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Specific Attachment of *Staphylococcus aureus* to Immobilized Fibronectin

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Staphylococcus aureus **cells have been shown to possess surface-associated proteins with affinity for soluble fibronectin. We have investigated the ability of these surface proteins to mediate attachment to immobilized fibronectin and collagen. Attachment was quantified by determination of bacterial ATP in a bioluminescence assay. The ability to attach to fibronectin- or collagen-coated plastic surfaces was investigated for four *S. aureus* strains: Cowan 1, Newman, SA113(83A), and Wood 46. Cells from the different strains varied in their attachment properties, but all cells except those of strain Wood 46 attached readily to substrates coated with fibronectin. Only cells from strain Cowan 1 attached reproducibly to collagen-coated substrates in the absence of fibronectin. The attachment of cells from strain SA113(83A) to fibronectin-coated surfaces was shown to be dependent on time, fibronectin concentration, and bacterial growth phase. Soluble fibronectin or NH_2-terminal fibronectin fragment (M_r, 29,000) disturbed the attachment to surfaces coated with fibronectin bound to denatured collagen type I. The attachment process to such substrates was also effectively inhibited by preincubating the substrate with fibronectin-binding proteins isolated from *S. aureus* Newman and SA113 (83A) and purified with affinity chromatography.**

The first step in many bacterial infections is specific attachment to host tissue (2). Some pathogenic gram-negative bacteria are known to produce surface proteins which bind to specific carbohydrates on the eucaryotic cell surface. These interactions are important in promoting bacterial attachment to the epithelia of the urinary tract (19, 39) and other mucosal surfaces (25). Another type of interaction thought to influence virulence and immune response is the binding of plasma proteins by *Staphylococcus aureus* and group A, C, and G streptococci (4, 26, 28, 29). This coating of the cell surface by host proteins has been proposed to mask the bacterial cell and influence the host's ability to produce an effective immune response (26, 43). Furthermore, specific interactions between bacteria and components of the extracellular matrix have been described, including binding of fibronectin to *S. aureus* (13, 31, 33), other staphylococcal species (41), and group A, C, and G streptococci (35, 36, 40), binding of fibronectin and laminin to *Escherichia coli* (10, 37), and binding of laminin to *S. aureus* (22).

Fibronectin is a glycoprotein with an apparent molecular weight of 440,000 consisting of two similar chains interlinked by disulfide bonds (53). It is present in a soluble form in plasma and in interstitial fibers (20). Recent studies revealed heterogeneity of mRNAs coding for fibronectin produced by rat hepatocytes (34), offering the possibility of functionally different subpopulations of this molecule. Fibronectin can be split with trypsin, cathepsin G (44), or plasmin, and binding regions for different macromolecules such as collagen, fibrinogen, and glycosaminoglycans have been identified (for a recent review, see reference 53). Binding regions for eucaryotic and for bacterial cells have also been identified. One staphylococcal binding domain was found at the NH_2 terminal of the fibronectin molecule (27), and an additional binding site was reported for staphylococci and streptococci (14).

Fibronectin was shown to mediate adherence of staphylococci to endothelial cells (48) and may influence phagocytosis of *S. aureus* by polymorphonuclear cells (6, 8, 17, 18, 32). The significance of fibronectin as an opsonin has been disputed, however (5, 43, 47).

In an earlier study we demonstrated that heat-resistant but trypsin-sensitive fibronectin-binding proteins could be isolated from lysostaphin lysates of *S. aureus* Cowan 1 (33). Lysates obtained from bacteria digested with trypsin before treatment with lysostaphin were depleted of fibronectin-binding proteins. The latter finding was interpreted as a surface location of the fibronectin-binding proteins recovered from nontrypsinized bacteria.

The present report concerns the attachment of *S. aureus* cells to immobilized fibronectin. The characteristics of the reaction were found to be compatible with a role of fibronectin in specific attachment of *S. aureus* to structures such as denatured collagen on which fibronectin can be immobilized. Additional physiologically relevant attachment mechanisms for staphylococci were suggested from the finding that cells from *S. aureus* Cowan 1 attached to collagen type I also in the absence of fibronectin. Other observations indicated a role of fibronectin-binding proteins on the staphylococcal cell surface in the specific attachment of *S. aureus* to fibronectin-coated surfaces. The ability of staphylococci to attach to immobilized fibronectin was dependent on the bacterial growth phase.

MATERIALS AND METHODS

Reagents. Lysostaphin, bovine serum albumin (BSA; fraction V), DNase I, RNase A, trypsin (type III S), soybean trypsin inhibitor (type I S), benzamidine hydrochloride, *N*-hydroxysuccinimidobiotin, and phenylmethylsulfonyl fluoride were obtained from Sigma Chemical Co. (St. Louis, Mo.); nicotinamide was from Mann Research Laboratories,

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(New York, N.Y.); thiamine was from U.S. Biochemical Corp. (Cleveland, Ohio); and tryptic soy broth was from Oxoid Ltd. (Basingstoke, Hants, United Kingdom). Rabbit immunoglobulin G (IgG) was purified from rabbit serum by affinity chromatography on protein A-Sepharose (Pharmacia, Uppsala, Sweden). CNBr-activated Sepharose 4B and Percoll were also purchased from Pharmacia. Coupling of proteins to CNBr-activated Sepharose was performed according to the manufacturer's recommendations. ATP monitoring kits were from LKB-Wallac (Bromma, Sweden) and perhydrol, D-biotin, calcium pantothenate, and other chemicals of reagent grade were from E. Merck AG (Darmstadt, Federal Republic of Germany). Double-distilled water was used throughout all procedures. Highly purified protein A was donated by J. Sjöqvist, Department of Medical and Physiological Chemistry, University of Uppsala, Uppsala, Sweden; human plasma fibronectin, isolated as described by Vuento and Vaheri (50), and the NH_2-terminal fibronectin fragment (M_r, 29,000), purified from plasmin-digested fibronectin with DEAE-cellulose and molecular sieve chromatography, were supplied by S. Johansson of the same department or were prepared in our laboratory. Native rat skin collagen type I was isolated (30) and, when applicable, denatured at 50°C for 20 min.

Bacterial strains. *S. aureus* Cowan 1, Newman, Wood 46, and SA113(83A) were used in the study. Cells from these strains were defined concerning their interactions with soluble fibronectin (40) and IgG (41a). Bacteria were kept on deep agar at −70°C, cultured on blood agar, and subsequently grown in tryptic soy broth (16). The tryptic soy broth medium was supplemented (4 ml/liter) with a solution containing calcium pantothenate (2 mg/ml), thiamine (1 mg/ml), D-biotin (0.01 mg/ml), and nicotinamide (1.2 mg/ml), all dissolved in PBS-D (0.14 M NaCl, 2.7 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 8 mM Na_2HPO_4, 1.5 mM NaH_2PO_4, pH 7.4). Exponentially growing cells were harvested at an A_{540} of 0.2 (2 to 4 h of culture) unless otherwise specified, washed in PBS-D, and resuspended in the same medium to the indicated concentrations. Cells in the stationary growth phase were harvested after 12 to 16 h. Live bacteria were used in all experiments unless otherwise stated.

Coating of plastic surfaces. Adhesion experiments were performed in Microtiter plates (Dynatech, Denkendorf, Federal Republic of Germany) passively coated at room temperature with solutions of denatured collagen type I (3 mg/ml, overnight), fibronectin (0.05 mg/ml, 3 h), or BSA (10 mg/ml, 20 min). Coating with native collagen type I (3 mg/ml) was performed at 4°C overnight. The volume added to each well was 50 μl, and all proteins were dissolved in PBS (0.13 M NaCl, 8.35 mM Na_2HPO_4, 1.65 mM NaH_2PO_4). A denatured collagen-fibronectin complex was formed by incubating a solution of fibronectin (0.05 mg/ml) on a plastic surface precoated with denatured collagen as described above. After each coating procedure the surfaces were washed four times with cold PBS-D to remove unbound protein.

Attachment assay. Bacterial suspensions were added to protein-coated plates and incubated for the desired time at 4°C with slow agitation (6 cycles per min). The plates were then washed four times with cold PBS-D to remove unattached bacteria. Bacterial attachment was determined by the quantification of bacterial ATP bound to the plates in comparison with the amount initially added to each of the same plates. For this purpose, bacteria were lysed by the addition of trichloroacetic acid at a final concentration of 1.25%. A portion of each sample was mixed with ATP-monitoring reagent, and the ATP content of the lysates was then determined by the firefly luciferase assay (23, 24). All determinations were made at room temperature with a Luminometer 1250, a potentiometric recorder (LKB-Wallac), and the procedure recommended by the manufacturer. The amount of ATP versus the bacterial count calculated from absorbance and CFU (the standard curve) was linear within the experimental ranges used. The ATP content per bacterium did not vary with the growth phase for any particular *S. aureus* strain and was similar for the various strains used. The detection limit of the method was approximately 10^4 *S. aureus* cells (10 fmol of ATP).

Inhibition experiments. Fibronectin, the NH_2-terminal fibronectin fragment (M_r, 29,000), rabbit IgG, and BSA were investigated as to ability to inhibit bacterial attachment to denatured collagen or to fibronectin immobilized on denatured collagen. Bacteria were preincubated in tubes with the relevant protein solution end over end for 2 h at 4°C. The tubes were pretreated with BSA to minimize nonspecific bacterial attachment to their walls. Bacteria were then seeded, still in the presence of the added protein, on plates coated as described above, and bacterial attachment was determined. When samples were incubated in solutions with a protein concentration of ≥0.1 mg/ml, the ATP level increased, and this required correction. The effects of various concentrations of purified staphylococcal fibronectin-binding proteins and of staphylococcal protein A were studied by adding the respective substances to plates coated as described above. The plates were incubated for 2 h at 20°C and then washed four times with cold PBS-D before the bacteria were added.

Binding of ^{125}I-fibronectin to staphylococci. Binding was measured as described earlier (33). Heat-treated (90°C for 20 min) staphylococcal cells (10^9) were incubated end over end at 20°C with 10^5 cpm of ^{125}I-fibronectin, iodinated by the chloramine T method, in 0.5 ml of 0.1% albumin in PBS-D. At the desired time, 100 μl of the mixture was applied on a Percoll gradient and centrifuged. The supernatant was discarded, and the pellet was analyzed in a gamma counter.

Biotinylation of proteins. Fibronectin and standard proteins of high (Bio-Rad Laboratories, Richmond, Calif.) or low (Pharmacia) molecular weight were dissolved in 0.1 M $NaHCO_3$ (pH 9.0) to a total protein concentration of 1.0 mg/ml and dialyzed against the same buffer overnight. To 0.5 ml of each protein solution, 60 μl of *N*-hydroxysuccinimidobiotin in dimethyl sulfoxide was added, and the mixture was incubated at 20°C for 4 h. The reaction was terminated by dialyzing against PBS with 0.02% sodium azide at 4°C overnight. The migration of the biotinylated standard proteins in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was identical with that of their nonbiotinylated counterparts.

Purification of fibronectin-binding proteins from *S. aureus*. The isolation of staphylococcal cell wall components with affinity for fibronectin was described earlier (33). The same procedure was used in this study except that bacteria were lysed in a buffer containing 50 mM benzamidine, 2 mM phenylmethylsulfonyl fluoride, and 10 mM EDTA to reduce protease activity. Heat-inactivated (90°C for 20 min) lysostaphin cell digests of *S. aureus* Newman or SA113(83A), harvested from stationary cultures, were subjected to precipitation by ammonium sulfate (35 to 65% saturation), passed through an IgG-Sepharose column (to deplete the material of protein A), and then subjected to affinity chromatography on fibronectin-Sepharose. Fibronectin-binding components were eluted from the fibronectin-Sepharose by treatment with a low-pH buffer (0.1 M glycine, 0.5 M NaCl,

pH 3). In accordance with earlier findings (33), the activity of the purified components was sensitive to trypsin treatment, indicating that the activity resided in protein.

The purification of the fibronectin-binding proteins was measured employing an enzyme-linked avidin-biotin technique. Biotin-labeled (see above) soluble fibronectin was shown to bind specifically to ammonium sulfate-precipitated proteins from lysostaphin-digested *S. aureus* cells. The inhibition of this binding by fibronectin-binding proteins from staphylococcal cells was used to assay the amount of this binding activity. Microtiter plates (Dynatech) were coated overnight with 50 μl of ammonium sulfate (35 to 65% ammonium sulfate saturation)-precipitated staphylococcal protein dissolved in PBS-azide to a concentration of 50 μg/ml. Remaining protein-binding sites on the plastic surfaces were blocked by the addition of 0.5% BSA in PBS-azide. The samples (40 μl) to be assayed were then added in serial dilutions. A solution (20 μl) containing biotin-labeled fibronectin was subsequently added at a concentration of 20 μg/ml in PBS-D (with 0.5% Tween 20) in a total volume of 50 μl per well. The plates were incubated at 4°C for 4 h, and the amount of bound fibronectin was determined by incubation with avidin covalently linked to alkaline phosphatase. Bound enzyme was determined by registration of color development after the addition of *p*-nitrophenol (1 mg/ml) at pH 9.8, measured at 405 nm in a Titertek multiscan spectrophotometer. The plates were washed as described previously (12) between each step. Suitable concentrations of biotinylated fibronectin and avidin-alkaline phosphatase had previously been determined in titration experiments.

Treatment of bacteria with trypsin. To investigate the location of the fibronectin-binding proteins, bacterial cells were digested with trypsin before lysostaphin treatment and ammonium sulfate precipitation. Bacteria from stationary cultures of strains Newman and SA113(83A) were harvested, washed three times with cold PBS, and incubated with trypsin (50 μg/ml) for 40 min at 20°C. The degradation was terminated by adding soybean trypsin inhibitor (100 μg/ml) and incubating the solution at 20°C for 10 min. The cell pellet was washed twice with cold PBS and lysed as described above. The lysostaphin digests were subsequently precipitated with the ammonium sulfate procedure, subjected to SDS-PAGE, and then transferred to nitrocellulose sheets (see below) for detection of remaining fibronectin-binding capacity. The enzyme-linked avidin-biotin system revealed only minute amounts of fibronectin-binding proteins in the lysates from trypsin-treated bacteria.

SDS-PAGE. Fibronectin-binding proteins were investigated with SDS-PAGE on 10 to 15% polyacrylamide gradient slab gels (3). The samples were dialyzed against water, lyophilized, and dissolved in sample buffer (4% SDS, 4% sucrose, 0.0625 M Tris hydrochloride [pH 8.8]) before electrophoresis. The samples were then reduced by adding β-mercaptoethanol to a final concentration of 10% and boiled for 3 min. Protein bands were visualized by silver staining (52).

Blotting procedure. Fibronectin-binding proteins were detected after electrophoretic transfer (200 mA, 24 h) of the proteins separated by SDS-PAGE to nitrocellulose sheets (membrane filters, BA 83, 0.2 μM; Schleicher and Schuell, Dassel, Federal Republic of Germany) in a buffer consisting of 20 mM Tris hydrochloride, 150 mM glycine, and 20% methanol. After blotting, the nitrocellulose sheets were incubated with 3% BSA in PBS for 1 h at 20°C to inhibit nonspecific binding. The filters were then incubated with fibronectin (200 μg/ml in PBS containing 0.1% BSA) and washed four times (0.15 M NaCl–20 mM Tris hydrochloride [pH 7.4] in the first and last washing bath and the same buffer containing 0.1% Tween 20 in the other two washes). A specific rabbit anti-human fibronectin serum diluted 1/50 was used in the following incubation, and after washing (as described above) the nitrocellulose sheets were incubated with protein A-peroxidase conjugate (Bio-Rad) and washed as before. The proteins were visualized with horseradish peroxidase color development reagent (Bio-Rad). The migration of biotin-labeled standard proteins in SDS-PAGE was visualized after electrophoretic transfer of the protein bands (as described above) to nitrocellulose sheets and incubation of the latter with streptavidin-peroxidase (Amersham International, Amersham, United Kingdom). All incubations were performed at 20°C for 90 min.

RESULTS

Staphylococcal attachment to immobilized fibronectin and collagen. Cells from *S. aureus* Newman, Cowan 1, and SA113(83A) harvested during the exponential growth phase were found to attach to surfaces coated with fibronectin. Surfaces formed by the immobilization of fibronectin on denatured collagen offered the most effective substrate for bacterial attachment, and fibronectin markedly enhanced bacterial attachment to surfaces coated with denatured collagen (Fig. 1). When harvested in the stationary growth phase, cells from strain SA113(83A) did not attach to any of the fibronectin-coated surfaces (Fig. 1). *S. aureus* Cowan 1 attached to native and denatured collagen type I irrespective of the growth phase. Cells from the other three tested strains only showed background attachment to surfaces coated with either native or denatured collagen. Attachment of staphylococcal cells to uncoated plastic tissue culture plates was of the same order of magnitude as the maximal attachment to fibronectin-coated surfaces, but this interaction was not studied further.

Bacterial attachment to surfaces coated with BSA was defined as the background level. The data shown in Fig. 1 were from one representative experiment. Five independent experiments of this type were performed in the present study. The degree of attachment varied among these experiments, but the above-described general pattern was reproducible.

For further, more detailed investigations we used *S. aureus* SA113(83A), because cells from this strain showed a high difference between attachment in the presence or absence of fibronectin.

Time course of attachment to fibronectin. The kinetics by which cells of strains SA113(83A) attached to fibronectin immobilized on denatured collagen proved to be biphasic, with a decreased velocity after 90 min followed by a second reaction for up to 240 min (Fig. 2). The second reaction may have reflected a tendency of the staphylococcal cells to aggregate (21), and microscopy indeed revealed bacterial aggregates after long incubation times (data not shown).

Cells of strain SA113(83A) did not attach to denatured collagen within the 240-min time period of the experiment (Fig. 2).

Proportion of total staphylococcal cells able to attach to fibronectin. The possibility that a subpopulation within SA113(83A) cells was responsible for the observed attachment to immobilized fibronectin was explored. Logarithmically growing cells were incubated on fibronectin as described above, and after 90 min unattached bacteria were removed and reseeded on a fresh fibronectin-coated plate

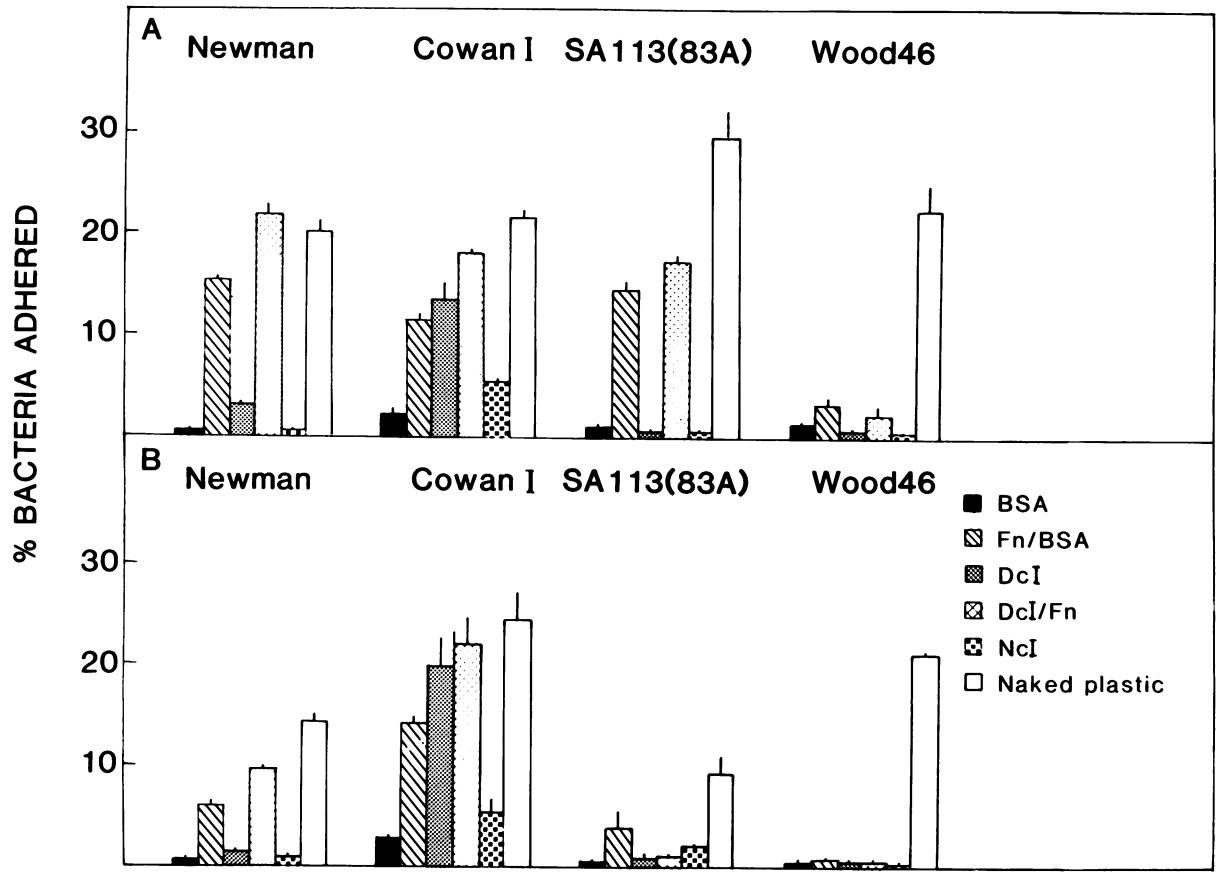

FIG. 1. Percent attachment of staphylococcal cells from four strains to six surfaces. (A) Bacteria harvested in the exponential growth phase (at an absorbance at 540 nm of 0.19 to 0.23). (B) Bacteria harvested in the stationary growth phase (at an absorbance at 540 nm of 7.3 to 9.7). Surface coatings: Fn/BSA, fibronectin and then BSA; DcI, denatured collagen type I; DcI/Fn, DcI and then fibronectin; NcI, native collagen type I. Samples of 50 μl of bacteria were seeded on each well in the following cell concentrations (per milliliter): Newman, (A) 5×10^7 and (B) 1.4×10^8; Cowan I (A) 6×10^7 and (B) 3×10^7; SA113(83A), (A) 1×10^8 and (B) 2×10^8; Wood 46, (A) 2×10^8 and (B) 2×10^8. The bacterial suspensions used in attachment assay showed an A_{540} of between 0.195 and 0.207. Levels are means of five independent determinations; standard deviations are indicated.

(Fig. 3). After four such passages, 76% of the initially added bacterial ATP was recovered as ATP bound to the fibronectin-coated surfaces. The unattached bacteria from the last incubation represented 15% of the initially added ATP. Thus, the assay achieved recovery of 91% of the initial addition.

Dependence on fibronectin concentration. The attachment of strain SA118(83A) cells was affected by concentration of fibronectin in the solutions used to coat plastic dishes (Fig. 4). Maximal attachment was achieved on plates preincubated with solutions containing ≥50 μg of fibronectin per ml (Fig. 4).

Dependence on bacterial growth phase. The relationship between bacterial growth phase and capacity to interact with fibronectin was studied with cells of strain SA113(83A). A constant number of cells, as determined by absorbance at 540 nm, was withdrawn from a growing culture at predetermined times. The absolute numbers of cells in these samples were later established from counts of CFU and measurements of ATP. Binding of soluble ^{125}I-fibronectin to staphylococcal cells and attachment of the bacteria to denatured collagen in the presence or absence of fibronectin were determined (Fig. 5). The ability to interact with soluble or with immobilized fibronectin showed a time-related decrease throughout the bacterial growth cycle (Fig. 5), indicating similar regulatory processes involved in binding of soluble fibronectin by staphylococcal cells and in attachment of cells to immobilized fibronectin. At no stage in the growth cycle could cells of strain SA113(83A) attach to immobilized denatured collagen (Fig. 5).

Inhibition of attachment to fibronectin-coated surfaces by soluble fibronectin and by amino-terminal fibronectin fragment. Exponentially growing cells from strain SA113(83A) attached to immobilized fibronectin in a reaction that was inhibited by soluble fibronectin (Fig. 6). Inhibition was observed only above a certain critical concentration of fibronectin; lower concentrations of fibronectin stimulated binding. The attachment of bacteria to denatured collagen was stimulated by fibronectin within a certain concentration range (Fig. 6A). The NH_2-terminal fibronectin fragment also inhibited the attachment of strain SA113(83A) cells to fibronectin-coated surfaces but had no stimulatory effect on the attachment to denatured collagen (Fig. 6B). The presence of BSA (Fig. 6C) or rabbit IgG (data not shown) in the incubation media did not affect attachment.

Characterization of staphylococcal fibronectin-binding proteins. Soluble staphylococcal fibronectin-binding proteins purified from *S. aureus* Newman and SA113(83A) as de-

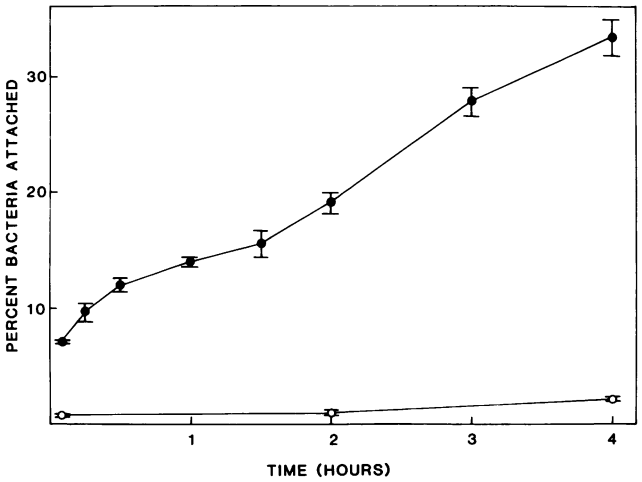

FIG. 2. Time course of bacterial attachment to fibronectin immobilized on denatured collagen (●) and to denatured collagen (○). *S. aureus* SA113(83A) cells were harvested in exponential growth phase. The values express percentages of attached bacteria relative to the total amount added (10^9 bacteria per ml); means of two independent experiments with total ranges are indicated.

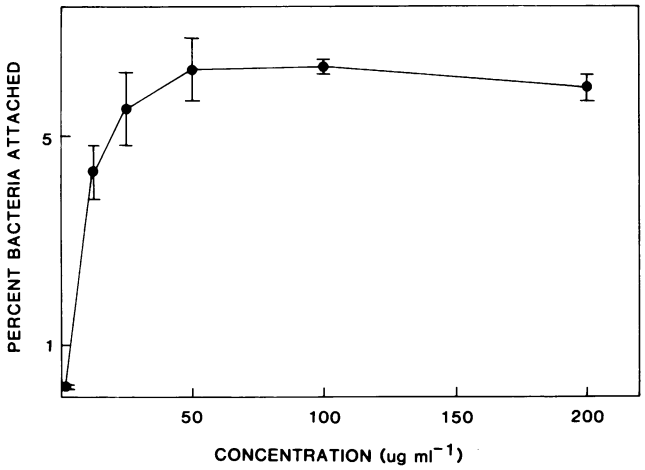

FIG. 4. Effect of fibronectin concentration on the attachment of exponentially growing *S. aureus* SA113(83A) cells to denatured collagen incubated with solutions containing increasing concentrations of fibronectin. Bacteria were allowed to attach for 90 min at 4°C in PBS-D. Attachment is expressed as the percentage of bound bacteria relative to the total number added (10^9/ml); means and total ranges from triplicate incubations are shown.

scribed in Materials and Methods were analyzed with SDS-PAGE, with blotting experiments, and for the ability to inhibit the attachment of staphylococci to immobilized fibronectin.

Analysis by SDS-PAGE and silver staining of gels revealed several protein bands in the material eluted from fibronectin-Sepharose (Fig. 7). The protein pattern was similar for material originating from the two staphylococcal strains (Fig. 7, lanes C and D), although more of the material originating from strain Newman had been applied to the gel. Most of the protein bands, especially in the low-molecular-weight range present in the unfractionated lysates (Fig. 7, lanes E and F), were lacking in the corresponding purified material. After separation by SDS-PAGE of material that had been fractionated on fibronectin-Sepharose, proteins were electrophoretically transferred to nitrocellulose sheets and subsequently incubated with soluble fibronectin. Bands capable of binding the soluble ligand could be visualized (Fig. 8, lanes E and F). When unfractionated lysates were

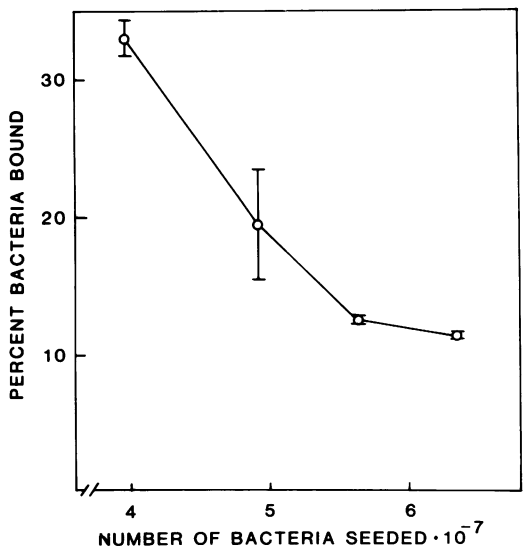

FIG. 3. Attachment of *S. aureus* SA113(83A) cells harvested from cultures in the exponential growth phase as a function of the number of bacteria seeded on dishes coated with fibronectin immobilized on denatured collagen type I. After 90 min of incubation, unattached bacteria were removed and reseeded on new, identically coated dishes; four passages were performed. Values express bound bacteria as percentages of the total added; means and total ranges from triplicate incubations are shown.

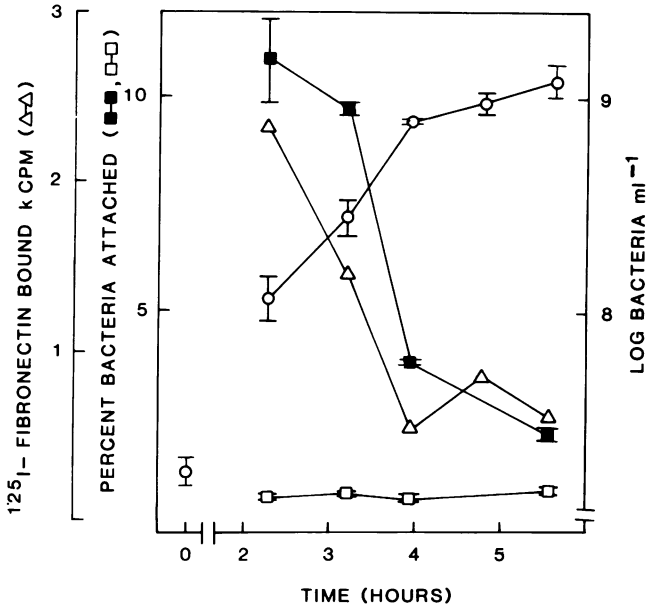

FIG. 5. Dependence on growth phase of the interaction of *S. aureus* SA113(83A) with fibronectin. Bacteria were harvested from cultures at the indicated points of time (○), and binding of soluble ^{125}I-fibronectin (△) and the attachment to denatured collagen in the presence (■) or absence (□) of fibronectin were determined. The concentration of the bacterial suspensions used for attachment was $3 \times 10^8 \pm 0.3 \times 10^8$/ml. Means and ranges are indicated.

analyzed with the same procedure, a similar pattern was obtained (Fig. 8, lanes C and D), but fibronectin-binding proteins with M_rs in the range of 68,000 to 103,000 were depleted after purification. High-molecular-weight fibronectin-binding protein bands with M_rs of approximately 185,000 were detected.

When the above analyses were done on lysates from bacterial cells of strain Wood 46 and from trypsin-treated cells of strains Newman and SA113(83A), these lysates were found to lack detectable fibronectin-binding protein bands (data not shown).

Inhibition of attachment to immobilized denatured collagen-fibronectin by soluble staphylococcal fibronectin-binding proteins. When the fibronectin substrates were incubated with staphylococcal fibronectin-binding protein isolated from SA113(83A) or Newman before the addition of bacteria, the attachment of cells from strain SA113(83A) was inhibited (Fig. 9A). This inhibitory effect was strongly dependent on concentration, with a narrow cutoff interval. Preincubation of the substrate with staphylococcal protein A was without effect (Fig. 9B).

DISCUSSION

This study concerned the mechanisms of attachment of cells from four strains of *S. aureus* to immobilized fibronectin and collagen. Cells from strains SA113(83A) and Newman showed a marked attachment preference for surfaces on which fibronectin had been immobilized. Cells from *S. aureus* Cowan 1 attached well, but strain Wood 46 attached poorly, to surfaces coated with fibronectin or collagen. Cells from strain Cowan 1 attached to surfaces coated with both denatured as well as native collagen type I even in the absence of fibronectin. All cells were found to attach to uncoated plastic dishes. Simple digestion of the matrix by staphylococcal proteases and subsequent attachment of bacteria to the underlying plastic seems unlikely. This conclusion was drawn from the observations that denatured collagen or BSA blocked attachment to plastic dishes of cells from strains SA113(83A) and Newman and that the presence of fibronectin was a prerequisite for the attachment of these strains. Furthermore, the studies on the inhibitory effect of soluble fibronectin on attachment (Fig. 6) indicated that, at least in high concentrations, soluble fibronectin did not trigger proteolytic activity sufficiently to enable bacteria to attach to free plastic surfaces generated after a possible degradation of the matrix. The possibility of such degradation after, or possibly in response to, an initial attachment to fibronectin cannot be excluded.

The attachment to fibronectin was studied further with cells from SA113(83A). Most bacterial cells within a given population of this strain expressed ability to attach to fibronectin (Fig. 3). The percentage of cells that attached was dependent on the number of bacteria seeded. The maximum number of bacteria that attached per surface area for any particular strain tended to be constant when above a saturation level.

Proteins purified by affinity chromatography on fibronectin-Sepharose from lysostaphin digests of *S. aureus* Newman and SA113(83A) readily inhibited the attachment process (Fig. 9). In agreement with earlier findings (33), we believe that staphylococcal protein A was not responsible for the observed activity, since passage of the lysates through an IgG-Sepharose column did not remove the inhibitory activity. Furthermore, purified protein A in high concentrations did not inhibit attachment.

That fibronectin-binding activity of the purified material was not destroyed by the purification procedure was ascertained in two ways. First, the enzyme-linked avidin-biotin assay showed that the purified proteins retained their fibronectin-binding activity. Second, purified material that was separated on SDS-PAGE and subsequently transferred to nitrocellulose bound intact native fibronectin (Fig. 8). SDS-PAGE analysis showed the purified fibronectin-binding proteins to contain several protein bands. The heterogeneity as to apparent molecular weights most probably was due to proteolytic degradation during the preparation procedure,

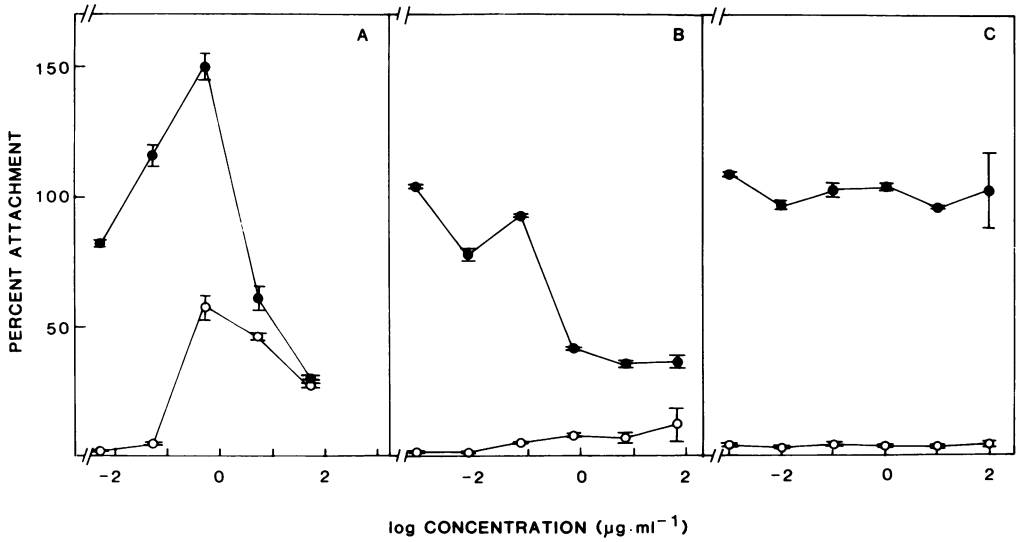

FIG. 6. Inhibition of the attachment of *S. aureus* SA113(83A) by soluble fibronectin, the NH_2-terminal fibronectin fragment, and BSA. Bacteria (7×10^8/ml) was seeded on surfaces to which fibronectin had been immobilized on denatured collagen (●) or on surfaces coated with denatured collagen (○). Inhibitors: A, fibronectin; B, the 29-kilodalton amino-terminal fibronectin fragment; C, BSA. Bacteria were preincubated with the relevant proteins for 2 h at 4°C in indicated concentrations and then seeded on substrate and allowed to attach for 90 min at 4°C. Shown is the relative attachment when 100% is the value in the absence of inhibitors. Ranges within triplicate incubations are indicated.

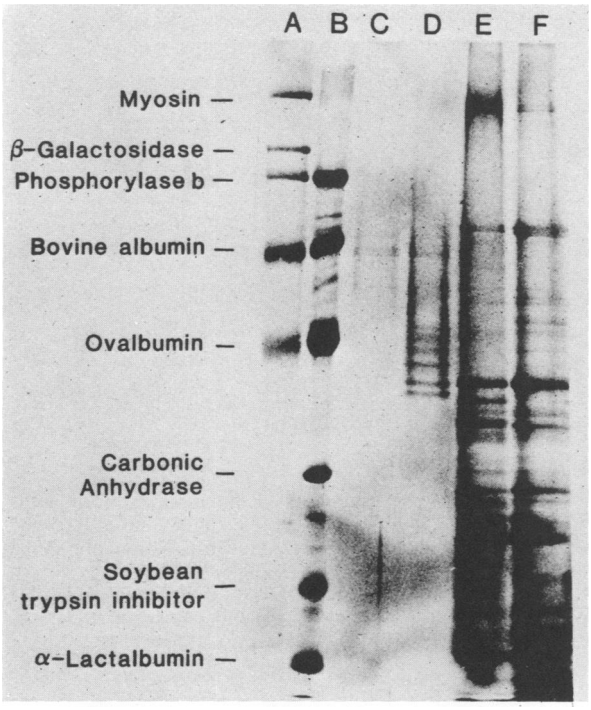

FIG. 7. SDS-PAGE of cell wall material from staphylococci. All samples were reduced. Molecular weight standards (M_r): in lane A, myosin (200,000), β-galactosidase (116,250), phosphorylase *b* (92,500), BSA (66,200), and ovalbumin (45,000) from Bio-Rad; in lane B, rabbit muscle phosphorylase (94,000), bovine serum albumin (67,000), chicken ovalbumin (43,000), bovine erythrocytes carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and bovine milk α-lactalbumin (14,400) from Pharmacia. Other lanes: C, fibronectin-binding proteins from *S. aureus* SA113(83A), 50 μl of a protein solution with A_{280} of 0.075; D, fibronectin-binding proteins from strain Newman, 50 μl of a protein solution with A_{280} of 0.3; E, ammonium sulfate-precipitated material from lysostaphin digests of cells, 50 μg of protein from strain SA113(83A); F, as in E, but from strain Newman (both lysates were passed through an IgG-Sepharose column before electrophoresis).

although protease inhibitors were added to the lysis buffer. This assumption is favored by the finding that staphylococcal proteases, including staphylococcal V8 protease (1), degraded the purified high-molecular-weight fibronectin-binding protein (G. Fröman, personal communication). Furthermore, the fibronectin molecule has been reported to have more than one staphylococcal binding domain (14). Hence several staphylococcal fibronectin-binding proteins may add to the observed heterogeneity. The observation that the protein bands, visualized by silver staining, bound fibronectin in blotting experiments indicated the purification procedure to be specific for fibronectin-binding proteins (Fig. 7 and 8). The observed inhibitory effect on attachment thus was most likely due to the ability of the proteins to bind fibronectin. The band with M_r 185,000 was of the highest molecular weight detectable in any fibronectin-binding protein in repeated experiments. This band may correspond to a native staphylococcal fibronectin receptor which is proteolytically degraded during the processing of bacteria. In other studies (9), fibronectin-binding proteins with apparent molecular weights 197,000 and 60,000 were isolated from sonicated staphylococcal cells. The procedure for isolating proteins differed between these studies and ours, which may explain the observed differences in the degree of proteolytic degradation.

The yields per cell of purified fibronectin-binding proteins differed between strains Newman and SA113(83A) when both were harvested from stationary cultures, with a reproducably fivefold-higher yield from strain Newman. When fibronectin-binding proteins were purified from logarithmically growing cultures of strain SA113(83A), however, the yield per cell increased (data not shown). The maximal yield of fibronectin-binding proteins from strain SA113(83A) nevertheless was greater from stationary cultures, due to the much higher numbers of bacteria that could then be handled. We therefore chose stationary cultures as starting material for purification in the present study. The yield differences between stationary cultures and logarithmically growing cultures of SA113(83A) were paralleled by differences in ability to attach to fibronectin-coated surfaces (Fig. 1 and 5). The decrease seen both in the attachment of cells from SA113(83A) to fibronectin-coated surfaces and in the yield of fibronectin-binding proteins from these cells may be explained either by reduced synthesis of fibronectin-binding proteins late in the growth cycle or by proteolytic removal of such components. Alternatively, the bacteria may produce capsular material (54) late in the growth cycle, which material could act as a steric hindrance to the binding of fibronectin to the cell surface. This lends further support to the concept that the purified fibronectin-binding proteins were involved in the attachment reaction.

Lysates of trypsin-treated bacteria were found to be devoid of fibronectin-binding proteins (data not shown). This

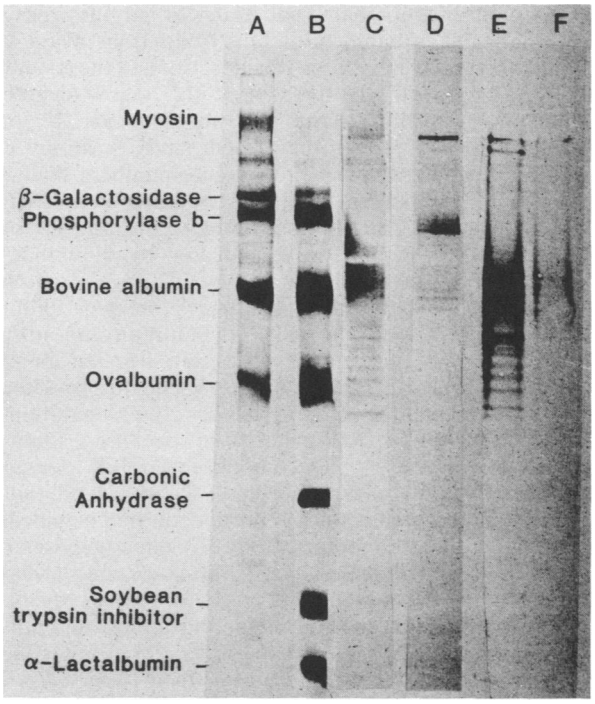

FIG. 8. Fibronectin binding to staphylococcal proteins transferred to nitrocellulose after separation on SDS-PAGE. Lanes A and B show biotinylated molecular weight standards (as in Fig. 7). Other lanes: C, fibronectin binding to ammonium sulfate-precipitated material from strain Newman; D, corresponding material from SA113(83A); E and F, fibronectin binding to purified fibronectin-binding proteins from Newman and SA113(83A), respectively. The amounts of protein applied to the gel are as in Fig. 7; lysates were passed through an IgG-Sepharose column before electrophoresis.

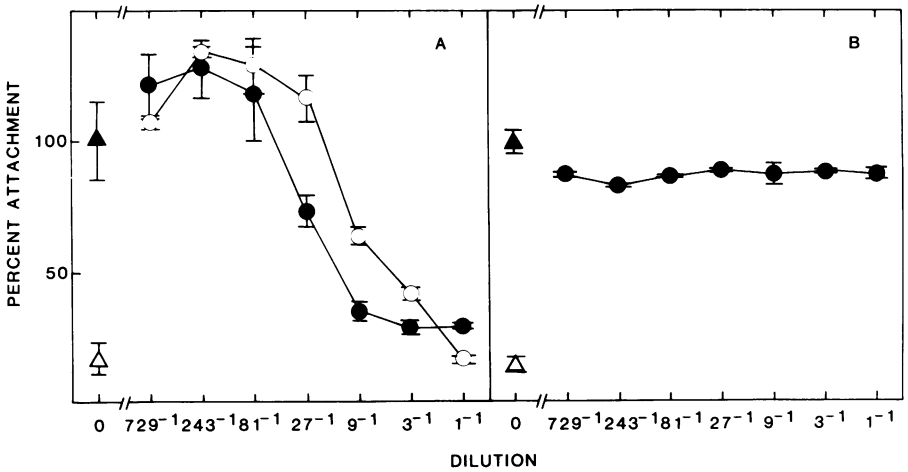

FIG. 9. Influence of purified fibronectin-binding proteins on the attachment of *S. aureus* SA113(83A) to immobilized fibronectin. (A) Proteins isolated from strains SA113(83A) (○) and Newman (●), showing absorbances of the undiluted samples at 280 nm of 0.05 and 0.2, respectively. (B) Staphylococcal protein A. The concentration of undiluted sample was 1 mg/ml. Surfaces coated with a fibronectin-denatured collagen complex were incubated with the respective bacterial surface protein for 2 h at 20°C before the seeding of bacteria at (A) 8×10^7/ml or (B) 7×10^8/ml. Bacteria were allowed to attach for 90 min at 4°C in PBS-D. The percentage of bound bacteria is relative to 100% binding in the absence of inhibitor. Staphylococcal attachment to denatured collagen (△) and to fibronectin-denatured collagen (▲) in the absence of staphylococcal fibronectin-binding proteins is also indicated. Ranges from duplicate incubations are shown.

observation was in analogy with our earlier study (33) of the binding of soluble fibronectin by staphylococci. Together the results indicated a surface location of the staphylococcal fibronectin-binding proteins.

Several recent reports dealt with the attachment of *S. aureus* to fibronectin-coated substrates of various types (45, 46, 49). Specific attachment of cells from strain Wood 46 to fibronectin-coated substrates was described in these studies. In comparison with the present study, a low degree of attachment in absolute terms was observed: ca. 10^5 organisms per cm^2 (45, 46). In the present study *S. aureus* cells of strain Newman attached in absolute numbers that were higher by more than a magnitude as compared with strain Wood 46 (Fig. 1). The contrast between our and the other (45, 46, 49) results may be explainable by differences in methodology and in definitions of attachment. Alternatively, there may be cell surface differences between different substrains of Wood 46 (51). In ammonium sulfate-precipitated material from lysostaphin-digested bacteria from stationary cultures of *S. aureus* Wood 46, we detected no fibronectin-binding protein bands after separation on SDS-PAGE followed by transfer to nitrocellulose (data not shown). This accords with the observed low degree of attachment for this strain. Earlier (41) we found that *S. aureus* Wood 46 bound soluble fibronectin to a significantly lower degree than did strain Cowan 1. Collectively the data show that a subpopulation of *S. aureus* Wood 46 carries fibronectin-binding proteins or, possibly, that all organisms in a population carry these proteins, but in low amounts.

The presence of soluble intact fibronectin at low concentrations (≤1 mg/ml) stimulated attachment (Fig. 6A). This finding can be explained by an additional binding of fibronectin to the collagen substrate in spite of the high fibronectin concentrations used in the coating of the collagenous surface (Fig. 4). The NH_2-terminal fibronectin fragment, lacking the collagen-binding domain, did not at any concentration stimulate attachment, a finding speaking in favor of the above assumption. Fibronectin-fibronectin interactions may also add to the stimulatory effect on attachment. Nevertheless, both the NH_2-terminal fragment as well as high concentrations (>10 μg/ml) of intact fibronectin clearly inhibited the attachment of staphylococcal cells to fibronectin.

Cells from strain Cowan 1 attached to collagenous substrates also in the absence of fibronectin. This is in agreement with a recent report demonstrating attachment of cells from *S. aureus* Cowan 1 and 52A5 to surfaces coated with the basal membrane collagen type IV (49). Furthermore, *S. aureus* cells have been shown to possess receptors for laminin (22). Thus, cells from *S. aureus* have several different possibilities to interact with components of the extracellular matrix.

Eucaryotic cell adhesion has been shown to be mediated by specific adhesive proteins, and a number of cell adhesion molecules have been characterized (7). In accordance with earlier studies (2, 17, 25, 39), the present study indicates that similar molecules exist in procaryotes, exemplified here by the attachment of *S. aureus* cells to fibronectin-coated surfaces. Data presented herein indicate that the attachment of cells from *S. aureus* SA113(83A) to immobilized fibronectin is a specific receptor-ligand interaction, a concept supported by the inhibition of the reaction by soluble fibronectin (Fig. 6) as well as by solubilized fibronectin-binding staphylococcal proteins (Fig. 9) (2).

During preparation of this manuscript, two independent reports on binding of collagen to *S. aureus* appeared. These reports (11, 38) are in agreement with the findings reported here. In addition, Kuusela et al. (15) recently reported on the attachment of cells from *S. aureus* Cowan 1 to fibronectin immobilized on glass cover slips. This attachment could not be inhibited by soluble fibronectin, which is in contrast to our findings. The reason for this discrepancy is not clear, but it may in part be due to conformation differences of fibronectin when immobilized on different surfaces.

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LITERATURE CITED

1. **Arvidsson, S. O.** 1983. Extracellular enzymes from *Staphylococcus aureus*, p. 745–808. *In* C. S. F. Easmon and C. Adlam (ed.), Staphylococci and staphylococcal infections. Academic Press, Inc., London.
2. **Beachey, E. H.** 1981. Bacterial adherence: adhesion receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Infect. Dis. **143**:325–345.
3. **Blobel, G., and B. Dobberstein.** 1975. Transfer of proteins across membranes. J. Cell Biol. **67**:835–851.
4. **Blobel, H., W. Schaeg, and J. Brüchler.** 1979. Demonstration and inactivation of staphylococcal clumping factor. p. 487–491. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections. Gustav Fischer Verlag, Stuttgart, Federal Republic of Germany.
5. **Brown, R. A.** 1983. Hypothesis: Failure of fibronectin as an opsonin in the host defense system: a case of competitive self inhibition? Lancet **ii**:1058–1060.
6. **Czop, J. K., J. L. Kadish, and F. Austen.** 1981. Augmentation of human monocyte opsonin-independent phagocytosis by fragments of human plasma fibronectin. Proc. Natl. Acad. Sci. USA **78**:3649–3653.
7. **Damsky, C. H., K. A. Knudsen, and C. A. Buck.** 1985. Integral membrane glycoproteins in cell-cell and cell-substratum adhesion, p. 1–64. *In* R. J. Ivatt (ed.), The biology of glycoproteins. Plenum Publishing Corp., New York.
8. **Eriksen, H. O., F. Espersen, and I. Clemmensen.** 1984. Opsonic activity of fibronectin in the phagocytosis of *Staphylococcus aureus* by polymorphonuclear leukocytes. Eur. J. Clin. Microbiol. **3**:108–112.
9. **Espersen, F., and I. Clemmensen.** 1982. Isolation of a fibronectin-binding protein from *Staphylococcus aureus*. Infect. Immun. **37**:526–531.
10. **Fröman, G., L. M. Switalski, A. Faris, T. Wadström, and M. Höök.** 1984. Binding of *Escherichia coli* to fibronectin. J. Biol. Chem. **23**:14899–14905.
11. **Holderbaum, D., R. A. Spech, and L. A. Ehrhart.** 1985. Specific binding of collagen to *Staphylococcus aureus*. Collagen Rel. Res. **5**:261–276.
12. **Klareskog, L., R. Holmdahl, E. Larsson, and H. Wigzell.** 1983. Role of T-lymphocytes in collagen II induced arthritis in rats. Clin. Exp. Immunol. **51**:117–125.
13. **Kuusela, P.** 1978. Fibronectin binds to *Staphylococcus aureus*. Nature (London) **276**:718–720.
14. **Kuusela, P., T. Vartio, M. Vuento, and E. B. Myhre.** 1984. Binding sites for streptococci and staphylococci in fibronectin. Infect. Immun. **45**:433–436.
15. **Kuusela, P., T. Vartio, M. Vuento, and E. B. Myhre.** 1985. Attachment of staphylococci and streptococci on fibronectin, fibronectin fragments, and fibrinogen bound to a solid phase. Infect. Immun. **50**:77–81.
16. **Landvall, P.** 1978. Dialysis culture for the production of extracellular protein A from *Staphylococcus aureus* A 676. J. Appl. Bacteriol. **44**:151–158.
17. **Lanser, M. E., and T. M. Saba.** 1981. Fibronectin as a co-factor necessary for optimal granulocyte phagocytosis of *Staphylococcus aureus*. RES J. Reticuloendothel. Soc. **30**:415–424.
18. **Lee, D. A., J. R. Hoidal, C. C. Clawson, P. G. Quie, and P. K. Peterson.** 1983. Phagocytosis by polymorphonuclear leukocytes of *Staphylococcus aureus* and *Pseudomonas aeruginosa* adherent to plastic, agar or glass. J. Immunol. Methods **63**:103–114.
19. **Leffler, H., and C. Svanborg-Edén.** 1981. Glycolipid receptors for uropathogenic *Escherichia coli* on human erythrocytes and uroepithelial cells. Infect. Immun. **34**:920–929.
20. **Linder, E., A. Vaheri, E. Rouslahti, and J. Wartiovaara.** 1975. Distribution of fibroblast surface antigen in the developing chick embryo. J. Exp. Med. **142**:41–49.
21. **Ljungh, Å., S. Hjertén, and T. Wadström.** 1985. High surface hydrophobicity by autoaggregating *Staphylococcus aureus* isolated from human infections studied with the salt aggregation test. Infect. Immun. **47**:522–526.
22. **Lopes, J. D., M. dos Reis, and R. R. Brentani.** 1985. Presence of laminin receptors in *Staphylococcus aureus*. Science **229**: 275–277.
23. **Ludwicka, A., B. Jansen, T. Wadström, and G. Pulverer.** 1984. Attachment of staphylococci to various synthetic polymers. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. **256**: 479–489.
24. **Lundin, A.** 1982. Application of firefly luciferase, p. 29–45. *In* M. Serio and M. Pazzagli (ed.). Luminescent assay: perspectives in endocrinology and clinical chemistry. Raven Press, New York.
25. **McNeish, A. S., J. Flemming, P. Turner, and M. Evans.** 1975. Mucosal adherence of human enteropathogenic *Escherichia coli*. Lancet **ii**:946–948.
26. **Miörner, H., E. Myhre, L. Björck, and G. Kronvall.** 1980. Specific binding of human albumin, fibrinogen, and immunoglobulin G on surface characteristics of bacterial strains as revealed by partition experiments in polymer phase systems. Infect. Immun. **29**:879–885.
27. **Mosher, D. F., and R. A. Proctor.** 1980. Binding and factor $XIII_a$-mediated cross-linking of a 27 kilodalton fragment of fibronectin to *Staphylococcus aureus*. Science **209**:927–929.
28. **Muller, H. P., and H. Blobel.** 1983. Absorption of human α-macroglobulin with selected strains of streptococci. Med. Microbiol. Immunol. **172**:33–39.
29. **Myhre, E. B., O. Holmberg, and G. Kronvall.** 1979. Immunoglobulin binding structures on bovine group G streptococci different from type III Fc receptors on human group G streptococci. Infect. Immun. **23**:1–7.
30. **Öbrink, B.** 1972. Non-aggregated tropocollagen at physiological pH and ionic strength: a chemical and physico-chemical characterization of tropocollagen isolated from the skin of lathyritic rats. Eur. J. Biochem. **25**:563–572.
31. **Proctor, R. A., D. F. Mosher, and P. J. Olbrantz.** 1982. Fibronectin binding to *Staphylococcus aureus*. J. Biol. Chem. **257**:14788–14794.
32. **Proctor, R. A., E. Prendergast, and D. F. Mosher.** 1982. Fibronectin mediates attachment of *Staphylococcus aureus* to human neutrophils. Blood **59**:681–687.
33. **Rydén, C., K. Rubin, P. Speziale, M. Höök, M. Lindberg, and T. Wadström.** 1983. Fibronectin receptors from *Staphylococcus aureus*. J. Biol. Chem. **258**:3396–3401.
34. **Schwartzbauer, J. E., J. W. Tamkun, I. R. Lemischka, and R. O. Hynes.** 1984. Three different fibronectin mRNAs arise by alternative splicing within the coding region. Cell **35**:421–431.
35. **Simpson, W. A., D. L. Hasty, J. M. Mason, and E. H. Beachey.** 1982. Fibronectin-mediated binding of group A streptococci to human polymorphonuclear leukocytes. Infect. Immun. **37**: 805–810.
36. **Speziale, P., M. Höök, L. M. Switalski, and T. Wadström.** 1984. Fibronectin binding to a *Streptococcus pyogenes* strain. J. Bacteriol. **157**:420–427.
37. **Speziale, P., M. Höök, and T. Wadström.** 1982. Binding of the basement membrane protein laminin to *Escherichia coli*. FEBS Lett. **146**:55–58.
38. **Speziale, P., M. Höök, and T. Wadström.** 1985. Binding of type II collagen to *Staphylococcus aureus*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. **14**:(Suppl.):191–196.
39. **Svanborg-Edén, C., R. Freter, L. Hagberg, R. Hull, S. Hull, H. Leffler, and G. Schoolnik.** 1982. Inhibition of experimental ascending urinary tract infection by an epithelial cell-surface receptor analogue. Nature (London) **298**:560–562.
40. **Switalski, L. M., Å. Ljungh, C. Rydén, K. Rubin, M. Höök, and T. Wadström.** 1982. Binding of fibronectin to the surface of group A, C and G streptococci isolated from human infections. Eur. J. Clin. Microbiol. **1**:381–387.
41. **Switalski, L. M., C. Rydén, K. Rubin, Å. Ljungh, M. Höök, and T. Wadström.** 1983. Binding of fibronectin to *Staphylococcus* strains. Infect. Immun. **42**:628–633.

41a. **Uhléu, M., B. Guss, B. Nilsson, F. Götz, and M. Lindberg.** 1984. Expression of the gene encoding protein A in *Staphylococcus aureus* and coagulase-negative staphylococci. J. Bacteriol. **159:**

713–719.
42. **Unni, S. K., and B. L. Middlebrook.** 1983. Streptococcal rheumatic carditis. Microbiol. Rev. **47**:97–120.
43. **Van de Water, L., A. T. Destree, and R. O. Hynes.** 1983. Fibronectin binds to some bacteria but does not promote their uptake by phagocytic cells. Science **220**:201–204.
44. **Vartio, T., H. Sheppa, and A. Vaheri.** 1981. Susceptibility of soluble and matrix fibronectins to degradation by tissue proteinases, mastcell chymase and cathepsin G. J. Biol. Chem. **256**: 471–477.
45. **Vaudaux, P. E., R. Suzuki, F. A. Waldvogel, J. J. Morgenthaler, and U. E. Nydegger.** 1984. Foreign body infection: role of fibronectin as a ligand for the adherence of *Staphylococcus aureus*. J. Infect. Dis. **150**:546–551.
46. **Vaudaux, P. E., F. A. Waldvogel, J. J. Morgenthaler, and U. E. Nydegger.** 1984. Adsorption of fibronectin onto polymethylmethacrylate and promotion of *Staphylococcus aureus* adherence. Infect. Immun. **4**:768–774.
47. **Verbrugh, H. A., P. K. Peterson, D. E. Smith, B. T. Nguyen, J. R. Hoidal, B. J. Wilkinson, J. Verhoef, and L. T. Furcht.** 1981. Human fibronectin binding to staphylococcal surface protein and its relative inefficiency in promoting phagocytosis by human polymorphonuclear leukocytes, monocytes, and alveolar macrophages. Infect. Immun. **33**:811–819.
48. **Vercellotti, G. M., D. Lussenhop, P. K. Peterson, L. T. Furcht, J. B. McCarthy, H. S. Jacob, and C. F. Moldow.** 1984. Bacterial adherence to fibronectin and endothelial cells: a possible mechanism for bacterial tissue tropism. J. Lab. Clin. Med. **103**:34–43.
49. **Vercellotti, G. M., J. B. McCarthy, P. Lindholm, P. K. Peterson, H. S. Jacob, and L. T. Furcht.** 1985. Extracellular matrix proteins (fibronectin, laminin, and type IV collagen) bind and aggregate bacteria. Am. J. Pathol. **120**:13–21.
50. **Vuento, M., and A. Vaheri.** 1979. Purification of fibronectin from human plasma by affinity chromatography under nondenaturing conditions. Biochem. J. **183**:331–337.
51. **Wadström, T.** 1983. Biological effects of cell damaging toxins, p. 671–704. *In* C. S. F. Easmon and C. Adlam (ed.), Staphylococci and staphylococcal infections, vol. 2. Academic Press, Inc., London.
52. **Wray, W., T. Boulikas, V. Wray, and R. Hancock.** 1981. Silver staining of proteins in polyacrylamide gels. Anal. Biochem. **118**:197–203.
53. **Yamada, K. M.** 1983. Cell surface interactions with extracellular materials. Annu. Rev. Biochem. **52**:41–49.
54. **Yoshida, K., M. Takahashi, T. Ohtomo, Y. Minegishi, Y. Ichiman, and K. Haga.** 1979. Application of fluorescent antibody for detecting capsular substances in *Staphylococcus aureus*. J. Appl. Bacteriol. **46**:147–152.