Direct Binding of the Platelet Integrin \(\alpha_{\text{IIb}}\beta_3\) (GPIIb-IIIa) to Talin

EVIDENCE THAT INTERACTION IS MEDIATED THROUGH THE CYTOSOLIC DOMAINS OF BOTH \(\alpha_{\text{IIb}}\) AND \(\beta_3\)*

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As a consequence of platelet activation and fibrinogen binding, glycoprotein (GP)IIb-IIIa (integrin \(\alpha_{\text{IIb}}\beta_3\)) becomes associated with the cytoskeleton. Although talin has been suggested to act as a linkage protein mediating the attachment of GPIIb-IIIa to actin filaments, direct binding of GPIIb-IIIa to this cytoskeletal protein has not been demonstrated. In the present study, we examined the interaction of GPIIb-IIIa with purified talin using a solid-phase binding assay. Soluble GPIIb-IIIa bound in a time- and dose-dependent manner to microtiter wells coated with talin but not with BSA. Time course studies demonstrated that steady-state binding was achieved after 4-5 h incubation at 37°C. Binding isotherms with varying concentrations of GPIIb-IIIa showed that half-saturation binding was achieved at approximately 15 nm GPIIb-IIIa. At saturation, there was 211 ± 8 fmol of GPIIb-IIIa bound per well containing 117 ± 10 fmol of immobilized talin. Besides binding to immobilized talin, GPIIb-IIIa also bound to talin captured by the anti-talin monoclonal antibody 8d4. Moreover, the interaction of GPIIb-IIIa to 8d4-captured talin was blocked by mAb1082, a monoclonal antibody raised against a synthetic peptide encompassing the entire cytoplasmic sequence of GPIIb. The interaction of talin with the cytosolic domain of GPIIb-IIIa was further investigated using peptide-coated wells. Purified talin was found to bind to both synthetic peptides corresponding to the cytosolic sequences of GPIIb (P2b) and GPIIa (P3a). As expected, the binding of talin to P2b-coated wells was specifically blocked by mAb1082. Thus, these results demonstrate direct binding of GPIIb-IIIa to talin and suggest a role of the cytosolic sequences of both GPIIb and GPIIa in mediating this interaction.

Integrins are a widely distributed family of heterodimeric transmembrane proteins that mediate cell adhesion by binding to extracellular adhesive proteins (1). In addition, integrins are thought to mediate the attachment of actin filaments to the cell membrane presumably by binding to intracellular cytoskeletal proteins (2). The most prominent platelet membrane glycoprotein GP IIb-IIIa\(^1\) (integrin \(\alpha_{\text{IIb}}\beta_3\)) plays a central role in hemostasis and thrombosis by acting as a receptor for several adhesive proteins including fibrinogen, fibronectin, von Willebrand factor, and vitronectin (3, 4). Furthermore, it has been demonstrated that following the binding of adhesive proteins to GP IIb-IIIa on activated platelets, this receptor complex becomes associated with the platelet cytoskeleton (5-9). This process has been implicated in several post-occupancy events including clot retraction (10), protein tyrosine phosphorylation (11, 12), as well as receptor clustering (13) and redistribution (14, 15). Although extracellular interactions between adhesive proteins and GPIIb-IIIa have been well characterized (16), the mechanisms mediating interactions between the cytosolic domains of GP IIb-IIIa and cytoskeletal proteins have not been clearly defined. Nevertheless, in other cell types such as endothelial cells and fibroblasts, several cytoskeletal proteins including talin, vinculin, and \(\alpha\)-actinin have been suggested to provide linkages between integrins and actin filaments (2). Morphological studies have demonstrated that these cytoskeletal proteins are concentrated in focal adhesions where integrins (e.g. \(\alpha_{\beta_3}\)) cluster and interact with proteins in both the extracellular matrix and cytoskeleton (2).

Several biochemical studies have suggested an interaction between talin and the cytosolic domains of the \(\beta\) subunits of integrins (17, 18). Furthermore, interactions between talin and vinculin (19–21), as well as between vinculin and \(\alpha\)-actinin (22), have also been reported. Since \(\alpha\)-actinin is an actin cross-linking protein, this leads to the speculation that actin filaments are anchored to membrane bound integrins via a chain of protein-protein interactions involving talin, vinculin, and \(\alpha\)-actinin. However, recent studies have suggested alternative mechanisms of cytoskeletal attachment of integrins in which talin (23) or \(\alpha\)-actinin (24, 25) directly links integrins to actin filaments. In addition, the recent findings that vinculin contains a cryptic actin-binding site raise the possibility that talin and vinculin mediate the binding of integrins to actin filaments in a manner independent of \(\alpha\)-actinin (26).

Regarding the attachment of GP IIb-IIIa to the platelet cytoskeleton, it has been shown that in resting platelets, GP IIb-IIIa associates with a membrane skeleton containing talin and vinculin. As a result of platelet aggregation, there is a concomitant redistribution of GP IIb-IIIa and cytoskeletal proteins to the cross-linked actin filaments in the cytoplasmic cytoskeleton (9). In a preliminary report utilizing ligand blotting techniques, it has been suggested that talin interacts with the cytoplasmic domain of GP IIa (27). More recently, specific binding of \(\alpha\)-actinin to purified GP IIb-IIIa adsorbed onto microtiter wells or incorporated into phospholipid vesicles has been reported (24).

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\(1\) The abbreviations used are: GP, glycoprotein; PAGE, polyacrylamide gel electrophoresis; BSA, bovine serum albumin; mAb, monoclonal antibody.
Using a solid-phase binding assay, we further examine the interaction of GPIIb-IIIa with talin, as well as the role of the cytoplasmic domain of GPIIb-IIIa in mediating interaction with this cytoskeletal protein.

MATERIALS AND METHODS

Peptides and Antibodies—Peptide sequences were represented by the single-letter amino acid code (28). A 41-amino acid peptide (P3a, HDERKFKEEERAKWDTANNPLYEASTFTSTNTYRGT) corresponding to residues 722-762 of GPIIa and its scrambled variant (sc-P3a, FEEETIKTPFDHPHRLAARERTNISKGYTKTKEFLTAAPOA), as well as a scrambled peptide of the cytoplasmic sequence of GPIIb (sc-P2b, DKFRGPVPKEEELDREG), were generous gifts of Dr. Leslie V. Parise of the University of North Carolina, Chapel Hill. The following peptides were synthesized by solid-phase synthesis using an ABI model 431 peptide synthesizer or were purchased from the Peninsula Laboratories, Inc. A 21-amino acid peptide (CKVGFFKRNRPPLEEDDEEGE) corresponding to the GPIIb cytoplasmic sequence was also synthesized. The peptide was desalted by reverse phase liquid chromatography on a C18 preparative column, eluted with a gradient of 0.1% acetic acid and 0.1% TFA to 0.1% acetic acid and 60% TFA, and lyophilized. The purity of the peptide was verified by reversed-phase HPLC and MALDI-TOF mass spectrometric analysis. The peptide was then sent to the University Microscopy Facility of the University of Alabama at Birmingham for conjugation to keyhole limpet hemocyanin using the Waters Pico Tag kit (Waters, Milford, MA). The Mass Spectrum of the conjugated product confirmed the retention of the peptide sequence. The conjugate was then stored at -20°C.

Production of Anti-peptide Monoclonal Antibodies Directed Against the GPIIb Cytoplasmic Sequence—P2b was coupled to keyhole limpet hemocyanin using m-maleimidobenzoic acid N-hydroxysuccinimide ester (35) and used as immunogen for BALB/c mice. Immunization and elicitation of antibody responses (36) were performed as described previously (37, 38). Washed outdated platelets were solubilized in lysis buffer containing 10 mM HEPES, pH 7.5, 0.15 mM NaCl, 1 mM CaCl2, 1 mM MgCl2, 0.1 mM leupeptin, 10 mM N-ethylmaleimide, 1 mM phenylmethanesulfonyl fluoride, and 50 mM octyl glucoside. The platelet lysate was applied to GRGDSK-coupled Sepharose 4B and incubated at 4°C overnight. After washing unbound proteins with column buffer (same as the lysis buffer except 0.1% octyl glucoside), GPIIb-IIIa was eluted with 1.7 mM H2, (Fig. 2A, lane 1). Since we and other investigators (37, 38) found that a subpopulation of affinity purified GPIIb-IIIa existed as a high molecular weight complex with actin filaments, we employed gel filtration to purify further GPIIb-IIIa for binding studies. Thus, the H2 eluate was applied to a Sephacryl S-300 High Resolution (HR) column (1.5 × 95 cm) and eluted with 10 mM HEPES, pH 7.5, 0.15 mM NaCl, 1 mM CaCl2, 1 mM MgCl2, and 25 mM octyl glucoside as shown in Fig. 2A, lane 2, a small fraction (<10%) of GPIIb-IIIa was eluted with actin in the void volume, and further elution yielded monomeric GPIIb-IIIa (lane 3) with a Stokes radius of 61 Å as previously reported (37, 39). To determine the concentration of purified GPIIb-IIIa, proteins were resolved by SDS-PAGE, and the Coomassie Blue-stained bands were quantitated by densitometric scanning using the Discovery Series densitometer and Quantity One software package (pdi Inc., Huntington Station, NY) with bovine serum albumin (BSA) as standards. Purified GPIIb-IIIa was stored at 4°C and used within a week of isolation.

Talin was purified from Triton X-100 extracts of outdated human platelets by RGD affinity chromatography followed by molecular sieving on Sephacryl S-300 HR gel. Proteins were analyzed by SDS-PAGE and silver staining. Lane 1, H2 eluate of RGD affinity column; lane 2, void volume fraction of Sephacryl S-300 HR column; lane 3, purified GPIIb-IIIa eluted from the Sephacryl S-300 column. B, talin was purified from Triton X-100 extracts of outdated human platelets, subjected to SDS-PAGE, and detected by Coomassie Blue staining (lane 1) or by immunoblotting with the anti-talin monoclonal antibody B4 (lane 2). 

FIG. 1. Western blot analysis of mAb10B2. Proteins in whole platelet lysates (lanes 1, 2, 4, and 5) or RGD affinity purified GPIIb-IIIa (lane 3) were separated on 7% acrylamide gels under nonreducing conditions or 12% acrylamide gels under reducing conditions. After transferring onto nitrocellulose filters, proteins were immunoblotted with the following: lane 1, PMI-1 and PMI-2 showing the migration of intact GPIIb and GPIIIa, respectively; lane 4, anti-V41 showing the migration of the GPIIb light chain; lanes 2, 3, and 5, mAb10B2.

FIG. 2. Purification of GPIIb-IIIa and talin. A, GPIIb-IIIa was purified from octyl glucoside extracts of outdated human platelets by RGD affinity chromatography followed by molecular sieving on Sephacryl S-300 HR gel. Proteins were analyzed by SDS-PAGE and silver staining. Lane 1, H2 eluate of RGD affinity column; lane 2, void volume fraction of Sephacryl S-300 HR column; lane 3, purified GPIIb-IIIa eluted from the Sephacryl S-300 column. B, talin was purified from Triton X-100 extracts of outdated human platelets, subjected to SDS-PAGE, and detected by Coomassie Blue staining (lane 1) or by immunoblotting with the anti-talin monoclonal antibody B4 (lane 2).
by incubation with $^{125}$I-labeled species-specific secondary antibodies followed by autoradiography.

Solid-phase Binding Assay of GPIIb-IIIa to Talin—Microtiter wells (Immulon 2 removewell strips) were coated with talin (45 μg/ml) for 48 h at 4°C and then blocked with 3% BSA overnight at 4°C. In some studies, talin was captured by immobilized 8d4 by incubating talin (150 μg/ml) for 2 h at 37°C with 8d4-coated wells. After two washes with a buffer containing 10 mM HEPES, 0.15 mM NaCl, 1 mM CaCl$_2$, 1 mM MgCl$_2$, 25 mM octyl glucoside, pH 7.4, followed by two additional washes with the same buffer without octyl glucoside, purified GPIIb-IIIa was added and incubation proceeded at 37°C for 4–5 h. Unbound GPIIb-IIIa was then removed, and the washing procedure described above was repeated. Bound receptor was detected by incubation with $^{125}$I-labeled mAb15 (50 nM) for 1 h at 22°C. The wells were then washed extensively with Tris-buffered saline (TBS, 10 mM Tris, 0.15 mM NaCl, pH 7.4) containing 0.05% Tween 20, and bound radioactivity was detected by $\gamma$-counting. Background absorption of $^{125}$I-labeled mAb15 to talin-coated wells, which had not been incubated with GPIIb-IIIa, was measured in parallel and was subtracted from the total amount of bound labeled antibody. To quantitate the amounts of adsorbed talin, the binding of $^{125}$I-labeled 8d4 (67 nM) to control wells was determined. In inhibition studies, GPIIb-IIIa was preincubated with the indicated antibodies for 1 h at 37°C before the receptor was added to the wells.

RESULTS

Characterization of the Binding of GPIIb-IIIa to Talin—As an initial approach to examine the interaction of GPIIb-IIIa with talin, we purified both proteins (see Fig. 2 in "Materials and Methods") and developed a solid-phase binding assay in which soluble GPIIb-IIIa was allowed to bind to talin immobilized onto microtiter wells. In order to minimize modification of GPIIb-IIIa which might affect binding, we detected unlabeled bound receptor with a saturating concentration of $^{125}$I-labeled mAb15. Assuming that mAb15 binds to GPIIb-IIIa with a 1:1 stoichiometry, this method allows us to quantitate directly the amounts of bound receptor. As shown in Fig. 3, preincubation of talin-coated wells with GPIIb-IIIa resulted in an increased binding of $^{125}$I-labeled mAb15 as compared with control wells incubated with the vehicle buffer of GPIIb-IIIa. In contrast, no detectable difference was observed with BSA-coated wells incubated with or without GPIIb-IIIa. These results suggest that GPIIb-IIIa bound specifically to talin immobilized onto microtiter wells. In subsequent experiments, we routinely measured the amounts of nonspecific absorption of mAb15 to talin- or BSA-coated wells, and these values were subtracted from the total amounts of bound antibody. Using this assay system, we then examined the time course of GPIIb-IIIa binding to immobilized talin (Fig. 4). At an input GPIIb-IIIa concentration of 10 nM, the binding of GPIIb-IIIa to immobilized talin was time-dependent and approached steady-state after 4–5 h incubation at 37°C. Again, no significant binding of GPIIb-IIIa to control BSA-coated wells was observed over this period. To characterize further the interaction of GPIIb-IIIa and talin, we performed binding isotherms with varying concentrations of GPIIb-IIIa. Fig. 5 shows that the dose-dependent binding of GPIIb-IIIa to talin was saturable, and half-saturation occurred at an input GPIIb-IIIa concentration of approximately 15 nM. Furthermore, as detected by $^{125}$I-labeled mAb15 binding, there was $211 \pm 8$ fmol of GPIIb-IIIa bound per well at saturating concentrations of GPIIb-IIIa.

It is generally agreed that coating of proteins onto plastic microtiter wells would induce conformational changes of the adsorbed proteins. In this regard, cryptic GPIIb-IIIa binding sites might have been exposed on immobilized talin. Therefore, we examined whether GPIIb-IIIa also bound to talin captured by immobilized 8d4, an anti-talin monoclonal antibody. To prevent the reduction of immobilized antibodies in these experiments, β-mercaptoethanol was removed from purified talin by dialysis prior to its addition to antibody-coated wells. After incubation at 37°C for 2 h, unbound talin was removed by washing, and the binding of GPIIb-IIIa to captured talin proceeded as described above. As shown in Fig. 6, without the preincubation of talin with 8d4-coated wells, minimal binding of GPIIb-IIIa was detected. However, the binding of talin to immobilized 8d4 caused a dramatic increase in GPIIb-IIIa binding. Parallel binding was also performed with control wells coated with purified normal mouse IgG, or with hVIN-1, an isotype-matched monoclonal antibody against vinculin. As expected, preincubation of talin to these wells coated with irrelevant antibodies did not cause any increase in GPIIb-IIIa binding. In three separate experiments, we consistently observed an increased binding of GPIIb-IIIa to talin captured by immo-

![Fig. 3. Binding of GPIIb-IIIa to immobilized talin or BSA.](image)

![Fig. 4. Time course of GPIIb-IIIa binding to talin.](image)

![Fig. 5. A binding isotherm of GPIIb-IIIa to talin.](image)

![Fig. 6. Binding of GPIIb-IIIa to immobilized 8d4.](image)
Talin binding—

Role of the Cytoplasmic Domain of GPIIb-IIIa in Mediating Talin Binding—In an attempt to examine whether the observed binding of GPIIb-IIIa to talin was mediated by the cytoplasmic domain of the receptor, we tested the effect of our newly developed anti-peptide monoclonal antibody (mAb10B2) directed against the cytoplasmic sequence of GPIIb (see Fig. 1 in “Materials and Methods”). In these experiments, purified mAb10B2 or normal mouse IgG was incubated with GPIIb-IIIa at 37 °C for 30 min before the receptor was allowed to bind to antibody-captured talin. Binding of GPIIb-IIIa to antibody-captured talin proceeded at 37 °C for 4 h, and bound receptor was detected with 125I-mAb15 (50 nM).

Purified GPIIb-IIIa was incubated with 1 μM of the indicated peptide at 4 °C for 48 h. After blocking with BSA, talin (150 μg/ml) was added, and binding proceeded at 37 °C for 4 h. Bound talin was detected with 125I-labeled 8d4 (67 nM). A, wells were coated with vehicle buffer (1), P3a (2), or scr-P3a (3). B, wells were coated with scr-P2b (1) or P2b (2–4) were incubated at 37 °C for 1 h with phosphate-buffered saline (1 and 2). 1 μM mAb10B2 (3), or 1 μM normal mouse IgG (4) prior to the addition of talin. As expected, mAb10B2 blocked talin binding to P2b by 95%, whereas normal mouse IgG was completely ineffective.

**DISCUSSION**

Based on the co-localization of talin and integrins in focal adhesions, it is generally believed that talin plays a crucial role in the attachment of integrins to cytoskeleton (2). However, to date, the only biochemical evidence for a direct integrin-talin interaction was the reported elution profile shift toward a higher molecular weight complex during equilibrium gel filtration of the avian integrin complex (CSAT antigen) in the presence of talin (17, 18). Using highly purified GPIIb-IIIa and talin in the present study, we have developed a solid-phase binding assay and demonstrated direct interaction between GPIIb-IIIa and talin. In this assay, we observed that GPIIb-IIIa bound to immobilized talin as well as to talin captured by the anti-talin monoclonal antibody 8d4. Furthermore, the observed GPIIb-IIIa/talin interaction was mediated by the cytoplasmic domain of the receptor since it was inhibited by the anti-GPIIb cytoplasmic domain monoclonal antibody mAb10B2. Finally, the findings that talin bound directly to synthetic peptides corresponding to the cytoplasmic sequences of GPIIb (P2b) and GPIIa (P3a) in this assay, purified talin was incubated with microtiter wells coated with these peptides, and bound talin was detected with 125I-labeled 8d4. As controls, we also examined talin binding to wells coated with scrambled variants of these peptides (scr-P2b and scr-P3a) with the same amino acid compositions but arranged in random sequences. As shown in Fig. 7A, talin bound more effectively to wells coated with P3a (bar 2) as compared to control wells coated without peptide (bar 1) or with scr-P3a (bar 3). In two separate experiments, we observed that the binding of talin to the native P3a sequence was 50–60% more effective than to its scrambled sequence. Likewise, we observed that talin also bound to wells coated with P2b (Fig. 7B, bar 2). Again, much less binding was detected to control wells coated with the scr-P2b peptide (bar 1). To substantiate further the specificity of talin binding to P2b, we examined the inhibitory effect of mAb10B2. Thus, P2b-coated wells were preincubated with mAb10B2 (Fig. 7B, bar 3) or normal mouse IgG (bar 4) at 37 °C for 1 h prior to the addition of talin. As expected, mAb10B2 blocked talin binding to P2b by 95%, whereas normal mouse IgG was completely ineffective.

**Antibody inhibition of GPIIb-IIIa binding to talin**

Talin was allowed to bind to 8d4-coated wells as described in the legend of Fig. 6. Purified GPIIb-IIIa was incubated with 1 μM of the indicated antibody for 1 h at 37 °C prior to addition to wells with captured talin. Binding proceeded as described, and percent inhibition of binding was calculated relative to controls without antibody.

**Table I**

| Antibody inhibition of GPIIb-IIIa binding to talin |
|-----------------------------------------------|
| fmol %                                   |
|------------------|------------------|
| **Control**      | 12.9 ± 0.9       |
| mAb10B2          | 6.7 ± 0.7        |
| Normal mouse IgG | 12.0 ± 0.7       |
a membrane skeleton fraction containing talin, vinculin, spectrin, and other signaling molecules. Following thrombin-induced platelet aggregation, GPIIb-IIIa and talin, among other proteins in the membrane skeleton, become associated with the actin filaments in the cytoplasmic cytoskeleton (9). Our findings that talin bound directly to the cytoplasmic domain of GPIIb-IIIa provide evidence that it acts as a linkage protein between GPIIb-IIIa and short actin filaments in the membrane skeleton. Furthermore, it is tempting to speculate that thrombin-induced redistribution of talin in platelets would facilitate the interaction of this cytoskeletal protein with GPIIb-IIIa. Nonetheless, it should be noted that our results do not exclude the involvement of other cytoskeletal proteins in the membrane skeleton fraction, and further studies are required to define their role in mediating GPIIb-IIIa attachment to the cytoskeleton.

To demonstrate GPIIb-IIIa/talin interaction, we initially measured the binding of soluble GPIIb-IIIa to immobilized talin. However, coating of talin onto microtiter wells might change the protein conformation and expose artifactual binding sites for GPIIb-IIIa. To eliminate this possibility, we examined the ability of GPIIb-IIIa to bind to talin captured by the anti-talin monoclonal antibody 8d4. The findings that GPIIb-IIIa interacts with 8d4-captured talin indicate that the GPIIb-IIIa interaction with talin is not affected by the use of immobilized talin. Furthermore, these results also rule out the possibility that GPIIb-IIIa also binds to 8d4 antibodies to the C-terminal region of the GPIIb-IIIa integrin cytoplasmic domain (25), thereby exhibiting a lower affinity interaction with talin. Alternatively, the scr-P3a peptide used in the present study may retain some sequence similarity to the native sequence, thereby exhibiting a lower affinity interaction with talin. Nonetheless, our results confirm earlier observations that a talin binding site(s) is present within the cytoplasmic domains of integrin β subunits (18, 44) and further substantiate functional studies in which truncation and certain point mutations of the GPIIa cytoplasmic sequence were shown to abolish cell spreading as well as the recruitment of GPIIb-IIIa and talin to focal adhesions (45, 46).

Besides interacting with the GPIIa cytoplasmic sequence, we observed that talin also binds to immobilized P2b peptide corresponding to the entire GPIIb cytoplasmic sequence. The specificity of this interaction was demonstrated by the failure of a scrambled version of P2b to support talin binding and by specific inhibition of talin binding to P2b caused by the anti-GPIIb cytoplasmic domain monoclonal antibody mAb10b2. Therefore, these results indicate that a talin-binding site is also present within the β subunit of this integrin. In support of this finding, Pavalko and Otey (47) noted in a review article that talin binds to synthetic peptides corresponding to the cytoplasmic tails of α4 and α5 but not α3. However, it is noteworthy that besides the highly conserved GFFKR sequence near the membrane, there is no apparent homology among the cytoplasmic domains of these integrin α subunits. Thus, it is not clear whether the diverse cytoplasmic sequences of α1, α5, and α6 interact with the same or different regions on talin. Recently, it has been suggested that the cytoplasmic sequences of GPIIb and GPIIIa associate with each other to form a defined tertiary structure (48). Since talin interacts with the cytoplasmic domains of both α and β subunits of certain integrins, it is conceivable that interaction of the two cytoskeletal tails in the native receptor would constitute a high affinity binding site for talin.

Platelet stimulation activates GPIIb-IIIa resulting in high affinity binding of macromolecular ligands such as fibrinogen (49, 50). Moreover, it has been shown that small ligand-mimetic peptides are also capable of activating GPIIb-IIIa (51). In this regard, RGD affinity purified GPIIb-IIIa was found to exist in the active conformer with high affinity fibrinogen-binding function (51, 52). It is interesting to determine whether the observed talin binding function of GPIIb-IIIa is confined to the active population of receptor isolated by this procedure. Therefore, whether conformational changes in GPIIb-IIIa also modulate its binding affinity to talin remains to be established. Similarly, thrombin activation of platelets leads to an increased phosphorylation of talin (53), and it would be interesting to determine whether phosphorylation would affect the affinity of talin for interaction with GPIIb-IIIa.

In summary, we have developed a rapid and sensitive solid-phase binding assay to detect direct interaction between GPIIb-IIIa and talin. Moreover, we demonstrated that purified talin binds to the cytoplasmic sequences of both α and β subunits of this integrin. This assay system would be useful for further studies to examine the interaction between talin and other integrins, as well as for the identification of specific determinants within the cytoplasmic domains of integrins mediating interaction with this cytoskeletal protein.

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Interaction of GPIIb-IIIa with Talin

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