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Platelet Receptor Recognition Domains on the α Chain of Human Fibrinogen: Structure–Function Analysis†

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Received September 1, 1988; Revised Manuscript Received December 1, 1988

ABSTRACT: We have previously shown that the α chain of human fibrinogen interacts directly with ADP-activated human platelets [Hawiger, J., Timmons, S., Kloczewiak, M., Strong, D. D., & Doolittle, R. F. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 2068]. Now, we report that platelet receptor recognition domains are localized on two CNBr fragments of the human fibrinogen α chain. They encompass residues 92–147 and 518–584, which inhibit ^{125}I-fibrinogen binding to ADP-stimulated platelets. The inhibitory CNBr fragment α92–147 contains the RGD sequence at residues 95–97. Synthetic peptides encompassing this sequence were inhibitory while peptide 99–113 lacking the RGD sequence was inactive. The synthetic peptide RGDF, corresponding to residues α95–98, inhibited the binding of ^{125}I-fibrinogen to ADP-treated platelets (IC_{50} = 2 μM). However, the peptides containing sequence RGDF, with residues preceding Arg^{95} or following Phe^{98}, were less inhibitory. It appears that the sequence α95–98 constitutes a platelet receptor recognition domain which is constrained by flanking residues. The second inhibitory CNBr fragment, α518–584, also contains the sequence RGD at positions 572–574. Synthetic peptides overlapping this sequence were inhibitory, while peptides lacking the sequence RGDS were not reactive. Thus, another platelet reactive site on the α chain encompasses residues 572–575 containing sequence RGDS. In conclusion, the platelet receptor recognition domains on the human fibrinogen α chain in the amino-terminal and in the carboxy-terminal zones contain the ubiquitous cell recognition sequence RGD shared with other known adhesive proteins. Together with a unique domain at the carboxy-terminal end of the γ chain, they endow human fibrinogen with six sites for interaction with activated platelets.

Human fibrinogen, a clottable and adhesive protein, is composed of three pairs of nonidentical chains (α, β, γ) linked by a series of disulfide bonds and arranged in three main structural domains: one central E and two distal D (Doolittle, 1984). We showed that both the γ chain and the α chain bear sites interacting with receptors on ADP-activated platelets (Hawiger et al., 1982). The γ-chain site has been subsequently pinpointed to the last 12 carboxy-terminal residues (Kloczewiak et al., 1984). Structure–function analysis of modified peptides patterned on the carboxy-terminal segment, γ 400–411, indicates that the continuous 12 amino acid sequence is required for optimal reactivity toward platelet receptors [see second of three papers in this issue (Kloczewiak et al., 1989)].

† This work was supported by Research Grant HL-33014 from the National Institutes of Health, U.S. Public Health Service.

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0006-2960/89/0428-2909$01.50/0

Localization of the site interacting with platelet receptors on the α chain was more elusive. Early experiments of Niewiarowski and colleagues (Niewiarowski et al., 1977) indicated that plasmin-treated fibrinogen rapidly loses its ability to support platelet aggregation, which was attributed to the loss of the carboxy-terminal segment of the α chain. However, the critical role of the carboxy-terminal portions of the α chain was subsequently questioned by Holt et al. (1979). In view of our previous findings that isolated α chains from human fibrinogen were reactive with ADP-activated platelets (Hawiger et al., 1982), we embarked on systematic studies of cyanogen bromide (CNBr)-derived fragments of human fibrinogen α chain. Using CNBr fragments and a series of overlapping synthetic peptide analogues, we were able to establish the locale of two separate platelet receptor recognition domains on the α chain of human fibrinogen. Both domains contain the sequence RGD identified previously as "the cell recognition site" of fibronectin (Pierschbacher & Ruoslahti, 1984). Synthetic peptide analogues containing the sequence RGD inhibited platelet aggregation and binding of fibrinogen, von Willebrand factor (vWF),[1] and fibronectin to activated platelets (Gartner & Bennet, 1985; Plow et al., 1985, 1987; Gardner & Hynes, 1985; Ginsberg et al., 1985; Haverstick et al., 1985).

We show that the α-chain-derived fragments of human fibrinogen containing sequence RGD are reactive with fibrinogen receptors (glycoproteins IIb and IIIa) on ADP-activated human platelets and that the context of sequence RGD in the α chain influences its receptor reactivity.

Materials and Methods

Human fibrinogen (Kabi) was purchased from Helena Laboratories, Beaumont, TX, and ^{125}I from Amersham. Bovine albumin for platelet preparation was reagent grade from ICN Biomedicals (Costa Mesa, CA). Boc-amino acids were from Vega (Tuscon, AZ) or Peninsula (San Carlos, CA). Reagents for peptide synthesis and purification were of high-pressure liquid chromatography (HPLC) quality.

Preparation of Human Platelet-Rich Plasma and Platelets Separated from Plasma Proteins. Blood was obtained from healthy volunteers who were fasting on the morning of blood collection and had abstained from taking aspirin or any other medication during the preceding 10 days. It was centrifuged at 160*g* for 15 min to obtain platelet-rich plasma (PRP), and platelets were separated from plasma proteins by albumin gradient gel filtration using 5 mM HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] balanced salt buffer, pH 7.35, with 0.1% dextrose and 0.35% albumin (Timmons & Hawiger, 1978).

Preparation of Human Fibrinogen and Its Chains. Low-solubility fibrinogen was prepared from Kabi fibrinogen (Timmons & Hawiger, 1986). Polypeptide chains of fibrinogen were purified as described (Hawiger et al., 1982; Cottrell & Doolittle, 1976).

CNBr Cleavage of α Chain and Isolation of Fragments. Purified α chain was degraded in CNBr in 70% formic acid for 6 h. The digest was freeze-dried, resuspended in 10% acetic acid, and separated on a preparative HPLC column (VYDAC Microsphere C_4 300-Å pore, 7-μm particle column, 2.5 × 25 cm) equilibrated with 0.05% trifluoroacetic acid (TFA). The solvent was 80% acetonitrile in 0.05% TFA. All fractions eluted in distinct peaks were freeze-dried twice. Material was dissolved in H_2O and tested for inhibitory activity toward binding of ^{125}I-fibrinogen to ADP-activated platelets. Fractions displaying inhibitory activity were refractionated on the HPLC column (VYDAC Microsphere C_4 300-Å pore, 7-μm particle column, 1 × 25 cm) equilibrated with 0.05% TFA. Peptides eluted with the same solvent system as in the previous step were freeze-dried twice, dissolved in H_2O, and tested for their inhibitory activity in the ^{125}I-fibrinogen binding assay as described below. Fractions showing ≥10% inhibition of ^{125}I-fibrinogen binding were applied to an analytical HPLC column (VYDAC Microsphere 300-Å pore, 7-μm particle column, 0.4 × 25 cm) equilibrated with 0.05% TFA. Chromatographically homogeneous fractions which produced inhibition of ^{125}I-fibrinogen binding were subjected to amino acid sequencing. The concentration of peptides at all steps of purification procedure was determined on the basis of amino acid analysis.

Peptide Synthesis and Purification. Peptides were synthesized by solid-phase methods (Barany & Merrifield, 1980) either manually (Kloczewiak et al., 1984) or with a Biosearch 9500 peptide synthesizer. Peptides were cleaved from the resin with HF and purified by using liquid chromatography and HPLC according to the procedures described by Kloczewiak et al. (1984).

Amino Acid Analysis and Sequencing. Amino acid composition was determined on a Beckman automated analyzer after hydrolysis of the peptide samples at 150 °C for 50 min with 6 N HCl (Pure Ultrex Baker Chemical) or in a 6 N HCl/CH_3CH_2COOH (50:50 v/v) mixture at 105 °C for 16 h. The amino acid sequencing of isolated peptides was determined in a Applied Bioscience automated gas-phase sequencer (Hunkapiller et al., 1983).

Platelet Aggregation. Aggregation was measured photometrically in a Payton dual-channel aggregometer (Payton Associates, Buffalo, NY) after addition of ADP (5 μM) using a percentage of maximum transmission (T_{max}) and rate ("slope value") which represented the change in 1 min along a tangent line to the steepest increase in light transmission (Hawiger et al., 1982; Timmons & Hawiger, 1986).

Binding Assay. Purified fibrinogen was iodinated with ^{125}I by using the standard iodine monochloride method (Macfarlane, 1963). The specific radioactivity of ^{125}I-fibrinogen was 3×10^{-7} cpm/mg. Human platelets, separated from plasma proteins and suspended in HEPES-balanced salt buffer, pH 7.35, were mixed with given concentrations of test peptide for 5 min. Then, ^{125}I-fibrinogen (0.15 μM) was added and followed by ADP (5 μM). The binding experiments were done at room temperature without stirring in a final volume of 0.5 mL which contained 1×10^8 platelets (Kloczewiak et al., 1984). Inhibition curves of ^{125}I-fibrinogen binding to platelets by α-chain peptides with an exponential function fitted to them were derived from at least three experiments. The inhibition curve for a tested fragment derived from the α chain or synthetic peptide was run in parallel with a known active synthetic peptide, i.e., RGDS and/or γ 400–411, used as a comparative control.

Results

Platelet Receptor Reactivity of CNBr Fragments of the α Chain of Human Fibrinogen. The α chain of human fibrinogen has 10 methionine residues, and their cleavage with CNBr results in 11 fragments (Doolittle et al., 1979). Initial fractionation of the mixture of CNBr fragments on a preparative HPLC column resulted in several peaks. All of them were examined for inhibition of ^{125}I-fibrinogen binding after

[1] Abbreviations: Boc, *tert*-butyloxycarbonyl; GPIIb–IIIa, glycoproteins IIb and IIIa; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC, high-pressure liquid chromatography; IC_{50}, concentration of peptide causing 50% inhibition of binding; PRP, platelet-rich plasma; vWF, von Willebrand factor.

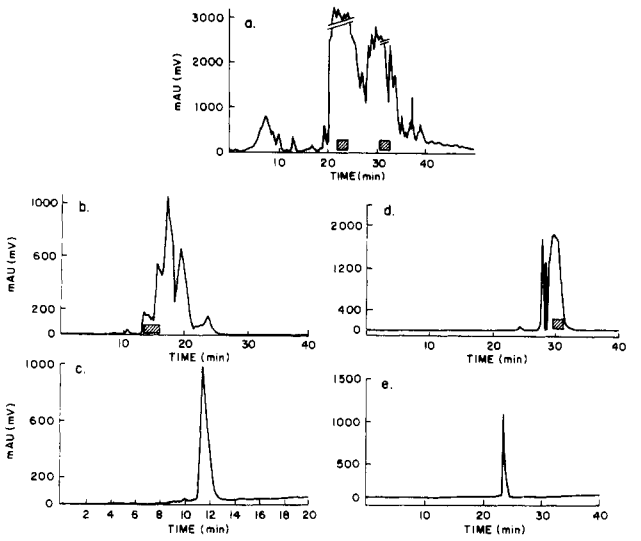

FIGURE 1: (a) Preparative HPLC column fractionation of CNBr-cleaved fragments of human fibrinogen α chain. The flow rate was 5 mL/min. The solvent (B) was 80% acetonitrile in 0.05% TFA, and the gradient was 0.20% B for 1 min progressing to 100% B in 50 min. All fractions were freeze-dried twice and dissolved in H_2O. They were then assayed for their inhibitory effect toward binding of ^{125}I-fibrinogen (0.15 μM) to human platelets (10^8/0.5 mL) stimulated with ADP (5 μM). Fractions showing inhibition ≥10% of binding of ^{125}I-fibrinogen in three independent experiments are indicated by hatched bars. (b and d) "Semipreparative" HPLC column fractionation of the peptides obtained in the prior step as indicated by hatched bars (a). The gradient was 0–100% B for 40 min; the flow rate was 2 mL/min. The obtained fractions were freeze-dried, dissolved in H_2O, and analyzed for their inhibitory effect in the ^{125}I-fibrinogen binding assay. The inhibitory fractions are marked by hatched bars. (c and e) Analytical HPLC separation of the peptides which were obtained in the prior step and are indicated as hatched bars (b and d). The flow rate was 1 mL/min, and the gradient was 0–100% B for 40 min.

freeze-drying and redissolving in aqueous buffer. All the peaks from preparative HPLC were rechromatographed on analytical HPLC and retested for inhibitory activity toward binding of ^{125}I-fibrinogen to human platelets. Two major peaks contained fractions possessing inhibitory activity toward binding of ^{125}I-human fibrinogen to ADP-activated platelets in three independent experiments (Figure 1a). These two active peaks were purified further by using the same type of HPLC column (Figure 1b,d). The inhibitory fractions were isolated, and their further separation on an analytical HPLC column produced single peaks (Figure 1c,e). Amino acid analysis and sequencing of material in these peaks indicated the presence of CNBr fragments encompassing residues 92–147, and residues

Table I: Analysis of Synthetic Peptide Analogues of α92–147 for Their Inhibition of Binding of ^{125}I-Fibrinogen to ADP-Treated Human Platelets

peptide	IC_{50} (μM)[a]
α92–113, EILRGDFSSANNRDNTYNRVSE	25
α92–98, EILRGDF	10
α95–113, RGDFSSANNRDNTYNRVSE	10
α95–98, RGDF	2
α95–100, RGDFSS	70
α99–113, SSANNRDNTYNRVSE	*b*

[a] Concentration of peptide causing 50% inhibition of binding of ^{125}I-fibrinogen (0.15 μM) to human platelets (10^8/0.5 mL) stimulated with ADP (5 μM). [b] Inactive at 300 μM.

α 518–584 in the α chain of human fibrinogen. Purified CNBr fragment α92–147 caused 26% inhibition of binding of ^{125}I-fibrinogen at 33 μM (the highest concentration tested). Purified CNBr fragment α518–584 caused 45% inhibition of binding of ^{125}I-fibrinogen at 86 μM (the highest concentration tested). Other CNBr fragments of the α chain did not show inhibitory activity ≥10% toward binding of ^{125}I-fibrinogen to activated platelets. In particular, the largest CNBr fragment, CNI(α241–476), and its more soluble derivative (α241–424), were inactive (results not shown).

Synthetic Peptide Analogues of α92–147. This fragment composed of 56 amino acids is a part of the "coiled coil" region of the α chain with a predominant, computer-predicted, helical segment (Doolittle et al., 1979). However, the amino-terminal part of this fragment has a random-coil structure in a computer-predicted conformation and contains the sequence RGD. Therefore, our strategy was aimed at this part of α92–147 (Figure 2). The overlapping synthetic peptides were assayed for biological activity by measuring the inhibition of ^{125}I-fibrinogen binding to ADP-activated platelets. The inhibition curves are shown in Figure 3, and the results are in Table I. It can be seen that 22-residue and 19-residue peptides (α92–113 and α95–113) were inhibitory with IC_{50} = 25 and 10 μM, respectively. However, the adjoining 15-residue peptide (α99–113) was inactive at 300 μM. Since the difference between inhibitory and noninhibitory peptides is the sequence RGDF (α95–98), we tested this tetrapeptide and found it more inhibitory (IC_{50} = 2 μM) than other longer peptides. Moreover, as shown in Figure 3, adding the two flanking residues Ser^{99} and Ser^{100} to the carboxy-terminal end of RGDF reduced its activity 35-fold (IC_{50} = 70 μM). On the other hand, addition of the flanking residues Gln^{92}, Ile^{93}, and Leu^{94} at the amino-terminal end caused only a 5-fold change in reactivity of the peptide α92–98 (IC_{50} = 10 μM). Since peptide α99–113 was inactive, we did not synthesize, purify,

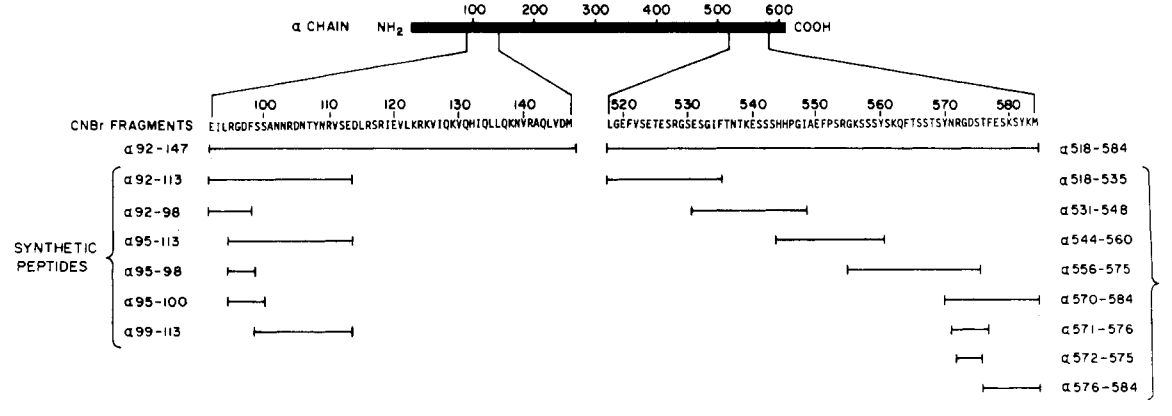

FIGURE 2: Identification of platelet receptor recognition domains in CNBr fragments of human fibrinogen α chain. The sequence of both CNBr fragments that were inhibitory toward platelet receptors is given. Overlapping synthetic peptides, designed to identify recognition domains for platelet receptors within inhibitory CNBr fragments, are depicted.

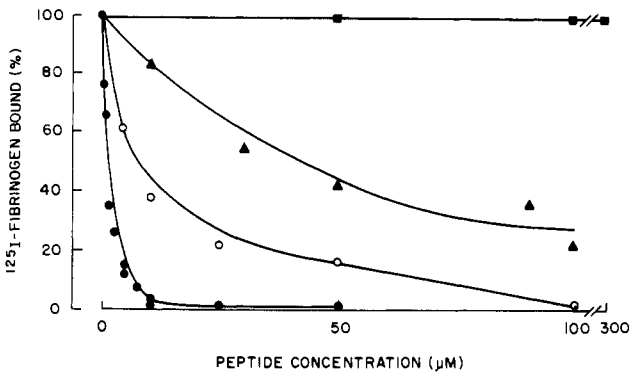

FIGURE 3: Inhibition curves for ^{125}I-fibrinogen binding to ADP-treated platelets by selected synthetic peptide analogues of CNBr α92–147: (●) α95–98; (○) α92–98; (▲) α95–100; (■) α99–113.

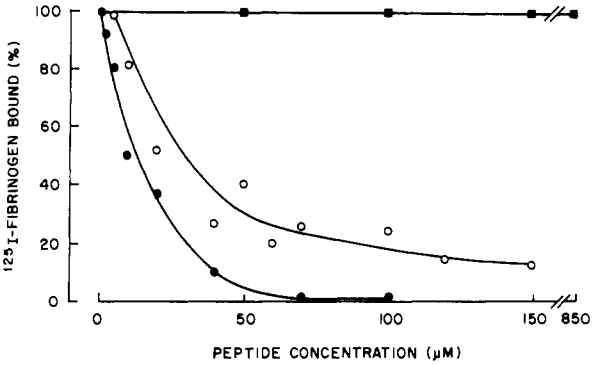

FIGURE 4: Inhibition curves for ^{125}I-fibrinogen binding to ADP-treated platelets by selected synthetic peptide analogues of CNBr α518–584: (●) α572–575; (○) α571–576; (■) α576–584.

Table II: Analysis of Synthetic Peptide Analogues of α518–584 for Their Inhibition of Binding of ^{125}I-Fibrinogen to ADP-Treated Human Platelets

peptide	IC_{50} (μM)[a]
α518–535, LGEFVSETESRGSESGIF	inactive at 300 μM
α531–548, SGIFTNTKESSSHHPGI	inactive at 300 μM
α544–560, HHPGIAEFPSRGKSSSY	inactive at 300 μM
α556–575, KSSSYSKQFTSSTSYNRGDS	17
α570–584, YNRGDSTFESKSYKM	40
α571–576, NRGDST	30
α572–575, RGDS	10
α576–584, TFESKSYKM	inactive at 850 μM

[a] Concentration of peptide causing 50% inhibition of binding of ^{125}I-fibrinogen (0.15 μM) to human platelets (10^8/0.5 mL) stimulated with ADP (5 μM).

and test peptides beyond this sequence.

Synthetic Peptide Analogues of α518–584. The 67-residue CNBr fragment 518–584 is relatively rich in serine, glycine, and threonine and has a computer-predicted random-coil conformation (Doolittle et al., 1979). Within this fragment, the sequence RGD is located at residues 572–574, and our aim was to synthesize a series of overlapping peptides encompassing residues 518–584 (Figure 2). As shown in Table II and in Figure 4, three peptides encompassing residues 518–560 were inactive, whereas peptide α556–575 was inhibitory in the ^{125}I-fibrinogen binding assay with IC_{50} = 17 μM (Figure 4). Overlapping peptide α570–584 was also inhibitory (IC_{50} = 40 μM). However, another overlapping peptide (α576–582) was not inhibitory at 850 μM. Since the difference between these two peptides was sequence YNRGDS, we synthesized peptide NRGDST (α571–576), which was inhibitory at 35 μM. The shorter peptide RGDS (α572–575) was more inhibitory (IC_{50} = 10 μM). These experiments indicate that the sequence RGDS is responsible for platelet receptor reactivity of the CNBR fragment α518–584 and that residues flanking the sequence RGDS influence its platelet reactivity.

Discussion

The dimeric structure of fibrinogen is important for its cell agglutinating function and requires specialized domains recognizing cellular receptors [for a review, see Hawiger (1987)]. From the outset of our efforts to map the fibrinogen molecule for domains responsible for its interaction with receptors on activated human platelets, we realized that both α and γ chains are involved (Hawiger et al., 1982). Identification of the sequence Arg-Gly-Asp (RGD) as a "cell adhesion" site of fibronectin by Pierschbacher and Ruoslahti (1984) was followed by experiments in which the synthetic peptide corresponding to this sequence in the α chain of human fibrinogen was used to inhibit binding of ^{125}I-fibrinogen and platelet aggregation (Gartner & Bennet, 1985; Plow et al., 1985, 1987).

Our present studies provide the initial evidence that 2 out of 11 fragments of native α chain, obtained by its cleavage with CNBr, interact with activated human platelets and block their receptors for fibrinogen. Our experimental strategy was based on analysis of CNBr-derived fragments α92–147 and α518–584 by synthesizing a series of overlapping peptides starting with the amino terminus of each CNBr fragment reactive with platelet receptors. Having found a synthetic peptide which was reactive, followed by the next one which was nonreactive, we assumed that subsequent sequences will be noninhibitory. Therefore, our series of overlapping peptides ended with the nonactive peptide α99–113 in the case of the CNBr fragment α92–147 and with peptide α576–584 in the case of CNBr fragment α518–584. These two CNBr fragments, α92–147 and α518–584, and a series of overlapping peptide analogues provide a direct structural basis for the functional role of sequences RGDF (α95–98) and RGDS (α572–575) in the interaction of human fibrinogen α chain with receptors on activated platelets.

The presence of two domains on the α chain that recognize platelet receptors and of one domain previously identified by us on the carboxy-terminal segment of the γ chain (Kloczewiak et al., 1984) brings to six the number of sites on the dimeric fibrinogen molecule that are available for engagement with platelet receptors. It is apparent then that one molecule of fibrinogen bridging two platelets is trivalent in regard to each platelet, assuring a higher affinity due to the higher than one valency level (Hawiger, 1987).

However, both domains in the α chain are prone to enzymatic extirpation by plasmin. The sequence RGDS (α572–575) is located between two plasmin-sensitive bonds which are attacked early during plasmin action on fibrinogen or fibrin. The segment containing sequence α95–98 is clipped off by plasmin during its intermediate stage of fibrinogen cleavage (Doolittle, 1981; Francis & Marder, 1987). Fragment X, lacking the RGDS sequence, weakly supports platelet aggregation (Niewiarowski et al., 1977), but it still binds tightly to platelets, presumably via the remaining receptor recognition domains on the α chain (residues 95–98) and the γ chain (residues 400–411) (Peerschke & Galanakis, 1987; Plow et al., 1987). These considerations are important because it is necessary to ascertain how these two sequences, containing RGD as the ubiquitous cell adhesion site on the α chain of human fibrinogen, contribute to its cell recognition function. Apparently, two α-chain domains are unable to compensate for the functionally abnormal γ chain in fibrinogen Paris I or for the defective function of γ′ or variant B of human γ

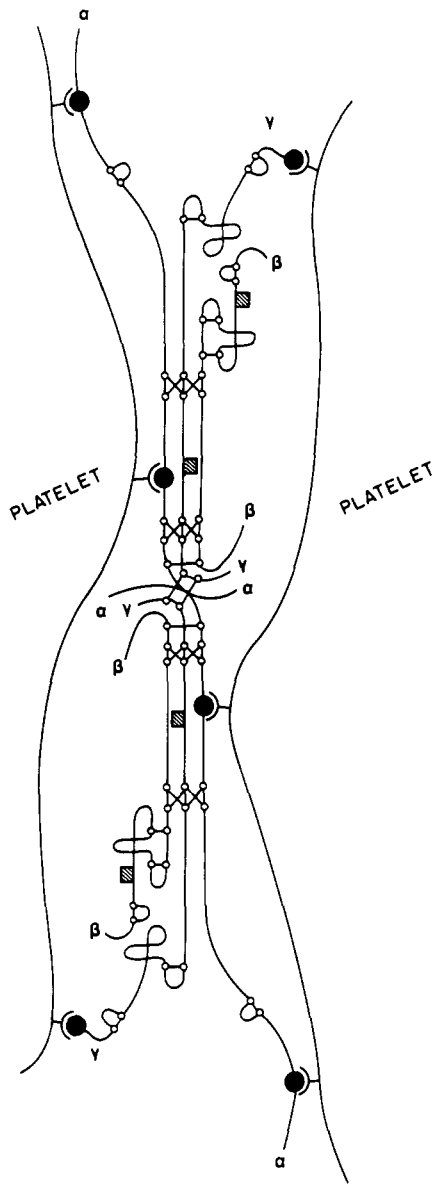

FIGURE 5: Proposed model of interaction of human fibrinogen with human platelets. The fibrinogen molecule composed of three pairs of nonidentical chains (α, β, and γ) is arranged in an antiparallel configuration. The hatched boxes represent carbohydrates on β and γ chains. The platelet receptor recognition domains marked as black dots encompass sequences 95–98 and 572–575 on the α chain and sequence 400–411 on the γ chain. One molecule of fibrinogen can be engaged in trans and cis interaction with platelet receptors made of GPIIb–IIIa (see text for details and references).

chain due to alternative splicing (Harfenist et al., 1984; Peerschke et al., 1986; Denninger et al., 1987). The latter displays a significant decrease in binding to activated platelets. It appears that the γ-chain domain not only confers ligand specificity on human fibrinogen but also, together with the two α-chain domains, is essential for the optimal platelet binding function of fibrinogen.

Both α-chain domains are located in the areas of computer-predicted random-coil structure. In the case of α95–98, this structure is preceded and followed by a α-helix region, and in the case of α572–575, it is followed by a region of α helix (Doolittle et al., 1979). The importance of the sequence context in which RGD residues are located is illustrated by our experiments showing constraints on the platelet receptor reactivity of tetrapeptides RGDF and RGDS by adding flanking residues (see Figures 3 and 4). Although the sequence RGD is positioned in the triple-helical structure of collagen (Dedhar et al., 1987), other adhesive proteins containing the sequence RGD as a cell adhesion site (e.g., fibronectin) appear to have it in the β-turn conformation (Ruoslahti & Pierschbacher, 1987). This may favor segmental mobility which contributes to a proper positioning of the recognition domain of the ligand versus the complementary domain in the receptor (Hawiger, 1987).

Our data led us to expand the existing model of the fibrinogen–platelet receptor interaction (Figure 5). The proposed model takes into account information derived from this study and other studies (Shirasawa & Chandler, 1969; Kloczewiak et al., 1984; Shainoff et al., 1987). We depict the fibrinogen molecule composed of three pairs of nonidentical chains arranged in an antiparallel configuration (Hoeprich & Doolittle, 1983). The fibrinogen molecule is positioned in a parallel mode with regard to the plane of the platelet membrane to accommodate the molecular dimensions of fibrinogen to the observed 100–200-Å distance between two adjoining platelets in an aggregate (Shirasawa & Chandler, 1969; Shainoff et al., 1987). Such parallel rather than perpendicular arrangement accommodates six domains of fibrinogen in apposition with their receptors on two platelets. Whether the α-chain carboxy-terminal zone extends outward or is folded back toward the amino-terminal zone (Weisel et al., 1985) may have some bearing on the domain located at α95–98. This issue can be settled when more evidence will be forthcoming from molecular electron microscopy of fibrinogen that is bound to activated platelet receptors.

Our model focuses on the position of human fibrinogen recognition domains for platelet receptors and on the trans-acting ("bridging") function of fibrinogen with regard to platelets. However, this model accommodates also the potentially important cis-acting function of fibrinogen. Binding of one fibrinogen molecule to two or three receptors, i.e., GPIIb–IIIa complexes, positioned along the long axis (475 Å) of fibrinogen can contribute to the observed clustering of GPIIb–IIIa (Isenberg et al., 1987). Moreover, it may enhance the conformational change in GPIIb–IIIa initiated by the agonist-mediated event that switches the platelet membrane GPIIb–IIIa from a nonbinding to a binding mode. A conformational shift in isolated GPIIb–IIIa complexes was induced by the RGD peptide and the γ-chain decapeptide (Parise et al., 1987). The occupancy of GPIIb–IIIa by fibrinogen is necessary for Na^+/H^+ exchange observed in epinephrine-treated platelets (Banga et al., 1986; Sweatt et al., 1986). Impairment of a cis-acting function of fibrinogen in platelets incubated at 4 °C, or in glutaraldehyde-fixed platelets stimulated with ADP, can be responsible for the apparent dichotomy between the binding of fibrinogen to such platelets and their ability to aggregate (Peerschke, 1981).

Taken together, by identifying two platelet receptor recognition domains on the α chain of human fibrinogen, we have expanded the functional map of this adhesive protein and have moved closer to understanding the relationship between the α chain and the γ chain of human fibrinogen in its interaction with GPIIb–IIIa on human platelets. This knowledge has also been applied to the design of "hybrid" peptides encompassing both α- and γ-chain domains (Timmons et al., 1989).

ACKNOWLEDGMENTS

The technical assistance of Mitchel Schwaber in some of these experiments is appreciated. We thank Barbara Maki for her excellent assistance in the preparation of the manuscript and Alvin Brass for the expert rendering of the illustrations.

Registry No. α92–147, 119413-65-9; α92–113, 119336-76-4; α92–98, 119336-77-5; α95–113, 119336-78-6; α95–98, 110697-46-6;

α95–100, 119336-79-7; α99–113, 119336-80-0; α518–584, 84135-10-4; α518–535, 119336-81-1; α531–548, 119336-82-2; α544–560, 119336-83-3; α556–575, 119336-84-4; α570–584, 119336-85-5; α571–576, 110697-41-1; α572–575, 91037-65-9; α576–584, 119336-86-6.

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