The platelet integrin, glycoprotein IIb-IIIa (GPIIb-IIIa), is a calcium-dependent heterodimer that binds fibrinogen, von Willebrand factor, and fibronectin after platelet activation. We examined GPIIb-IIIa alone and bound to these ligands by electron microscopy after rotary shadowing with platinum/tungsten. We found, as observed previously, that in the presence of detergent and 2 mM Ca\(^{2+}\), GPIIb-IIIa consists of an 8 X 12-nm head with two 18-nm flexible tails extending from one side. We also found that in the presence of EDTA, GPIIb-IIIa dissociates into two similar comma-shaped subunits, each containing a portion of the globular head and a single tail. Using monoclonal antibodies to GPIIb, GPIIIa, and the GPIIb-IIIa heterodimer, we found that the tails contained the carboxyl termini of each subunit, while the nodular head was composed of amino-terminal segments of both subunits. Electron microscopy of GPIIb-IIIa bound to fibrinogen revealed a highly specific interaction of the nodular head of GPIIb-IIIa with the distal end of the trinodular fibrinogen molecule and with the tails of GPIIb-IIIa extended laterally at an angle of \(\approx 98^\circ\) with respect to the long axis of fibrinogen. When a GPIIb-IIIa was bound to each end of a single fibrinogen, the tails were oriented to opposite sides of fibrinogen, enabling fibrinogen to bridge two adjacent platelets. Electron microscopy of GPIIb-IIIa bound to fibronectin revealed GPIIb/IIIa-binding sites approximately two-thirds of the distance from the amino terminus of each end of the fibronectin molecule, while GPIIb-IIIa was found to bind to von Willebrand factor protomers along a rod-like region near the central nodule of the molecule.

The platelet glycoprotein IIb-IIIa complex (GPIIb-IIIa)\(^1\), a member of the integrin family of cell adhesion receptors, is required for platelet aggregation (1). Because of the role played by platelet aggregation in hemostasis and thrombosis, there has been considerable interest in the structure and function of GPIIb-IIIa. The GPIIb-IIIa complex is a calcium-dependent heterodimer (2). GPIIb, the \(\alpha\) subunit of the heterodimer, has an apparent molecular weight of 140,000 and dissociates into an \(M_2\), 125,000 heavy chain and an \(M_1\), 23,000 light chain following disulfide bond reduction (2). GPIIIa, the \(\beta\) subunit of the heterodimer, is a single chain protein with an apparent unreduced molecular weight of 95,000 that increases to 110,000 following disulfide bond reduction (2). The GPIIb-IIIa heterodimer, but neither of its individual subunits alone, contains binding sites for fibrinogen, von Willebrand factor, fibronectin, and vitronectin that are exposed by platelet stimulation (1, 3). Platelet aggregation is thought to occur when one or more of these proteins bind to GPIIb-IIIa on adjacent stimulated platelets, cross-linking the activated platelets into aggregates. Experimental and clinical evidence suggests that under physiologic conditions, soluble fibrinogen and von Willebrand factor are the proteins that mediate platelet aggregation (4). However, GPIIb-IIIa also may be involved in the initial attachment of platelets to damaged blood vessels (5), and fibronectin and vitronectin binding to GPIIb-IIIa may be involved in this process.

GPIIb-IIIa, like other integrins, recognizes the amino acid sequence Arg-Gly-Asp (RGD) when it is presented in the proper context (6). Each of the proteins that bind to activated GPIIb-IIIa contain RGD sequences (3). Furthermore, likely sites of RGD binding, located in the amino-terminal portions of GPIIb and GPIIIa, have been determined by cross-linking RGD-containing peptides to the intact heterodimer (7, 8). GPIIb-IIIa also interacts with peptides that correspond to the carboxyl terminus of the \(\gamma\) chain of fibrinogen and are not related to RGD (9-11). Moreover, it has been observed, but not explained, that the binding of fibrinogen \(\gamma\) chain peptides and the RGD-containing peptides to GPIIb-IIIa is mutually exclusive (12). To understand better the interaction of GPIIb-IIIa with its protein ligands, we have examined GPIIb-IIIa and the complexes it forms with fibrinogen, von Willebrand factor, and fibronectin by electron microscopy.

**EXPERIMENTAL PROCEDURES**

*Protein Purification*—GPIIb-IIIa heterodimers were isolated from detergent extracts of human platelets by affinity chromatography (13). Outdated human platelets obtained from the Penn-Jersey Red Cross were washed twice with a buffer containing 150 mM NaCl, 14 mM sodium citrate, and 0.7 mM glucose, pH 6.5, and disrupted by nitrogen cavitation. Undisrupted debris was removed by centrifugation at 30,000 \(\times\) g. Protein purification was accomplished by a second centrifugation at 30,000 \(\times\) g. The particulate fraction was then extracted with a 0.05 M Tris buffer, pH 7.3, containing 1% Triton X-100, 10 mM benzamidine, 12.5 mg/ml leupeptin, 2 mg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, and 1 mM N-carbobenzoxy-L-glutamyl-L-tyrosine. Following adjustment of its calcium concentration to 1 mM, the extract was applied to a 5-ml column of Sepharose 4B containing the murine monoclonal

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antibody SSA6 specific for GPIIb (14). After washing the column with 50 ml of 0.05 M Tris, pH 7.3, containing 60 mM n-octyl β-D-glucopyranoside, GPIIb-IIIa heterodimers were eluted with 1 M glycine buffer, pH 4.0, containing 60 mM n-octyl β-D-glucopyranoside. The pH of the eluted fractions was immediately restored to 7.3 with 1 M NaOH, and fractions containing the highest protein concentrations, as estimated by absorbance at 280 nm, were pooled. The protein composition of the pooled fractions was assessed by SDS-polyacrylamide gel electrophoresis as previously described (13) (Fig. 1).

The fibrinogen used in these studies was human band I fibrinogen kindly provided by Dr. Jose Martinez of the Cardeza Foundation, Thomas Jefferson University, Philadelphia. The purity of the fibrinogen and the integrity of its Aα chains were confirmed by SDS-polyacrylamide gel electrophoresis (Fig. 1). Human plasma fibronectin was a gift from Dr. Steven Albelda of the Pulmonary Section, Hospital of the University of Pennsylvania, Philadelphia. Human von Willebrand factor was kindly provided by Dr. Harvey Gralnick of the National Institutes of Health, Bethesda.

Binding Reactions—Binding of purified GPIIb-IIIa to one of its purified protein ligands or to a monoclonal antibody was performed by incubating 0.5 mg/ml samples of each in a 0.05 M Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl, 2 mM CaCl2 and 60 mM n-octyl β-D-glucopyranoside at either 4°C for 18 h or 37°C for 1.5 h. Identical results were obtained under each set of conditions. For experiments involving dissociated GPIIb-IIIa, 4 mM EDTA was substituted for the CaCl2. In selected experiments, n-octyl β-D-glucopyranoside was removed by extensive dialysis. Prior to rotary shadowing for electron microscopy, samples were diluted 10 times with the volatile buffer described below.

Electron Microscopy—Rotary-shadowed samples were prepared using a modification of standard procedures (15) by spraying a dilute solution of molecules in a volatile buffer (0.05 M ammonium formate) and glycerol (30–50%) onto freshly cleaved mica and shadowing with platinum-tungsten in a vacuum evaporator (Denton Vacuum Co., Cherry Hill, NJ) (16). Each experiment was carried out 12–30 times, and all specimens were examined in a Philips 400 electron microscope (Philips Electronic Instruments Co., Mahwah, NJ), operating at 80 kV and at a magnification of ×60,000.

RESULTS

Electron Microscopy of the Glycoprotein IIb-IIIa Complex in the Presence or Absence of Detergent—To confirm and extend the results of a previous ultrastructural study of GPIIb-IIIa, we purified GPIIb-IIIa from extract of human platelets and examined the complex by electron microscopy after rotary shadowing with platinum-tungsten. Similar to results published previously by Carrel et al. (17), we found that individual GPIIb-IIIa complexes, examined in the presence of 2 mM Ca2+ and the detergent n-octyl β-D-glucopyranoside displayed a globular head and two tails extending from one side of the head (Fig. 2). At least 90% of more than 500 images examined had this appearance; the majority of the remainder showed globular structures that often appeared as if the tails were wrapped around the head. After correction for the layer of metal on the most commonly observed structures, the average dimensions of the globular heads were 12 ± 2 nm by 8 ± 1.5 nm. Each tail had a contour length of 18 ± 3 nm and was ∼2 nm in diameter, although the techniques used preclude exact measurements of structures as thin as the tails. Furthermore, we found that many of the tails terminated in a small globular region ∼4 nm in diameter. In approximately two-thirds of the

![Fig. 1. SDS-polyacrylamide gel electrophoresis of purified platelet GPIIb-IIIa and human fibrinogen.](attachment://fig1.png)

**Fig. 1.** SDS-polyacrylamide gel electrophoresis of purified platelet GPIIb-IIIa and human fibrinogen. Human platelet GPIIb-IIIa was isolated as described under “Experimental Procedures.” Human band I fibrinogen was kindly provided by Dr. Jose Martinez. Ten μg of GPIIb-IIIa and 6 μg of fibrinogen were electrophoresed on a 0.1% SDS-10% polyacrylamide slab gel which was stained with Coomassie Brilliant Blue. Molecular weight standards (Bethesda Research Laboratories) were electrophoresed concurrently. Lane 1, GPIIb-IIIa; lane 2, human band I fibrinogen demonstrating the presence of predominantly intact Aα chains and a doublet in the γ chain region composed of γ1 and γ2 chains.

![Fig. 2. A gallery of electron microscope images of rotary-shadowed GPIIb-IIIa complexes.](attachment://fig2.png)

**Fig. 2.** A gallery of electron microscope images of rotary-shadowed GPIIb-IIIa complexes. **Rows a-f.** GPIIb-IIIa in the presence of the detergent octyl glucoside. Each individual GPIIb-IIIa complex has a globular head and two long tails extending from one side. Some molecules show a smaller nodule at the end of each tail. The average size of the globular head is about 12 × 8 nm, while the tails are about 18 nm long, and the globular regions at the tips of the tails are about 4 nm. In **rows a and b**, the tails are roughly parallel or splayed (∼66% of molecules), while in **row c** they are touching each other at their ends to yield a signet ring shape (∼34% of molecules). **Rows d-f** present some examples of fields of complexes to illustrate the variety of appearances seen. **Row g** contains electron microscope images of GPIIb-IIIa in the absence of detergent. Under these conditions, GPIIb-IIIa complexes aggregate to form rosettes with their tails pointed inward and interacting and their nodular heads at the periphery. This result suggests that the carboxyl-terminal, hydrophobic transmembrane domains, which would interact in the absence of detergent, are located in the tails. **Bar,** 100 nm.
images, the tails were either parallel to each other or splayed apart (Fig. 2, a, b, d, and e). However, in the remainder, the ends of the tails appeared to touch (Fig. 2, c and f), giving a “signet ring” appearance to the complex. Images of GPIIb-IIIa were also obtained after removal of detergent by dialysis (Fig. 2g). In the absence of detergent, GPIIb-IIIa formed “rosettes” with the ends of the tails interacting and pointed inward, while the globular heads made up the outer perimeter of the rosette. These images suggest that the ends of the tails are predominantly hydrophobic and the globular heads are hydrophilic.

Electron Microscopy of Glycoprotein IIb-IIIa in the Presence of EDTA—The GPIIb-IIIa heterodimer, in contrast to other integrins, is calcium dependent and in the presence of calcium chelators like EDTA, dissociates into its two subunits. Carrell et al. (17) reported that in the presence of EDTA, GPIIb is an 8 × 10-nm ellipsoid structure and GPIIIa is a 20–30-nm filamentous rod. Because these conclusions are not consistent with the structure of GPIIb and GPIIIa surmised from examination of their primary structures (18, 19), we repeated the electron microscopy experiments after incubating our preparations of purified GPIIb-IIIa preparations with 4 mM EDTA for 2 h at 21 °C. In the presence of EDTA, the GPIIb-IIIa structures we observed previously were replaced by “comma-shaped” structures consisting of a small globular head and a single tail and with a total length of 24–27 nm (Fig. 3a). Since the preponderance of observed structures had this shape and our technique insures that all components in the solution will be present on the electron microscope grid, it is likely that GPIIb and GPIIIa are similar in appearance at this level of resolution. Following brief treatment with EDTA, we observed structures in which two adjacent subunits were joined at the ends of their tails (Fig. 3b), suggesting that they originated as signet ring structures and that the calcium-dependent interaction that formed the signet rings involved the globular heads. Furthermore, these images indicate that the globular head of GPIIb-IIIa is composed of portions of GPIIb and GPIIIa and that each of the tails of the intact complex is contributed by one of the subunits. They also argue against the possibility that selective retention of either GPIIb or GPIIIa on the electron microscopy grid accounts for the “similarity” in their appearance. About 85% of the structures observed in EDTA are comma-shaped; most of the rest of the images were undissociated GPIIb-IIIa complexes. Occasionally, we observed globular or ball-shaped structures, similar to those reported previously for GPIIb (17), but under conditions where the native structure of GPIIb-IIIa was less well maintained. Thus, it is likely that the shape of the individual subunits of GPIIb-IIIa is more fragile than that of the heterodimer, and the ball-like structures are subunits whose tails have rolled up around the heads.

Monoclonal Antibody Binding to the Glycoprotein IIb-IIIa Heterodimer—To confirm our conclusions regarding the topography of the GPIIb-IIIa complex, we performed electron microscopy after incubating the GPIIb-IIIa complex with each of three well-characterized monoclonal antibodies (Fig. 4). The monoclonal antibody B1B5 recognizes an epitope located near the carboxyl terminus of GPIIb heavy chain (18). We found that B1B5 bound near the tip of only one of the two tails of a GPIIb-IIIa complex, confirming that the carboxyl terminus of GPIIb is located in this structure. The monoclonal antibody SSA6 binds exclusively to unreduced GPIIIa. Although the location of the epitope for this antibody has not been determined, SSA6 does react with an Mr 66,000 chymotrypsin fragment of GPIIIa that is composed predominantly of the carboxyl-terminal portion of the molecule (20). Again, we found that SSA6 bound to only one of the two tails of GPIIb-IIIa, indicating this contains the carboxyl-terminal segment of GPIIIa. The monoclonal antibody A2A9 binds only to the intact GPIIb-IIIa heterodimer and, in contrast to B1B5 and SSA6, inhibits the interaction GPIIb-IIIa with its protein ligands (13). Moreover, the rate of A2A9 binding increases following platelet activation, suggesting that the epitope for this antibody is influenced by the conformational change that GPIIb-IIIa undergoes after platelet activation. We found that A2A9 binds to the globular head of the GPIIb-IIIa complex on the side opposite the tails. This supports our conclusion that the globular head is composed of portions of both GPIIb and GPIIIa and suggests that this part of GPIIb-IIIa undergoes the conformational change that exposes the heterodimer’s ligand-binding sites.

Electron Microscopy of Glycoprotein IIb-IIIa Bound to Fibrinogen—Binding of soluble proteins to GPIIb-IIIa in intact

Fig. 4. Monoclonal antibody binding to GPIIb-IIIa in the presence of octyl glucoside. Monoclonal antibody B1B5 (indicated by arrows) is specific for an epitope near the carboxyl terminus of the GPIIb heavy chain and binds to one of the tails of intact GPIIb/IIIa. Monoclonal antibody SSA6 (indicated by arrows) reacts with a 66-kDa chymotrypsin fragment of GPIIIa composed primarily of the carboxyl-terminal portion of the molecule and also binds to one of the tails of intact GPIIb/IIIa. Monoclonal antibody A2A9 (indicated by arrows) interacts exclusively with an epitope present on the intact GPIIb-IIIa heterodimer and inhibits the interaction of GPIIb-IIIa with its ligands. A2A9 binds to the nodular head of GPIIb-IIIa on the side opposite the tails. Bar, 50 nm.

Fig. 3. GPIIb-IIIa in the presence of EDTA and octyl glucoside. In the absence of calcium, GPIIb-IIIa dissociates into two comma-shaped structures, as illustrated in a. Several globular structures with their tail curled around the head and structures with a less prominent globular domain are also shown. In b, examples are given of two comma-shaped structures with the tips of their tails interacting. These images likely arose as signet ring structures that were in the process of dissociating. Bar, 100 nm.
platelets requires platelet activation (1). However, a portion of the GPIIb-IIIa isolated from platelet extracts appears to acquire this activity (21–23). Therefore, to obtain images of isolated GPIIb-IIIa bound to fibrinogen, we incubated aliquots of purified GPIIb-IIIa with purified fibrinogen in the presence of 2 mM Ca\(^{2+}\). Initially, we performed the incubations in the absence of detergent because of concern about possible effects of detergent on the binding reaction. The incubation solution was then sprayed onto freshly cleaved mica and rotary shadowed. As seen in Fig. 5d, GPIIb-IIIa molecules were present as rosettes, many of which contained a fibrinogen molecule whose end nodule was associated with globular head of a single GPIIb-IIIa complex. These experiments indicate that it is possible to obtain complexes of isolated GPIIb-IIIa and soluble fibrinogen and that images of these complexes have an identifiable structure. However, because the GPIIb-IIIa rosettes are the projection of complex three-dimensional structures, it is difficult to determine accurately the orientation of the bound fibrinogen with respect to the GPIIb-IIIa.

To obtain more detailed images of individual GPIIb-IIIa heterodimers associated with fibrinogen, we repeated the incubations in the presence of detergent. The results of these experiments are seen in Fig. 5, a–c. We found that, in more than two dozen experiments in the presence of 60 mM octylglucopyranoside, 5–11% of the GPIIb-IIIa in the incubation mixture was bound to fibrinogen, either at one or both ends of the fibrinogen molecule. As we observed in the absence of detergent, we found that GPIIb-IIIa interacted with fibrinogen exclusively via its globular head. Furthermore, the orientation of GPIIb-IIIa with respect to fibrinogen was specific, and in about 85% of images that showed association, it was the same (the remainder of the images will be discussed in detail below). In most images, the lateral aspect of the head of GPIIb-IIIa was in contact with an end nodule of fibrinogen and the tails of the GPIIb-IIIa extended laterally and away from the longitudinal axis of fibrinogen. Although the flexibility of both fibrinogen and the tails of GPIIb-IIIa resulted in some variety in the appearance of the complexes (Fig. 5, a–c), measurements based on the average orientation of the tails in each image indicate that the angle of GPIIb-IIIa with respect to the long axis of fibrinogen is 98° ± 14° (S.D.). Moreover, while the majority of the tails of GPIIb-IIIa bound to fibrinogen were parallel or splayed, ≈15% of the GPIIb-IIIa retained a signet ring configuration in comparison to ≈34% for unbound GPIIb-IIIa.

When GPIIb-IIIa heterodimers were bound to each end of a single fibrinogen, the GPIIb-IIIa retained their specific orientation with respect to fibrinogen, with their tails pointing in opposite directions from fibrinogen. Furthermore, ≈82% of the images displayed a “handedness” of binding such that if GPIIb-IIIa was bound to the left end of a fibrinogen, its tails pointed up, while tails of the GPIIb-IIIa molecules bound to the right end pointed down. This handedness was apparent since films containing shadowed structures were always viewed and photographed from the same direction in the electron microscope. This observation is consistent with the hypothesis that specific domains of each of these molecules mediate their interaction, and also suggests that the molecules maintain a preferential orientation with respect to the mica surface (24). While this orientation may arise from fibrinogen’s interactions with surfaces, in our experiments the orientation was observed only because of the GPIIb-IIIa binding.

Because a minority of the GPIIb-IIIa complexes in the incubation mixture were bound to fibrinogen, we attempted several maneuvers to enhance the interaction. First, GPIIb-IIIa was isolated from platelets previously incubated with 1 unit/ml thrombin, assuming that an active conformation of GPIIb-IIIa might be retained during the isolation procedure. Second, isolated GPIIb-IIIa was incubated with the peptide RGDS at a concentration of 1 mM in order to induce an active conformation in GPIIb-IIIa before fibrinogen was added, as previously reported by Du et al. (25). In neither case was a significant increase in the number of GPIIb-IIIa-fibrinogen complexes observed. We also tested the specificity of the interaction of GPIIb-IIIa with fibrinogen by performing incubations in the presence of either 4 mM EDTA or 1 mM RGDS, conditions known to prevent fibrinogen binding to activated platelets. Under both sets of conditions, no GPIIb-IIIa-fibrinogen complexes were observed, supporting the notion that the complexes we examined were specific.

In approximately 85% of the images of GPIIb-IIIa bound to fibrinogen, GPIIb-IIIa was associated with the distal ends of the fibrinogen molecule. These images were obtained under conditions in which the protein concentration was sufficiently dilute that particles were spread evenly on the surface of the

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**Fig. 5. Rotary-shadowed preparations of fibrinogen binding to GPIIb-IIIa.** Rows a–c, interactions in the presence of octyl glucoside. GPIIb-IIIa is recognized by an appearance similar to that seen in Fig. 2, while an example of a 47-nm long trinodular fibrinogen molecule with no GPIIb-IIIa bound is indicated by the arrow in row a. Row a, GPIIb-IIIa complexes are bound to each end of fibrinogen molecules. Note the orientation of the GPIIb-IIIa tails, yielding a roughly 2-fold symmetry of the ligand-receptor complex. Rows b and c, a single GPIIb-IIIa is bound to each fibrinogen. Images were chosen to illustrate the variety of appearances observed. Note the similarity of the orientation of the tails with respect to the long axis of fibrinogen in most images, in spite of the flexibility of both molecules. In most images, the GPIIb-IIIa tails are parallel or splayed; the tips are touching each other in less than 15% of images. 82% of images have the same handedness; at the far right in row c is an example of a complex that has the opposite orientation. Row d, fibrinogen binding to GPIIb-IIIa in the absence of detergent. The GPIIb-IIIa rosettes visualized are identical to those seen in Fig. 2, but each also contains a fibrinogen molecule, having the typical trinodular configuration, bound at the periphery of the rosette. Again, the images here illustrate the variety of appearances observed, but the overall orientation of fibrinogen with respect to GPIIb-IIIa is similar to that above, where the GPIIb-IIIa can be seen more clearly. Tracings of the shadowed molecules in rows a–c have been added to aid in the interpretation of these electron micrographs. Bar, 100 nm.
mica and the probability of molecules lying side-by-side by chance was low. In the remaining 15% of the images, possible GPIIb-IIIa binding involved a variety of other positions around the fibrinogen molecule. The interaction of GPIIb-IIIa with fibrinogen is thought to involve the carboxyl termini of the fibrinogen γ chains located at the distal ends of fibrinogen (16), and our images of GPIIb-IIIa associated with the distal ends of fibrinogen are consistent with this hypothesis. GPIIb-IIIa is also thought to interact with fibrinogen via one or both RGD sequences present in the Aα chain. One RGD sequence is located in the middle of a coiled-coil rod region in the amino-terminal portion of the chain and the other near the end of a flexible appendage that contains the Aα chain’s carboxyl terminus (16, 26). We did not observe statistically significant binding of GPIIb-IIIa at a location consistent with position of the amino-terminal RGD. The location of the carboxyl-terminal RGD is problematical since the domains that contain this sequence can interact with each other to form a globular region near or adjacent to the central domain of fibrinogen (16, 27, 28) or may project 20–23 nm from the ends of fibrinogen as free-swimming appendages (29). Therefore, it is possible that in those images in which GPIIb-IIIa was not bound to the ends of fibrinogen, binding could have involved the RGD site near the carboxyl end of the Aα chain. We searched for images of GPIIb-IIIa and fibrinogen in which GPIIb-IIIa, although not clearly bound to fibrinogen, was present within a radius of 23 nm of the distal fibrinogen nodule; however, we did not find GPIIb-IIIa within this area at a frequency that was statistically significant.

Electron Microscopy of Glycoprotein IIb-IIIa Bound to Fibronectin or von Willebrand Factor—Fibronectin and von Willebrand factor are also GPIIb-IIIa ligands, and we obtained images of GPIIb-IIIa bound to each by incubating the heterodimer with the purified proteins for 18 h at 4 °C in the presence of 2 mM Ca²⁺ prior to spraying and shadowing. Fibronectin and von Willebrand factor are both flexible molecules and the images of their association with GPIIb-IIIa were considerably more complex than those of GPIIb-IIIa and fibrinogen. By electron microscopy, fibronectin appears as a long thin molecule with several kinks. GPIIb-IIIa complexes (indicated here by an arrow), yielding a complex fibrous network. The ends of von Willebrand factor protomers extend from the cluster of GPIIb-IIIa complexes, indicating that the central part of the protomer is interacting with GPIIb-IIIa. Note that some GPIIb-IIIa complexes bound to fibronectin or von Willebrand factor appear to have their tails wrapped around their heads. Tracings of the shadowed molecules in rows a–c have been added to aid in the interpretation of these images. Bar, 100 nm.

The Shape of the Glycoprotein IIb-IIIa Complex and the Disposition of Its Polypeptide Chains—We found that GPIIb-IIIa heterodimers isolated and visualized in the presence of 2 mM Ca²⁺ consisted of 8 × 12-nm globular heads with two adjacent 18-nm flexible tails extending from one side of the head. This result is similar to that reported by Carrell and co-workers (17), as well as to the images of the fibronectin receptor reported by Nermut et al. (34) and a smooth muscle integrin reported by Kelly et al. (35). The complete amino acid sequences of GPIIb and GPIIIa have been determined, and the carboxyl-terminal segments of each contain a single
hydrophobic transmembrane domain, while their amino-terminal segments are extracellular and hydrophilic (18, 19). Thus, our images, including those obtained using monoclonal antibodies specific for GPIIb, GPIIIa, and the GPIIb-IIIa heterodimer and those obtained in the absence of detergent, indicate that the tails of the GPIIb-IIIa represent the carboxyl-terminal segments of each subunit that are embedded in the platelet membrane, while the nodular head is composed of the amino-terminal segments of each subunit and extends well above the cell surface. These conclusions are also supported by the structures we observed following dissociation of the GPIIb-IIIa heterodimer into its component subunits with EDTA. In the presence of EDTA, GPIIb-IIIa appeared as individual 24–27-nm comma-shaped structures, each containing a portion of the globular head and a single flexible tail. This result differs from that of Carrel et al. (17) who concluded that in the presence of EDTA, GPIIb is an ellipsoid structure and GPIIIa a filamentous rod. Although a minority of the structures we visualized in the presence of EDTA had these configurations, further analysis indicated that the ellipsoids were produced when a tail encircled a globular head and the filamentous rods are similar to comma-shaped structures in which the globular portion is not as prominent.

The topography of GPIIb and GPIIIa have been surmised from analyses of their primary structures (18, 19) and their disulfide bond arrangement (36, 37). The amino-terminal 95% of GPIb is extracellular (18). Because residues 558–747 are not antigenic and are resistant to proteolysis, it is possible that they are folded into a globular domain that is less accessible to solvent (36). Thus, GPIIb residues 1–747 likely constitute the nodular head of GPIb, with its remaining 261 residues comprising its tail. The number of residues assigned to the head of GPIIb is roughly consistent with the size of the head we observed, but the number of residues assigned to the tail would produce an extended polypeptide more than twice the observed length of the tail. This suggests that the tail also exists in a folded conformation. The amino-terminal 95% of GPIIIa is also extracellular and contains a cysteine-rich, protease-resistant amino-terminal domain, a putative ligand-binding domain, and a cysteine-rich and protease-resistant core (37). These three regions extend from residues 1–622 and constitute the globular head of GPIIIa, with the remaining 140 residues comprising its tail. Again, the number of residues comprising the head is consistent with its observed size, but the size of the tail is less than expected for an extended polypeptide chain.

The Interaction of Glicycoprotein IIb-IIIa with Fibrinogen, Fibronectin, and von Willebrand Factor—Although the GPIIb-IIIa present in the membranes of intact platelets requires platelet activation to interact with soluble proteins, we found, as have others (21–23), that approximately 5–10% of the GPIIb-IIIa isolated from platelets acquires this ability. While we cannot be absolutely certain that this interaction occurred in solution, rather than after one or the other protein was adherent to the mica surface, the spraying methodology we employed made it unlikely that significant binding occurred after the proteins were adherent. Thus, following incubation of GPIIb-IIIa with its ligands, the protein solution was vaporized onto a mica surface at a protein concentration sufficiently dilute (≈5–50 μg/ml) to result in a even distribution of single molecules on the mica surface and in a manner such that the small drops of vaporized solution formed a thin liquid film on the surface. Moreover, experiments in which fibrinogen was applied first to the mica and then overlayed with GPIIb-IIIa failed to produce satisfactory images. Similarly, we cannot be certain that the interaction of isolated GPIIb-IIIa with ligands is equivalent to that of GPIIb-IIIa on activated platelets. However, isolated GPIIb-IIIa does bind selectively to fibrinogen, and this binding can be inhibited by peptides and antibodies in a manner that is similar to the inhibition of fibrinogen binding to GPIIb-IIIa in platelets (21–23). Furthermore, we found that GPIIb-IIIa bound to fibrinogen assumed a specific configuration. Thus, from the viewpoint of structural biology, our results argue strongly that the binding we observed in vitro is physiologically relevant.

When two GPIIb-IIIa molecules were bound to one fibrinogen molecule, they were related by a 2-fold axis of symmetry through the center of fibrinogen, i.e., rotation of 180° about this axis moves one GPIIb-IIIa into the other one. This arrangement is consistent with the proposed approximate 2-fold molecular symmetry of the dimeric fibrinogen molecule (16, 38). Furthermore, this arrangement is such that when fibrinogen is bound to one GPIIb-IIIa complex on the platelet surface, the binding site for GPIIb-IIIa on the opposite end of the fibrinogen is oriented away from the platelet surface and toward a GPIIb-IIIa complex on another platelet. This configuration also suggests that the receptor-ligand complexes have a preferred orientation with respect to the surface on which they lie. The orientation of fibrinogen with respect to GPIIb-IIIa is also consistent with previous estimates of the distance between the surfaces of aggregated platelets. Electron microscope studies of platelet aggregates report the distance between the surfaces of opposed platelets as ≈25 (39) to 50 nm (40). Measurements from our studies and studies by Parise and Phillips (41) suggest that GPIIb-IIIa extends ≈18–20 nm above the platelet surface. Thus, if fibrinogen, with a molecular length of ≈47 nm, binds to GPIIb-IIIa at an average angle of 98°, this measured distance between the surface of adjacent platelets would be ≈43–47 nm, a value nearer the measured distance than a distance of ≈83–87 nm if the angle between fibrinogen and GPIIb-IIIa were 180°.

Previous studies have suggested that fibrinogen binding to GPIIb-IIIa involves the carboxyl-terminal amino acids of the fibrinogen γ chain and either of two regions of the fibrinogen Aα chain that contain an RGD sequence (3, 42). The carboxyl terminus of the γ chain is located in the distal nodular domain of fibrinogen (16), while the Aα chain RGD sequences occur at residues 55–79 located between the central and end fibrinogen nodular domains in a coiled-coil region and at residues 572–574 located near the carboxyl terminus of the Aα chain in a flexible protuberance whose position in soluble fibrinogen is uncertain (29). Biochemical data indicate that the interaction of peptides corresponding to these regions of fibrinogen with GPIIb-IIIa is mutually exclusive (12), and it has been difficult to incorporate these topographically distant regions of fibrinogen into a realistic model depicting the interaction of fibrinogen with GPIIb-IIIa. Moreover, there is evidence that fibrinogen lacking the flexible protuberance of the Aα chain retains the ability to interact with GPIIb-IIIa (43). In most of the images of GPIIb-IIIa and fibrinogen we observed, the interaction of these proteins involved the distal ends of fibrinogen. No association between GPIIb-IIIa and fibrinogen was observed at a position consistent with the location of RGD 95–97. Although the position in three dimensions of the portion of the fibrinogen a chain containing RGD 572–574 is uncertain, there is evidence that, under certain conditions, it can be either a free-swimming appendage extending from each end of fibrinogen or folded back upon the central domain (16, 26–28). Because interactions of GPIIb-IIIa with such a flexible domain could produce images with a variety of appearances, it is possible that these complexes are present, but a maximum of 15% of ligand-receptors would be of this form. Thus, our studies indicate that the stable interactions of GPIIb-IIIa with fibrinogen involve predominantly the distal end domain of fibrinogen and, by inference, the carboxyl...
Electron Microscopy of GPIb-IIIa

Fig. 7. Schematic summary of the interaction of GPIb-IIIa with fibrinogen. A computer graphic representation of a fibrinogen molecule with a GPIb-IIIa complex bound to each end is shown. The tails of GPIb-IIIa extend laterally with respect to the long axis of fibrinogen. In normal platelets, the tails of the GPIb-IIIa complex at each end of fibrinogen would be embedded in the membranes (represented here as a patch of lipid bilayer) of different platelets. The carboxyl termini of the two γ chains (γC) of fibrinogen are located at the ends of the molecule. The amino-terminal αa RGD sequences in fibrinogen are located in the middle of coiled-coil regions represented here as rods. The RGD sequences in the carboxyl termini of the fibrinogen αa chains (αC) are present in αa chain extensions that are located in a region adjacent to the central domain of the molecule.

residues 1744–1746 near its carboxyl terminus that is believed to be its site of interaction with GPIb-IIIa (44). As expected from the multimeric nature of von Willebrand factor, we found multiple GPIb-IIIa complexes associated with von Willebrand factor multimers. Each GPIb-IIIa bound near the center of a von Willebrand factor promotor, the location predicted for GPIb-IIIa binding from analysis of the von Willebrand factor primary sequence.

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