The glycoprotein IIb-IIIa complex (GP IIb-IIIa) is a platelet cell-surface receptor for fibrinogen and fibronectin. A carboxyl-terminal decapeptide of the fibrinogen γ-chain (Leu-Gly-Glu-Ala-Lys-Gln-Ala-Gly-Asp-Val (LGGAKQAGDV)) and a tetrapeptide (Arg-Gly-Asp-Ser (RGDS)) from the fibrinogen α-chain and the fibronectin cell-binding domain appear to mediate the binding of these ligands to GP IIb-IIIa. The present study was designed to examine the effects of these and related peptides on the structure of purified platelet GP IIb-IIIa. Treatment of GP IIb-IIIa with various synthetic peptides affected the glycoprotein so that GP IIb became a substrate for hydrolysis by thrombin. The order of potency of these peptides was as follows: RGDS > LGGAKQAGDV > KGDS > RGES. This is the same order of potency in which these peptides inhibit fibrinogen binding to platelets. This effect was time-, temperature-, and concentration-dependent; RGDS induced a half-maximal effect at ~60 μM. In addition, RGDS, but not RGES, decreased the intensity of the intrinsic protein fluorescence of GP IIb-IIIa. Finally, the decapeptide or RGDS decreased the sedimentation coefficient of GP IIb-IIIa from 8.5 to 7.7 or 7.4 S, respectively, whereas RGES had a minimal effect. This decrease was accompanied by an increase in the Stoke’s radius from 74 to 82 Å with RGDS or 85 Å with the decapeptide, indicating a peptide-induced unfolding of the GP IIb-IIIa complex. This change in conformation may be related to changes in the distribution and function of GP IIb-IIIa on the platelet surface that occur when adhesive proteins or peptides from the GP IIb-IIIa binding domains of these proteins bind to GP IIb-IIIa.

Fibrinogen binds specifically to the glycoprotein IIb-IIIa complex (GP IIb-IIIa) on activated platelets (1, 2) and, by its bivalent nature, is believed to mediate platelet aggregation. The proposed sites on fibrinogen that adhere to GP IIb-IIIa are a decapeptide (Leu-Gly-Glu-Ala-Lys-Gln-Ala-Gly-Asp-Val (LGGAKQAGDV)) at the carboxy terminal of the γ-chain (3, 4), a tetrapeptide (Arg-Gly-Asp-Ser (RGDS)) toward the carboxyl terminal of the α-chain, and/or a tripeptide (Arg-Gly-Asp (RGD)) toward the amino terminus of the α-chain (5). The decapeptide and RGDS inhibit fibrinogen binding to activated platelets (3, 4, 6) or to purified GP IIb-IIIa (2). Affinity columns composed of these peptides also bind GP IIb-IIIa in a specific manner (7). These findings indicate that the fibrinogen and peptide binding sites are contained within the GP IIb-IIIa structure. In addition, either peptide inhibits the binding of other RGDS-containing ligands (e.g., fibrinectin and von Willebrand factor) to platelets (4, 8–10) or to purified GP IIb-IIIa (11). The reason that the decapeptide inhibits the binding of fibrinectin or von Willebrand factor (which do not contain the decapeptide sequence) is not understood, although it has been proposed that the binding sites for these two peptides overlap (4, 7).

Several recent reports have suggested that occupancy of specific sites on GP IIb-IIIa affects its distribution and function on the platelet surface. Isenberg et al. (12) found that binding of fibrinogen, RGDS, or the γ-chain decapeptide induces clustering of platelet-associated GP IIb-IIIa. Banga et al. (13) reported that the apparent occupancy of GP IIb-IIIa by fibrinogen is necessary to maintain Na+/H+ exchange in epinephrine-stimulated platelets. Finally, GP IIb-IIIa associates with the platelet cytoskeleton under conditions where fibrinogen is secreted from and bound to the platelet (14). Thus, it is possible that occupancy of specific sites on GP IIb-IIIa changes its structure or conformation, thereby signaling these events. However, the effect of ligand binding on the conformation of GP IIb-IIIa is completely unknown. In the present study, therefore, we examined the effect that synthetic peptides derived from fibrinogen or fibronectin have on the conformation of purified GP IIb-IIIa. Our results demonstrate that RGDS and the decapeptide from the fibrinogen γ-chain induce a profound and specific change in the conformation of purified GP IIb-IIIa. This conformational change may be related to fibrinogen- or peptide-induced changes in the distribution and function of GP IIb-IIIa observed in whole platelets.

**MATERIALS AND METHODS**

**Purification of Glycoprotein IIb-IIIa**—The GP IIb-IIIa heterodimer complex was purified from outdated human platelets by the method

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of Fitzgerald et al. (15), except that a 5-ml gelatin-Sepharose (Sigma) affinity chromatography step was included to remove contaminating fibronectin. The purified GP IIb-IIIa in buffer A (20 mM Tris, 0.1% Triton X-100, 0.15 M NaCl, 1 mM CaCl₂, 0.05% NaN₃, pH 7.4) was stored at -80° C until use.

Peptide incubations. Triton X-100 was removed from GP IIb-IIIa and replaced with the detergent C₈E₇ (dodecyl)octaethylene glycol monooether, Nikko Chemical Co., Tokyo, Japan), which does not absorb significant amounts of light at 285 nm. The detergent exchange was accomplished by adsorbing GP IIb-IIIa to an O-dithiylaminoethyl (DEAE) column (-0.5-ml packed volume) in buffer A containing 0.05 M NaCl; washing the column with ~30 ml of the same buffer but with 0.5% C₈E₇ in place of Triton X-100 (buffer B), and eluting GP IIb-IIIa with buffer B containing 0.5 M NaCl. The eluted GP IIb-IIIa was pooled and dialyzed into buffer B containing 0.15 M NaCl.

Incubation Conditions—Purified GP IIb-IIIa in polypropylene Eppendorf tubes was incubated in buffer A with the concentrations of glycoprotein, peptides, or EDTA indicated in the figure legends. Incubations were performed at 37° C and pH 7.4 for various times. For experiments involving thrombin hydrolysis, incubations were continued after an additional hour at 37° C after thrombin (1.7 units/ml) or buffer had been added. Previous studies indicated that this concentration of thrombin maximally hydrolyzed GP IIb, within 1 h (16). Addition of thrombin diluted the peptide or EDTA concentration by no more than 20%. Peptide and EDTA concentrations given represent those before thrombin addition. (The thrombin was kindly provided by John W. Fenton II, New York State Department of Health, Albany, NY).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis and Densitometry—Samples for analysis were dissolved in a reducing buffer such that the final concentrations of the components were 2% sodium dodecyl sulfate (SDS) (w/v), 5% β-mercaptoethanol (v/v), 10% glycerol (v/v), 0.002% Bromphenol Blue (w/v), and 62.5 mM sodium dodecyl sulfate (SDS) (w/v), 5% 8-mercaptoethanol (v/v), and 62.5 mM Tris-HCl, pH 6.8, and were incubated at 100 °C for 5 min before electrophoresis. Samples were electrophoresed on 7.5% SDS-polyacrylamide gels (17), which were subsequently stained with Coomassie Brilliant Blue R-250. The amount of protein in the bands of interest was measured by densitometry (Hoefer GS 300 Scanning Densitometer interfaced with an Apple IIe computer).

Sedimentation Coefficients—Sedimentation coefficients (s₂₀[w]) were determined by the method of Martin and Ames (18). Samples (290 μl of 0.7-1.0 mg/ml of GP IIb-IIIa) were cooled to 4 °C after the 37° C incubation. They were then mixed with the internal standards, catalase (s₂₀[w] = 11.3 S (100 μg in 50 μl)) and bovine serum albumin (s₂₀[w] = 4.6 S (100 μg in 50 μl)) (total volume now 390 μl) and layered into 48.5-ml linear 5-25% sucrose gradients, which had been prepared in buffer A. The gradients were centrifuged at 4 °C in a Beckman SW 55 Ti rotor at 63,000 rpm for 17 h in a Beckman model ultracentrifuge set at a = 9 × 10⁻¹¹ rad/s (this is approximately equivalent to a 7.5-h run at 55,000 rpm, kₑₑₑ = 390,000). Gradients were drained from the bottom, and 35 or 36 fractions were collected. The bottoms of the drained centrifuge tubes were washed with 0.25 ml of 0% SDS to recover any adhering protein. Positions of GP IIb, GP IIIa, or protein standards in each fraction were quantitated by densitometry of SDS-polyacrylamide gels (see previous section). The peak positions of the standard and sample proteins were determined by triangulation.

Stoke’s Radii—The Stoke’s radii (R) were estimated by the method of Clarke (19). A 1.15×1.05-cm Sephadryl S-300 column was pre-equilibrated at 4 °C with buffer A and calibrated with blue dextran (exclusion volume (Ve) = 64.4 ml), β-mercaptoethanol (total volume (Vₜ) = 146.5 ml), thyroglobulin (R₂ = 85 Å and elution volume (Ve) = 73.7 ml) and catalase (R₂ = 52.2 Å and Ve = 97.1 ml). Glycoprotein IIb-IIIa (1.5 ml of 0.7-1.0 mg/ml), treated as indicated in Table 1, was cooled to 4 °C before column loading. The column flow rate was 23 ml/h, and each fraction was collected for 1.5 min into preweighed tubes. Positions of GP IIb-IIIa and protein standards were located using the Bio-Rad protein assay (Bio-Rad), supplemented with SDS-polyacrylamide gel electrophoresis in initial experiments. The Stoke’s radius of GP IIb-IIIa was determined from its partition coefficient (K₀), i.e. (Vₜ/Vₑ)/(Vₑ/Vₜ) relative to a plot of the Stoke’s radius of the standard proteins versus their partition coefficient.

Fluorescence Spectroscopy—Fluorescence emission spectra of glycoprotein IIb-IIIa at room temperature using a SPEX Fluorolog Model 112 spectrofluorometer. The 285-nm (0.9-nm bandwidth) excitation beam was provided by a 150-W Xenon arc lamp and a single-grating monochromator. Emission intensity was corrected for variations in the excitation intensity with a rhodamine B quantum counter. Spectra were scanned from 300 to 400 nm (2.25-nm bandwidth) with a double-grating monochromator and have not been corrected for the wavelength dependence of the detection system. The emission intensity at 2-mm intervals was integrated for 10 s by point counting, using a Hamamatsu R928 photomultiplier tube. The optical densities of the GP IIb-IIIa solutions studied ranged from 0.1 to 0.2 at 285 nm for a 1-cm pathlength. Addition of peptides, EGTA, or CaCl₂ solutions gave the expected changes in the optical density, as predicted from the dilution factor (<10%). The background intensities due to light scattering by the buffer solutions without protein were subtracted from all spectra. No time-dependent changes in the fluorescence intensity at 350 nm were observed when the glycoprotein solutions were illuminated at 285 nm (0.9-nm bandwidth). However, increasing the excitation intensity, i.e. using greater bandwidths, did result in bleaching of the intrinsic protein fluorescence. Therefore, these higher excitation intensities and illumination of the samples between spectra were avoided.

Synthetic Peptides—The decapeptide LGGAQAGDV was custom-synthesized by Vega Biotechnologies (Tucson, AZ). The tetrapeptides RGDS, RGES, and KGDS were purchased from Peninsula Laboratories (Belmont, CA). Custom-synthesized and purified RGDS and RGES were also obtained from Biosearch (San Rafael, CA). Identical peptides from either source gave comparable results. Concentrations of peptides in solution for each experiment were determined from the total amino acid content (20).

RESULTS

Previous studies demonstrated that thrombin-sensitive sites are exposed on GP IIb, following treatment of GP IIb-IIIa with Ca²⁺ chelators (16); Ca²⁺ chelation changes the structure of GP IIb-IIIa by dissociating it into monomers (GP IIb and GP IIIa). We therefore used thrombin hydrolysis as an initial measure of structural changes induced in GP IIb-IIIa by synthetic peptides. When the untreated GP IIb-IIIa complex was reduced and observed on SDS-polyacrylamide gels after staining with Coomassie Brilliant Blue, GP IIb and GP IIIa appeared in a ratio of ~0.9:1 and migrated with apparent molecular weights of 116,000 and 108,000, respectively (21) (Fig. 1, lane 1). As seen in Fig. 1, lane 2, GP IIb, of the GP IIb-IIIa complex is not normally readily accessible to hydrolysis by thrombin; a high concentration of thrombin (1.7 units/ml for 1 h at 37 °C) hydrolyzed only 10% of the GP IIb, relative to the control (Fig. 1, lane 1). Thrombin did not appear to hydrolyze GP IIIa. However, pretreatment of GP IIb-IIIa with RGDS altered the GP IIb-IIIa complex so that 60% of GP IIb, was hydrolyzed by thrombin. Products of this hydrolysis were observed in positions immediately above and below that of GP IIIa (dotted arrows in Fig. 1, lane 3). Similar results were obtained with the fibrogenin γ-chain decapetide (data not shown). In contrast, RGES, a peptide that lacks the ability to inhibit fibrinogen binding to platelets (6, 10), did not expose thrombin-sensitive sites on GP IIb, (Fig. 1, lane 4) relative to its control (Fig. 1, lane 2). Separate experiments demonstrated that RGES did not inhibit the catalytic activity of thrombin (data not shown). For comparison, the GP IIb-IIIa complex was dissociated into monomers with EDTA and then treated with thrombin. Note that the catalytic products generated when thrombin was added to EDTA-treated glycoprotein (Fig. 1, lane 5) were distinct from those occurring with RDGS-treated glycoprotein (Fig. 1, lane 3).

The order of potency of the peptides in exposing thrombin-sensitive sites on GP IIb, was as follows: RDGS > the decapeptide LGGAQAGDV > KGDS > RGES (Fig. 2). This is the same order of potency in which these peptides inhibit fibrinogen binding to platelets (4, 10). The concentrations of

3 Glycoprotein IIb consists of two disulfide-linked subunits: GP IIb, (Mₛ = 116,000 under reducing conditions) and GP IIIa, (Mₛ = 25,000).
The observed hydrolysis of GP IIb, with any given time of incubation, was 50% or fibronectin binding to platelets or to purified GP IIb-IIIa by 50%. The time course of this effect was relatively slow: half of the RGDS and the fibrinogen decapeptide that induced 50% of the observed hydrolysis of GP IIb, with any given time of incubation were ~60 and 140 μM, respectively. These concentrations are similar to those necessary to inhibit fibronectin or fibronectin binding to platelets or to purified GP IIb-IIIa by 50% (4, 6, 8, 10).

The effect of peptides on the exposure of thrombin-sensitive sites on GP IIb, was time-dependent as well, as observed over a 25-h period (Fig. 3). The same order of potency was maintained as observed in Fig. 2, i.e. RGDS > KGDS > RGES. The time course of this effect was relatively slow: half of the

**Table I**

| Hydrodynamic parameter | Time of incubation | Peptides added* |
|------------------------|--------------------|-----------------|
|                        | Peptide (μM)       | None            | RGDS | LGGAKQAGDV |
| s20w (S)               |                    |                 |       |             |
| 1                      | 8.4 ± 0            | 8.4*b           | 8.1 ± 0 | 8.5 ± 0.1 |
| 6                      | 8.5 ± 0.1          | 8.1*c           | 7.4 ± 0.1 | 7.7 ± 0.3 |
| R, (Å)                 | 74 ± 0.7           | ND              | 82 ± 1.4 | 85 ± 0.6 |
| M* estimatesd          | 297,400 ± 4,500    | ND              | 286,900 ± 6,200 | 309,500 ± 12,300 |

* Peptide concentration was 2 mM.
* n = 1.
* ND, not determined.

d Anhydrous M* estimates that include bound detergent. Calculated from the equation $M^*_s = \frac{6 \pi N R_s s_{20w}^w}{1 - \gamma^w P_{RH,s}^w}$, where $N$ is Avogadro’s number, $\eta$ is assumed to be 0.01 g/cm-s, $R_s$ is expressed in meters, $s_{20w}$ is expressed in 1 x 10^-15 s, $\rho_{20w}$ is assumed to be 1 g/cc, and $\gamma^* = \frac{\gamma_s + \delta_p + \Delta \gamma}{1 + \gamma_s + \delta}$, where $\gamma_s$ is assumed to be 0.74 cc/g (23), $\gamma_p$ (for Triton X-100) = 0.908 cc/g (24), $\delta$ (for carbohydrate content) is assumed to equal 0.6 cc/g, $\delta_0 = 0.3$ g/g (25), and $\delta = 0.15$ g/g (26). The value for $\delta_0$ was assumed to remain constant with or without peptide treatment. The $M_s$ standard deviations were calculated based on rules for propagation of errors as described by Skoog and West (27).

**Fig. 1.** Effects of Arg-Gly-Asp-Ser (RGDS), Arg-Gly-Glu-Ser (RGES), and EDTA on the hydrolysis of glycoprotein (GP) IIb, by thrombin as observed by SDS-polyacrylamide gel electrophoresis. Glycoprotein IIb-IIIa (0.7-1.0 mg/ml) was incubated at 37 °C for the times indicated and cooled to 4 °C for the determination of hydrodynamic parameters. Each experimentally determined parameter represents the average of two to three values ± S.D. except where indicated.

GP IIb, became accessible to hydrolysis by thrombin after a 6- to 7-h preincubation with 0.3 mM RGDS. The decapptide exhibited a similar time dependency, as measured over 5.5 h (data not shown).

Preincubation of GP IIb-IIIa with peptides at various temperatures followed by a 1-h incubation with thrombin at 37 °C demonstrated that this change in GP IIb-IIIa was also temperature-dependent. At 37 °C, RGDS readily converted GP IIb, to a thrombin substrate (Figs. 1-3). At 22 °C this effect was much reduced and at 4 °C it was undetectable (data not shown).

Peptide-induced structural changes in GP IIb-IIIa were also detected by measuring intrinsic peptide fluorescence (Fig. 4). When GP IIb-IIIa was treated for 30 min at room temperature with either RGES (Fig. 4, curve 1), RGDS (Fig. 4, curve 3), or buffer (curve not shown), nearly identical fluorescence spectra were observed, with a broad, featureless maximum at ~355 nm. These fluorescence spectra are consistent with emission from tryptophan residues that are predominantly...
Peptides May Be Due to Several Types of Structural Changes in the Presence and Absence of Glycoprotein IIb-IIIa

Peptides May Be Due to Several Types of Structural Changes in the Presence and Absence of Glycoprotein IIb-IIIa. Glycoprotein IIb-IIIa (0.3 mg/ml) was incubated at 37°C for various times in the presence of 0.3 mM RGES, RGDS, or KGDS. The last hour of each incubation included thrombin (1.7 units/ml) or an equivalent volume of buffer for the control. The amount of remaining GP IIb was determined by densitometry of SDS-polyacrylamide gels.

Exposed to an aqueous environment, but the spectra also most likely include the combined emission of aromatic residues from many separate microenvironments. However, when GP IIb-IIIa was incubated with RGDS for 4 h at 37°C, i.e. under conditions known to change its susceptibility to thrombin hydrolysis, the intensity of the fluorescence spectrum decreased, with no shift in the emission peak (Fig. 4, curve 4).

This result was specific for RGDS: no such change occurred with RGES (Fig. 4, curve 2) or buffer (curve not shown) under identical conditions. The RGDS-induced change in fluorescence is consistent with the quenching of fluorescence from tryptophan residues exposed to an aqueous environment; this apparent quenching was not accounted for by an increase in light scattering or by bleaching of GP IIb-IIIa (see "Materials and Methods").

Dissociation of the GP IIb-IIIa complex by a 5-min treatment with 2 mM EGTA at 37°C (22) resulted in a fluorescence spectrum distinct from that induced by RGDS. The EGTA treatment caused a larger decrease in fluorescence intensity, with a shift in the emission peak to the blue by ~5 nm (Fig. 4, curve 6) relative to the control (curve 5). These changes are consistent not only with a greater quenching of fluorescence from tryptophan in an aqueous environment, but also with the transfer of some tryptophan residues to a more hydrophobic environment.

The exposure of thrombin-susceptible sites on GP IIb and the quenching of intrinsic protein fluorescence by the active peptides may be due to several types of structural changes in GP IIb-IIIa, including an unfolding of the GP IIb-IIIa complex, a dissociation of the complex into free GP IIb and GP IIIa subunits (distinct from the dissociation induced by Ca²⁺ chelation), and dimer (or greater) formation of GP IIb-IIIa complexes. We attempted to distinguish among these possibilities by measuring the sedimentation coefficient and Stoke's radius of the glycoprotein to estimate its molecular weight in the presence and absence of peptides. Glycoprotein IIb-IIIa was incubated with a relatively high concentration of peptide (2 mM) to ensure that maximal changes occurred for the particular time of treatment used. Preincubation of GP IIb-IIIa with RGDS decreased the sedimentation coefficient from a control value of 8.4 to 8.1 S after 1 h and to 7.4 S after 6 h, again demonstrating the time dependency of this change. Similar changes were observed upon treatment with the fibrinogen γ-chain decapptide (i.e. s₂₀,w = 7.7 S after a 6-h incubation) but to a lesser extent with RGES (Table I). This decrease in sedimentation coefficient was accompanied by an increase in the Stoke's radius of GP IIb-IIIa from 74 to 82 Å with RGDS or to 85 Å with the fibrinogen γ-chain decapptide, indicating that the molecular weight of the glycoprotein did not change appreciably as a result of peptide treatment. This apparent lack of change in the glycoprotein molecular weight suggests that the peptides cause the GP IIb-IIIa complex to unfold rather than to dissociate or form dimers or larger aggregates.

**DISCUSSION**

The present study establishes that occupancy of the adhesive protein binding sites on purified GP IIb-IIIa affects its structure. This was demonstrated by the increased susceptibility of GP IIb, to hydrolysis by thrombin, the decreased
intrinsic fluorescence of GP IIb-IIIa, and the altered hydrodynamic parameters of the glycoprotein. Synthetic peptides rather than whole adhesive plasma proteins were used in this study because of the complications of measuring physical changes in GP IIb-IIIa in the presence of another large protein. The change in structure of GP IIb-IIIa induced by peptides is distinctly different from that induced by chelation of Ca²⁺; different hydrolytic products were formed upon thrombin hydrolysis; different fluorescence spectra resulted; and the molecular weights, as estimated from the hydrodynamic parameters, are consistent with peptide-treated GP IIb-IIIa remaining as a heterodimer complex rather than dissociating to form monomers (as occurs with chelating agents (21)) or aggregating. Thus, the most likely explanation for the change in glycoprotein structure is that peptide binding causes the GP IIb-IIIa heterodimer complex to unfold. This type of structural change can be defined as a change in protein conformation.

It is possible that the peptide-induced changes in conformation observed with purified GP IIb-IIIa also occur with platelet-associated GP IIb-IIIa. This conformational change could provide a mechanism whereby these peptides or ligands containing these sequences signal specific cellular events. For example, Isenberg et al. (12) recently observed that fibrinogen, RGDS, or the fibronectin γ-chain decapeptide, but not RGES, causes clustering of GP IIb-IIIa on the surface of ADP-treated platelets, as demonstrated by an immunoelectron microscopic surface replica technique. The peptide RGDS, but not fibrinogen, also induced this change on the surface of resting platelets, presumably because the peptide is small enough to have access to GP IIb-IIIa. A second cellular event that may be initiated by a ligand-induced change in the conformation of GP IIb-IIIa is a change in membrane permeability to ions. Banga et al. (13) found that occupancy of GP IIb-IIIa by fibrinogen was necessary for Na⁺/H⁺ exchange to occur in epinephrine-stimulated platelets; an anti-GP IIb-IIIa monoclonal antibody or the absence of fibrinogen prevented this exchange. These findings suggest that occupancy of binding sites on GP IIb-IIIa, and thereby a change in its conformation, may signal Na⁺/H⁺ exchange or other events to occur.

The time course for the change in conformation of purified GP IIb-IIIa appears to be slower than the time necessary to produce clustering of the glycoprotein in platelets or Na⁺/H⁺ exchange. While this may indicate that the conformational change is unrelated to these two events, several considerations suggest that they may be related. First, it is probable that peptide binding to GP IIb-IIIa causes subtle conformational changes immediately; the techniques used in the present study may only measure the more profound changes in structure that occur at later stages. Second, purified GP IIb-IIIa may change its conformation more slowly than GP IIb-IIIa in the platelet membrane. Alternatively, the change in GP IIb-IIIa conformation that we observed may relate to a situation in which the platelet is in contact with a ligand (e.g., fibrinogen) for a long period, as in a thrombus, rather than to the more rapid events that occur upon platelet stimulation.

A separate implication of the present study is related to the mechanism by which RGDS and the fibronectin γ-chain decapeptide inhibit ligand binding to GP IIb-IIIa. Several recent reports imply that these two peptides may bind to identical or closely associated sites on GP IIb-IIIa. First, both peptides inhibit the binding of fibrinogen, fibronectin, and von Willebrand factor to platelets, even though the decapeptide sequence is not found within that of fibronectin or von Willebrand factor (4, 9). Next, these peptides inhibit ligand binding in a competitive or partially competitive manner, suggesting direct competition for a specific site on GP IIb-IIIa (4, 6, 8). Also, Lam et al. (7) recently reported that the decapeptide elutes GP IIb-IIIa that is bound to an RGDS affinity column and vice versa. A possible explanation for these findings is that each peptide binds to the same or overlapping sites on GP IIb-IIIa, directly inhibiting the binding of the other peptide or fibrinogen. The present study provides an alternative explanation. Since each peptide has an effect on the conformation of GP IIb-IIIa, the binding sites for each peptide may be distant from one another but affected by the conformational change. Such a proposal would explain why two peptides having little structural similarity are able to inhibit the binding of one another (7) and fibrinogen (4, 6) or fibronectin (4, 8) in an apparently competitive manner. Knowledge of the precise location of peptide binding sites on GP IIb-IIIa will help to clarify their mechanism of action.

Although the present study demonstrates that the active peptides expose thrombin-sensitive sites on GP IIb, this result does not necessarily mean that the peptide binding sites are contained within GP IIb. It is possible that these peptides could bind to any one of the glycoprotein subunits (GP IIb, GP IIb₃, or GP IIIa) to change the exposure or conformation of GP IIb. A recent study by Santoro and Lawing (28) shows that the fibrinogen γ-chain peptide modified to contain a cross-linker becomes cross-linked to GP IIb, whereas an RGDS-containing peptide with a cross-linker becomes cross-linked to both GP IIb and GP IIIa, and each peptide inhibits cross-linking of the other at not entirely identical binding sites. A peptide-induced conformational change in GP IIb-IIIa could account for this inhibition.

In conclusion, our results suggest that GP IIb-IIIa has the potential to signal secondary events in platelet function through a specific conformational change. Two important questions remain from the present study: first, whether ligands that contain the decapeptide sequence and/or RGDS (e.g., fibrinogen or fibronectin) are themselves capable of changing the conformation of GP IIb-IIIa; and second, whether a change in conformation occurs with platelet-associated GP IIb-IIIa, in addition to purified GP IIb-IIIa. Answers to these questions will be important in determining a role for GP IIb-IIIa in transmembrane signaling.

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