Identification of the Binding Site for Fibrinogen Recognition Peptide \(\gamma^{383-395}\) within the \(\alpha_M\)I-Domain of Integrin \(\alpha_M\beta_2^*\)

Received for publication, November 8, 2000, and in revised form, January 8, 2001
Published, JBC Papers in Press, January 19, 2001, DOI 10.1074/jbc.M010174200

Valentin P. Yakubenko†, Dmitry A. Solovjov‡, Li Zhang§, Vivien C. Yee‡, Edward F. Plow‡, and Tatiana P. Ugarova‡¶

From the †Joseph J. Jacobs Center for Thrombosis and Vascular Biology and the Department of Molecular Cardiology, Lerner Research Institute, Cleveland, Ohio 44195 and the ¶J. Holland Laboratory, American Red Cross, Rockville, Maryland 20855

The leukocyte integrin \(\alpha_M\beta_2\) (Mac-1, CD11b/CD18) is a cell surface adhesion receptor for fibrinogen. The interaction between fibrinogen and \(\alpha_M\beta_2\) mediates a range of immune responses. The sequence \(\gamma^{383-395}\)TKIIFNRTLIG\(\), P2-C, within the \(\gamma\)-module of the D-domain of fibrinogen, is a recognition site for \(\alpha_M\beta_2\) and \(\alpha_M\beta_2\). We have now identified the complementary sequences within the \(\alpha_M\)I-domain of the receptor responsible for recognition of P2-C.

The strategy to localize the binding site for P2-C was based on distinct P2-C binding properties of the three structurally similar I-domains of \(\alpha_M\beta_2\), \(\alpha_M\beta_2\), and \(\alpha_M\beta_2\), i.e. the \(\alpha_M\)I- and \(\alpha_M\)I-domains bind P2-C, and the \(\alpha_M\)I-domain did not bind this ligand. The \(\gamma^{383-395}\)KM261 sequence, which forms a loop \(\beta_2\)-\(\delta_5\) and an adjacent helix \(\delta_5\) in the three-dimensional structure of the \(\alpha_M\)I-domain, was identified as the binding site for P2-C. This conclusion is supported by the following data: 1) mutant cell lines in which the \(\alpha_M\)I-domain segments \(\gamma^{385-402}\) and \(\gamma^{385-402}\) were switched to the homologous \(\alpha_M\)I-domain segments failed to support adhesion to P2-C; 2) synthetic peptides duplicating the \(\gamma^{385-402}\) and \(\gamma^{385-402}\) sequences directly bound the D fragment and P2-C derivative, \(\gamma^{384-402}\), and this interaction was blocked efficiently by the P2-C peptide; 3) mutation of three amino acid residues within the \(\gamma^{385-402}\) segment, Phe\(^{386}\), Asp\(^{387}\), and Pro\(^{390}\), resulted in the loss of the binding function of the recombinant \(\alpha_M\)I-domains; and 4) grafting the \(\alpha_M\)I(\(\gamma^{385-402}\)) segment into the \(\alpha_M\)I-domain converted it to a P2-C-binding protein. These results demonstrate that the \(\alpha_M\)I(\(\gamma^{385-402}\)) segment, a site of the major sequence and structure difference among \(\alpha_M\)I, \(\alpha_M\)I, and \(\alpha_M\)I-domains, is responsible for recognition of a small segment of fibrinogen, \(\gamma^{383-395}\), by serving as ligand binding site.

Integrin \(\alpha_M\beta_2\) participates in the attachment of leukocytes to the endothelial lining of blood vessels and the subsequent transmigration of adherent cells during immune-inflammatory responses (1–3). The engagement of fibrinogen (Fg)\(^1\) by \(\alpha_M\beta_2\) on the surface of leukocytes and by intercellular adhesion molecule-1 (ICAM-1) on the endothelium may play a role in mediating the adhesion of leukocytes to the vessel wall (4, 5) and in facilitating their subsequent extravasation across the endothelial monolayer (6). In addition, the binding of deposited fibrinogen or fibrin to \(\alpha_M\beta_2\) may mediate leukocyte adhesion at extravascular sites of inflammation (7–9).

In previous studies, Altieri et al. (10, 11) demonstrated that a peptide (designated P1), corresponding to residues 190–202 of the \(\gamma\)-chain of the D-domain of Fg, was recognized by \(\alpha_M\beta_2\). However, when residues key to the recognition of P1 by \(\alpha_M\beta_2\)-bearing cells were mutated in the \(\gamma\)-module, \(\gamma^{148-411}\), this recombinant fragment was as active as its wild-type counterpart in supporting \(\alpha_M\beta_2\)-mediated adhesion (12). This observation led to the search for additional \(\alpha_M\beta_2\) recognition sites within the \(\gamma\)-chain, and ultimately the P2 peptide, corresponding to \(\gamma^{377-395}\), was identified (12). Indeed, in comparative analyses, P2 was 10–15-fold more potent than P1 in inhibiting adhesion of the \(\alpha_M\beta_2\)-expressing cells to the D fragment of Fg.

Further analyses of the adhesion-promoting activity of overlapping peptides showed that its COOH-terminal part, \(\gamma^{383-395}\)TKMIIFNRTLIG\(\), designated P2-C, was the primary site of its biological activity (12). Recently, a second leukocyte integrin, \(\alpha_M\beta_2\), which is highly homologous to \(\alpha_M\beta_2\), was demonstrated to bind to the \(\gamma\)-module and P2-C peptide (13), and soluble P2-C peptide efficiently blocked the \(\alpha_M\beta_2\)-mediated adhesion.

Within the heterodimeric \(\alpha_M\beta_2\) receptor, the I-domain, a region of ~200 amino acid residues, inserted in the \(\alpha_M\) subunit, contributes broadly to the recognition of ligands by \(\alpha_M\beta_2\) (14) and specifically to the binding of Fg to this integrin (14, 15). In addition to Fg, this region also was implicated in the binding of ICAM-1 (15), iC3b (16), and neutrophil inhibitory factor, NIF (17, 18). We have shown previously that P2 interacts with the recombinant \(\alpha_M\)I-domain and that NIF partially blocked this interaction (12).

Recent studies suggested that overlapping but not identical sites are involved in the recognition of iC3b, NIF, and Fg (19). However, although the binding sites for iC3b and NIF in the \(\alpha_M\)I-domain were mapped extensively (20–23), the recognition site for Fg has not been studied. Recently, sequences key to the binding of NIF and iC3b to the \(\alpha_M\)I-domain were mapped using a homolog scanning mutagenesis strategy (22, 24). This approach is based upon the structural similarity of the I-domains of \(\alpha_M\) and \(\alpha_M\), and the differences in their ligand recognition; i.e. the crystal structures of the I-domains of \(\alpha_M\) and \(\alpha_M\) are very similar (25–28), but only the
I-domain of α₄ binds with high affinity (17, 18). Fg, together with NIF and iC3b, does not bind to α₂β₁, suggesting that differences in the structure of the α₂β₁- and α₁I-domains may be responsible for their distinction in ligand binding specificity. In this study, we have sought to localize the binding site for the P2-C sequence of Fg within the α₁I-domain. The strategy developed was based on the differences in the binding of P2-C to the α₂β₁-, α₂I-, and α₁I-domains and involved several independent approaches, including screening of mutant cells, synthetic peptides, site-directed mutagenesis, and the gain-of-function analyses. The binding site for P2-C was localized within the segment α₄Lys245-Arg261, a site of the major structure divergence between α₂L-, α₂I-, and α₁I-domains. The grafting of this segment into the α₁I-domain converted it to the P2-C-binding protein. Thus, a small amino acid sequence, P2-C, with a defined structure within a crystallized domain of fibrinogen (29) is shown to interact with a small segment that also has a defined structure within the α₁I-domain.

**EXPERIMENTAL PROCEDURES**

**Proteins, Peptides, and Monoclonal Antibodies**—Human kidney 293 cells expressing wild-type and the mutant forms of the α₂β₁ receptor were described and characterized in detail previously (19, 22). These cells were grown as adherent monolayers in Dulbecco’s modified Eagle’s medium/F-12 medium (BioWhittaker, Walkersville, MD), supplemented with 10% fetal bovine serum, 25 mM HEPES, and antibiotics. Human Fg was purified from fresh human blood by differential ethanol precipitation (30) or obtained from Enzyme Research Laboratories (South Bend, IN). The D100 (Mr 98,000) was produced by digestion of human Fg with plasmin (Enzyme Research Laboratories, South Bend, IN) and purified as described (31). The D98 fragment (Mr 98,000) was produced by digestion of the D100 with plasmin. This fragment lacks 5–15 amino acid residues from the COOH terminus of the γ-chain and will be described elsewhere. 5D₁₆ was biotinylated with EZ-link Sulfo-NHS-LC-Biotin (Pierce) according to the manufacturer’s instructions. P1 (γ₁90–202), P2 (γ₂57–395), P2’ (γ₂53–395), H19 (γ₃40–357), H20 (γ₃56–374), L10 (γ₄02–411), and H19 (γ₄02–411) peptides were synthesized and purified as described (12). In addition, an analog of P2-C, P2-Ce, a peptide with the extended COOH-terminal end, γ₈₃₈–402, was also synthesized to test for direct binding to α₂β₁-domain peptides. The following peptides duplicating selected sequences within the α₂β₁-domain were synthesized: 1PHDFIR (20), P350TQLGRRGTIIHGR (21), P305TQL (21), Lys245Arg261, 245KFGDP-PLG (22), Lys245γ253, 245DPHEL (22), Lys245γ253, 245KFGDP-PLG (22), Lys245γ253, 245DPHEL (22), Lys245γ253, and 213FITGARK (22). The numbers indicate the positions of the residues within the α₂β₁ subunit (numbered according to Arnaout et al. (32)). NIF (a gift from Covias International, San Diego) was labeled with EZ-link Sulfo-NHS-LC-Biotin according to the manufacturer’s protocol. mAbs OKM1, 44a, and 904 were obtained from ATCC (Rockville, MD). mAb 4b (3-2) was a generous gift from Dr. B. Kudryk, the New York Blood Center, and mAb 4A5 (84) was obtained from Dr. G. Matsueda (Bristol-Myers Squibb). Expression of Recombinant α₂β₁, α₂I-, and α₁I-Domains and Site-directed Mutagenesis—The I-domains were expressed as fusion proteins with glutathione S-transferase (GST) and purified from soluble fractions of Escherichia coli lysates by affinity chromatography. The

**Mutations in the Lysγ²⁴⁵—Arg²⁶¹ sequence of the recombinant α₁I-domain and nucleotide sequences used for their constructing**

| Mutant | Mutagenic primer |
|--------|-----------------|
| 1K245/1G247A | GTCAATCGGATGAGGACAGGCTTTGATGACGACGAGAG |
| 2D235A/P273T | CTTGGGATAGCTGGCAGCCTTGCTAGAG |
| 3K245/1G247A/D254A/P257A | CCGATGAGGACAGGCTTTGATGACGACGAGAG |
| 4F246R | CTATTGGAATGGCAGTTCGCTTGGG |
| 5D254A | GCCCTGAGGACAGGCTTTGATGACGACGAGAG |
| 6P275A | GGATGATGACGACGCTGGCAGCCTTGCTAGAG |
| 7D526A | GAGGATGATGACGACGCTGGCAGCCTTGCTAGAG |

*The number indicates the position of amino acids in the full-length α₄ subunit (32). The lowercase letters indicate the mutagenic bases.*

**Generation of the α₂β₁ (Lysγ²⁴⁵—Arg²⁶¹)I-Domain Chimera**—The segment corresponding to the sequence Alaγ²⁴⁵—Arg²⁶¹ within the α₁I was exchanged to the homologous segment of the α₂I-Domain Lysγ²⁴⁵—Arg²⁶¹. The segment switch was created by oligonucleotide-directed mutagenesis using polymerase chain reaction. The construction of the chimera was based on the observation that oligonucleotide sequence corresponding to α₂β₁ (Lysγ²⁴⁵—Arg²⁶¹) contains a unique restriction site for EcoRI (SauI), whereas the second site for this enzyme is present between 4760 and 4761 of the pGEX-4T-1 sequence. To switch the P25C γLysγ⁴⁵—Argγ²⁶¹ from α₂β₁ to α₁I, the mutagenic primers were designed to contain the cDNA fragments coding for the full-length of α₁L and α₁R, respectively. The primers used for the α₁I-Domain were 5’TGGCATGACGAGGCTTTGATGACGACGAGAG and 5’TGGATCCGGATGAGGACAGGCTTTGATGACGACGAGAG. The primers used for the α₂β₁-Domain were 5’CAGGAAGGACAGGCTTTGATGACGACGAGAG and 5’CTTTGGAATGGCAGTTCGCTTGGG (forward) and 5’TGGCTTGGCCACGCTTCAATGAGCATAGA (reverse). The underlined nucleotides are BamHI and NotI recognition sequences that were introduced in the primers. The fragments were digested with BamHI and NotI and cloned in the expression vector pGEX-4T-1 (Amersham Pharmacia Biotech). The accuracy of the DNA sequence was verified by sequencing. The construct was transformed in E. coli strain BL-21(DE3)pLysS competent cells, and expression was induced by adding 1 mM isopropyl-1-thio-β-D-galactopyranoside for 3–5 h at 37 °C. To express the α₁I-Domain, the following primers were used to amplify a cDNA fragment encoding the α₁I-Domain (residues Gluγ¹³²—Argγ²⁶¹) and a segment randomly primed from the cDNA fragment of the U937 monocytic cell line 5’AGGCTCAGGATGAGGACGAGGCTTTGATGACGACGAGAG (forward) and 5’CATCTGCAATTTGGCCACGCTTTGCTGATGC (reverse). The product was digested with BamHI and NotI and cloned into pGEX-4T-1. The plasmid was transformed in E. coli BL-21(DE3)pLysS cells, and the correctness of the α₁I-Domain insertion was confirmed by sequencing. The α₁I-Domain was expressed and purified from the cell lysates as a fusion protein with GST under the conditions used for the α₂β₁-Domain.

Site-directed mutagenesis of the α₂β₁-Domain was performed by using QuickChange™ mutagenesis kit (Strategene, San Diego). The pGEX-4T-1 construct containing DNA encoding the α₂β₁-Domain was modified by site-directed mutagenesis using two mutagenic primers containing the desired mutations. The mutations introduced in the α₂β₁-Domain and the primers used are listed in Table I. The oligonucleotide primers, each complementary to opposite strands of the vector, were extended during temperature cycling by using PfuTurbo™ DNA polymerase. Following temperature cycling, the product was treated with DpnI endonuclease to digest the parental DNA template. The nicked vector DNA incorporating the desired mutations was then transformed into the Epicurian coli XXL-1 Blue supercompetent cells, and cDNA from individual bacterial clones was analyzed by sequencing. The E. coli BL-21(DE3)pLysS host cells were then transformed with the mutant plasmids, and the mutant α₁I-Domains were prepared as described above for the recombinant wild-type α₁I-Domain. The immunoactivity of wild-type and mutant recombinant I-Domains was analyzed by enzyme-linked immunosorbent assay with mAbs 44a and 904 using our standard protocol (35).

**TABLE I**

| Mutant | Mutagenic primer |
|--------|-----------------|
| 1K245A/G247A | GTCAATCGGATGAGGACAGGCTTTGATGACGACGAGAG |
| 2D235A/P273T | CTTGGGATAGCTGGCAGCCTTGCTAGAG |
| 3K245A/G247A/D254A/P257A | CCGATGAGGACAGGCTTTGATGACGACGAGAG |
| 4F246R | CTATTGGAATGGCAGTTCGCTTGGG |
| 5D254A | GCCCTGAGGACAGGCTTTGATGACGACGAGAG |
| 6P275A | GGATGATGACGACGCTGGCAGCCTTGCTAGAG |
| 7D526A | GAGGATGATGACGACGCTGGCAGCCTTGCTAGAG |

*The number indicates the position of amino acids in the full-length α₄ subunit (32). The lowercase letters indicate the mutagenic bases.*
domain was modified by polymerase chain reaction using PfuTurbo DNA polymerase with the following cycling parameters: 95°C for 30 s, 55°C for 1 min, 68°C for 11.5 min. Following temperature cycling, the product was treated with Dpsol to digest the parental DNA template. The linear product was purified and digested with EcoRI to produce the P2-C fragments with cohesive ends. These fragments were ligated and transformed into Epicurian Coli XL1-Blue supercompetent cells. The accuracy of the DNA sequence and the correctness of the I-domain direction were verified by sequencing. The E. coli BL21 (DE3) pLYS8 cells were transformed with the mutated plasmid, and the chimeric molecule was prepared following the procedure described above for the wild-type and mutant I-domains.

**Flow Cytometry—** FACS analyses were performed to assess the expression of αββ2 on the surface of the cells transfected with wild-type and mutant forms of the receptor. The cells were harvested, and 10⁶ cells were incubated with α-specific mAbs OKM1 or 44A at 15 μg/200 μl of cell suspension for 30 min at 4°C. The cells were then washed and incubated with fluorescein isothiocyanate-goat anti-mouse IgG (at a 1:1,000 dilution) for additional 30 min at 4°C. Finally, the cells were washed and analyzed in a FACS Star (Beckton Dickinson, Mountain View, CA). Populations of cells expressing a similar amount of the receptor were selected by FACS. A clonal population of each mutant was typically used in the subsequent experiments.

Peptide assays—The wells of tissue culture plates (Costar, Cambridge, MA) were coated with 6.1, 12.5, 25, 50, and 100 μg/ml P2-C peptide or 1, 5, and 25 μg/ml of D100 fragment for 3 h at 37°C. The amount of peptides immobilized onto the wells was measured by utilizing radiolabeled peptides (12). The coated wells were postcoated with 0.5% polyvinylpyrrolidone for 1 h at 22°C. After washing, bound I-domains were detected with an anti-GST mAb (Upstate Biotechnology, Lake Placid, NY) at a 1:5,000 dilution.

**RESULTS**

Binding of Wild-type Recombinant αIIb-β3, αIIb-β1, and αIIb-β1-β3 Domains to the P2-C Peptide of Fg—In previous studies, we have demonstrated that the P2-C peptide binds directly to the recombinant αIIb-β3 domain (12). αIIb-β1 also recognizes P2-C (13); but the role of the αIIb-β1 domain in this interaction was not evaluated, and the recognition of P2-C by the αIIb-β1 domain had not been tested. Therefore, the three I-domains were expressed as GST fusion proteins and tested for their ability to interact with the immobilized P2-C peptide. As shown in Fig. 1, the recombinant αIIb-β3 domain exhibited a dose-dependent and saturable binding to the P2-C peptide similar to that of the recombinant αIIb-β3 domain. In contrast, the recombinant αIIb-β1 domain did not interact with P2-C even at the highest concentration of the αIIb-β1 domain added (200 μg/ml maximal testable concentration). The binding characteristics of the isolated I-domains parallel the binding properties of the corresponding intact receptors on cell surfaces, i.e. the αIIb-β3 and αIIb-β1-expressing cells adhere to Fg and P2-C, whereas the αIIb-β3-bearing cells do not (see Figs. 2 and 3). Thus, these data confirm that binding of P2-C to two highly homologous integrins, αIIb-β3 and αIIb-β1, is mediated by αIIb-β3 and αIIb-β1-expressing cells adhere to Fg and P2-C, whereas the αIIb-β1-bearing cells do not.

Binding of P2-C to Mutant Cell Lines—As the first step to define the binding site for P2-C within the αIIb-β3 domain, mutant cell lines, each expressing a mutant αIIb-β3 in which a short αIIb-β3-domain sequence was replaced for the corresponding region of the αIIb-β3-domain, were tested for their adhesion to immobilized...
mobilized P2-C or D100 fragment. These mutant cell lines have been used previously to examine the binding of NIF and iC3b to coated with different concentrations of P2-C. Adhesion of $\alpha_M(K_{231}NAF)$-expressing cells to H19 is shown for comparison (dashed line). After 25 min at 37 °C in a humidified atmosphere containing 5% CO₂, the nonadherent cells were removed by three washes with HBSS, and the amount of adherent cells was determined using the fluorescent dye CyQuant as described under “Experimental Procedures.” Data are expressed as a percentage of added cells and are the mean ± S.E. of four individual experiments. The actual amounts of P2-C and H19 immobilized onto the plastic wells were determined as described (12) and are shown on the abscissa.

The adhesion of each mutant was measured with increasing concentrations of immobilized P2-C and D100 fragment to determine the maximal level of adhesion. In all cases where a cell line bearing a mutant receptor did adhere to either substrate, the adhesion was dependent upon the concentration of the immobilized ligand and reached a plateau. This maximal adhesion was compared with that of the cells expressing wild-type $\alpha_M\beta_2$ and mock-transfected cells in the same experiment to allow normalization of results. The cell lines exhibiting adhesion similar to or greater than the wild-type $\alpha_M\beta_2$ cells were identified as “positive” mutants. The pattern of cell adhesion to P2-C obtained for wild-type $\alpha_M\beta_2$ and a representative mutant, $\alpha_M(K_{231}NAF)$, is shown in Fig. 2. Adhesion of $\alpha_M(K_{31}NAF)$ to P2-C was dose-dependent and saturable with ~70% of added cells adherent to P2-C at the plateau. Adhesion of wild-type and mutant cells to a control peptide, H19, was tested and was found to be less than 5% of added cells. Also, as the essential control, the $\alpha_M\beta_2$-expressing cells adhered poorly to either adhesive substrates, P2-C and D100 (−10–15% of added cells) consistent with lack of interaction of $\alpha_M\beta_2$ with Fg. All negative mutants did not adhere to P2-C; adhesion of these cells was similar to that of mock-transfected cells.

The results of adhesion of 16 mutants to P2-C and the D100 fragment are summarized in Fig. 3, A and B. The data are expressed as the percent adhesion of the wild-type $\alpha_M\beta_2$-expressing cells to each substrate. Substitutions for the following regions of $\alpha_M$-I domain abrogated adhesion: $\alpha_M(Pro^{147}Arg^{152})$, $\alpha_M(Pro^{201}Gly^{207})$, $\alpha_M(Arg^{208}Lys^{217})$, $\alpha_M(K^{245}FG)$, and $\alpha_M(Glu^{253}Arg^{261})$. These cell lines form a group of negative mutants. The lack of adhesion of these mutants was not the result of decreased surface expression of the receptor because there was no correlation between adhesion and expression. Specifically, although surface expression of $\alpha_M(R^{206}K^{217})$ was 2.0 lower than that of the wild-type $\alpha_M\beta_2$ cells, adhesion was decreased 6-fold. On the other hand, surface expression of the $\alpha_M(K^{31}FG)$ was 1.2-fold higher than that of the cells expressing the wild-type $\alpha_M\beta_2$ cells, but adhesion was abrogated completely. Surface expression of two other negative mutants, $\alpha_M(P^{145}E^{150})$ and $\alpha_M(E^{253}R^{261})$, was very similar to that of the wild-type receptor.

The following mutants supported the same or higher levels of adhesion to P2-C compared with the cells expressing the wild-type $\alpha_M\beta_2$ receptor (positive mutants): $\alpha_M(M^{165}T^{169})$, $\alpha_M(E^{162}L^{170})$, $\alpha_M(E^{178}T^{186})$, $\alpha_M(Q^{162}S^{197})$, $\alpha_M(K^{211}NAF^{234})$, deletion mutant $\alpha_M(D^{481}PLGY^{525})$, $\alpha_M(R^{281}E^{287})$, and $\alpha_M(Q^{280}E^{294})$. Adhesion of dE262G and $\alpha_M(13998)$ was partially affected: the maximal level of adhesion to P2-C reached 64 ± 5% and 83 ± 7%, respectively, of wild-type $\alpha_M\beta_2$ cells. These two receptors were classified as “intermediate” mutants. $\alpha_M(D^{273}K^{279})$ was the only mutant to exhibit differential recognition of P2-C and the D fragment; it supported adhesion to P2-C effectively (65%) but mediated adhesion to D100 poorly (15%).

Interaction of P2-C and D Fragment with the $\alpha_M$-I Domain Peptides—In subsequent analyses, we focused on the five neg-
ative mutants. Peptides corresponding to the wild-type αM-I-domain sequences were synthesized and tested for their ability to interact with the D fragment and P2-C. Because the sequences within two of the negative mutants, αM(Pro201-Gly207) and αM(Arg208-Lys217), were contiguous, one linear peptide spanning Pro201-Lys217 was prepared. Also, the peptide, Lys245-Tyr252 containing the sequence of the negative mutant αM(K445FG) was extended at its COOH terminus to include the D245PLGY252 sequence. Thus, four peptides were synthesized: Pro147-Arg152, Pro201-Lys217, Lys245-Tyr252, and Glu253-Arg261. In these experiments, we used a derivative of the D98 fragment, D99, which has a higher affinity for αMβ2. D99 contains the entire P2-C but differs from D100 in the length of the constituent γ-chain, which terminates at γ397/405 in D99 compared with the intact γ11 in D100. These αM-I-domain peptides were immobilized onto microtiter plates, and the binding of biotinylated D98 fragment to them was assessed. As shown in Fig. 4A, two peptides, Lys245-Tyr252 and Glu253-Arg261, bound D99 effectively. The immobilized Pro201-Lys217 peptide exhibited low binding of D99, and Pro147-Arg152 and control peptide Phe223-Aas232 did not bind the fragment.

The interaction of the D98 fragment with Lys245-Tyr252 and Glu253-Arg261 was characterized further. The binding of D98 to immobilized Lys245-Tyr252 and Glu253-Arg261 was effectively inhibited by P2-C and P2 (γ377–395), which contains the P2-C sequence (Fig. 4B; inhibition of D98 binding to Glu253-Arg261 by P2 is shown). In addition, P1 (γ190–202) inhibited the binding of D99 to Lys245-Tyr252 and Glu253-Arg261 (Fig. 4B; the effect of P1 on the binding to Glu253-Arg261 is shown). Two control peptides duplicating fibrinogen sequences γ340–357 (H19) and γ350–374 (H20) did not affect the interaction. P1 inhibited the interaction as efficiently as P2, consistent with ability of this peptide to compete with P2 for binding to αMβ2 (12). The interaction of the D98 with immobilized αM-I-domain peptides was cation-independent; in fact, the higher level of binding was observed in the absence than in the presence of 1 mM Ca2+ or Mg2+ (Table II). Interestingly, NIF did not inhibit binding of the D98 fragment to the immobilized Lys245-Tyr252 and Glu253-Arg261 at concentrations as high as 100 μg/ml (Table II), although it efficiently inhibited adhesion of the αMβ2-expressing cells to the D fragment and P2-C peptide at a concentration as low as 0.1 μg/ml (not shown). These data suggest that NIF does not interact with the I-domain peptides, which are capable of binding P2-C and the D99 fragment. Indeed, when direct binding of biotinylated NIF to the immobilized αM-I-domain peptides was tested, NIF did not bind to Lys245-Tyr252 and Glu253-Arg261 peptides (not shown).

In addition, we were able to detect direct binding of P2-C to the immobilized αM-I-domain peptides. Using a derivateive peptide P2-Ce, γ384–402, which contains an epitope for the reporting mAb 4-2, we demonstrated that P2-Ce efficiently bound to immobilized Lys245-Tyr252 and Glu253-Arg261 in a dose-dependent and saturable manner (Fig. 5). The binding of P2-Ce to Pro201-Lys217 was low, whereas Pro147-Arg152 and control peptide Phe223-Aas232 did not bind P2-Ce. Similar to the interaction of the whole D98 fragment, the binding of P2-Ce to Lys245-Tyr252 and Glu253-Arg261 was cation-independent (Table II). To exclude further the possibility that the binding of P2-Ce to immobilized Lys245-Tyr252 and Glu253-Arg261 was nonspecific, we tested two control fibrinogen peptides, γ402–411 (L10) and γ400–411 (H12). These peptides duplicate sequences that reside in close proximity to P2-Ce (γ384–402) and interact with platelet integrin αMβ2. These peptides contain an epitope at γ405–411 recognized by the mAb 4A5 (34). L10 and H12 did not bind to the immobilized αM-I-domain peptides as judged by the lack of the mAb 4A5 immunoreactivity, providing further

---

**Fig. 4.** Binding of the D98 fragment to the peptides from within the αM-I-domain. Panel A, 10 μg/ml biotinylated D98 fragment in 50 mM Tris-HCl, pH 7.5, and 0.05% Tween 20 was added to the wells coated with 0.1 ml/well 100 μM each Pro147-Arg152, Pro201-Lys217, Lys245-Tyr252, Glu253-Arg261, and Phe223-Aas232 and incubated for 2.5 h at 37 °C. After washing, the bound D98 fragment was detected using streptavidin conjugated to alkaline phosphatase and p-nitrophenyl phosphate for disclosure. Panel B, 10 μg/ml biotinylated D98 in 50 mM Tris-HCl buffer, pH 7.5, with 0.05% Tween 20 was mixed with different concentrations of P2 (γ377–395) ( ), P1 (γ190–202) ( ), H19 (γ340–354) ( ), and H20 (γ350–374) ( ) and added to the wells coated with 100 μM Glu253-Arg261 peptide for 2 h at 37 °C. The binding of the D98 was determined as above. Data are expressed as a percentage of the D98 binding in the absence of peptides. Shown in panels A and B are the representative experiments of three to five independent determinations.

---

Evidence for the specificity of the interaction between P2-Ce and two αM-I-domain peptides, Lys245-Tyr252 and Glu253-Arg261. Thus, the D99 and P2-C-derivative bound strongly to the same site for P2-Ce. Guided by the crystal structure of the αM-I- and αI-I-domains, the whole contiguous Lys245-Tyr252 segment was switched from the αM-I-domain into the counterpart region of the αI-I-domain. The appropriate DNA sequence of the entire mutated I-domain was confirmed, and the chimeric I-domain was expressed as a GST fusion protein. The functional consequence of this switch is shown in Fig. 6A. The P2-C peptide was immobilized onto microtiter plates, and the binding of the chimeric αI(αM(Lys245-Arg261))I-domain molecule and the pa-
Effect of cations and NIF on the binding of D98 fragment and the P2-C derivative, γ384–402, to immobilized Glu253–Arg261

10 μg/ml biotinylated D98 fragment in 50 mM Tris-HCl buffer, pH 7.5, containing 1 mM Ca2+, 1 mM Mg2+, 0.05% Tween 20 or in 50 mM Tris-HCl, pH 7.5, 0.05% Tween 20 without cations was added to the wells coated with 100 μM Glu253–Arg261 and incubated for 2.5 h at 37 °C. After washing, bound D98 was detected with streptavidin conjugated to alkaline phosphatase and nitrophenyl phosphate. The effect of 100 μg/ml NIF on the binding of the D98 fragment in the absence of cations was tested in parallel. The inhibitory effect of 10 μM each P2-C and P1 (both tested without cations) is shown for comparison. The binding of the γ384–402 was detected by using the mAb 4–2 as described in Fig. 5. The data shown are the mean values (± S.D.) of the absorbance at 405 nm of a representative experiment done in triplicate.

Table II

| Added ligand | Ca2+ + Mg2+ | No cations | +NIF | + P2-C | + P1 |
|--------------|-------------|------------|------|--------|------|
| D98 fragment | 0.45 ± 0.05 | 1.56 ± 0.08 | 1.46 ± 0.03 | 0.41 ± 0.1 | 0.22 ± 0.05 |

FIG. 5. Binding of the P2-Ce peptide to the αMα1-dI-domain peptides. Different concentrations of P2-Ce (γ384–402) in 50 mM Tris-HCl, pH 7.5, with 100 mM NaCl and 0.05% Tween 20 were added to the wells coated with 100 μM solutions of Pro147–Arg152 (○), Pro201–Lys217 (△), Lys245–Tyr252 (▽), Glu253–Arg261 (●), and Phe223–Asn232 (■) and incubated for 3 h at 37 °C. After washing, the mAb 4–2 at 1:1,000 dilution was added for 1 h at 37 °C. To detect the binding of the mAb 4–2, the second goat anti-mouse IgG was added, and its binding was determined by reaction with p-nitrophenyl phosphate, measuring absorbance at 405 nm.

FIG. 6. Binding of wild-type αM1-domain and αM(αM1-Lys245–Arg261)1-chimeric I-domain to P2-C. Panel A, different concentrations of wild-type αM1- (●) and αM(αM1-Lys245–Arg261)1- (□) domains as fusions with GST in 20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM Ca2+, 1 mM Mg2+, 0.05% Tween 20, and 5% glycerol were added to the wells coated with 50 μg/ml P2-C and incubated for 3 h at 22 °C. After washing, bound I-domains were detected by anti-GST mAb (1:5,000 dilution). After washing, goat anti-mouse IgG conjugated to alkaline phosphatase was added for 1 h, and the binding of the I-domains was measured by reaction with the p-nitrophenyl phosphate. Background binding to BSA or polyvinyl alcohol was subtracted. Panel B, inhibition of the chimeric I-domain binding by P2-C. 50 μg/ml chimera was mixed with different concentrations of P2-C (●) or H19 (□) and then added to the wells coated with P2-C. The binding of the I-domain was determined as in panel A. Results are presented as a percentage of maximal chimeric binding in the absence of P2-C.

Arg261 segment meet both criteria, being exposed and identical in the MI-domain; 2) given the similarities in the X(I-domain binding by P2-C. 50 μg/ml chimera was mixed with different concentrations of P2-C (●) or H19 (□) and then added to the wells coated with P2-C. The binding of the I-domain was determined as in panel A. Results are presented as a percentage of maximal chimeric binding in the absence of P2-C.

rental α1I-domain was tested. Whereas the parental α1I-domain does not bind to P2-C, the grafting of the αM segment imparted the P2-C binding function to the chimeric I-domain. Although the affinity of the interaction could not be assessed accurately from the experimental format used, the concentration of the immobilized P2-C required to obtain 50% of the chimeric I-domain binding was similar to that for wild-type αM1-domain, i.e., ~12 μg/ml for the chimera compared with ~20 μg/ml for the wild-type αM1-domain. The interaction of the chimeric molecule with P2-C was blocked by soluble P2-C, thus confirming specificity (Fig. 6B).

Identification of Residues within the αM(Lys245–Arg261) Segment Critical for P2-C Binding—The residues within the αM(Lys245–Arg261) segment, responsible for the P2-C binding, were identified by site-directed mutagenesis. The selection of residues for mutational analyses was based on the following considerations: 1) the side chains of residues that participate in direct docking of P2-C should be exposed on the hydrated surface of the α1I-domain; 2) given the similarities in the binding function between αM and α1I-domains, it is likely that the residues that interact with P2-C should be identical or conserved between two I-domains; 3) because the switch of the DPLGY252 segment did not affect adhesion of mutant cell line, this sequence was not included in mutational analyses.

Nine residues, Lys245–Phe246–Gly247–Glu253–Asp254–Pro257–Glu258–Asp260, and Arg261 are exposed on the surface of the αM1-domain in the Lys245–Arg261 stretch, and eight residues are identical in the αM(Lys245–Arg261) and αM(Lys262–Ala277) sequences (see Fig. 7A). Thus, only 5 of the 17 residues in Lys245–Arg261 segment meet both criteria, being exposed and identical between αM and αM(Lys245–Arg261) in the P2-C binding function and the Arg261 segment segment was used to introduce the αM1-domain (Fig. 7A), and the capability of mutant proteins to interact with the immobilized P2-C was tested (Fig. 7B). The binding of mutants containing...
The solvent-exposed residues in the right part of the figure are underlined. The residues mutated in the MI-domain are illustrated in the lower part of panel A. Panel B, different concentrations of wild-type (WT) MI-1, MI-2, and MI-3 domains and mutant MI-domains as fusions with GST were added to microtiter wells coated with 50 μg/ml P2-C and incubated for 3 h at 22 °C. The binding of the recombinant I-domains was detected as in Fig. 6. The binding of each mutant reached the maximal level at 100 μg/ml of the added I-domain, and results are presented as a percentage of the binding attained with wild-type MI-domain. The dashed line indicates the maximal level of binding achieved with wild-type MI-domain, and the dotted line is drawn at the level of binding attained with the recombinant MI-domain.

**Discussion**

In this study, we have identified key elements of the binding site for a small amino acid sequence of Fg, γ383–395 (P2-C), within the αMl-domain of αMβ2. The strategy to define the ligand binding site was based on the difference in the P2-C binding properties of the αM1-, αM2-, and αM3-domains and entailed four complementary approaches. In the first approach, a series of homolog-scanning mutants, used previously to map the binding regions for NIF, iC3b, and Candida albicans (22, 24, 37), were screened for adhesion to P2-C and D100 fragment of Fg. In these mutants, 16 segments from the MI-domain were replaced with the corresponding segments from the homologous αM-domain, which does not bind Fg. Because all of these swapped segments are located at the hydrated surface of the αMl-domain, they should identify candidate sequences for interaction with the ligand. Five mutants lacked the ability to support adhesion to P2-C and D100: αMl(147-R152), αM2(P201-G207), αM3(R208-K217), αM(250-R265), and αM(255-R261). Therefore, these αM-domain segments may be critical for binding of these ligands. Alteration of three other regions, dE262G, Asp273-Lys279, and Phe297-Thr307, resulted in the partial loss of adhesive function. These segments may play an accessory role in ligand binding. Thus, the initial insight provided by these mutant receptors indicated that the P2-C binding interface within the αM-domain was composed of several nonlinear sequences. Based on the crystal structure of the αM-domain (25), the segments critical for recognition encompass a portion of helix 1, the loop between helix 3 and helix 4, the small 246–255, K207 segment in the loop between helix 5 and β-strand D, and the entire helix 5 (Fig. 8, left). These sequences form an almost continuous stretch on the upper face of the αM-domain (colored in different shades of green in Fig. 8, left).

The second approach entailed the use of synthetic peptides duplicating the sequences of the critical segments in the MI-domain. These analyses showed that two of four critical segments, αMl(226–235, K207) and αMl(250–265, R261), may contain amino acid residues that participate directly in binding the P2-C sequence of Fg because the peptides that duplicated Lys245-Tyr252 and Glu253-Arg261 bound P2-C. Although the peptide Pro147Arg152 did not interact with the Fg derivatives, and Pro201-Lys217 interacted weakly, the negative results do not exclude a role for these peptides in binding function; the immunobilized peptide may simply not support the appropriate conformation for recognition by the ligand. The Lys245-Tyr252 and Glu253-Arg261 sequences are contiguous, and the entire αM-domain (Lys245-Tyr252, Glu253-Arg261) sequence might serve as the primary binding site for P2-C. Of note, this segment is the most divergent between the αM- and MI-domains in terms of sequence homology and folding (Fig. 8, right). In fact, αM lacks most of helix 5, which is formed by residues Tyr252-Arg261 of αMl (27, 28). In addition, the loop βD-α5 is longer in αMl and assumes a different conformation. Therefore, this difference between αMl and αM could account for inability of αMl to bind Fg. To obtain direct evidence that the βD-α5 loop-α5 helix in

**Fig. 7. Binding of the recombinant wild-type I-domains and αMl-domain mutants to P2-C.** Panel A, alignment of the αM1-, αM2-, and αM3-domain sequences. The αMl(Lys245-Arg261) sequence was aligned with human αM(Lys242-Ala257) and αM(Ala246-Asp256) sequences using the NCBI Blast program. The solvent-exposed residues in the αM(245-Arg261) are in bold, and the residues identical between αM1 and αM2 are underlined. The residues mutated in the MI-domain are illustrated in the lower part of panel A. Panel B, different concentrations of wild-type (WT) MI-1, MI-2, and MI-3 domains and mutant MI-domains as fusions with GST were added to microtiter wells coated with 50 μg/ml P2-C and incubated for 3 h at 22 °C. The binding of the recombinant I-domains was detected as in Fig. 6. The binding of each mutant reached the maximal level at 100 μg/ml of the added I-domain, and results are presented as a percentage of the binding attained with wild-type MI-domain. The dashed line indicates the maximal level of binding achieved with wild-type MI-domain, and the dotted line is drawn at the level of binding attained with the recombinant MI-domain.
Fig. 8. Binding site for P2-C in the \(\alpha_I\)-domain. Left, ribbon model of the \(\alpha_I\)-domain based upon its crystal structure (25), code 1JLM. The \(245^{KFG}\) in the \(\beta\)-a5 loop and \(254^{Glu253}Arg^{261}\) in the helix a5 are bright green. The segments \(201^{Pro201}-Arg^{152}\) and \(201^{Pro201}-Lys^{217}\) are light green. The numbers indicate the positions of segments. The side chains of residue mutations that were found to affect the P2-C binding, \(246^{Phe246}\), \(254^{Asp254}\), and \(255^{Pro257}\) are shown in purple. \(\beta\)-Strands and helix assignments are shown. Right, ribbon model of the \(\alpha_I\)-domain, based upon its crystal structure, code 1LFA (27), shown for comparison. The region homologous to \(\alpha_I\(245^{Lys245}Arg^{261}\) is light blue. The models were drawn using the computer program Molscript, Bobscript and Raster (44–46).

The \(\alpha_I\)-domain constitutes the functional binding site for the Fg ligand, the third approach entailed grafting of the entire \(245^{KFG}\) segment into the corresponding region of the \(\alpha_I\)-domain. As demonstrated in Fig. 6, this manipulation impaired P2-C binding capacity to the chimeric molecule, and the binding affinity of the chimeric receptor for P2-C was very similar to that of wild-type \(\alpha_I\)-domain. Thus, the role of the \(\beta\)-a5 loop-a5 helix in P2-C binding, which initially was inferred from the loss-in-function experiments, was verified by the gain-in-function approach.

Finally, the implementation of the fourth method, site-directed mutagenesis, served the dual purpose. First, it provided the independent confirmation that the \(245^{Lys245}Arg^{261}\) segment is important for P2-C binding because mutations of the three residues resulted in the significant loss of P2-C binding. Second, it implicated \(246^{Phe246}\), \(254^{Asp254}\), and \(255^{Pro257}\) as contact residues. Taken together, the four approaches substantiated independently the role of the \(\beta\)-a5 loop-a5 helix in the P2-C binding and provided evidence that three residues participate in ligand docking.

It is unclear why switches of the \(\alpha_M\(247^{Pro247}Arg^{152}\) and \(201^{Pro201}-Lys^{217}\) resulted in the loss of function given that two other unsubstituted segments, \(245^{Glu253}Arg^{261}\) and \(255^{Glu253}Arg^{261}\) could potentially support adhesion. The effect of the switches of \(\alpha_M\(247^{Pro247}Arg^{152}\) and \(201^{Pro201}-Lys^{217}\) on P2-C recognition could conceivably arise from changes of conformation of the ligand binding region residing in the \(\alpha_I\)-domain \(245^{Lys245}Arg^{261}\). In this regard, a subtle perturbation of the structure of \(201^{Pro201}-Lys^{217}\) and \(201^{Pro201}-Arg^{152}\) mutants was suggested previously by an altered reactivity with the conformation-dependent mAb 24 (22). In addition to the MlDAS motif (25), which is known to be required for the normal binding function (21, 38), single point mutations in the regions outside MlDAS also may abrogate ligand binding by altering conformation (21, 38, 39). For example, alanine substitution of \(248^{Asp248}\) or \(252^{Tyr252}\), the residues that are not exposed on the surface of the \(\alpha_I\)-domain, eliminated the binding of mutants to iC3b (21), suggesting that a structural alteration might have been involved in the loss of binding function. Thus, even relatively small perturbations in the folding of the I-domain can lead to gross alterations in binding affinities.

Although \(245^{Lys245}Arg^{261}\) resides in close proximity to the cation binding MlDAS motif, none of the residues in this sequence is directly involved in coordination of the divalent cation (25). Our results indicate that the binding of Fg derivatives, the D fragment and P2-C, to immobilized peptides duplicating \(245^{Lys245}Arg^{261}\) region was cation-independent. This finding is consistent with previous data showing that EDTA or mutation of Asp244, a residue which coordinates to the bound metal, only partially impairs the binding of Fg to the \(\alpha_I\)-domain although it abolished the binding of other ligands, including NIF and iC3b to the recombinant fragment (25). Another report also suggests that ligands can bind to I-domains independent of cations. Peptides duplicating \(\beta\)-a5 loop in the \(\alpha_I\)-domain or immediately preceding it bound to iC3b in a cation-independent manner (16). Thus, at least in the case of P2-C, its binding to the \(\alpha_I\)-domain does not occur through direct interaction with the metal ion as was proposed (25). This conclusion is further supported by the fact that P2-C sequence in Fg does not contain a candidate acidic residue to provide a missing coordination to the metal. At the same time, P2-C does contain an arginine residue, Arg260, which could displace cation, a model suggested from the crystal structure of the \(\alpha_I\)-domain (40).

The sequence \(245^{Lys245}Tyr^{252}\) which overlaps with the identified Fg-binding region \(245^{Lys245}Arg^{261}\), was implicated previously in the binding of iC3b. Deletion of \(246^{Phe246}Tyr^{252}\) abolished rosetting of iC3b-coated erythrocytes with \(245^{Lys245}Arg^{261}\) (21). In addition, mutation of \(245^{Lys245}Ala^{245}Arg^{261}\) to \(245^{Lys245}Arg^{261}\) (24) and \(245^{Phe246}Tyr^{252}\) to \(245^{Lys245}Arg^{261}\) (24) also significantly reduced iC3b binding. However, although the binding site for iC3b overlaps with the Fg binding site, the contribution of this region in recognition of two ligands appears to be distinct. For example, deletion of \(245^{KFG}\) did not affect adhesion to P2-C or D100 fragment in our experiments, but it reduced to some extent iC3b binding (19).

The overlapping nature of the NIF and Fg binding sites within the \(\alpha_I\)-domain was suggested previously based on the ability of NIF to inhibit interaction of the \(\alpha_M\)-bearing cells with Fg (18, 19). The same segments which were identified as critical for Fg binding, \(245^{Pro247}Arg^{152}\), \(201^{Pro201}-Gly^{207}\), \(201^{Arg^{208}-Lys^{217}}\), and \(255^{Glu253}Arg^{261}\), also have been shown to participate in NIF binding (22). The significant differences in the binding of \(\alpha_I\)-domain mutants to these two ligands were: 1) switch of \(245^{KFG}\), which completely abrogated adhesion to Fg peptides, was not critical for NIF binding; and 2) deletion of \(245^{KFG}\), which affected NIF binding, was not detrimental for adhesion to Fg derivatives. The binding site for NIF was verified previously by grafting the identified segments into the \(\alpha_I\)-domain because these swaps imparted NIF binding capacity to the chimeric receptor (22). However, because the identified segments were grafted simultaneously, it is un-
certain whether all of these sequences contain NIF contact sites or if some may provide structural elements necessary to maintain a permissive conformation for NIF binding. That NIF did not inhibit the binding of the D fragment or P2-C to immobilized Lys^{245}-Tyr^{252} and Glu^{253}-Arg^{261} suggests that these segments of the αM-domain may not contain contact sites for NIF. Therefore, one possibility is that the primary binding site whereby integrins specify ligand recognition.

At the same time, affinity of the αM(Pro^{147}-Arg^{152}) and αM(Pro^{201}-Lys^{217}) decreased affinity for NIF 33-, 305- and 206-fold, respectively (22). In addition, Rieu et al. (20) demonstrated the importance of Gly^{143}, Asp^{149}, and Arg^{208}, which reside close or within αM(Pro^{147}-Arg^{152}) and αM(Pro^{201}-Lys^{217}), for NIF binding. The extension of this hypothesis is that NIF and P2-C do not compete for the same binding site on the αM-domain but rather that NIF blocks adhesion to Fg by steric interference with binding of Fg to stimulated leukocytes resides 7E3, which recognizes the active I-domain conformation and in the MIDAS face of the αM-domain.

In summary, we have determined the binding site for the Fg Binding Site within the αM-Domain 14003

REFERENCES

1. Anderson, D. C., Schmaleitig, F. C., Shearer, W., Becker-Freeman, K., Kohl, S., Smith, C. W., Tosi, M. F., and Springer, T. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 3215–2200.
2. Anderson, D. C., and Springer, T. A. (1987) Annu. Rev. Med. 38, 175–194.
3. Hogg, N., Stewart, M. P., Scarr, S. L., Newton, R., Shaw, J. M., Law, S. K., and Klein, N. (1999) J. Clin. Invest. 103, 97–106.
4. Languino, L. R., Plescia, J., Duperray, A., Brian, A. A., Plow, E. F., Geltosky, J. E., and Altieri, D. C. (1993) Cell 73, 1432–1443.
5. Sriramamoo, P., Languino, L. R., and Altieri, D. C. (1996) Blood 88, 3416–3423.
6. Languino, L. R., Duperray, A., Joganic, K. J., Fornar, M., Thornton, G. B., and Altieri, D. C. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1323–1327.
7. Bini, A., Fediglio, J. J., Jr., Mesa-Tejada, R., Kudryk, B., and Kaplan, K. L. (1989) Arteriosclerosis 9, 199–212.
8. Valenzuela, R., Sainoff, J. R., Dibello, P. M., Urbanic, D. A., Anderson, J. M., Matsueda, G. R., and Kudryk, B. J. (1992) Am. J. Pathol. 141, 861–880.
9. Wu, X., Helfrich, M. H., Horton, M. A., Feigen, L. P., and Lefkowith, J. B. (1994) J. Clin. Invest. 94, 925–936.
10. Altieri, D. C., Agbanyo, F. R., Plescia, J., Ginsberg, M. H., Edgington, T. S., and Mosesson, M. W. (1994) J. Biol. Chem. 269, 12119–12122.
11. Altieri, D. C., Plescia, J., and Plow, E. F. (1993) J. Biol. Chem. 268, 1847–1853.
12. Ugarova, T. P., Solovyov, D. A., Zhang, L., Leukoning, D. I., Yew, Y. C., Medved, L. V., and Plow, E. F. (1998) J. Biol. Chem. 273, 22519–22527.
13. Ugarova, T. P., and Yakubenko, V. P. (2001) Ann. N. Y. Acad. Sci., in press.
14. Diamond, M. S., Garcia-Aguilar, J., Bickford, J. K., Corbi, A. L., and Springer, T. A. (1999) J. Cell Biol. 120, 1031–1043.
15. Zhou, L., Lee, D. H., Plescia, J., Lao, C. Y., and Altieri, D. C. (1994) J. Biol. Chem. 269, 17075–17079.
16. Ueda, T., Rieu, P., Brayer, J., and Arnaout, M. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10680–10684.
17. Rieu, P., Ueda, T., Haruta, I., Sharma, C. P., and Arnaout, M. A. (1994) J. Cell Biol. 127, 2081–2091.
18. Muchowski, P. J., Zhang, L., Chang, E. R., Soule, H. R., Plow, E. F., and Moyle, M. (1994) J. Biol. Chem. 269, 26419–26423.
19. Zhang, L., and Plow, E. F. (1996) J. Biol. Chem. 271, 18211–18216.
20. Rieu, P., Sugimori, T., Griffith, D. L., and Arnaout, M. A. (1996) J. Biol. Chem. 271, 15858–15861.
21. McGuire, S. L., and Bajt, M. L. (1995) J. Biol. Chem. 270, 25666–25671.
22. Zhang, L., Plow, E. F., and Plow, E. F. (1993) J. Biol. Chem. 268, 127558–127560.
23. Li, R. R., Rieu, P., Griffith, D. L., Scott, D., and Arnaout, M. A. (1998) J. Cell Biol. 143, 1525–1534.
24. Zhang, L., and Plow, E. F. (1999) Biochemistry 38, 8604–8671.
25. Lee, J.-O., Rieu, P., Arnaout, M. A., and Liddington, R. (1995) Cell 80, 631–638.
26. Lee, J.-O., Bankston, L. A., Arnaout, M. A., and Liddington, R. C. (1995) Structure 3, 1335–1340.
27. Qu, A., and Lealby, D. J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10277–10281.
28. Qu, A., and Lealby, D. J. (1996) Structure 4, 931–942.
29. Yee, V. C., Pratt, K. P., Cote, H. C. F., LeTrong, I., Chung, D. W., Davé, E. W., Storza, W. L., and Plow, E. F. (1996) Structure 4, 125–130.
30. Doolittle, R. F., Schubert, D., and Schwartz, S. A. (1987) Arch. Biochem. Biophys. 119, 456–467.
31. Ugarova, T. P., and Budzynski, A. Z. (1992) J. Biol. Chem. 267, 13687–13693.
32. Arnaout, M. A., Gupta, S. K., Pierce, M. W., and Tenen, D. G. (1988) J. Cell Biol. 106, 2153–2158.
33. Procyk, R., Kudryk, B., Callender, S., and Blombach, B. (1991) Blood 77, 1469–1475.
34. Matsueda, G. R., and Bernatowicz, M. S. (1988) in Fibrinogen 3: Biochemistry, Biological Functions, Gene Regulation and Expression (Mosesson, M. W., Amrani, D., Siebenlist, K. R., and DiOrio, P., eds) pp. 133–136, Elsevier Science Publishers, Amsterdam.
35. Ugarova, T. P., Budzynski, A. Z., Shattil, S. J., Rggerini, Z. M., Ginsberg, M. H., and Plow, E. F. (1993) J. Biol. Chem. 268, 21080–21087.
36. DeGrado, W. F., and Summa, C. M. (1999) Annu. Rev. Biochem. 68, 779–819.
37. Forsyth, C. B., Plow, E. F., and Zhang, L. (1998) J. Immunol. 161, 6198–6205.
38. Michishita, M., Videm, V., and Arnaout, M. A. (1993) Cell 72, 857–867.
39. Edwards, C. P., Chumpe, M., Gonzalez, T., Wessinger, M. E., Spencer, S. A., Presta, L. G., Berman, P. W., and Bodary, S. C. (1995) J. Biol. Chem. 270, 12653–12660.
40. Nolte, M., Pepsinis, R. B., Venyaminov, S. Y., Kotelsianys, V., Gotwals, P. J., and Karpuas, M. (1999) FEBS Lett. 435, 379–385.
41. Orešič, G., Lang, W., and Karpusas, M. (1999) FEBS Lett. 435, 379–385.