Fibrin(ogen) Peptide Bβ 15–42 Inhibits Platelet Aggregation and Fibrinogen Binding to Activated Platelets†

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ABSTRACT: The binding of fibrinogen to activated platelets leads to platelet aggregation. Fibrinogen has multiple binding sites to platelet membrane glycoprotein IIb–IIIa complex. At least two well-defined sequences in fibrinogen, Arg-Gly-Asp sequence of Aα 95–97 and Aα 572–574 and γ 400–411, have been shown to interact with glycoprotein IIb–IIIa. A possible binding site on the amino-terminal end of fibrinogen to platelet glycoprotein IIb–IIIa has also been reported. In this paper the effect of synthetic peptides derived from the amino-terminal end of the Bβ chain on platelet aggregation and fibrinogen binding has been examined. Bβ 15–42 peptide inhibits platelet aggregation and ^{125}I-fibrinogen binding to activated platelets in a dose-dependent manner. Since Bβ 15–42 contains a previously identified fibrinogen binding site, Bβ 15–18, exposed by thrombin cleavage of native fibrinogen, we also examined the effect of Bβ 15–18, Bβ 19–42, and Bβ 1–14 (fibrinopeptide B) on platelet aggregation and fibrinogen binding. Synthetic fibrinopeptide B and Bβ 15–18 had no effect on platelet aggregation and fibrinogen binding while Bβ 19–42 retained the inhibitory effect. When fibrinogen is chromatographed on a column of agarose-bound Bβ 15–42, a cation-dependent retention of fibrinogen on the peptide column was observed, and fibrinogen was eluted from the column by Bβ 15–42 but not by Bβ 1–14. Under the same conditions, platelet glycoprotein IIb–IIIa was not retained in the column. Thus, the observed inhibitory effect is due to its interaction with fibrinogen rather than to platelet glycoprotein IIb–IIIa. These results show a previously unrecognized fibrinogen-derived peptide that inhibits platelet aggregation and fibrinogen binding.

Platelet membrane glycoproteins IIb and IIIa form a major heterodimeric complex on the surface of activated platelets and provide binding sites for fibrinogen (Gogstad et al., 1982; Bennett et al., 1982; Nachman & Leung, 1982), von Willebrand protein (Ruggeri et al., 1983; Nokes et al., 1984; DeMarco et al., 1986) and fibronectin (Plow & Ginsberg, 1981; Gardner & Hynes, 1985; Pytela et al., 1986) on activated platelets. In a series of elegant studies using isolated fibrinogen chains, cyanogen bromide fragments and synthetic peptides, Hawiger et al. (1982, 1987) and Kloczewiak et al. (1984) localized one of the binding sites to residues 400–411 in the carboxy-terminal end of the γ chain. In addition, the α chain of fibrinogen also contains two Arg-Gly-Asp sequences (Doolittle, 1979), and peptides containing these sequences also inhibit fibrinogen binding to activated platelets (Gartner & Bennett, 1985; Haverstick et al., 1985). Lam et al. (1987) have suggested that RGDS and γ 400–411 may share a common binding site on platelet Gp IIb–IIIa complex, since both GRGDSP and γ 400–411 will elute the platelet glycoprotein IIb–IIIa complex from Sepharose-bound GRGDSP. Neither RGDS nor γ 400–411 inhibits fibrinogen binding completely, suggesting the presence of additional binding site(s) in fibrinogen (Kloczewiak et al., 1984; Plow et al., 1984; Gartner & Bennett, 1985).

Recently, Thorsen et al. (1986a,b) suggested the presence of an additional binding site on the amino-terminal end of fibrinogen from studies utilizing the binding of ^{125}I-labeled fibrin(ogen) fragments to platelets and to immunoprecipitated glycoprotein IIb–IIIa complex. The experiments reported here were undertaken to determine whether Bβ 15–42 is involved in fibrinogen binding to platelets. This peptide is generated in vivo by cleavage of Bβ 14–15 by thrombin and of Bβ 42–43 by plasmin and has been identified in the peripheral blood (Weitz et al., 1986). Bβ 15–42 contains the sequence Gly-His-Arg-Pro (GHRP), Bβ 15–18, a fibrinogen binding site unmasked by thrombin cleavage of native fibrinogen that may be involved in fibrin polymerization (Olexa & Budzynski, 1980; Laudano & Doolittle, 1980). We have studied the effect of Bβ 15–18 and Bβ 19–42 on ADP- and thrombin-induced platelet aggregation and ^{125}I-fibrinogen binding to ADP-stimulated platelets. Peptide affinity columns were also prepared to elucidate the mechanism of the inhibitory activity of the peptide.

Experimental Procedures

Silicone oils (methylsilicone DC200 and diphenylsilicone DC550) were purchased from William F. Nye, Inc., Fairhaven, MA. Human thrombin was a gift of Dr. J. Fenton, New York State Department of Health, Albany, NY. Bio-Gel A-5 M and Affi-Gel 10 activated agarose beads were purchased from Bio-Rad Laboratories, Richmond, CA. Octyl glucoside was obtained from Calbiochem-Behring Diagnostics, San Diego, CA. PGE_1, ADP, and standard laboratory chemicals were from Sigma Chemical Co., St. Louis, MO. $Na^{125}I$ was from ICN Radiochemicals, Irvine, CA. Iodogen was from Pierce Chemical Co., Rockford, IL. Human fibrinogen was prepared from fresh plasma by the method of Kazal (1963) modified by the addition of ε-aminocaproic acid to a final concentration

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of 0.1 M in buffers and plasma (Martinez et al., 1974). The fibrinogen was passed through a column of gelatin–Sepharose to remove fibronectin. Fibrinogen was labeled with $Na^{125}I$ by the Iodogen method to a specific activity of 46 cpm/ng (Markwell et al., 1978). Outdated platelets were obtained from the American Red Cross, Portland, OR, and detergent extracts of platelet membrane containing glycoproteins IIb and IIIa were prepared by Triton X-114 extraction and further purified by DEAE-cellulose chromatography as described by Newman et al. (1983).

Peptide Synthesis. The amino acid sequence of Bβ 1–42 was obtained from Blomback (1978). Bβ 1–14, Bβ 15–42, Bβ 15–18 (GHRP), Bβ 19–42, GRGDSP, and GRGESP peptides were synthesized with an automatic peptide synthesizer (Model 403A, Applied Biosystems). After cleavage by HF, the resins were washed with dichloromethane and ethyl acetate and then extracted with 5% acetic acid. The extracts were analyzed on a reverse-phase HPLC (C-18) column. GHRP, Bβ 19–42, GRGDSP, and GRGESP were found to be >95% homogeneous, and Bβ 1–14 was >97% homogeneous. The Bβ 15–42 was also sequenced with a gas-phase sequencer (Model 470A, Applied Biosystems) and found to be ~70% homogeneous with ~25% deletion peptide (less one histidine near the N-terminal). A second batch of Bβ 15–42 was >90% homogeneous. Prior to use the peptides were dissolved at 5 mM concentration in a buffer consisting of 150 mM NaCl and 50 mM sodium phosphate, pH 7.35. In some experiments the peptides were also dissolved in Waymouth's medium, pH 7.35 (Irving Scientific, Santa Ana, CA).

Platelet Preparation. Blood was collected from healthy volunteers after informed consent approved by the Human Subjects Review Committee of the University of Washington was obtained. Platelet-rich plasma (PRP) was obtained by differential centrifugation of human blood at 1000*g* for 4 min at room temperature. The blood was previously anticoagulated with 1/10 volume of 3.8% trisodium citrate, pH 6.5. For washing the platelets, PGE_1 was added to the PRP to a final concentration of 1 μM, and the platelets were sedimented by centrifugation at 1000*g* for 12 min at room temperature. The platelet pellet was resuspended in platelet washing buffer (113 mM NaCl, 4.3 mM K_2HPO_4, 4.2 mM $Na_2HPO_4 \cdot 7H_2O$, 24.4 mM NaH_2PO_4, and 5.5 mM dextrose, pH 6.5) and washed 3 times as described by Baenziger and Majerus (1974) for binding studies and twice for platelet aggregation. The platelets were suspended in a buffer consisting of 140 mM NaCl, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.42 mM NaH_2PO_4, 0.5 mM glucose, 5 mM HEPES, and 0.3% bovine serum albumin (Sigma Chemical Co., St. Louis, MO), pH 7.35, at a platelet concentration of (6–8) × 10^8/mL. Platelets were also prepared by gel filtration (Tangen et al., 1973) on an agarose column (Bio-Gel A-50 M, Bio-Rad, Richmond, CA). Seven milliliters of PRP was applied to a plastic column (2.4 × 23) in modified Tyrode's buffer (140 mM NaCl, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.42 mM NaH_2PO_4, 5 mM HEPES, 0.1% dextrose, 0.05 mM $CaCl_2$, 0.98 mM $MgCl_2$, 0.3% bovine serum albumin, pH 7.35) (Tangen et al., 1973; Santoro, 1983). The gel-filtered platelets were used within 1 h of venipuncture.

Platelet Aggregation. Platelet aggregation was performed with a four channel aggregometer (Monitor IV Plus, Helena Laboratories, Beaumont, TX). The reaction mixture typically consisted of 150 μL of washed or gel-filtered platelet suspension and 125 μL of Waymouth's medium or modified Tyrode's buffer containing various concentration of the peptides. The reaction mixture was incubated for 3 min at 37 °C with stirring at 1000 rpm in the aggregation cuvette. Human thrombin or ADP was added in a volume of 25 μL to give a final concentration of 1.0 unit/mL thrombin or 10 μM ADP. The final concentration of the platelets was ~3 × 10^8/mL.

Binding Studies. Binding studies were performed at room temperature in polypropylene tubes (No. 38368, Stockwell Scientific, Walnut Creek, CA). Suspensions of platelet (150 μL) in platelet aggregation buffer were mixed with various concentration of ^{125}I-fibrinogen, and calcium chloride was added to a final concentration of 1 mM. Platelets were stimulated with ADP to a final concentration of 10 μM. The mixture was incubated for 5 min without stirring. At the end of the incubation period, the entire contents of the incubation mixture were layered onto silicone oil in microsedimentation tubes (No. 72.702, Sarstedt, West Germany) and spun at 10000*g* for 3 min at room temperature in an Eppendorf Model 5412 centrifuge. Solutions of DC200 and DC500 were mixed in ratios of 1:5 before use. After sedimentation, the tips of the centrifuge tubes containing the platelet pellet were sliced, and the bound fibrinogen and free fibrinogen were counted separately in a Searle counter (Searle Analytic Inc., Chicago, IL). Since 10 mM EDTA completely inhibits fibrinogen binding to platelet Gp IIb–IIIa, nonspecific binding was determined in parallel experiments in the presence of 10 mM EDTA. Nonspecific binding was also determined in the presence of a tenfold excess of unlabeled fibrinogen and was similar to that in 10 mM EDTA (Figure 5). Total binding in these experiments represented 4.5–7% of the total ligand input. Nonspecific binding was less than 10% of the total binding. The effect of various peptides on fibrinogen binding was determined by preincubating peptides with platelet suspensions for 3 min before the addition of ADP.

Affinity Chromatography of Fibrinogen and Platelet Glycoprotein IIb–IIIa on Agarose-Bound Bβ 15–42 Peptide. Bβ 15–42 was coupled to activated agarose beads (Affi-Gel 10, Bio-Rad Laboratories, Richmond, CA) according to the manufacturer's instructions (~15 mg/mL of agarose). The affinity column was equilibrated in PBS, pH 7.0, containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM PMSF (column buffer). Fibrinogen (5 mg) was applied to the column (4-mL total bed volume) in the same buffer. Unbound fibrinogen was washed with 60 mL of the column buffer. Bound fibrinogen was eluted by washing the column sequentially with 5 mL of column buffer containing 1.5 mM Bβ 1–14 and 25 mL of column buffer followed by 5 mL of column buffer containing 1.5 mM Bβ 15–42 and 25 mL of column buffer. Five-milliliter fractions were collected, and the fractions were dialyzed against distilled H_2O, lyophilized, and analyzed on 7.5% SDS–PAGE under reducing conditions. In another experiment the bound fibrinogen was eluted in PBS containing 10 mM EDTA.

Platelet glycoproteins IIb and IIIa from 5–10 units of platelets were applied to the same column in the presence of 100 mM octyl glucoside in PBS, pH 7.0, containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM PMSF. The column was washed with 60 mL of the buffer containing 25 mM octyl glucoside in PBS containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM PMSF (column buffer), and the bound proteins were then eluted by washing the column sequentially with 5 mL of column buffer containing 1.5 mM Bβ 1–14 and 25 mL of column buffer followed by 5 mL of buffer containing 1.5 mM Bβ 15–42 and 25 mL of column buffer. Five-milliliter fractions were collected sequentially, dialyzed, lyophilized, and analyzed on 7.5% SDS–PAGE under reducing conditions as above. Platelet glycoproteins IIb and IIIa were also chromatographed on a column of agarose-bound GRGDSP with

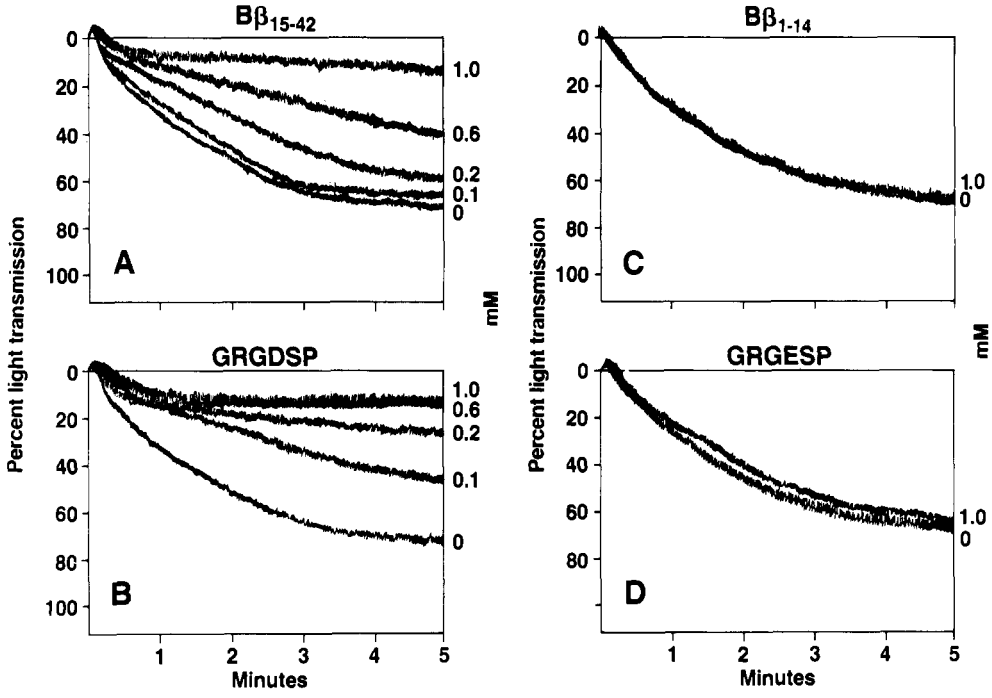

FIGURE 1: Effect of synthetic peptides on platelet aggregation. Platelets (3×10^8 platelets/mL) were stimulated by 1 unit/mL thrombin in the presence of various concentrations of the peptides. (Panel A) Effect of various concentrations of Bβ 15–42; (panel B) effect of various concentrations of GRGDSP; (panel C) effect of Bβ 1–14; (panel D) effect of GRGESP.

elutions by GRGDSP and GRGESP as positive and negative controls, respectively, as described by Lam et al. (1987).

RESULTS

Inhibition of Platelet Aggregation by Bβ 15–42 Peptide. As shown in Figure 1, there is a dose-dependent inhibition of thrombin-induced platelet aggregation in the presence of Bβ 15–42 peptide with a 50% inhibition at 0.6 mM. Under the same conditions GRGDSP also showed a dose-dependent inhibition with 50% inhibition occurring at 0.1 mM. Bβ 15–42 almost completely inhibited platelet aggregation at 1 mM. Bβ 1–14 and GRGESP up to 1.0 mM had no effect on platelet aggregation induced by thrombin.

Bβ 15–42 contains GHRP (Bβ 15–18), a fibrinogen binding site exposed by thrombin cleavage of native fibrinogen (Laudano & Doolittle, 1980; Olexa & Budzynski, 1980). This sequence in Bβ 15–42 might interact with the D domain, thus interfering with γ 400–411 function. To exclude this possibility, we investigated the effect of Bβ 15–18 (GHRP) and Bβ 19–42 on thrombin-induced platelet aggregation. As noted in Figure 2, GHRP up to the 1.0 mM level tested had no inhibitory effect on thrombin-induced platelet aggregation. The inhibitory effect was retained by Bβ 19–42, which lacked the GHRP sequence.

Since washed platelets responded less well to ADP, we used gel-filtered platelets to study the effects of peptides on ADP-induced platelet aggregation. As shown in Table I, Bβ 15–42 inhibited almost completely ADP-induced aggregation of gel-filtered platelets [$(1.5–4) \times 10^8$/mL] at 0.8 mM. GRGDSP caused 90% inhibition of ADP-induced platelet aggregation at less than or equal to 0.4 mM. Both GRGESP and Bβ 1–14 had no effect on ADP-induced platelet aggregation. Gel-filtered platelets were used within an hour of venipuncture, and as noted before (Kaplan et al., 1981), no exogenous fibrinogen was necessary for ADP-induced platelet aggregation in our system.

Since the gel-filtered platelets at high platelet concentrations ($>3 \times 10^8$/mL) may cause release of α-granule contents more readily than washed platelets, we repeated the ADP-induced

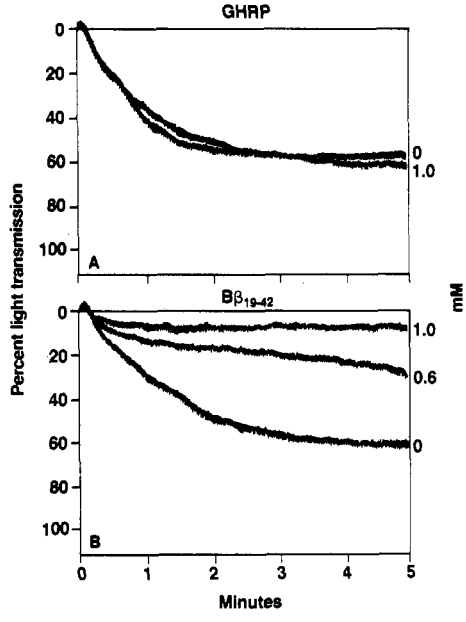

FIGURE 2: Effect of GHRP and Bβ 19–42 on platelet aggregation. Platelets (3×10^8/mL) were stimulated with 1 unit/mL thrombin in the presence of various concentration of the peptide. (Panel A) Effect of various concentrations of GHRP; (panel B) effect of various concentrations of Bβ 19–42.

platelet aggregation of gel-filtered platelets at lower platelet concentrations [$(0.5–0.75) \times 10^8$/mL] with exogenous fibrinogen added. As seen in Table II even at the lower platelet concentration, platelet aggregation still occurred in the absence of exogenous fibrinogen. Addition of 100 nM fibrinogen enhanced platelet aggregation by 32%–45% as compared to platelet aggregation in the absence of exogenous fibrinogen. ADP-induced aggregation in the presence of exogenous fibrinogen was still inhibited by Bβ 15–42.

Inhibition of ^{125}I-Fibrinogen Binding by Bβ 15–42. Fibrinogen binds specifically in a saturable manner to platelets stimulated with 10 μM ADP. Incorporation of 0.4 mM Bβ 15–42 in the incubation mixture inhibited fibrinogen binding

Table I: Effect of Synthetic Peptides on ADP-Induced Platelet Aggregation[a]

peptide	concn of peptide (mM)	LTU after 2 min donor 1	donor 2	donor 3
Bβ 15–42	0.2	92	69	76
	0.4	63	31	50
	0.6	20	11	15
	0.8		3	9
Bβ 1–14	0.2	95	100	89
	0.4	85	100	91
	0.6	95	98	95
	0.8		97	93
GRGDSP	0.2		12.5	28
	0.4		3	3
	0.6		1.6	2
	0.8		1.0	1
GRGESP	0.2		100	91
	0.4		95	87
	0.6		95	90
	0.8		100	89
no peptide		95	100	95

[a] A total of 150 μL of gel-filtered platelet suspensions (3×10^8/mL to 8×10^8/mL) was incubated with various concentrations of the peptides in 125 μL at 37 °C for 3 min, and 25 μL of ADP was added to give a final concentration of 10 μM. Aggregation was recorded for 5 min on a four-channel aggregometer (Helena Laboratories, Beaumont, TX). The aggregometer was calibrated with a suspension of gel-filtered platelets [0 light transmission unit (LTU)] and buffer alone (100 light transmission units). The results were from gel-filtered platelets of three different donors. The final platelet concentrations used range from 1.5×10^8/mL to 4×10^8/mL. No exogenous fibrinogen was added in platelet aggregation.

Table II: Effect of Synthetic Peptides on ADP-Induced Platelet Aggregation at Low Platelet Concentration[a]

peptide	concn of peptide (mM)	LTU after 2 min donor 1	donor 2
Bβ 15–42	0.1	94	97
	0.4	43	50
	0.6	35	32
	1.0	17	20
Bβ 1–14	0.1	98	94
	0.4	95	90
	0.6	98	85
	1.0	89	91
GRGDSP	0.1	21	40
	0.4	10	18
	0.6	11	17
	1.0	7	5
GRGESP	0.1	87	95
	0.4	90	93
	0.6	91	90
	1.0	95	92
no peptide		98	90
no peptide and no fibrinogen		74	62

[a] A total of 150 μL of gel-filtered platelet suspensions [(1–1.5) × 10^8/mL] was incubated with various concentrations of the peptide in 125 μL of modified Tyrode's buffer containing 100 nM fibrinogen at 37 °C for 3 min, and 25 μL of ADP was added to give a final concentration of 10 μM. Aggregation was done exactly as in Table I. The results were from gel-filtered platelets of two different donors. The final platelet concentrations used range from 0.5×10^8/mL to 0.75×10^8/mL.

(Figure 3). When these data were analyzed by double-reciprocal plots (Figure 4), the plots intersected at the ordinate, consistent with the interpretation that Bβ 15–42 decreased the affinity of fibrinogen toward platelets without affecting the total number of binding sites available.

Effect of Various Peptides and Unlabeled Fibrinogen on the Binding of ^{125}I-Fibrinogen to Activated Platelets. The ability of various synthetic peptides to inhibit fibrinogen binding to ADP-stimulated platelets was measured at a fixed

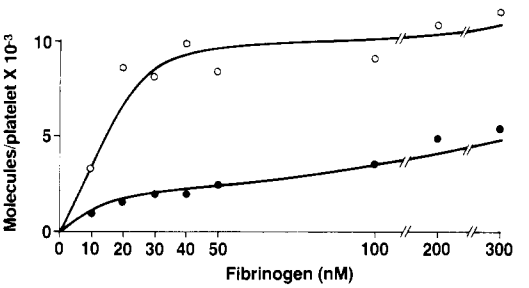

FIGURE 3: Inhibition of ^{125}I-fibrinogen binding to ADP-stimulated platelets by Bβ 15–42. Various concentrations of ^{125}I-fibrinogen were incubated with suspensions of washed platelets (4×10^8 platelets/mL) at 25 °C in the presence or absence of Bβ 15–42. The platelets were then stimulated with 10 μM ADP. After 5 min, specific fibrinogen binding was measured as described under Experimental Procedures. Specific ^{125}I-fibrinogen binding measured in the absence (○) or in the presence (●) of 0.4 mM Bβ 15–42.

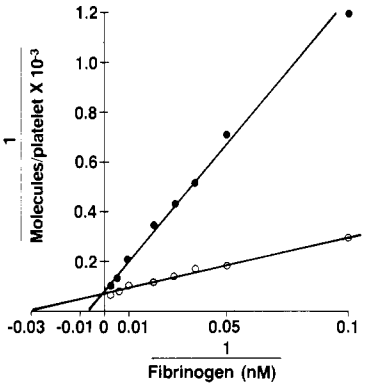

FIGURE 4: Double-reciprocal plots of ^{125}I-fibrinogen binding to ADP-stimulated platelets by Bβ 15–42. The data from Figure 3 were converted to a double-reciprocal plot. Binding isotherms: specific ^{125}I-fibrinogen binding measured in the absence (○) or in the presence (●) of 0.4 mM Bβ 15–42.

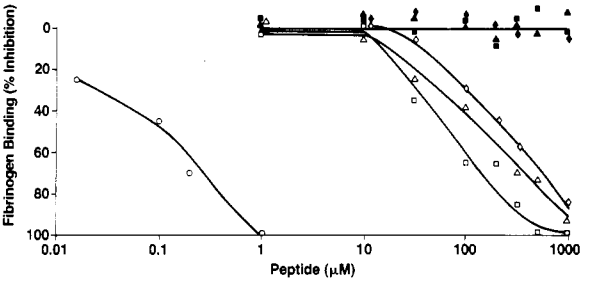

FIGURE 5: Effect of various peptides on ^{125}I-fibrinogen binding to ADP-stimulated platelets. Aliquots of washed platelets (4×10^8 platelets/mL) were incubated with 0.1 μM ^{125}I-fibrinogen and various concentrations of peptides at 25 °C. The platelets were stimulated with 10 μM ADP, and specific ^{125}I-fibrinogen binding to platelets was measured as described under Experimental Procedures. There were 15 000 molecules of ^{125}I-fibrinogen bound per platelet in the absence of any peptides, and this was considered 0% inhibition. Unlabeled fibrinogen (○), Bβ 1–14 (▲), Bβ 15–42 (△), GRGESP (■), GRGDSP (□), Bβ 15–18 (♦), and Bβ 19–42 (◇).

concentration of ^{125}I-fibrinogen (0.1 μM). Various concentrations of the peptides were compared with the effect of unlabeled fibrinogen. As seen in Figure 5, a tenfold excess of unlabeled fibrinogen almost completely inhibited the binding of ^{125}I-fibrinogen. Under identical conditions, half-maximal inhibition of binding occurred with Bβ 15–42 and GRGDSP at 180 and 75 μM, respectively. GRGESP and Bβ 1–14 did not inhibit fibrinogen binding to platelets up to 1 mM concentration tested. GHRP was also not inhibitory up to 1 mM concentration tested, whereas Bβ 19–42 retained the inhibitory activity with half-maximal inhibition at 380 μM.

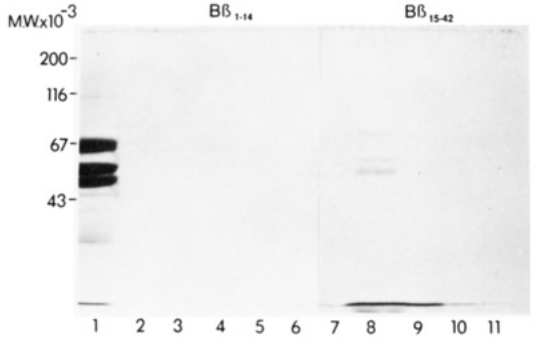

FIGURE 6: Sequential elution of bound fibrinogen from agarose-bound Bβ 15–42. Human fibrinogen in PBS, pH 7.0, containing 1 mM $CaCl_2$, 1 mM $mgCl_2$, and 1 mM PMSF was applied to a Bβ 15–42–agarose column. Fibrinogen was eluted from the affinity column by 1.5 mM Bβ 1–14 followed sequentially by 1.5 mM Bβ 15–42. (Lane 1) Fibrinogen; (lanes 2–6) elutions with Bβ 1–14; (lanes 7–11) subsequent elutions with Bβ 15–42.

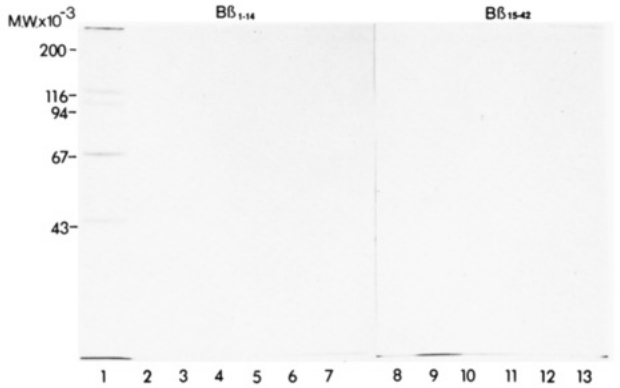

FIGURE 7: Sequential elution of bound platelet glycoproteins IIb and IIIa from agarose-bound Bβ 15–42. Platelet glycoproteins IIb and IIIa in PBS, pH 7.0, containing 100 mM octyl glucoside, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM PMSF were applied to the column and eluted sequentially by 1.5 mM Bβ 1–14 peptide followed by 1.5 mM Bβ 15–42 peptide. (Lane 1) Platelet extract containing Gp IIb and IIIa applied to the column; (lanes 2–7) elutions with Bβ 1–14; (lanes 8–13) subsequent elutions with Bβ 15–42.

Affinity Chromatography of Fibrinogen and Platelet Glycoproteins IIb and IIIa on Agarose-Bound Bβ 15–42. As seen in Figure 6, fibrinogen bound to Bβ 15–42 immobilized on agarose was eluted with Bβ 15–42. This interaction appears to be specific since Bβ 1–14 did not elute fibrinogen from the column. Fibrinogen binding to Bβ 15–42–agarose was cation dependent since 10 mM EDTA was able to elute fibrinogen from the column (data not shown). Human albumin did not bind to the column under similar conditions. When platelet glycoprotein IIb–IIIa was applied to the column under the same conditions in the presence of 100 mM octyl glucoside, no retention of glycoprotein IIb–IIIa could be demonstrated (Figure 7). However, some faint bands corresponding to the molecular weight region of fibrinogen chains were seen in elution with Bβ 15–42, but not with Bβ 1–14. This may be derived from some contaminating platelet fibrinogen in the platelet glycoprotein IIb–IIIa preparation. Under similar conditions the same preparation of platelet glycoprotein IIb–IIIa bound to a column of agarose-bound GRGDSP and could be eluted specifically by GRGDSP peptide. Neither Bβ 15–42 nor GRGESP eluted the bound glycoprotein IIb–IIIa from agarose-bound GRGDSP.

Discussion

Two distinct peptide sequences in fibrinogen, γ 400–411 and the RGD sequences, have been shown to be binding sites for platelet glycoprotein IIb–IIIa complex, and peptides containing these sequences have an inhibitory effect in platelet aggregation and ^{125}I-labeled fibrinogen binding studies (Kloczewiak et al., 1984; Gartner & Bennett, 1985; Lam et al., 1987; Haverstick et al., 1985). There are two RGD sequences in the α chain of fibrinogen, Aα 95–97 and Aα 572–574. Recent data suggest the proximal RGD may be the more important of the two in fibrinogen–platelet interactions (Peerschke & Galanakis, 1987; Plow et al., 1987). The apparent K_d values of both RGD and γ 400–411 are severalfold less than that of intact fibrinogen. This suggests the presence of additional binding sites, or the already known binding sites (RGD and/or γ 400–411) must be held in a restricted conformational state in order to be recognized by the Gp IIb–IIIa complex in activated platelets. A similar mechanism was originally suggested by Humphries et al. (1987) for fibronectin interaction with its receptor.

Niewiarowski et al. (1977, 1981) provided evidence that fragment X, an early plasmin-cleaved product of fibrinogen, was less potent in promoting ADP-induced platelet aggregation than intact fibrinogen and suggested that the C-terminal part of Aα chain and/or the N-terminal part of the Bβ chain was required for platelet aggregation. Thorsen et al. (1986a,b) recently suggested that there may be a binding site on the N-terminal end of fibrinogen.

There were conflicting reports on the possible involvement of the amino-terminal region of fibrinogen in platelet aggregation using fragments E (Marguerie et al., 1982; Tomikawa et al., 1982). Since Bβ 15–42 is easily cleaved by plasmin and may be lacking in fragment E derived from plasmin digestion of fibrinogen, we entertained the possibility that Bβ 15–42 may be involved in platelet aggregation. We show that Bβ 15–42 inhibited thrombin- and ADP-induced platelet aggregation as well as fibrinogen binding to ADP-stimulated platelets.

To further define the mechanism of inhibition of platelet aggregation and fibrinogen binding, we examined the interaction of fibrinogen and platelet glycoproteins IIb and IIIa on agarose-bound Bβ 15–42. A specific cation-dependent interaction of fibrinogen with Bβ 15–42 could be demonstrated. We could not demonstrate a specific interaction of platelet glycoprotein IIb–IIIa with the peptide under conditions in which the previously described interaction of glycoprotein IIb–IIIa with GRGDSP–agarose was demonstrated. Thus the inhibitory effect of Bβ 15–42 appears to be mediated by its interaction with fibrinogen. Our study corroborates studies using fibrinogen chain monomers which did not implicate Bβ chain as a potential binding site on fibrinogen for platelet Gp IIb–IIIa (Hawiger et al., 1982; Nachman et al., 1984). Recently, Thorsen et al. (1987) have further localized that new binding site on fibrinogen to platelet glycoprotein IIb–IIIa on the γ chain of the N-terminal disulfide knot of the fibrinogen molecule.

Thrombin-induced release of fibrinopeptide A and B exposes polymerization sites on the α and β chains (Doolittle, 1981; Olexa & Budzynski, 1980), and synthetic tripeptides corresponding to the α chain amino-terminal sequence, Gly-Pro-Arg, and the β chain amino-terminal sequence, Gly-His-Arg, have been shown to bind to the D domain of fibrinogen (Laudano & Doolittle, 1978, 1980). GPR inhibits fibrin polymerization (Laudano & Doolittle, 1978) as well as fibrinogen binding to platelets (Plow & Marguerie, 1982). The inhibitory effect of Bβ 15–42 on platelet aggregation and fibrinogen binding is not due to GHRP, since GHRP has no effect on fibrinogen binding and platelet aggregation up to the 1 mM concentration tested while Bβ 19–42 still retains its inhibitory effect although less active than Bβ 15–42. GHRP

may confer a special conformational state to Bβ 15–42 for its full activity. Adjacent amino acids have been shown to influence the potency of RGD peptides (Plow et al., 1987). Thus, we have presented evidence for a previously unrecognized sequence in the amino-terminal end of fibrinogen, Bβ 15–42, that inhibited thrombin- and ADP-induced platelet aggregation as well as fibrinogen binding to ADP-stimulated platelets, by its interaction with fibrinogen. The precise sequence to which Bβ 15–42 binds in fibrinogen and its relation to fibrin polymerization remain to be determined.

Fibrinogen degradation products (FDPs) have been shown to inhibit thrombin, ADP-, epinephrine-, and collagen-induced platelet aggregation (Stachurska et al., 1970; Jerushalmy & Zucker, 1966; Wintrobe et al., 1981) with low molecular weight FDPs (<5000 daltons) being most inhibitory (Stachurska et al., 1970). Bβ 15–42 is generated in vivo and has been identified in peripheral blood and urine (Weitz et al., 1986; Morozumi et al., 1987). We suggest Bβ 15–42 to be one of many FDPs that, when pathologically elevated in vivo in sepsis, trauma, burn, and other conditions, may cause platelet dysfunction by inhibiting fibrinogen interaction with platelet glycoprotein IIb–IIIa.

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