Factor XI Interacts with the Leucine-rich Repeats of Glycoprotein Ibα on the Activated Platelet*

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Factor XI (FXI) binds specifically and reversibly to high affinity sites on the surface of stimulated platelets (Kd app of ~10 nm; Bmax of ~1,500 sites/platelet) utilizing residues exposed on the Apple 3 domain in the presence of high molecular weight kininogen and Zn2+ or prothrombin and Ca2+. Because the FXI receptor in the platelet membrane is contained within the glycoprotein Ibα subunit of the glycoprotein Ib-IX-V complex (Baglia, F. A., Badellino, K. O., Li, C. Q., Lopez, J. A., and Walsh, P. N. (2002) J. Biol. Chem. 277, 1662–1668), we utilized moccarnahgin, a cobra venom metalloproteinase, to generate a fragment (His1–Glu282) of glycoprotein Ibα containing the leucine-rich repeats of the NH2-terminal globular domain and excludes the macroglycopeptide portion of glycocalcin, the soluble extracytoplasmic portion of glycoprotein Ib. This fragment was able to compete with FXI for binding to activated platelets (Kf of 3.125 ± 0.25 nm) with a potency similar to that of intact glycocalcin (Kf of 3.72 ± 0.30 nm). However, a synthetic glycoprotein Ibα peptide, Asp268–Asp287, containing a thrombin binding site had no effect on the binding of FXI to activated platelets. Moreover, the binding of 125I-labeled thrombin to glycocalcin was unaffected by the presence of FXI at concentrations up to 10−5 μM. The von Willebrand factor A1 domain, which binds the leucine-rich repeats, inhibited the binding of FXI to activated platelets. Thus, we examined the effect of synthetic peptides of each of the seven leucine-rich repeats on the binding of 125I-FXI to activated platelets. All leucine-rich repeat (LRR) peptides derived from glycoprotein Ibα were able to inhibit FXI binding to activated platelets in the following order of decreasing potency: LRR7, LRR1, LRR4, LRR5, LRR6, LRR3, and LRR2. However, the leucine-rich repeat synthetic peptides derived from glycoprotein Ibβ and Toll protein had no effect. We conclude that FXI binds to glycoprotein Ibα at sites comprising the leucine-rich repeat sequences within the NH2-terminal globular domain that are separate and distinct from the thrombin-binding site.

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Human factor XI (FXI), a homodimeric coagulation protein, circulates in plasma as a complex with a non-enzymatic cofactor high molecular weight kininogen (HK) (1–7). FXI can bind specifically and reversibly to high affinity sites on the surface of stimulated platelets in the presence of HK (and Zn2+ ions) or prothrombin (and Ca2+ ions) (8, 9). We have demonstrated previously that the Apple 3 domain of FXI mediates the binding of FXI to platelets (10, 11), activated platelets promote optimal rates of FXI activation by thrombin in the presence of HK or prothrombin (8), and FXI binds to the platelet glycoprotein Ibα (GP) Ib-IX-V complex (12) in platelet membrane lipid rafts (13), promoting its activation by thrombin (12, 13).

The platelet GPIb-IX-V complex is a large plasma membrane complex (~25,000 copies/platelet) comprising four polypeptide chains, GPIbα, GPIbβ, GPIX, and GPV, arranged in a stoichiometry of 2:2:2:1, respectively (14). This receptor is responsible for platelet adhesion to the site of injury, a function that it carries out by binding the von Willebrand factor (vWF). The GPIb-IX-V complex also binds thrombin with high affinity. Glycocalcin is the soluble form of the extracellular portion of GPIbα, which contains the NH2-terminal globular domain as well as the macroglycopeptide region. GPIbα, GPIbβ, GPIbIX, and GPIbV each contain an extracellular domain, a single transmembrane helix, and a short cytoplasmic tail. The binding sites within the complex for both vWF and thrombin reside within the first 300 amino acids of GPIbα (15). GPIbα is also a member of a family of proteins containing leucine-rich repeat (LRR) sequences including a large number of proteins that are principally involved in mediating protein-protein interactions (16). These LRR sequences are typically 22–28 amino acids long and occur in tandem repeats that are commonly flanked by disulfide loop structures (16). The interaction between vWF and GPIbα is mediated by the A1 domain of vWF and the NH2-terminal domain of the GPIbα chain (17–21). The x-ray crystal structures of the vWF A1 domain and GPIbα have been reported (20, 21), as have those of the GPIbα-thrombin complex (22, 23). The GPIbα NH2-terminal and COOH-terminal regions have been implicated in contributing to vWF binding (24, 25), whereas thrombin binding is centered around an anionic region containing three tyrosine residues

1 The abbreviations used are: FXI, human factor XI; HK, high molecular weight kininogen; GPIbα, glycoprotein Ibα; LRR, leucine-rich repeat; vWF, von Willebrand factor; PFACK, prolyl-phenylalanil-alanyl-arginyl-chloromethyl-ketone; BSA, bovine serum albumin; ABE, anion binding exosite.
(Tyr276, Tyr278, and Tyr279) that are post-translationally sulfated in the native receptor. This modification is essential for GPIb binding to thrombin and may contribute to the interaction with vWF (26, 27).

Thus, the biological relevance and structural determinants of GPIb interactions with thrombin and the A1 domain of vWF are relatively well understood. In contrast, although GPIb appears to comprise the platelet receptor and physiological locus for the FXI colocalization with thrombin leading to initiation of the consolidation pathway of blood coagulation (8–13), relatively little is known about the structural determinants of FXI interactions with the GPIb-IX-V complex and its colocalization with thrombin. The present studies demonstrate that FXI and thrombin bind GPIbα at distinct sites, which suggest that GPIbα may allow the colocalization of FXI and thrombin for efficient activation of FXI on the platelet surface.

**EXPERIMENTAL PROCEDURES**

**Materials**—Human FXI, human prothrombin, and human HK were purchased from Hematologic Technologies Inc. (Essex Junction, VT). Ristocetin was purchased from Sigma. Human thrombin (2,800 NIH units/mg) was purchased from Enzyme Research Laboratories (South Bend, IN). Methyl silicon oil (1 DC-200) and Hi phenyl silicon oil (125 DC-550) were purchased from William F. Nye Inc. (Fairhaven, MA). Carrier-free Na\(^{125}\)I was from Amersham Biosciences. The thrombin receptor agonist peptide SFLLRN-amide and the LRR peptides (20–24 amino acids) (Table I) were synthesized at the Protein Chemistry Facility of the University of Pennsylvania on the Applied Biosystems 430A Synthesizer, and reverse-phase high performance liquid chromatography was used to purify it to >99% homogeneity (Foster City, CA). The potent thrombin inhibitor prolyl-phenylalanyl-arginyl-chloromethylketone (PPACK) was purchased from Calbiochem (Indianapolis, IN). Active site-inhibited thrombin was prepared by incubation of a 10-fold excess of PPACK with α-thrombin for 1 h at 37 °C, and this mixture was dialyzed with Spectrophor tubing (3,500 M, cut off; Spectrum Medical Industries, Los Angeles, CA) overnight in phosphate-buffered saline at
FIG. 3. Effect of the mocarhagin fragment and synthetic peptides on the binding of 125I-FXI to glyocalcin in a solid phase assay. The effects of the mocarhagin fragment and synthetic peptides were examined, including the mocarhagin fragment His1–Glu282 (△), Thr266–Asp287 (○), and Asp269–Pro280 (●). 

125I-FXI (22 nM) was incubated with either the mocarhagin fragment or synthetic peptides, and the assay was performed as described under “Experimental Procedures.” Glycocalcin was bound to the wells of microtiter plates the amount of 125I-FXI bound was <0.1% of the control value, and the maximum variation of counts per minute bound for each experimental observation was <2% of the total counts per minute bound. One hundred percent binding of 125I-FXI represents binding in the absence of fragment or synthetic peptides (mean = 29,122 cpm). Nonspecific binding, as represented by 125I-FXI binding to wells coated with BSA (mean = 492 cpm), was subtracted from the total binding at each data point. Results shown represent the mean ± S.E. of three experiments, each done in duplicate.

5 °C. All purified proteins appeared homogeneous after SDS-PAGE.

Radiolabeling of FXI—Purified FXI and PPACK-thrombin were radiolabeled with 125I by a minor modification (8) of the Iodogen method to a specific activity of ~5 × 10^6 cpm/µg and ~1 × 10^7 cpm/µg, respectively. The radiolabeled FXI retained >98% of its biological activity.

Protein Analysis—Protein concentrations were determined by the Bio-Rad dye-binding assay according to the instructions provided by the manufacturer (Bio-Rad Laboratories).

Preparation of Washed Platelets—Platelets were prepared from normal donors as described (9, 11, 28). Platelet-rich plasma obtained from citrated human blood was centrifuged, and the platelets were suspended in calcium-free Hepes-Tyrode’s buffer (126 mM NaCl, 2.7 mM KCl, 1 mM MgCl2, 0.38 mM NaH2PO4, 5.6 mM dextrose, 6.2 mM sodium Heps, 8.8 mM Heps-free acid, and 0.1% bovine serum albumin (BSA)), pH 6.5, and gel-filtered on a column of Sepharose 2B equilibrated in calcium-free Hepes-Tyrode’s buffer, pH 7.2. Platelets were counted electronically (Coulter Electronics, Hialeah, FL).

Preparation of Glyocalcin and Mocarhagin Fragment His1–Glu282 from Human Platelets—Glyocalcin was extracted from human platelets and purified as described previously (29). The mocarhagin fragment His1–Glu282 was prepared from intact glyocalcin and purified as described previously (30).

Platelet Binding Experiments—Platelets were pre-warmed to 37 °C and incubated at a concentration of 1 × 10^9/ml in calcium-free Hepes-Tyrode’s buffer, pH 7.3, in a 1.5-ml Eppendorf plastic centrifuge tube with a mixture of radiolabeled FXI, divalent cations, a thrombin receptor (PAR-1) activation peptide (SFLLRN-amide) as a platelet agonist (9, 10, 28), and HK or other proteins as designated in the legends to Figs. 1, 5, and 6. All incubations were performed at 37 °C without stirring after an initial mixing of the reaction mixture. At various added FXI concentrations, aliquots were removed (100 µl) and centrifuged through a mixture of silicone oils as described (8–10, 28). In competition binding experiments, the concentration of the competitor that displaced 50% of the bound 125I-FXI (IC50) was determined by plotting the amount of competitor ligand bound to platelets versus the amount of competitor ligand added. The Ks was calculated using the equation IC50 = (1 ± [s]/Ks)Ks, where [s] is the concentration of 125I-FXI used in these experiments (held constant at 22 nM) and the Ks was the value (~10 nM) determined from direct binding experiments.

Solid Phase Binding of 125I-FXI or 125I-PPACK Thrombin to Glyocalcin—We utilized a modification of the method of DeCristofaro et al. (31) to examine the binding of 125I-FXI to platelet-bound glyocalcin. Wheat germ lectin (10 µg/ml) was coated on the wells of a solid phase plate (96-well polystyrene trays; Immulon high protein capacity binding) and incubated overnight at 4 °C in 50-mM carbonate buffer, pH 9.5. The remaining binding capacity of the sample wells was blocked by incubation for 2 h with 1% BSA in Hepes-buffered saline (150 mM NaCl and 3.5 mM Hepes, pH 7.2). After aspiration of the BSA solution, purified glyocalcin was added to the wells at a concentration of 20 µg/ml and incubated at 4 °C for 1 h. After aspiration, the 125I-FXI or 125I-PPACK-thrombin was applied to the wells and incubated for 1 h at 37 °C. Each sample and blank well were washed with Hepes-buffered...
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saline seven times for 1 min each, dried, and counted in a 1470 Wallac Wizard gamma counter.

**Sulfated Tyrosine-containing Peptides—** Solid phase synthesis of sulfated tyrosines in peptides Thr266–Asp287 and Asp269–Pro280 (Table I) was accomplished by the method of Kitagawa et al. (32).

**Expression of Wild-type and the Type 2B Mutant A1 Domain of vWF—** The 508–709 recombinant fragments of vWF with the wild-type sequence or the Ile546Val type 2B mutation were expressed and purified as described previously (33, 34).

**RESULTS**

The Effect of Glycocalcin and Mocarhagin Fragments on the Binding of FXI to Activated Platelets—Previous studies have determined that the FXI binding site on platelets is in the GPIbα subunit of the GPIb-IX-V complex for the following reasons: 1) because two GPIbα ligands, S22 (a monoclonal antibody agonist the NH2 terminal of GPIbα) and bovine vWF, inhibit FXI binding to platelets; 2) because FXI was shown by surface plasmon resonance to bind specifically to glycocalcin in a Zn2+-dependent fashion; and 3) because glycocalcin could promote FXI activation by thrombin, another GPIbα ligand (12). It has been determined that a sulfated tyrosine/anionic sequence, Tyr754–Glu758, of GPIbα comprises a binding site for vWF and thrombin (21, 22, 24–27) and that the LRRs of GPIbα are important for binding vWF (26, 27). To determine the binding site for FXI in GPIbα, we utilized mocarhagin, a cobra venom metalloproteinase, that cleaves GPIbα at Glu352–Asp383 to generate a fragment, His3–Glu312, which contains the LRRs and the sulfated tyrosine/anionic sequences Tyr276–Glu282 and excludes the carbohydrate-rich macroglycopeptide. We examined the effect of glycocalcin and the His3–Glu312 fragment on the binding of FXI to activated platelets. Fig. 1 shows that the His3–Glu312 fragment competed for binding sites on FXI-activated platelets with an IC50 similar to that for glycocalcin (10 nM ± 0.65), suggesting that FXI binds to the NH2-terminal portion of glycocalcin. Also, neither of the two synthetic peptides with sulfated tyrosines (Thr266–Asp287 and Asp269–Pro280) had any effect on the binding of FXI to activated platelets, suggesting that FXI does not bind to the anionic domain containing sulfated tyrosines that is utilized for binding thrombin and vWF.

**FXI Binding to Glycocalcin or the Mocarhagin Fragment in the Presence of ZnCl2—** To confirm the notion that FXI is binding to the NH2-terminal region of GPIbα, we performed direct binding experiments in a solid phase assay. Fig. 2 shows that FXI binds to the fragment His1–Glu282 with the same Kd(app) (~10 nM ± 0.9) as intact glycocalcin in the presence of ZnCl2, whereas in the absence of ZnCl2 no FXI-binding to either glycocalcin or the His1–Glu312 fragment was observed. This confirms the conclusion that the NH2-terminal globular domain of GPIbα mediates the binding of FXI to GPIb-IX-Va.

**The Effect of the His3–Glu312 Fragment and Sulfated Peptides on the Binding of FXI to Glycocalcin—** To further confirm our findings on the effect of the His3–Glu312 fragment and sulfated peptides on FXI binding to activated platelets, we examined their effect on the binding of FXI to glycocalcin in a solid phase assay. Fig. 3 shows that the His3–Glu312 fragment inhibited the binding of FXI to glycocalcin with an IC50 of 20 nM ± 1.8, whereas the sulfated peptides Thr266–Asp287 and Asp269–Pro280 had no effect.

**The Effect of FXI on the Binding of Thrombin to Glycocalcin—** Because both FXI and thrombin bind to the NH2-terminal globular domain of GPIbα and because FXI interacts with thrombin through anion-binding exosite I (35), we determined whether thrombin and FXI share a common binding site on GPIbα. Fig. 4 shows that FXI at concentrations up to 4 × 10−5 M does not inhibit the binding of thrombin to glycocalcin, which suggests that FXI binds glycocalcin at sites separate and distinct from those bound by thrombin.

**The Effect of PPACK Thrombin on the Binding of FXI to Glycocalcin—** Because FXI did not compete with thrombin for binding to glycocalcin, we examined the effect of PPACK thrombin on the binding of FXI to glycocalcin in the presence of 25 μM ZnCl2. Our results show that PPACK thrombin at concentrations up to 3 × 10−5 M does not affect the binding of FXI to glycocalcin (data not shown), confirming the conclusion that FXI and thrombin bind to GPIbα at separate and distinct sites.

**The Effects of the A1 Domain (Wild-type and I546V Type 2B Mutant) of vWF on the Binding of FXI to Activated Platelets—** It has been determined that an interaction between the vWF-A1 domain and platelet GPIbα occurs in the presence of high shear stress or when vWF becomes immobilized on a surface, but not under static conditions in free solution (36). This interaction can be induced by exogenous non-physiologic modulators such as ristocetin (37, 38) and by fluid shear stress (39, 40), or it can be the consequence of selected mutations in the A1 domain (such as the I546V vWD type 2B mutant). Thus, the I546V A1 domain mutant binds with high affinity to GPIbα in the absence of modulators. To further investigate the FXI–GPIbα interaction, we asked whether the A1 domain of vWF affects FXI binding to activated platelets. Fig. 5 demonstrates that the vWF-A1 domain preparations (i.e. both the wild-type A1 domain and I546V mutant) inhibit FXI binding to activated platelets in the presence of ristocetin with an IC50 value of 416 nM ± 39 K (K of 130 ± 12 nM) for the wild-type A1 domain and an IC50 of 52 nM ± 4.9 K (K of 16.3 ± 1.5 nM) for the vWF-A1 domain with the I546V mutation. The wild-type vWF-A1 domain does not bind GPIbα unless a modulator such as ristocetin is present.

**FIG. 5. The effect of A1 domain vWF (wild-type) and vWF-A1 2B mutant I546V on the binding of FXI to activated platelets.** The effects of vWF-A1 domains were examined, including wild-type A1 domain vWF (○), wild-type A1 domain vWF and ristocetin (1.5 μg/ml) (X), the vWF-A1 2B mutant I546V (+), and the vWF-A1 2B mutant I546V and ristocetin (1.5 μg/ml) (♀). FXI (22 nm), gel-filitered platelets (1 × 108 platelets/ml), ZnCl2 (25 μM), CaCl2 (2 mm), the thrombin receptor activation peptide (25 μM), and HK (42 μM) were incubated for 30 min at 37 °C either with the designated peptide at the indicated concentration or with buffer solution. Aliquots were removed and centrifuged as described under “Experimental Procedures.” Each point is the average of triplicate determinations. When [125I]-FXI was incubated with platelets at time 0 (at the start of the incubation), the amount of [125I]-FXI bound was <1% of the control value, and the maximum variation of counts per minute bound for each experimental observation was <2% of the total counts per minute bound. One hundred percent binding of FXI represents an average of 98,910 cpm bound, whereas 0% binding to FXI represents 0% bound after subtracting 150 cpm, representing the control in which [125I]-FXI was incubated with platelets at time 0.
ent. Thus, as expected in the absence of ristocetin, the wild-type A1 domain did not compete with FXI for binding to activated platelets. In contrast, the vWF type 2B mutant does also contain the LRRs, which have been implicated in regulating the binding of vWF to platelet GPIb-IX-V (16). Consequently, a single amino acid substitution of proline for Leu129 within the fifth LRR is associated with the congenital bleeding disorder Bernard-Soulier syndrome in close relatives.

The Effect of LRRs on the Binding of FXI to Activated Platelets—We have determined that bovine vWF, which binds to GPIbα in the absence of ristocetin, inhibits the binding of FXI to activated platelets (12). Because vWF interacts with the leucine-rich repeat sequences 1, 2, 3, and 4 and may also interact with the sulfated tyrosine/anionic sequence (Tyr276–Glu282) of GPIbα, we determined whether FXI also interacts with the LRRs of GPIbα. Synthetic peptides corresponding to the sequence of each LRR (20–24 amino acids) were prepared (Table I) and examined for their effects on the binding of FXI to activated platelets. Fig. 6 shows that all LRR peptides were able to inhibit FXI binding to activated platelets in the following order of decreasing potency: LRR7 (Leu177–Ala200), IC₅₀ of 2 × 10⁻⁸ M; LRR1 (Leu53–Val176, IC₅₀ of 2.5 × 10⁻⁸ M); LRR4 (Leu105–Glu128, IC₅₀ of 4.5 × 10⁻⁸ M); LRR5 (Leu129–Lys152, IC₅₀ of 6 × 10⁻⁸ M); LRR6 (Leu152–Thr176, IC₅₀ of 7.5 × 10⁻⁹ M); LRR3 (Leu129–Val144, IC₅₀ of 9 × 10⁻⁸ M); and LRR2 (Leu60–Thr81, IC₅₀ of 1.25 × 10⁻⁷ M). However, a scrambled peptide composed of the same amino acids present in LRR7 did not inhibit the binding of FXI to the activated platelets (Tables I and II and Fig. 6).

Finally, neither the LRR sequences from GPIbβ (41) nor the Toll protein (Drosophila-derived protein) (42) were able to inhibit FXI binding to activated platelets (Fig. 6 and Table I), thereby confirming the specificity of FXI interaction with the LRR of GPIbα.

**DISCUSSION**

The platelet GPIb-IX-V complex is involved in several activities crucial to normal platelet function, including the initial adhesion of platelets to the exposed subendothelium, the regulation of certain cytoskeletal properties such as actin polymerization, and the response of platelets to low concentrations of thrombin (14). FXI interacts with the GPIb-IX-V complex on the platelet surface, and this interaction promotes thrombin-catalyzed FXI activation (12). We have also demonstrated that FXI can interact with lipid rafts on the platelet surface, and this interaction is mediated through the GPIb-IX-V complex (13). The evidence that the FXI binding site on activated platelets consists of the GPIbα subunit is as follows. 1) Bernard-Soulier platelets, lacking the GPIb-IX-V complex, are deficient in FXI binding. 2) Two GPIbα ligands, SZ2 (a monoclonal antibody) and bovine vWF, inhibit FXI binding to platelets; and 3) FXI interacts specifically with glycoplicacin by surface plasmon resonance (12). It has been determined that the 45-kDa NH₂-terminal domain of GPIbα is involved in binding thrombin primarily through a stretch of negatively charged residues including sulfated tyrosines spanning amino acids Gly268–Glu282 (15). The NH₂-terminal portion of GPIbα (His³–Glu²⁸²) also contains the LRxs, which have been implicated in regulating the binding of vWF to platelet GPIb-IX-V (16). Consistent with this conclusion, a single amino acid substitution of proline for Leu¹²⁹ within the fifth LRR is associated with the congenital bleeding disorder Bernard-Soulier syndrome in which GPIbα is expressed in a dysfunctional form that does not bind vWF (43). In the present study we aimed to determine the subdomains within GPIbα that bind FXI and regulate the activation of FXI by thrombin on the platelet surface.

**FIG. 6.** The effect of LRR synthetic peptides on the binding of FXI to platelets. The effects of LRR synthetic peptides derived from sequences in GPIbα, GPIbβ, and the Toll protein were examined, including GPIbα LRR 36–59 (♀), GPIbα LRR 60–81 (♂), GPIbα LRR 82–104 (ahrungen), GPIbα LRR 105–128 (♀), GPIbα LRR 129–152 (♂), GPIbβ LRR 153–176 (♀), GPIbα LRR 177–200 (♂), GPIbβ LRR 177–200 scrambled (♀), and Toll protein LRR 35–58 (♀). GPIbα LRR 49–52 (♀), ZnCl₂ (25 mM), CaCl₂ (2 mM), the thrombin peptide (25 μM), and HK (42 mM) were incubated for 30 min at 37 °C either with the designated peptide at the indicated concentration or with buffer solution. Aliquots were removed and centrifuged as described under "Experimental Procedures." Each point is an average of triplicate determinations. 125I-FXI was incubated with platelets at time 0 (at the start of the incubation), the amount of 125I-FXI bound was <1% of the control value, and the maximum variation of counts per minute bound for each observation was <2% of total counts per minute bound. One hundred percent binding of FXI represents an average of 99,565 cpm bound, whereas 0% binding of FXI represents 0% bound after subtracting 198 cpm, representing the control in which labeled FXI was incubated with platelets at time 0.

**TABLE I**

| Protein   | Derived sequence | Peptide sequence |
|-----------|------------------|------------------|
| GPIbα     | LRR1, 36–59      | L H L S E N L L Y T F S A L T L P Y T R L Q T  |
| GPIbβ     | LRR2, 60–81      | L N L D R A E L T K Q V D G T L P Y V G T   |
| GPIbα     | LRR3, 82–104     | L D L S H N Q L Q S L P L G Q T L P A L T V |
| GPIbα     | LRR4, 105–128    | L D V S F N R L T S L P L G A R L G L G E L Q E |
| GPIbα     | LRR5, 129–152    | L Y L K G N E L K T L P G L T L P K L R E K |
| GPIbα     | LRR6, 153–176    | L S L A N N L T E L P A G L L N G L E N L D T |
| GPIbα     | LRR7, 177–200    | L L L Q E N S L Y T I P K G F G G S H L L P P F A |
| GPIbβ     | 266–287          | T L D G E D T D T L Y a D Y a Y P E E D T E G D |
| GPIbα     | 269–280          | D E G D T D L Y a D Y a Y P |
| GPIbβ     | Scrambled LRR7, 177–200 | A Q F L P L H I L E P L N S G K L T Y F G L F |
| GPIbβ     | LRR, 35–58       | L Y L T G N N L T A L P G L L D A L P A R T |
| Toll protein | LRR, 361–384   | L L E H Q V N L L S L D L S N N R L T H L S G D |

*O-sulfated.

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Our data support the conclusion that FXI interacts with a site (distinct from the thrombin-binding site) within the NH2-terminal globular domain of GPIbα containing the LRR. The evidence supporting this conclusion is as follows. 1) The mocularhagin fragment H1–Glu282, as does glycocalcin (IC50 ~10 nM), competes for FXI binding sites within the intact receptor complex on activated platelets (Fig. 1). 2) FXI binds directly to the GPIbα fragment H1–Glu282 and to glycocalcin with almost identical affinity (Kd ~ 1 ± 0.1 nm). Thus, in the structure of the GPIb-vWF-A1 complex, the vWF-A1 domain (wild-type) – ristocetin, leading to optimal rate of thrombin-catalyzed FXI activation. 3) Recent work has demonstrated a LRR motif in the form of an M-shaped arrangement of three tandem β-hairpins (29–30), each containing a hydrophobic hairpin region (265–280). The NH2-terminal β-hairpin has two antiparallel strands with a disulfide (Cys4–Cys17) bridge at the base. The two β-strands present on the concave face of GPIbα are on LRR3 and LRR4. The eight LRRs fold into a characteristic arc shape with parallel β-sheets on the concave face. The structure also demonstrates a LRR motif in the form of an M-shaped arrangement of three tandem β-turns. A negatively charged binding surface on the LRR concave face and the anionic region are implicated in a two-step mechanism of binding to vWF-A1, which can be regulated by an unmasking mechanism involving a conformational change of a key loop. Thus, in the structure of the GPIb-vWF-A1 complex, the vWF-A1 domain forms extensive contacts with the leucine-rich repeat residues on the concave surface of GPIbα and a loop (from Val227 to Ser241) termed the “β-switch” that interacts with vWF-A1 to form a continuous β-sheet (20). By contrast, in the GPIbα-thrombin complex (22, 23) the β-switch region extends away from the LRR domain in a conformation resembling that of unliganded GPIbα (19). This conformation favors intimate contacts with thrombin. Because FXI does not bind to the anionic region (Thr266–Asp287) of GPIbα (Figs. 1 and 3), the active site. This model does not represent the dimeric form of FXI, which is not required for GPIb binding.

The GPIbα structure reveals a large patch of negatively charged amino acids (Glu14, Asp18, Glu40, Asp63, Asp83, Asp106, Glu128, Glu151, and Asp175) across the concave surface formed by the LRR sequences and the continuous β-strands of the NH2-terminal hairpin loop that form contact sites for interaction with vWF-A1 (19–21). The experiment in Fig. 6 demonstrated that FXI interacts with a specific manner. This model does not represent the dimeric form of FXI, which is not required for GPIb binding.

FIG. 7. Schematic diagram. Schematic diagram of the ternary complex formed between FXI (blue), thrombin (green), and glycoprotein Ib (shown as the leucine-rich repeats, LRR, and the anionic region) on the platelet surface. Three important contact sites on thrombin are labeled as follows: site 1, anionic binding exosite I; site 2, anion binding exosite II; and site 3, the active site. This model does not represent the dimeric form of FXI, which is not required for GPIb binding.

### Table II

The effect of various peptides and fragments on the binding of factor XI to activated platelets

| Peptide or protein | Protein     | IC50 (nM) | Kd (nM) |
|-------------------|-------------|-----------|---------|
| vWF-A1 domain     | vWF human   | 416 ± 39  | 130 ± 12|
| vWF-A1 domain     | GPIbα       | No effect up to 400 nM |
| vWF-A1 domain I546V type 2B mutant | GPIbα | 52 ± 4.9 | 16.35 ± 1.5 |
| vWF-A1 domain I546V type 2B mutant | GPIbα | 40 ± 5.8 | 18.8 ± 2.0 |
| Glycocalcin       | GPIbα       | 12 ± 1.0  | 7.81 ± 0.8 |
| LRR, 36–59        | GPIbα       | 25 ± 1.8  | 7.81 ± 0.8 |
| LRR, 60–81        | GPIbα       | 125 ± 9.1 | 39.06 ± 4.1 |
| LRR, 82–104       | GPIbα       | 90 ± 8.4  | 28.125 ± 3.0 |
| LRR, 105–128      | GPIbα       | 45 ± 5.6  | 14.06 ± 1.5 |
| LRR, 129–152      | GPIbα       | 60 ± 6.2  | 18.75 ± 1.9 |
| LRR, 153–176      | GPIbα       | 75 ± 6.9  | 23.43 ± 2.4 |
| LRR, 177–200      | GPIbα       | 20 ± 1.2  | 6.25 ± 0.70 |
| Thr105–Asp129     | GPIbβ       | No effect up to 1 × 10−6 M |
| Asp129–Pro140     | GPIbα       | No effect up to 1 × 10−6 M |
| LRR, 177–200 (scrambled) | GPIbα | No effect up to 1 × 10−6 M |
| LRR, 35–58        | GPIbα       | No effect up to 1 × 10−6 M |
| LRR, 361–384      | Toll protein| No effect up to 1 × 10−6 M |
FXI binding to activated platelets, suggesting that FXI may interact with a concave surface formed by the LRR. However, the fact that LRR1 and LRR7 are the most potent inhibitors of FXI binding to platelets suggests that the major interaction of FXI with GPIb involves LRR1 and LRR7. Unlike the vWF-A1 domain interaction with GPIb, the anionic region does not appear to be involved in FXI interaction with GPIb, because the sulfated peptides (Thr266–Asp287 and Asp266–Pro280) do not inhibit either FXI interaction with activated platelets or with glycolicin (Figs. 1 and 3). Because the vWF-A1 domain inhibits FXI interaction with activated platelets (Fig. 5), it is possible that FXI may interact with some of the same negative amino acids to which vWF binds in the LRRs of GPIb. We have determined the specific amino acids within the FXI-A complex 3 domain that interact with platelets and with GPIb (28, 44). A logical extension of the present study will be to identify the amino acid residues within the LRRs of GPIb that interact with the Apple 3 domain of FXI.

In agreement with our finding that FXI interacts with the LRRs of GPIb, the anionic region does not inhibit FXI interaction with activated platelets (Fig. 5), it is possible that FXI may interact with some of the same negative amino acids to which vWF binds in the LRRs of GPIb. We have determined the specific amino acids within the FXI-A complex 3 domain that interact with platelets and with GPIb (28, 44). A logical extension of the present study will be to identify the amino acid residues within the LRRs of GPIb that interact with the Apple 3 domain of FXI.

REFERENCES

1. Baglia, F. A., Badellino, K. O., Ho, D. H., Dasari, V. R., and Walsh, P. N. (2000) J. Biol. Chem. 275, 31954–31962
2. Binder, B., Krug, G., and Vukovich, T. (1975) Thromb. Diath. Haemorrh. 34, 354
3. Brunner, T., La Porta, C., Reddigari, S. R., Salerno, V. M., Kaplan, A. P., and Silverberg, M. (1993) Blood 81, 580–586
4. Galliani, D., and Broze, G. J., Jr. (1991) Science 253, 909–912
5. Galliani, D., and Broze, G. J., Jr. (1995) Semin. Thromb. Hemostasis 21, 396–404
6. Kaplan, A. P., Silverberg, M., Dunn, J. T., and Miller, G. (1981) Ann. N. Y. Acad. Sci. 370, 253–260
7. Thompson, R. E., Mandle, R. Jr., and Kaplan, A. P. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4862–4866
8. Baglia, F. A., and Walsh, P. N. (1998) Biochemistry 37, 2271–2281
9. Greenwald, J. S., Heek, M. J., Ercal, E., Walsh, P. N., and Griffin, J. H. (1986) Biochemistry 25, 3884–3890
10. Baglia, F. A., and Walsh, P. N. (2000) J. Biol. Chem. 275, 20514–20519
11. Baglia, F. A., Jameson, B. A., and Walsh, P. N. (1995) J. Biol. Chem. 270, 6734–6740
12. Baglia, F. A., Badellino, K. O., Li, C. Q., Lopez, J. A., and Walsh, P. N. (2002) J. Biol. Chem. 277, 1662–1668
13. Baglia, F. A., Shrimpton, C. N., Lopez, J. A., and Walsh, P. N. (2003) J. Biol. Chem. 278, 21744–21750
14. Lopez, J. A. (1994) Blood Coagul. Fibrinolysis 5, 97–119
15. De Marco, L., Mazzuccato, M., Masetti, A., Fenton, J. W. D., and Ruggeri, Z. M. (1991) J. Biol. Chem. 266, 23776–23783
16. Hess, D., Schaller, J., Rickli, E. E., and Clemetson, K. J. (1991) Eur. J. Biochem. 199, 389–393
17. Cruz, M. A., Handin, R. I., and Wise, R. J. (1993) J. Biol. Chem. 268, 21238–21245
18. Emsley, J., Cruz, M., Handin, R., and Liddington, R. (1998) J. Biol. Chem. 273, 10396–10401
19. Uff, S., Clemetson, J. M., Harrison, T., Clemetson, K. J., and Emsley, J. (2002) J. Biol. Chem. 277, 35657–35663
20. Huizinga, E. G., Tsuji, S., Romijn, R. A., Schiphorst, M. E., de Groot, P. G., and Sixma, J. J. (2000) Science 297, 1176–1179
21. Dumas, J. J., Kumar, R., McDonagh, T., Sullivan, F., Stahl, M. L., Somers, W. S., and Mosyak, L. (2004) J. Biol. Chem. 279, 23327–23334
22. Celikel, R., McClintock, R. A., Roberts, J. R., Mendolicchio, G. L., Ware, J., Varughese, K. I., and Ruggeri, Z. M. (2003) Science 301, 218–221
23. Dumas, J. J., Kumar, R., Seehra, J., Somers, W. S., and Mosyak, L. (2003) Science 301, 222–226
24. Cauwenberghs, N., Vanhoorelbeke, K., Vauterin, S., Westra, D. F., Romo, G. M., Turner, N. T., McIntire, L. V., and Lopez, J. A. (2001) Blood 98, 652–660
25. Scholer, Y., Romo, G. M., Dong, J. F., Schade, A. McIntire, L. V., Kenoy, D., Whistock, J., McBernt, M. C., Lopez, J. A., and Andrews, R. K. (2000) Blood 95, 903–910
26. Dong, J. F., Schade, A. J., Gao, S., Romo, G. M., Turner, N. T., McIntire, L. V., and Lopez, J. A. (2001) J. Biol. Chem. 276, 16690–16694
27. Marchese, P., Murata, M., Mazzuccato, M., Pradella, P., De Marco, L., Ware, J., and Ruggeri, Z. M. (1995) J. Biol. Chem. 270, 8571–8578
28. Baglia, F. A., and Walsh, P. N. (2000) Biochemistry 39, 316–322
29. Romo, G. M., Dong, J. F., Schade, A. J., Gardner, E. E., Kanas, G. S., Li, C. Q., McIntire, L. V., Berndt, M. C., and Lopez, J. A. (1999) J. Exp. Med. 190, 803–814
30. Ward, C. M., Andrews, R. K., Smith, A. I., and Berndt, M. C. (1996) Biochemistry 35, 4929–4938
31. De Cristofaro, R., De Candia, E., Landolfi, R., Rutella, S., and Hall, S. W. (2001) Biochemistry 40, 13289–13273
32. Katagawa, K., Aida, C., Fujiwara, H., Yagami, T., Futaki, S., Kogire, M., Ida, J., and Inoue, K. (2001) J. Org. Chem. 66, 1–10
33. Mihara, H., Yoshikawa, A., Zumerman, T. S., and Ruggeri, Z. M. (1989) J. Biol. Chem. 264, 17361–17367
34. Celikel, R., Ruggeri, Z. V., and Varughese, K. I. (2000) Nat. Struct. Biol. 7, 851–854
35. Yun, T. H., Baglia, F. A., Myles, T., Navaneetham, D., Lopez, J. A., Walsh, P. N., and Leung, L. L. (2003) J. Biol. Chem. 278, 48112–48119
36. Miyata, S., Goto, S., Federici, A. B., Ware, J., and Ruggeri, Z. M. (1996) J. Biol. Chem. 271, 9046–9053
37. Howard, M. A., and Firkin, B. G. (1971) J. Biol. Chem. 246, 261–269
38. Scott, J. P., Montgomery, R. R., and Retzinger, G. S. (1991) J. Biol. Chem. 266, 8149–8155
39. Peterson, D. M., Stathopoulos, N. A., Giorgio, T. D., Hellums, J. D., and Moake, J. L. (1987) Blood 69, 625–628
40. Goto, S., Salomon, D. R., Ikeda, Y., and Ruggeri, Z. M. (1995) J. Biol. Chem. 270, 23352–23361
41. Lopez, J. A., Chang, D. W., Fujikawa, K., Hagen, F. S., Dave, E. W., and Roth, G. J. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2135–2139
42. Hashimoto, C., Hudson, C., and Krutzsch, H. (1991) P1097 (abstr.)
Factor XI Interacts with the Leucine-rich Repeats of Glycoprotein Ibα on the Activated Platelet

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