SdrG, a Fibrinogen-binding Bacterial Adhesin of the Microbial Surface Components Recognizing Adhesive Matrix Molecules Subfamily from *Staphylococcus epidermidis*, Targets the Thrombin Cleavage Site in the Bβ Chain*

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*Staphylococcus epidermidis* is an important opportunistic pathogen and is a major cause of foreign body infections. We have characterized the ligand binding activity of SdrG, a fibrinogen-binding microbial surface component recognizing adhesive matrix molecules from *S. epidermidis*. Western ligand blot analysis showed that a recombinant form of the N-terminal A region of SdrG bound to the native Bβ chain of fibrinogen (Fg) and to a recombinant form of the Bβ chain expressed in *Escherichia coli*. By analyzing recombinant truncates and synthetic peptide mimetics of the Fg Bβ chain, the binding site for SdrG was localized to residues 6–20 of this polypeptide. Recombinant SdrG bound to synthetic 25-amino acid peptide β1–25 representing the N terminus of the Fg Bβ chain with a *K*<sub>D</sub> of 1.4 × 10<sup>−7</sup> M as determined by fluorescence polarization experiments. This was similar to the apparent *K*<sub>D</sub> (0.9 × 10<sup>−7</sup> M) calculated from an enzyme-linked immunosorbent assay where SdrG bound immobilized Fg in a concentration-dependent manner. SdrG could recognize fibrinopeptide B (residues 1–14), but with a substantially lower affinity than that observed for SdrG binding to synthetic peptide β1–25 and β6–20. However, SdrG does not bind to thrombin-digested Fg. Thus, SdrG appears to target the thrombin cleavage site in the Fg Bβ chain. In fact, SdrG was found to inhibit thrombin-induced fibrinogen clotting by interfering with fibrinopeptide B release.

Coagulate-negative staphylococci are important opportunistic pathogens that are particularly associated with foreign body infections in humans. *Staphylococcus epidermidis* is the most common pathogenic species of coagulate-negative staphylococci and accounts for 74–92% of the infections caused by this group of staphylococci (1).

The molecular pathogenesis of most infections is complex and involves multiple microbial factors and host components but is generally initiated by the adherence of the microbe to host tissues. Bacterial adherence involves specific surface components called adhesins. Bacterial pathogens, such as staphylococci that live in the extracellular space of the host, target extracellular matrix components, including fibrinogen (Fg) and fibrinectin, for adherence and colonization. This process is mediated by a subfamily of adhesins that have been termed microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) (2). *Staphylococcus aureus* expresses multiple MSCRAMMs, of which several have been characterized in some detail (for a recent review see Ref. 3).

In addition to *S. epidermidis*, *S. aureus* also causes serious foreign body infections. *S. aureus* appears to adhere to the biomaterial through an indirect mechanism. Upon implantation, the foreign body rapidly becomes coated with host proteins derived primarily from plasma with Fg being a dominant component. *S. aureus* appears to adhere to the absorbed proteins rather than to the biomaterial itself using adhesins of the MSCRAMM family (4, 5). At least four of the *S. aureus* MSCRAMMs recognize Fg. Two of these MSCRAMMs, CfrA and ClfB (clumping factors A and B), have Fg-binding A regions followed by a long segment of Ser-Asp (SD) dipeptide repeats. The other two Fg-binding MSCRAMMs contain a similar ligand-binding A region followed by a fibronectin-binding motif that is repeated five times (6). Because the fibronectin binding activity was identified first, these two MSCRAMMs are known as FnbpA and FnbpB (fibronectin-binding proteins A and B) (7, 8). Studies have demonstrated the importance of CfrA and ClfB in the adherence of *S. aureus* to plasma-coated biomaterials. *S. aureus* mutants deficient in one or both of these MSCRAMMs exhibited an impaired ability to adhere to plasma-coated catheters *in vivo* or *ex vivo* (9, 10).

For *S. epidermidis*, adherence to foreign bodies could involve both specific and nonspecific processes. The bacteria may initially associate directly with the foreign body through nonspecific interactions, whereas the later stages of adherence may involve more specific interactions between bacterial adhesins and host ligands. *S. epidermidis* expresses polysaccharide ad-
hesins including polysaccharide adhesin and polysaccharide intercellular adhesin, which are encoded by the ica locus (11, 12). In addition, we (13) and others (14) have recently shown that S. epidermidis contains surface proteins structurally related to S. aureus MSCRAMMs. Two of these S. epidermidis proteins, called SdrF and SdrG (serine-aspartate repeat proteins F and G), have features typical of Gram-positive bacterial proteins that are anchored to the cell wall. Both proteins show significant amino acid sequence homology to ClfA and ClfB proteins, called SdrF and SdrG (serine-aspartate repeat proteins), can bind Fg and specifically recognize the B chain of this molecule. In the current study, we have localized the SdrG-binding site in the Fg B chain to the N-terminal segment of this polypeptide, proximal to the thrombin cleavage site. In fact, we have demonstrated that SdrG inhibits thrombin-induced fibrin clot formation by interfering with the release of fibrinopeptide B.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Growth Conditions—Escherichia coli strain JM101 was used for plasmid cloning. E. coli strain Top10 (Stratagene) was used for protein expression. Strains harboring plasmids were grown in Lennox L broth (Sigma) or on Lennox L agar (Sigma) supplemented with 100 μg/ml ampicillin.

Polymerase Chain Reaction Amplification of the sdrG Gene Fragment—The gene fragment encoding the entire A region was amplified by polymerase chain reaction (PCR) using S. epidermidis K28 genomic DNA as a template. The oligonucleotide primers used were 5′-CCCGATCC-GAGGAGAATACAGTACAAGACG-3′ and 5′-CCCGTACCCGATTATTTC-TCAGAGCGAAGTACAGG-3′. The restriction enzyme cleavage sites (underlined) BamHI and KpnI were incorporated into the forward and reverse primers, respectively. The reactions were carried out using a Perkin-Elmer DNA thermocycler. The reactions contained 50 ng of template DNA, 100 pmol of forward and reverse primers, 20 mM MgSO4, 10 mM KCl, 10 mM (NH4)2SO4, 0.1% Triton X-100, 25 mM of each dNTP, and 5 units of Pfu DNA polymerase (Stratagene). Amplification was performed at 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 4 min for 25 cycles.

Cloning of sdrG into the Expression Plasmid—The amplified sdrG fragment was digested with BamHI and KpnI and ligated into the expression plasmid pQE30 (Qiagen Inc.) that had been digested with the same enzymes, yielding the construct pSdrG (50–597). The recombinant protein rSdrG (50–597) expressed from this plasmid contains a N-terminal extension of six histidine residues (His tag).

Expression and Purification of Recombinant MSCRAMM Protein—E. coli transformed with pSdrG (50–597) was grown for 2 h at 37 °C for an additional 3 h. The bacteria were pelleted and resuspended in phosphate-buffered saline (PBS), pH 7.5 (140 mM NaCl, 270 mM KCl, 430 mM Na2HPO4, 147 mM KH2PO4) and frozen overnight at −20 °C. Bacterial cells were thawed and mechanically lysed by using a French pressure cell (SLM Amnico). Cell debris was removed by centrifugation and filtration through a 0.45-μm filter membrane. The supernatant containing the recombinant protein was applied to a Ni2+–charged (87.5 mM) 5-ml Hi Trap chelating column (Amersham Pharmacia Biotech) connected to a fast protein liquid chromatography system. The column was equilibrated with buffer A (0.1 mM NaCl, 10 mM Tris-Cl, pH 8.0) before the application of the filtered lysate. The column was then washed with 10 bed volumes of buffer A containing 5 mM imidazole. Bound protein was eluted with a continuous linear gradient of imidazole (0–120 mM; total volume, 160 ml) in buffer A. Fractions were monitored for protein binding to the column. Fractions containing rSdrG (50–597) were identified by SDS-PAGE (16). These fractions were pooled and dialyzed against PBS, pH 7.5. The dialyzed protein was then applied to a Q-Sepharose column (Amersham Pharmacia Biotech) equilibrated with 25 mM Tris-Cl, pH 8.0. The bound protein was eluted with a continuous linear gradient of NaCl (0–0.5 M; total volume, 160 ml) in 25 mM Tris-Cl, pH 8.0. Fractions containing the purified rSdrG (50–597) were identified by determining the absorbance at 280 nm and by SDS-PAGE. The truncated A region of ClfA was purified as previously reported (17).

Synthetic Peptides—The synthetic Fg peptides β1-25, β1-25S, β6-25, and β6-25S were custom ordered from Research Genetics, and the FpA and FpB (fibrinopeptides A and B) were from Bachem. Peptides β6-20 and β11-20 were synthesized in our laboratory using a multiple peptide synthesizer by Advanced Chemtech. For the following peptides the residue numbers are given and the sequence follows (residue 1 corresponds to the first residue of the mature Bβ chain): peptide β1-25, composed of the first 25 amino acid residues of the N terminus of the Bβ chain of Fg (QGVNDNEEGFFSARGHRPLDKKREE); peptide β1-20 (QGVNDNEEGFFSARGHRPLDKKREE); peptide β6-25 (NEEGFFSARGHRPLDKKREE); peptide β6-25S (NEEGFFSARGHRPLDKKREE); peptide β6-20, a scrambled version of peptide β1-25 (FSERDLHQGEFNPVFENADKR); peptide β6-20 (NEEGFFSARGHRPLDKKREE); peptide β11-20 (FSARGHRPLDKKREE); FpA (ADSEEGGDFLAGGGYR), and FpB (QGVNDNEEGFFSARGHRPLDKKREE). Peptides were purified by HPLC and analyzed by matrix-assisted laser desorption ionization mass spectrometry.

ELISA—Microtiter plates (Immulon 4, Dynatech Laboratories Inc.) were coated with 1 μg of Fg (Enzyme Research Labs) in PBS, pH 7.5, for 18 h at 4 °C. The plates were washed three times with PBS, 0.05% Tween 20 (PBST) and blocked with 1% (w/v) bovine serum albumin (BSA) for 1 h at room temperature. The plates were washed three times with PBST and rSdrG (50–597), diluted into PBS, and added to the wells, and the plate was incubated for 1 h at room temperature. The plates were washed three times with PBST and bound rSdrG (50–597) was detected by adding a 1:2000 dilution of an anti-His tag monoclonal antibody (CLONTECH) in PBST, 0.1% BSA. The plates were incubated for 1 h at room temperature and then washed three times with PBST. A 1:2000 dilution of goat anti-mouse alkaline phosphatase-conjugated polyclonal antibodies (Bio-Rad) in PBST, 0.1% BSA were added to the
was washed three times with PBST, and 50 Fg-coated wells. The plate was incubated at 37 °C for 30 min. The plate was washed three times with PBST and developed with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium (Bio-Rad) in carbonate:bicarbonate buffer (14 mM Na2CO3, 25 mM NaH2PO4, pH 9.0, at room temperature for ~30 min. The plates were read at 405 nm using an ELISA plate reader (Thermomax microplate reader, Molecular Devices).

In the inhibition experiments, 50 nM rSdrG (50–597) in PBS was preincubated with the indicated amounts of selected peptides for 1 h at room temperature. The sample mixtures were added to the Fg-coated wells, and bound rSdrG (50–597) was detected as described above. The plate with thrombin-digested Fg, the plate was coated with Fg and blocked as described above. The plate was washed three times with PBST, and 50 μl of 1.0 NIH unit/ml of thrombin was added to the Fg-coated wells. The plate was incubated at 37 °C for 20 min. The plate was washed three times with PBST, and 50 μl of 1.0 NIH unit/ml of hirudin (Sigma) was added to the wells and incubated at 37 °C for 30 min. The plate was washed three times with PBST and blocked with 1% BSA for 1 h at room temperature. After washing three times with PBST, 100 μl of biotin labeled rSdrG (50–597) (25–1000 nM) or a rSdrG (50–597)/hirudin (1.0 NIH unit/ml) mixture was added to the wells and incubated for 1 h at room temperature. The plate was washed three times with PBST and a 1:5000 dilution of streptavidin-alkaline phosphatase conjugated (Roche Molecular Biochemicals) in PBST, 0.1% BSA was added to the wells for 1 h at room temperature. The plate was washed three times with PBST and developed as described above.

Construction of Fg Bβ Chain Truncates—An E. coli strain harboring plasmid p668, which contains the cDNA for the Fg Bβ chain, was kindly provided by Dr. Susan T. Lord (University of North Carolina, Chapel Hill, NC). The 1525-base pair fragment from p668 was subcloned into the plasmid pQE30 to produce recombinant mature Bβ chain with a C-terminus His tag. Additional Bβ chain constructs (see Fig. 3A) were made by subcloning into either pQE30 or pGEX-KG (Amersham Pharmacia Biotech) to produce recombinant proteins with a C-terminal His tag or glutathione S-transferase fusion.

Western Ligand Blot Analysis—Whole E. coli lysates harboring each respective Fg Bβ chain construct were fractionated by SDS-PAGE, and the separated proteins were transferred to nitrocellulose membrane with a semi-dry transfer cell (Bio-Rad). The membrane was incubated overnight with 5% (w/v) nonfat dry milk in PBS, pH 7.5, at 4 °C to saturate nonspecific binding sites. After blocking, the membrane was washed three times with PBST and then incubated with biotin labeled rSdrG (50–597) (0.5 μM) for 1 h at room temperature. rSdrG (50–597) was treated with EZ Link-sulfo-NHS-LC-biotin (Sigma) according to the manufacturers’ instructions. After more washing with PBST, the blot was incubated with a 1:5000 dilution of streptavidin-alkaline phosphatase conjugated for 1 h at room temperature and developed with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium (Bio-Rad) in carbonate/bicarbonate buffer (14 mM Na2CO3, 36 mM NaHCO3, 5 mM MgCl2, 6H2O, pH 9.8) for ~15 min at room temperature.

RESULTS

Expression and Purification of Recombinant SdrG A Region—To characterize the ligand binding activity of SdrG, a recombinant form of the putative ligand-binding A region (residues 50–597) (Fig. 1C) was expressed in E. coli with a N-terminal His tag. This protein construct, rSdrG (50–597), was purified by metal chelate affinity chromatography followed by ion-exchange chromatography. The purity of the recombinant protein was confirmed by SDS-PAGE analysis, where it migrates with an apparent molecular mass of ~97 kDa (Fig. 1B). This is larger than the theoretical molecular mass of 63.7 kDa predicted from the primary amino acid sequence of this protein. Analysis of rSdrG (50–597) by matrix-assisted laser desorption ionization mass spectrometry indicated a molecular mass of 63.3 kDa. Aberrant migration in SDS-PAGE has also been observed with recombinant MSCRAMMs derived from S. aureus and may be explained by the hydrophilic nature of these proteins (9, 13).

SdrG Binds the Fg Bβ Chain—SdrG is closely related to the recently described fibrinogen binding MSCRAMM Fbe (15). Therefore, we initially examined the ligand binding specificity of SdrG for Fg in an ELISA. In this assay, rSdrG (50–597) bound immobilized Fg but failed to bind to other immobilized proteins (9, 13).
extracellular matrix proteins such as fibronectin, collagen types I and IV, vitronectin, laminin, and thrombospondin (data not shown). Binding of increasing concentrations of rSdrG (50–597) to absorbed Fg exhibited saturation kinetics (Fig. 2). Together these observations demonstrate the specificity of the SdrG-Fg interaction. Furthermore, biotin-labeled rSdrG (50–597) recognized the Bβ chain but not the Aα or γ chains of Fg when analyzed by Western ligand blotting (data not shown).

Localization of the SdrG Binding Site in the Fg Bβ Chain—The observation that rSdrG binds the Fg Bβ chain fractionated under reducing and denaturing conditions in Western ligand blot analysis suggests that the MSCRAMM recognizes a specific linear amino acid sequence in the Bβ chain. To explore this possibility and locate the SdrG-binding site, a recombinant mature Fg Bβ chain and a series of truncated forms of the Bβ chain expressed in E. coli were analyzed by Western ligand blot. The recombinant Bβ chain constructs were expressed as either His tag or glutathione S-transferase fusion proteins (Fig. 3A). The fractionated proteins were transferred to a supporting membrane and probed with biotin labeled rSdrG (50–597) (Fig. 3B). rSdrG (50–597) recognized the mature recombinant Bβ chain (residues 1–462) as well as the recombinant truncates encompassing residues 1–341, 1–220, 1–195, and 1–95. However, rSdrG (50–597) failed to bind to the two recombinant truncates that lacked the N-terminal 25 amino acid residues of the Bβ chain, rβ (25–95) and rβ (25–195) (Fig. 3B). These observations demonstrate that rSdrG (50–597) recognizes a linear sequence in Fg and suggests that this site lies within the N-terminal region of the Bβ chain.

Inhibition of rSdrG (50–597) Binding to Fg by Synthetic Peptides—To further define the rSdrG (50–597) binding site in the Fg Bβ chain, we used a peptide mimetic approach. A series of peptides representing segments of the N-terminal region of the Fg Bβ chain were synthesized and tested for their ability to inhibit the binding of rSdrG (50–597) to Fg in an ELISA (Fig. 4A). In Fig. 4A, peptides β1–25 and β6–25 were shown to inhibit the binding of rSdrG (50–597) to Fg in a concentration-dependent manner, whereas the scrambled version of β1–25, peptide β1–25S, did not interfere with the binding of rSdrG (50–597) to Fg. Effective inhibition of rSdrG (50–597) binding to Fg was also observed with peptide β6–20 and, to a somewhat lesser degree, with β1–20. Peptide β11–20 was essentially inactive in this assay (Fig. 4B).

The thrombin cleavage sites in Fg lie between residues 14 (Arg) and 15 (Gly) in the Bβ chain and between 16 (Arg) and 17 (Gly) in the Aα chain. Upon cleavage of Fg by thrombin the fibrinopeptides FpA and FpB are sequentially released. The fibrinopeptides were examined as inhibitors of rSdrG (50–597) binding to Fg in an ELISA. FpB inhibited the binding of rSdrG (50–597) in a concentration-dependent manner, but this peptide was at least 10-fold less active than the synthetic peptide β1–25 (Fig. 4C). FpA was essentially inactive and behaved similarly to the scrambled peptide β1–25S. Taken together, this suggest that rSdrG (50–597) recognizes a linear amino acid sequence in the Bβ chain located within residues 6–20. This recognition site appears to overlap the thrombin cleavage site in this polypeptide.

rSdrG Binding to Thrombin-digested Fg—The rSdrG-binding site seems to lie within close proximity to the thrombin cleavage site; therefore, we investigated whether rSdrG (50–597) could bind to Fg in which the thrombin cleavage site was abolished. Fg-coated microtiter wells were pretreated with thrombin or thrombin plus hirudin (which inhibits thrombin activity) to remove FpB and destroy the cleavage site. The ability of rSdrG (50–597) to bind to this thrombin-digested Fg was significantly impaired (Fig. 5), suggesting that the thrombin cleavage site residues Bβ Arg14 and Gly15 and residues within FpB (1–14) are essential for rSdrG (50–597) to bind Fg.

Determination of Equilibrium Dissociation Constants ($K_D$)—An equilibrium dissociation constant ($K_D$) for the interaction of rSdrG (50–597) with the Fg Bβ chain peptide β1–25 was determined. By analyzing the binding of increasing concentrations of rSdrG (50–597) to the fluorescein-labeled β1–25 peptide in a fluorescence polarization assay, rSdrG (50–597) binding to the labeled peptide exhibited saturation kinetics with a $K_D$ of 1.4 ± 0.01 × 10^{-7} M (Fig. 6A). To demonstrate the specificity of this interaction, the binding of rSdrG (50–597) to the labeled β1–25 peptide was measured in the presence of increasing amounts of unlabeled peptide (β1–25) or scrambled peptide (β1–25S). The unlabeled β1–25 peptide but not peptide β1–25S inhibited binding of rSdrG (50–597) to the fluorescein-labeled β1–25 peptide, in a concentration-dependent manner (Fig. 6B). The apparent $K_D$ determined for the binding of rSdrG (50–597) to the fluorescein-labeled peptide β1–25 is similar to the apparent $K_D$ (9.0 × 10^{-7} M) for the interaction of rSdrG (50–597) with immobilized, intact Fg as determined by ELISA (Fig. 2).

rSdrG (50–597) Inhibits Thrombin-induced Fibrin Clot For-
In the final stages of the blood coagulation cascade, thrombin cleaves Fg releasing the fibrinopeptides and producing fibrin monomers. These fibrin monomers then polymerize to form a fibrin clot (20). The localization of the SdrG-binding site described above raises the possibility that rSdrG (50–597) may be able to inhibit thrombin-induced fibrin clot formation, perhaps by directly competing with thrombin for binding to the N terminus of the Bβ chain of Fg or by binding to a proximal site and sterically blocking the proteolytic attack of thrombin on the Bβ chain. To test this hypothesis, we designed a fibrin clot inhibition assay in which 3.0 μM Fg, 0–6.0 μM rSdrG (50–597), and 1.0 NIH unit/ml of thrombin were incubated, and the formation of a fibrin clot was monitored by measuring the increase in optical density at 405 nm. Fig. 7 shows that rSdrG (50–597) inhibited fibrin clot formation in a concentration-dependent manner, whereas BSA had no effect. This suggests that rSdrG (50–597) can interfere with thrombin activity by binding to a site in the Fg Bβ chain that is proximal to or overlaps the binding site for thrombin.

Analysis of Fibrinopeptide B Release by HPLC—The release of FpA and FpB from the N terminus of the Aα and Bβ chains of Fg by thrombin can be monitored and quantitated by high performance liquid chromatography (19, 21, 22). We examined the effect of rSdrG (50–597) on fibrinopeptide release by measuring the peak areas of FpA and FpB, as detected by HPLC. The HPLC chromatograms shown in Fig. 8 show the expected fibrinopeptide release following digestion of Fg with thrombin superimposed with the fibrinopeptide release when Fg and thrombin are incubated with rSdrG (50–597). A significant decrease in the amount of FpB release was shown with a 1:1 ratio of rSdrG (50–597) to Fg (Table I), whereas a 5:1 ratio was effectively able to inhibit the release of FpB (Fig. 8). This effect was seen at incubation times of 15 and 60 min. There was no apparent interference of FpA release by rSdrG (50–597).

DISCUSSION

In this study, we have shown that SdrG binds the N terminus of the Bβ chain of Fg with a high degree of specificity. The binding of SdrG to an N-terminal Fg peptide exhibits a $K_d$ of $1.4 \times 10^{-7}$ M, which is significantly lower than the $K_d$ determined for the binding of ClfA to a γ chain peptide ($2.0 \times 10^{-5}$).
Thus, SdrG appears to have a higher affinity for its respective synthetic Fg peptide target compared with the S. aureus MSCRAMM. The $K_D$ determined for the binding of SdrG to the synthetic peptide $\beta_1$–25 was calculated to be $1.4 \pm 0.01 \times 10^{-7}$ M. Binding of the fluorescein-labeled $\beta_1$–25 to rSdrG(50–597) in the presence of increasing concentrations of unlabeled $\beta_1$–25 (●) or the scrambled Bβ chain peptide $\beta_1$–25S (▲). The values are the means of duplicate reactions.

Several studies have examined the role of Fg-binding MSCRAMMs from S. aureus as virulence factors in animal models. Strains in which the genes encoding ClfA or ClfB have been inactivated are less virulent compared with the wild type strain in a rat model of catheter-induced endocarditis (23, 24). These results suggest that ClfA- and ClfB-mediated adherence is required for the maximum virulence potential of S. aureus to be expressed. ClfB has been shown to promote S. aureus adherence to ex vivo hemodialysis tubing, further confirming that ClfB contributes to bacterial attachment to biomaterials coated with host proteins (9). In a recent study, Stutzmann Meier et al. (25) showed that heterologous expression of ClfA on Strepto-
coccus gordonii, which is generally considered a nonvirulent bacterium, rendered this organism pathogenic in a rat endocarditis model. With the discovery that SdrG is a Fg-binding MSCRAMM expressed by S. epidermidis, the possibility arises that SdrG can act as a virulence factor in S. epidermidis-induced infections and plays a role similar to that of the Fg-binding MSCRAMMs in S. aureus-induced infections.

We have mapped the binding site of rSdrG (50–597) in the Fg ββ chain to a linear sequence in the N-terminal region of this polypeptide. Peptide β6–20 is a potent inhibitor of the binding of rSdrG (50–597) to Fg, whereas FpB (1–14) has poor inhibitory activity. Because peptide β6–20 but not β11–20 is recognized by this MSCRAMM, the N-terminal border of the binding site must lie between residues 6 and 11 of the ββ chain. The observation that rSdrG (50–597) is unable to bind to thrombin-digested Fg, whereas FpB (1–14) has poor inhibitory activity. Because peptide β6–20 but not β11–20 is recognized by this MSCRAMM, the N-terminal border of the binding site must lie between residues 6 and 11 of the ββ chain. The observation that rSdrG (50–597) is unable to bind to thrombin-digested Fg, i.e., the fibrinopeptides are absent, suggests that the C terminus of this binding site is located between residues 14 and 20 of the ββ chain.

It is striking that many of the identified staphylococcal MSCRAMMs appear to specifically recognize Fg, although the sites targeted in Fg by these proteins vary. CIfA, FnbpA, and FnbpB of S. aureus all recognize the C terminus of the Fg γ chain (6, 27). CIfB from S. aureus targets an as yet unidentified site in the Aα chain (9), and SdrG is here shown to bind to the N terminus of the ββ chain. Thus, these MSCRAMMs use a conserved region to bind different sites in Fg. Furthermore, the MSCRAMMs appear to target sites in Fg that are important in the molecular physiology of this key component of hemostasis. The C terminus of the γ chain is recognized by the platelet integrin αIIbβ3, and CIfA is a potent inhibitor of Fg-induced platelet aggregation (26, 27). Here, we show that the binding site in the ββ chain for rSdrG (50–597) appears to overlap the thrombin cleavage site and that rSdrG (50–597) can interfere with fibrin clot formation by inhibiting the thrombin-induced release of FpB. Fg may play an important role in the host’s defense against microbial infections, and interfering with this function gives the bacteria an advantage and the ability to survive in a hostile environment. One such potential advantage may be related to the observed chemotactic activity of FpB for human peripheral blood leukocytes (28–30). We have shown that rSdrG (50–597) can prevent the release of FpB; thus one can speculate that the reason S. epidermidis possesses a protein that can bind to this region of the Fg ββ chain is to prevent the release of chemotactic elements. This may reduce the influx of phagocytic neutrophils and help to ensure the survival of the bacteria in the host.

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REFERENCES

1. Garrett, D. O., Jochimsen, E., Murfitt, K., Hill, B., McAllister, S., Nelson, P., Spera, R. V., Sall, R. K., Tenover, F. C., Johnston, J., Zimmer, B., and Levin, W. R. (1999) Infect. Control Hosp. Epidemiol. 20, 167–170
2. Patti, J. M., and Hoik, M. (1994) Curr. Opin. Cell Biol. 6, 752–758
3. Foster, T. J., and Hoik, M. (1998) Trends Microbiol. 6, 484–488
4. Gallianni, S., Viot, M., Cremieux, A., and Van der Auwera, P. (1994) J. Lab. Clin. Med. 123, 685–692
5. Vaudaux, P., Pittet, D., Haeberli, A., Huggler, E., Nydegger, U. E., Lew, D. P., and Waldvogel, F. A. (1989) J. Infect. Dis. 150, 805–875
6. Wallin, K. R., Gurusiddappa, S., and Hoik, M. (2000) J. Biol. Chem. 275, 13863–13871
7. Flock, J. I., Froman, G., Jonsson, K., Gass, B., Signas, C., Nilsson, B., Raucci, G., Hoik, M., Wadstrom, T., and Lindberg, M. (1987) EMBO J. 6, 2351–2357
8. Froman, G., Switalski, L., Speciale, P., and Hoik, M. (1987) J. Biol. Chem. 262, 6564–6571
9. Ni Eidhin, D., Perkins, S., Francois, P., Vaudaux, P., Hoik, M., and Foster, T. J. (1998) Mol. Microbiol. 30, 245–257
10. Vaudaux, P. E., Francois, P., Proctor, R. A., McDevitt, D., Foster, T. J., Albrecht, R. M., Lew, D. P., Wabers, H., and Cooper, S. L. (1998) Infect. Immun. 66, 585–590
11. McKenney, D., Huhner, J., Muller, E., Wang, Y., Goldmann, D. A., and Pier, G. B. (1988) Infect. Immun. 66, 4711–4720
12. Crumpton, S. E., Gerke, C., Schnell, N. F., Richards, W. W., and Gotz, F. (1999) Infect. Immun. 67, 5427–5433
13. McCrea, K. W., Hartford, O., Davis, S., Ni Eidhin, D., Lina, G., Speciale, P., Foster, T. J., and Hoik, M. (2000) Microbiology 146, 1535–1546
14. Nilsson, M., Frykberg, L., Flock, J. I., Lei, P., Lindberg, M., and Gass, B. (1998) Infect. Immun. 66, 2666–2673
15. Lei, P., Palma, M., Nilsson, M., Gass, B., and Flock, J. I. (1999) Infect. Immun. 67, 4525–4530
16. Laemmli, U. K. (1970) Nature 227, 680–685
17. McDevitt, D., Francois, P., Vaudaux, P., and Foster, T. J. (1995) Mol. Microbiol. 16, 897–904
18. O’Connell, D. P., Nanavaty, T., McDevitt, D., Gurusiddappa, S., Hoik, M., and Foster, T. J. (1998) J. Biol. Chem. 273, 6821–6829
19. Molin, J. L., Gurusiddappa, S., Binnie, C. G., and Lord, S. T. (2000) J. Biol. Chem. 275, 25239–25246
20. Herrick, S., Blanc-Brude, O., Gray, A., and Laurent, G. (1999) Int. J. Biochem. Cell Biol. 31, 741–746
21. Ng, A. S., Lewis, S. D., and Shafer, J. A. (1993) Methods Enzymol. 222, 341–358
22. Haverkate, F., Koopman, J., Kluft, C., Mannucci, P. M., and Moreillon, P. (1998) Thromb. Haemostasis 80, 131–135
23. Moreillon, P., Meng, J., Francioni, P., McDevitt, D., Foster, T. J., Francioni, P., and Vaudaux, P. (1995) Infect. Immun. 63, 4738–4743
24. Moreillon, P., and Moreillon, M. P., and Moreillon, P. (2001) Infect. Immun. 69, 657–664
25. Albrecht, R. M., Lew, D. P., Wabers, H., and Cooper, S. L. (1995) Infect. Immun. 63, 5427–5446
26. Stutzmann, M., and Flock, J. I. (2000) Theor. Biol. Med. Model. 752–758
27. Kopp, E., Hoek, M., and Vaudaux, P. (1999) Infect. Immun. 68, 5443–5446
28. Vaudaux, P., Gurusiddappa, S., and Hoik, M. (1997) Eur. J. Biochem. 247, 416–424
29. Vaudaux, P., Gurusiddappa, S., and Hoik, M. (1997) Eur. J. Biochem. 247, 416–424
30. Senior, R. M., Skogen, W. F., Griffin, G. L., and Wilner, G. D. (1986) J. Clin. Invest. 77, 1014–1019
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