, a correlation between altered function and altered cytoskeletal association has been noted (23, 27, 28). Although it is well established that GP Ib-IX associates with the cytoskeleton, the importance of the cytoplasmic domain of this receptor in regulating ligand binding or ligand-induced transmembrane signaling has not been investigated.

In the present study, we expressed full-length and truncated forms of the GP Ib-IX complex in cultured cells. The results show that either all or part of the amino acid sequence Trp-570 to Ser-590 in the cytoplasmic domain of GP Ib, is necessary for mediating association of the entire GP Ib-IX complex with the cytoskeleton. Although GP Ib-IX complex lacking this cytoplasmic region of GP Ib, did not associate with the cytoskeleton, it was incorporated into a complex with the other subunits and was inserted into the membrane of cultured cells. The truncation of the cytoplasmic domain that ablated the interaction of the receptor with the cytoskeleton had no detectable effect on the binding of adhesive ligand. However, cells expressing truncated receptor showed a very different morphology following adhesion to immobilized von Willebrand factor than did cells expressing full-length receptor. These results show that the cytoplasmic domain of GP Ib, is absolutely required for attachment of the GP Ib-IX complex to the cytoskeleton and suggest that the cytoplasmic domain of GP Ib, plays an important role in mediating initial interaction of platelets with the subendothelium at a site of injury (1–4). Several lines of evidence have shown that the von Willebrand factor-GP Ib-IX interaction can initiate transmembrane signaling (9–12) leading to activation of intracellular pathways, release of platelet granule contents, and aggregation of platelets.
in regulating von Willebrand factor-induced transmembrane signaling.

MATERIALS AND METHODS

Cell Culture and Transfections—Chinese hamster ovary (CHO) cells (American Type Culture Collection, Rockville, MD) or a melanoma cell line (29) kindly provided by Dr. C. Cunningham (Brigham Women's Hospital, Boston, MA) were transfected with the cDNA coding for full-length or truncated GP Ibα, GP Ibβ (gifts from Dr. José López of the Veteran's Affairs Medical Center, Houston, TX), and GP IX (a gift from Dr. David Roth of Seattle Veterans Administration Hospital, Seattle, WA) as described previously (21, 30). Cells that expressed high levels of GP Ib-IX were selected by several rounds of fluorescence-activated cell sorting (FACS).

Mutagenesis—A fragment of DNA containing the SV40 enhancer, adenovirus major late promoter, and the entire coding sequence and 3′-untranslated region of GP Ibα was inserted into the KS-BlueScript II vector (Stratagene, La Jolla, CA) and used as the template for introducing stop codons at five different locations in the cytoplasmic domain of GP Ibα, utilizing Stratagene's Double TakeTM double-stranded mutagenesis system. The mutagenic primers that were used included 5′-GGGAGGACGGAATTGACATG-3′ (m545), 5′-CCTCTAGAGGTACGAAGGAGGT-3′ (m559), 5′-CTCTCTCTGATGACCTAAC-3′ (m574), 5′-GCCCCCACTTGTAATGACATG-3′ (m591), and 5′-GTGAGCACTAGTATTGGCCACACCTC-3′ (m605) (Operon Technologies, Inc., Alameda, CA). The mutations of interest were verified by sequence analysis.

Association of the GP Ib-IX Complex with the Cytoskeleton—Cells were harvested with EDTA, lysed in a Triton X-100 containing buffer, and soluble and insoluble fractions isolated by centrifugation as described previously (21). Samples were analyzed on one-dimensional 5–15% SDS-polyacrylamide gels by the method of Laemmli (31) as described previously (16). GP Ibα, GP Ibβ, and the Triton X-100-soluble and -insoluble fractions isolated by centrifugation as described previously (21) were analyzed on Western blots (32) using 10 μg/ml monoclonal antibody IOP42b (AMAC, Inc., Westbrook, ME) or polyclonal antibodies against the GP Ib-IX complex (33) followed by horseradish peroxidase-labeled secondary antibodies. The chemiluminescence was detected on autoradiographs using Amersham's ECL system (Amersham Int., Buckinghamshire, United Kingdom).

Flow Cytometry and Cell Sorting—Cells were harvested with EDTA, washed twice with PBS, and incubated with 5 μg/ml fluorescein isothiocyanate (FITC)-conjugated AN51 (DAKO, Carpinteria, CA), a monoclonal antibody to GP Ibα, or SZ1 (Amac Inc., Westbrook, ME), a monoclonal antibody that recognizes an epitope on the GP Ib-IX complex (5). The cells were rocked in the dark at room temperature for 30 min, centrifuged at 800 rpm for 10 min, and then washed twice with PBS. Samples were then resuspended in 500 μl of PBS and flow cytometric analysis performed on a modified FACS 440 (Becton Dickinson, Mountain View, CA) cell sorter equipped with CICERO electronics and CPU (Cytometry, Fort Collins, CO) and with a single argon laser (Coherent, Mountain View, CA). All experiments were performed using the 488-nm laser line set at 400 mW with triggering and thresholding done on forward light scatter. Forward and side light scatter emission were detected through 488/10 nm band-pass filters, and fluorescence emission was collected through a 530/30 nm band-pass filter. In most cases, the 5% of cells with the highest level of fluorescence intensity were isolated in a three-drop sort packet directed into medium.

Binding of von Willebrand Factor to GP Ib-IX—Transfected CHO cells (5 × 105/sample) were harvested by treatment with EDTA for 10 min, washed once with PBS, and resuspended in 400 μl of PBS, 1% bovine serum albumin. Cells were incubated with 0.5 μg/ml von Willebrand factor (gift of Dr. J. Joel Mann, Baylor College of Medicine, Houston, TX) in the presence or absence of 5 μg/ml botrocetin (Pentapharm, Basel, Switzerland) for 1 h at 4 °C with gentle agitation. Samples were washed twice with PBS, fixed in 0.5% paraformaldehyde (Sigma) for 30 min at room temperature, and washed twice again in PBS. To detect von Willebrand factor binding samples were then incubated with anti-factor VIII anti-factor VIII (Jackson Immunoresearch Laboratories, West Grove, PA) followed by FITC-conjugated goat anti-rabbit IgG (Vector Laboratories, Inc., Burlingame, CA) for 30 min at 4 °C, washed twice with PBS, resuspended in 500 μl of PBS, and analyzed by flow cytometry.

For Scatchard analysis, von Willebrand factor was labeled with 125I by the lactoperoxidase method and various concentrations incubated with transfected cells as described previously (30). Samples were rocked at room temperature for 1 h, aliquots sedimented through sucrose cushions, and the amount of 125I-labeled von Willebrand factor associated with the sedimented cells measured. Preliminary experiments used excess unlabeled von Willebrand factor to determine nonspecific binding. In subsequent experiments nonspecific binding was determined by measuring 125I-labeled von Willebrand factor that bound to cells which had not been transfected with GP Ib-IX and subtracting the counts from those of the corresponding transfected samples. The two methods of determining nonspecific binding gave comparable results; the experiments in the present study were performed using the latter method for determining nonspecific binding.

Immunofluorescence Microscopy—Transfected cells were mixed with 5 μg/ml botrocetin and plated on Lab-Tek tissue culture chamber slides (Nunc, Inc., Naperville, IL) that had been coated with 10 μg/ml von Willebrand factor and saturated with 3% bovine serum albumin. After 2 h at 37 °C, cells were fixed, lysed, labeled with polyclonal antibodies that had been raised against the GP Ib-IX complex (33) followed by FITC-labeled anti-rabbit IgG (Vector Laboratories) as described previously (21, 30). Fluorescence microscopy was performed on an inverted microscope (DIAPHOT-TMD, Nikon, Japan).

RESULTS

Expression of GP Ib-IX Containing Truncated GP Ibα, in the Plasma Membrane of Cultured Cells—The approach that was used to determine whether the cytoplasmic domain of GP Ib-IX regulates the function of the receptor was to express full-length and truncated forms of the complex in cultured cells. Because previous work with synthetic peptides had shown that peptides based on sequences in the cytoplasmic domain of the GP Ibα subunit could bind to actin-binding protein in vitro (22), we focused on truncations in the cytoplasmic domain of the GP Ibα subunit of the complex. Stop codons were introduced into the cDNA for GP Ibα by site-directed mutagenesis at Gin-545, Ser-559, Thr-570, Gin-591, or Tyr-605. Fig. 1 illustrates the resulting truncated forms of GP Ibα; m545, m559, m570, m591, and m605. Truncated forms of GP Ibα were expressed in CHO cells or melanoma cells along with full-length GP Ibα and GP IX. We have shown previously that full-length GP Ibα associates with the other components of the GP Ib-IX complex and is inserted into the membrane of these cultured cells (21, 30). In the present study, analysis of the cells by flow cytometry (Fig. 2) and examination by immunofluorescence (data not shown) revealed that each of the truncated forms of GP Ibα was also inserted into the membranes of these cells. In order to be able to compare the function of the various forms of GP Ibα, cells were sorted by FACS analysis until populations of cells expressing comparable amounts of the various forms of GP Ibα in their membranes were obtained (Fig. 2).

The Cytoplasmic Domain of GP Ibα Mediates the Interaction of the Entire GP Ib-IX Complex with the Cytoskeleton—To determine whether the truncated forms of GP Ibα could associate with the cytoskeleton, transfected CHO cells were lysed with

![Fig. 1. Map of GP Ibα mutations. Stop codons were introduced into the cytoplasmic domain of GP Ibα, cDNA by single base pair changes at Gin-545 (C to T at base pair 1729), Ser-559 (C to A at base pair 1766), Thr-570 (G to A at base pair 1800), Gin-591 (C to T at base pair 1861), and Tyr-605 (C to A at base pair 1905) to generate the m545, m559, m570, m591, and m605 constructs, each coding for a different truncated form of GP Ibα. The solid bars in the lower portion of the figure represent synthetic peptides previously assayed in in vitro binding studies (22).](http://www.jbc.org/doi/fig)
pellet, as was GP Ib-IX in the intact cell, they show that the cytoplasmic domain is absolutely required for this interaction.

To determine whether the COOH-terminal region of the cytoplasmic domain of GP Ib-IX was necessary for association of the other components of the GP Ib-IX complex with the cytoskeleton, detergent-insoluble and -soluble fractions from CHO cells expressing GP Ib-IX that contained either full-length GP Ib-IX or the truncated m559 form (lacking 52 amino acids) were analyzed by Western blots using polyclonal antibodies that recognized all three subunits of the GP Ib-IX complex. Fig. 3, full-length GP Ib-IX and the various truncations of GP Ib-IX were analyzed on SDS gels in the presence and absence of reducing agent; like full-length GP Ib-IX, GP IX, and the various truncations of GP Ib-IX, Platelets (1 x 10^9/ml) solubilized in SDS-containing buffer were included for reference. The GP Ib-IX-reactive band with a slightly lower molecular weight than GP Ib-IX that is indicated with an arrow is assumed to be GP Ib-α that had been cleaved at a protease-sensitive site in the extracellular amino-terminal region of the protein (see Ref. 34).

To determine whether the COOH-terminal region of the cytoplasmic domain of GP Ib-IX was necessary for association of the other components of the GP Ib-IX complex with the cytoskeletal fraction, detergent-insoluble and -soluble fractions from CHO cells expressing GP Ib-IX that contained either full-length GP Ib-IX or the truncated m559 form (lacking 52 amino acids) were analyzed on Western blots using polyclonal antibodies that recognizes all three subunits of the GP Ib-IX complex. Fig. 4 shows that release of GP Ib-α from the detergent-insoluble fraction was accompanied by release of the other two components of the GP Ib-IX complex. Although these results do not exclude the possibility that GP Ib-α or GP IX may play a role in mediating the interaction of the GP Ib-IX complex with the cytoskeleton in the intact cell, they show that the cytoplasmic domain of GP Ib-α is absolutely required for this interaction.

To determine whether the truncation of GP Ib-α that prevented its association with the cytoskeleton had an effect on its incorporation into the GP Ib-IX complex, detergent-insoluble Triton X-100 and the insoluble cytoskeletal proteins isolated by centrifugation as described previously (21, 30). GP Ib-α was detected on Western blots. As shown in Fig. 2, full-length GP Ib-α was recovered with the detergent-insoluble cytoskeletal pellet, as was GP Ib-α in which the carboxyl-terminal 6 (m605) or 20 (m591) amino acids were missing. In contrast, GP Ib-α lacking 41 (m570), 52 (m59), or 66 (m545) amino acids was present primarily in the detergent-soluble fraction and therefore not associated with the cytoskeleton. Transfected cells were also analyzed by dual-label immunofluorescence to determine whether expressed GP Ib-IX colocalized with the cells’ endogenous actin-binding protein. The results provided further evidence that truncated GP Ib-α lacking 41 or more carboxyl-terminal amino acids did not associate with actin-binding protein while GP Ib-α lacking 20 COOH-terminal amino acids or less did (data not shown).

To determine whether the COOH-terminal region of the cytoplasmic domain of GP Ib-α was necessary for association of the other components of the GP Ib-IX complex with the cytoskeletal fraction, detergent-insoluble and -soluble fractions from CHO cells expressing GP Ib-IX that contained either full-length GP Ib-α or the truncated m559 form (lacking 52 amino acids) were analyzed on Western blots using polyclonal antibodies that recognizes all three subunits of the GP Ib-IX complex. Fig. 4 shows that release of GP Ib-α, from the detergent-insoluble fraction was accompanied by release of the other two components of the GP Ib-IX complex. Although these results do not exclude the possibility that GP Ib-α or GP IX may play a role in mediating the interaction of the GP Ib-IX complex with the cytoskeleton in the intact cell, they show that the cytoplasmic domain of GP Ib-α is absolutely required for this interaction.
GP Ib-IX-Cytoplasmic Domain

results demonstrate that even when the cytoplasmic domain of GP Ib-IX is truncated and the GP Ib-IX complex is not associated with the cytoskeleton, the complex is still capable of binding ligand with the same affinity as full-length cytoskeleton-associated receptor.

The Cytoplasmic Domain of GP Ibα Regulates the Morphology of Transfected Cells Spreading on von Willebrand Factor-coated Slides—To determine whether the association of GP Ib-IX with the cytoskeleton is important in directing ligand-induced transmembrane signaling, the way in which transfected cells spread following GP Ib-IX-induced adhesion to von Willebrand factor-coated slides was studied. Cells expressing either full-length GP Ib-IX or the m545 truncation were seeded on von Willebrand factor-coated chamber slides and incubated at 37°C for 2 h in the presence or absence of botrocetin. The cells were examined by phase microscopy; in each chamber 300 cells were examined and the number of cells that were adherent and spreading recorded (Table I). The cells were subsequently fixed, stained for GP Ib-IX, and the morphology of the adherent cells determined by immunofluorescence (Fig. 7). Nontransfected cells failed to adhere to von Willebrand factor-coated slides whether botrocetin was present or not (data not shown). In the absence of botrocetin, only a few of the transfected cells adhered to the slides; the few that did adhere remained round and failed to spread (Table I; Fig. 7, panels A and C). In contrast, if botrocetin was present transfected cells adhered to the von Willebrand factor-coated slides (Table I); adhesion occurred whether the GP Ib-IX complex contained a truncated cytoplasmic domain or not. In both cases adhesion resulted in transmembrane signaling as shown by the fact that the adherent cells extended pseudopodia and spread over the von Willebrand-factor-coated surface (Table I and Fig. 7, panels B and D). However, examination of the morphology of the spreading cells revealed marked differences in the cells expressing full-length complex compared to those expressing truncated GP Ib-IX. Cells expressing the full-length complex extended only a few short, blunt protrusions (Fig. 7, panel B). In comparison, cells expressing m545 had a much more spread morphology and extended numerous thin, multi-branched, fin-
ger-like projections. These projections occurred around the entire periphery of the cell (Fig. 7, panel D).

**DISCUSSION**

The GPIb-IX complex is one of the major platelet membrane receptors and is responsible for mediating the initial interaction of platelets with the subendothelium at a site of injury (1–4). Following interaction of GPIb-IX with its adhesive ligand, von Willebrand factor, transmembrane signaling is initiated and intracellular pathways leading to the secretion of granule contents and aggregation are induced (9–12). The way in which binding of von Willebrand factor to the GPIb-IX complex induces intracellular changes is not known. In the present study, we have investigated the importance of the cytoplasmic domain of GPIbα in regulating the function of the complex. We show that truncation of the carboxyl-terminal half of the cytoplasmic domain of GPIbα does not affect incorporation of GPIbα into the GPIb-IX complex, insertion of the complex into the membrane, or binding of von Willebrand factor to the extracellular domain of the complex. However, truncation of this cytoplasmic domain results in a GPIb-IX complex that cannot associate with the cytoskeleton in transfected cells. Moreover, cells expressing complex with truncated GPIbα cytoplasmic domain showed a very different shape following adhesion to immobilized von Willebrand factor than did cells expressing full-length GPIbα.

Previous work has demonstrated that the link between GPIb-IX and the platelet cytoskeleton is actin-binding protein (16–21). Furthermore, in vitro binding studies utilizing synthetic peptides derived from the sequence of the cytoplasmic domains of GPIbα and GPIbβ showed that sequences in the cytoplasmic domain of the GPIbα subunit could bind to purified actin-binding protein (22). In particular, a hydrophilic amino acid sequence from Thr-536 to Phe-568 in GPIbα was most effective, while the adjacent Trp-570 to Ala-588 sequence bound to a lesser extent. Because of potential problems involving the binding of synthetic peptides to purified proteins in in vitro assays, it was necessary to identify sequences that were involved in mediating the interaction with the cytoskeleton in an intact cell. Based on our previous results with the synthetic peptides, we chose to focus on GPIbα as the subunit most likely to be involved in mediating the interaction. The present study shows that GPIbα missing 20 amino acids (truncated at Gln-591) or less is still able to associate with the cytoskeleton while GPIbα missing 41 or more COOH-terminal amino acids (truncated at Trp-570) loses its ability to do so. Thus, it appears that all or part of the Trp-570 to Ser-590 sequence in the cytoplasmic domain of GPIbα is essential to maintaining the cytoskel-
etal association in an intact cell, either by interacting with submembranous actin-binding protein or perhaps by binding and subsequently conferring stability or a favorable secondary structure to the adjacent Thr-356 to Phe-368 region, making it also amenable to interaction with actin-binding protein.

Truncation of the cytoplasmic domain of a number of different cell adhesion molecules has been shown to affect the ligand binding properties of the receptors. In several cases, a correlation between altered function and an inability to associate with the cytoskeleton has been suggested. For example, truncation of the cytoplasmic domain of the β3 integrin subunit eliminated its incorporation into focal contacts and eliminated its ability to promote adhesion of cultured cells (23). Deletions in the carboxy half of the cytoplasmic domain of E-cadherin caused it to lose its ability both to associate with the cytoskeleton and to promote cell-cell adhesion, while mutations in other regions of the cytoplasmic domain had no effect on either property (27, 28). In other studies, the ability of another cadherin, L-CAM, to mediate cell aggregation was abolished (41) following treatment of transfected cells with cytochalasin D, an agent that disrupts cytoskeletal microfilaments. In the case of GP Ib-IX, it has been suggested that association of the GP Ib-IX complex with actin-binding protein in the platelet membrane skeleton might be responsible for maintaining the uniform distribution of the receptor in the platelet membrane and that this distribution might in turn be important in regulating the binding of von Willebrand factor multimers (38, 42). However, in the present study using transfected cells we found no evidence that association of the complex with the cytoskeleton regulates its ability to bind adhesive ligand. The number of binding sites per cell was slightly different on the cells in which the cytoplasmic domain of GP Ibα was truncated but the difference could be accounted for entirely by a difference in the number of receptors in the membrane; the affinity of the interaction was the same whether the cytoplasmic domain of GP Ibα was truncated or not. Thus, in transfected cells GP Ib-IX complex containing a truncated form of GP Ibα that could not associate with the cytoskeleton was able to bind von Willebrand factor just as well as GP Ib-IX containing full-length subunit. These findings suggest a difference between GP Ib-IX and other adhesion receptors. However, the distribution of GP Ib-IX could be maintained by the cytoskeleton differently in cultured cells than it is in platelets. Furthermore, in order to induce binding of soluble von Willebrand factor to GP Ib-IX in cultured cells, modulators such as ristocetin or botrocetin (21, 30, 39, 40) were used. It is conceivable that if it were possible to determine the effect of the cytoplasmic domain mutations in a more physiological system (e.g. shear-induced binding of von Willebrand factor to platelets or platelets adhering to von Willebrand factor in the extraacellular matrix) a different result would be obtained. Future studies using transgenic mice in which the receptor is truncated may help to resolve these issues. The identification of the subunit that mediates the interaction of the entire GP Ib-IX complex with the cytoskeleton and of truncations that ablate the interaction while still allowing the complex to be inserted into the membrane should prove useful in allowing the design of such experiments.

To investigate the possibility that the cytoplasmic domain of GP Ib-IX regulated events that occurred following von Willebrand factor binding to the complex, we allowed transfected cells to settle onto von Willebrand factor-coated slides. Nontransfected cells showed little adhesion, those that adhered remained round. Similarly, in the absence of botrocetin transfected cells showed little adhesion; those that did adhere did not spread. In contrast, when botrocetin was present, cells that expressed GP Ib-IX adhered and spread. Even when the GP Ib-IX complex was not associated with the cytoskeleton, the cells spread showing that neither the interaction with the cytoskeleton nor the interaction of the carboxyl-terminal half of the cytoplasmic domain of GP Ibα with other intracellular proteins is needed to transmit the signals that allow this ligand-induced change in cell behavior. However, the morphology of the spreading cells was very different between the two transfected cell types. Cells containing full-length GP Ibα extended a few broad pseudopods. Cells expressing truncated GP Ibα extended numerous finger-like projections around the entire cell. Because non-transfected cells did not spread, the cellular morphologies observed were the specific result of GP Ib-IX-von Willebrand factor interactions. These results indicate that the reorganization of the cytoskeleton resulting from ligand-receptor interactions was regulated in an altered way in the cells in which the cytoplasmic domain of the receptor was truncated compared to those in which it was not.

In platelets, the major spreading that occurs following adhesion of platelets to exposed extracellular matrix is thought to occur as a consequence of αIIBβ3-induced transmembrane signaling (43–45). Ligand-αIIBβ3 interactions have been shown to induce cytoskeletal reorganizations (46, 47) and the incorporation of signaling molecules into the newly formed integrin-cytoskeletal complexes has been studied in detail (47, 48–52). In contrast, dramatic spreading of platelets does not appear to be induced as a direct consequence of GP Ib-IX-matrix interactions (43–45) and little consideration has been given to the possibility that GP Ib-IX-von Willebrand factor interactions induce cytoskeletal reorganizations. However, a recent publication (12) suggests that signaling molecules are induced to associate with the cytoskeleton following GP Ib-IX-ligand interactions. The present study indicates that cytoskeletal reorganizations are also induced; ligand-receptor interactions resulted in a compact cell morphology and the formation of only a few broad protrusions. In platelets, GP Ib-IX-von Willebrand factor interactions are known to induce the stable attachment of platelets to the extracellular matrix (43–45); the cytoskeletal reorganizations detected in the present study may stabilize the GP Ib-IX-induced adhesion of platelets to the extracellular matrix while subsequent αIIBβ3-induced signaling results in spreading of the platelets over the surface.

The GP Ib-IX-induced cytoskeletal reorganizations occurred even in the absence of the COOH-terminal 52 amino acids of GP Ibα. However, in the absence of these amino acids a very different cytoskeletal reorganization was induced. Because the cells exhibited a much more irregular morphology with many finger-like projections around the cells in the absence of GP Ibα COOH-terminal domain, we propose that this cytoplasmic domain has a restraining effect on von Willebrand factor-induced cytoskeletal reorganizations, regulating both the location and extent of the reorganizations. Because truncation of the cytoplasmic domain also prevented association of the GP Ib-IX complex with the cytoskeleton, it is possible that it is the association of the complex with the cytoskeleton that allows it to directly exert an influence on the ligand-induced reorganization of the cytoskeleton. Previously, we have shown that in platelets the β-subunit of the GP Ib-IX complex is phosphorylated on serine 166 (53) and have provided evidence that this phosphorylation is involved in inhibiting the polymerization of actin within platelets (54). Thus, it is conceivable that the β-subunit of GP Ib-IX can regulate the extent and location of actin polymerization and that it is unable to do this if the glycoprotein complex is not associated with the cytoskeleton. Another possibility is that the cytoplasmic domain of GP Ibα associates with molecules that are involved in the transmission of signals across the GP Ib-IX complex and in the absence of
this association a regulatory pathway is lost. One candidate is 14.3.3, a protein that has recently been found to co-isolate with GP Ib-IX from platelet lysates (55) and to associate with the COOH-terminal region of GP Ibα. Further experiments will be needed to investigate these possibilities.

Acknowledgments—We are grateful to Bill Hyun for FACS analyses and cell sorting, Kwok Keung Poon and Susanne Zuerbig for technical assistance, Philippe Gascard for critical review of the manuscript, and Al Averbach for editorial assistance. Al Averbach for editorial assistance.
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*J. Biol. Chem.* 1996, 271:11581-11587.
doi: 10.1074/jbc.271.19.11581

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