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Inhibition of fibrinogen binding to human platelets by the tetrapeptide glycyl-L-prolyl-L-arginyl-L-proline

(structure–function relationships/receptor–ligand interactions)

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ABSTRACT **The role of fibrinogen as a cofactor in platelet aggregation is mediated by its binding to platelet receptors that are induced by stimuli such as ADP. In the present study, we demonstrate that the tetrapeptide glycyl-L-prolyl-L-arginyl-L-proline inhibits the interaction of fibrinogen with its platelet receptor. The primary effect of the peptide was on the extent rather than on the rate of fibrinogen binding. Significant inhibition occurred at a 1:1 molar ratio of peptide to fibrinogen and reached maximal levels at 100:1 ratio. The inhibition was dependent upon fibrinogen concentration and occurred in the presence of calcium or magnesium. The peptide inhibited the binding of fibrinogen to platelets with exposed receptors, suggesting that it interfered directly with the ligand–receptor interaction. Fibrinogen binding supported by epinephrine and thrombin as well as ADP was inhibited by the peptide. Fibrinogen-dependent aggregation of washed platelets by ADP was abolished by a 30-fold molar excess of the peptide. The tetrapeptide is an analog of the amino-terminal sequence of the α-chain of fibrin and has been shown to inhibit fibrin polymerization [Laudano, A. P. & Doolittle, R. F. (1978) *Proc. Natl. Acad. Sci. USA* 75, 3085–3089]. A peptide corresponding to the natural sequence, glycyl-L-prolyl-L-arginyl-L-valyl-L-valine, was also capable of inhibiting fibrinogen binding to the platelet. These results suggest that common structural features within fibrinogen may serve a dual function by permitting the molecule to participate in both platelet aggregation and fibrin formation.**

The participation of fibrinogen in both platelet aggregation and fibrin formation establishes a bifunctional role for the molecule in hemostasis. The contribution of the molecule in platelet aggregation is mediated by its interaction with specific receptor sites on human platelets (1, 2). These binding sites are not expressed by the resting platelet but are induced as a result of ADP stimulation (1–5). Approximately 38,000 fibrinogen molecules may be bound to ADP-stimulated platelets with a dissociation constant of 5×10^{-7} M (3). A variety of other stimuli may also support fibrinogen binding, and mobilization of platelet-associated ADP may in part be involved in receptor induction (6–8). Based on correlations between fibrinogen binding and platelet function, it appears that the interaction of the molecule with the cell leads directly to platelet aggregation and that dissociation of platelet-bound fibrinogen results in disaggregation (1–9).

Fibrinogen is a hexamer consisting of three pairs of nonidentical polypeptide chains (Aα, Bβ, and γ chains) covalently linked in a dimeric structure by clusters of disulfide bonds (reviewed in ref. 10). Although the reactions and molecular sites involved in fibrin formation and stabilization have been extensively characterized, the intramolecular loci that permit the molecule to interact with the platelet have been more elusive. The capacity of fibrinogen to support platelet aggregation is rapidly lost in association with plasmic degradation. Intermediate fragment X supports aggregation only weakly, whereas later degradation products, Y, D, and E, are ineffective (11). Paralleling this loss of functional activity, Niewiarowski *et al.* reported that fragment X binds weakly to the platelet, whereas fragments Y and D fail to bind (9). These observations led these workers to propose that the plasmin-sensitive carboxy-terminal region of the Aα chain may mediate fibrinogen binding to the platelet, but they found only minimal binding of a 45,000 M_r fragment derived from this region to the platelet (12). In contrast to these results, Tomikawa *et al.* (13) reported that both fragments E and D inhibit ADP-induced platelet aggregation, suggesting that the platelet-binding site in fibrinogen may be retained within the plasmin-resistant core of the molecule. As still another alternative, Holt *et al.* (14) suggested that the plasmin-sensitive amino-terminal aspect of the Bβ chain was required for platelet-aggregating activity. Other data suggested that the carbohydrate moieties and glycopeptide regions of fibrinogen may mediate its binding to the platelet (15), but asialofibrinogen is as effective as fibrinogen in supporting platelet aggregation (16, 17). Thus, multiple and inconsistent leads as to the identity of the loci within fibrinogen recognized by the platelet have been forwarded.

In this study, we report that a nonglycosylated tetrapeptide, Gly-Pro-Arg-Pro, inhibits both fibrinogen binding and platelet aggregation. This peptide corresponds in part to the amino-terminal sequence of the α-chain of fibrin and is an analog of the polymerization sites involved in fibrin polymer formation (18–20).

MATERIALS AND METHODS

Fibrinogen Isolation and Peptide Synthesis. Fresh human blood was collected into acid/citrate/dextrose containing hirudin and aprotinin and the fibrinogen was isolated by ether (21) or by ethanol precipitation (22). The fibrinogen was radiolabeled with carrier-free $Na^{125}I$ to a specific activity of ≈1 μCi/μg (1 Ci = 3.7×10^{10} becquerels). The characteristics of the labeled and nonlabeled fibrinogen have been reported in detail (1, 23). Gly-Pro-Arg-Pro was synthesized by Peninsula Laboratories (San Carlos, CA) and purified in our laboratory by HPLC by using a μBondapak C_{18} column (Waters Associates). Solvent A was 0.1% H_3PO_4 adjusted to pH 3.0 with KOH, and solvent B was 60% acetonitrile/40% solvent A. A 20-min linear gradient from 0–45% solvent B was applied at 1 ml/min, and the elution was monitored at 212 nm. A single major peak eluting at 11.55 min was collected and utilized throughout the study. The amino acid composition of a 24-hr hydrolysate (6 M HCl at 110°C *in vacuo*) of this peak was 1.0:2.0:0.96 for Gly/

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Pro/Arg. Gly-Pro-Arg-Val-Val was also synthesized commercially and purified by HPLC. Under the conditions indicated above, this peptide eluted from the C_{18} column at 10.15 min, and its identity was verified by amino acid analysis. Reductive methylation of Gly-Pro-Arg-Pro was performed as follows. The peptide (3 mg) was dissolved in 1.0 ml of 0.2 M sodium borate buffer (pH 9.0) at 4°C and 0.4 mg of sodium borohydride was added. This was followed by four 2-μl additions of 18% formaldehyde at 5-min intervals. Separation of methylated from nonmodified peptide was achieved by HPLC. Under the conditions described above, the modified peptide eluted at 12.77 min and the extent of modification was 88%. When subjected to amino acid analysis, the modified peptide contained ≤6% free Gly, whereas Arg and Pro residues were not altered.

Platelets. Platelets were isolated from fresh human blood drawn into acid/citrate/dextrose by differential centrifugation and gel filtration in a divalent ion-free Tyrode's buffer (pH 7.2) containing 2% bovine serum albumin (3). In selected experiments, the platelets were labeled with ^{51}Cr or [^{3}H]serotonin in the platelet-rich plasma as described (3). The aggregation of washed platelets was measured in 0.7-cm diameter siliconized glass cuvettes at 37°C in a dual channel aggregometer (DP117E, Scienco, Morrison, CO). To 0.4 ml of the platelet suspension at 2×10^8 cells per ml in Tyrode's buffer, fibrinogen, peptide, divalent ions, and ADP were added to bring the final volume of the mixture to 0.42 ml. The sensitivity was adjusted with a 1/3 dilution of the platelet suspension.

Platelets were fixed with paraformaldehyde as follows. To platelets at 1–2 × 10^9 cells per ml in divalent ion-free Tyrode's buffer containing 2% albumin, paraformaldehyde (Polyscience, Warrington, PA) was added from a freshly prepared 4% stock solution in 0.1 M phosphate buffer (pH 7.2) to a final concentration of 0.5%. After 30 min at 22°C, the paraformaldehyde was neutralized by addition of an equal volume of 20 mM NH_4Cl in 0.15 M NaCl/0.3 M Tris, pH 7.2, and the platelets were washed by centrifugation or gel filtration. To obtain ADP-fixed platelets, the cells were stimulated with 12.5 μM ADP for 5 min prior to addition of the paraformaldehyde.

Binding Analysis. The binding of ^{125}I-labeled fibrinogen (^{125}I-fibrinogen) to platelets was measured by centrifugation through sucrose as described (1, 3). Platelets, at a final concentration of 1×10^8 cells per ml, were stimulated with ADP (12.5 μM) in the presence of ^{125}I-fibrinogen and calcium or magnesium (1 mM). The peptide was dissolved in Tyrode's buffer and added to a platelet suspension immediately prior to ^{125}I-fibrinogen addition. At selected time points, triplicate 50-μl aliquots were removed from the incubation mixture, layered on top of 0.4 ml of 20% sucrose, and centrifuged for 2.5 min at 11,750 rpm in a Beckman Microcentrifuge B. The radioactivity within the tip was determined, and the number of fibrinogen molecules bound per platelet was calculated from the specific activity of the ligand. All binding assays were performed at 22°C without stirring. Platelet lysis and the release reaction were assessed on the basis of ^{51}Cr and [^{3}H]serotonin recovery. These were measured under the same conditions as the binding assay in the presence of nonlabeled fibrinogen. Imipramine at a final concentration of 2 μM was included in experiments to measure serotonin release.

RESULTS

The capacity of Gly-Pro-Arg-Pro to alter the interaction of ^{125}I-fibrinogen with washed human platelets was evaluated. In the initial experiment, the binding of ^{125}I-fibrinogen to ADP-stimulated platelets in the presence or absence of the peptide was assessed with respect to time (Fig. 1). In the absence of the peptide, ^{125}I-fibrinogen was bound to the ADP-stimulated platelets in a time-dependent reaction, and equilibrium was reached within approximately 20 min at 22°C. With the peptide present in a 30-fold molar excess of ^{125}I-fibrinogen, the number of fibrinogen molecules specifically bound at equilibrium was decreased by 50%. The presence of the peptide did not influence the nonspecific background as estimated from the residual binding of ^{125}I-fibrinogen in the presence of a 50-fold excess of nonlabeled fibrinogen. With the peptide present or absent, the nonspecific binding was <10% of the total binding. Half-maximal binding with or without the peptide present was attained at approximately 7 min, suggesting that the peptide influenced the extent of the interaction rather than the rate constant of association.

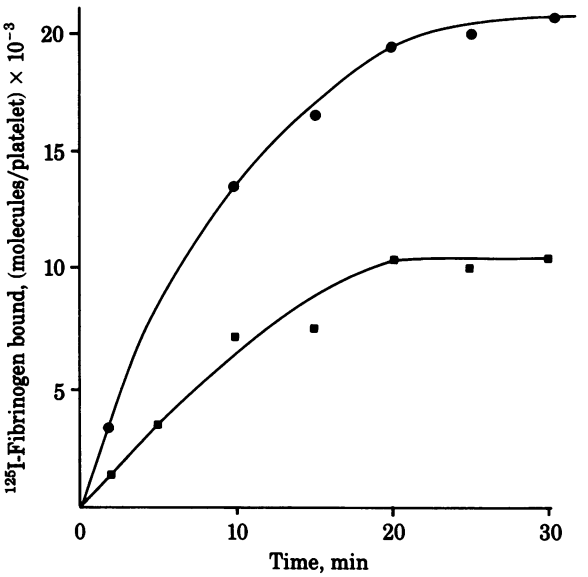

FIG. 1. The effect of the Gly-Pro-Arg-Pro peptide on the binding of ^{125}I-fibrinogen to ADP-stimulated platelets in the presence (■) or absence (●) of the peptide as a function of time. Washed human platelets (10^8 per ml) in Tyrode's buffer (pH 7.2) containing 2% albumin and 1 mM calcium were stimulated with 12.5 μM ADP with ^{125}I-fibrinogen at a final concentration of 0.7 μM and the peptide at a 30-fold molar excess. Specific fibrinogen binding is shown and was derived by subtracting the binding of ^{125}I-fibrinogen observed in the presence of a 50-fold excess of nonlabeled fibrinogen (nonspecific) from that obtained in the absence of nonlabeled fibrinogen.

The inhibition of ^{125}I-fibrinogen binding by varying concentrations of Gly-Pro-Arg-Pro is illustrated in Fig. 2. The peptide and ^{125}I-fibrinogen were added to the platelets prior to ADP addition in molar ratios ranging from 0.5:1 to 900:1, and ^{125}I-fibrinogen binding to the platelet was measured 30 min after the addition of 12.5 μM ADP. The inhibitory capacity of the peptide increased linearly from ratios of 1:1 to 100:1 and reached a plateau at ≈60% inhibition. At all peptide concentrations below the 900:1 ratio, the recovery of ^{51}Cr-labeled and [^{3}H]serotonin-labeled platelets was ≥95%, indicating that the peptide did not induce platelet lysis or dense granule secretion.

The inhibitory effect of the peptide as a function of fibrinogen concentration is considered in the *Inset* to Fig. 2. The peptide was present at a final concentration of 1.8×10^{-4} M, which represented a 100-fold molar excess of the highest ^{125}I-fibrinogen concentration used. It is apparent that the inhibition of fibrinogen binding to the ADP-stimulated platelets by the peptide was dependent upon fibrinogen concentration, and the inhibition increased with increasing fibrinogen levels. Additional data derived in characterizing the inhibitory effect of the peptide included the following observations: (*i*) The inhibition by

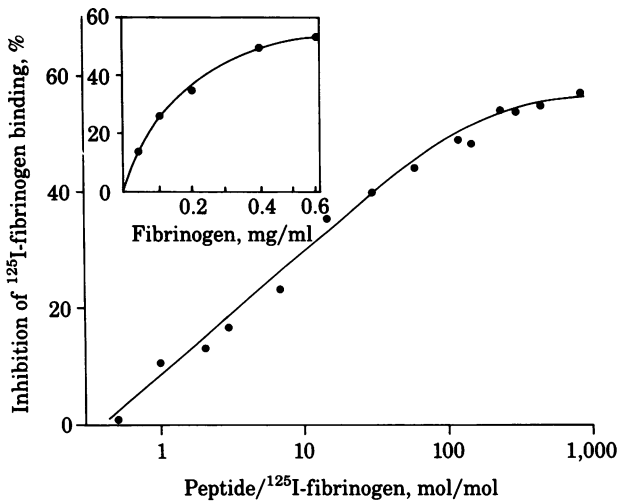

FIG. 2. The effect of increasing concentrations of Gly-Pro-Arg-Pro on the binding of ^{125}I-fibrinogen to ADP-stimulated platelets. The conditions of the binding assay are the same as in Fig. 1, and binding was at 30 min. Percent inhibition was calculated relative to a control lacking the peptide. (*Inset*) ^{125}I-Fibrinogen concentrations were varied with the peptide present at a concentration of 0.18 mM.

the peptide of ^{125}I-fibrinogen binding was consistent with different platelet preparations. With fibrinogen present at a final concentration of 0.7 μM and the peptide in a 30-fold molar excess, the inhibition of binding was 39.6 ± 9% (±SEM) with platelets from seven individual donors. (*ii*) The free amino acids comprising the peptide were noninhibitory even when present in a 1,000-fold excess of fibrinogen. The failure of free arginine to inhibit the interaction is particularly pertinent because this amino acid has been reported to inhibit fibrinogen binding to platelets (24). (*iii*) Preincubation of the peptide with ^{125}I-fibrinogen, platelets, or ADP-stimulated platelets for 30 min did not affect the extent of inhibition observed upon addition of the missing components to the incubation mixture. (*iv*) The capacity of the peptide to inhibit fibrin formation and polymerization induced by thrombin (18, 20) could be the basis of its inhibition of fibrin(ogen) binding to the platelet if contaminating thrombin were present in the binding assay. This possibility was excluded by showing that the thrombin inhibitor hirudin, at a concentration of 2 units/ml, did not inhibit fibrinogen binding or affect the inhibitory capacity of the peptide. (*v*) As shown in Table 1, similar inhibition of ^{125}I-fibrinogen binding by the peptide was observed in the presence of either calcium or magnesium. Furthermore, at 1 or 5 mM calcium, the extent of inhibition was the same, suggesting that the inhibition did not reflect an effect on the available divalent ion concentration.

The interaction of fibrinogen with the platelet involves an initial induction of the receptor by ADP followed by binding of fibrinogen to the cell. To segregate the induction from the binding reaction, ADP-stimulated platelets with induced receptors were fixed with paraformaldehyde. As shown in Fig. 3*A*, ^{125}I-fibrinogen was bound to cells fixed with paraformaldehyde after stimulation with ADP, and this binding was unaffected by readdition of ADP or by ADP scavengers such as phosphocreatine/creatine phosphokinase (shown) or apyrase (not shown). In contrast, when nonstimulated platelets were fixed, they failed to bind ^{125}I-fibrinogen (even if ADP was added after fixation). In comparing the interaction of ^{125}I-fibrinogen with ADP-fixed and nonfixed platelets, the kinetics, affinity, and divalent ion requirements were similar. These findings are consistent with ADP-fixed platelets expressing exposed fibrinogen receptors as suggested by Peerschke and Zucker (25). Inhibition of the interaction between ^{125}I-fibrinogen and ADP-fixed platelets by varying concentrations of Gly-Pro-Arg-Pro was assessed after a 30-min incubation (Fig. 3*B*). Significant inhibition was observed as the ratio of peptide to fibrinogen increased from 1:1 to 100:1, and a maximal inhibition of 25% was obtained at a 250:1 ratio. With ADP-stimulated but nonfixed platelets from the same donor, the inhibition pattern was

Table 1. Effect of divalent ions on inhibition of fibrinogen binding to platelets by the peptide

Divalent ion	^{125}I-Fibrinogen bound, molecules/platelet		Inhibition of fibrinogen binding, %
	Without peptide	With peptide	
Ca^{2+}, 1 mM	21,400	10,500	50.9
Ca^{2+}, 5 mM	14,200	7,270	48.8
Mg^{2+}, 1 mM	17,200	8,300	51.7

Binding measured at 30 min with Gly-Pro-Arg-Pro present at a 30-fold molar excess of ^{125}I-fibrinogen.

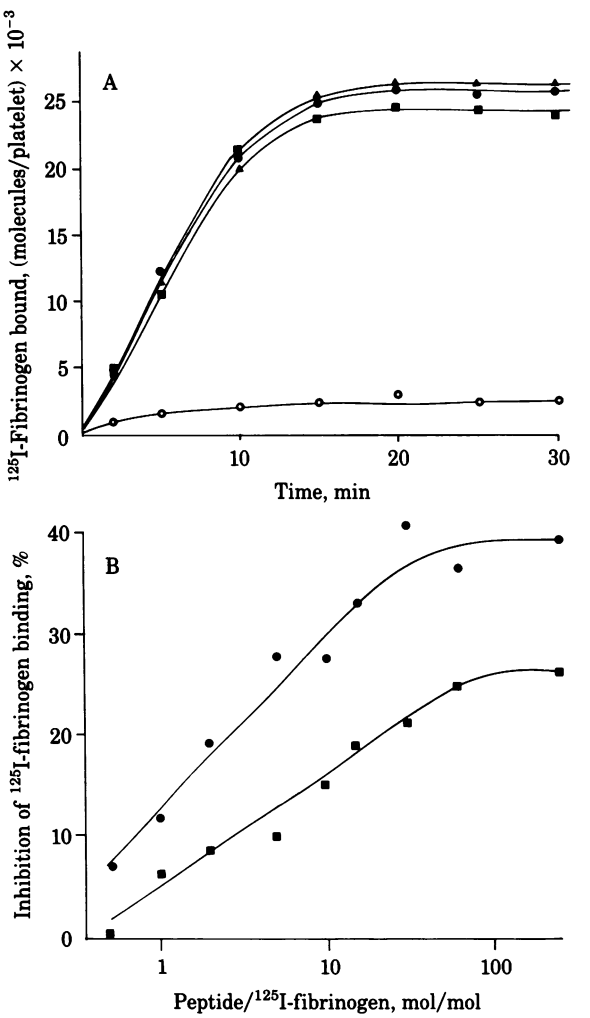

FIG. 3. Inhibition of ^{125}I-fibrinogen binding to paraformaldehyde-fixed platelets by Gly-Pro-Arg-Pro. (*A*) Fibrinogen-binding properties of the fixed cells. ADP-fixed platelets (●) were stimulated with 12.5 μM ADP for 5 min prior to fixation. The effect of an additional 12.5 μM ADP (▲) or the ADP scavenger system, phosphocreatine/creatine phosphokinase (■), added to the ADP-fixed platelets is shown, as is the fibrinogen binding to nonstimulated fixed platelets (○). (*B*) Inhibition of ^{125}I-fibrinogen binding by increasing concentrations of Gly-Pro-Arg-Pro to ADP-fixed (■) and nonfixed ADP-stimulated platelets (●) from the same donor.

generically similar but more extensive. Similar results were observed with five different preparations of ADP-fixed platelets.

Paralleling the inhibition of fibrinogen binding by the peptide, an effect of Gly-Pro-Arg-Pro on ADP-induced platelet aggregation was observed (Fig. 4). When the peptide was present in a 10-fold molar excess of fibrinogen, platelet shape change and the initial rate of platelet aggregation induced by ADP were unaltered, but the maximal change in light transmission was decreased by ≈50%. At a 30-fold molar excess, the peptide completely inhibited the platelet aggregation, but shape change was not affected.

Stimuli other than ADP can support the binding of fibrinogen to platelets, and the capacity of Gly-Pro-Arg-Pro to inhibit fibrinogen binding induced by epinephrine and thrombin as well as by ADP was compared. The inhibition produced by a 30-fold molar excess of the peptide was 37.3%, 54.2%, and 48.3% with ADP-, epinephrine-, and thrombin-stimulated platelets, respectively. These values were derived when equilibrium binding of ^{125}I-fibrinogen with each stimulus had been attained: 30 min for thrombin (8) and ADP (1, 3) and 120 min for epinephrine (6). With each stimulus, the extent but not the rate of ^{125}I-fibrinogen binding was affected by the peptide.

In preliminary experiments, we have examined the inhibitory capacity of selected peptide analogs. Gly-Pro-Arg-Pro is similar to the sequence Gly-Pro-Arg-Val-Val which is found in the amino-terminal region of the Aα chain of fibrinogen. In a 30-fold molar excess of ^{125}I-fibrinogen, Gly-Pro-Arg-Val-Val produced 32% inhibition compared to 40% inhibition by Gly-Pro-Arg-Pro. The maximal inhibition by Gly-Pro-Arg-Val-Val was 45% as compared to 60% for Gly-Pro-Arg-Pro. Methylation of the glycyl residue of Gly-Pro-Arg-Pro resulted in complete abrogation of inhibitory capacity (no inhibition of binding in a 60-fold molar excess of ^{125}I-fibrinogen).

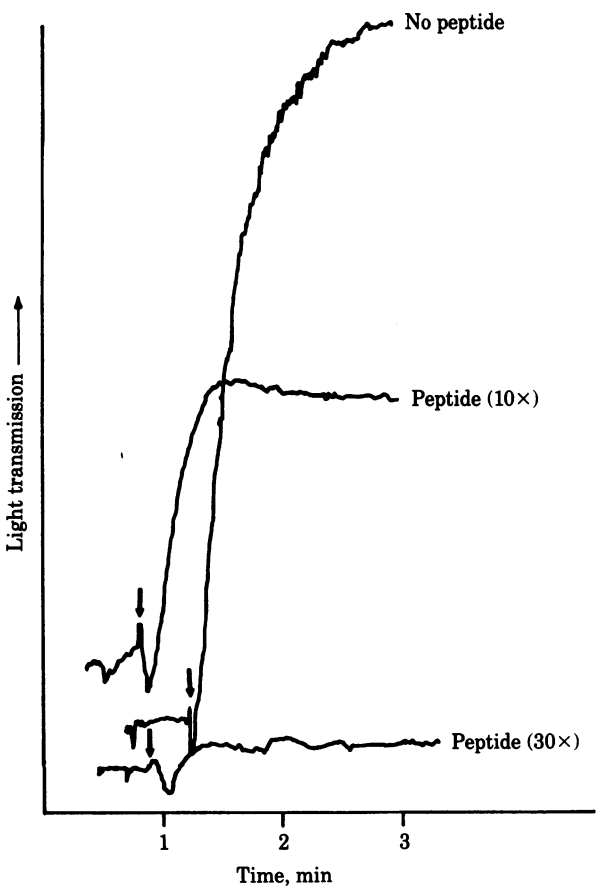

FIG. 4. The effect of Gly-Pro-Arg-Pro on ADP-induced aggregation of washed human platelets. Each vertical arrow indicates the time of addition of 3 μM ADP to 2 × 10^8 platelets per ml in Tyrode's buffer (pH 7.2) containing 2% albumin, 1 mM calcium, and 0.7 μM fibrinogen and with the peptide absent or present in 10- or 30-fold molar excess of fibrinogen.

DISCUSSION

In this study, the Gly-Pro-Arg-Pro peptide has been shown to inhibit the binding of fibrinogen to washed human platelets. The primary effect of the peptide was on the extent of fibrinogen binding, but the rate of interaction was not altered. Significant inhibition of binding was observed at molar ratios of peptide to fibrinogen as low as 1:1, and maximal inhibition was attained at 100:1 ratio. From the postulated interrelationship between fibrinogen binding and platelet aggregation, an inhibition of the interaction of the molecule with its platelet receptor by the tetrapeptide should result in an attenuation of aggregation. This was born out experimentally as a 30-fold molar excess of peptide abolished platelet aggregation. Because this concentration of peptide produced only a 40% inhibition of fibrinogen binding, this suggests that a significant proportion of the fibrinogen receptors must be occupied to produce macroscopic platelet aggregation as detected in the aggregometer.

The inhibition of fibrinogen binding to the platelet could arise from an effect of the peptide on platelet stimulation, induction of the fibrinogen receptor, or a direct interference with the binding of the molecule to its exposed receptor. Several lines of evidence suggest that the peptide may directly block the binding of fibrinogen to the cell. From the platelet-aggregation patterns, it was apparent that platelet shape change, which requires the interaction and stimulation of the cell by ADP, was not affected by the peptide, but platelet aggregation, which requires the binding of fibrinogen to the cell, was inhibited. In addition, the peptide inhibited fibrinogen binding supported by thrombin, epinephrine, and ADP—platelet agonists which interact and stimulate with the platelet by diverse mechanisms. Therefore, platelet stimulation does not appear to be the primary target of the inhibitory effect of the peptide. Additionally, the peptide inhibited fibrinogen binding to ADP-fixed platelets. With these cells, fibrinogen binding is independent of platelet stimulation and receptor induction, and the inhibitory effect of the peptide suggests the direct interaction of fibrinogen with the cell is affected. Because the inhibition by Gly-Pro-Arg-Pro with ADP-fixed cells was less extensive than with nonfixed cells, the peptide may also secondarily alter receptor induction.

Certain trivial explanations for the inhibitory effect of the peptide have been excluded. First, inhibition of fibrinogen binding was not attributable to an effect on the divalent ion concentration. Second, the inhibition did not result from an effect on fibrin formation by thrombin *per se*. The thrombin inhibitor hirudin did not interfere with fibrinogen binding to the platelet and did not alter the inhibitor capacity of the peptide. This is consistent with our previous data indicating that the molecule bound to the platelet is fibrinogen and not fibrin based on fibrinopeptide A content (1). Third, propyl- or arginyl-containing peptides are not nonspecific inhibitors of fibrinogen binding, as methylation of the amino-terminal glycyl residue of the Gly-Pro-Arg-Pro resulted in the complete abrogation of inhibitory activity. The requirement for an unmodified amino-group for the inhibition of fibrinogen binding to platelets is paralleled by a same requirement for the inhibition of fibrin polymerization by the peptide (26).

Gly-Pro-Arg-Pro is an analog of the sequence Gly-Pro-Arg-

Val located within the Aα chain of fibrinogen. This sequence becomes the new amino-terminal aspect of the α-chain of fibrin when fibrinopeptide A is cleaved from fibrinogen by thrombin. The observation that Gly-Pro-Arg-Val-Val also inhibited fibrinogen binding to the platelet strongly suggests that the inhibitory effect of Gly-Pro-Arg-Pro rests upon its homology with the natural sequence of fibrinogen. Within the context of this homology, two potential mechanisms may be considered to explain the inhibitory effect of the peptide on fibrinogen binding to the platelet. First, the peptide may be homologous to the platelet binding site within fibrinogen. This would place the recognition site for the platelet within the E (amino-terminal) domain of fibrinogen. Second, the peptide may bind to fibrinogen and inhibit its capacity to associate with the platelet. As an analog of the fibrin polymerization sites, Gly-Pro-Arg-Pro has been shown to bind to two or three sites within the D (carboxy-terminal) domain of fibrinogen (18–20). If this is the mechanism of inhibition, the platelet-binding site within fibrinogen would be postulated to reside in the D domain. The observation that the inhibitory effect of the peptide increased with increasing fibrinogen concentrations would seem to favor an inhibitory mechanism involving the binding of the peptide to fibrinogen rather than to the platelet receptor. The affinity constant for the binding of the peptide to fibrinogen has been estimated to be approximately $5 \times 10^4\ M^{-1}$ (18–20). Under the conditions of the platelet-binding assay, a 10-fold molar excess of peptide to ^{125}I-fibrinogen should result in ≈32% of the ligand being associated with peptide, and the inhibition of binding was 36%. However, at higher concentrations of the peptide this direct relationship was not maintained. Prolyl residues may exist in *cis*- or *trans*-conformations (27), and their configuration in the peptide may affect these stoichiometric considerations. It must also be considered that multiple sites within the D and E domains of fibrinogen may interact with the platelet, and the inhibition by the peptide may result from a combination of the effects cited above. This would be analogous to the participation of multiple sites in fibrin polymerization (28).

The failure of Gly-Pro-Arg-Pro to produce complete inhibition of fibrinogen binding warrants consideration. Because the affinity of fibrinogen for the platelet is two orders of magnitude greater than that of the peptide for fibrinogen, it may not be possible for the peptide to completely inhibit fibrinogen binding to the platelet. Alternatively, receptor heterogeneity with the peptide blocking the interaction of fibrinogen with only one receptor system could also result in partial inhibition. Because Gly-Pro-Arg-Val-Val produced a maximal inhibition of 45% as contrasted to the 60% maximum produced by Gly-Pro-Arg-Pro, the former interpretation rather than receptor heterogeneity is favored.

All the proposed interpretations for basis of the inhibitory activity of the peptides imply that the sites within fibrinogen recognized by the platelet reside within the plasmin-resistant D:E core, yet the direct binding of D or E fragments to platelets has not been demonstrable. This may be explained in part by the nature and heterogeneity of these plasmic degradation products. With extensive plasmic digestion in the absence of calcium, the D fragment loses a polymerization site located in the carboxy-terminal region of the γ-chain (29) and the E fragment may lose the amino-terminal region of the Aα chain containing Gly-Pro-Arg (30). In support of the role of the D or E domains (or both), Tomikawa *et al.* (13) found that both derivatives inhibited ADP-induced platelet aggregation. Although the precise mechanism of the inhibitory effect of the peptide remains to be established, the data suggest that common structural features within fibrinogen permit the molecule to participate both in fibrin polymerization and in platelet aggregation. The expression of multiple functional activities through a single structurally conserved region of the molecule (19) is highly attractive from the standpoint of evolutionary development.

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1. Marguerie, G. A., Plow, E. F. & Edgington, T. S. (1979) *J. Med. Chem.* **254**, 5357–5363.
2. Bennett, J. S. & Vilaire, G. (1979) *J. Clin. Invest.* **64**, 1393–1401.
3. Marguerie, G. A., Edgington, T. S. & Plow, E. F. (1980) *J. Biol. Chem.* **255**, 154–161.
4. Peerschke, E. I., Zucker, M. B., Grant, R. A., Egan, J. J. & Johnson, M. M. (1980) *Blood* **55**, 841–847.
5. Harfenist, E. J., Packham, M. A. & Mustard, J. F. (1980) *Blood* **56**, 189–198.
6. Plow, E. F. & Marguerie, G. A. (1980) *J. Biol. Chem.* **255**, 10971–10977.
7. Hawiger, J., Parkinson, S. & Timmons, S. (1980) *Nature (London)* **283**, 195–197.
8. Plow, E. F. & Marguerie, G. A. (1980) *Blood* **56**, 553–555.
9. Niewiarowski, S., Budzynski, A. Z., Morinelli, T. A., Brudzynski, T. M. & Stewart, G. J. (1981) *J. Biol. Chem.* **256**, 917–925.
10. Doolittle, R. F., Watt, K. W. K., Cottrell, B. A., Strong, D. D. & Riley, M. (1979) *Nature (London)* **280**, 464–468.
11. Niewiarowski, S., Budzynski, A. Z. & Lipinski, B. (1977) *Blood* **49**, 635–644.
12. Budzynski, A. Z., Joseph, R. T., Olexa, S. A. & Niewiarowski, S. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 4043 (abstr.).
13. Tomikawa, M., Iwamoto, M., Soderman, S. & Blombäck, B. (1980) *Thromb. Res.* **19**, 841–855.
14. Holt, J. C., Mahmoud, M. & Gaffney, P. (1979) *Thromb. Res.* **16**, 427–435.
15. Soria, J., Soria, C., Bertrand, O. & Samama, M. (1978) *Biochem. Biophys. Res. Commun.* **82**, 442–450.
16. Coller, B. S. (1979) *Blood* **53**, 325–332.
17. Harfenist, E. J., Packham, M. A. & Mustard, J. F. (1980) *Thromb. Res.* **20**, 353–358.
18. Laudano, A. P. & Doolittle, R. F. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3085–3089.
19. Laudano, A. P. & Doolittle, R. F. (1980) *Biochemistry* **19**, 1013–1019.
20. Laudano, A. P. & Doolittle, R. F. (1981) *Science* **212**, 457–459.
21. Kekwick, R. A., McKay, M. E., Nance, M. H. & Record, B. R. (1955) *Biochem. J.* **60**, 671–683.
22. Doolittle, R. F., Schubert, D. & Schwartz, S. A. (1967) *Arch. Biochem. Biophys.* **118**, 456–467.
23. Marguerie, G. A. & Plow, E. F. (1981) *Biochemistry* **20**, 1074–1080.
24. Kinlough-Rathbone, R. L., Chahil, A., Perry, D. W., Packham, M. A. & Mustard, J. F. (1979) *Blood* **54**, Suppl. 1, 249 (abstr.).
25. Peerschke, E. A. & Zucker, M. B. (1981) *Blood* **57**, 663–670.
26. Hsieh, K., Mudd, M. S. & Wilner, G. D. (1981) *J. Biol. Chem.* **24**, 322–327.
27. Liakopoulou-Kyriakides, M. & Galardy, R. E. (1979) *Biochemistry* **18**, 1952–1957.
28. Olexa, S. A. & Budzynski, A. Z. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1374–1378.
29. Olexa, S. A. & Budzynski, A. Z. (1981) *J. Biol. Chem.* **256**, 3544–3549.
30. Wallen, P. (1971) *Scand. J. Haematol. Suppl.* **13**, 3–14.