

## Identification of a *Staphylococcus aureus* Extracellular Matrix-Binding Protein with Broad Specificity

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A staphylococcal surface protein capable of binding several extracellular matrix glycoproteins was purified as a result of our attempts to identify a receptor(s) for bone sialoprotein (BSP) on *Staphylococcus aureus* cells. Proteins from different staphylococcal strains were solubilized in sodium lauryl sulfate, separated by polyacrylamide gel electrophoresis, blotted onto Immobilon P membranes, and probed with <sup>125</sup>I-BSP. Several bacterial proteins bound the radiolabeled ligand, and various strains expressed different repertoires of BSP-binding proteins. Major BSP-binding proteins with apparent *M<sub>s</sub>* of 72,000 or 60,000 were present on most strains, and these proteins were further studied. The 72- and 60-kDa proteins were preferentially expressed when bacteria were cultured in Luria broth compared with when they were cultured on tryptic soy broth, and the abundance of the proteins could be correlated to an increased <sup>125</sup>I-BSP binding. Both the 72-kDa and the 60-kDa proteins were solubilized by extraction of cells with 1 M LiCl and were purified by cation-exchange chromatography. Amino acid composition analysis of the purified 72-kDa protein indicated a high content of lysine (11.9%) and hydrophobic amino acids (28.0% combined). In Western ligand blotting (immunoblotting) experiments, the 72-kDa protein bound not only BSP but also radiolabeled fibronectin, fibrinogen, vitronectin, thrombospondin, and, to some extent, collagen. Addition of the purified 60-kDa protein to *S. aureus* cells did not inhibit binding of the different ligands but in some cases resulted in an augmentation of the binding of <sup>125</sup>I-ligand. Purified 60-kDa protein could hemagglutinate sheep erythrocytes at a concentration of 61 μg/ml. The agglutination reaction was inhibited by high concentrations of fucose, mannose, or melibiose. These data suggest that the purified proteins may serve as bacterial receptors with broad specificity for matrix glycoproteins and that the proteins may act as carbohydrate-binding proteins.

*Staphylococcus aureus* is a major human pathogen and is associated with infections such as bacterial arthritis (5), osteomyelitis (28, 37), and acute infectious endocarditis (25). Staphylococci can also cause pneumonia and tracheobronchitis in immunocompromised patients and patients with cystic fibrosis (26).

The initial step in an infectious disease is often the adhesion and colonization of host tissue surfaces. A number of bacterial cell surface structures have been identified as mediators of host tissue adherence. Previous studies of gram-negative bacteria indicated that adhesion of these bacteria to tissues is mediated primarily through lectins present on pili or fimbriae (12, 20). These lectins recognize and bind to glycoproteins and glycolipids present on host cell surfaces. Gram-positive bacteria, on the other hand, appear to adhere primarily to extracellular matrix molecules such as fibronectin (FN) via specific cell surface receptors in what are believed to be protein-protein interactions. Also, some gram-negative bacteria can recognize and bind to extracellular matrix proteins (reviewed in reference 10).

The *S. aureus* FN receptor is probably the best-characterized bacterial receptor for an extracellular matrix protein (4, 13, 18, 31). Two very similar and closely spaced staphylococcal genes coding for FN-binding proteins have been

identified (13). The FN-binding activity is located to a segment composed of a 38-amino-acid motif which is repeated three times in both proteins (13, 18, 31). The receptor binds to the N-terminal domain of FN, which is not known to contain any carbohydrate. Binding of FN to staphylococcal cells therefore appears to involve primarily a protein-protein interaction. Staphylococcal cells may also express receptors for thrombospondin (TSP), bone sialoprotein (BSP), fibrinogen (FBG), collagen, and vitronectin (VN) (1, 7–10, 30, 33, 35). Expression of receptors for matrix proteins with specific tissue distribution may partly explain the tissue tropism of infections caused by various staphylococcal strains. Thus, staphylococcal strains isolated from patients with septic arthritis express a collagen receptor, and the presence of this receptor appears to be necessary and sufficient for bacteria to adhere to cartilage (34). Osteomyelitis-derived strains also had a high binding capacity for BSP which was on average five times higher than that expressed by strains isolated from patients with endocarditis (29).

In the present study, we screened several *S. aureus* isolates from different sources for the presence of bacterial proteins capable of binding BSP. BSP is a glycoprotein found only in the bone matrix and dentin (3), and the ability to bind BSP may be a factor which targets *S. aureus* cells to bone tissue (28–30). The data presented here describe a 72-kDa protein and a 60-kDa protein which have BSP-binding activity and are present on many strains. These proteins, which are released from bacterial cells by treatment with 1 M LiCl and purified by cation-exchange chro-

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matography, bind not only BSP but also FN, TSP, VN, and FBG. Furthermore, the 60-kDa protein can agglutinate sheep erythrocytes (SRBC) possibly via a lectin-like activity, which may account for its ability to bind a number of matrix glycoproteins. Hence, the 72- and 60-kDa proteins may represent a general adhesin mediating or augmenting staphylococcal adherence to a variety of extracellular matrices.

## MATERIALS AND METHODS

**Bacteria and growth conditions.** The *S. aureus* clinical isolates were from the Department of Orthopedics, Bowman Gray School of Medicine, Winston-Salem, N.C., or the Clinical Pathology Laboratory, School of Medicine, University of Alabama at Birmingham, Birmingham (34). These isolates include X50151, 87/8, and 87/12 from synovial fluid; F44947 isolated from sputum; M65051 isolated from a soft tissue infection; strain Cowan (33) isolated from a septic arthritis patient; and 88/4, an osteomyelitis isolate. Strain 024, an osteomyelitis isolate, has been described previously (30). Strain Newman (4) was obtained from the Department of Bacteriology, College of Veterinary Medicine, Uppsala, Sweden, and *S. aureus* #574 was obtained from the U.S. Food and Drug Administration.

Glycerol stocks were made from overnight cultures in tryptic soy broth (TSB; Difco, Detroit, Mich.) and were stored at  $-20^{\circ}\text{C}$ . *S. aureus* cultures were started by inoculation from glycerol stocks into either TSB or Luria broth (LB; GIBCO BRL). After overnight growth at  $37^{\circ}\text{C}$  with agitation, cells were harvested by centrifugation ( $3,600 \times g$ , 15 min) and resuspended in phosphate-buffered saline (PBS: 10 mM phosphate, 0.14 M NaCl, 0.02% sodium azide, pH 7.4). Cell density was adjusted to  $8 \times 10^9$  cells per ml by using a reference standard curve relating optical density at 600 nm to cell number. The cells were kept on ice until used.

**Preparation and iodination of ligands.** The ligands used for this study were obtained from the following sources. BSP, isolated from a rat osteogenic cell line (19), was a gift from L. Fisher of the National Institutes of Health, Bethesda, Md. Collagen type II isolated from chicken sternum (27) was provided by R. Mayne, Department of Cell Biology, University of Alabama at Birmingham. FN and the 29-kDa N-terminal FN fragment were isolated from porcine plasma as described elsewhere (2). Human FBG was obtained from KabiVitrum, Stockholm, Sweden, and further purified by passage through gelatin-Sepharose to remove contaminating FN (16). TSP purified from fresh human platelets (22) was provided by J. Murphy-Ullrich, Department of Pathology, University of Alabama at Birmingham. Human VN was purchased from Sigma Chemical Co. (St. Louis, Mo.) or Collaborative Biomedical Products (Bedford, Mass.).

Iodination of ligands was conducted by either the chloramine T method of Hunter (11) or the lactoperoxidase method (21) using Enzymobeads (Bio-Rad Corp, Richmond, Calif.). The estimated specific activities of the iodinated ligands ranged from  $2.0 \times 10^6$  to  $4.4 \times 10^7$  cpm/ $\mu\text{g}$ .

**Binding of ligands to bacteria.** The binding of radiolabeled ligands to bacterial cells was quantitated essentially as described by Fröman et al. (4). Tubes (75 by 12 mm, 4.5 ml; Sarstedt, Newton, N.C.) were precoated with 5% (wt/vol) bovine serum albumin (BSA; Sigma) in PBS on an end-over-end mixer for a minimum of 1 h to reduce nonspecific binding to the plastic surface. The binding buffer used was either 0.1% (wt/vol) BSA in PBS containing 0.1% Tween 80 (ligand binding buffer I) or 10 mM HEPES (*N*-2-hydroxyethylpiper-

azine-*N'*-2-ethanesulfonic acid), pH 7.4, containing 137 mM NaCl, 5 mM KCl, 0.7 mM  $\text{MgSO}_4$ , 1.2 mM  $\text{CaCl}_2$ , and 0.1% BSA (ligand binding buffer II [24]). In either case,  $8 \times 10^8$  cells were added to  $5 \times 10^4$  cpm of  $^{125}\text{I}$ -labeled ligand in a final volume of 0.5 ml of binding buffer. Tubes containing the incubation samples were rotated end over end at room temperature for 90 min. The reaction was stopped by the addition of 3 ml of ice-cold PBS containing 0.1% Tween 80 (PBS-Tween), followed by centrifugation. The supernatant was aspirated, and the radioactivity associated with the bacterial cell pellet was quantitated in a gamma counter (LKB, Turku, Finland). Some binding assays were conducted in the presence of 10 mM EDTA or 0.2 M mannose.

**Solubilization of *S. aureus* proteins.** (i) **Solubilization in SDS.** Overnight cultures of *S. aureus* cells were harvested by centrifugation, and bacteria were suspended in PBS to 1/10 of the original volume. All cell suspensions were adjusted by addition of PBS to the same optical density at 600 nm. The cells were boiled in the presence of 2% sodium dodecyl sulfate (SDS) and 5% 2-mercaptoethanol for 3 min and loaded onto SDS-polyacrylamide gel electrophoresis (PAGE) gels.

(ii) **Lysostaphin digestion.** To solubilize *S. aureus* cell surface proteins, cells were digested with lysostaphin (Sigma) as previously described (4). The residual cells were removed by centrifugation. The solubilized surface proteins recovered in the supernatant were further analyzed.

(iii) **Trypsin digestion.** Overnight cultures of *S. aureus* cells were harvested by centrifugation ( $3,600 \times g$ , 15 min) and resuspended in 1/10 volume of PBS and adjusted to the same optical density at 600 nm. The cells were incubated with 25  $\mu\text{g}$  of trypsin (Sigma) per ml for 1 h at  $37^{\circ}\text{C}$ . The reaction was stopped by adding 50  $\mu\text{g}$  of soybean trypsin inhibitor (Sigma) per ml as described elsewhere (32). The residual cells were collected by centrifugation and used in binding assays.

(iv) **Lithium chloride extraction.** Overnight bacterial cultures were harvested by centrifugation ( $3,600 \times g$ , 15 min) and resuspended in 1/10 volume of 1 M LiCl, pH 6.0. The cell suspension was incubated at  $45^{\circ}\text{C}$  for 2 h with gentle agitation. Subsequently, the cells were removed by centrifugation ( $3,600 \times g$ , 15 min), and the supernatant containing solubilized proteins was collected.

**Detection of ligand-binding components by Western ligand blotting (immunoblotting).** Proteins were separated by SDS-PAGE using gradient gels of 3 to 15% acrylamide and the buffer system of Laemmli (15). Proteins were transferred to Immobilon P (Millipore, Bedford, Mass.) membranes with the Bio-Rad Trans-Blot apparatus and the transfer buffer of Towbin et al. (36). Additional protein-binding sites on the membranes were blocked by incubation for 60 min in PBS containing 3% (wt/vol) BSA. Membranes were then incubated overnight at  $4^{\circ}\text{C}$  with gentle agitation in a solution containing  $10^3$  cpm of  $^{125}\text{I}$ -labeled ligand in ligand binding buffer I. Subsequently, the membranes were washed extensively with 0.1% Tween 20 in PBS, air dried, and exposed to Fuji RX-100 X-ray film for 4 to 16 h at  $4^{\circ}\text{C}$ .

**Purification of ligand-binding components.** Protein was precipitated from the LiCl extracts of *S. aureus* cells cultured in LB by the addition of ammonium sulfate to a final concentration of 60% (wt/vol), followed by gentle stirring overnight at  $4^{\circ}\text{C}$ . The precipitated protein was recovered by centrifugation ( $15,000 \times g$ , 30 min), and the pellet was resuspended in a minimal volume of 10 mM Tris-HCl, pH 7.5. In preparation of further purification steps, protein was dialyzed against three changes of 10 mM Tris-HCl (pH 7.5) buffer at  $4^{\circ}\text{C}$ .

The dialyzed protein solution was clarified by centrifuga-

tion and subjected to cation-exchange chromatography on a Mono-S column (Pharmacia, Uppsala, Sweden) fitted on a Pharmacia FPLC system. The column was equilibrated in 10 mM Tris-HCl, pH 7.5, and bound protein was eluted with a linear gradient of up to 1 M NaCl in 10 mM Tris-HCl, pH 7.5. The eluate was monitored by measuring the  $A_{280}$ , and protein-containing fractions were analyzed by SDS-PAGE. Ligand-binding components were detected by Western ligand blotting. Peak fractions containing ligand-binding activity were pooled and stored at  $-20^{\circ}\text{C}$ .

**Hemagglutination of SRBC with purified protein.** The hemagglutination titration was conducted as previously described for the *Sophora japonica* lectin (20). Serial twofold dilutions of a 1-mg/ml stock solution of purified protein were made with PBS as the diluent in a microtiter plate. The final volume of the diluted protein was 100  $\mu\text{l}$ . An equal volume of a 2% suspension of SRBC (Colorado Serum Co., Denver) was added to the protein dilutions. After incubation at room temperature for 1 h, the agglutination was scored. The microtiter round-bottom well containing no protein resulted in the SRBC forming a button, whereas agglutination results in a mat of SRBC on the bottom of the microtiter well. The titer was defined as the lowest dilution which caused agglutination of the erythrocytes. Inhibition of hemagglutination was tested by addition of serial twofold dilutions of the following carbohydrates: 0.1 M lactose, 0.1 M mannose, 0.1 M melibiose, 0.05 M L-fucose, 0.1 M N-acetyl-D-glucosamine, and 0.05 M N-acetyl-D-galactosamine (E-Y Laboratories Inc., San Mateo, Calif.) in PBS.

**Amino acid composition analysis of purified protein.** Purified protein was analyzed for amino acid composition by the Protein Chemistry Core Facility of the University of Alabama at Birmingham Cancer Center on an Applied Biosystems 420A derivatizer, 130A separation system, and 920A data analysis module.

## RESULTS

**Several *S. aureus* proteins bind BSP.** Initial characterization of the binding of BSP to *S. aureus* Newman and #574 indicated that the amounts of radiolabeled ligand bound to the bacterial cells were not, in our hands, affected by the presence of 1.2 mM  $\text{CaCl}_2$ , 0.7 mM  $\text{MgSO}_4$ , and 10 mM EDTA in the binding buffer as reported in other studies (30). The growth media used to culture bacteria affected the BSP-binding capacity, particularly that of strain #574. When grown in LB, cells of strain #574 bound as much as 50% more freshly labeled BSP than did cells cultured in TSB. Strain Newman bound larger amounts of  $^{125}\text{I}$ -BSP than did strain #574, and the BSP-binding capacity of the former strain was less affected by the growth media used. Treatment of bacterial cells with trypsin and/or lysostaphin resulted in a 50% reduction of the amount of ligand bound, suggesting that bacterial surface proteins are at least partially responsible for the binding of  $^{125}\text{I}$ -BSP.

To assess whether the interaction of BSP with the bacterial cells is mediated by specific protein components, whole-cell lysates of the various *S. aureus* strains were separated by SDS-PAGE and subjected to Western ligand blotting with  $^{125}\text{I}$ -BSP (Fig. 1). The repertoire of BSP-binding proteins varied among different strains. All strains analyzed contained a BSP-binding protein of approximately 32 kDa and two smaller BSP-binding proteins of approximately 18 and 14 kDa. The largest protein species which bound BSP was approximately 85 kDa and is prevalent in strains F44947, 87/2, #574, and 024. A 72-kDa BSP-binding protein was

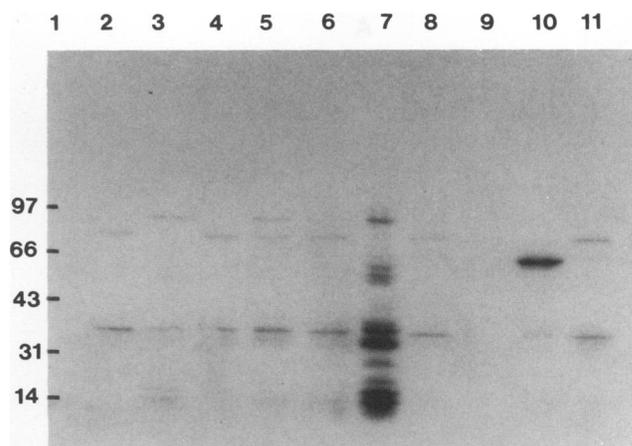


FIG. 1. Autoradiogram of Western ligand blotting of *S. aureus* strains with  $^{125}\text{I}$ -BSP. Proteins in lysates of *S. aureus* cells cultured in TSB were separated by SDS-PAGE on 3 to 15% acrylamide gels and transferred to an Immobilon P membrane. Additional protein-binding sites on the membrane were blocked by incubation in a solution of PBS containing 3% (wt/vol) BSA for 60 min. The membrane was then incubated overnight at  $4^{\circ}\text{C}$  in  $10^5$  cpm of  $^{125}\text{I}$ -BSP in ligand binding buffer I. The membrane was washed in PBS-Tween 20, air dried, and exposed to Fuji RX-100 X-ray film overnight. Lane 1, low-molecular-weight markers (Bio-Rad) (indicated on the left in thousands). The *S. aureus* isolates are in the remaining lanes as follows: lane 2, X50151; lane 3, F44947; lane 4, 87/8; lane 5, 87/12; lane 6, #574; lane 7, 024; lane 8, Cowan; lane 9, M65051; lane 10, Newman; and lane 11, 88/4.

observed in strains X50151, 87/8, 87/2, #574, Cowan, and 88/4. A 60-kDa BSP-binding protein was predominant in strain Newman and also observed in strains Cowan, M65051, and 88/4.

**Solubilization and purification of BSP-binding proteins from *S. aureus*.** Cells of strain #574 were grown overnight in LB and TSB and boiled in SDS, and solubilized proteins were separated by SDS-PAGE. The results of these experiments showed that two major proteins with  $M_r$ s of 72,000 and 60,000 are preferentially expressed by cells grown in LB (Fig. 2A, lanes 2 and 5). Western ligand blotting showed that the two proteins are major BSP-binding proteins (Fig. 2B, lane 2). Our studies were therefore focused on attempts to isolate and characterize these proteins. Extraction of cells with 1 M LiCl resulted in the preferential solubilization of the 72-kDa protein from cells grown in LB or TSB (Fig. 2A), and Western ligand blotting showed that this protein bound  $^{125}\text{I}$ -BSP. The LiCl extract of cells grown in LB also contained three additional BSP-binding proteins with apparent molecular masses of 180, 140, and 60 kDa.

Since LiCl apparently solubilized the major BSP-binding proteins from strain #574, this extraction method was also used to solubilize protein from strain Newman cultured in LB. Solubilized proteins were recovered by ammonium sulfate precipitation and fractionated on a Mono-S cation-exchange column fitted on a FPLC system. The column was eluted with a NaCl gradient, and the protein concentrations of the eluted fractions were estimated by analyzing the  $A_{280}$ . The two major peaks of protein eluted between 0.7 and 0.8 M NaCl and were found to each contain an apparently homogenous 60-kDa protein. The purified protein was subject to amino acid composition analysis (Table 1) which revealed a high molar percentage of lysine (11.9%), aspar-

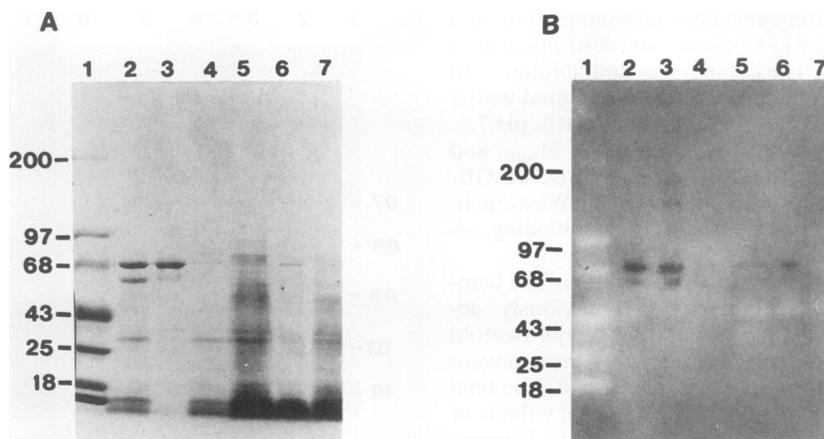


FIG. 2. SDS-PAGE and Western ligand blots of *S. aureus* #574 cellular extracts after growth in TSB or LB. Gel electrophoresis (A) and Western ligand blotting (B) were conducted as outlined in Materials and Methods and the legend to Fig. 1. Lanes 1 contain prestained molecular weight standards (Bio-Rad) (indicated on the left in thousands). Samples in lanes 2, 3, and 4 are from *S. aureus* cultured in LB, and those in lanes 5, 6, and 7 are from cells cultured in TSB. Lanes 2 and 5, *S. aureus* cells solubilized in SDS; lanes 3 and 6, LiCl-released proteins; lanes 4 and 7, the protein in *S. aureus* cell residues after LiCl extraction. (A) Gel stained with Coomassie blue; (B) autoradiogram after <sup>125</sup>I-BSP binding.

agine-aspartic acid (combined: 16.9%), and hydrophobic amino acids (28.0%).

The 72-kDa protein was purified from the Food and Drug Administration strain #574 grown in LB by the same purification protocol as described above for the purification of the 60-kDa protein from strain Newman. The purified proteins were checked for binding activity by the Western ligand blot assay. The purified 60-kDa protein from strain Newman and the purified 72-kDa protein from strain #574 both bound <sup>125</sup>I-BSP, as demonstrated by Western ligand blotting (Fig. 3). The relationship between the isolated 60-kDa protein from *S. aureus* Newman and the 72-kDa protein from *S. aureus* #574 is presently unclear.

**The purified proteins are general binders of extracellular matrix glycoproteins.** The ligand binding specificity of the 72-kDa protein purified from strain #574 was further analyzed. Various radiolabeled matrix glycoproteins, including

FN, FBG, collagen, VN, and TSP, were used to probe Western blots of whole *S. aureus* #574 cells, LiCl extracts, and the extracted residues of cells grown in both LB and TSP. Remarkably similar patterns (Fig. 4) were obtained regardless of which ligand was used to probe the membranes, although the intensity on the autoradiogram varied considerably. The binding of ligands such as VN and TSP was more dramatic than that of collagen. The two higher-molecular-weight proteins in the LiCl extracts which bound BSP (Fig. 2) also appear to bind both <sup>125</sup>I-labeled VN and TSP. There is more ligand binding associated with the samples prepared from cells cultured in LB, and the major ligand-binding protein has an *M<sub>r</sub>* of 72,000 and is solubilized by extraction of bacteria with 1 M LiCl. Taken together, these data suggest that the same bacterial protein is capable of binding all the different ligands tested. When the purified

TABLE 1. Amino acid composition of the 60-kDa protein purified from *S. aureus* Newman

Amino acid	mol% <sup>a</sup>
Asparagine-aspartic acid.....	16.9
Glutamine-glutamic acid.....	6.8
Serine.....	10.3
Glycine.....	5.1
Histidine.....	1.5
Arginine.....	3.3
Threonine.....	7.7
Alanine.....	4.8
Proline.....	2.2
Tyrosine.....	6.0
Valine.....	6.0
Methionine.....	ND
Cysteine.....	ND
Isoleucine.....	7.3
Leucine.....	7.5
Phenylalanine.....	2.4
Lysine.....	11.9
Tryptophan.....	ND

<sup>a</sup> ND, not determined.

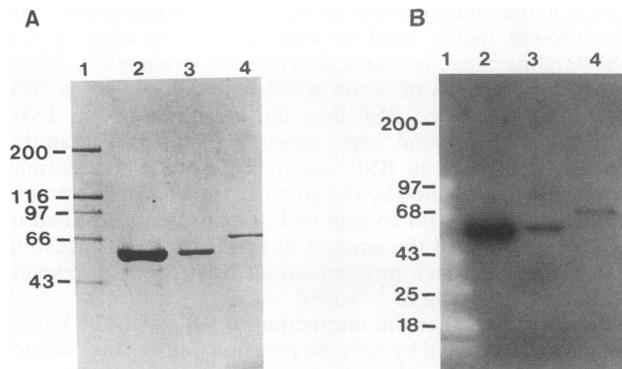


FIG. 3. Gel electrophoresis and Western ligand blot with <sup>125</sup>I-BSP of purified proteins from *S. aureus* Newman and #574. (A) SDS-PAGE of proteins purified by FPLC. The gel is stained with Coomassie blue. (B) Western ligand blotting with <sup>125</sup>I-BSP of the same preparations. Lanes 1, high-molecular-weight (A) and prestained molecular weight (B) markers (indicated on the left in thousands). Lanes 2 and 3, the two major protein peaks from FPLC of LiCl-extracted material from strain Newman; lane 4, protein isolated from *S. aureus* #574.

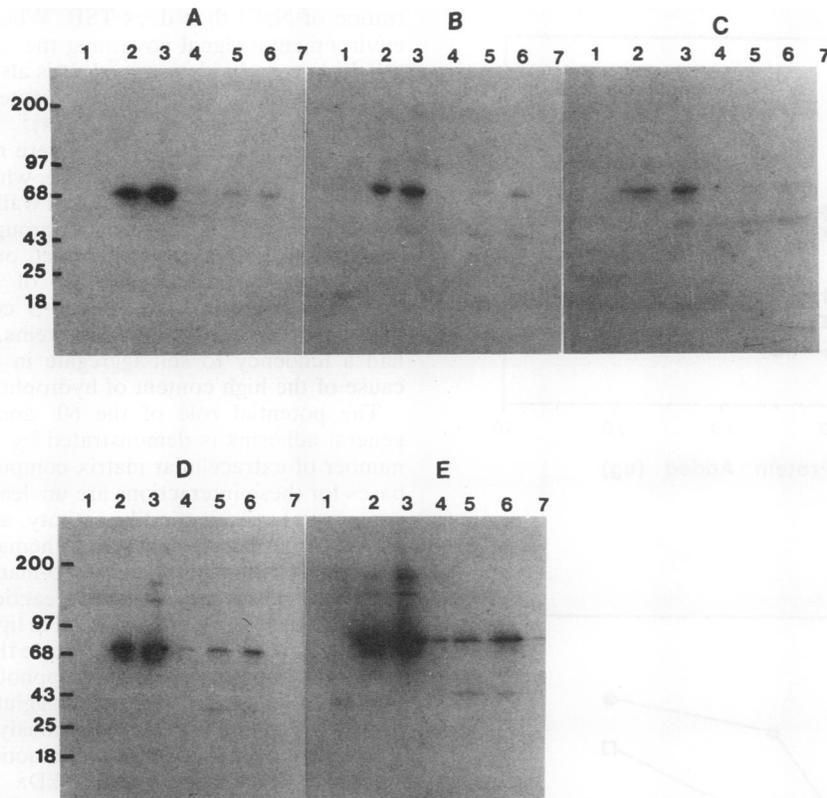


FIG. 4. Binding of  $^{125}\text{I}$ -ligands to *S. aureus* #574 cellular protein extracts after culture in TSB or LB. *S. aureus* cells grown in TSB or LB were extracted and subjected to Western ligand blotting as described in Materials and Methods and the legend to Fig. 1. Lanes were as described in the legend to Fig. 2. Ligands were as follows:  $^{125}\text{I}$ -FN (A),  $^{125}\text{I}$ -FBG (B),  $^{125}\text{I}$ -collagen (C),  $^{125}\text{I}$ -VN (D), and  $^{125}\text{I}$ -TSP (E).

60-kDa protein from strain Newman and the 72-kDa protein from #574 were tested, both proteins bound all radiolabeled ligands, demonstrating a broad binding specificity for these proteins (data not shown). It is noteworthy that the 210-kDa FN (4, 31) or the 135-kDa collagen (24, 35) receptors could not be detected in Western ligand blots.

If the purified proteins represent soluble forms of the primary receptor for each of the ligands tested, then the isolated proteins would be expected to competitively inhibit ligand binding to intact bacteria. Binding assays in which increasing amounts of 60-kDa protein purified from strain Newman were added to incubation mixtures containing *S. aureus* cells and different radiolabeled ligands were therefore conducted. The results of these experiments show (Fig. 5) that in the case of the 29-kDa FN fragment, there was a slight decrease in the amount of iodinated ligand bound to bacterial cells when purified 60-kDa protein was added. For all of the other ligands assayed, enhancement of ligand binding to the bacterial cells was observed with increasing amounts of 60-kDa protein added. The binding of TSP and that of VN were increased up to 13- and 15-fold, respectively, over the amount of radiolabeled ligand bound in the absence of added 60-kDa protein. The observed maximal enhancement of collagen, BSP, FN, and FBG binding, was 1.2-, 1.5-, 2.8-, and 4.3-fold, respectively, above the level observed with no addition of 60-kDa protein. The extent of ligand binding enhancement caused by addition of the 60-kDa protein varied from one experiment to another, and the molecular basis for this effect remains unclear.

**The isolated 60-kDa protein is an agglutinin.** The ability of

the purified proteins to bind several unrelated extracellular matrix glycoproteins suggested that this protein may act as a lectin. The protein was therefore tested for the ability to hemagglutinate SRBC. The results of these experiments showed that the 60-kDa protein at concentrations of 61  $\mu\text{g}/\text{ml}$  and higher agglutinated the SRBC (data not shown). This interaction could be inhibited by the addition of L-fucose (0.0125 M), D-mannose (0.05 M), and melibiose (0.025 M) but was not affected by lactose (0.1 M), N-acetyl-D-glucosamine (0.1 M), or N-acetyl-D-galactosamine (0.05 M). Taken together, these results suggest that the 60-kDa protein may function as an agglutinating lectin and may recognize and bind to carbohydrate structures present in the different matrix glycoproteins.

## DISCUSSION

In the study presented in this communication, we have used Western ligand blotting to detect ligand-binding proteins in *S. aureus*. A surprisingly large number of BSP-binding proteins were detected in various *S. aureus* isolates. The metabolic and structural relationships between these proteins are presently unclear.

Strains Newman and #574 expressed major BSP-binding proteins with apparent molecular masses of 60 and 72 kDa, respectively. Both proteins were selectively solubilized by extraction with 1 M LiCl and were purified by identical purification protocols. Furthermore, preliminary peptide mapping of these two proteins gave similar fragments (17a). These data suggest that the 60-kDa protein of strain Newman

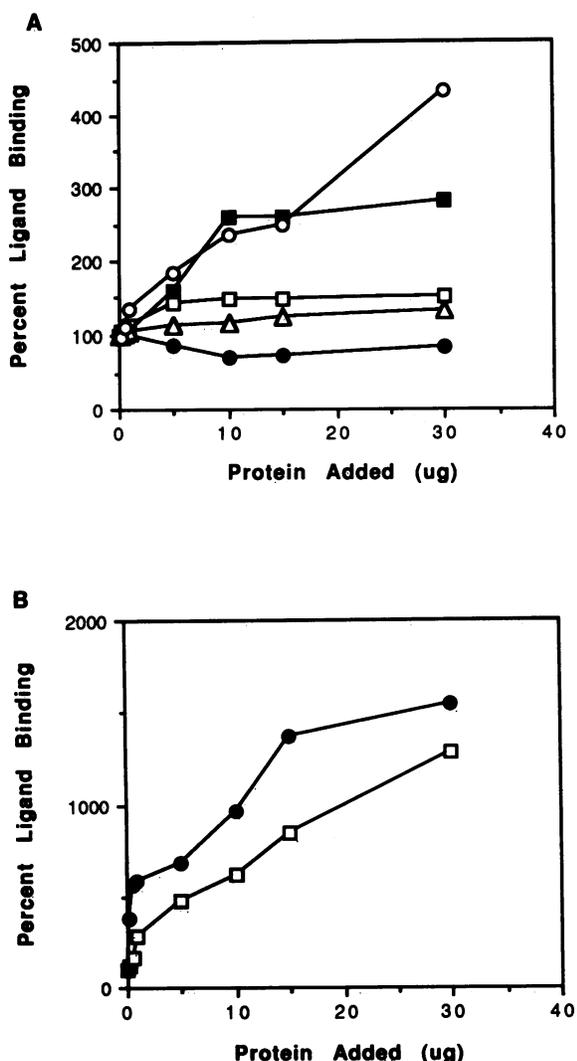


FIG. 5. Effect of purified 60-kDa protein on the ability of *S. aureus* #574 to bind  $^{125}\text{I}$ -ligand. The indicated amount of 60-kDa protein purified from strain Newman was mixed with  $5 \times 10^4$  cpm of various  $^{125}\text{I}$ -labeled ligands in an assay volume of 0.5 ml of ligand binding buffer and incubated for 30 min on an end-over-end mixer at room temperature. Bacterial cells ( $8 \times 10^8$ ) were added, and after 90 min of mixing at room temperature, unbound ligand was diluted by the addition of 3 ml of ice-cold PBS containing 0.1% (vol/vol) Tween 80. After centrifugation at  $1,350 \times g$  for 20 min, the supernatant was aspirated and the radioactivity associated with the bacterial pellet was quantified with an LKB gamma counter. Results are expressed as a percentage of the radioactivity bound by cells in the absence of added protein (which was set to 100%). (A) □, BSP; ■, FN; ●, 29-kDa N-terminal FN fragment; △, collagen; ○, human fibrinogen. (B) □, TSP; ●, VN.

and the 72-kDa protein of strain #574 are closely related. When strain #574 was grown in LB medium it bound more  $^{125}\text{I}$ -BSP and appeared to express more of the 72-kDa protein than did cells grown in TSB. These observations suggest that the 72-kDa protein mediates binding of BSP to *S. aureus* cells.

The enhanced expression of the 72-kDa protein in LB-grown cultures may be a consequence of the higher osmolarity of the medium. LB contains a twofold-higher concen-

tration of NaCl than does TSB. Whether osmolarity is the environmental signal governing the expression of this protein has yet to be determined. It is also unclear whether the expression of the 60- and 72-kDa proteins is regulated by the accessory gene regulator (*agr*) (14).

The 60- and 72-kDa proteins were released from bacteria through extraction with 1 M LiCl, which suggests that they are not firmly anchored in the cell wall or membrane but are associated with the cell surface through an unknown mechanism. The LiCl extract contained only minor amounts of other proteins, and purification of either protein was a one-step procedure on a Mono-S column, owing to the highly basic nature of these proteins. The purified protein had a tendency to self-aggregate in solution, perhaps because of the high content of hydrophobic amino acids.

The potential role of the 60- and 72-kDa proteins as general adhesins is demonstrated by their ability to bind a number of extracellular matrix components. The molecular bases for these interactions are unclear. The 60-kDa protein appears to have a lectin-like activity, as demonstrated by the ability of the purified protein to hemagglutinate SRBC in a reaction sensitive to L-fucose, D-mannose, and melibiose. Although the hemagglutination reaction is characteristic of lectins, it has also been attributed to lipid moieties and highly basic proteins (20). Our data indicate that the purified protein is both highly basic and hydrophobic. Therefore, these properties could explain the hemagglutination activity of the protein. Further studies are obviously needed to define the mechanism of matrix-protein interaction.

Neither the 60- nor the 72-kDa protein inhibited the binding of radiolabeled ligands to bacterial cells when added to incubation mixtures. Instead, a concentration-dependent stimulation of bacterial binding of some ligands was observed. This effect could be explained if the isolated protein, perhaps through aggregation, acted as a multivalent ligand binder. Large ligand-*S. aureus* protein aggregates could be formed and subsequently could bind to the cells. Such a mechanism would be facilitated if the ligands contained several binding sites for the bacterial proteins.

The data presented describe cell surface proteins on *S. aureus* cells which may act as general adhesins and bind a number of extracellular matrix components. A general adhesive mechanism has recently been suggested for *Treponema denticola* with respect to the binding of FN, laminin, and FBG via two proteins with apparent molecular masses of 53 and 72 kDa (6). Furthermore, a surface protein related to glyceraldehyde-3-phosphate-dehydrogenase and present on *Streptococcus pyogenes* cells seems to have a broad ligand-binding specificity (17, 23). The *S. aureus* proteins described in this study may represent a similar binding mechanism and could as such represent an important general virulence determinant.

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