Glycoprotein IIb-IIIa and RGD(S) Are Not Important for Fibronectin-Dependent Platelet Adhesion Under Flow Conditions

By Patricia F.E.M. Nievelstein and Jan J. Sixma

Previous studies have indicated that activated blood platelets interact with fibronectin through binding of fibronectin to the glycoprotein IIb-IIIa complex (GPIIb-IIIa). The cell attachment site of fibronectin with its crucial arg-gly-asparagine (RGD) sequence is involved in these bindings. We studied the importance of these interactions for the fibronectin dependence of platelet adhesion under flow conditions. An RGDS-containing hexapeptide (GRGDSP) was compared with a nonreactive control peptide (GRGESP). The GRGDSP-peptide inhibited thrombin-induced aggregation and adhesion under static conditions at 0.1 mmol/L. This concentration had no effect on platelet adhesion to nonfibrillar collagen type I in flow. GRGDSP at 1 mmol/L had a significant inhibitory effect at 1.500 s⁻¹, but not at the lower shear rates of 800 and 300 s⁻¹ where platelet adhesion is also fibronectin dependent. On the matrix of cultured human umbilical vein endothelial cells, 1 mmol/L GRGDSP had no effect on platelet adhesion.

FIBRONECTIN is a large multifunctional, adhesive glycoprotein with a wide distribution in vivo. It consists of a series of relatively protease-resistant functional domains, and many laboratories have been able to isolate and define fragments of the molecule involved in various interactions, including those with collagen, fibrin, actin, glycosaminoglycans, and staphylococci. Large fragments of fibronectin supporting fibroblast attachment have been identified, and a chymotryptic 120-kd fragment of plasma fibronectin displaying this activity was characterized. The use of overlapping peptides and cDNA and sequencing studies localized the cell attachment activity to a tetrapeptide, l-arginylglycyl-l-aspartyl-l-serine. Synthetic peptides containing this RGD-sequence inhibited fibronectin-dependent fibroblast attachment, and the same sequence was demonstrated to inhibit fibronectin binding to platelets as well. With specific antibodies, the complex of glycoprotein IIb and IIIa (GPIIb-IIIa) of the activated blood platelets has been shown to act as surface binding site for fibronectin. These platelet glycoproteins could be isolated by affinity binding to fragments of fibronectin or to the active-site peptide (RGDS). Recently, a direct effect of RGDS on purified GPIIb-IIIa was shown by inducing a profound and specific change in the conformation of this glycoprotein complex.

From these and other studies, it is clear that under static conditions fibronectin binds through the RGD-sequence to GPIIb-IIIa on the activated platelet membrane. Furthermore, glycoprotein Ic-IIa (GPIc-IIa) was recently shown to act as an activation-independent fibronectin receptor. This protein complex is partly homogenous to VLA5, the fibronectin receptor found on many other cells.

The initial response to vascular injury is adhesion of platelets to human subendothelium. This adhesion depends on the presence of von Willebrand factor (vWF) and fibronectin in the subendothelium and in plasma. In vitro studies have shown that the extracellular matrix of endothelial cells or purified collagen is a reliable model to study platelet adhesion, and adhesion to these surfaces also depends on the presence of vWF and fibronectin. In the present study, we focus on fibronectin. The purpose of this study was to investigate whether fibronectin platelet interaction under flow conditions is also mediated by arg-gly-asparagine (RGD) containing sequences interacting with glycoprotein IIb-IIIa.

MATERIALS AND METHODS

Collagen. Collagen type I was isolated from human umbilical arteries as previously described. Purity and identity was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The collagen was dissolved in 50 mmol/L acetic acid (1 mg/mL) and sprayed on glass coverslips to a quantity of 30 μg collagen/cm² as previously described.

Endothelial cell matrix. Cell matrices were derived from human umbilical vein endothelial cells as previously described. In some experiments, the endothelial cell matrix (ECM) was incubated with antifibronectin F(ab')₂ fragments or nonimmune rabbit F(ab')₂ fragments. Incubations were performed at room temperature for 60 minutes with 1.25 mg/mL antibody in phosphate-buffered saline (PBS) or overnight with 0.78 mg/mL.

Characteristics of peptides and monoclonal antibodies. A hexapeptide gly-arg-gly-asparagine-ser-pro, (GRGDSP) was tested (mol wt 667 daltons), including the RGDS sequence which promotes cell attachment activity. As a control, a peptide was used in which aspartic
Fibronectin was isolated from human plasma by affinity chromatography on gelatin-Sepharose as previously described. The diagnosis of Glanzmann's thrombasthenia type I relied on the absence of platelet aggregation with collagen, epinephrine, and ADP and normal aggregation with ristocetin. The absence of GPIIb and GPIIIa was demonstrated by two-dimensional electrophoreses of platelet proteins labeled by 125I with iodogen as previously described.

Preparation of fibronectin-free plasma and purification of fibronectin. Fibronectin was isolated from human plasma by affinity chromatography on gelatin-Sepharose as previously described.

Radiolabeling of fibronectin. Fibronectin was radiolabeled with Na125I (Iodine-125, Amersham International, Buckinghamshire, England) by the iodogen procedure according to the manufacturer's instructions. The amount of free 125I, measured after precipitating bound 125I with 20% (wt/vol) trichloroacetic acid was 0.5% of total radioactivity. The specific activity of 125I fibronectin was 0.054 μCi/μg.

Inhibition of binding of 125I-fibronectin to thrombin-stimulated platelets. Washed platelets were resuspended in Tyrode's solution with 0.1% glucose and 0.2% bovine serum albumin (BSA) (Boseral, Organon Teknika, Oss, The Netherlands), pH 7.4, to an amount of 2 × 10⁶/μL. Platelets were incubated with a dilution of 1 to 100 as compared with the amount of plasma or plasma substitute. F(ab')2 fragments of rabbit antiserum against human plasma fibronectin were purchased lyophilized (Cappel Laboratories, Cochranville, PA) and reconstituted in 0.02 mol/L PBS, pH 7.3. Protein concentration was 12.5 mg/mL.

Characterization of the Glanzmann patient. The diagnosis of Glanzmann's thrombasthenia type I relied on the absence of platelet aggregation with collagen, epinephrine, and ADP and normal aggregation with ristocetin. The absence of GPIIb and GPIIIa was demonstrated by two-dimensional electrophoreses of platelet proteins labeled by 125I with iodogen as previously described.

Perfusion conditions. Perfusion was carried out in a flat perfusion chamber as previously described. Part of the perfusion system was performed with whole blood and part were performed with reconstituted blood. For reconstituted blood, platelets were aspirin treated and washed in Krebs-Ringer solution as previously described and resuspended in plasma or plasma substitute to a platelet count of 190,000/μL plasma. Washed RBCs were added to a hematocrit of 0.4. In perfusions with platelets with Glanzmann's thrombasthenia, aspirin treatment was omitted. Perfusion was performed for 5 minutes at 37°C at wall shear rates ranging from 300 s⁻¹ to 1,500 s⁻¹.

Evaluation. Platelet deposition on collagen was determined by counting in radioactivity in a gamma counter. Platelet adhesion to endothelial cell matrix was determined morphometrically with a light microscope connected to an image analyzer (Quantimet 720, Imago, Royston, UK), as previously described.

Aggregation studies. Washed platelets resuspended in platelet-poor plasma (PPP, 200,000/μL) were stirred at 900 rpm at 37°C and stimulated with 0.5 U/mL thrombin (Sigma, St Louis) with continuous monitoring of light transmission in a Payton dual channel aggregometer (Payton, Scarborough, Canada). Responses were traced in the presence of 100 and 200 μmol/L GRGDSP or GRGESP.

Static adhesion assay. Immuno wells were coated overnight at 4°C with 20 μg/mL purified fibronectin in PBS. Platelets were washed and labeled with 111In as described above. They were resuspended in Tyrode's solution with 0.1% glucose and 0.2% BSA, pH 7.35, to ~200,000/μL/mL. The wells were washed three times with PBS and incubated with the platelet suspension in the presence of 0.1, 0.5, or 1 mmol/L GRGDSP of GRGESP for 1 hour at 37°C. The wells were washed again three times with PBS, and the amount of platelets was determined by counting 111In in a gamma counter.

Statistical analysis. Significance of differences between means was calculated with Student's t test.

RESULTS

Inhibition by GRGDSP of thrombin-induced platelet aggregation. Addition of 0.1 or 0.2 mmol/L GRGDSP to washed platelets, stimulated with 0.5 U/mL thrombin, significantly reduced the degree and rate of platelet aggregation as compared with the same concentration of control peptide (data not shown). In accordance with previous reports, the effect was concentration dependent. No effect on shape change was observed.

Concentration-dependent inhibition of GRGDSP on platelet adhesion in a static system. Adhesion of nonactivated 111In-labeled platelets to fibronectin-coated surfaces was studied with different concentrations of GRGDSP or control peptide (GRGESP) added. Addition of 0.1, 0.5, or 1 mmol/L GRGDSP, respectively, gave mean adhesions of 62%, 49%, and 37% as compared with the same concentrations of the control. All these differences were significant within a 95% confidence interval. Absolute values are presented in Table 1.

Effect of GRGDSP on platelet adhesion under flow conditions. The effect of GRGDSP on platelet adhesion was studied on the matrix of cultured endothelial cells, which has been shown to be a good model of the vessel wall, and on

<table>
<thead>
<tr>
<th>Concentration of Added Peptide (mmol/L)</th>
<th>111In Platelets x 10⁵/Well</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>GRGSIP</td>
</tr>
<tr>
<td>0.5</td>
<td>14.8 ± 0.7</td>
</tr>
<tr>
<td>1.0</td>
<td>14.8 ± 0.7</td>
</tr>
<tr>
<td>No addition</td>
<td>13.9 ± 0.6</td>
</tr>
</tbody>
</table>

Immuno wells coated overnight at 4°C with 20 μg/mL purified fibronectin in PBS, pH 7.4, were incubated for 1 hour at 37°C with 111In-labeled platelets (1 × 10⁶/mL in Tyrode's solution, pH 7.25) with 0.1, 0.5, or 1 mmol/L GRGDSP or control peptide (GRGESP). After incubation, the wells were washed three times with PBS (pH 7.4) and the number of adhered platelets was counted. Adhesion to wells coated only with albumin was <0.01. Values are mean ± SEM (n = 8) subtracted by the background level of 0.95 ± 0.1 obtained by incubating with PBS. P values of the comparison of the peptides at different molarity were <.001.
surfaces coated with nonfibrillar vascular collagen type I. Incubation of the ECM with antifibronectin and perfusion with fibronectin-depleted plasma has demonstrated the fibronectin dependence of platelet adhesion on this surface. Dependence on fibronectin for adhesion to nonfibrillar collagen I was made evident by perfusion with fibronectin-depleted plasma. The results are summarized in Tables 2 and 3. GRGDSP 1 mmol/L had no effect on adhesion to ECM at all shear rates. It inhibited adhesion to nonfibrillar collagen I significantly at a shear rate of 1,500 s⁻¹ (P < .001).

Dependence of thrombasthenic platelets on fibronectin in the matrix of human vascular endothelial cells. Normal platelets or platelets from patients with Glanzmann's thrombasthenia resuspended in fibronectin-free plasma were perfused over an ECM in which fibronectin was blocked with anti-fibronectin F(ab')₂ fragments. Controls were incubated with aspecific F(ab')₂ fragments. Results are summarized in Table 4. Preincubation of the matrix with antifibronectin resulted in a similar decrease of platelet deposition as compared with controls for normal platelets and thrombasthenic platelets. This indicates that thrombasthenic platelets are also dependent on fibronectin for optimal adhesion to the matrix of cultured endothelial cells.

Effect of addition of anti–GPIIb-IIIa to the perfusate on platelet adhesion to an ECM with or without fibronectin. The relative importance of GPIIb-IIIa at different wall shear rates for platelet deposition on ECM was investigated by perfusion with the perfusate with anti–GPIIb-IIIa (EDU3). Results are shown in Table 5. At relatively low wall shear rate (300 s⁻¹), no effect of preincubation with anti–GPIIb-IIIa was found. At higher shear rates, however, platelet deposition was lower than with anti–GPIIb-IIIa controls except at the highest shear rate of 1,800 s⁻¹; this can be ascribed to the relatively low normal values obtained on ECM at this shear rate. (P values for 800, 1,300 and 1,800 s⁻¹ are <.01, <.01, and <.2, respectively). The effect was strongest at 1,300 s⁻¹. At this wall shear rate, the relative importance of GPIIb-IIIa for fibronectin-dependent platelet deposition on ECM was further investigated. Perfusates consisted of normal plasma with addition of anti–GPIIb-IIIa or the same amount of control ascites. The ECM was preincubated with antifibronectin F(ab')₂ fragments or with nonimmune F(ab')₂ fragments. Results are summarized in Table 6. This experiment clearly shows the dependence of platelet adhesion on fibronectin and GPIIIa-IIIb. Furthermore, it shows that blockage of GPIIb-IIIa does not eliminate the dependence of platelet adhesion on fibronectin.

### Table 2. Platelet Deposition on Nonfibrillar Human Collagen Type I: Effect of Addition of 0.1 and 1 mmol/L GRGDSP to Perfusate

<table>
<thead>
<tr>
<th>Addition to Fibronectin-Depleted Plasma</th>
<th>Platelet Deposition (10⁶ Platelets × 10²/cm²)</th>
<th>Wall Shear Rate (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>300</td>
<td>800</td>
</tr>
<tr>
<td>Buffer</td>
<td>1.8 ± 0.3</td>
<td>3.6 ± 0.9</td>
</tr>
<tr>
<td>Fibronectin + buffer</td>
<td>13.2 ± 1.9</td>
<td>16.5 ± 0.6</td>
</tr>
<tr>
<td>Fibronectin + 0.1 mmol/L GRGESP</td>
<td>13.1 ± 1.2</td>
<td>16.4 ± 1.7</td>
</tr>
<tr>
<td>Fibronectin + 0.1 mmol/L GRGDSP</td>
<td>12.6 ± 1.5</td>
<td>14.4 ± 1.0</td>
</tr>
<tr>
<td>Fibronectin + 1 mmol/L GRGESP</td>
<td>13.1 ± 0.7</td>
<td>15.2 ± 2.1</td>
</tr>
<tr>
<td>Fibronectin + 1 mmol/L GRGDSP</td>
<td>11.4 ± 1.0</td>
<td>11.7 ± 1.1</td>
</tr>
</tbody>
</table>

Nonfibrillar human collagen type I (30 μg/cm²) was perfused for 5 minutes with reconstituted blood at wall shear rates of 300, 800, and 1,500 s⁻¹. Synthetic peptides GRGESP and GRGDSP were added to fibronectin-depleted plasma in a 110-fold (0.1 mmol/L) or a 1,100-fold molar excess (1 mmol/L) compared to the added amount of purified fibronectin (200 μg/mL plasma). When fibronectin or peptides were omitted, the same volume of buffer was added. Values are mean ± SEM; n = 4.

### Table 3. Platelet Deposition on Endothelial Cell Matrix: Effect of Addition of 1 mmol/L GRGDSP to the Perfusate

<table>
<thead>
<tr>
<th>Wall Shear Rate (s⁻¹)</th>
<th>Addition to Fibronectin-Depleted Plasma</th>
<th>Platelet Deposition (% Surface Coverage)</th>
<th>Control</th>
<th>Antifibronectin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ECM Preincubation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>None</td>
<td>25.4 ± 3.2</td>
<td>10.1 ± 1.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 mmol/L GRGESP</td>
<td>23.9 ± 2.3</td>
<td>10.1 ± 1.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 mmol/L GRGDSP</td>
<td>24.5 ± 1.2</td>
<td>9.9 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>None</td>
<td>26.1 ± 1.3</td>
<td>8.7 ± 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 mmol/L GRGESP</td>
<td>30.0 ± 3.0</td>
<td>5.1 ± 0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 mmol/L GRGDSP</td>
<td>29.8 ± 1.5</td>
<td>10.7 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>1,500</td>
<td>None</td>
<td>28.8 ± 2.6</td>
<td>6.4 ± 1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 mmol/L GRGESP</td>
<td>24.3 ± 2.8</td>
<td>7.0 ± 1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 mmol/L GRGDSP</td>
<td>25.4 ± 4.8</td>
<td>6.3 ± 1.5</td>
<td></td>
</tr>
</tbody>
</table>

The endothelial cell matrix was preincubated overnight with antifibronectin F(ab')₂ fragments (0.78 mg/mL PBS) or with the same concentration of aspecific F(ab')₂ fragments. Subsequently, perfusions were performed for 5 minutes with reconstituted blood at wall shear rates of 300, 800, and 1,500 s⁻¹. Synthetic peptides, GRGESP or GRGDSP, were added to fibronectin-depleted plasma in a concentration of 1 mmol/L, 10 minutes before perfusion, and incubation was performed at 37°C. As control, perfusions were performed without addition of peptides. Values are mean ± SEM; n = 4.
The endothelial cell matrix was preincubated for 1 hour at room temperature with antifibronectin F(ab')2 fragments (500 μg/mL PBS) or with the same concentration of isotype-matched (IgG) F(ab')2 fragments. Subsequently, perfusions were performed with either normal platelets or platelets of a patient with Glanzmann’s thrombasthenia resuspended in fibronectin-depleted plasma for 5 minutes at wall shear rates of 300 and 1,300 s⁻¹. Values are mean ± SEM; n = 4.

suggesting that GPIIb-IIIa is not the receptor for fibronectin involved in adhesion under flow conditions.

**Inhibition of the binding of ¹²⁵I-fibronectin to thrombin-stimulated platelets by anti-GPIIb-IIIa.** The ability of the anti–GPIIb-IIIa antibodies EDU3 and 7E3 to block GPIIb-IIIa was confirmed by binding studies with ¹²⁵I-fibronectin. An amount of 140,000 ± 16,000 fibronectin molecules bound to thrombin-stimulated platelets. Incubation of the platelets with EDU3 or 7E3 resulted in reduction to 19% or 5% of the maximal fibronectin binding. Unstimulated platelets bound <1% of the added fibronectin.

**DISCUSSION**

The purpose of the present study was to determine whether fibronectin binding to GPIIb-IIIa is involved in platelet adhesion to substrates under flow conditions. We used a RGD(S)-containing hexapeptide, GRGDSP, and a hexapeptide GRGESP as a negative control. The reactivity of GRGDSP was validated by its effect on thrombin-induced platelet aggregation. For comparison, we also studied the effect of GRGDSP on platelet adhesion to fibronectin in a static system (Table 1). In both systems, we found an already prominent effect at the lowest concentration of 0.1 mmol/L. Increasing the concentration to 0.5 and 1.0 mmol/L showed a dose-dependent increase in inhibition, but most of the inhibition was already observed at 0.1 mmol/L. These data compare well with what has been published on the inhibitory effect of RGD-containing peptides on adhesion of baby hamster kidney cells.8,12 and blood platelets in a static system.14 These data are also in good agreement with binding studies of ¹²⁵I-fibronectin to thrombin-activated platelets in which half-maximal inhibition of binding was found at 10 to 20 μmol/L.14

In contrast, under flow condition, we found no significant effect of GRGDSP at concentrations up to 1 mmol/L on ECMs at low and high wall shear rate and on purified collagen at 300 s⁻¹. At 800 s⁻¹, no significant inhibition was observed on collagen at 0.1 mmol/L, but inhibition (although still not significant) had become visible at 1 mmol/L. At 1,500 s⁻¹, 60% inhibition was observed at 1 mmol/L. Dependence on fibronectin for platelet adhesion on ECM has been shown by the use of antifibronectin F(ab')2 fragments (Table 3). These results are in agreement with previous results from this laboratory.23 A decreased adhesion to nonfibronillar collagen type I at 300 s⁻¹, after perfusion with fibronectin-depleted plasma, compared with control perfusions with fibronectin-containing perfusates also clearly shows the fibronectin dependence of this surface at 300 s⁻¹ (Table 2). In a separate study, we showed that fibronectin dependence was still observable at shear rates down to 10 s⁻¹.30 GRGDSP at a concentration of 0.1 mmol/L fully blocks fibronectin binding to GPIIb-IIIa on activated platelets.34 This concentration or a tenfold increase had little or no effect on platelet adhesion in a flow system to ECM at all shear rates and to nonfibrillar collagen type I at 300 s⁻¹. This suggests that RGD-mediated binding of fibronectin is not involved in platelet adhesion under these flow conditions.

The inhibitory effect of GRGDSP at higher shear rates on adhesion to collagen at first appears not to be in accordance with this idea. Several observations, however, indicate that GPIIb-IIIa, as receptor for RGD-containing proteins, may become important for adhesion at high shear rates. First, a monocolonal to GPIIb-IIIa specifically inhibiting binding of vWF to GPIIb-IIIa on activated platelets inhibited platelet adhesion at high shear rates.32 Second, blood of patients with Glanzmann’s thrombasthenia showed an adhesion defect at high shear rates.33 A similar defect was seen with Mabs to GPIIb-IIIa. Third, the dodecapeptide mimicking, the carboxy-terminal end of the γ chain of fibrinogen that inhibits binding of fibrinogen, fibronectin, and vWF to GPIIb-IIIa inhibited platelet adhesion at high shear rates.36,37 The fibrinogen γ-chain dodecapeptide and RGD(S) are complete competitive inhibitors of each other in their association with GPIIb-IIIa36,38 and both can change the conformation of purified GPIIb-IIIa.16 Although they compete for the same receptor,29 their binding sites on GPIIb-IIIa are related, but not identical.40 These data indicate that the inhibitory effect of GRGDSP at higher shear rates may be due to its

**Table 4. Comparison of Effect of Antifibronectin on Adhesion of Normal and Thrombasthenic Platelets to Endothelial Cell Matrix**

<table>
<thead>
<tr>
<th>Endothelial Cell Matrix Preincubation</th>
<th>Platelet Adhesion (% Surface Coverage) [Wall Shear Rate (s⁻¹)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>300</td>
</tr>
<tr>
<td>Control Normal</td>
<td>10.7 ± 1.2</td>
</tr>
<tr>
<td>Antifibronectin Normal</td>
<td>6.9 ± 1.1</td>
</tr>
<tr>
<td>Control Patient</td>
<td>11.1 ± 1.2</td>
</tr>
<tr>
<td>Antifibronectin Patient</td>
<td>6.7 ± 0.9</td>
</tr>
</tbody>
</table>

The extracellular matrix of human vascular endothelial cells was perfused with whole blood after incubation of the perfusate for 5 minutes at room temperature with dilution of 1:100 of anti–GPIIb-IIIa (EDU3) or a control ascites. Perfusions were performed for 5 minutes at wall shear rates of 300, 800, 1,300, and 1,800 s⁻¹. Values are mean ± SEM; n = 4.

**Table 5. Platelet Deposition on Extracellular Matrix of Human Vascular Endothelial Cells: Effect of Addition of Anti–GPIIb-IIIa at Different Wall Shear Rates**

<table>
<thead>
<tr>
<th>Perfusate Preincubation</th>
<th>Platelet Adhesion (% Coverage) [Wall Shear Rate (s⁻¹)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>300</td>
</tr>
<tr>
<td>Control</td>
<td>9.9 ± 1.1</td>
</tr>
<tr>
<td>Anti–GPIIb-IIIa</td>
<td>9.2 ± 0.7</td>
</tr>
</tbody>
</table>

The extracellular matrix of human vascular endothelial cells was perfused with whole blood after incubation of the perfusate for 5 minutes at room temperature with dilution of 1:100 of anti–GPIIb-IIIa (EDU3) or a control ascites. Perfusions were performed for 5 minutes at wall shear rates of 300, 800, 1,300, and 1,800 s⁻¹. Values are mean ± SEM; n = 4.
inhibitory effect on the binding of various proteins to GPIIb-IIIa. Synthetic RGD-containing peptides of vWF inhibit platelet adhesion to collagen at a wall shear rate of 2,600 s⁻¹. Involvement of RGD sequences in fibrogen is less evident. The RGDS and RGDV sequences in its α chain are probably of minor importance for its interaction with GPIIb-IIIa. The inhibitory effect of the dodecapeptide from the γ chain of fibrogen in the perfusion system may be caused by preventing binding of the main proteins involved in platelet adhesion, vWF, and fibronectin to the RGDS binding site, which is related to the dodecapeptide binding site either by overlap or conformational linkage. No inhibition by GRGDSP of adhesion to an ECM was observed. This is at present unexplained, but it is important to remember that adhesion to this matrix involves several other factors apart from vWF and fibronectin. The effect of an inhibitory peptide may therefore be less evident.

To determine further whether fibronectin binding to GPIIb-IIIa is involved in platelet adhesion, we also used blood of a patient with Glanzmann's thrombasthenia and Mabs against GPIIb-IIIa. The patient had type I, Glanzmann's disease completely lacking GPIIb and IIIa on two-dimensional O'Farrell gels stained with silver. Based on similar mol wt and some properties, GPIIb-IIIa is probably identical to the receptor described by Giancotti and colleagues, which has the characteristics of the fibronectin receptor of nucleated cells, and VLA 5. Both are members of the RGD-directed family of receptors. These results are in agreement with part of our current data which demonstrate that there must be a second binding site on platelets for fibronectin. Preliminary studies with an antibody directed against the avian fibronectin receptor, which reacts primarily with the β-subunits of the fibronectin receptor of human cells (gift from Dr K. M. Yamada) showed no effects on adhesion to ECM in our flow system (data not shown). Further studies with specific antibodies should be performed to clarify the role of the GPIIb-IIIa complex in adhesion of blood platelets in flow. Our observation that GRGDSP does not inhibit fibronectin-dependent adhesion in flow argues against GPIIb-IIIa as fibronectin receptor because GPIIb-IIIa is RGD-directed. One would have to assume that GPIIb-IIIa is much less sensitive to inhibition or that interactions with other domains on fibronectin may serve as backup system.

The two binding systems for fibronectin have parallelism with vWF. VWF binds to activated platelets through GPIIb-IIIa and a carboxy-terminal domain containing RGD. Interaction of an amino-terminal domain of vWF with GPIb on the platelets is the primary mechanism involved in adhesion, but this mechanism becomes apparent only when vWF is adsorbed to a surface or in the presence of ristocetin or after desialylation. One may postulate therefore that the platelet binding site for fibronectin involved in adhesion reacts with fibronectin only when fibronectin has first adsorbed to a surface that perhaps has collagen as the native binding site.

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Glycoprotein IIb-IIIa and RGD(S) are not important for fibronectin-dependent platelet adhesion under flow conditions

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