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Cloning, Expression, and Nucleotide Sequence of a *Staphylococcus aureus* Gene (*fbpA*) Encoding a Fibrinogen-Binding Protein

AMBROSE I. CHEUNG,[1]* STEVEN J. PROJAN,[2]† REVA E. EDELSTEIN,[1] AND VINCENT A. FISCHETTI[1]

Laboratory of Bacterial Pathogenesis and Immunology, The Rockefeller University, New York, New York 10021,[1] and Public Health Research Institute, New York, New York 10016[2]

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Septicemia due to *Staphylococcus aureus* often begins as a focal infection (e.g., colonized wounds or catheters) from which the organism gains access to the bloodstream. On the basis of recent data from this laboratory, it is likely that *S. aureus* colonizes catheters and endothelium by using a fibrinogen-binding protein to mediate adhesion to fibrinogen-coated surfaces. To characterize the fibrinogen-reactive protein, we screened a λZap library of *S. aureus* DB, a clinical isolate, for clones that were reactive with fibrinogen. Of 100,000 plaques screened, 3 were found to react with fibrinogen on immunoblots. Plasmid DNA prepared from clones 14, 30, and 36, upon digestion with *Eco*RI, which released the insert, revealed fragments of 4.6, 3.6, and 3.2 kb, respectively. To identify the cloned protein expressed in *E. coli*, cells were fractionated into periplasmic, membrane, and cytoplasmic fractions. Expression studies of clone 14, which comprised approximately two-thirds of the mature molecule, including the C terminus, revealed a 34-kDa fibrinogen-reactive protein in both the periplasmic and membrane fractions. This protein, designated FbpA, could be partially purified on a fibrinogen column. By using both clones 14 and 36 as templates, the complete DNA sequence of the fibrinogen-binding protein was obtained, yielding a molecule with a predicted size of 69,991 Da. Although sequence analysis revealed a high degree of homology with coagulase, there is a unique sequence of 11 amino acids that is not found in three known coagulases as well as two recently cloned fibrinogen-binding proteins. This unique sequence shares homology with a cell wall anchor motif found in other gram-positive surface proteins.

Septicemia due to *Staphylococcus aureus* is often the consequence of a localized infection (e.g., infected wounds or catheters) from which the organism gains access to the bloodstream (36). Once the bacteria are in the bloodstream, patients are at an increased risk of developing endocarditis and other metastatic complications, especially patients with underlying valvular abnormalities (e.g., congenital or rheumatic heart diseases) (37). Although the use of newer antimicrobial agents has been able to control some of these infections, the recent emergence of increased resistance to antistaphylococcal antibiotics (including fluoroquinolones) has made many currently available antibiotics ineffective and is already posing important public health problems (10, 25). Thus, there is a renewed interest in understanding pathogenic mechanisms in an attempt to identify targets that may be amenable to therapeutic intervention.

The mechanisms by which *S. aureus* initializes colonization at host tissues are not completely understood. However, one can reasonably assume that the bacteria must adhere to surfaces (e.g., the catheter and its corresponding wound) before invasion can occur. Studies from our laboratory have established that fibrinogen can act as a bridging molecule in the adherence of *S. aureus* to catheters (6) and endothelial cells (9). In addition, inflammatory mediators such as tumor necrosis factor alpha can significantly promote adherence of *S. aureus* to cultured human endothelium in vitro (8). These studies indicated that the fibrinogen binding receptor on *S. aureus* is a significant adherence factor to both catheters and endothelium under both normal and stimulated conditions.

The biochemical isolation of the fibrinogen binding receptor of *S. aureus* has been reported in several studies (2, 13, 34). Although these investigations agree on the proteinaceous nature of the receptor, the molecular sizes of the putative proteins differ widely among studies. Using a genetic approach, two research groups have recently cloned and sequenced two different fibrinogen-binding protein genes that do not appear to share sequence similarity with each other (3, 24). In this study, we report the identification of yet another gene from an *S. aureus* genomic library that also encoded a fibrinogen-reactive component. Expression studies of one of the clones in an *Escherichia coli* host revealed a 34-kDa fibrinogen binding fragment which was partially purified on a fibrinogen column. Detailed DNA and amino acid sequence analysis of the complete molecule revealed an interesting molecular architecture that differs from other Gram-positive surface proteins. Within this protein is a unique stretch of 11 amino acids containing a motif that shares homology with a cell wall anchor motif found in other gram-positive surface proteins.

MATERIALS AND METHODS

Bacteria, plasmids, and phage. Strain DB which had been phenotypically characterized (7) was used in the construction of a genomic library. *E. coli* Sure (Stratagene, La Jolla, Calif.) was the host cell for the λZap vector. Clones 14 and 36 were phagemids derived from fibrinogen-reactive plaques containing the insert. A pBluescript phagemid, pAC8, an *S. aureus* protein A clone, was derived from a λZap genomic library of DB.

Media and antibiotics. Unless otherwise indicated, NZY broth and LB (29) were used for the growth of *E. coli* strains.

Preparation of affinity-purified goat antifibrinogen conjugate. Goat anti-human fibrinogen antibody (Cappel, West Chester, Pa.) was affinity purified on a fibrinogen column as previously described (9). The fibrinogen column was prepared with glutardialdehyde-activated beads (Boehringer Mannheim, Indianapolis, Ind.) as described in the manufacturer's insert (6). The monospecificity of the affinity-purified goat anti-human fibrinogen immunoglobulin G was verified

* Corresponding author. Mailing address: Laboratory of Bacterial Pathogenesis, The Rockefeller University, 1230 York Ave., New York, NY 10021. Phone: (212) 327-8163. Fax: (212) 327-7584. Electronic mail address: cheunga@rockvax.rockefeller.edu.

† Present address: American Cyanamid, Lederle Laboratories, Pearl River, NY 10965.

by an immunoblot using purified fibrinogen and plasma as antigens (9). The protein concentration of the affinity-purified antibody was determined by the bicinchoninic acid protein reagent assay (Pierce Chemicals, Rockford, Ill.). Affinity-purified goat antifibrinogen antibody was conjugated to bovine intestinal alkaline phosphatase (Sigma, St. Louis, Mo.) as described by Voller et al. (35).

Construction and screening of the staphylococcal genomic library. A genomic library of strain DB was constructed with the λZap vector that had been digested with *Eco*RI and dephosphorylated (Stratagene cloning kit). DB chromosomal DNA was extracted from lysostaphin-lysed cells as previously described (7, 32). Genomic DNA was sheared with a 26-gauge syringe and subjected to gel filtration on Sepharose CL2B to remove fragments smaller than 1 kb. Fractions containing 4- to 5-kb fragments were pooled, treated with T4 polymerase to produce blunt ends, methylated with *Eco*RI methylase (New England Biolabs, Boston, Mass.), inserted into the *Eco*RI site of the λZap vector with *Eco*RI linkers, and packaged in vitro with Gigapack packing extracts (Stratagene). Over 90% of the recombinant phages produced white plaques when plated on *lac* mutant host strain Sure in the presence of isopropyl-β-D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-inolyl-β-D-galactopyranoside (X-Gal).

For the screening of fibrinogen-reactive plaques, recombinant phage was incubated with the *E. coli* host on NZY agar (29) at 42°C for 3.5 h to allow plaque formation and then induced with IPTG at 37°C for gene expression. Following transfer to duplicate nitrocellulose filters (82 mm in diameter; Schleicher & Schuell, Keene, N.H.), the filters were blocked with 10 ml of TNT (10 mM Tris with 0.15 M NaCl and 0.05% Tween 20) containing 1% bovine serum albumin (BSA) for 1 h at room temperature (RT) and then incubated at 37°C for 3 h with ≈25 μg of fibrinogen (Sigma 4883). This fibrinogen preparation had been purified over a protein A-Sepharose column to remove contaminating immunoglobulin G and was found to be essentially free of contaminants as determined by a silver-stained sodium dodecyl sulfate (SDS)-polyacrylamide gel. The filters were then washed once with TNT containing 0.1% BSA and 0.1% Nonidet P-40 and twice with TNT with 0.1% BSA for 5 min each. Affinity-purified goat antifibrinogen antibody-alkaline phosphatase conjugate diluted 1:1,000 in TNT with 1% BSA was then incubated with the filter for 1 h at RT. After the filters were washed twice with TNT containing 0.1% BSA and 0.1% Nonidet P-40 and three times with TNT with 0.1% BSA for 5 min each, fibrinogen-reactive plaques were visualized with 5-bromo-4-chloro-3-indolyl phosphate as a substrate as described by Blake et al. (1). A λZap vector with a pBR322 insert plated on *E. coli* or *E. coli* cells alone served as negative controls. Positive clones detected by fibrinogen-antifibrinogen conjugate were confirmed by allowing plaques on duplicate filters to react with [^{125}I]fibrinogen. This screening procedure was similar to the method described above except that [^{125}I]fibrinogen (100,000 cpm) was used in place of the cold fibrinogen. Following reaction, the nitrocellulose filters were washed twice with TNT containing 0.1% BSA and 0.1% Nonidet P-40 and three times with TNT containing 0.1% BSA for 5 min each and finally subjected to autoradiography. Purified plaques were isolated by rescreening each positive clone at least four times.

DNA sequencing of the fibrinogen-reactive clones. By infecting the *E. coli* host strain simultaneously with an f1 helper phage (R408) and the λZap vector containing inserts, pBluescript phagemids with inserts were generated when plated on LB-ampicillin agar (according to the Stratagene cloning kit instructions). Plasmids for sequencing were purified from *E. coli* by equilibrium centrifugation in cesium chloride-ethidium bromide gradients (29). By using both T3 and T7 primers flanking the insert, plasmid sequencing of clones 14 and 36 was performed with the Sequenase kit (U.S. Biochemicals, Cleveland, Ohio) (30). On the basis of the initial sequence data, additional primers were obtained for sequencing from within the insert.

Southern blot hybridization. Southern blot hybridization was performed with random-primed samples of gel-purified DNA fragments as probes (14, 29). Briefly, chromosomal DNA digested with restriction enzymes was resolved on 0.7% TBE gels (29) and transferred onto Hybond-N$^+$ membranes (Amersham, Arlington Heights, Ill.) (14). DNA probes were labeled with ^{32}P ([α-^{32}P]dCTP; Amersham) by using the random-primed DNA labeling kits (Boehringer Mannheim). The membrane was then hybridized with the ^{32}P-labeled DNA probe at 65°C overnight and washed twice with 2× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.7]) (24) with 0.1% SDS at RT for 10 min each and once with 1× SSPE with 0.1% SDS at 65°C for 15 min. The membrane was then subjected to autoradiography with an intensifying screen at −70°C.

Expression of the fibrinogen-reactive protein of *S. aureus* in *E. coli*. One of the fibrinogen-binding clones, clone 14, was evaluated for the location of the expressed fibrinogen-binding protein in *E. coli* by methods previously established (15). *E. coli* cells containing this clone were grown in 10 ml of LB with 50 μg of ampicillin per ml at 37°C until the optical density at 600 nm reached 1.0. Cells were collected by centrifugation at 7,000 × *g* for 10 min and resuspended in 1.25 ml of ice-cold TSE buffer (100 mM Tris, pH 8.0, containing 20% sucrose and 5 mM EDTA). Lysozyme was added to a final concentration of 0.5 mg/ml, and the sample was iced for 20 min. For whole-cell lysate, 0.25 ml of this suspension was removed and 7.5 μl of Triton X-100 and 50 μl of DNase solution (10 mM $MgCl_2$ with 100 μg of DNase per ml) were added. The sample was frozen (−70°C) and thawed twice.

Magnesium chloride was added to the remaining 1 ml of cell suspension (50 mM final concentration) to stabilize the spheroplasts, which were then pelleted at 7,000 × *g* for 15 min. The supernatant was filtered through a 0.45-μm-pore-size Millipore membrane to obtain the periplasmic fraction.

To lyse the spheroplasts, 0.25 ml of DNase solution was added to the pellet along with 0.75 ml of water. The disrupted spheroplasts were aspirated vigorously several times with a Pasteur pipette and frozen and thawed twice as described above. The lysate generated by this treatment was centrifuged at 49,000 × *g* for 1 h. The supernatant filtered through a 0.45-μm-pore-size membrane was designated the cytoplasmic fraction, while the pellet reconstituted in TSE buffer was the membrane fraction.

Coagulase test. Coagulase tests were performed with periplasmic extracts of the fibrinogen-reactive clone and coagulase plasma (Difco, Detroit, Mich.) as described in the manufacturer's insert. The periplasmic extracts were serially diluted twofold.

SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblot analysis. Cellular extracts (10 μl each) were separated on SDS–9% polyacrylamide gels by the method of Laemmli (22). Molecular weight standards from Bethesda Research Laboratories (Gaithersburg, Md.) or Pharmacia (Piscataway, N.J.) were run concurrently in adjacent wells. After electrophoresis, the gel was either stained with silver (Pierce Chemicals) or transferred onto nitrocellulose (33). After transfer, the nitrocellulose filters were allowed to react with fibrinogen followed by affinity-purified goat antifibrinogen-alkaline phosphatase conjugate and a reactive substrate as described above in the screening procedure. In some experiments, the filters were incubated with [^{125}I]fibrinogen (250,000 cpm), washed, and autoradiographed as described above.

To detect protein A in the cell extracts, chicken anti-protein A antibody (Accurate Chemicals, Westbury, N.Y.) diluted 1:3,000 in blocking buffer (10 mM Tris with 0.5 M NaCl and 0.05% Tween 20, pH 8.2) was incubated with the blot for 2 h at RT. This was followed by incubation with a rabbit anti-chicken immunoglobulin G–alkaline phosphatase conjugate (Jackson Immunoresearch, West Grove, Pa.) diluted 1:5,000 in blocking buffer for 1 h, and then the mixture was processed for band visualization as previously described (5).

Partial purification of the fibrinogen-reactive protein of *S. aureus*. In an attempt to purify the fibrinogen-reactive protein from clone 14, periplasmic extracts from 4 liters of culture were prepared as previously described (15). Briefly, cells were harvested by centrifugation (7,000 × *g* for 20 min) and resuspended in 75 ml of TSE buffer containing 37.5 mg of lysozyme. After incubation on ice for 20 min, $MgCl_2$ was added to a final concentration of 50 mM and the spheroplasts were sedimented at 7,000 × *g* for 30 min. The periplasmic fraction was aspirated from the supernatant, filtered through a 0.45-μm-pore-size membrane, and immediately added to a column (2.5 by 20 cm) containing fibrinogen immobilized on a solid support. The solid-phase fibrinogen was prepared by mixing 25 mg of fibrinogen and 5 g of glutardialdehyde beads as previously described (6). The column was then sealed and allowed to rotate at 4°C overnight. After collecting the fall through, the column was washed with 150 ml of phosphate-buffered saline (PBS) followed by 150 ml of PBS with 0.5 M NaCl. The fibrinogen-reactive protein was then eluted from the column by rotating the beads with 10 ml of 3 M potassium thiocyanate at RT for 20 min and then collected. In preliminary studies, an alternate elution procedure with 0.1 M glycine (pH 3.0) was not successful. Fractions from the column were concentrated in a Centricon 10 filter (Amicon, Danver, Mass.) and analyzed by SDS-PAGE and immunoblots with fibrinogen as described above.

Computer analysis of sequence data. DNA and protein sequence analyses and comparison with database sequences were conducted with the Sequence Analysis Software Package from the Genetics Computer Group (University of Wisconsin, Madison) (11). The deduced amino acid sequence of the putative protein (accession no. U20794) was compared by the algorithm of Pearson and Lipman (TFASTA implementation of GenBank) (26).

RESULTS

Isolation of fibrinogen-reactive clones. A λZap library of strain DB, a clinical isolate, was screened for clones that were reactive with fibrinogen. Of 100,000 plaques screened, three clones, designated 14, 30, and 36, were found to be highly reactive with both [^{125}I]fibrinogen and fibrinogen-antifibrinogen conjugate on immunoblots. Subclones containing the pBluescript phagemids together with the insert were subsequently generated in *E. coli* Sure. Plasmid DNA of clones 14, 30, and 36, prepared by the alkaline lysis method and digested with *Eco*RI, revealed DNA fragments of 4.6, 3.6, and 3.2 kb, respectively. Using the 4.6-, 3.6- and 3.2-kb fragments as separate probes, Southern blot analysis of *Eco*RI digests of these clones indicated that they hybridized to each other (data not shown). Notably, these clones did not hybridize with the *Eco*RI fragment of pAC8, a protein A probe of strain DB, thus eliminating the possibility of a false-positive reaction between an expressed protein A gene product and goat antifibrinogen an-

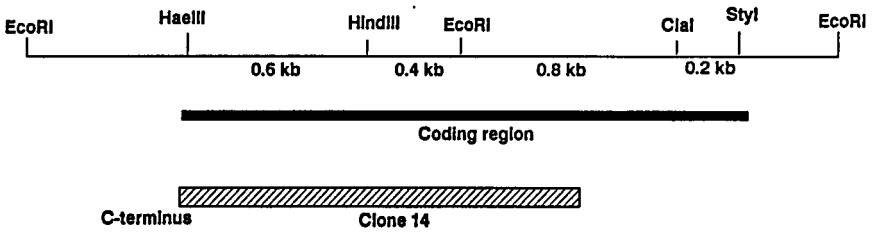

FIG. 1. Restriction map of the 3.2-kb insert of clone 36. The coding region for the entire *fbpA* gene (≈2 kb) and that of clone 14 are depicted within this insert.

tibody conjugate during the screening procedure. A restriction map of one of the clones (clone 36) which contained an intact open reading frame (see sequencing data below) is shown in Fig. 1. Further analysis indicated that clone 14 comprised approximately two-thirds of the mature molecule, including the C terminus.

Expression studies of the fibrinogen-reactive protein of *S. aureus*. On the basis of restriction analysis, clones 14 and 30 were similar. Although clone 36 was found to contain the complete gene as determined by sequence analysis (see below), expression of the fibrinogen-reactive protein with this clone was found to be difficult. However, a culture of clone 14 when grown to late stationary phase (i.e., optical density at 600 nm, >1.5) also resulted in a significantly decreased yield in fibrinogen-reactive protein. This finding can be explained either by toxicity of this protein on *E. coli* or by increased proteolytic breakdown during stationary phase. Because of this finding, we elected to evaluate the expression of the partial protein produced by clone 14 grown to early stationary phase (optical density at 600 nm, 1.0).

Western blots (immunoblots) of different fractions from cells carrying clone 14 probed with either [^{125}I]fibrinogen or fibrinogen-antifibrinogen conjugate indicated that a 34-kDa binding protein was found in the whole-cell, periplasmic, and membrane fractions (Fig. 2) but not in the cytoplasmic fraction (not shown). In contrast, a crude lysate of an *E. coli* clone containing a pBluescript phagemid with a pBR322 insert did not react with fibrinogen (Fig. 2). In all fractions, there was also a higher-molecular-weight band, possibly a dimer, which also reacted with fibrinogen. The fibrinogen-reactive band was not protein A since it did not react with affinity-purified chicken anti-protein A antibody.

To confirm the binding specificity of this protein to fibrinogen, periplasmic preparations, which contained fewer contaminating bands, were harvested from 4 liters of *E. coli* cells and applied to an affinity column containing fibrinogen-linked beads. The cloned protein of interest, as analyzed by silver stain and Western blots with [^{125}I]fibrinogen and fibrinogen-antifibrinogen conjugate, was found in precolumn fractions and the 3 M potassium thiocyanate eluant. However, it was not found in the fall through or in the acid eluant (glycine, pH 3.0) (Fig. 3). Although we did not purify the protein to homogeneity in this one-step procedure as seen in the stained gel (Fig. 3B), these results point to the binding specificity of this protein to fibrinogen. Notably, the periplasmic extract of clone 14 did not contain any coagulase activity as assayed by clotting reactions with rabbit plasma.

Sequence analysis of the fibrinogen-reactive protein. The complete sequence of the gene for the fibrinogen-binding protein, designated *fbpA*, is shown in Fig. 4. The sequence revealed an open reading frame of 1,935 nucleotides. The sequence has a GC content of 34.7%, in contrast to 30% in the staphylococcal genome (12). The higher GC content is attributable to the carboxyl-terminal half of the molecule (39.7%). Putative ribosomal binding sites and translation start are indicated in Fig. 4. The first 26 amino acids have features characteristic of a bacterial signal peptide (19). On the basis of the predicted cleavage site, the mature protein has a predicted size of 69,991 Da with a deduced pI of 6.5. Additional analysis also

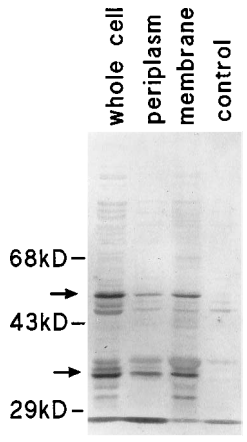

FIG. 2. Western blot of cellular fractions of clone 14 probed with fibrinogen followed by antifibrinogen antibody conjugate. The control contains crude lysate of an *E. coli* clone with a pBR322 insert in λZap. The arrows indicate a 34-kDa reactive band together with an upper band which may be a dimer. Results of a duplicate blot probed with [^{125}I]fibrinogen were similar.

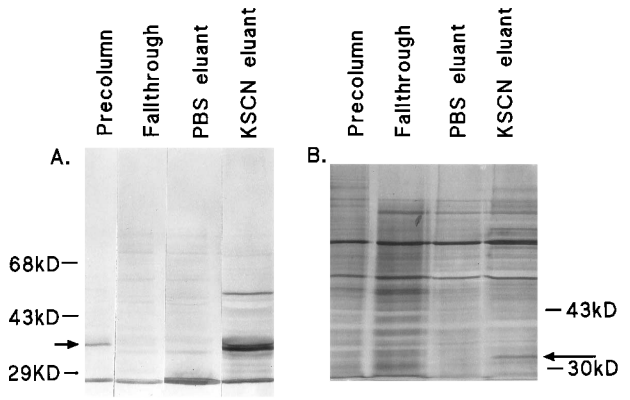

FIG. 3. Western blot (A) and silver-stained gel (B) of the periplasmic extract of clone 14 fractionated in a fibrinogen column. The Western blot was probed with fibrinogen-antifibrinogen conjugate. The short arrow indicates the fibrinogen-reactive band from the crude lysate positive control. The long arrow marks a 34-kDa protein that reacts with fibrinogen (KSCN eluant lane in panel A) and is eluted with 3 M potassium thiocyanate but not with PBS with 0.5 M NaCl nor by acid elution (not shown).

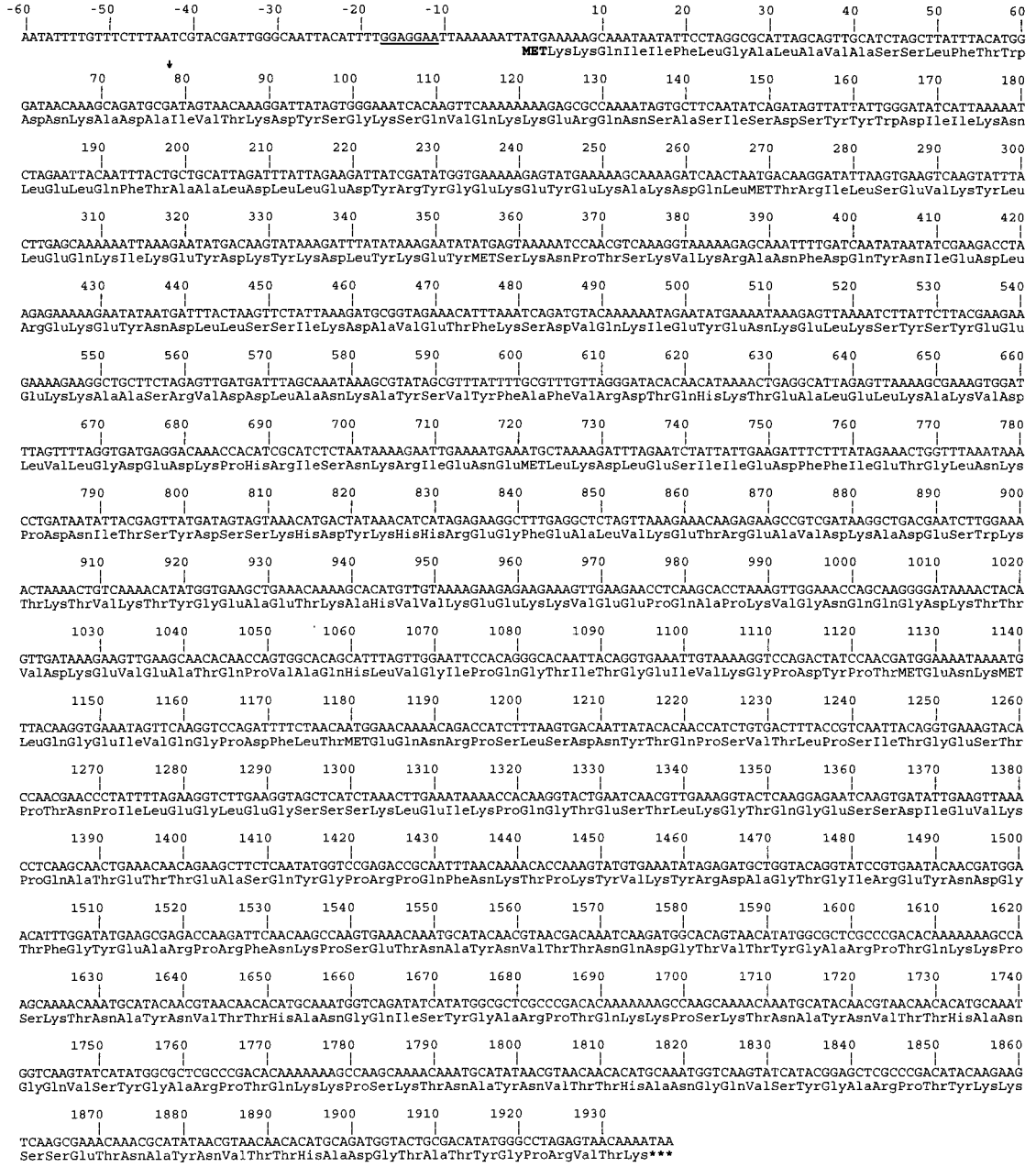

FIG. 4. Nucleotide sequence of and predicted amino acid sequence from *fbpA*. The stop codon (***) is indicated. The Shine-Dalgarno sequence (GGAGGAA) is underlined and is 10 bp upstream from the translation start (ATG). The first 26 amino acids have features characteristic of a bacterial signal peptide. On the basis of this cleavage site (↓), the predicted molecular size of the mature protein is 69,991 kDa with a deduced pI of 6.5.

revealed that clone 14 is an in-frame fusion comprising residues 229 to 645.

Analysis of the deduced amino acid sequence revealed three distinct domains in this protein. With the exception of residues 27 to 58, the N-terminal half (residues 59 to 297) of the protein is primarily α-helical as predicted by the Garnier algorithm (18) (Fig. 5). Two areas (residues 59 to 194 and 264 to 297) within the α-helical portion of the molecule reveal a 7-residue periodicity in which residues in positions a and d in the heptad motif abcdefg are either hydrophobic or nonpolar. This finding suggests that this region of the molecule is in a coiled-coil conformation (23). The second domain, between residues 326 and 505, is a proline- and glycine-rich region (20%), in which 17 residues are proline and 19 residues are glycine within this 180-amino-acid sequence. This contrasts to the N-terminal portion of the molecule, where only 3% of the residues are either proline or glycine, while the remaining carboxyl portion (residues 506 to 645) reveals a composition of 14% proline or glycine residues. The C-terminal domain (residues 506 to 645) consists of five tandem repeats of 27 amino acids each, followed by 5 terminal amino acids (PRVTK). Divergence is observed mainly in the end repeats (Fig. 5). Conformational analysis indicated that this repeat region is predominantly β-sheet.

Comparison of this protein sequence with others in the GenBank database revealed significant homology to the three pub-

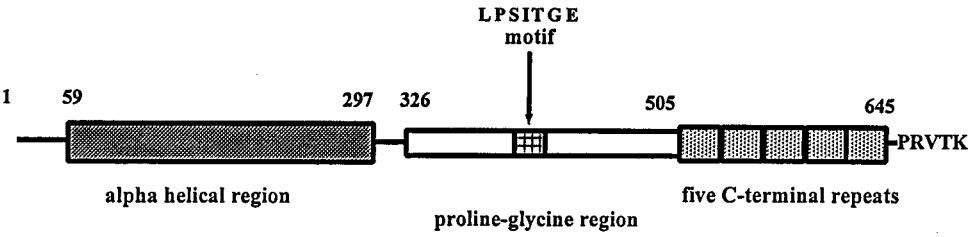

FIG. 5. A diagrammatic representation of FbpA as deduced from the predicted amino acid sequence. Within the broad proline-glycine region is an unusual sequence (LPSITGE) that shares homology with the classic cell wall anchor motif (LPXTGX) found in other gram-positive surface proteins.

lished *S. aureus* coagulase sequences from *S. aureus* 8325-4, BB, and 213 (20, 21, 27). As seen in Fig. 6, with the exception of residue 7 of the *fbpA* protein, the N-terminal 33 amino acid residues which represent the leader peptides of all four sequences are identical and therefore likely to possess identical sequence cleavage sites (Fig. 4). Comparison of the sequence of FbpA with those of the coagulases of strains 8325-4, BB, and 213 revealed 56.2, 73.2, and 56.2% identity from residues 1 to 422 and 93.9, 95.1, and 96.9% identity from residues 423 to 645, respectively. The latter region of identity corresponds to the homologous regions (residues 423 to 504) as well as five repeated units in the *fbpA* sequence. Like the *fbpA* protein, the C termini of the coagulase sequences of strains 8325-4, BB, and 213 are composed of repeating units of 27 homologous, but not identical, amino acids followed by the terminal sequence PRVTK (Fig. 6). However, the number of repeating units differs among the strains.

Although the FbpA sequence displays features that are common to the coagulase sequence, a careful comparison revealed a unique stretch of 11 amino acids between residues 409 and 419 (SVTLPSITGES) located in the center of the proline-glycine-rich region (Fig. 6). Of interest is the fact that except for the inserted isoleucine, the sequence LPSITGE shares homology with a cell wall anchor motif (LPXTGX) found in nearly all gram-positive surface proteins (16, 31). However, no complete identity with this heptad motif was found among sequences in the GenBank database.

We also compared the FbpA sequence with those of two recently sequenced fibrinogen-binding proteins from *S. aureus* (19 and 130 kDa) (3, 24). Remarkably, there is no sequence similarity between the *fbpA* protein described here and the 130-kDa clumping factor protein (24). In contrast, the 19-kDa clumping factor protein (FibA) described by Bóden and Flock (3) revealed similarity to a 42-amino-acid sequence present in our protein (Fig. 7) as well as other coagulases.

DISCUSSION

Recent studies from our laboratory have established that fibrinogen can act as a bridging molecule in the adherence of *S. aureus* to both catheters and endothelial cells (6, 8, 9). On the basis of this finding, it appears that the fibrinogen-binding protein(s) is an important adherence factor that confers on *S. aureus* the ability to adhere to fibrinogen adsorbed onto catheters and cultured endothelium. However, because *S. aureus* secretes a variety of proteases in culture (12), previous attempts to purify this protein directly from whole-cell lysates have resulted in either a low yield or proteolytic fragments (13, 34). As an alternative, we have taken a molecular approach to screen for fibrinogen-reactive clones in a λZap expression library. Several fibrinogen-reactive clones were subsequently sequenced and characterized.

Sequence analysis revealed that the *fbpA* protein reported here shares significant sequence similarity with staphylococcal coagulases. Recent studies by Bodén and Flock have also suggested the presence of at least three different forms of fibrinogen-binding proteins in strain Newman (87-, 60-, and 19-kDa proteins), two of which possess coagulase activity (2, 4). The production of two fibrinogen-binding coagulases (87- and 60-kDa proteins) is growth phase dependent. In contrast, the 19-kDa protein (designated FibA) is constitutively expressed during the entire growth cycle and does not exhibit coagulase activity. Analysis of the 19-kDa FibA protein disclosed a stretch of 41 amino acids in the N terminus (3) that shows sequence similarity to a region in the C-terminal segment of our protein as well as that of coagulases (Fig. 7). This raises an intriguing possibility of a common fibrinogen-binding motif among this group of proteins. However, the validity of this hypothesis will require additional experimental confirmation.

Expression studies of clone 14 which encompassed residues 229 to 645 of the mature molecule indicated that it lacked coagulase activity but bound to free fibrinogen as well as to fibrinogen immobilized on a column. These data support the notion that the amino-terminal third of the molecule is not required for fibrinogen binding. However, the N-terminal sequence of the classically described coagulase molecule has been shown to be the region that complexes with prothrombin to convert fibrinogen to fibrin (12, 28). Whether this requirement for coagulase activity is also true for FbpA will require confirmation with the intact protein. In hybridization studies of strain DB chromosomal digests cut with several unique enzymes and then probed with an intact gene from clone 36, we were not able to demonstrate an additional coagulase gene in this strain (unpublished observations). On the basis of these findings, it appears that FbpA belongs to a family of coagulase-like proteins. Nevertheless, a unique 11-amino-acid sequence (residues 409 to 419) that shares homology with a cell wall anchor motif (LPXTGX) in a variety of gram-positive surface proteins is present in FbpA but not in any coagulase sequences described. This may have implications for how some coagulase-like proteins with fibrinogen-binding properties are anchored to the staphylococcal cell wall (16, 31).

In comparison with previously isolated fibrinogen-binding proteins, cysteine residues are absent in FbpA as well as the 19- and 130-kDa fibrinogen-binding proteins (3, 24) but not the 60-kDa protein biochemically isolated by Usui (34). The isoelectric points also differ widely among the reported fibrinogen-binding proteins. The 130-kDa protein has a predicted pI of 3.22 which probably results from a 308-residue region consisting of dipeptide repeats of mostly aspartic acid and serine near the C terminus (24). In contrast, the mature FbpA has a deduced pI of 6.5 while the 19- and 60-kDa proteins each have a deduced pI of ≈10 (3, 34). Thus, it seems likely that the FbpA protein differs from the previously described fibrinogen-binding proteins.

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Strains
          1                                                    50
DB        MKKQIIfLGA LAVASSLFTW DNKADAIVTK DYSgkSqVqk KerqNsasIS
8325-4    MKKQIISLGA LAVASSLFTW DNKADAIVTK DYSgkSqVNa gSK.NGtLId
213       MKKQIISLGA LAVASSLFTW DNKADAIVTK DYSkeSrVNE KSK.kGatVS
BB        MKKQIISLGA LAVASSLFTW DNKADAIVTK DYSkeSrVNE nSK.yGtLIS

          51                                                   100
DB        DsYyWdIikn LElQFtaAlD LLEdYrYGEK eYekAKDqLM TRILsEvkYL
8325-4    sRYlnsalyy LEdyiiyAig LtnkYeYGDn iYKEAKDRLL ekVLrEDQYL
213       DYYyWkIidS LEaQFtgAiD LLEnYkYGDp iYKEAKDRLM TRVLGEDQYL
BB        DWYlkgrltS LEsQFinAlD iLEtYhYGEK eYKDAKDkLM TRILGEDQYL

          101                                                  150
DB        LEqKikeYDk YKdlYKeYms kNPTSK.vKr AnFdqYNiED LreKEYNDLL
8325-4    LERKksqYED YKqwYanYKK ENP.rtdLKM AnFhkYNlEE LsMKEYNELq
213       LkkKideYEl YKKwYKssnK nt......nM ltFhkYNlyn LTMnEYNDiF
BB        LERKkvqYEE YKKlYqkYKe ENPTSKgLKL ktFdqYtiED LTMrEYNELt

          151                                                  200
DB        sSiKDAVEtF ksDVqkIEye NkELKsYsyE EEkKAasrVd DLankaysvy
8325-4    DaLKrAlDDF hrEVKdIkdK NsDLKtFnaa EEDKATkeVy DLvseidtlv
213       nSLKDAVyqF nKEVKeIEhK NvDLKqFdkD gEDKATkeVy DLvseidtlv
BB        ESLKsAVkDF eKDVekIEnq hhDLKpFtdE mEEKATsrVd DLankaysvy

          201                                                  250
DB        fAFvRDtqhk teAlELkAKv DLVLGDeDkP HrIsNkRIen EMlkDLeSII
8325-4    vsYygDkdyg ehAkELrAKl DLILGDtDnP HkItNERIkK EMIdDLnSII
213       vtYyaDkdyg ehAkELrAKl DLILGDtDnP HkItNERIkK EMIdDLnSII
BB        fAFvRDtqhk teAlELkAKv DLVLGDeDkP HrIsNERIeK EMIkDLeSII

          251                                                  300
DB        EDFFiETglN kPdnITsYDs sKHdYKhHrE ...gFEaLVk ETreAVdkAD
8325-4    DDFFmETkqN rPksITkYnp ttHNYKtnSD NKPnFDkLVe ETkkAVKEAD
213       DDFFmETkqN rPnsITkYDp tKHNFKekSE NKPnFDkLVe ETkkAVKEAD
BB        EDFFiETglN kPgnITsYDs sKHhYKnHSE ...gFEaLVk ETreAVanAD

          301                                                  350
DB        ESWKTKTVKt YGEaETKahV VKEEKKVEEP QAPKVgNQQg dKTTvdKevE
8325-4    DSWKkKTVKK YGETETKSPV VKEEKKVEEP QAPKVdNQQE VKTTAGKAEE
213       ESWKnKTVKK YeETvTKSPV VKEEKKVEEP QlPKVgNQQE VKTTAGKAEE
BB        ESWKTKTVKK YGEsETKSPV VKEEnKVEDP QsPKfdNQQE VKTTAGKAEE

          351                                                  400
DB        aTQPVAQhLV gIPQGTITGE IVKGPDYPTM ENKmLQGEIV QGPDFLTMEQ
8325-4    TTQPVAQPLV KIPQGTITGE IVKGPEYPTM ENKTvQGEIV QGPDFLTMEQ
213       TTQPVAQPLV KIPQeTIyGE tVKGPEYPTM ENKTLQGEIV QGPDFLTMEQ
BB        TTQPVAQPLV KIPQGTITGE IVKGPEYPTM ENKTLQGEIV QGPDFpTMEQ
                     ↓          ↓
          401        ___________                               450
DB        nrPSLSDNYT QPsvtlpsit gesTptNPIL EGLEGSSSKL EIKPQGTEST
8325-4    sgPSLSnNYT nP..... ...pltNPIL EGLEGSSSKL EIKPQGTEST
213       nrPSLSDNYT QP..... ...TTpNPIL EGLEGSSSKL EIKPQGTEST
BB        sgPSLSDNYT QP..... ...TTpNPIL EGLEGSSSKL EIKPQGTEST

          451                                                  500
DB        LKGtQGESSD IEVKPQATET TEASQYGPRP QFNKTPKYVK YRDAGTGIRE
8325-4    LKGtQGESSD IEVKPQATET TEASQYGPRP QFNKTPKYVK YRDAGTGIRE
213       LKGiQGESSD IEVKPQATET TEASQYGPRP QFNKTPKYVK YRDAGTGIRE
BB        LKGiQGESSD IEVKPQATET TEASQYGPRP QFNKTPKYVK YRDAGTGIRE

          501                                                  550
DB        YNDGTFGYEA RPRFNKPSET NAYNVTTNQD GTVtYGARPT Q.........
8325-4    YNDGTFGYEA RPRFNKPSET NAYNVTThan GqVSYGARPT ykKPSETNAY
213       YNDGTFGYEA RPRFNKPSET NAYNVTTNQD GTVSYGARPT QNKPSETNAY
BB        YNDGTFGYEA RPRFNKPSET NAYNVTTNQD GTVSYGARPT QNKaSETNAY

          551                                                  600
DB        .......... .......... .......... .......... ..........
8325-4    NVTTHANGQV SYGARPTQ.. .......... .......... ..........
213       NVTTHANGQV SYGARPTQ.. .......... .......... ..........
BB        NVTTHANGQV SYGARPTQkk psetnaynvt thangqvsyg arptynkpse

          601                                                  650
DB        .......... .......... ..kKPSKTNA YNVTTHANGQ ISYGARPTQk
8325-4    .......... .......... ..nKPSKTNA YNVTTHgNGQ VSYGARqaQn
213       .......... .......... ..nKPSKTNA YNVTTHANGQ VSYGARPTQk
BB        tnaynvtthg ngqvsygarp tykKPSKTNA YNVTTHANGQ VSYGARPTQn

          651                                                  700
DB        KPSKTNAYNV TTHANGQVSY GARPTqKKPS kTNAYNVTTH ANGQVSYGAR
8325-4    KPSKTNAYNV TTHANGQVSY GARPTy.... .......... ..........
213       KPSKTNAYNV TTHANGQVSY GARPTyKKPS ETNAYNVTTH ANGQVSYGAR
BB        KPSeTNAYNV TTHANGQVSY GARPTqnKPS ETNAYNVTTH gNGQVSYGAR

          701                              730
DB        PTYKKsSeTN AYNVTTHADG TATYGPRVTK
8325-4    ...KKPSkTN AYNVTTHADG TATYGPRVTK
213       PTqKKPSeTN AYNVTTHADG TATYGPRVTK
BB        PTYnKPSkTN AYNVTTHADG TATYGPRVTK
```

FIG. 6. Sequence comparison of FbpA (strain DB) and coagulases from strains 8325-4, 213, and BB with the Pileup program under the Genetics Computer Group package. The arrows indicate the 11-amino-acid sequence that is unique to our protein. This sequence shares homology with a cell wall anchor motif found in other gram-positive cell wall proteins. However, there is no complete identity to this motif in the GenBank. Lowercase letters indicate amino acids lacking consensus; dots indicate gaps introduced to maximize alignment.

Previous studies have revealed that the fibrinogen-binding component of *S. aureus* is a cell wall constituent because it is absent in staphylococcal L forms (12). As a surface protein(s), it is also susceptible to proteolytic digestion (6). In reviewing

```
FibA      YQSRPKFNSTPKYIKFKHDYN-ILEFNDGTFEYGARPQFNKP  -72
          :  :: :: :::: :       : : ::::: : ::: ::::
FbpA      YGPRPQFNKTPKYVKYRDAGTGIREYNDGTFGYEARPRFNKP  -513
```

FIG. 7. Alignment of FibA (19 kDa) with FbpA. Colons indicate identical amino acids. The numbers indicate the position of the last amino acids displayed.

the molecular architecture of the C-terminal region of other gram-positive surface-anchored proteins, it is evident that they contain several conserved features (16, 17). At the C termini of these proteins, a charged tail (4 to 7 amino acids) is usually preceded by a highly hydrophobic membrane-spanning region (≈16 to 20 amino acids) followed by the hexamer LPXTGX, a proline-glycine-rich domain, and a repeat region (17). The C-terminal region of FbpA differs from that model (Fig. 5). In particular, the region preceding the stop codon lacks a charged tail and a hydrophobic membrane-spanning region. Instead, the five C-terminal amino acids (PRVTK) are preceded by five repeats of 27 amino acids each. In addition, the region N terminal to these repeats is composed of an extended proline-glycine region (residues 326 to 505), in the center of which is a unique sequence (LPSITGE) that shares homology with the cell wall anchor motif (LPXTGX) found in other gram-positive surface proteins.

Except for the LPXTGX motif, the C-terminal molecular architecture of FbpA is similar to that described for pneumococcal surface protein A (PspA) (38). The amino-terminal half of PspA, like that of FbpA, is α-helical and is consistent with an α-helical coiled-coil conformation (Fig. 5). This α-helical region of PspA is followed by a proline-rich domain and a repeat domain consisting of 10 20-amino-acid repeats. The C terminus of PspA, in analogy to FbpA, also lacks a membrane-spanning region and a charged tail. A potential mechanism by which PspA anchors to the bacterial cell surface was recently described (39). This novel anchoring mechanism involves choline-mediated interactions between the membrane-associated lipoteichoic acid and the C-terminal repeat region of PspA. As a consequence, the release of PspA from the cell surface can be effected by deletion of 5 of the 10 C-terminal repeat units (39). Whether similar anchoring mechanism exists for FbpA is not currently known.

In summary, we have cloned and sequenced a fibrinogen-reactive protein of *S. aureus*. In comparison with other gram-positive surface proteins, this protein has a distinct molecular architecture at the C terminus. Sequence analysis reveals a unique stretch of 11 amino acids near the C terminus that shares homology with a cell wall anchor motif found in a variety of gram-positive surface proteins. Whether this motif (LPSITGE) plays a role in anchoring proteins to the gram-positive cell surface remains to be determined.

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