The Peptide Glu-His-Ile-Pro-Ala Binds Fibrinogen and Inhibits Platelet Aggregation and Adhesion to Fibrinogen and Vitronectin

(43303)

T. KENT GARTNER[1] AND DONALD B. TAYLOR
Department of Biology, Memphis, State University, Memphis, Tennessee 38152

Abstract. **Antiplatelet agents are clinically useful as antithrombotic entities. The importance of antiplatelet agents led us to design, synthesize, and characterize a new antiplatelet peptide. This peptide is a presumptive mimic of a ligand binding site on the platelet fibrinogen receptor. Unlike peptides related to Arg-Gly-Asp-Ser and His-His-Leu-Gly-Gly-Ala-Lys-Gln-Ala-Gly-Asp-Val that bind to the fibrinogen receptor, this peptide binds to fibrinogen. The anticomplementarity hypothesis was used to design this presumptive peptide mimic of the vitronectin binding site on the fibrinogen receptor, glycoprotein IIb/IIIa complexes. The resulting peptide (Glu-His-Ile-Pro-Ala) has the characteristics of a fibrinogen binding site mimic: It binds fibrinogen and inhibits both the adhesion of platelets to fibrinogen and platelet aggregation. The peptide also inhibits the adhesion of platelets to vitronectin. The antiplatelet activity of this mimic peptide was dependent on its amino acid sequence, since closely related analogues were either inactive or less active inhibitors of platelet function than the original peptide. These results demonstrate that the peptide Glu-His-Ile-Pro-Ala has the characteristics expected of a mimic of a glycoprotein IIb/IIIa ligand binding site.** [P.S.E.B.M. 1991, Vol 198]

Antiplatelet agents are clinically important because they increase the success rate of other antithrombotic treatments such as angioplasty (1, 2). Therefore, peptides that inhibit the binding of fibrinogen (Fg) and other ligands to the platelet Fg receptor, the glycoprotein IIb/IIIa complex (GP IIb/IIIa), may have the potential of being clinically useful antiplatelet agents. In this regard, we recently presented data which demonstrate that the amino acid sequence Gly-Ala-Pro-Leu (GAPL, residues 309–312 of glycoprotein IIb) appears to be a Fg binding domain of the Fg receptor (3). The anticomplementarity hypothesis (4) was used to identify the GAPL sequence as a presumptive Fg binding domain (3). Here, these studies are extended to the ligand binding domain predicted by the Arg-Gly-Asp-Val (RGDV) sequence of vitronectin (Vn).

According to the anticomplementarity hypothesis, if the nucleotide sequence of the messenger RNA for the receptor binding domain of a ligand is known, this information can be used to predict the amino acid sequence of the ligand binding domain of the receptor, and vice versa (4). Although the theoretical basis for the anticomplementarity hypothesis is not understood, the hypothesis has been applied successfully to identifying the amino acid sequences of the cystatin C binding site on the fourth component of complement (5) and a fibrinogen binding domain of GP IIb (3). Because of these successes and the potential importance of antiplatelet peptides, the hypothesis was used in this study to predict a putative binding site for the RGDV sequence of Vn on GP IIb/IIIa (6), the assumption being that a peptide mimic of the presumptive Vn receptor ligand binding site might be an antiplatelet agent.

The rationale for this assumption is based on the fact that at least four ligands, Fg, fibronectin, Vn, and von Willebrand factor, bind to the Fg receptors on platelets (7). Synthetic peptides corresponding to the RGDX sequences of these ligands can inhibit platelet

[1] To whom correspondence and requests for reprints should be addressed at Department of Biology, Memphis State University, Memphis, TN 38152.

Received March 15, 1991. [P.S.E.B.M. 1991, Vol 198]
Accepted May 29, 1991.

0037-9727/91/1981-0649$3.00/0

aggregation by blocking the binding of Fg to its receptors (7). For example, a peptide corresponding to a presumed cell binding site of Vn (residues 45–48, RGDV) inhibits Fg binding to platelets (8). The simplest interpretation of the ability of RGDV to inhibit Fg binding to platelets is that it binds to the same site on GP IIb/IIIa as the α-chain of Fg. With this view in mind, the nucleotide sequence of the mRNA for the RGDVF (Phe) sequence of Vn was used as the basis for designing an antiplatelet peptide. According to the hypothesis, the resulting peptide should correspond functionally to a Vn binding site on GP IIb/IIIa.

Materials and Methods

Design of the Fibrinogen-Binding Peptide. The anticomplementarity hypothesis (4) was used to design a peptide that hypothetically corresponds to a Vn binding site on its receptor. Presumably, residues 45–48 (amino acid sequence RGDV) of Vn constitute at least part of the Vn binding site for GP IIb/IIIa and the Vn receptor (6). The nucleotide sequence for RGDVF in the mRNA for Vn is shown in Figure 1. The complement of this nucleotide sequence is labeled cRNA in Figure 1. Translation of this cRNA in the 5′–3′ direction generates a peptide with the amino acid sequence Glu-His-Ile-Pro-Ala (EHIPA). The anticomplementarity hypothesis predicts that this peptide corresponds functionally to a Vn binding domain of a Vn receptor.

Peptide Preparation. Peptides were synthesized in our laboratory using a PSS-80 automated peptide synthesizer (Applied Protein Technologies, Cambridge, MA), cleaved from the Merrifield resins with trifluoromethane sulfonic acid, and purified, with amino acid compositions and sequences confirmed, as described previously (3, 9, 10). Prior to use, 10 m*M* stock solutions of individual peptides were made in 0.01 *M* acetic acid and stored at −20°C.

Materials. Human α-thrombin was generously donated by Dr. J. W. Fenton II, New York State Department of Health, Albany, NY. Purified human Peak I fibrinogen was a kind gift from Dr. D. L. Amrani, Department of Medicine, University of Wisconsin, Milwaukee, WI. The Vn was a generous gift of Dr. Deane Mosher and Steve Bittorf, Department of Medicine, University of Wisconsin, Madison, WI. ADP, L-epinephrine, and (bovine serum albumin BSA) were from the Sigma Chemical Co. (St. Louis, MO). [^{14}C]-5-hydroxytryptamine binoxalate (1.7 GBq/mmol) was from Dupont NEN (Wilmington, DE). Sodium chromate (^{51}Cr, 250–500 mCi/mg Cr) was from the Amersham Corp. (Arlington Heights, IL). *t*-Butyloxycarbonyl amino acids and Merrifield resins for synthetic solid phase peptide synthesis were from Bachem Inc. (Torrance, CA) and Peninsula Labs (Belmont, CA). All other reagents were of analytical or reagent grade.

Antibodies. Rabbit anti-Fg sera were prepared as described (3). Sera were reconstituted in phosphate-buffered saline (8.77 g of NaCl, 1.15 g of Na_2HPO_4, and 0.2 g of KH_2PO_4/liter, pH 7.4) after $(NH_4)_2SO_4$ precipitation (40%). Anti-Fg IgG fractions were made monospecific by affinity chromatography (3). Both immune and preimmune antibodies against purified human Fg were titered as described (3).

Platelet Isolation. Whole blood obtained from aspirin-free human donors was drawn into acid citrate dextrose (39 m*M* citric acid, 75 m*M* sodium citrate, and 135 m*M* glucose, pH 4.5). Washed platelets were prepared and tested in aggregation studies as described (3, 11). Platelet-rich plasma (PRP) was obtained from blood collected in White's anticoagulant (0.249 g of sodium citrate and 0.245 g/liter of glucose, pH 6.4) (12). For aggregation studies, 0.3 ml of PRP was mixed with 0.15 ml of phosphate-buffered saline or Tyrode's solution and the indicated amounts of peptides were added prior to initiation of aggregation by ADP. For the ristocetin studies, 0.3 ml of PRP was mixed with 0.15 ml of phosphate-buffered saline or Tyrode's solution and the required amounts of ristocetin and peptides. Release of serotonin was estimated as described (13). Aggregation-driven release was determined by the method of Coller *et al.* (14).

Platelet Adhesion. The platelet adhesion assays were performed as described (3). Labeled platelets were stimulated, first with epinephrine (5 μM) for 5 min and then with ADP (30 μM) for 5 min, prior to use (3). Assays of platelet adhesion to Vn were performed as described with the following modifications. For these assays, the divalent cation Mn^{2+} (10 μM) was substituted for Mg^{2+} and Ca^{2+}, as suggested by Dr. T. J. Kunicki (personal communication). Also, adhesion was allowed to proceed for 90 min, not 30 min as with Fg, prior to termination of the assay. Two types of controls were used as indicators of nonspecific binding in these assays. Activated platelets were allowed to adhere to wells coated with BSA. As the second indicator of nonspecific binding, aliquots of platelets were incubated at 37°C at pH 7.8 in the presence of 5 m*M* EDTA for 15 min to irreversibly inactivate GP IIb/IIIa complexes, thereby minimizing adhesion to Fg mediated by its receptor (15). The value for this binding was subtracted from the total binding to give specific binding. Typically, 5–8% of the platelets present in the initial suspension adhered to the protein-coated wells.

Binding of Fibrinogen to the Peptide. Fibrinogen binding to EHIPA was characterized with a modified enzyme-linked immunoabsorbent assay (ELISA), as described (3, 16). Peptides were incubated in 96-well, flatbottomed, Pro-Bind ELISA microtiter plates (Falcon). After drying, washing, and blocking the wells with BSA, as described (3), each well was incubated with 10 μg/0.2 ml Peak 1 Fg for 2 hr at 37°C. After washing as described (3), 0.2 ml of a 1/200 dilution of monospe-

VN: ARG-GLY-ASP-VAL-PHE (RGDVF)

DNA: (3'-5') GCG-CCC-CTA-CAC-AAG (TEMPLATE STRAND)

mRNA: (5'-3') CGC-GGG-GAU-GUG-UUC

cRNA: (3'-5') GCG-CCC-CUA-CAC-AAG

PEPTIDE: GLU-HIS-ILE-PRO-ALA (EHIPA)

Figure 1. Design of the fibrinogen binding peptide. The method for translating the amino acid sequence of the receptor-binding domain of Vn (*RGD*VF) into a putative ligand binding site for the RGDVF sequence of Vn on GP IIb/IIIa. See Materials and Methods for details.

cific polyclonal anti-Peak 1 Fg IgG was added to each well and incubated for 1 hr at ambient temperature. After the described washing (3), goat anti-rabbit IgG horseradish peroxidase conjugate was used to measure Fg binding to the peptide. Specific binding was calculated as the difference between the total binding and the amount of binding of Fg to wells that were coated with BSA rather than EHIPA. All values are reported as specific binding unless otherwise noted. Further specificity of binding of EHIPA to Fg was determined by assaying the extent of BSA binding to the immobilized peptide using an ELISA. For these assays, casein, not BSA, was used to block the wells. An affinity purified anti-BSA rabbit IgG was used for these assays. All determinations were performed in triplicate. Statistical analyses were performed with the aid of the Statview 512^+ program and a Macintosh SE.

Results

A peptide with the sequence EHIPA was derived from the mRNA sequence for RGDV of Vn (see Materials and Methods and Fig. 1 for details). According to the anticomplementarity hypothesis, this peptide corresponds to a Vn binding domain of GP IIb/IIIa. Assuming that this peptide also corresponds to a Fg binding domain of GP IIb/IIIa, it might be able to bind Fg. Furthermore, if the peptide can bind Fg with a sufficient affinity, it might be able to inhibit Fg-dependent platelet function under appropriate conditions. These hypothetical functions of EHIPA were tested using three systems: platelet adhesion to Vn and Fg, platelet aggregation, and an ELISA evaluation of Fg binding.

The data presented in Figure 2 demonstrate that EHIPA specifically inhibits the adhesion of activated platelets to Vn. Notice that 1.2 m*M* EHIPA inhibited 54% of the specific adhesion of platelets to Vn. In contrast, the control peptides EHPIA, KHPIA, and EHGPA were without effect at this concentration. Thus, the ability of EHIPA to inhibit the adhesion of platelets to Vn is dependent upon the amino acid sequence of the peptide.

The hypothetical ability of the peptide EHIPA to bind Fg was also evaluated using the platelet adhesion assay. Consistent with this hypothesis, EHIPA at 1 m*M* inhibited 58% of the adhesion of the activated platelets to Fg (Fig. 3). This ability of EHIPA to inhibit the adhesion of platelets to Fg was concentration dependent (data not shown). In contrast, EHPIA, KHPIA, and APLSV, each at the same concentration as EHIPA, had little, if any, effect on adhesion. These results demonstrate that the ability of EHIPA to inhibit the adhesion of platelets to Fg is dependent upon the amino acid sequence of the peptide.

Since EHIPA can block the adhesion of platelets to Fg, the peptide was tested for the ability to inhibit platelet aggregation using both platelets in PRP stimu-

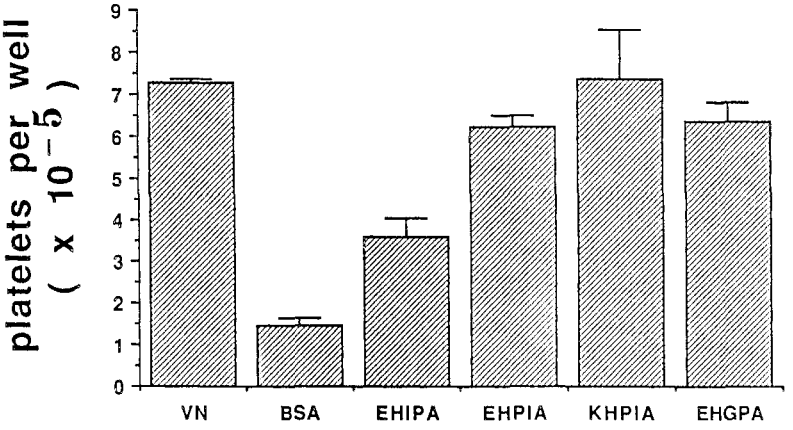

Figure 2. Inhibition of platelet adhesion to vitronectin by EHIPA. The adhesion of ^{51}Cr-labeled platelets to immulon-1 microtiter plates coated with Vn (0.2 ml of a 5 μg/ml solution) was determined as described in Materials and Methods. Platelets (1×10^7) were sequentially activated with epinephrine and ADP and assayed in the absence and presence of different peptides (1.2 m*M*). The number of platelets specifically bound per well is indicated on the ordinate. The bar labeled "VN" represents the extent of adhesion to the substrate in the absence of peptide. The bar labeled "BSA" represents the extent of adhesion to a BSA substrate. Error bars indicate SE of the data.

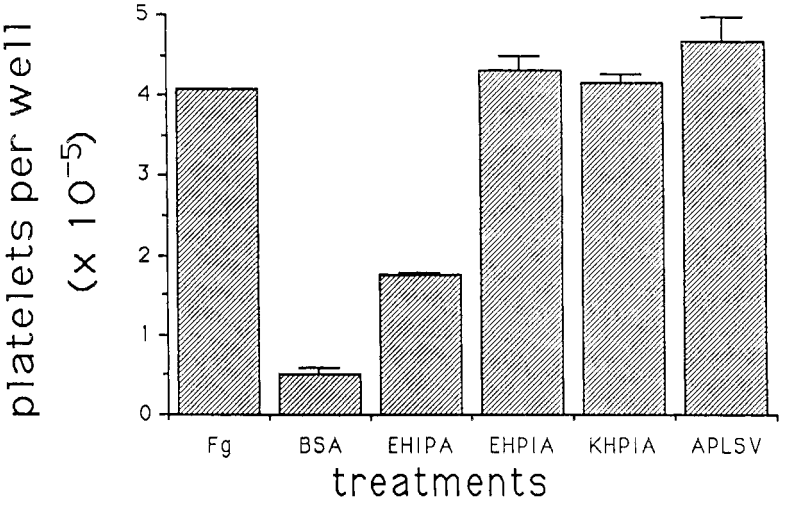

Figure 3. Inhibition of platelet adhesion to Fg by synthetic peptides. The adhesion of ^{51}Cr-labeled platelets in the presence of four different peptides (1 m*M*) was determined as described in Materials and Methods. The number of platelets specifically bound per well is indicated on the ordinate. The bar labeled "Fg" represents the extent of adhesion to the substrate in the absence of peptide. The bar labeled "BSA" represents the extent of adhesion to a BSA substrate. Error bars indicate SE of the data.

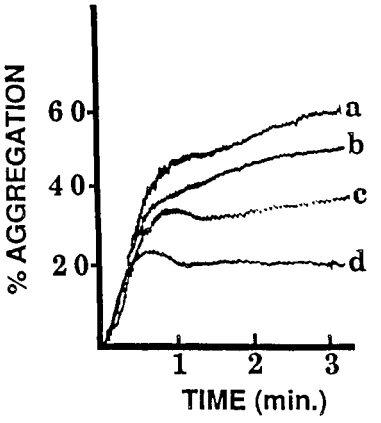

Figure 4. Effect of EHIPA on ADP-treated PRP. Platelets were activated with ADP (2.4 μm) in the presence or absence of peptide. Traces a through d correspond to platelet aggregation in the presence of: no addition, KHPIA (500 μm), EHPIA (630 μm), and EHIPA (400 μm), respectively.

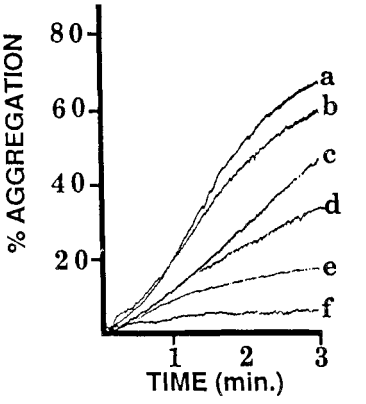

Figure 5. Inhibition of platelet aggregation by the peptide EHIPA. Washed platelets (2 × 10^8/ml) were stimulated with low-dose α-thrombin (0.054 units) in the presence and absence of increasing concentrations of peptide. Traces a through f correspond to: no addition, equal volume 0.01 *N* HoAc, EHIPA (100 μm), EHIPA (250 μm), EHIPA (400 μm), and EHIPA (500 μm), respectively).

lated with ADP and washed platelets stimulated with thrombin. The data in Figure 4 demonstrate that the ability of EHIPA to inhibit the aggregation of platelets in PRP was dependent upon the amino acid sequence of the peptide. These results reveal a similar dependence on amino acid sequence (EHIPA > EHPIA > KHPIA) as that required to inhibit the adhesion of platelets to Fg. The aggregation of washed platelets stimulated with thrombin was inhibited as a function of the concentration of the peptide present in the assay (Fig. 5). About 50% of the aggregation in response to 0.05 units/ml of thrombin was inhibited by 250 μM EHIPA.

Inhibition of platelet aggregation by EHIPA appears to result from a direct prevention of Fg binding to the platelets. This conclusion is supported by the data presented in Figure 6, which demonstrate that Fg binds to EHIPA in an ELISA. The binding of Fg approached saturation in wells coated with a solution containing 10 μg/ml of EHIPA (Fig. 6). Using this concentration of peptide to coat the well, Fg binding to EHIPA approached saturation when used at approximately 5 μg/ml in the fluid phase (data not shown). It is evident from the characterization of the ELISA that EHIPA and Fg bind to each other. The controls demonstrate that the binding of Fg to EHIPA is specific in the sense that BSA did not bind to EHIPA. Also, the first and second antibodies did not bind to EHIPA. These data demonstrate that the ability of EHIPA to inhibit the adhesion of platelets to Fg and platelet aggregation probably results from the ability of EHIPA to bind Fg.

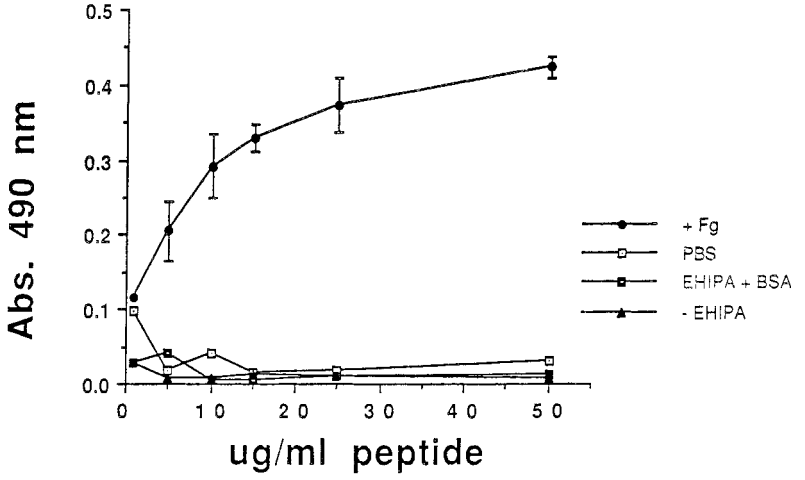

Figure 6. Binding of Fg by EHIPA. Microtiter wells were coated with the complementary peptide, EHIPA, in the indicated amounts. The bound Fg was determined by a modified ELISA, as described in Materials and Methods. The data points are average values of specifically bound Fg. Error bars indicate SE of the data.

Table I. Effect of EHIPA and EDTA on [^{14}C]-5-hydroxytryptamine Release from Platelets Treated with Low-Dose α-Thrombin[a]

Additions	EDTA	Release (cpm)	Percentage of aggregation
Tyrode's	−	675	60
Tyrode's	+	376	18
APLSV	−	632	56
EHIPA	−	246	18
APLSV	+	348	11
EHIPA	+	289	8

[a] Washed platelets (2.0×10^8/ml) in Ca^{2+}-free Tyrode's solution were stimulated at 37°C and secretion was measured as described in Materials and Methods. The average level of release and aggregation in response to 0.05 units of thrombin is indicated as Tyrode's solution without EDTA (e.g., Line 1) in the table. In some experiments, EDTA (5 m*M* final concentration) was included to assess whether inhibition of release by EHIPA occurs as a consequence of inhibition of aggregation-driven release at low levels of agonist. All peptides were tested at a final concentration of 400 μm. Results are the means of four experiments.

Discussion

The results described here demonstrate that the peptide EHIPA can bind Fg. This was demonstrated directly using ELISA and indirectly in platelet adhesion and aggregation studies. The ELISA using wells coated with the peptide EHIPA revealed the ability of this peptide to bind Fg. The binding of Fg to the wells was not dependent upon divalent cations (data not shown), but was dependent upon the concentration of the peptide present in the solution used to coat the wells, time, and temperature of incubation (data not shown). The ability to bind Fg is a characteristic expected of a presumptive peptide mimic of the Fg binding site of the Fg receptor.

The physiological relevance of the ability of EHIPA to bind Fg was tested by using the peptide in assays of platelet function. With this objective in mind, the peptide was tested as an inhibitor of the adhesion of platelets to Fg. Platelets were stimulated sequentially with epinephrine and ADP to activate them while minimizing fibrin formation. Under these conditions, EHIPA (1 m*M*) inhibited adhesion of the platelets to Fg by 58%. In contrast, the control peptides EHPIA, KHPIA, and APLSV were without effect at the same concentration (Fig. 5). Presumably, EHIPA inhibited the adhesion of platelets to Fg and Vn by blocking the binding of the ligands to the platelets.

Platelet aggregation was the second physiological assay used to test the peptides for function. The peptides described above were tested in this assay (Figs. 4 and 5). Once again, EHIPA was the most potent inhibitor of function. In this assay, it is assumed that the ability of EHIPA to inhibit platelet aggregation results from its ability to bind to Fg. The assumption is that Fg complexed with EHIPA is unable to support platelet aggregation. This assumption is supported by the observation that EHIPA inhibits the adhesion of platelets to Fg and binds to Fg (Figs. 5 and 6).

The data in Table I do not exclude the possibility that the peptide EHIPA inhibits platelet activation. However, inhibition of activation may not be the sole explanation for the ability of EHIPA to inhibit platelet aggregation, since the peptide does bind Fg and can directly inhibit the binding of Fg to platelets. This is evident from the ELISA data (Fig. 6) and the fact that EHIPA inhibited the adhesion of platelets to Fg (Fig. 3) and Vn (Fig. 2), even though those platelets were activated by two agonists for 10 min prior to exposure to the peptide. Thus, inhibition of adhesion of platelets to Fg and Vn was a postactivation event.

Finally, the specificity of the ability of EHIPA to inhibit platelet aggregation is revealed by the inability

of EHIPA to inhibit ristocetin- (17) generated agglutination of platelets in PRP (data not shown). This result means that EHIPA is not a general nonspecific inhibitor of direct platelet to platelet interaction. Thus, the behavior of EHIPA in the platelet aggregation assays is consistent with that of a peptide mimic of a ligand binding site of the Fg receptor.

Brentani *et al.* (18) and Pasqualini *et al.* (19) have reported that a peptide with the amino acid sequence GAVSTA is predicted using the anticomplementarity hypothesis and the nucleotide sequence of the mRNA for the GRGDSP sequence in human fibronectin. This peptide, but not a similar peptide (GATSTA), inhibited the binding of purified GP IIb/IIIa complexes to fibronectin. A comparison of the hydropathy plots of EHIPA and GAVSTA reveals that the plots are not congruent (data not shown). Thus, it may be that these two peptides correspond to different regions of the GP IIb/IIIa complex. Pasqualini *et al.* (19) noted that the amino acid sequence GVSS is predicted by the RGDS sequence in the α-chain of Fg. The hydropathy plots for GVSS and AVST (from GAVSTA) are nearly congruent (19). Interestingly, the sequence GVSS is near the amino terminus of GP IIIa as residues 9–12; this sequence is not proximal to the region of GP IIIa (residues 109–171) that was cross-linked by an RGDS-containing peptide (20).

The data presented here demonstrate the successful application of the anticomplementarity hypothesis in designing a peptide which has the potential of being an antithrombotic agent. The peptide behaves as a functional mimic of a Fg binding domain of the Fg receptor, despite the fact that the sequence EHIPA is not present in GP IIb/IIIa (21, 22). However, the hydropathy plots for the sequences GAPL, a sequence which appears to be a Fg binding domain of GP IIb (3),and IPAS (of EHIPAS, S being the next residue predicted for this sequence) are congruent (data not shown). Thus, GAPL and IPAS are hydropathically equivalent. Interestingly, the peptide characterized here works by a mechanism distinct from that of RGDS (13) and HHLGGAKQAGDV (23): It binds to Fg. Unlike RGDS- and HHLGGAKQAGDV-related peptides, this class of antithrombotic agent has the potential of being used as a local, as well as a systemic, antiplatelet agent. The antithrombotic potential of EHIPA has been demonstrated by Johnson *et al.* (24). They have demonstrated that EHIPA treatment of Fg-coated polyethylene conduits decreased the extent of platelet adhesion to these surfaces by approximately 50% (24).

We thank Dr. David Amrani for the gift of Peak 1 fibrinogen, Dr. John Fenton II for the α-thrombin, and Dr. Deane Mosher and Steve Bittorf for the vitronectin. This work was supported by Grant R15 HL42523 from the National Institutes of Health and by a grant from the American Heart Association, Tennessee Affilitate.

1. Harker LA. Antithrombotic therapy. In: Williams JW, Beutler E, Erslev AJ, Lichtman MA, Eds. Hematology, 4th ed. New York: McGraw-Hill, p1569, 1990.
2. Harker LA. Role of platelets and thrombosis in mechanisms of acute occlusion and restenosis after angioplasty. Am J Cardiol **60**:820–828, 1987.
3. Gartner TK, Taylor DB. The amino acid sequence gly-ala-pro-leu appears to be a fibrinogen binding site in the platelet integrin, glycoprotein IIb. Thromb Res **60**:291–309, 1990.
4. Bost KL, Blalock, JE. Preparation and use of complementary peptides: In: Conn PM, Ed. Methods in Enzymology. New York: Academic Press, Vol **168:** p16, 1989.
5. Ghiso J, Saball E, Leoni J, Rostagno A, Frangione B. Binding of cystatin C to C4: The importance of sense-antisense peptides in their interaction. Proc Natl Acad Sci USA **87**:1288–1291, 1990.
6. Suzuki S, Oldberg A, Hayman EG, Pierschbacher MD, Ruoslahti E. Complete amino acid sequence of human vitronectin deduced from cDNA. Similarity of cell attachment sites in vitronectin and fibronectin. EMBO J **4**:2519–2524, 1985.
7. Kieffer N, Phillips DR. Platelet membrane glycoproteins: Functions in cellular interactions. In: Palade GE, Alberts BM, Spudich JA, Eds. Annual Review of Cell Biology. California: Annual Reviews, Vol. **6:** p329, 1990.
8. Thiagarajan P, Kelly K. Exposure of binding sites for vitronectin on platelets following stimulation. J Biol Chem **263**:3035–3040, 1987.
9. Tam JP, Heath WF, Merrifield RB. Mechanisms for the removal of benzyl protecting groups in synthetic peptides by trifluoromethanesulfonic acid-trifluoroacetic acid-dimethyl sulfide. J Am Chem Soc **108**:5242–5251, 1986.
10. Bennett JS, Shattil SJ, Power JW, Gartner TK. Interaction of fibrinogen with its platelet receptor. J Biol Chem **263**:12948–12953, 1988.
11. Gartner TK, Walz DA. TFP inhibits the expression of the endogenous platelet lectin and secretion of α-granules. Thromb Res **29**:63–74, 1983.
12. Gerrard JM, Phillips DR, Rao GHR, Plow EF, Walz DA, Ross R, Harker LA, White JG. Biochemical studies of two patients with gray platelet syndrome-selective deficiency of platelet alpha granules. J Clin Invest **66**:102–109, 1980.
13. Gartner TK, Bennett JS. The tetrapeptide analogue of the cell attachment site of fibronectin inhibits platelet aggregation and fibrinogen binding to platelets. J Biol Chem **260**:11891–11894, 1985.
14. Coller BS, Peerschke EI, Scudder LE, Sullivan CA. A murine monoclonal antibody that completely blocks the binding of fibrinogen to platelets produces a thrombasthenic-like state in normal platelets and binds to glycoproteins IIb and/or IIIa. J Clin Invest **72**:325–338, 1983.
15. Zucker MB, Grant RA. Nonreversible loss of platelet aggregability induced by calcium deprivation. Blood **52**:505–514, 1978.
16. Engvall E, Perlman P. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay for immunoglobulin G. Immunochemistry **8**:871–875, 1971.
17. Muranog G, Bick RL. Basic concepts of hemostasis and thrombosis. In: Triplett D, Ed. Platelet Disorders. Boca Raton, FL: CRC Press, p101, 1980.
18. Brentani RR, Ribeiro SF, Potoncnjak P, Pasqualini R, Lopes JD, Nakaie CR. Characterization of the cellular receptor for fibronectin through a hydropathic complementarity approach. Proc Natl Acad Sci USA **85**:364–367, 1988.
19. Pasqualini R, Chamone DF, Brentani RR. Determination of the putative binding site for fibronectin on platelet glycoprotein IIb-IIIa complex through a hydropathic complementarity approach. J Biol Chem **264**:14566–14570, 1989.
20. D'Souza SE, Ginsberg MH, Burke TA, Lam SC-T, Plow EF.

Location of an arg-gly-asp recognition site within an integrin adhesion receptor. Science **242**:91–93, 1988.
21. Poncz M, Eisman R, Heidenreich R, Silver SM, Vilaire G, Surrey S, Schwartz E, Bennett JS. Structure of the platelet membrane glycoprotein IIb. Homology to the α-subunits of the vitronectin and fibronectin membrane receptors. J Biol Chem **262**:8476–8482, 1987.
22. Fitzgerald LA, Steiner B, Rall SC Jr, Lo S-S, Phillips DR. Protein sequence of endothelial glycoprotein IIIa derived from a cDNA clone. Identity with platelet glycoprotein IIIa and similarity to "integrin." J Biol Chem **262**:3936–3939, 1987.
23. Kloczewiak M, Timmons S, Lukas TJ, Hawiger J. Platelet receptor recognition site on human fibrinogen. Synthesis and structure-function relationship of peptides corresponding to the carboxy-terminal segment of the gamma chain. Biochemistry **23**:1767–1774, 1984.
24. Johnson PC, Taylor DB, Gartner TK. The peptide glu-his-ile-pro-ala, predicted by the codon sequence for arg-gly-asp-val in the vitronectin mRNA, diminishes platelet adhesion to fibrinogen coated polyethylene conduits. Blood **76**:511a, 1990.