

Molecular Interactions of Human Plasminogen with Fibronectin-binding Protein B (FnBPB), a Fibrinogen/Fibronectin-binding Protein from *Staphylococcus aureus**

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Staphylococcus aureus is a commensal bacterium that has the ability to cause superficial and deep-seated infections. Like several other invasive pathogens, *S. aureus* can capture plasminogen from the human host where it can be converted to plasmin by host plasminogen activators or by endogenously expressed staphylokinase. This study demonstrates that sortase-anchored cell wall-associated proteins are responsible for capturing the bulk of bound plasminogen. Two cell wall-associated proteins, the fibrinogen- and fibronectin-binding proteins A and B, were found to bind plasminogen, and one of them, FnBPB, was studied in detail. Plasminogen captured on the surface of *S. aureus*- or *Lactococcus lactis*-expressing FnBPB could be activated to the potent serine protease plasmin by staphylokinase and tissue plasminogen activator. Plasminogen bound to recombinant FnBPB with a K_D of 0.532 μM as determined by surface plasmon resonance. Plasminogen binding did not occur by the same mechanism through which FnBPB binds to fibrinogen. Indeed, FnBPB could bind both ligands simultaneously indicating that their binding sites do not overlap. The N3 subdomain of FnBPB contains the full plasminogen-binding site, and this includes, at least in part, two conserved patches of surface-located lysine residues that were recognized by kringle 4 of the host protein.

Staphylococcus aureus colonizes the anterior nares of ~25% of the healthy human population (1, 2). This commensal Gram-positive bacterium has the ability to cause a plethora of infections ranging from superficial skin abscesses to serious and potentially life-threatening invasive diseases such as osteomyelitis, endocarditis, and septic arthritis. Strains that are resistant to multiple antibiotics are associated with infections in hospitals. These are referred to as hospital or healthcare-associated

methicillin-resistant *S. aureus* (HA-MRSA),⁴ which have a propensity to cause bacteremia often associated with biofilm formation on indwelling medical devices (3, 4). Recently a global epidemic of MRSA has occurred in the community (community-associated MRSA (CA-MRSA)) exemplified by USA300 strains such as LAC (5). CA-MRSA strains have fewer antibiotic resistance determinants than HA-MRSA; they express a lower level of resistance to β -lactams, and they can survive on human skin and cause serious skin and soft tissue infections often requiring hospitalization.

The surface of *S. aureus* is decorated with proteins that are covalently anchored to the cell wall by sortases. During the process of secretion and anchoring to the cell wall peptidoglycan, the pre-proteins undergo post-translational changes both at the N terminus to remove the secretory signal sequence and at the C terminus where sortase recognizes the LPXTG motif, and it covalently links the COOH of the Thr to the amino group of the fifth Gly in the nascent cross-bridge of the peptidoglycan precursor lipid II. Transglycosylation joins the cell wall-associated (CWA) protein-linked precursor to peptidoglycan (6).

Fig. 1 shows the structural organization of two important CWA proteins, the fibronectin-binding proteins A and B (FnBPA and FnBPB), which are members of the microbial surface component recognizing adhesive matrix molecules (MSCRAMM) family. FnBPs are multifunctional proteins. The C-terminal fibronectin binding repeats are unstructured and form a flexible stalk-like region that projects the A domain away from the cell surface. The N-terminal A domain binds to fibrinogen by the “dock, lock, and latch” mechanism (7, 8) in a similar fashion to the archetypal MSCRAMM clumping factor A (9). A hydrophobic trench located between subdomains N2 and N3 accepts the C-terminal peptide of the fibrinogen γ -chain. A flexible region at the C terminus of N3 (the locking and latching peptide) undergoes a conformational change that locks the ligand in the trench and forms an additional β -strand in a

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⁴ The abbreviations used are: HA-MRSA, hospital/healthcare-associated methicillin-resistant *S. aureus*; CA-MRSA, community-associated MRSA; Clf, clumping factor; CWA, cell wall-associated; EACA, ϵ -aminocaproic acid; FBG, fibrinogen; FN, fibronectin; FnBPA, fibronectin-binding protein A; FnBPB, fibronectin-binding protein B; MSCRAMM, microbial surface component recognizing adhesive matrix molecules; PBST, PBS containing Tween 20; PLG, plasminogen; SAK, staphylokinase; rSAK, recombinant SAK; Sbi, immunoglobulin binding protein; t-PA, tissue plasminogen activator; rFnBPB, recombinant FnBPB.

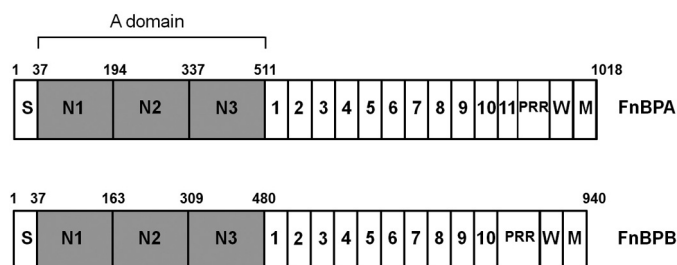


FIGURE 1. **Schematic diagram of FnBPA and FnBPB structure.** The primary translation products of FnBPA and FnBPB proteins contains a signal sequence (S) at the N terminus and a wall-spanning region (M) and sorting signal (W) at the C-terminal end. The N-terminal A region of FnBPA and FnBPB contains three separately folded subdomains, which are known as N1, N2, and N3. Structurally, N2 and N3 form IgG-like folds that combined bind ligands such as FBG by the dock, lock, and latch mechanism. Located distal to the A domain is an unfolded region that contains multiple tandemly arranged motifs (11 in FnBPA and 10 in FnBPB) that bind to the N-terminal type I modules of fibronectin and a proline-rich region.

β -sheet in subdomain N2. The A domains of FnBPA and FnBPB both bind to elastin, most probably involving dock lock and latch. They can also engage in homophilic interactions to form dimers, and when two FnBP molecules on neighboring cells interact, this can lead to cell accumulation during biofilm formation (10). The HA-MRSA strain BH1CC and the CA-MRSA strain LAC form biofilm that is dependent on FnBPs (11, 12).

Several pathogenic bacteria that cause invasive infections (*Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Borrelia burgdorferi*) can capture host plasminogen (PLG) and allow it to become activated to form the potent serine protease plasmin (13–19). Surface-bound plasmin enables bacteria to remove opsonins IgG and C3b, to degrade fibrin clots, and to promote bacterial spreading by cleaving tissue components (20–22).

Plasminogen is a 92-kDa zymogen that includes a PNA-apple N-terminal domain, five kringle domains (K1–5), and a serine proteinase catalytic domain (23). The kringle domains mediate interactions with fibrin clots and surface receptors from both eukaryotic and bacterial cells (24, 25). Proteolytic activation of PLG to plasmin occurs through cleavage of the Arg⁵⁶¹–Val⁵⁶² peptide bond in the catalytic domain by the tissue plasminogen activator (t-PA) and urokinase plasminogen activator. The cleavage results in the formation of active plasmin enzyme that contains a serine protease active site in the C-terminal region (23, 26, 27).

S. aureus can capture PLG on its cell surface where the zymogen can be activated by host t-PA and urokinase, or by staphylokinase, a zymogen activator encoded by a gene located on lysogenic bacteriophages in *S. aureus* (28). Surprisingly little is known about the surface-located proteins of *S. aureus* that capture PLG. The second immunoglobulin-binding protein Sbi and the extracellular fibrinogen-binding protein Efb both occur in the culture supernatant and are associated non-covalently with the cell wall and both can bind PLG (29). The membrane-bound manganese transporter MntC and the moonlighting cytoplasmic proteins enolase and triose-phosphate isomerase can also bind PLG (30–32). However, the biological relevance of these PLG-binding proteins has never been elucidated.

Surprisingly, the ability of CWA proteins to bind PLG has never been examined. Here, for the first time we have tested the

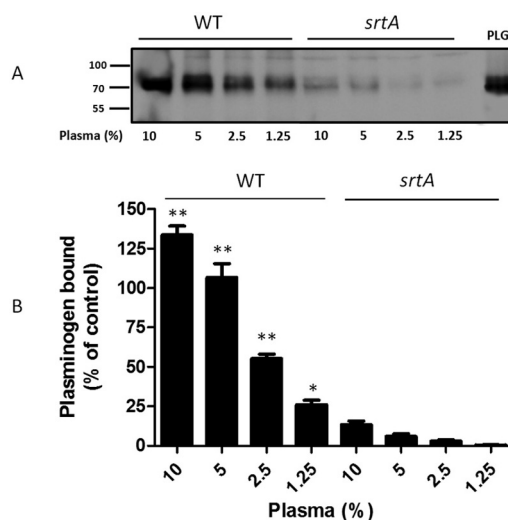


FIGURE 2. **Capture of PLG from human plasma by *S. aureus*.** A, *S. aureus* strain LAC and LAC *srtA* were mixed with different concentrations of human plasma for 60 min. Proteins that were bound to the cell surface were released by extraction buffer and separated by SDS-PAGE under non-reducing conditions and analyzed by Western immunoblotting. The membranes were probed with rabbit anti-human PLG followed by HRP-conjugated mouse anti-rabbit IgG and developed with the ECL Western blotting detection kit. B, densitometric analysis of PLG bound to *S. aureus* LAC and the sortase A mutant as reported in A. The band intensity was quantified relative to a sample of pure human PLG. The reported data are the mean values \pm S.D. from three independent experiments. Statistically significant differences are indicated (Student's *t* test; *, $p < 0.05$; **, $p < 0.01$).

ability of CWA proteins to bind PLG and allow it to be activated to form plasmin. Initially, a sortase A mutant was compared with the wild type and was found to bind much less PLG. The FnBPs were found to contribute to the PLG-binding phenotype and thereafter a detailed analysis of one of these, FnBPB was undertaken.

Results

S. aureus Cell Wall-anchored Proteins Bind PLG in Human Plasma—*S. aureus* cells are known to bind to human PLG, but the bacterial surface components responsible have not been identified. To determine whether cell wall-anchored proteins on the surface of *S. aureus* contribute to PLG binding, the wild-type strain LAC and a sortase A-deficient mutant were compared. Bacteria were incubated with different concentrations of human plasma, and proteins that were bound non-covalently to the cell surface were dissociated by addition of extraction buffer, separated by SDS-PAGE under non-reducing conditions, and analyzed by Western immunoblot probing with anti-PLG IgG. Bacterial cells captured a 90-kDa immunoreactive protein in a dose-dependent manner (Fig. 2A). Densitometric analysis of the blots showed that the wild-type strain captured at least 10-fold more PLG than the sortase A mutant (Fig. 2B). To exclude the possibility that the markedly reduced capture of PLG from plasma by the sortase A mutant is due to higher proteolytic activity, LAC and its *srtA* mutant were grown to exponential phase and were incubated with purified PLG, and the integrity of the protein in the supernatants was then examined by SDS-PAGE. As expected, the amounts of unbound PLG in the supernatant from *srtA* mutant were even higher than that from the parental strain (data not shown). It can be concluded

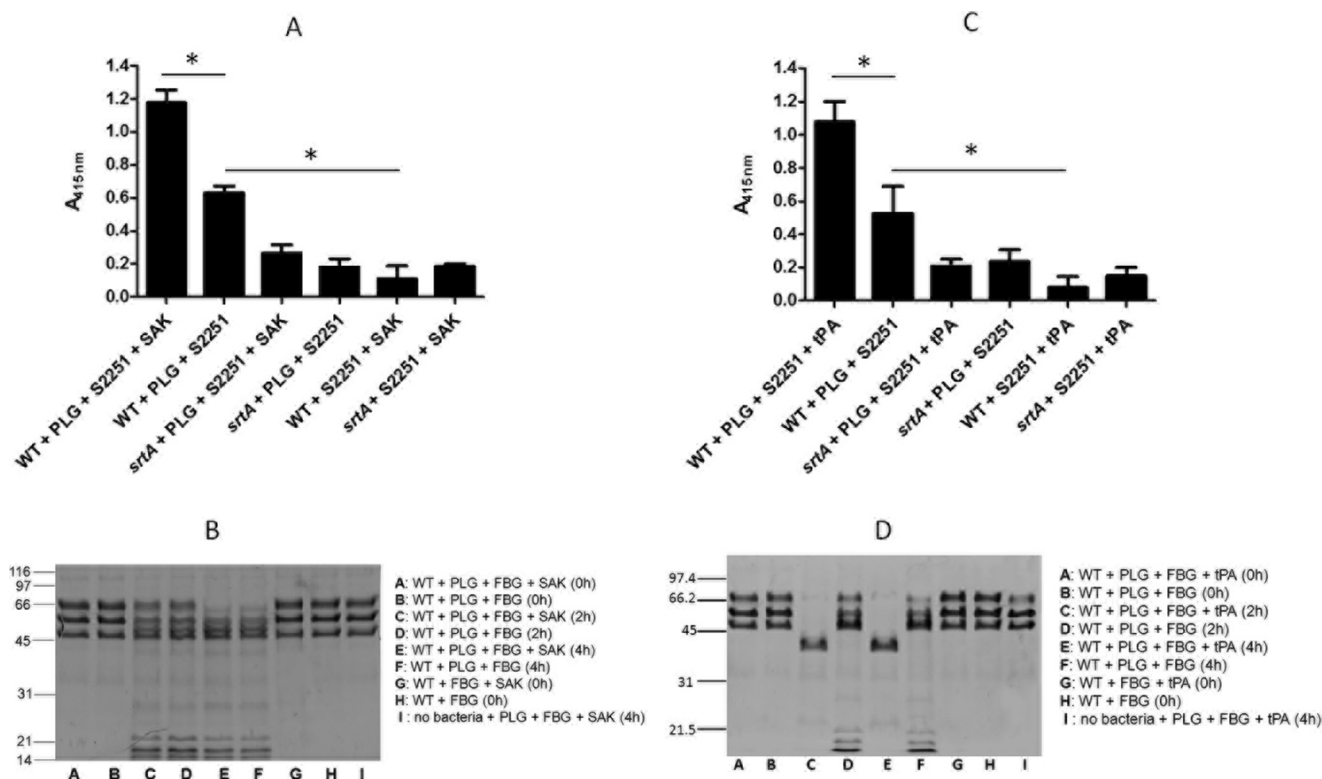


FIGURE 3. PLG captured by *S. aureus* LAC is functionally active. *S. aureus* LAC and the *srtA* mutant were immobilized on the surface of microtiter plates and then incubated with human PLG for 1 h at 37 °C. After washing, cells were incubated with recombinant SAK (A) or tPA (C) and S-2251 for 8 h, and the activity of plasmin was determined measuring the absorbance at 415 nm. Controls lacking PLG or activator were included. Data points are the means of three independent experiments each performed in triplicate \pm S.D. Statistically significant differences are indicated (Student's *t* test; *, $p < 0.05$). *S. aureus* LAC wild-type cells were also immobilized on microtiter plates and incubated with human PLG for 1 h at 37 °C. After washing, PLG was activated by addition of rSAK (B) or tPA (D) and the plasmin substrate FBG. Samples were incubated for 2 or 4 h, and FBG in the fluid phase was subjected to SDS-PAGE and stained with Coomassie Blue. The figure is the representative of three independent experiments.

that CWA proteins are the dominant PLG-binding proteins on the bacterial cell surface and that other surface-located proteins contribute minimally. To compare the plasmin and plasminogen binding activity of *S. aureus*, equal quantities of the purified PLG and plasmin were incubated with LAC cells. After washing, the bacteria-bound plasmin(ogen) was extracted from the staphylococcal surface, and the PLG and plasmin in the extracts were evaluated by ELISA. The results show that bacteria captured the same level of PLG and plasmin (data not shown).

PLG Bound to the Surface of *S. aureus* Can Be Activated—The ability of PLG bound to the surface of *S. aureus* LAC to be activated by exogenous or endogenous PLG activators was tested (Fig. 3). *S. aureus* LAC is lysogenized by a bacteriophage that carries the gene for SAK, a known activator of the zymogen PLG. Human tissue PLG activator was also tested. Bacterial cells (LAC and LAC *srtA*) were immobilized on the surface of microtiter wells and incubated with PLG and then incubated for 8 h with exogenously purified recombinant SAK (rSAK) or tPA and the chromogenic plasmin substrate S-2251. The substrate was consumed following incubation with the wild type, but minimal substrate consumption was seen with LAC *srtA* (Fig. 3, A and C). Controls where PLG was omitted did not have the ability to cleave the substrate. Interestingly, incubation of wild-type LAC with PLG and S-2251 alone showed a significantly higher cleavage of the substrate as compared with the controls. Conversely, although the level of substrate consump-

tion was significantly lower than that observed with samples with exogenous SAK or tPA added, the assays reached the same level if left for longer periods (data not shown).

These data show that PLG bound to the cell surface by CWA proteins can be activated efficiently by exogenously added rSAK or tPA (Fig. 3, A and C, respectively), and furthermore, the immobilized cells can release an endogenous PLG activator, presumably SAK. Plasmin is a central component of the fibrinolytic system and can actively cleave fibrin and FBG. To determine whether PLG captured by CWA proteins on the surface of *S. aureus* can be activated by SAK and cleave FBG, immobilized *S. aureus* LAC cells were incubated with PLG. After washing, rSAK and human FBG were added. The integrity of FBG was assessed by SDS-PAGE and staining with Coomassie Blue. After 2 and 4 h of incubation, FBG was progressively cleaved (Fig. 3B). Samples where rSAK was omitted also cleaved FBG, which was presumably due to secretion of endogenous SAK (Fig. 3B, tracks D and F). When cells were incubated with tPA rather than rSAK, FBG was digested more efficiently. In fact, all three FBG chains were hydrolyzed compared with the mild hydrolysis observed when PLG was activated by SAK. This is probably due to the different mechanisms of PLG activation promoted by tPA and SAK, respectively (Fig. 3D) (28).

Captured PLG Can Be Activated by Endogenously Expressed SAK—To determine whether cleavage of plasmin substrates S-2251 and whole FBG in the experiments described in Figs. 2

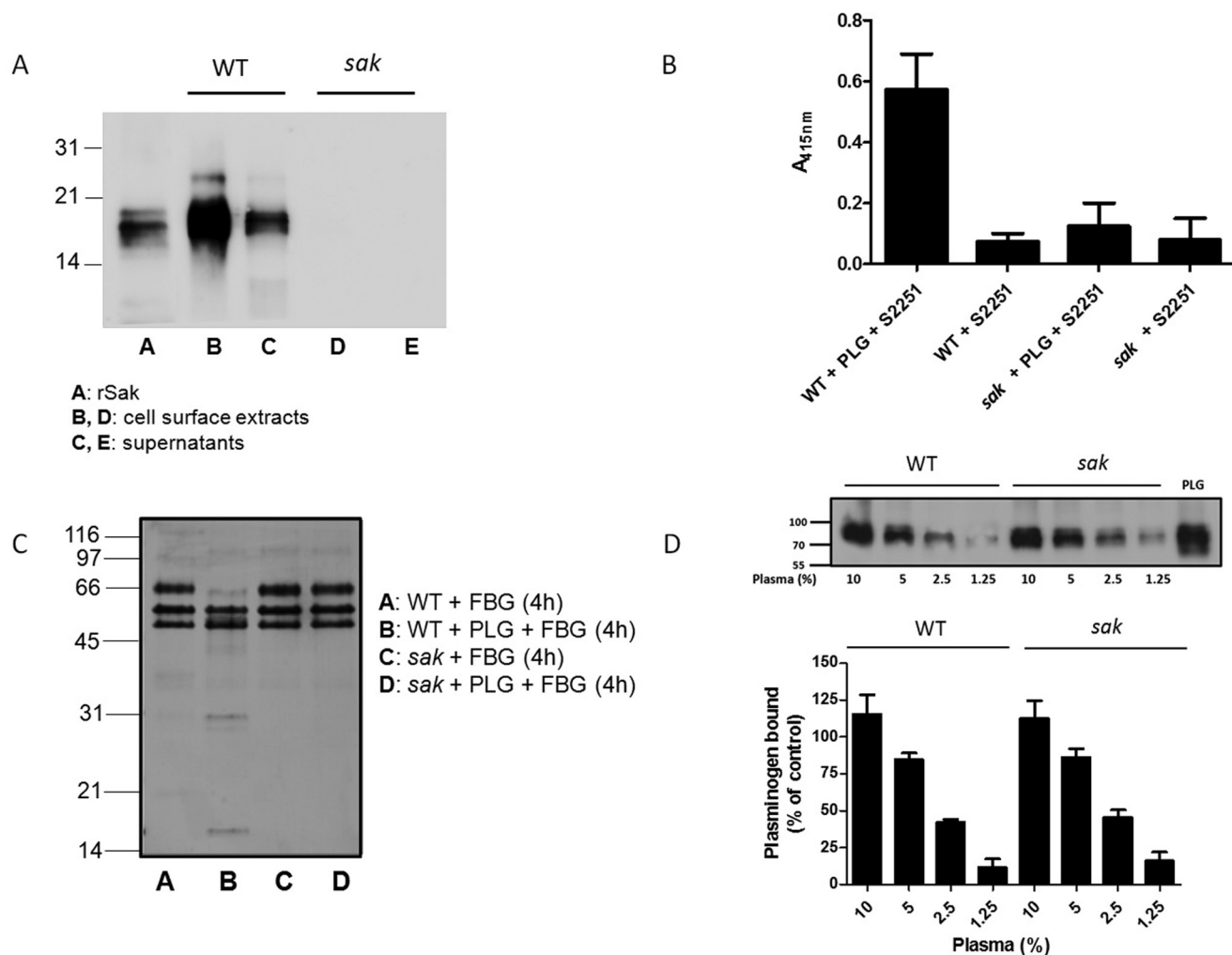


FIGURE 4. *S. aureus* cells-bound PLG can be activated by endogenous SAK. *A*, TCA-precipitated culture supernatants and proteins extracted from the cell surface were subjected to SDS-PAGE and Western immunoblot probing with mouse anti-rSAK IgG and HRP-conjugated rabbit anti-mouse IgG. *B*, *S. aureus* LAC and the *sak* mutant were immobilized on the surface of microtiter plates and then incubated with human PLG for 1 h at 37 °C. After washing, cells were incubated with S-2251 for 8 h, and the activity of plasmin was determined by measuring the absorbance at 415 nm. Controls lacking PLG were included. Data points are the means of three independent experiments each performed in triplicate \pm the S.D. *C*, *S. aureus* LAC wild-type and *sak* mutant cells were immobilized on microtiter plates and incubated with human PLG for 1 h at 37 °C. After washing, the plasmin substrate FBG was added. Samples were incubated for 2 or 4 h, and FBG in the fluid phase was subjected to SDS-PAGE and stained with Coomassie Blue. The figure is the representative of three independent experiments. *D*, *S. aureus* strain LAC and *sak* mutant cells were mixed with different concentrations of human plasma for 60 min. *Upper panel*, proteins that were bound to the cell surface were released with extraction buffer and separated by SDS-PAGE under non-reducing conditions and analyzed by Western immunoblotting. The membranes were probed with rabbit anti-human PLG followed by HRP-conjugated mouse anti-rabbit IgG. *Lower panel*, densitometric analysis of released PLG. The band intensities were quantified relative to a sample of pure human PLG. The reported data are the mean values from three independent experiments.

and 3, where exogenous rSAK or tPA was not present, was due to SAK expressed by the bacterium, a null mutation in the chromosomal *sak* gene of LAC was constructed (LAC *sak*). Culture supernatants and cell surface extracts were examined by SDS-PAGE and Western immunoblotting using anti-SAK IgG. SAK was present in the supernatant and is associated with the cell surface of LAC, and no SAK was detected in fractions from LAC *sak* (Fig. 4A). To determine whether endogenously expressed SAK is responsible for activating captured PLG, wild-type and mutant cells were compared (Fig. 4B). Cleavage of the substrate S-2251 was evident with wild-type cells, whereas no cleavage occurred with the *sak* mutant. Similarly, wild-type cells could capture and activate PLG and cleave FBG (Fig. 4C), whereas the *sak* mutant was defective. To demonstrate directly that LAC *sak* could still capture PLG, bacteria were incubated with dif-

ferent concentrations of plasma, and the amount of PLG captured was quantified by Western immunoblotting and densitometry (Fig. 4D). No difference in PLG bound to the wild-type or *sak* mutant cells was observed. Thus, it can be concluded that SAK secreted by *S. aureus* LAC in the supernatant or bound to the cell surface is capable of activating captured PLG.

Fibronectin-binding proteins A and B Bind PLG—The inability of the *srtA* mutant of *S. aureus* LAC to capture PLG indicates that one or more sortase-anchored cell wall-associated protein(s) is involved. To begin the process of identifying which of the plethora of CWA proteins can bind PLG, purified recombinant ligand binding domains of several CWA proteins were tested by far Western blotting. Proteins were subjected to SDS-PAGE, and Western blottings were probed with purified human PLG and anti-PLG IgG. Subdomains N2 and N3 of the

Plasminogen Interactions with *S. aureus* FnBPB

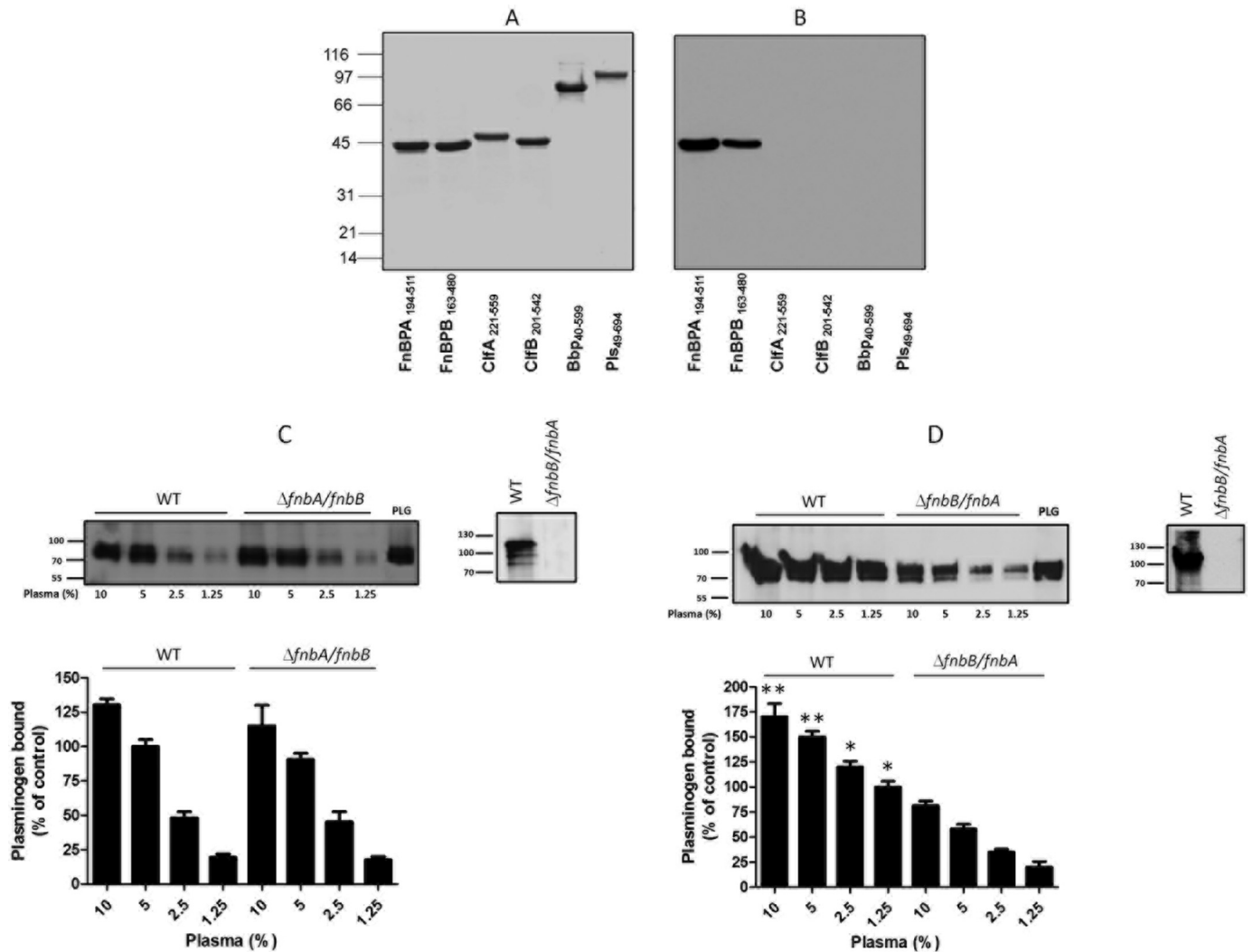


FIGURE 5. Recombinant cell wall-anchored proteins binding to PLG. Purified recombinant A domains of several CWA proteins of *S. aureus* were subjected to SDS-PAGE and either stained with Coomassie Blue (A) or transferred to a nitrocellulose membrane (B) and probed with human PLG followed by rabbit anti-PLG serum and then HRP-conjugated goat anti-rabbit IgG. C, *S. aureus* strains LAC and its *fnbA/fnbB* double mutant cells (upper panel) were mixed with different concentrations of human plasma for 60 min. Proteins that were bound to the cell surface were released by extraction buffer and separated by SDS-PAGE under non-reducing conditions and analyzed by Western immunoblotting. The membranes were probed with rabbit anti-human PLG followed by HRP-conjugated mouse anti-rabbit IgG and developed with the ECL Western blotting detection kit. D, similar experiment was performed with strain BH1CC and its *fnbA/fnbB* double mutant (upper panel). Densitometric analysis of PLG bound to the strains (lower panels of C and D). The band intensity was quantified relative to a sample of pure human PLG. The reported data are the mean values \pm S.D. from three independent experiments. In the insets the material released by treatment of bacteria with lysostaphin was subjected to Western blotting and probed with FN. Statistically significant differences are indicated (Student's *t* test; *, $p < 0.05$; **, $p < 0.01$).

N-terminal A regions of both fibronectin-binding proteins FnBPA and FnBPB bound PLG, whereas the A domains of clumping factors A and B (ClfA and ClfB), the bone sialoprotein-binding protein, and the plasmin-sensitive protein did not (Fig. 5B). To investigate whether FnBPA and FnBPB are the sole PLG-binding proteins of *S. aureus* LAC, a mutant that lacks both proteins was tested for PLG capture. No statistically significant difference between the wild type and the *fnbA fnbB* mutant was seen (Fig. 5C), which indicates that one or more CWA protein(s) in addition to FnBPA and FnBPB can bind PLG. Strain LAC expresses low levels of FnBPs compared with the HA-associated strain BH1CC (12), so we decided to determine whether FnBPs contribute to PLG capture when FnBPs are highly expressed. Strain BH1CC showed higher PLG binding than LAC (Fig. 5D), and BH1CC-bound PLG that was acti-

vated by either SAK or tPA cleaved more efficiently the artificial substrate S2251 compared with the LAC strain (data not shown). The *fnbA fnbB* mutant of BH1CC captured significantly less PLG than the parental strain (Fig. 5D) showing the importance of FnBPs to the phenotype. Likewise, *fnbA fnbB* mutants of 8325-4 and LS1 showed a significantly lower ability to capture PLG from plasma. Furthermore, the *fnbA fnbB* deletion mutant of LAC that was transformed with a plasmid bearing either the *fnbA* or *fnbB* gene, which overexpressed FnBPA or FnBPB, respectively, showed a much higher potential to capture plasma PLG than the parental strain (Table 1). All together these data indicate that FnBPA and FnBPB represent critical PLG receptors in *S. aureus*. In contrast, as reported for LAC, FnBPA and FnBPB apparently do not seem to be the predominant PLG-binding proteins in

TABLE 1

Capture of PLG from human plasma by *S. aureus* strains

S. aureus strains and their *fnbA/fnbB* mutants were mixed with 5% diluted human plasma for 60 min. Proteins that were bound to the cell surface were released by extraction buffer and separated by SDS-PAGE under non-reducing conditions and analyzed by Western immunoblotting. The membranes were probed with rabbit anti-human PLG followed by HRP-conjugated mouse anti-rabbit IgG and developed with the ECL Western blotting detection kit. The densitometric analysis of PLG bound to *S. aureus* strains was performed as reported under "Experimental Procedures." The band intensity was quantified relative to a sample of pure PLG (5 μ g, 100% intensity). Statistically significant differences between WT strains and their corresponding mutants are indicated (Student's *t* test; **, $p < 0.01$).

Bacterial strain	Band intensity (% PLG bound, mean \pm S.D.)
LAC	110 \pm 10
LAC Δ <i>fnbA/fnbB</i>	95 \pm 15
LAC Δ <i>fnbA/fnbB</i> pFNBA4	205 \pm 12 ^a
LAC Δ <i>fnbA/fnbB</i> pFNBB4	195 \pm 20 ^a
BH1CC	150 \pm 15
BH1CC Δ <i>fnbA/fnbB</i>	62 \pm 6 ^a
P1	115 \pm 12
P1 Δ <i>fnbA/fnbB</i>	96 \pm 8
8325-4	106 \pm 15
8325-4 Δ <i>fnbA/fnbB</i>	47 \pm 4 ^a
LS1	165 \pm 13
LS1 Δ <i>fnbA/fnbB</i>	58 \pm 9 ^a
SH1000	108 \pm 10
SH1000 Δ <i>fnbA/fnbB</i>	105 \pm 12

^a Statistically significant differences between WT strains and their corresponding mutants are indicated (Student's *t* test; $p < 0.01$).

strains P1 and SH1000, suggesting that additional CWA proteins are involved (Table 1).

Lactococcus lactis-expressing FnBPB Can Capture PLG—To study FnBPB in isolation from other *S. aureus* CWA proteins, a strain of *L. lactis*-expressing FnBPB from a gene cloned into the plasmid vector pNZ8037 was used. *L. lactis* pNZ8037::*fnbB* and the same strain carrying the empty vector were immobilized in microtiter wells and incubated with PLG, and following the washing steps, the chromogenic plasmin substrates S-2251 and rSAK (Fig. 6A) or tPA (Fig. 6C) were added. Cleavage of the substrate was observed, whereas the cells bearing the empty vector had no activity. It should be noted that this system was somewhat less active than *S. aureus* LAC cells. *L. lactis* (pNZ8037::*fnbB*) cells were also incubated with FBG and rSAK (Fig. 6B) or tPA (Fig. 6D). SDS-PAGE analysis showed that FBG was cleaved by the FnBPB-expressing cells and not by those carrying the empty vector. These experiments show that *L. lactis* cells normally lack the ability to capture PLG and that FnBPB expressed on the bacterial cell surface can capture PLG in a form that can be activated by exogenous PLG activators.

PLG and FBG Bind FnBPB at Distinct Sites—The A domain of FnBPB binds to FBG by the dock, lock, and latch mechanism. To determine whether PLG and FBG bind FnBPB at the same or distinct sites, rFnBPB(163–480) was immobilized onto the surface of microtiter wells and tested for binding to saturating concentrations of PLG in the presence of increasing amounts of FBG by ELISA-type assay. Bound PLG was detected with specific antibodies, and the amount of PLG bound did not change as the concentration of FBG added increased (Fig. 7A). A similar result was obtained when saturating concentrations of FBG were tested in the presence of increasing amounts of PLG (Fig. 7B). In both panels, dose-dependent binding of increasing amounts of either FBG or PLG to FnBPB was also reported. Together, these data indicate that PLG and FBG bind to distinct sites on the A domain of FnBPB without steric hindrance. The

failure of FBG to interfere with rFnBPB binding to PLG suggested that the ligands bind to different sites within the A domain of the protein. To investigate whether the dock, lock, and latch mechanism is involved in PLG binding and to further localize its binding site, variants of the recombinant A domain were tested for ligand binding in solid phase ELISA-type assays. A truncate lacking 17 residues at the C terminus of subdomain N3 that is involved in the locking and latching steps of the dock, lock, and latch mechanism (rFnBPB(163–463)) and a protein carrying amino acid substitutions in residues Asn-312 and Phe-314 of the FnBPB fibrinogen-binding trench (rFnBPB(163–480)-N312A/F314A) were tested for binding to FBG and PLG (Fig. 8, A–D). Neither the C-terminal truncate nor the trench mutant could bind FBG, but each bound PLG dose-dependently and saturably in a manner that was indistinguishable from rFnBPB(163–480). This confirms that PLG does not bind to the FnBPB A domain using the dock, lock, and latch mechanism. Finally, subdomains N2 and N3 were expressed separately (N2 subdomain rFnBPB(163–308) and N3 subdomain rFnBPB(309–480)) and tested for binding to PLG and FBG (Fig. 8, E and F). Neither subdomain N2 nor N3 bound FBG detectably, whereas subdomain N3 bound PLG dose-dependently and saturably with the same profile as N2N3 (rFnBPB(163–480)), which is also consistent with the two ligands binding to FnBPB at different non-overlapping sites. This suggests that the PLG-binding site of FnBPB is contained within the N3 subdomain.

Measurement of the Dissociation Constant of FnBPB Binding to PLG by Surface Plasmon Resonance—The affinity of the FnBPB for PLG was measured by surface plasmon resonance. Purified rFnBPB(163–480) was immobilized onto the surface of a dextran chip, and PLG was passed over the chip in concentrations ranging from 0.78 to 5 μ M (Fig. 9). The K_D for the interaction was $0.532 \pm 0.028 \mu$ M, an affinity that is nearly 4-fold higher than the interaction between FnBPB and FBG (33).

Localization of the Binding Sites within PLG and FnBPB—PLG binds to the N3 subdomain of FnBPB that was cloned from *S. aureus* 8325-4. There are seven isoforms of FnBPB N2N3 subdomains with amino acid sequence identities ranging from 61 to 85% (33). FnBPBs from strains 8325-4, LAC, and BH1CC have identical amino acid sequences and belong to isoform I. Each of the seven isoforms retains the ability to bind FBG, fibronectin, and elastin (33). The recombinant N2N3 subdomains of isoforms II–VII were purified and tested for their ability to bind to PLG in an ELISA-type assay. Each isoform bound PLG similarly to isoform I (Fig. 10A).

PLG binding to its natural ligands is attributed to strong affinity of kringle domain(s) for lysine-rich regions of target proteins. To investigate whether lysine residues are important in binding to FnBPB isoform I, PLG binding assays were performed with the recombinant bacterial protein in the presence of lysine and the lysine analog ϵ -aminocaproic acid (EACA). Both molecules caused dose-dependent and complete inhibition of the interaction, with EACA being far more potent (Fig. 10B).

The structure of the N3 subdomain of FnBPB isoform I was modeled on the x-ray crystal structure of the related protein

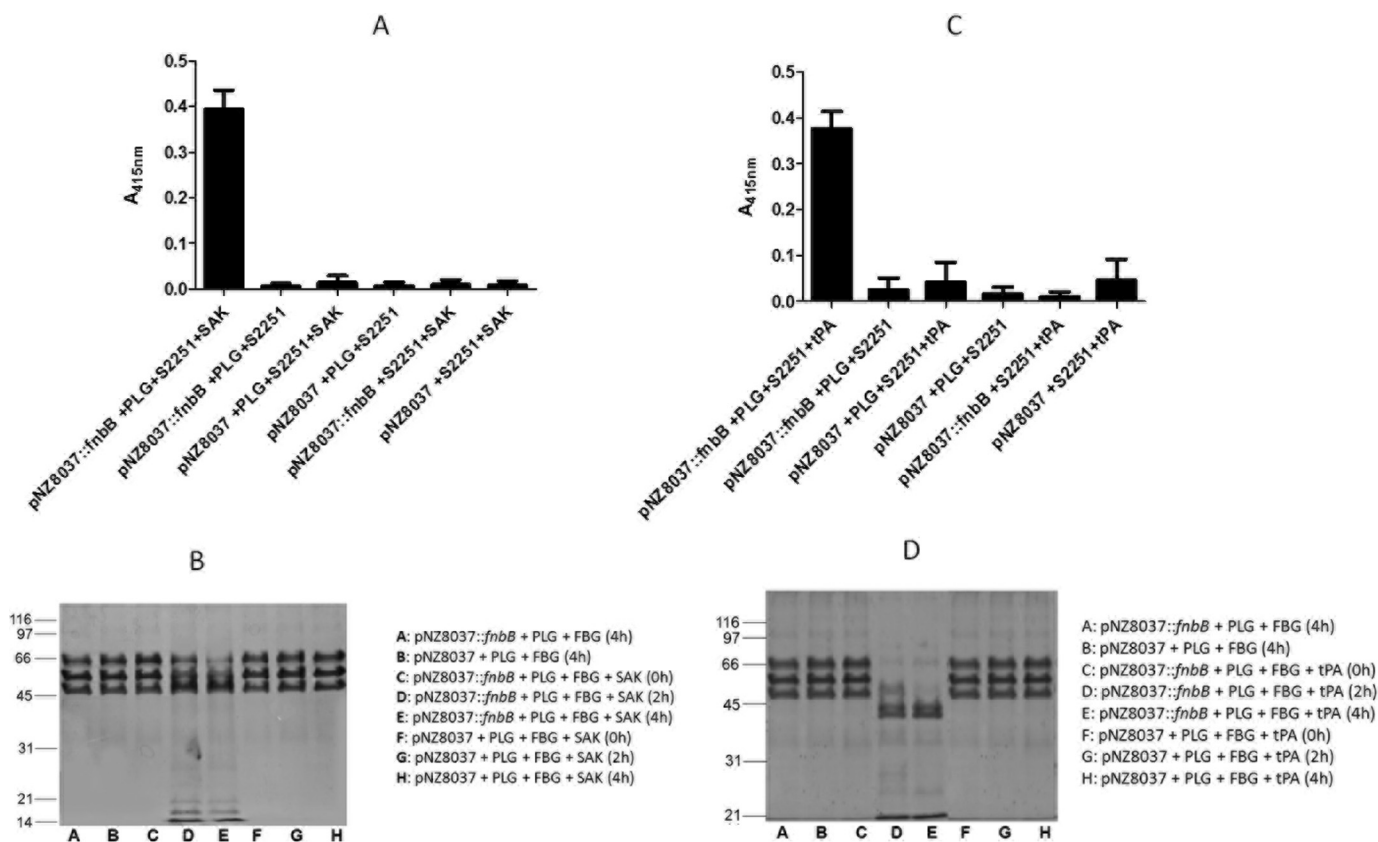


FIGURE 6. **Activation of PLG bound to *L. lactis*-expressing FnBPB.** *L. lactis*-expressing FnBPB (pNZ8037fnbB) and cells carrying the empty vector were immobilized in microtiter plates and incubated with PLG for 1 h at 37 °C. After washing, cells were incubated with rSAK (A) or tPA (C) and the chromogenic plasmin substrate S-2251 for 8 h. The activity of plasmin was determined measuring the absorbance at 415 nm. The data points are the means (\pm S.D.) of three independent experiments each performed in triplicate. Immobilized *L. lactis*-expressing FnBPB and cells carrying the empty vector were also incubated with PLG for 1 h at 37 °C. After washing, cells were incubated with FBG and rSAK (B) or tPA (D) for 2 and 4 h. Controls lacking rSAK or tPA were included (tracks A and B). The fluid was analyzed by SDS-PAGE followed by staining with Coomassie Blue.

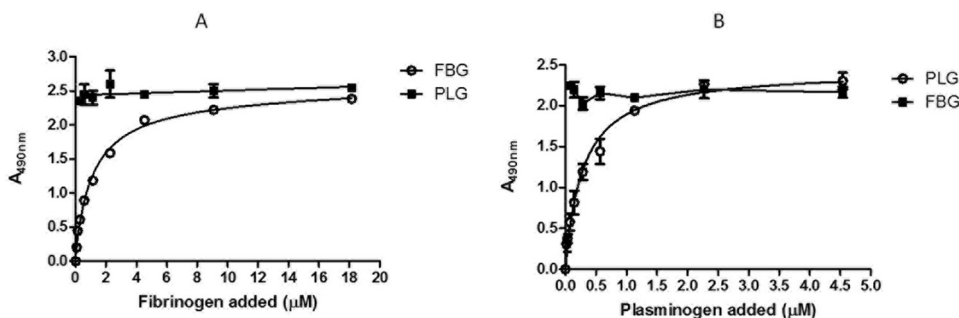


FIGURE 7. **Binding of FnBPB to PLG in the presence of FBG.** A, rFnBPB was immobilized on the surface of microtiter wells. Saturating concentrations of PLG were added along with increasing concentrations of FBG. Bound PLG was detected with rabbit anti-PLG followed by HRP-conjugated goat anti-rabbit IgG (full squares). In the same panel, binding of increasing amounts of FBG to the wells is also reported (open dots). Bound FBG was detected with mouse anti-FBG serum followed by HRP-conjugated rabbit anti-mouse IgG. The data points are the means \pm S.D. of three independent experiments each performed in triplicate. B, ELISA-type assay with rFnBPB immobilized on the surface of microtiter wells. Saturating concentrations of FBG were added along with increasing concentrations of PLG. Bound FBG was detected with mouse anti-FBG followed by HRP-conjugated rabbit anti-mouse IgG (full squares). The panel shows binding of increasing amounts of PLG to the wells (open dots). Bound proteins were detected with specific antibodies as in A. The data points are the means (\pm S.D.) of three independent experiments each performed in triplicate.

FnBPA. Amino acid sequence alignments of isotypes I–VII of FnBPB were used to identify conserved lysine residues in subdomain N3. The conserved lysine residues were visualized using the molecular model, and two conserved lysine-rich surface regions were identified. Alanine substitutions were isolated in site 1 (Lys-330, Lys-334, and Lys-362) and site 2 (Lys-342, Lys-374, and Lys-423) and then combined to form a double site 1-site 2 mutant. The variants were tested for their ability to

bind to PLG and FBG. Both site 1 and site 2 mutants showed a significant reduction in binding to PLG, a difference that was even greater when site 1 and site 2 substitutions were combined. In contrast, FBG binding was not reduced significantly (Fig. 10C). This is consistent with PLG and FBG binding to FnBPB A domain at different sites.

To determine which part of the PLG protein binds FnBPB, truncates comprising kringles 1–3 and 1–4, and mini-PLG,

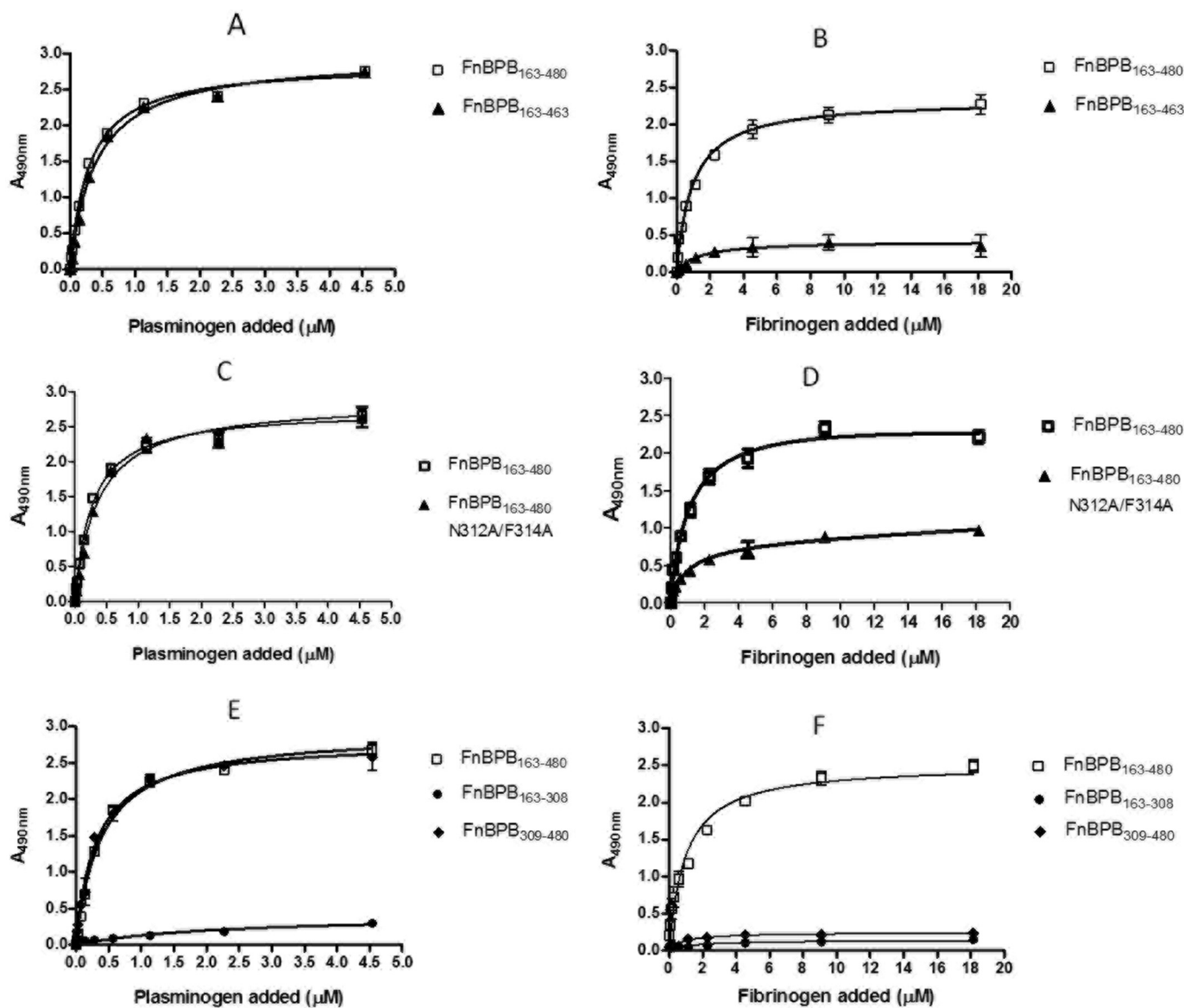


FIGURE 8. **PLG binding to FnBPB(163–480) and its derivatives.** Truncated variants of rFnBPB A domain were immobilized in microtiter plate wells and tested for binding to PLG (A, C, and E) or FBG (B, D, and E). Bound PLG was detected with rabbit antibodies to human PLG followed by HRP-conjugated goat anti-rabbit IgG. Bound FBG was detected with mouse antibodies to human FBG followed by HRP-conjugated rabbit anti-mouse IgG. Immobilized proteins: A and B, C-terminal truncate lacking the lock and latch regions (rFnBPB(163–463)); C and D, amino acid substitution mutant in the ligand-binding trench (rFnBPB(163–480) N312A/F314A); E and F, N2 and N3 subdomains rFnBPB(163–308) and rFnBPB(309–480). The data points are the means (\pm S.D.) of three independent experiments each performed in triplicate.

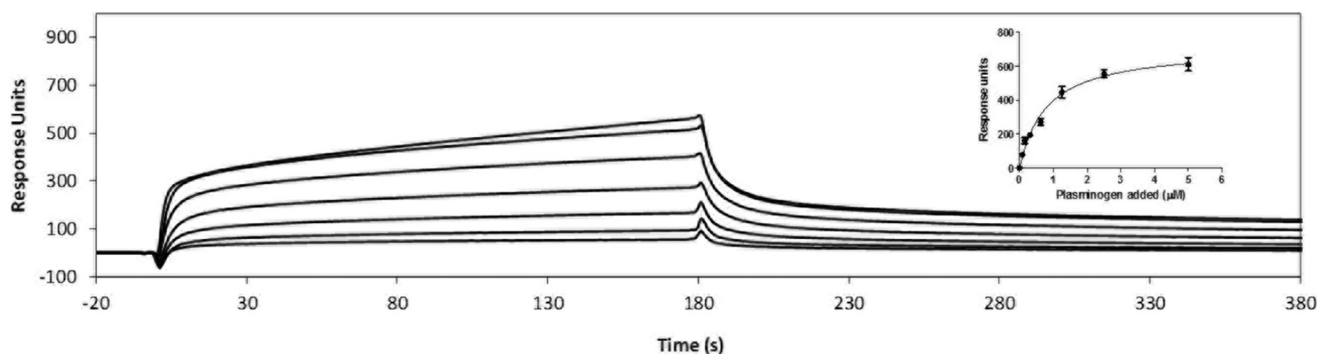


FIGURE 9. **FnBPB binds PLG with high affinity.** Surface plasmon resonance analysis of the interaction of rFnBPB(163–480) with PLG. Representative sensorgrams display binding of PLG to and dissociation from rFnBPB(163–480). The affinity was calculated from curve fitting to a plot of the response unit values against concentrations of PLG (inset). The figure shows one representative of three experiments.

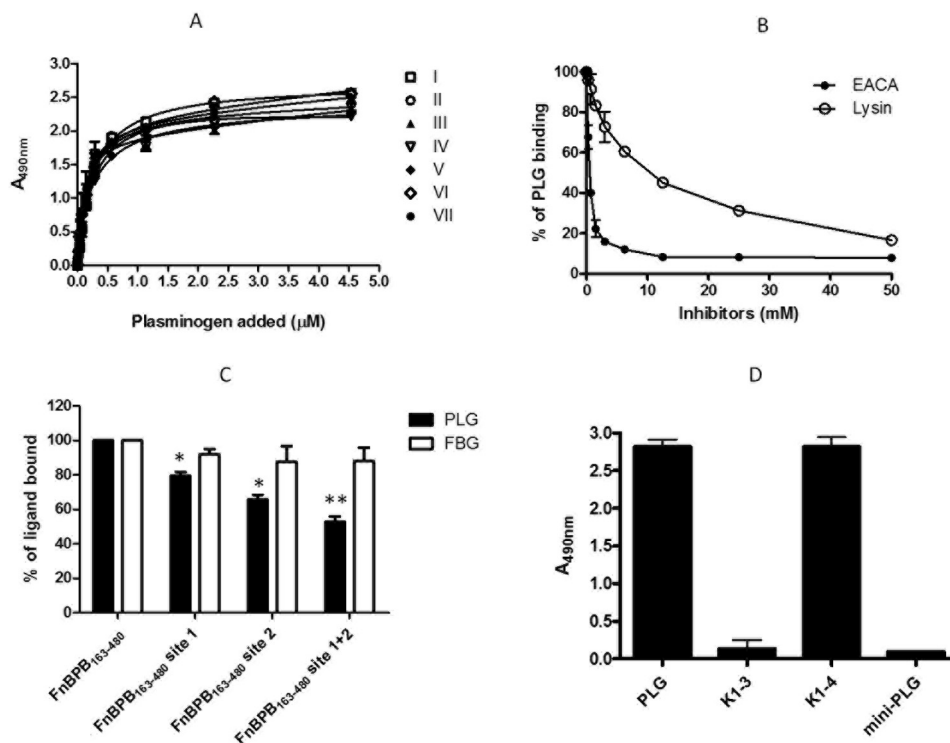


FIGURE 10. A, recombinant N2N3 subdomains of isoforms I–VII of FnBPB were immobilized in microtiter plate wells and tested for binding to PLG. Bound PLG was detected with rabbit antibodies to human PLG followed by HRP-conjugated goat anti-rabbit IgG. The data points are the means \pm S.D. of three independent experiments each performed in triplicate. B, rFnBPB(163–480) was immobilized onto microtiter plates and incubated with PLG in the presence of increasing concentrations of lysine and the lysine analog ϵ -aminocaproic acid. PLG bound to FnBPB was detected with a mouse polyclonal PLG antiserum and HRP-conjugated anti-rabbit IgG. Binding of PLG by FnBPB in the absence of inhibitor was set to 100%. Values represent the means of three separate experiments performed in triplicate, and error bars show S.D. C, microtiter plates coated with FnBPB(163–480) and its isoforms mutated at site 1, site 2, or both sites were incubated with PLG or FBG. Bound PLG was detected with rabbit antibodies followed by HRP-conjugated goat anti-rabbit IgG. Bound FBG was detected with mouse antibody followed by HRP-conjugated rabbit anti-mouse IgG. Binding of each ligand to the variants was expressed as percentage of the controls where binding of PLG or FBG to FnBPB(163–480) was set to 100%. The data points are the means \pm S.D. of three independent experiments each performed in triplicate. Statistically significant differences are indicated (Student's *t* test; *, $p < 0.05$; **, $p < 0.01$). D, microtiter plates coated with FnBPB(163–480) were incubated with equimolar concentrations of PLG or PLG fragments K1–K3, K1–K4, and mini-PLG. PLG and the fragments bound to FnBPB were detected by incubation of the plates with a rabbit PLG antibody followed by goat HRP-conjugated secondary IgG. Three independent experiments were conducted in triplicate, and graphs represent means \pm S.D.

which is composed of kringle 5 along with C-terminal residues, were tested for binding to the bacterial protein. Kringles 1–3 and kringle 5 failed to bind FnBPB, whereas kringles 1–4 bound similarly to full-length PLG. Thus, kringle 4 seems to be the sole binding domain within PLG for FnBPB (Fig. 10D).

Discussion

S. aureus, like other invasive pathogens such as *S. pneumoniae* (13, 14), *S. pyogenes* (15, 16), and *S. agalactiae* (17, 34, 35) can capture PLG from human plasma. Staphylococcal proteins that bind the human protein have been described previously, the secreted and wall-associated protein Sbi (29), the MntC (30), and the moonlighting cytoplasmic protein enolase (31), but the biological significance of these interactions is not clear because experiments were performed either with purified protein *in vitro* or with cell lysates.

Here, we have shown for the first time that sortase A-anchored cell wall-associated proteins are the dominant PLG-binding proteins on the *S. aureus* cell surface. A sortase A mutant of *S. aureus* LAC bound at least a 10-fold lower level of PLG than the wild type. It is noteworthy that PLG captured from human plasma could be activated by exogenously added tissue PLG activator t-PA and SAK.

Two CWA proteins, FnBPA and FnBPB, were shown to bind PLG *in vitro* and in the case of FnBPB when expressed on the surface of the surrogate host *L. lactis*. However, deletion mutants of *S. aureus* USA300 LAC, P1, and SH1000 lacking FnBPs bound the same level of PLG as the parental strain, although mutants of BH1CC, 8325-4, and LS1 showed at least a 50% reduction in PLG binding, indicating that one or more of the other CWA proteins are also important. Thus, the contribution of FnBPs to PLG capture will vary depending on strain background and the level of *fnbA fnbB* gene expression, something that is influenced by the growth conditions of the bacterium. To identify the repertoire of PLG binding CWA proteins and to determine their relative importance in the capacity of the bacterium to bind PLG, it would be necessary to express recombinantly all 23 sortase-anchored proteins and to test them by far Western blotting and/or ELISA. This would need to be backed up by the isolation of mutants defective in the candidate proteins, both singly and in combination.

The A domain of FnBPB was chosen for detailed analysis of PLG binding. This domain can bind to FBG by the dock, lock, and latch mechanism (8). We have shown that PLG and FBG bind FnBPB at distinct non-overlapping sites and that PLG

TABLE 2
Bacterial strainsAbbreviations used are as follows: Em^r, erythromycin resistance; Tc^r, tetracycline resistance; Cm^r, chloramphenicol resistance.

Bacterial strains	Relevant properties	Reference
<i>S. aureus</i>		
LAC	Community-associated MRSA of USA300 lineage	5
LAC Δ <i>fnbA fnbB</i>	Deletion of <i>fnbA</i> and <i>fnbB</i> genes isolated by allelic exchange	12
LAC Δ <i>fnbA fnbB</i> (pFNBA4)	Mutant transformed with plasmid-expressing FnBPA	12
LAC Δ <i>fnbA fnbB</i> (pFNBB4)	Mutant transformed with plasmid-expressing FnBPB	12
LAC <i>srtA</i>	Mutant of LAC defective in sortase A. Transduction of <i>srtA</i> ::Em ^r mutation from strain Newman into an erythromycin-sensitive derivative of LAC	This study
LAC <i>sak</i>	Mutant of LAC defective in staphylokinase. Transduction of <i>sak</i> ::Tn Em ^r from the Nebraska transposon mutant library strain JE2 <i>sak</i> ::Tn into an erythromycin-sensitive derivative of LAC	This study
8325-4	NCTC8325 cured of three prophages	39
8325-4 <i>fnbA fnbB</i>	<i>fnbA</i> ::Tc ^r <i>fnbB</i> ::Em ^r mutations isolated by allelic exchange	40
BH1CC	Hospital-associated MRSA	3
BH1CC <i>fnbA fnbB</i>	<i>fnbA</i> ::Tc ^r <i>fnbB</i> ::Em ^r mutations transduced from 8325-4 <i>fnbA fnbB</i>	3
P1	Rabbit passaged strain derived from ATCC25923	41
P1 <i>fnbA fnbB</i>	<i>fnbA</i> ::Tc ^r <i>fnbB</i> ::Em ^r mutations transduced from 8325-4 <i>fnbA fnbB</i>	42
LS1	Isolate from spontaneous outbreak of septic arthritis in mouse colony	43
LS1 <i>fnbA fnbB</i>	<i>fnbA</i> ::Tc ^r <i>fnbB</i> ::Em ^r mutations transduced from 8325-4 <i>fnbA fnbB</i>	44
SH1000	<i>rbsU</i> restored in 8325-4	45
SH1000 <i>fnbA fnbB</i>	<i>fnbA</i> ::Tc ^r <i>fnbB</i> ::Em ^r mutations transduced from 8325-4 <i>fnbA fnbB</i>	This study
<i>L. lactis</i>		
<i>L. lactis</i> NZ9800 (pNZ8037)	Expression vector with nisin-inducible promoter, Cm ^r	46
<i>L. lactis</i> NZ9800 (pNZ8037 <i>fnbB</i>)	<i>fnbB</i> gene cloned in pNZ8037 Cm ^r	47

binding does not involve the dock, lock, and latch mechanism. This is important because PLG and FBG are both components of blood plasma. FnBPB can simultaneously capture both PLG and FBG (a substrate for plasmin, once plasminogen becomes activated).

The PLG-binding site is confined to subdomain N3, and it does not overlap the FBG-binding site, and variants of the recombinant A domain with substitutions at two distinct conserved lysine-rich sites in subdomain N3 bound PLG with reduced affinity. X-ray crystal structure analysis of the FnBPB-PLG complex will provide molecular details of the interaction. The seven distinct isoforms of FnBPB each bound PLG with similar affinities in solid phase assays. This attests to the idea that PLG binding is an important property that has been retained during diversification of the N3 subdomain surface residues. It will be interesting to compare PLG binding by FnBPA and its seven isoforms.

Our data indicate that kringle 4 probably comprises the only binding domain within PLG for FnBPB and that this binds the two lysine-rich patches within subdomain N3 of FnBPB. Structural analysis of streptokinase binding to PLG showed that the bacterial zymogen activator bound in such a way as to avoid the kringle domains. SAK and streptokinase belong to the same protein superfamily even though they share little amino acid sequence similarity (36), so it seems likely that they interact with PLG at the same or a similar site leaving kringle 4 free to bind to FnBPB. It would be interesting to determine whether SAK can bind to and displace PLG from the FnBPB and stimulate the release of active plasmin or whether the host protease remains attached to the cell-bound MSCRAMM.

The presence of active plasmin on the surface of *S. aureus* cells most likely contributes to the pathogenesis of different infections. For example, degradation of host- or SAK-promoted fibrin clots could help bacteria to spread (37). Damage to subcutaneous tissue could contribute to the pathogenesis of skin soft tissue infections caused by CA-MRSA (38). Degradation of opsonins could contribute to the avoidance of neutro-

phil-mediated phagocytosis and killing (20–22). To investigate this further requires the employment of appropriate animal models, and to test the role of SAK in these processes requires the use of a transgenic mouse expressing human PLG.

In summary, we have shown that CWA proteins are the dominant bacterial receptors for capturing host PLG by *S. aureus*, and we have investigated in detail the mechanism of PLG binding by one such CWA protein FnBPB.

Experimental Procedures

Bacterial Strains and Culture Conditions—All strains are listed in Table 2 (39–47). *S. aureus* was grown in brain heart infusion broth (VWR International Srl, Milan, Italy) at 37 °C with shaking. *L. lactis* cells carrying the expression vector (pNZ8037) alone or harboring the *fnbB* gene (pNZ8037::*fnbB*) were grown overnight in M17 medium (Difco, Detroit, MI) supplemented with 10% lactose, 0.5% glucose, and chloramphenicol (10 µg/ml) at 30 °C without shaking. Cultures were diluted 1:100 in the above medium and allowed to reach exponential phase. Nisin (6.4 ng/ml) was added, and cultures were allowed to grow overnight at 30 °C without shaking. In those experiments, where a defined number of cells were used, bacteria were harvested from the cultures by centrifugation, washed and suspended in PBS, and counted in a Petroff-Hausser chamber. *Escherichia coli* TOPP3 transformed with vector pQE30 (Stratagene, La Jolla, CA) or derivatives were grown in Luria agar and Luria broth (VWR) containing 100 µg/ml ampicillin.

Plasmid and DNA Manipulation—Plasmid DNA (Table 3) was isolated using the Wizard® Plus SV miniprep kit (Promega, Madison, WI), according to the manufacturer's instructions, and finally transformed into *E. coli* TOPP3 cells using standard procedures (48). Transformants were screened by restriction analysis and verified by DNA sequencing (Eurofins Genomics, Milan, Italy). Chromosomal DNA was extracted using the bacterial genomic DNA purification kit (Edge Biosystems, Gaithersburg, MD). DNA encoding residues 163–480 of FnBPB incorporating alanine substitutions in site 1 (residues Lys-330,

TABLE 3

Plasmids

Abbreviation used is as follows: Amp^R ampicillin resistance.

Plasmid	Features	Marker	Source/Reference
pQE30	<i>E. coli</i> vector for the expression of hexa-His-tagged recombinant proteins	Amp ^R	Qiagen
pQE30::rFnBPB(163–480)	pQE30 derivative encoding the N2N3 subdomain of FnBPB from <i>S. aureus</i> 8325–4	Amp ^R	8
pQE30::rFnBPB(163–480) site 1	pQE30 derivative encoding the N2N3 incorporating alanine substitutions in site1 (residues Lys-330, Lys-334, and Lys-362)	Amp ^R	This study
pQE30::rFnBPB(163–480) site 2	pQE30 derivative encoding the N2N3 incorporating alanine substitutions in site2 (residues Lys-342, Lys-374, and Lys-423)	Amp ^R	This study
pQE30::rFnBPB(163–480) site 3	pQE30 derivative encoding the N2N3 incorporating alanine substitutions site 1 and 2 substitutions combined	Amp ^R	This study
pQE30::rFnBPB(163–463)	pQE30 derivative encoding residues 163–463 of FnBPB from <i>S. aureus</i> 8325–4	Amp ^R	8
pQE30::rFnBPB(163–480) N312A/F314A	pQE30 derivative encoding the N2N3 subdomain of FnBPB from <i>S. aureus</i> 8325–4 with mutations encoding the changes N312A and F314A	Amp ^R	8
pQE30::rFnBPB(163–308)	pQE30 derivative encoding the N2 subdomain of FnBPB from <i>S. aureus</i> 8325–4	Amp ^R	This study
pQE30::rFnBPB(309–480)	pQE30 derivative encoding the N3 subdomain of FnBPB from <i>S. aureus</i> 8325–4	Amp ^R	This study

Lys-334, and Lys-362), site 2 (Lys-342, Lys-374, and Lys-423), or site 1 and 2 substitutions combined was synthesized as g-Block® double-stranded DNA fragments by Integrated DNA Technologies (Coralville, IA). Primers rFnBPB(163–480)F and rFnBPB(163–480)R (Table 4) were used to amplify the sequence for cloning into pQE30. Restriction digests and ligations were carried out using enzymes from New England Biolabs (Ipswich, MA) according to the manufacturer's protocols. Oligonucleotides were purchased from Integrated DNA Technologies (Leuven, Belgium) and are listed in Table 4. DNA purification was carried out using the Wizard®SV gel and PCR clean-up system (Promega).

Expression and Purification of Recombinant Proteins—Recombinant proteins FnBPB(163–308), FnBPB(309–480), and FnBPB(163–463) latch truncated were expressed from pQE30 (Qiagen, Chatsworth, CA) in *E. coli* TOPP3 (Stratagene). Overnight starter cultures were diluted 1:50 in Luria broth containing ampicillin (100 µg/ml) and incubated with shaking until the culture reached an optical density at 600 nm (*A*₆₀₀) of 0.4–0.6. Recombinant protein expression was induced by addition of isopropyl 1-thio-β-D-galactopyranoside (0.5 mM) and continued for 2 h. Bacterial cells were harvested by centrifugation and frozen at –80 °C and purified from cell lysates by Ni²⁺-affinity chromatography on a HiTrap chelating column (GE Healthcare). Recombinant FnBPB isotypes (33), FnBPB(163–480) N312A/F314A trench mutant (8), FnBPA(194–511) (49), ClfA(221–559) (50), ClfB(201–542) (51), bone sialoprotein-binding protein(40–599) (52), and plasmin-sensitive protein(49–694) (53), were each previously expressed with His₆ N-terminal affinity tags using *E. coli* vectors and purified on a HiTrap chelating column (GE Healthcare) by Ni²⁺-chelate chromatography as described above. The *E. coli* clone expressing recombinant SAK protein was generously provided by Dr. S. H. M. Rooijackers, University Medical Center Utrecht, The Netherlands, and the SAK protein was purified according to the methods described for isolation of CHIPS by De Haas *et al.* (54). Protein purity was assessed to be 98% by SDS-PAGE, Coomassie Brilliant Blue staining, and den-

sitometry analysis. A bicinchoninic acid protein assay (Pierce) was used to measure concentrations of purified proteins.

Proteins—Human PLG was purified from plasma by affinity chromatography on a Lys-Sepharose column (55). Human FBG was obtained from Calbiochem. FBG was further purified on a gelatin-Sepharose column to remove contaminating fibronectin (FN). Human FN was purified from plasma by a combination of gelatin- and arginine-Sepharose affinity chromatography. To exclude the presence of trace amounts of contaminants, affinity-purified FN was spotted onto nitrocellulose membranes at different concentrations and overlaid with anti-FBG and anti-PLG antibodies (56). The purity of the proteins was assessed by 7.5–12.5% SDS-PAGE and Coomassie Brilliant Blue staining. Kringle 1–3 and Kringle 1–4 were purchased from Sigma and MyBiosource (San Diego), respectively. The mini-PLG (residues Val⁴⁴²-Asn⁷⁹⁰) was obtained by digestion of PLG with porcine pancreatic elastase (Sigma), as described previously (57, 58). Unless stated otherwise, all other reagents were purchased from Sigma.

Human Plasma—Normal human plasma was prepared from freshly drawn blood obtained from healthy volunteers with informed consent and permission of the ethical board of the University of Pavia (permit no. 19092013). After centrifugation, the plasma fraction was frozen in aliquots and stored at –20 °C.

Antibodies—Polyclonal antiserum against purified PLG was raised in a rabbit by routine immunization procedures using purified human PLG as antigen. Polyclonal antisera against human FBG or recombinant SAK were raised in mice using purified or recombinant SAK as antigens. Purification of rabbit or mouse antibodies from sera was performed by affinity chromatography using protein G-Sepharose columns (GE Healthcare, Buckinghamshire, UK). The anti-human FN rabbit polyclonal IgG was purchased from Pierce. Rabbit anti-mouse or goat anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Dako Cytomation (Glostrup, Denmark).

ELISA-type Solid-phase Binding Assays—The ability of immobilized proteins (FnBPB(163–480) and its derivatives) to

TABLE 4

Primers

F is forward, and R is reverse.

Primer	Sequence (5'–3') ^a	5'-Restriction site
rFnBPB(163–480) F	<u>CGGGGATCCGCATCGGAACAAAAACAATAC</u>	BamHI
rFnBPB(163–480) R	<u>AATCCCGGGTTACTTTAGTTTATCTTTGCC</u>	SmaI
rFnBPB(163–308) F	<u>GGGGGATCCGGTACAGATGTAACAAATAAAG</u>	BamHI
rFnBPB(163–308) R	<u>CTCCCCGGGCTATTGAATATTAATATTTTGCTAA</u>	SmaI
rFnBPB(309–480) F	<u>CCCGGATCCTATTTAGGTGGAGTTAGAGATAAT</u>	BamHI
rFnBPB(309–480) R	<u>AATCCCGGGTTACTTTAGTTTATCTTTGCCG</u>	SmaI

^a Restriction sites used for cloning are underlined.

bind to soluble ligands (PLG or FBG) was determined using ELISA. Microtiter wells were coated overnight at 4 °C with 0.5 μg/well of the appropriate protein in 0.1 M sodium carbonate, pH 9.5. The plates were washed with 0.5% (v/v) Tween 20 in PBS (PBST). To block additional protein-binding sites, the wells were treated for 1 h at 22 °C with bovine serum albumin (BSA, 2% v/v) in phosphate-buffered saline (PBS). The plates were then incubated for 1 h with the appropriate amounts of the ligand. After several washings with PBST, 0.5 μg of the specific anti-ligand rabbit/mouse IgG in BSA (1% v/v) was added to the wells and incubated for 90 min. The plates were washed incubated for 1 h with HRP-conjugated anti-rabbit or anti-mouse IgG diluted 1:1000. After washing, *o*-phenylenediamine dihydrochloride was added, and the absorbance at 490 nm was determined using an ELISA plate reader.

To identify PLG kringle(s) involved in FnBPB(163–480) binding, microtiter plates were coated with 0.5 μg of FnBPB(163–480) per well and incubated with PLG, PLG fragments K1-K3, K1-K4, or mini-PLG (1 μM). Proteins bound to FnBPB(163–480) were detected by mouse PLG antibody followed by goat HRP-conjugated secondary IgG.

The effect of lysine and lysine analog EACA on FnBPB(163–480)/PLG interaction was examined incubating recombinant FnBPB(163–480) immobilized onto microtiter plates (0.5 μg/well) with 1 μg of purified PLG in the presence of increasing concentrations of lysine or EACA. PLG bound to FnBPB was detected with mouse polyclonal PLG antiserum and HRP-conjugated anti-rabbit IgG.

SDS-PAGE and Western Immunoblotting and Far Western Immunoblotting—Samples for analysis by SDS-PAGE were boiled for 3 min in sample buffer (0.125 M Tris-HCl, 4% (w/v) SDS, 20% (v/v) glycerol, 10% (v/v) β-mercaptoethanol, 0.002% (w/v) bromophenol blue) and separated by 10–15% (w/v) PAGE. The gels were stained with Coomassie Brilliant Blue (Bio-Rad). For Western immunoblotting, proteins were subjected to SDS-PAGE and electroblotted onto a nitrocellulose membrane (GE Healthcare), and the membrane was blocked overnight at 4 °C with 5% (w/v) skim milk (Sigma) in PBS. Blotted proteins were probed with rabbit polyclonal antibody against human PLG (1:5000) or mouse polyclonal antibody against SAK (5 μg/ml) for 1 h at 22 °C. Following washes with PBST, the membrane was incubated for 1 h with either HRP-conjugated goat anti-rabbit IgG (1:10,000) or rabbit anti-mouse IgG (1:10,000). Finally, blots were developed using the ECL Advance Western blotting detection kit (GE Healthcare), and an ImageQuant™ LAS 4000 mini-biomolecular imager (GE Healthcare) was used to capture images of the bands. When recombinant staphylococcal proteins were subjected to SDS-PAGE and far Western

blotting, membranes were incubated with 1 μg/ml PLG in PBST for 1 h at 22 °C and washed, and the complexes were detected with anti-PLG antibody as reported above.

Preparation of Cell Surface-bound Proteins and TCA-precipitated Culture Supernatants—*S. aureus* USA300 was grown to exponential phase and washed, and ~1 × 10⁸ cfu were incubated for 1 h with the indicated concentrations of human plasma. Cells were harvested by centrifugation, washed with PBS, treated with extraction buffer (125 mM Tris-HCl, pH 7.0, containing 2% SDS) (10 μl/mg wet weight of pellet) (59), for 3 min at 95 °C, and then centrifuged at 10,000 × *g* for 3 min. The supernatants were subjected to 10% PAGE under non-reducing conditions and transferred to a nitrocellulose membrane. The membrane was incubated with rabbit anti-PLG IgG followed by HRP-conjugated secondary antibody. The band intensities were quantified relative to the PLG sample (5 μg, 100% intensity) with the Quantity One software (Bio Rad, Milan, Italy).

To detect SAK on the surface of *S. aureus* USA300 LAC and LAC *sak*, bacteria were treated with extraction buffer, heated at 95 °C for 3 min, and subjected to 15% SDS-PAGE and Western blotting. SAK was detected by incubation with mouse anti-SAK IgG followed by a rabbit HRP-conjugated secondary antibody. Bacteria grown to exponential phase were removed from cultures by centrifugation at 4000 × *g* for 5 min, and the supernatant was concentrated by treatment with 20% trichloroacetic acid (TCA) (v/v) at 4 °C for 30 min. The precipitates were collected by centrifugation at 10,000 × *g* for 10 min, washed with acetone, dissolved in the sample buffer, boiled for 3 min and finally subjected to 15% SDS-PAGE and Western blotting. SAK was detected as reported above.

Release of Cell Wall-anchored Proteins from *S. aureus* and Detection of Fibronectin Binding Activity—To release cell wall-anchored proteins from *S. aureus*, bacteria were grown to an A₆₀₀ of 0.4 to 0.6, harvested by centrifugation at 7000 × *g* at 4 °C for 15 min, washed three times with PBS, and resuspended to an A₆₀₀ of 40 in lysis buffer (50 mM Tris-HCl, 20 mM MgCl₂, pH 7.5) supplemented with 30% raffinose. Cell wall proteins were solubilized from *S. aureus* by incubation with lysostaphin (200 μg/ml) at 37 °C for 20 min in the presence of protease inhibitors (Complete Mini; Roche Applied Science). Protoplasts were recovered by centrifugation at 6000 × *g* for 20 min, and the supernatants were taken as the wall fraction. The material was adsorbed on IgG-Sepharose column to remove protein A and subjected to SDS-PAGE and Western blotting incubating the membrane with human FN (5 μg/ml) and a rabbit anti-FN antibody (2 μg/ml).

Plasminogen Interactions with *S. aureus* FnBPB

Surface Plasmon Resonance Analysis of PLG Binding to FnBPB(163–480)—To estimate the affinity of the interaction between PLG and FnBPB(163–480), surface plasmon resonance was conducted using a BIAcore X-100 instrument (GE Healthcare). FnBPB(163–480) was covalently immobilized on dextran matrix CM5 sensor chip surface by using an FnBPB(163–480) solution (30 $\mu\text{g}/\text{ml}$ in 50 mM sodium acetate buffer, pH 5) in a 1:1 dilution with *N*-hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride. The excess of active groups on the dextran matrix was blocked using 1 M ethanolamine, pH 8.5. On another flow cell, the dextran matrix was treated as described above but without any ligand to provide an uncoated reference flow cell. The running buffer used was PBS containing 0.005% (v/v) Tween 20. A 2-fold linear dilution series (0.078–5 μM) of PLG in running buffer was passed over the ligand at the flow rate of 10 $\mu\text{l}/\text{min}$, and all the sensorgrams were recorded at 22 °C. Assay channel data were subtracted from reference flow cell data. The response units at steady state were plotted as a function of PLG concentration and fitted to the Langmuir equation to yield the K_D of the PLG-FnBPB(163–480) interaction.

Effect of Bacteria Bound PLG on the Cleavage of Chromogenic Substrate S-2251 and Degradation of Human Fibrinogen—*S. aureus* LAC (5×10^7 cells) isolated from exponential phase or *L. lactis*-expressing FnBPB were suspended in PBS and immobilized onto microtiter wells at 37 °C overnight. After several washings with PBST, the cells were incubated for 1 h with human PLG (10 $\mu\text{g}/\text{well}$) in PBS at 37 °C. After washing with PBST, bacterially bound PLG was activated by addition of recombinant SAK (15 $\mu\text{g}/\text{ml}$) or 27 nM tPA, incubated with 0.6 mM chromogenic substrate S-2251 (H-D-valyl-L-leucyl-L-lysine-*p*-nitroaniline dihydrochloride; Chromogenix), dissolved in 100 mM HEPES, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mg/ml PEG 8000 for 8 h, and the reaction followed spectrophotometrically at 415 nm.

Alternatively, surface-coated cells of *S. aureus* LAC or *L. lactis* were incubated for 90 min at 37 °C with PLG (1 $\mu\text{g}/\text{well}$) diluted in PBS. After several washings with PBST, FBG (10 $\mu\text{g}/\text{well}$) together with tPA (27 nM) or rSAK (15 $\mu\text{g}/\text{ml}$) in 100 mM HEPES, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mg/ml PEG 8000 were added to the wells, and the mixtures were incubated at 22 °C for the indicated times. The reaction was stopped by the addition of reducing sample buffer and boiling for 3 min. Thereafter, the samples were separated by 12.5% SDS-PAGE, and degradation of FBG was evaluated by Coomassie Blue staining.

Statistical Methods—Continuous data were expressed as means and standard deviations. Two group comparisons were performed by Student's *t* test. One-way analysis of variance, followed by Bonferroni's post hoc tests, was exploited for comparison of three or more groups. Analyses were performed using Prism 4.0 (GraphPad). Two-tailed *p* values of 0.05 were considered statistically significant.

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References

1. Lowy, F. D. (1998) *Staphylococcus aureus* infections. *N. Engl. J. Med.* **339**, 520–532
2. van Belkum, A., Melles, D. C., Nouwen, J., van Leeuwen, W. B., van Wamel, W., Vos, M. C., Wertheim, H. F., and Verbrugh, H. A. (2009) Co-evolutionary aspects of human colonisation and infection by *Staphylococcus aureus*. *Infect. Genet. Evol.* **9**, 32–47
3. O'Neill, E., Pozzi, C., Houston, P., Humphreys, H., Robinson, D. A., Loughman, A., Foster, T. J., and O'Gara, J. P. (2008) A novel *Staphylococcus aureus* biofilm phenotype mediated by the fibronectin-binding proteins, FnBPA and FnBPB. *J. Bacteriol.* **190**, 3835–3850
4. Vergara-Irigaray, M., Valle, J., Merino, N., Latasa, C., García, B., Ruiz de Los Mozos, I., Solano, C., Toledo-Arana, A., Penadés, J. R., and Lasa, I. (2009) Relevant role of fibronectin-binding proteins in *Staphylococcus aureus* biofilm-associated foreign-body infections. *Infect. Immun.* **77**, 3978–3991
5. Diep, B. A., Gill, S. R., Chang, R. F., Phan, T. H., Chen, J. H., Davidson, M. G., Lin, F., Lin, J., Carleton, H. A., Mongodin, E. F., Sensabaugh, G. F., and Perdreau-Remington, F. (2006) Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. *Lancet* **367**, 731–739
6. Foster, T. J., Geoghegan, J. A., Ganesh, V. K., and Höök, M. (2014) Adhesion, invasion and evasion: the many functions of the surface proteins of *Staphylococcus aureus*. *Nat. Rev. Microbiol.* **12**, 49–62
7. Loughman, A., Sweeney, T., Keane, F. M., Pietrocola, G., Speziale, P., and Foster, T. J. (2008) Sequence diversity in the A domain of *Staphylococcus aureus* fibronectin-binding protein A. *BMC Microbiol.* **8**, 74
8. Burke, F. M., Di Poto, A., Speziale, P., and Foster, T. J. (2011) The A domain of fibronectin-binding protein B of *Staphylococcus aureus* contains a novel fibronectin binding site. *FEBS J.* **278**, 2359–2371
9. Deivanayagam, C. C., Wann, E. R., Chen, W., Carson, M., Rajashankar, K. R., Höök, M., and Narayana, S. V. (2002) A novel variant of the immunoglobulin fold in surface adhesins of *Staphylococcus aureus*: crystal structure of the fibrinogen-binding MSCRAMM, clumping factor A. *EMBO J.* **21**, 6660–6672
10. Geoghegan, J. A., Monk, I. R., O'Gara, J. P., and Foster, T. J. (2013) Subdomains N2N3 of fibronectin-binding protein A mediate *Staphylococcus aureus* biofilm formation and adherence to fibrinogen using distinct mechanisms. *J. Bacteriol.* **195**, 2675–2683
11. Pozzi, C., Waters, E. M., Rudkin, J. K., Schaeffer, C. R., Lohan, A. J., Tong, P., Loftus, B. J., Pier, G. B., Fey, P. D., Massey, R. C., and O'Gara, J. P. (2012) Methicillin resistance alters the biofilm phenotype and attenuates virulence in *Staphylococcus aureus* device-associated infections. *PLoS Pathog.* **8**, e1002626
12. McCourt, J., O'Halloran, D. P., McCarthy, H., O'Gara, J. P., and Geoghegan, J. A. (2014) Fibronectin-binding proteins are required for biofilm formation by community-associated methicillin-resistant *Staphylococcus aureus* strain LAC. *FEMS Microbiol. Lett.* **353**, 157–164
13. Agarwal, V., Kuchipudi, A., Fulde, M., Riesbeck, K., Bergmann, S., and Blom, A. M. (2013) *Streptococcus pneumoniae* endopeptidase O (PepO) is a multifunctional plasminogen- and fibronectin-binding protein, facilitating evasion of innate immunity and invasion of host cells. *J. Biol. Chem.* **288**, 6849–6863
14. Bergmann, S., Schoenen, H., and Hammerschmidt, S. (2013) The interaction between bacterial enolase and plasminogen promotes adherence of *Streptococcus pneumoniae* to epithelial and endothelial cells. *Int. J. Med. Microbiol.* **303**, 452–462
15. Berge, A., and Sjöbring, U. (1993) PAM, a novel plasminogen-binding protein from *Streptococcus pyogenes*. *J. Biol. Chem.* **268**, 25417–25424
16. Sanderson-Smith, M. L., Downton, M., Ranson, M., and Walker, M. J. (2007) The plasminogen-binding group A streptococcal M protein-related protein Prp binds plasminogen via arginine and histidine residues. *J.*

- Bacteriol.* **189**, 1435–1440
17. Six, A., Bellais, S., Bouaboud, A., Fouet, A., Gabriel, C., Tazi, A., Dramsi, S., Trieu-Cuot, P., and Poyart, C. (2015) Srr2, a multifaceted adhesin expressed by ST-17 hypervirulent Group B *Streptococcus* involved in binding to both fibrinogen and plasminogen. *Mol. Microbiol.* **97**, 1209–1222
 18. Fuchs, H., Wallich, R., Simon, M. M., and Kramer, M. D. (1994) The outer surface protein A of the spirochete *Borrelia burgdorferi* is a plasmin(ogen) receptor. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12594–12598
 19. Önder, Ö., Humphrey, P. T., McOmber, B., Korobova, F., Francella, N., Greenbaum, D. C., and Brisson, D. (2012) OspC is potent plasminogen receptor on surface of *Borrelia burgdorferi*. *J. Biol. Chem.* **287**, 16860–16868
 20. Attali, C., Durmort, C., Vernet, T., and Di Guilmi, A. M. (2008) The interaction of *Streptococcus pneumoniae* with plasmin mediates transmigration across endothelial and epithelial monolayers by intercellular junction cleavage. *Infect. Immun.* **76**, 5350–5356
 21. Fulde, M., Rohde, M., Hitzmann, A., Preissner, K. T., Nitsche-Schmitz, D. P., Nerlich, A., Chhatwal, G. S., and Bergmann, S. (2011) SCM, a novel M-like protein from *Streptococcus canis*, binds (mini)-plasminogen with high affinity and facilitates bacterial transmigration. *Biochem. J.* **434**, 523–535
 22. Rooijackers, S. H., van Wamel, W. J., Ruyken, M., van Kessel, K. P., and van Strijp, J. A. (2005) Anti-opsonic properties of staphylokinase. *Microbes Infect.* **7**, 476–484
 23. Law, R. H., Caradoc-Davies, T., Cowieson, N., Horvath, A. J., Quek, A. J., Encarnacao, J. A., Steer, D., Cowan, A., Zhang, Q., Lu, B. G., Pike, R. N., Smith, A. I., Coughlin, P. B., and Whisstock, J. C. (2012) The x-ray crystal structure of full-length human plasminogen. *Cell Rep.* **1**, 185–190
 24. Chavakis, T., Athanasopoulos, A., Rhee, J. S., Orlova, V., Schmidt-Wöll, T., Bierhaus, A., May, A. E., Celik, I., Nawroth, P. P., and Preissner, K. T. (2005) Angiostatin is a novel anti-inflammatory factor by inhibiting leukocyte recruitment. *Blood* **105**, 1036–1043
 25. Dubocq, C., Genoud, V., Parborell, M. F., and Kordich, L. C. (1997) Impaired clot lysis by rt-PA catalyzed mini-plasminogen activation. *Thromb. Res.* **86**, 505–513
 26. Castellino, F. J., and Powell, J. R. (1981) Human plasminogen. *Methods Enzymol.* **80**, 365–378
 27. Bhattacharya, S., Ploplis, V. A., and Castellino, F. J. (2012) Bacterial plasminogen receptors utilize host plasminogen system for effective invasion and dissemination. *J. Biomed. Biotechnol.* **2012**, 482096
 28. Peetermans, M., Vanassche, T., Liesenborghs, L., Lijnen, R. H., and Verhamme, P. (2015) Bacterial pathogens activate plasminogen to breach tissue barriers and escape from innate immunity. *Crit. Rev. Microbiol.* **2015**, 1–17
 29. Koch, T. K., Reuter, M., Barthel, D., Böhm, S., van den Elsen, J., Krafczy, P., Zipfel, P. F., and Skerka, C. (2012) *Staphylococcus aureus* proteins Sbi and Efb recruit human plasmin to degrade complement C3 and C3b. *PLoS ONE* **7**, e47638
 30. Salazar, N., Castiblanco-Valencia, M. M., da Silva, L. B., de Castro, Í., Monaris, D., Masuda, H. P., Barbosa, A. S., and Arêas, A. P. (2014) *Staphylococcus aureus* manganese transport protein C (MntC) is an extracellular matrix- and plasminogen-binding protein. *PLoS ONE* **9**, e112730
 31. Antikainen, J., Kuparinen, V., Lähteenmäki, K., and Korhonen, T. K. (2007) Enolases from Gram-positive bacterial pathogens and commensal lactobacilli share functional similarity in virulence-associated traits. *FEMS Immunol. Med. Microbiol.* **51**, 526–534
 32. Furuya, H., and Ikeda, R. (2011) Interaction of triosephosphate isomerase from *Staphylococcus aureus* with plasminogen. *Microbiol. Immunol.* **55**, 855–862
 33. Burke, F. M., McCormack, N., Rindi, S., Speziale, P., and Foster, T. J. (2010) Fibronectin-binding protein B variation in *Staphylococcus aureus*. *BMC Microbiol.* **10**, 160
 34. Wiles, K. G., Panizzi, P., Kroh, H. K., and Bock, P. E. (2010) Skizzle is a novel plasminogen- and plasmin-binding protein from *Streptococcus agalactiae* that targets proteins of human fibrinolysis to promote plasmin generation. *J. Biol. Chem.* **285**, 21153–21164
 35. Buscetta, M., Firon, A., Pietrolola, G., Biondo, C., Mancuso, G., Midiri, A., Romeo, L., Galbo, R., Venza, M., Venza, I., Kaminski, P. A., Gominet, M., Teti, G., Speziale, P., Trieu-Cuot, P., and Beninati, C. (2016) PbsP, a cell wall-anchored protein that binds plasminogen to promote hematogenous dissemination of Group B *Streptococcus*. *Mol. Microbiol.* **101**, 27–41
 36. Parry, M. A., Zhang, X. C., and Bode, I. (2000) Molecular mechanisms of plasminogen activation: bacterial cofactors provide clues. *Trends Biochem. Sci.* **25**, 53–59
 37. Loof, T. G., Goldmann, O., Naudin, C., Mörgelin, M., Neumann, Y., Pils, M. C., Foster, S. J., Medina, E., and Herwald, H. (2015) *Staphylococcus aureus*-induced clotting of plasma is an immune evasion mechanism for persistence within the fibrin network. *Microbiology* **161**, 621–627
 38. Wang, X., Towers, S., Panchanathan, S., and Chowell, G. (2013) A population based study of seasonality of skin and soft tissue infections: implications for the spread of CA-MRSA. *PLoS ONE* **8**, e60872
 39. Novick, R. (1967) Properties of a cryptic high-frequency transducing phage in *Staphylococcus aureus*. *Virology* **33**, 155–166
 40. Greene, C., McDevitt, D., Francois, P., Vaudaux, P. E., Lew, D. P., and Foster, T. J. (1995) Adhesion properties of mutants of *Staphylococcus aureus* defective in fibronectin-binding proteins and studies on the expression of *fnb* genes. *Mol. Microbiol.* **17**, 1143–1152
 41. Sherertz, R. J., Carruth, W. A., Hampton, A. A., Byron, M. P., and Solomon, D. D. (1993) Efficacy of antibiotic-coated catheters in preventing subcutaneous *Staphylococcus aureus* infection in rabbits. *J. Infect. Dis.* **167**, 98–106
 42. Roche, F. M., Downer, R., Keane, F., Speziale, P., Park, P. W., and Foster, T. J. (2004) The N-terminal A domain of fibronectin-binding proteins A and B promotes adhesion of *Staphylococcus aureus* to elastin. *J. Biol. Chem.* **279**, 38433–38440
 43. Bremell, T., Lange, S., Svensson, L., Jennische, E., Gröndahl, K., Carlsten, H., and Tarkowski, A. (1990) Outbreak of spontaneous staphylococcal arthritis and osteitis in mice. *Arthritis Rheum.* **33**, 1739–1744
 44. Palmqvist, N., Foster, T., Fitzgerald, J. R., Josefsson, E., and Tarkowski, A. (2005) Fibronectin-binding proteins and fibrinogen-binding clumping factors play distinct roles in staphylococcal arthritis and systemic inflammation. *J. Infect. Dis.* **191**, 791–798
 45. Horsburgh, M. J., Aish, J. L., White, I. J., Shaw, L., Lithgow, J. K., and Foster, S. J. (2002) σ B modulates virulence determinant expression and stress resistance: characterization of a functional rsbU strain derived from *Staphylococcus aureus* 8325-4. *J. Bacteriol.* **184**, 5457–5467
 46. de Ruyter, P. G., Kuipers, O. P., and de Vos, W. M. (1996) Controlled gene expression systems for *Lactococcus lactis* with the food-grade inducer nisin. *Appl. Environ. Microbiol.* **62**, 3662–3667
 47. Fitzgerald, J. R., Loughman, A., Keane, F., Brennan, M., Knobel, M., Higgins, J., Visai, L., Speziale, P., Cox, D., and Foster, T. J. (2006) Fibronectin-binding proteins of *Staphylococcus aureus* mediate activation of human platelets via fibrinogen and fibronectin bridges to integrin GPIIb/IIIa and IgG binding to the Fc γ RIIIa receptor. *Mol. Microbiol.* **59**, 212–230
 48. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., pp. 175–177, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
 49. Keane, F. M., Loughman, A., Valtulina, V., Brennan, M., Speziale, P., and Foster, T. J. (2007) Fibrinogen and elastin bind to the same region within the A domain of fibronectin-binding protein A, an MSCRAMM of *Staphylococcus aureus*. *Mol. Microbiol.* **63**, 711–723
 50. O'Connell, D. P., Nanavaty, T., McDevitt, D., Gurusiddappa, S., Höök, M., and Foster, T. J. (1998) The fibrinogen-binding MSCRAMM (clumping factor) of *Staphylococcus aureus* has a Ca²⁺-dependent inhibitory site. *J. Biol. Chem.* **273**, 6821–6829
 51. Mulcahy, M. E., Geoghegan, J. A., Monk, I. R., O'Keefe, K. M., Walsh, E. J., Foster, T. J., and McLoughlin, R. M. (2012) Nasal colonisation by *Staphylococcus aureus* depends upon clumping factor B binding to the squamous epithelial cell envelope protein lorricin. *PLoS Pathog.* **8**, e1003092
 52. Vazquez, V., Liang, X., Horndahl, J. K., Ganesh, V. K., Smeds, E., Foster, T. J., and Hook, M. (2011) Fibrinogen is a ligand for the *Staphylococcus aureus* microbial surface components recognizing adhesive matrix molecules (MSCRAMM) bone sialoprotein-binding protein (Bbp). *J. Biol. Chem.* **286**, 29797–29805
 53. Roche, F. M., Meehan, M., and Foster, T. J. (2003) The *Staphylococcus aureus* surface protein SasG and its homologues promote bacterial adher-

Plasminogen Interactions with *S. aureus* FnBPB

- ence to human desquamated nasal epithelial cells. *Microbiology* **149**, 2759–2767
54. de Haas, C. J., Veldkamp, K. E., Peschel, A., Weerkamp, F., Van Wamel, W. J., Heezius, E. C., Poppelier, M. J., Van Kessel, K. P., and van Strijp, J. A. (2004) Chemotaxis inhibitory protein of *Staphylococcus aureus*, a bacterial antiinflammatory agent. *J. Exp. Med.* **199**, 687–695
55. Deutsch, D. G., and Mertz, E. T. (1970) Plasminogen: purification from human plasma by affinity chromatography. *Science* **170**, 1095–1096
56. Speziale, P., Visai, L., Rindi, S., and Di Poto, A. (2008) Purification of human plasma fibronectin using immobilized gelatin and Arg affinity chromatography. *Nat. Protoc.* **3**, 525–533
57. Christensen, U., and Mølgaard, L. (1992) Positive co-operative binding at two weak lysine-binding sites governs the Glu-plasminogen conformational change. *Biochem. J.* **285**, 419–425
58. Váli, Z., and Patthy, L. (1982) Location of the intermediate and high affinity ω -aminocarboxylic acid-binding sites in human plasminogen. *J. Biol. Chem.* **257**, 2104–2110
59. Hussain, M., Becker, K., von Eiff, C., Schrenzel, J., Peters, G., and Herrmann, M. (2001) Identification and characterization of a novel 38.5-kilodalton cell surface protein of *Staphylococcus aureus* with extended-spectrum binding activity for extracellular matrix and plasma proteins. *J. Bacteriol.* **183**, 6778–6786

Molecular Interactions of Human Plasminogen with Fibronectin-binding Protein B (FnBPB), a Fibrinogen/Fibronectin-binding Protein from *Staphylococcus aureus*

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