Platelet Receptor Recognition Domain on the γ Chain of Human Fibrinogen and Its Synthetic Peptide Analogues†

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

ABSTRACT: We have shown previously that the domain recognizing receptors on activated human platelets is located on the human fibrinogen γ chain between residues 400 and 411 [Kloczewiak, M., Timmons, S., Lukas, T. J., & Hawiger, J. (1984) *Biochemistry 23*, 1767]. To study the correlation between the structure of this segment of the γ chain and its reactivity toward receptors on ADP-activated human platelets, we designed a series of analogues containing replacements at 9 out of 12 positions. A double substitution of the normal His^{400}-His^{401} sequence by Ala-Ala reduced the inhibitory potency of the dodecapeptide 3-fold. When Lys^{406} was replaced by Arg, the inhibitory potency of the dodecapeptide decreased 15 times. On the other hand, substitution of Ala^{408} with Arg increased the inhibitory potency of the dodecapeptide 6-fold. A drastic decrease in the reactivity of the dodecapeptide toward platelet receptors was observed when Val^{411} was replaced by leucine or cysteine or tyrosine. A 3-fold decrease in reactivity was noted when Val^{411} was substituted with phenylalanine. Amidation of the carboxy-terminal Val^{411} also produced a significant decrease in dodecapeptide reactivity. With seven residues (His^{400}, His^{401}, Leu^{402}, Lys^{406}, Gln^{407}, Asp^{410}, and Val^{411}) preserved, substitution of the intervening five amino acids with nonpolar leucine or polar serine, increasing or decreasing the hydrophobicity of the dodecapeptide, reduced more than 16-fold its inhibitory potency. Rabbit antibody Fab fragments directed against the human fibrinogen γ-chain peptide encompassing residues 385–411 inhibited 50% of ^{125}I-fibrinogen binding at a 2:1 stoichiometry with regard to ^{125}I-fibrinogen. In vivo infusion of dodecapeptide with a native sequence into rabbit mesenteric artery caused reversible inhibition of hemostatic platelet thrombus formation.

The essential role of the γ chain in the interaction of human fibrinogen with receptors on activated platelets has been established [for a review, see Hawiger (1987)]. The carboxy-terminal segment of the γ chain encompassing residues 400–411 was pinpointed by us as the platelet receptor recognition domain. This was done through the use of "native" peptide isolated from CNBr-cleaved human fibrinogen γ chain (Kloczewiak et al., 1982) and truncated synthetic peptides (Kloczewiak et al., 1984). Peptides shorter than 12 residues were less reactive, exemplified by the fact that the heptapeptide γ400–406 was not reactive while the pentapeptide QAGDV (γ407–411) was 6 times less reactive than the dodecapeptide. Interestingly, human fibrinogen that contains the elongated variant B or γ′ of the γ chain, resulting from alternative splicing, has an impaired ability to interact with platelet receptors (Crabtree, 1987; Harfenist et al., 1984; Peerschke et al., 1986). Thus, these studies led to the realization that the continuous sequence of 12 amino acid residues between positions 400 and 411 in the predominant A variant of the γ chain of human fibrinogen is required for its interaction with human platelet receptors. On the other hand, Plow et al. (1984) reported that the decapeptide γ402–411 is as reactive as the dodecapeptide γ400–411 in inhibition of binding of ^{125}I-fibrinogen to human platelets and that shorter peptides were not active.

Following our previous experiments with "truncated" peptides (Kloczewiak et al., 1984), we have synthesized a series of analogues containing amino acid replacements at positions 400, 401, 406, 408, and 411. Such analogues should be of considerable value in understanding the role of individual residues in the interaction of the γ chain with human platelet receptors. We were particularly interested in the role of valine as the carboxy terminus of the predominant variant A of human fibrinogen γ chain. Finally, we applied monospecific antipeptide antibody to assess the overall function of the γ-chain domain in the interaction of human fibrinogen with platelet receptors, and we tested the synthetic dodecapeptide in vivo using the rabbit mesenteric artery infusion system.

Materials and Methods

All the procedures employed in this study have been fully described in previous publications from this laboratory, including the preparation and labeling of fibrinogen (Timmons & Hawiger, 1986), the isolation of cyanogen bromide cleaved fragment γ385–411, and the preparation of rabbit antibody Fab fragments (Kloczewiak et al., 1983, 1984), as well as synthesis of peptides, their purification and analysis, and the experimental system for binding ^{125}I-fibrinogen to platelets and platelet aggregation (Kloczewiak et al., 1984; Hawiger et al., 1989). In vivo infusion of peptides into rabbit superior mesenteric artery circuit and bleeding time measurements were done as described by Sakon and Hawiger (1984).

Calculation of Peptide Polarity, Hydrophobicity, Hydropathy, and Mean Solvent Accessibility. These characteristics were determined by using computerized software based on the values reported for amino acid side chains for polarity (Zimmerman et al., 1968), hydrophobicity (Nozaki & Tanford,

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

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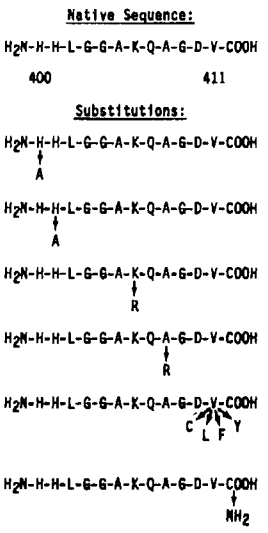
0006-2960/89/0428-2915$01.50/0

Table I: Design of Synthetic Peptide Analogues of Human Fibrinogen γ_A Chain in Which "Key" Residues Are Substituted



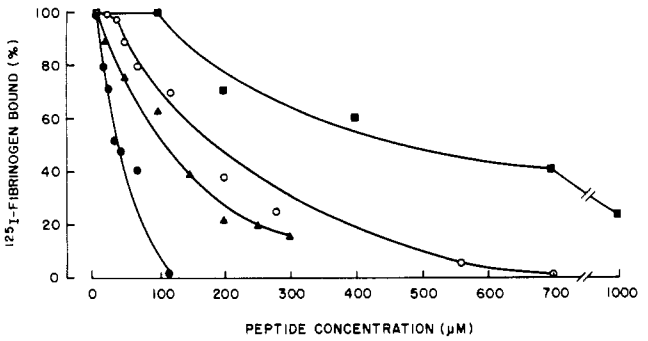


FIGURE 1: Synthetic peptide analogue inhibition curves of ^{125}I-fibrinogen (0.15 μM) binding to human platelets (10^8/0.5 mL) stimulated by ADP (5 μM). The following peptide analogues of γ400–411 are shown: HHLGGAKQAGDV (●); AALGGAKQAGDV (▲); HHLGGAKQAGDV-NH_2 (○); HHLGGARQAGDV (■).

1971), hydropathy (Kyte & Doolittle, 1982), and the mean solvent accessibility (Lesser et al., 1987).

RESULTS

Choice of Analogues of the Platelet Receptor Recognition Domain of the Human Fibrinogen γ Chain (γ400–411). To examine which residues are essential for the reactivity of the γ-chain domain encompassing residues 400–411 (γ400–411) toward human platelet receptors, we made sequence replacements in 400–411 that would be expected to induce significant changes in the reactivity of the peptide analogue toward platelet receptors. Of particular interest was the role of His^{400} and His^{401} and of Val^{411}. The latter as the carboxy terminus of the γ chain contrasts in its hydrophobic character from the rest of the receptor recognition domain. Table I shows the design of synthetic peptide analogues of human γ400–411 in which selected residues are replaced or modified. In the "middle" of sequence γ400–411, two replacements introduced arginine in positions 406 and 408. While preserving residues His^{400}, His^{411}, Leu^{402}, Lys^{406}, Gln^{407}, Asp^{410}, and Val^{411}, we have replaced intervening amino acids with large nonpolar leucine or small polar serine to examine the role of the hydrophobic/hydrophilic character of analogues in their receptor reactivity.

Inhibitory Activity of the Analogues. The purified analogues of γ400–411 were assayed for biological activity toward

Table II: Comparison of Inhibitory Activity of Synthetic Peptides toward ^{125}I-Fibrinogen Binding of ADP-Treated Human Platelets

peptide	IC_{50} (μM)[a]
H_2NHHLGGAKQAGDVCOOH	30
H_2N*AA*LGGAKQAGDVCOOH[b]	95
H_2NHHLGGA*R*QAGDVCOOH	460
H_2NHHLGGAKQ*R*GDVCOOH	0
H_2NHHLGGAKQAGD*VNH*$_2$	180
*Ac*HHLGGAKQAGD*VNH*$_2$	85
H_2NHHLGGAKQAGD*L*COOH	>400[c]
H_2NHHLGGAKQAGD*Y*COOH	>500[c]
H_2NHHLGGAKQAGD*C*COOH	>500[c]
H_2NHHLGGAKQAGD*F*COOH	100

[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] The italicized residues represent substitutions or modifications of residues in sequence γ400–411. [c] The highest concentration tested which did not cause 50% inhibition of binding of ^{125}I-fibrinogen.

Table III: Characteristics of Synthetic Peptide Analogues of γ400–411 with Predominantly Hydrophobic and Hydrophilic Residues

peptide	binding inhibition, IC_{50} (μM)	polarity average	hydrophobicity average	hydropathy average	accessibility
HHLGGAKQAGDV	28	17.18	0.84	−0.57	0.72
HHL*QLL*KQ*LL*DV[a]	>500[b]	17.52	1.40	0.20	0.75
HH*SQSS*KQ*SS*DV	>500[b]	18.16	0.52	−1.05	0.67

[a] Italicized residues represent substitutions in sequence γ400–411. [b] The highest concentration tested which did not cause 50% inhibition of binding of ^{125}I-fibrinogen.

receptors on ADP-activated human platelets by measuring the inhibition of binding of ^{125}I-fibrinogen. The inhibition curves for selected analogues are shown in Figure 1, and results expressed as the concentration of peptide causing 50% inhibition of binding (IC_{50})[1] are in Table II. It can be seen that replacement of selected residues results in a significant decrease in the inhibitory activity of the analogues. Thus, replacements of His^{400} and His^{401} by Ala resulted in a 3-fold decrease, while replacement of Lys^{406} by Arg caused a 15-fold drop in reactivity of the analogue as compared to peptide γ400–401. However, when Arg replaced Ala^{408}, the resulting analogue gained 6 times on inhibitory potency. Amidation of Val^{411} resulted in a 6-fold decrease in dodecapeptide reactivity, while replacement of valine by phenylalanine, tyrosine, leucine, or cysteine caused a 3–16-fold decrease in the inhibitory potency of the analogues.

Analogues with Predominantly Nonpolar and Polar Residues. The sequence of human fibrinogen γ chain encompassing residues 400–411 is more hydrophilic than the γ chain, in general, and the preceding segment (γ385–394) in particular (Kloczewiak et al., 1984). However, the essential role of Val^{411} led us to examine the role of hydrophobicity in receptor recognition function. Two analogues were designed in which residues His^{400}, His^{401}, Leu^{402}, Lys^{406}, Gln^{407}, Asp^{410}, and Val^{411} were preserved and five intervening residues were replaced by a large nonpolar amino acid, leucine, or a small polar residue, serine (Table III). The leucine-rich analogue had similar polarity, but was more hydrophobic than native γ400–411. Its inhibitory potency toward binding of ^{125}I-fibrinogen to platelets was reduced more than 16-fold. However, the serine-rich analogue, of higher polarity and lower hydrophobicity than the "native" sequence of γ400–411, was also not inhi-

[1] Abbreviations: GPIIIb–IIIa, glycoproteins IIb and IIIa; HPLC, high-pressure liquid chromatography; IC_{50}, concentration of peptide causing 50% inhibition of binding; vWF, von Willebrand factor.

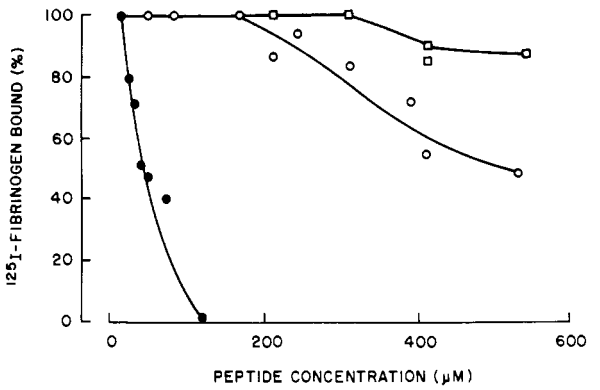


FIGURE 2: Inhibitory effect of synthetic peptide analogues of increased or decreased hydrophobicity on ^{125}I-fibrinogen (0.15 μM) binding to ADP-stimulated human platelets (10^8/0.5 mL): HHLGGAKQAGDV (●); HHLQLLKQLLDV (○); HHSQSSKQSSDV (□).

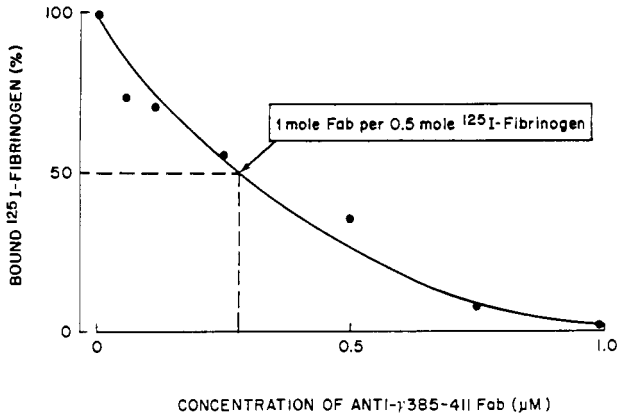


FIGURE 3: Inhibition of binding of ^{125}I-labeled human fibrinogen to ADP-stimulated human platelets by monovalent Fab antibody fragments against peptide γ385–411. Binding assay contained platelets separated from plasma proteins (10^8/0.5 mL) and ^{125}I-labeled human fibrinogen (0.15 μM) and increasing concentrations of anti-γ385–411 antibody Fab fragment. Binding of ^{125}I-fibrinogen observed in the absence of anti-γ385–411 antibody represents 100%. Control rabbit nonimmune IgG Fab fragments were noninhibitory. (See text for details.)

bitory (IC_{50} > 500 μM) (Figure 2).

Inhibition of ^{125}I-Fibrinogen Binding by Antipeptide γ385–411 Fab Antibody. To assess the role of the carboxy-terminal segment of the γ chain of human fibrinogen in its interaction with receptors on activated platelets, we prepared the monospecific Fab antibody fragments directed against the carboxy-terminal fragment (residues 385–411) derived from the γ chain by CNBr cleavage. This fragment was purified by high-pressure liquid chromatography (HPLC) and was inhibitory toward fibrinogen receptors on ADP-activated platelets (Kloczewiak et al., 1982). The monovalent Fab antibody fragment was used previously by us to block aggregation of ADP-treated platelets mediated by a polyvalent conjugate of synthetic peptide γ397–411 (Kloczewiak et al., 1984). In the present experiments, we tested the effect of increasing concentrations of antipeptide γ385–411 Fab fragments on the binding of ^{125}I-fibrinogen. As shown in Figure 3, 50% inhibition of ^{125}I-fibrinogen binding was observed at 0.28 μM Fab antibody fragment. This corresponds to a stoichiometry of 2:1 with regard to the ^{125}I-fibrinogen used in this binding experiment (0.15 μM). Nonimmune rabbit IgG Fab fragment did not inhibit binding of ^{125}I-fibrinogen at 80-fold excess (not shown). Because each molecule of fibrinogen contains two γ chains with respective carboxy-terminal domains available for Fab antibody fragments, the observed

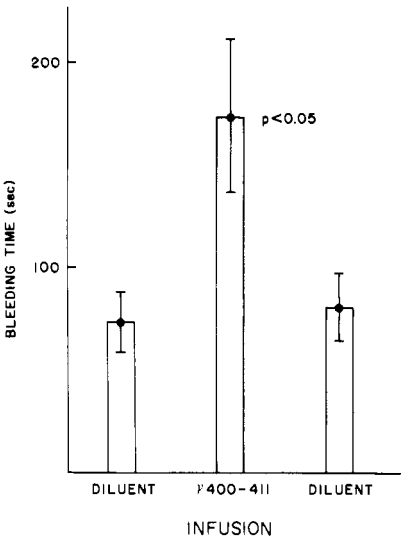


FIGURE 4: Effect of dodecapeptide γ400–411 infused into rabbit mesenteric artery on hemostatic platelet plug formation in vivo as measured by a prolongation of the bleeding time. Each bar represents the mean ± SE of three experiments. The interval between infusion of dodecapeptide and diluent was 20 min.

stoichiometry of inhibition is remarkably close to predicted values.

In Vivo Inhibitory Activity of Dodecapeptide γ400–411 toward Hemostatic Plug Formation. Dodecapeptide γ400–411 was inhibitory in vitro toward rabbit platelets and inhibited ADP-induced aggregation of rabbit platelet-rich plasma (IC_{50} = 192 μM). Using the previously described rabbit mesenteric artery infusion system (Sakon & Hawiger, 1984), we examined the effect of dodecapeptide (γ400–411) on formation of a hemostatic plug by measurement of prolongation of the time required to arrest the bleeding from cuts performed on small branches of the mesenteric artery. Infusion of diluent through the mesenteric canula with a constant-volume Harvard pump at a flow rate of 0.11 mL/min did not adversely affect the bleeding time, which was 73 ± 16 s, not different from that measured in nonperfused animals. Infusion of dodecapeptide γ400–411, at a concentration in the infusate of 1 mM for 40 min, caused a significant prolongation of bleeding time, which was 177 ± 37 s ($p < 0.05$). The estimated dilution of infused dodecapeptide between the catheter and the sites of bleeding ranged from 4 to 12 times. When infusion of dodecapeptide was stopped and diluent was infused again into the same vascular bed, the inhibitory effect of dodecapeptide was reversed within 20 min, and bleeding time returned to the level observed before dodecapeptide infusion (Figure 4). These experiments indicate that dodecapeptide γ400–411 was active in vivo, causing reversible inhibition of the initial platelet thrombus during hemostatic plug formation.

Discussion

The essential role of the carboxy-terminal segment encompassing 12 residues (dodecapeptide γ400–411) of the γ chain of human fibrinogen in its interaction with receptors on activated platelets is supported by experiments with analogues of the native sequence encompassing residues 400–411 in the γ chain and experiments with antipeptide antibody against γ385–411. This antibody is also effective in blocking the interaction of human platelets with fibrinogen absorbed to a synthetic polymer surface while several monoclonal antibodies toward different epitopes of human fibrinogen were not inhibitory (Shiba et al., 1988). The peptide γ400–411 reversibly inhibited the formation of a hemostatic plug in a rabbit mesenteric artery. In related experiments, using whole human

blood pumped through a Baumgartner chamber at controlled flow conditions, the synthetic peptide γ400–411 and peptide RGDS inhibited platelet thrombus formation. Control peptide was inactive in vitro and ex vivo (Weiss et al., 1989).

Our data with 12-mer analogues are consistent with previously reported experiments in which a series of truncated synthetic peptides was used (Kloczewiak et al., 1984). Interestingly, while truncated dodecapeptide without His^{400} and His^{401} was 3 times less reactive in that study, identical loss of reactivity was observed in the present study when His^{400} and His^{401} were replaced by alanine. Thus, His^{400} and His^{401} constitute important residues for the reactivity of dodecapeptide (γ400–411) toward human platelet receptors. These data differ from observations made by Plow et al. (1984), who showed no difference in inhibitory potency between dodecapeptide γ400–411 and decapeptide γ402–411.

The role of Lys^{406} is underscored by its replacement with arginine, leading to a drastic drop in dodecapeptide reactivity. The inhibitory potency (IC_{50} = 500 μM) of this analogue is similar to the truncated peptide QAGDV, determined previously (Kloczewiak et al., 1984). The sequence AGD of this pentapeptide resembles sequence RGD constituting the "cell adhesion site" of fibronectin (Peerschbacher & Ruoslahti, 1984). When Ala was replaced by Arg, the resulting tetrapeptide RGDV was more inhibitory (Timmons et al., 1989), and dodecapeptide with substitution of Arg for Ala^{408} was 6 times more inhibitory than native γ400–411. A similar result was obtained by Ruggeri et al. (1986).

Val^{411} as the carboxy terminus of human fibrinogen γ chain is particularly important in the interaction of this chain with receptors on activated human platelets. Negative charge seems to play a role as well as the nonpolar bulk of valine. This was established when the carboxyl group of valine was replaced with an amino group, thereby reducing the reactivity of the dodecapeptide. Likewise, when large nonpolar amino acids, such as leucine and phenylalanine, replaced valine, or when intermediately polar tyrosine was used, inhibitory potency was diminished. It appears that valine, present at the carboxy-terminal end, provides a hydrophobic "key" which positions the rest of dodecapeptide within the receptor's "lock".

This "hydrophobic key" is preceded by the sequence which is more hydrophilic than the average human γ chain (Kloczewiak, 1984). Whether the character of the dodecapeptide is changed toward more hydrophobicity or more hydrophilicity, while preserving His^{400}, His^{401}, Leu^{402}, Lys^{406}, Gln^{407}, Asp^{410}, and Val^{411}, the resulting analogues lose their reactivity toward platelet receptors. These experiments substantiate the point that not only preserved residues but also the spatial structure of the "native" sequence determines the receptor reactivity of the γ chain of human fibrinogen. The replacement of Gly^{403} and Gly^{404} seems not to be solely responsible for loss of reactivity because we have shown previously that heptapeptide γ405–411 without these two glycine residues was not less inhibitory than decapeptide γ402–411 (Kloczewiak et al., 1984). Nevertheless, the carboxy-terminal segment of human fibrinogen chain encompassing residues γ400–411 has a predicted β-turn conformation (Jolles et al., 1978) and the addition of a helix-permissive Gln in position 403 could also contribute to reduced reactivity of analogues with the platelet receptors.

The carboxy-terminal segment of the γ chain of human fibrinogen has no apparent sequence homology with α and β chains (Doolittle et al., 1979). Although the γ chain of human fibrinogen has a 28% identity with human β-thromboglobulin (Doolittle, 1981) and sheep κ casein (Jolles et al., 1978), neither protein contains a segment showing strong homology to γ400–411. Among adhesive proteins, such as von Willebrand factor (vWF), vitronectin, and fibronectin, known to interact with the human platelet glycoprotein IIb–IIIa complex (GPIIb–IIIa) [for a review, see Hawiger (1987)], vWF at residues 1118–1129 has 5 out of 12 amino acids homologous with dodecapeptide γ400–411 (Titani et al., 1986). We have shown previously that fibrinogen and vWF competitively inhibit each other with regard to receptors on ADP-activated platelets, and dodecapeptide γ400–411 inhibited binding of ^{125}I-vWF (Timmons et al., 1984). Plow et al. (1984) showed the same also with regard to fibronectin. The partial homology between human fibrinogen γ400–411 and human vWF residues 1118–1129 may not contribute to the cross-inhibition of these two proteins with regard to receptors on ADP-stimulated platelets because antibody against vWF peptide encompassing sequence RGD blocked its binding to human platelets (Berliner et al., 1988). While the sequence RGD is ubiquitous among the known adhesive and many nonadhesive proteins (Ruoslahti & Pierschbacher, 1987), the γ-chain sequence 400–411 borne by fibrinogen seems to be uniquely designed for platelet receptors. It remains to be seen whether this sequence is involved in the interaction of fibrinogen with receptors belonging to the integrin superfamily present on other eukaryotic cells.

ACKNOWLEDGMENTS

The excellent assistance of Barbara Maki in the preparation of the manuscript is appreciated.

Registry No. H_2N-HHLGGAKQAGDV-COOH, 89105-94-2; H_2N-AALGGAKQAGDV-COOH, 119336-87-7; H_2N-HHLGGARQAGDV-COOH, 119336-88-8; H_2N-HHLGGAKQRGDV-COOH, 105151-58-4; H_2N-HHLGGAKQAGDV-COOH, 119336-89-9; Ac-HHLGGAKQAGDV-COOH, 119336-90-2; H_2N-HHLGGAKQAGDL-COOH, 119336-91-3; H_2N-HHLGGAKQAGDY-COOH, 119336-92-4; H_2N-HHLGGAKAQAGDC-COOH, 119336-93-5; H_2N-HHLGGAKQAGDF-COOH, 119336-94-6; HHLQLLKQLLDV, 119366-23-3; HHSQSSKQSSDV, 119336-95-7.

REFERENCES

Berliner, S., Niiya, K., Roberts, J. R., Houghten, R. A., & Ruggeri, Z. M. (1988) *J. Biol. Chem. 263*, 7500.

Crabtree, G. R. (1987) in *The Molecular Basis of Blood Diseases* (Stomatoyannopoulos, G., Nienhuis, A. W., Leder, P., & Majerus, P. W., Eds.) pp 631–661, W. B. Saunders, Philadelphia.

Doolittle, R. F. (1981) *Science 214*, 149.

Doolittle, R. F., Watt, K. W. K., Cottrell, B. A., Strong, D. D., & Riley, M. (1979) *Nature 280*, 464.

Harfenist, E. J., Packham, M. A., & Mustard, J. F. (1984) *Blood 64*, 1163.

Hawiger, J. (1987) in *Hemostasis and Thrombosis. Basic Principles and Clinical Practice* (Colman, R. W., Hirsh, J., Marder, V. J., & Salzman, E. W., Eds.) 2nd ed., pp 182–209, J. B. Lippincot, Philadelphia.

Hawiger, J., Kloczewiak, M., Bednarek, M. A., & Timmons, S. (1989) *Biochemistry* (first of three papers in this issue).

Jolles, P., Loucheux-Lefebvre, M.-H., & Henschen, A. (1978) *J. Mol. Evol. 11*, 271.

Kloczewiak, M., Timmons, S., & Hawiger, J. (1982) *Biochem. Biophys. Res. Commun. 107*, 181.

Kloczewiak, M., Timmons, S., & Hawiger, J. (1983) *Thromb. Res. 29*, 249.

Kloczewiak, M., Timmons, S., Lukas, T. J., & Hawiger, J. (1984) *Biochemistry 23*, 1767.

Kyte, J., & Doolittle, R. F. (1982) *J. Mol. Biol. 157*, 105.

Lesser, G. J., Lee, R. H., Zehfus, M. H., & Rose, G. D. (1987) in *Protein Engineering* (Oxender, D. L., & Fox, C. F., Eds.) pp 175–179, Alan R. Liss Jr., Inc., New York.
Nozaki, Y., & Tanford, C. (1971) *J. Biol. Chem. 246*, 2211.
Peerschke, E. I. B., Francis, C. W., & Marder, V. J. (1986) *Blood 67*, 385.
Pierschbacher, M. D., & Ruoslahti, E. (1984) *Nature 309*, 30.
Plow, E. F., Srouji, A. H., Meyer, D., Marguerie, G., & Ginsberg, M. H. (1984) *J. Biol. Chem. 259*, 5388.
Ruggeri, Z. M., Houghten, R. A., Russell, S. R., & Zimmerman, T. S. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 5708.
Ruoslahti, E., & Pierschbacher, M. D. (1987) *Science 238*, 491.
Sakon, M., & Hawiger, J. (1984) *Trans. Assoc. Am. Physicians 97*, 311.
Shiba, E., Lindon, J., Kushner, L., Kloczewiak, M., Hawiger, J., Matsueda, G., Kudryk, B., & Salzman, E. W. (1988) *Circulation 78*, II-662.
Timmons, S., & Hawiger, J. (1986) *Trans. Assoc. Am. Physicians 99*, 226.
Timmons, S., Kloczewiak, M., & Hawiger, J. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 4935.
Timmons, S., Bednarek, M. A., Kloczewiak, M., & Hawiger, J. (1989) *Biochemistry* (third of three papers in this issue).
Titani, K., Kumar, S., Takio, K., Ericsson, L. H., Wade, R. D., Ashida, K., Walsh, K. A., Chopek, M. W., Sadler, J. E., & Fujikawa, K. (1986) *Biochemistry 25*, 3171.
Weiss, H. J., Hawiger, J., Ruggeri, Z. M., Turitto, V. T., Thiagarajan, P., & Hoffman, T. (1989) *J. Clin. Invest. 83*, 288.
Zimmerman, J. M., Eliezen, N., & Simhal, R. (1986) *J. Theor. Biol. 21*, 170.

Antiplatelet "Hybrid" Peptides Analogous to Receptor Recognition Domains on γ and α Chains of Human Fibrinogen†

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

ABSTRACT: Platelet receptor recognition domains are located on the γ and α chains of human fibrinogen. The former encompasses residues 400–411 [Kloczewiak, M., Timmons, S., Lukas, T. J., & Hawiger, J. (1984) *Biochemistry 23*, 1767], and the latter is present in two loci on the α chain (α95–97 and α572–574) [Hawiger, J., Kloczewiak, M., Bednarek, M. A., & Timmons, S. (1989) *Biochemistry* (first of three papers in this issue)]. Peptide γ400–411 (HHLGGAKQAGDV) inhibited aggregation of ADP-treated platelets mediated not only by γ-chain but also by α-chain multimers. Peptide α572–575 (RGDS) inhibited aggregation of platelets mediated by α-chain as well as γ-chain multimers. These results indicate that the platelet receptor for fibrinogen is isospecific with regard to the domain present on α and γ chains. Subsequent "checkerboard" analysis of combinations of γ400–411 and α572–575 showed that the inhibitory effect toward binding of ^{125}I-fibrinogen was additive rather than synergistic. Next, a series of "hybrid" peptides was constructed in which the α-chain sequence RGDF (α95–98) replaced the carboxy-terminal segment of γ408–411. The dodecapeptide HHLGGAKQRGDF was inhibitory with concentration, causing 50% inhibition of binding (IC_{50}) at 6 μM, 5 times more potent than γ400–411. The shorter peptides AKQRGDF and KQRGDF were also more inhibitory than γ400–411. The second series of hybrid peptides was constructed with the α-chain sequence RGDS preceding the sequence of γ400–411 or sequence RGDV following it. The hybrid peptides YRGDSQHLGGAKQAGDV and HHLGGAKQAGDVGRGDV had the same reactivity toward platelet receptors as γ400–411. Alternatively, the hybrid peptide HHLGGAKQAGDSRGDV was 3 times more potent (IC_{50} = 10 μM). Hybrid peptides mimicking the γ-chain domain represent a new class of inhibitors with enhanced potency toward the platelet receptor for fibrinogen.

The recognition of human platelet receptors by fibrinogen plays a key role in the binding of this adhesive protein to activated platelets (Hawiger, 1987). We have shown that both the γ chain and the α chain of human fibrinogen directly interact with ADP-activated human platelets (Hawiger et al., 1982). The γ-chain domain is formed by a sequence of 12 carboxy-terminal residues, γ400–411 (Kloczewiak et al., 1984, 1989). The amino acid sequence of the α chain contains two loci at residues 95–97 and 572–574 reactive with human platelet receptors (Hawiger et al., 1989). Since there is no apparent homology between the carboxy-terminal segments of the γ chain and the α chain (Doolittle, 1984), the issue arose whether γ and α chains interact with separate receptors on human platelets or different sites of the same receptor.

The human platelet receptor for fibrinogen has been identified as the glycoprotein IIb–IIIa (GPIIb–IIIa)[1] heterodimer

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

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[1] Abbreviations: GPIIb–IIIa, glycoproteins IIb and IIIa; IC_{50}, concentration of peptide causing 50% inhibition of binding.

0006-2960/89/0428-2919$01.50/0