Interactions between staphylococci and components of the extracellular matrix mediate attachment of the bacteria to host tissues and organs and define an important mechanism leading to colonization, invasion, and formation of metastatic abscesses. We have previously demonstrated a specific binding interaction between Staphylococcus aureus and elastin, one of the major protein components of the extracellular matrix. Available evidence suggests that this association is mediated by a 25-kDa elastin-binding protein on the surface of S. aureus (EbpS). To study the molecular structure and function of EbpS, the gene encoding EbpS was cloned, sequenced, and expressed in Escherichia coli. DNA sequence data indicate that the ebpS open reading frame consists of 606 base pairs and encodes a novel polypeptide with a predicted molecular mass of 23,345 daltons and pI of 4.9. A polyclonal antibody raised against recombinant EbpS interacted with the native 25-kDa cell surface EbpS and inhibited staphylococcal elastin binding. Furthermore, recombinant EbpS bound specifically to immobilized elastin and inhibited binding of S. aureus to elastin. A degradation product of recombinant EbpS lacking the first 59 amino acids of the molecule and a C-terminal fragment of CNBr-cleaved recombinant EbpS, however, did not interact with elastin. Together, these results confirm that EbpS is the cell surface molecule mediating binding of S. aureus to elastin. The inability of truncated forms of recombinant EbpS to bind to elastin suggests that the elastin binding site in EbpS is contained in the first 59 amino acids of the molecule.

The extracellular matrix (ECM) is a ubiquitous structure that contributes to the architecture, elasticity, and rigidity of virtually all vertebrate tissues and organs. Within the last several decades, additional biological activities of the ECM have been identified. Distinct components of the ECM have been found to mediate one or several cellular events such as adhesion, proliferation, and regulation of gene expression (1-4). These cell-ECM interactions, in turn, direct many physiological and pathological processes including development, wound healing, and tumor cell metastasis (5-7). It is now known that cell surface ECM receptors are key mediators of these biological events. Many ECM receptors belong to a family of dimeric receptor complexes called integrins (8, 9), although nonintegrin ECM receptors have been identified (10). In addition to eukaryotic cells, various pathogenic bacteria also interact specifically with the host ECM through cell surface ECM-binding molecules. ECM-binding molecules of pathogenic bacteria belong to a group of proteins known collectively as adhesins or microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and are widely believed to play important roles in key steps of disease pathogenesis (11, 12).

Among the many important pathogenic bacteria, few are as efficient in developing multiple resistance to antibiotics and causing a wide spectrum of diseases as Staphylococcus aureus. S. aureus is one of the causative agents of diseases such as infective endocarditis, osteomyelitis, aortitis, pneumonia, and scalded skin syndrome (13-15). Furthermore, several strains of S. aureus tend to extravasate into the circulation to cause bacteremia and subsequent formation of metastatic abscesses. These properties imply that S. aureus is capable of interacting with host tissue components. Consistent with this notion is the finding that S. aureus binds specifically to major ECM components such as collagen (16), fibronectin (17), laminin (18), proteoglycans (19), and elastin (20). Importantly, there is increasing evidence supporting a relationship between the ability of S. aureus to bind to ECM and its pathogenicity. For example, most clinical isolates of S. aureus demonstrate binding to fibronectin, and mutant strains defective in fibronectin binding have a decreased ability to colonize damaged heart valves in animal models of endocarditis (21). S. aureus binding to collagen has been implicated in osteomyelitis and septic arthritis (22), in which expression of the collagen adhesin has been found to be both necessary and sufficient for bacterial attachment to the type II collagen-rich cartilage. It has also been demonstrated in a murine experimental arthritis model that greater than 70% of animals injected with collagen adhesin-positive strains developed septic arthritis, whereas less than 25% of animals challenged with isogenic mutant strains.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U48826.

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*This work was supported by National Institutes of Health Grants HL 26499 and HL 41926 (to R. P. M.) and AR 20553 (to J. R.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: ECM, extracellular matrix; EbpS, elastin-binding protein of S. aureus, rEbpS, recombinant EbpS; MSCRAMM, microbial surface component recognizing adhesive matrix molecules; Ni2+-NTA, nickel nitritoacetic acid; TSB, tryptic soy broth; PCR, polymerase chain reaction; kb, kilobase(s); PAGE, polyacrylamide gel electrophoresis.
lacking the collagen adhesin developed clinical symptoms of the disease (23). In addition, invasive S. aureus interacts with the basement membrane component laminin, whereas noninvasive Staphylococcus epidermidis shows no binding (18). Taken together, these observations indicate that S. aureus-ECM interactions are playing critical roles in targeting host tissues for attachment, colonization, and invasion.

Elastin is an important structural protein whose primary physiological role is to confer the property of reversible elasticity to tissues and organs (24). Elastin expression is highest in the lung, skin, and blood vessels, but elastin is widely expressed at lower levels in most mammalian tissues. In a previous study we showed that S. aureus binds to elastin with properties of reversibility, saturation, and specificity that suggested the presence of an elastin-binding protein on the bacterial surface (20). Using affinity chromatography techniques we were able to isolate a 25-kDa cell surface elastin-binding protein (named EbpS for elastin-binding protein of S. aureus) that has been proposed to facilitate binding of the bacteria to elastin-rich host ECM. EbpS is structurally distinct from the mammalian cell surface elastin-binding protein and exhibits different binding specificity in terms of sequence recognition. Whereas the mammalian elastin-binding protein recognizes the hexapeptide sequence YXVAY located in the C-terminal half of elastin (25), EbpS binds to a region in the N-terminal one-third of the molecule. In this study, we report the cloning, sequencing, and expression of ebpS. Our results confirm that EbpS is the cell surface molecule mediating binding of S. aureus cells to elastin and that EbpS is a novel protein. Characterization of elastin binding activity by various constructs of EbpS suggests that the N-terminal 59 amino acids of the molecule play an important role in elastin recognition.

**EXPERIMENTAL PROCEDURES**

**Materials—** Restriction endonucleases, calf intestinal alkaline phosphatase, T4 DNA ligase, T4 polynucleotide kinase, isopropyl-β-D-thiogalactopyranoside, 5-bromo-4-chloro-3-indolyl-β-D-galactoside, Wizard MiniPrep plasmid purification kits, and HindIII-digested λ DNA markers were purchased from Promega (Madison, WI). DNA-free RNAse was obtained from Boehringer Mannheim. Luria-Bertani (LB) medium and LB agar medium capsules were from BIO 101 (La Jolla, CA). Tryptic soy broth (TSB) was obtained from Remel (Lenexa, KS). Na125I, [γ-32P]ATP, and [α-32P]CTP were from ICN (Costa Mesa, CA); Papain and preparations of human thyroid peroxidase were purchased from Pierce. Rapid-hyb buffer and Rediprime DNA labeling system were obtained from Amersham Corp. Chroma Spin-10 columns were purchased from Clontech (Palo Alto, CA). QIAexpress vector kit type IV and the Midi-Prep plasmid purification kit were obtained from Qiagen. Taq polymerase cycle was provided by FMC Products. Competent DH5α cells were transformed with the ligated material, and different dilutions were plated out on LB agar medium plates supplemented with chloramphenicol (20 μg/ml), isopropyl-β-D-thiogalactopyranoside (0.5 mm); and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (40 μg/ml) for antibiotic and bluewhite selections. White colonies were collected and propagated overnight, and the Wizard plasmid Mini-prep was used to isolate plasmid DNA from cells. Purified plasmids were digested with EcoRI and screened by Southern blotting using the radiolabeled oligonucleotide probe.

The cloned 4.2-kb fragment was digested with HindIII and Hind1, yielding a 2.6-kb fragment, which was subcloned into pBluescript KS+ and pUC19. The 2.6-kb fragment was also used as a probe in Southern analyses with S. aureus genomic DNA. The insert was digested using the EcoRI/mung bean nuclease system (Stratagene, La Jolla, CA) to generate two sets of nested deletions. Multiple clones covering both strands in their entirety were sequenced by the Sanger dyeoxyxynucleotide chain termination method as modified for Taq polymerase cycle sequencing using an ABI 373A automated DNA sequencer. Sequence data were assembled and discrepancies resolved using the Wisconsin Package (Genetics Computer Group, Madison, WI). The primary sequence of ebpS as shown in Fig. 2 has been assigned the GenBank accession number U48260. The expression of ebpS in E. coli and CNBr Cleavage of Recombinant EbpS—A 2.6-kb HindII/Hind1 fragment in pBluescript KS+ (30 ng) served as the template, and PCR reactions were performed with a Perkin-Elmer thermocycler using standard reagents. The open reading frame of ebpS was PCR-amplified using the sense oligonucleotide, 5'-G5TGGGATCCATAGAAAGGAAG-3', and the antisense oligonucleotide, 5'-G5CAAAGCTTGCCTGATACGCCACAAATT-3'. The sense oligonucleotide contained a BamHI site (underlined), and A of the two ATG codons was changed to G (in boldface lettering) to avoid internal initiation of translation as recommended by Qiagen. The antisense oligonucleotide contained a HindIII cleavage site (underlined). The exact conditions for amplification were 94°C for 1 min, followed by 30 cycles at 94°C for 30 s, 70°C for 30 s, and 72°C for 30 s. The PCR product was digested with BamHI and HindII and gel-purified. This material was ligated to pQE-30 that had been digested with BamHI and HindII and treated with calf intestinal alkaline phosphatase. Competent M15 cells were transformed with the ligation product and selected by ampicillin (100 μg/ml) and kanamycin (20 μg/ml), and antibiotic-sensitive colonies were screened for recombinant protein expression. After obtaining several positive clones, ideal conditions for maximum expression were examined.

Based on results from these studies, the following protocol was used routinely for medium scale purification of recombinant EbpS (rEbpS). A stock culture of the clone was grown overnight in 10 ml of LB medium supplemented with ampicillin and kanamycin. On the following day, this culture was added to 100 ml of fresh LB media with antibiotics. Cells were allowed to regrow until the A600 value reached 0.8 (~3 h). Expression was then induced with 1 mM isopropyl-β-D-thiogalactopy-

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15804 Gene for S. aureus Elastin-binding Protein
anaseide for 4 h at 37 °C. The cells were pelleted by centrifugation (5000 × g), resuspended in 15 ml of buffer A (8 mM urea, 100 mM NaH2PO4, 10 mM Tris-HCl, pH 8), and vortexed gently for 15 min. The lysed cells were centrifuged at 15,000 × g for 20 min at 4 °C, and the supernatant was transferred to a tube containing 4 ml of nickel nitriatoc acid resin (Ni2+-NTA) pre-equilibrated with buffer A. The mixture was incubated for 30 min at room temperature with gentle agitation, transferred to a disposable polypropylene column, and washed consecutively with 100 ml of buffer A and 100 ml of buffer B (same as buffer A except pH 6). The tightly bound recombinant protein was eluted with 10 ml of buffer C (same as buffer A, but pH 4). The eluted material was dialyzed twice against 4 liters of 10 mM Tris-HCl, pH 7.5, and the concentration of protein in the dialysate was determined by UV spectrophotometry based on the number of Tyr and Trp residues in rEbpS (1 × A280 = 2.68 mg/ml). The yield of purified rEbpS under these conditions was approximately 5 mg/100 ml of induced culture.

To generate CNBr-cleaved fragments, 500 μg of rEbpS was incubated in the dark for 24 h at room temperature with 1 mg of CNBr in 200 μl of 70% formic acid. At the end of incubation, the sample was diluted with 14 ml of deionized H2O and Speed-vac dried. The dried material was resuspended in 10 ml of deionized H2O and redried in 100-μg aliquots.

Generation of Rabbit Anti-rEbpS Polyclonal Antibodies—Preimmune sera were collected, and New England White rabbits were injected with 1 ml of purified rEbpS (20 μg) mixed 1:1 with complete Freund’s adjuvant. Booster injections (20 μg) mixed 1:1 with incomplete Freund’s adjuvant were given at 5, 7, 10, 14, and 19 weeks. Sera were tested by Western immunoblotting using rEbpS.

IgG fractions were purified from immune and preimmune sera by either caprylic acid precipitation (27) or protein A affinity chromatography. For generation of an antibody affinity resin, approximately 100 mg of anti-rEbpS IgG were covalently coupled to 5 ml of Affi-Gel 10 according to the manufacturer’s instructions. To generate anti-rEbpS Fab fragments, 50 mg of lyophilized IgG was reacted overnight at 37 °C with 2 ml of immobilized papain in 5 ml of papain digestion buffer (20 mM NaH2PO4, 20 mM cysteine-HCl, 10 mM EDTA, pH 6.5). Fab fragments were separated from undigested IgGs and free Fc fragments by protein A affinity chromatography.

Binding of Radiolabeled rEbpS Constructs to Immobilized Elastin Peptides—Preparation and coupling of elastin peptides to Affi-Gel 10 were as described previously (20). Both rEbpS (20 μg) and CNBr-cleaved rEbpS (80 μg) were iodinated with 300 μCi of Na125I by the IO-DEN GEN method. The specific activities were approximately 2.3 × 106 and 1.2 × 106 cpm/μg for rEbpS and CNBr-cleaved rEbpS, respectively. Radiolabeled rEbpS (45 ng) in 1.5 ml of binding buffer (50 mM Tris, 500 mM NaCl, 2 mM CaCl2, 0.1 mg/ml bovine serum albumin) was preincubated with 1 ml of the elastin peptide affinity resin for 2 h at room temperature in the absence or presence of 2 ng of unlabeled elastin peptides. The mixture was transferred to disposable polypropylene columns and washed with binding buffer by gravity flow until radioactivity of the flow-through reached background. Bound rEbpS was eluted with 3 ml of 1% SDS buffer, spin-concentrated, and analyzed by 10% SDS-PAGE and autoradiography. Binding of radiolabeled CNBr-cleaved rEbpS to immobilized elastin was assessed similarly, except 80 ng of the starting material was used, and bound material was visualized by 12% SDS-PAGE and autoradiography.

Detection of the Native 25-kDa Cell Surface-labeled EbpS with Anti- rEbpS Antibodies—Surface-labeled extracts from S. aureus cells were prepared by lysozyme digestion as described previously (20). Approximately 107 cpm of surface-labeled extract was first absorbed with 3 ml of packed Affi-Gel 10 resin for 2 h at room temperature. The unbound supernatant was collected and incubated with 1 ml of the anti-rEbpS IgG affinity resin in the absence or presence of 2 mg of unlabeled rEbpS for 2 h at room temperature in 2 ml of binding buffer. The mixtures were transferred to disposable columns and washed with binding buffer until flow-through reached background radioactive levels. Bound cell surface-labeled molecules were eluted from the column by 3 ml of 1% SDS buffer, spin-concentrated, and analyzed by 15% SDS-PAGE and autoradiography.

Other Procedures—Purification and radiolabeling of full-length recombinant human elastin and cellular elastin binding assays were performed as described previously (20). Automated amino acid sequence and composition analyses were carried out in our laboratory with the Applied Biosystems 473A protein sequencer and the Bedmass Protein Mass Spectrometer (PerSeptive Biosystems). Gene analysis was done using the Applied Biosystems 473A protein sequencer and the Bedmass Protein Mass Spectrometer. Sequence and composition analyses were carried out in our laboratory with the Applied Biosystems 473A protein sequencer and the Bedmass Protein Mass Spectrometer.
The mature protein has a predicted molecular mass of 23,344.7 daltons and an acidic pI of 4.9. Accordingly, the protein has a preponderance of acidic amino acids Asp (10.9%) and Glu (11.9%) and is devoid of Cys residues. Garnier analysis predicted a secondary structure that is 58.4% α-helical and 23.8% coiled-coil. The BLAST network service of the National Institutes of Health was used to search for sequence homologies. The December 1, 1995 releases of the Brookhaven Protein Data Bank, GenBank™, EMBL Data Library, and SWISS-PROT protein sequence data base and the translated coding sequence of GenBank™ were used for comparison. No significant homologies were found between reported sequences in these data bases and the primary sequence of ebpS.

Expression of ebpS in E. coli—We studied whether the cloned gene encodes an elastin-binding protein by expressing ebpS in E. coli. The PCR-amplified ebpS open reading frame was expressed in E. coli as a fusion protein containing six His residues attached to the N terminus. rEbpS purified from three different positive clones by Ni²⁺-NTA affinity chromatography was fractionated by 10% SDS-PAGE and stained with Coomassie Brilliant Blue R-250 (lanes B–D). The migration pattern of the size standard is shown in lane A.

To evaluate the integrity of rEbpS, the N-terminal sequence of full-length rEbpS, as well as internal sequences from a degradation product and two fragments generated by CNBr cleavage, were determined by protein microsequencing. Altogether, unambiguous sequences were obtained for 58 residues, and they matched perfectly with the predicted sequences (Fig. 2, underlined sequences). Furthermore, amino acid and mass spectrometry analyses indicated that the composition of rEbpS and actual molecular mass of rEbpS agree with the predicted data. These results indicate that the correct protein has been expressed and that overestimation of the molecular mass is due to aberrant migration in SDS-PAGE.

Elastin Binding Activities of rEbpS Constructs—To investigate whether rEbpS interacts specifically with elastin, elastin peptide affinity chromatography was performed with radiolabeled rEbpS. Labeled rEbpS was incubated with the elastin peptide affinity resin for 2 h at room temperature in the absence or presence of excess unlabeled elastin peptides. The mixture was then washed extensively with buffer until radioactivity in the wash reached background levels. Bound material was eluted with 1% SDS buffer and analyzed by SDS-PAGE and autoradiography. The starting material for this experiment had been stored for 1 week at 4°C after purification with Ni²⁺-NTA chromatography and, as can be seen in Fig. 4, was partially degraded (lane B). This turned out to be fortu-
tested by incubating radiolabeled elastin with *S. aureus*. Binding to radiolabeled elastin was abrogated at the highest concentration tested in a concentration-dependent manner. In contrast, Fab fragments from immune IgGs inhibited binding of recombinant *EbpS* (lane D). Peptide microsequencing was employed to verify correct cleavage and to identify which band corresponded to the N- and C-terminal fragments (Fig. 2). These results suggest that the first 59 amino acids in *EbpS* play a critical role in elastin recognition.

To determine whether the N-terminal region of *EbpS* contains the elastin binding site, elastin binding properties of CNBr-cleaved *EbpS* fragments were examined. *EbpS* contains a single internal Met residue at position 125 such that cleavage with CNBr would generate two fragments. In agreement with the predicted sequence, two dominant bands were detected in CNBr-cleaved *EbpS*. Peptide microsequencing was employed to verify correct cleavage and to identify which band corresponded to the N- and C-terminal fragments (Fig. 2, underlined). When elastin binding activity of these fragments was assayed with elastin peptide affinity chromatography, only the N-terminal fragment bound to the peptide affinity resin, supporting the conjecture that the elastin binding site is contained in the first 59 amino acids of *EbpS*.

Effects of *EbpS* on *S. aureus* Binding to Elastin—If *EbpS* is the cell surface molecule responsible for elastin binding at the cellular level, then an active form of soluble *EbpS* should interfere with *S. aureus* binding to elastin. This hypothesis was tested by incubating radiolabeled elastin with *S. aureus* cells in the absence or presence of various concentrations of unlabeled *EbpS* as can be seen in Fig. 5, *EbpS* inhibited binding of labeled elastin in a concentration-dependent manner. *S. aureus* binding to radiolabeled elastin was abrogated at the highest concentration of *EbpS* (19 μM). The control polyhistidine fusion protein mouse dihydrofolate reductase did not influence binding at 26 μM. These results demonstrate that *EbpS* inhibits cellular elastin binding and that the polyhistidine domain of *EbpS* does not affect elastin binding.

Expression of *EbpS* on the Cell Surface of *S. aureus*—The ability of *EbpS* to interact directly with elastin and to inhibit cellular elastin binding strongly suggest that *EbpS* is the cell surface protein mediating *S. aureus* binding to elastin. To provide further evidence that *EbpS* is on the bacterial surface, affinity chromatography was performed with surface-labeled *S. aureus* extracts and immobilized anti-*EbpS* IgG. *S. aureus* cells were surface-labeled by the IODO-GEN method, and extracts were prepared by lysostaphin digestion. Approximately 107 cpm of this material was exposed to a porcine IgG affinity resin to remove surface-labeled protein A, and the nonbound fraction was incubated with the anti-*EbpS* IgG affinity resin for 2 h at 25 °C. After washing extensively with binding buffer, bound cell surface molecules were eluted with 1% SDS buffer and were analyzed by SDS-PAGE and autoradiography. As shown in Fig. 6, preabsorption with the porcine IgG resin removed surface-labeled protein A from the starting material (compare 50 kDa band in lanes A and B). Of the remaining numerous surface-labeled proteins, a 35- and 25-kDa protein associated with the anti-*EbpS* IgG affinity resin (lane C). To determine the specificity of binding, the same experiment was performed in the presence of excess unlabeled *EbpS*. Binding of the surface 25-kDa protein, but not the 35-kDa protein, to immobilized anti-*EbpS* IgG was inhibited by unlabeled *EbpS* (not shown). Densitometric scanning of the bands revealed that the band intensity for the 25- and 35-kDa proteins decreased by 64 and 7%, respectively, in the presence of excess unlabeled *EbpS*. These results indicate that the 25-kDa protein is cell surface *EbpS* and that the 35-kDa protein is interacting nonspecifically with the agarose affinity support of the elastin peptide affinity resin.

To test whether antibodies to *EbpS* would block binding to elastin, *S. aureus* cells were incubated with radiolabeled elastin in the absence or presence of immune or preimmune Fab fragments. As shown in Fig. 7, Fab fragments from immune IgGs inhibited binding of *S. aureus* to radiolabeled elastin in a concentration-dependent manner. In contrast, Fab fragments from preimmune antibodies had no effect on binding at the two concentrations tested.

**DISCUSSION**

Cell surface components of pathogenic bacteria play important roles in facilitating the organism's survival in the hostile environment. However, the mechanisms by which these proteins interact with host proteins are not well understood. The present study has provided evidence that *EbpS* is a cell surface protein mediating *S. aureus* binding to elastin. To further explore the role of *EbpS* in elastin recognition, the elastin binding site was identified and confirmed by various methods. The results presented here suggest that *EbpS* plays a critical role in elastin recognition, which could have important implications for the development of strategies to inhibit *S. aureus* infection.
environment of the host. For Gram-positive bacteria, these surface molecules are used in pathogenic processes such as in evading host immune responses (30), digesting host carbohydrates to expose host attachment sites (31, 32), capturing host enzymes to digest host tissues (33), and binding host tissue determinants to establish a firm basis for colonization (34). Cell surface adhesins and MSCRAMMs interact with host ECM components and participate in the colonization of and extravasation through tissues and organs. We have demonstrated previously that S. aureus binds specifically to elastin and have identified a cell surface elastin-binding protein (EbpS) that mediates the S. aureus-elastin interaction. On the basis of these findings, EbpS has been proposed to be the elastin MSCRAMM.

Several independent criteria indicate that EbpS is the surface protein mediating S. aureus binding to elastin. First, rEbpS binds specifically to immobilized elastin and inhibits binding of S. aureus cells in a dose-dependent manner. These results establish that EbpS is an elastin-binding protein that is functionally active in a soluble form. Second, Fab fragments of an antibody raised against rEbpS inhibit binding of S. aureus to elastin. This suggests that the topology of EbpS is such that the elastin binding site is accessible to interact with the ligand and is not embedded in the cell wall or membrane domains. Third, immunoochemical analysis found that the antibody to rEbpS recognizes the native, 25-kDa protein expressed on the surface of S. aureus cells.

Cloning and detailed characterization of ebpS show that it exists as a single copy gene in the S. aureus genome. The 606-base pair gene encodes a protein with a predicted molecular mass of 23 kDa that is highly acidic at neutral pH. Expression of ebpS in E. coli produces a protein of 26 kDa, as determined by mass spectrometry, that migrates as a 45-kDa protein on SDS-PAGE. The exact cause of this aberrant migration is unknown, although anomalous migration in SDS-PAGE is frequently observed for Gram-positive cell surface proteins and for polyhistidine fusion proteins (35–39). In some cases, the anomalous behavior of Gram-positive surface proteins on SDS-PAGE has been attributed to the presence in the protein of multiple repetitive domains (35, 36) or to a high proline content (37, 38). Other factors must account for the abnormal migration of EbpS, however, since it contains no repetitive domains and proline makes up only about 4% of the total amino acid residues.

The aberrant migration of rEbpS on SDS-PAGE suggests an answer to a question raised during our initial characterization of EbpS. In that study, we found two forms of the protein: a functionally active 40-kDa form of EbpS that was only detected intracellularly and the 25-kDa form present on the cell surface. Our current findings suggest that full-length, native EbpS, with a predicted size of 23 kDa, may be migrating in SDS-PAGE as the 40-kDa intracellular precursor, and that the 25-kDa surface form of EbpS is actually a smaller form of the molecule processed at the C terminus. This conclusion is supported by amino acid sequencing showing that both the 25- and 40-kDa proteins have identical amino acid sequences over 20 residues at their amino terminus, by the presence of a single gene for EbpS in the S. aureus genome, and by the observation that the 26-kDa rEbpS migrates anomalously as a 45-kDa protein on SDS-PAGE. The significance of C-terminal processing of EbpS is unknown. One possibility, however, is that the processing event may be important for targeting EbpS to the cell surface. Because EbpS lacks an N-terminal signal peptide and other known sorting and anchoring signals, this proposed intracellular processing event may facilitate surface targeting. In fact, C-terminal signal peptides have been identified in several bacterial proteins (40), and alternative means of anchoring proteins to the cell surface have been reported in Gram-positive bacteria (41).

The mechanism of EbpS expression on the bacterial cell surface is still unclear. Several surface proteins of Gram-positive bacteria have been found to share common motifs involved in sorting, transporting, and anchoring to the cell surface (42). These motifs include a cleaved signal peptide, which is followed by the ligand binding extracellular N-terminal domain, a Pro-rich region thought to span the cell wall, a conserved LPXTGX hexapeptide sequence, a hydrophobic membrane-spanning domain, and a charged C-terminal tail. A recent study by Schnee- wind et al. (43) has shown that protein A of S. aureus is cleaved after the threonine residue of the LPXTGX sequence and is anchored to the cell wall via amide linkage of the carboxyl group of threonine to a free amino group on the pentaglycine peptide moiety of the staphylococcal peptidoglycan. Apart from the putative localization of the elastin binding site to the extracellular N-terminal domain and identification of a charged C-terminal tail, EbpS contains none of the other common surface protein motifs. EbpS is not unique in this regard, however,
since several other Gram-positive surface proteins lack conserved structures. Examples include streptococcal proteins such as the fibronectin/fibrinogen-binding protein (44), albumin-binding protein (38), and the plasmin receptor (45). Like Ebps, these proteins are all smaller than the majority of Gram-positive surface proteins with common structural motifs, and in the case of the streptococcal plasmin receptor, the internal methionine residue is cleaved in the mature protein (45), similar to what is found with Ebps.

It is now apparent that interactions between pathogenic bacteria and host ECM components play an important role in disease pathogenesis. However, molecular structure-function analyses for most ECM adhesins have not been performed despite the obvious potential of developing effective prophylactic and therapeutic agents based on information derived from these studies. In cases where information is available, the primary ligand binding site has been found to be contained in the N-terminal extracellular domain (36, 46, 47). Consistent with this observation is our finding that truncated fragments of rEbps lacking the first 59 amino acids do not bind elastin, confirming that the elastin binding site in Ebps is also contained within the extracellular N-terminal domain. Furthermore, preliminary studies show that a recombinant construct of Ebps containing only this 59-amino-acid domain directly binds to elastin and inhibits binding of S. aureus to elastin. Similar to other domains in Ebps, this putative elastin binding region lacks homology with sequences reported to various data bases. Additional studies using recombinant constructs, synthetic peptides, and domain-specific antibodies are in progress to further define the elastin binding site in Ebps.

Acknowledgments—We thank Drs. Michael Caparon, William Parks, and Robert Senior for valuable comments concerning the manuscript. Technical assistance was provided by Clarina Tisdale and Benjamin Mecham.

REFERENCES

