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Staphylococcus saprophyticus Hemagglutinin Binds Fibronectin

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Attachment of microorganisms to host tissue is regarded as an important step in the pathogenesis of infections. *Staphylococcus saprophyticus* adheres to various epithelial cells and hemagglutinates sheep erythrocytes. The hemagglutinin has been identified, but a human target for this surface protein is still not known. In our report, we show that hemagglutinating strains of *S. saprophyticus* bind to immobilized fibronectin, whereas nonhemagglutinating strains do not. Bacterial binding was inhibited by antibody to the hemagglutinin but not by antibody to Ssp, another surface protein of *S. saprophyticus*. The purified hemagglutinin but not other surface proteins bound biotin-labeled fibronectin. Binding was saturable and could be inhibited by unbound hemagglutinin, unlabeled fibronectin, and by antibody to the hemagglutinin. We thus conclude that the hemagglutinin of *S. saprophyticus* may act as a fibronectin receptor in the human host. Heparin, the D3 peptide, or Arg-Gly-Asp-Ser (RGDS) containing peptides did not inhibit binding of fibronectin to the hemagglutinin, indicating that the binding site is different from that of *Staphylococcus aureus* or *Treponema pallidum*.

Attachment of microorganisms to host tissues is commonly regarded as the crucial initial step in the pathogenesis of microbial infections. In many bacterial species, binding to extracellular matrix proteins (e.g., fibronectin, vitronectin, laminin, thrombospondin, and collagen) has been described (1, 4, 11, 13, 15, 19–24, 27, 28, 31, 35–39, 42). In some species, the receptors on the bacterial cell or the binding sites on the matrix proteins have been identified or characterized (13, 19, 22, 24, 40). Binding to matrix proteins is thought to mediate bacterial adhesion to host tissues. In addition, proteins deposited on the bacterial surface may protect the microorganism from being recognized by the host defense mechanisms (11, 28, 43).

Fibronectin is a 440- to 480-kDa matrix protein consisting of two subunits (25, 29). The molecule interacts with cells of the macroorganism through a cell attachment sequence (Arg-Gly-Asp [RGD]) which is recognized by receptors (integrins) on the eucaryotic cell (32). Many bacteria bind to the 29-kDa N-terminal domain of fibronectin (42). Binding to *Staphylococcus aureus* requires the presence of all five type I modules (38) of the N-terminal part of fibronectin. However, interactions with other parts of the molecule have also been reported. Interestingly, *Treponema pallidum* binds to the RGD sequence of fibronectin (40), and *Yersinia enterocolitica* specifically attaches to a splicing variant of fibronectin, i.e., cellular fibronectin, but the target sequence has not been identified. In *Streptococcus pyogenes*, a fibronectin-binding protein seems to be the major adhesin (13); however, a contribution of lipoteichoic acids to fibronectin binding has also been reported (1). Attachment to other components of the extracellular matrix has been found in several microorganisms. Binding of laminin was found to be a virulence factor of *Toxoplasma gondii* (4) and *Streptococcus gordonii* (37). In *S. aureus*, a surface protein that binds various matrix proteins has been described recently (23).

Staphylococcus saprophyticus, an important cause of urinary tract infections, binds fibronectin (39) and laminin (27). In earlier contributions, we reported the identification and isolation of two surface proteins of *S. saprophyticus*, Ssp (8), a 95-kDa protein which may be involved in adhesion to epithelial cells and is produced by many strains, and the hemagglutinin (10), a 160-kDa protein. *S. saprophyticus* hemagglutinates sheep erythrocytes only, and it has been suggested that a carbohydrate structure (*N*-acetyl-lactosamine) serves as the receptor on the erythrocyte (12). However, another group reported different specificities (2). A human target for binding of the two proteins has not yet been found. Here we report the binding of human fibronectin to the 160-kDa surface protein of *S. saprophyticus*.

MATERIALS AND METHODS

Strains. Clinical isolates of *S. saprophyticus* were from women with symptomatic urinary tract infections and have been characterized in detail previously (9). *S. saprophyticus* CCM883 and *S. aureus* Cowan I (ATCC 12598) were used as reference strains. *S. saprophyticus* CCM883, 7108, and 9520 were hemagglutination positive; strains 9325 and 11635 were negative (9). All strains except CCM883 and 1711 produce Ssp. Strain 1711 was derived from strain 9325 by chemical mutagenesis as described previously (7).

Fibronectin. Fibronectin was purified from spent culture medium of human fibroblasts by affinity chromatography on gelatin-agarose (Sigma, Munich, Germany) as described by Engvall and Ruoslahti (5). Briefly, culture medium was diluted 1:3 in phosphate-buffered saline (PBS; 10 mM Na_2HPO_4-NaH_2PO_4, 130 mM NaCl [pH 7.2]) and applied to a gelatin-agarose column (1.6 by 5.0 cm). The column was washed with 10 column volumes of PBS, and the fibronectin was eluted with urea (8 M urea, 50 mM Tris [pH 8.0]). Purity was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10), and identity was confirmed on Western blots (immunoblots) (10) developed with anti-human fibronectin (Sigma). In some of the protein gels, we also used gel sample buffer without 2-mercaptoethanol. For control purposes, all experiments were also conducted with purified fibronectin purchased from Boehringer (Mannheim, Germany). Purchased fibronectin and fibronectin purified from hu-

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man fibroblasts always yielded comparable results. Unless stated otherwise, only those results obtained with purified fibronectin are reported in this article.

Biotin labeling. Fibronectin was labeled with *N*-hydroxysuccinimide–biotin as described previously (3). Briefly, 200 μg of fibronectin was labeled in 2 ml of PBS containing 1 mM sulfo–*N*-hydroxysuccinimide–biotin (Serva, Heidelberg, Germany). The mixture was incubated (1 h, room temperature) with occasional shaking. The reaction was stopped by the addition of 1 M Tris buffer (pH 7.5) to a final concentration of 50 mM. Fibronectin was separated from unreacted sulfo–*N*-hydroxysuccinimide–biotin by size exclusion chromatography (Sephadex G-25, PD10 columns [Pharmacia, Freiburg, Germany], equilibrated and eluted with PBS).

***S. saprophyticus* surface proteins and antisera.** The 95-kDa fibrillar surface protein (Ssp) (8) and the 160-kDa hemagglutinin were prepared and purified as described previously (10). The crude preparations always contain a 31-kDa polypeptide with unknown function which can be purified by the same procedure used for the purification of Ssp and the hemagglutinin (8, 10). This protein was used as a control in some binding studies. Antisera were raised in rabbits, and immunoglobulin G (IgG) fractions were prepared by affinity chromatography on protein G-Sepharose (8).

Binding of bacteria to immobilized fibronectin. Fibronectin and bovine serum albumin (BSA; Sigma) were diluted in carbonate buffer (50 mM Na_2CO_3-$NaHCO_3$ [pH 9.5]) to 50 μg/ml. Microtiter plates (F-96; Nunc, Wiesbaden, Germany) were coated with 5 μg of protein per well (16 h, 4°C). The wells were washed (three times with PBS) and incubated with PBS-Tween (PBS containing 0.5% Tween 20 and 0.05% NaN_3; 1 h, 37°C). Bacteria were grown in P broth (7) in two consecutive cultures of 6 and 20 h (37°C, with shaking). Cells from 10 ml were pelleted, washed with cold PBS containing NaN_3 (0.05%), and adjusted to an A_{600} of 6.0 in PBS-Tween. These suspensions were diluted 1:2 with PBS-Tween, applied to the coated microtiter wells, and incubated (3 h, 37°C). In some experiments, the densities of the bacterial suspensions were varied. It had been determined in preliminary experiments that NaN_3 will inhibit growth of *S. saprophyticus* during the incubation steps. The bacterial suspensions were carefully aspirated, and the wells were washed with PBS-Tween. The plates were dried, and the adherent bacterial films were measured photometrically (405 nm). Binding experiments were also done in the presence of BSA (5 mg/ml) and antibody to Ssp or the hemagglutinin.

Periodate treatment. Fibronectin immobilized to microtiter wells was deglycosylated by mild periodate treatment as described previously (44). Concentrations of sodium *meta*-periodate were varied from 10 to 40 mM. Periodate (in 50 mM sodium acetate buffer [pH 5.5]) was added to coated wells and incubated (1.5 h, room temperature, in the dark). Wells were washed twice (acetate buffer), glycine (1% in acetate buffer) was added, and incubation was continued (30 min, room temperature). After two washes (PBS), the wells were incubated with PBS-Tween (1 h, 37°C), and adherence tests with whole bacteria were done as described above. Wells treated with the same procedure but using buffers without periodate served as controls. Soluble fibronectin was treated with 40 mM periodate (in sodium acetate buffer, 1 h, in the dark), glycine was added (final concentration, 1%), and incubation was continued for 30 min. Unreacted compounds were removed by gel filtration (PD-10 columns equilibrated with PBS), and fibronectin was labeled as described above.

Binding of labeled fibronectin to *S. saprophyticus* surface proteins. (i) Dot blot. Purified surface proteins (the hemagglutinin and Ssp), the 31-kDa protein, and BSA were spotted onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany; 1 to 3 μg per spot). Free binding sites were blocked with PBS-Tween, the membranes were incubated with biotin-labeled fibronectin (3 μg/ml, 2 h, 37°C) and washed, and binding was detected with alkaline phosphatase-labeled avidin (Sigma; 1:10,000 in PBS). The reaction was visualized with the substrates used in the Western blotting procedure (10). In some experiments, we used biotin-labeled BSA to check for nonspecific binding.

(ii) Enzyme-linked immunosorbent assay. Microtiter plates (U-96; Greiner, Nürtingen, Germany) were coated with purified hemagglutinin (1 μg per well; carbonate buffer; 14 h, 4°C). The wells were washed with PBS and incubated with PBS-Tween (1 h, 37°C). One hundred microliters of labeled fibronectin (1.7 μg/ml) was added. After incubation (3 h, 37°C), the wells were washed with PBS-Tween, and bound fibronectin was detected with alkaline phosphatase-labeled avidin and *p*-nitrophenol phosphate (Sigma) as the substrate. After 10 min, the absorption was determined (A_{405}). In some experiments, the concentrations of the hemagglutinin used for coating or the concentrations of the labeled fibronectin were varied.

(iii) Inhibition experiments. Inhibitors (unlabeled fibronectin, the hemagglutinin, antibody to the hemagglutinin, D3 peptide [Sigma], RGDS, RGD [Sigma], GRGDSP, GRGESP [Telios, San Diego, Calif.], VGSE [Bachem, Heidelberg, Germany], heparin [Sigma], glucose, mannose, *N*-acetylglucosamine, galactose, fucose, and methyl-α-D-mannopyranoside [Sigma]) were incubated together with the labeled fibronectin in concentrations given in Results.

Binding of labeled fibronectin to whole bacterial cells. Bacteria were grown, washed, and adjusted to an optical density at 600 nm (OD_{600}) of 100 as described above. A sample (200 μl) was pelleted, resuspended in a solution of labeled fibronectin (1 mg/ml in PBS-Tween), and incubated (1.5 h, room temperature, with constant shaking). After being washed three times (PBS-Tween), the cells were resuspended in the gel sample buffer (10), heated (20 min, 80°C), and centrifuged, and a sample (25 μl) of the supernatant was subjected to SDS-PAGE. Proteins were blotted onto nitrocellulose membranes, and biotin-labeled proteins were detected with labeled avidin by using an immunoblot procedure described previously (10).

Hemagglutination. Hemagglutination and hemagglutination inhibition with fibronectin, anti-hemagglutinin antibody, and BSA were done as described previously (10).

Statistics. Statistical calculations were done with the INSTAT program (GraphPad, San Diego, Calif.). Median values were compared with the Wilcoxon two-sample test.

RESULTS

Purification of fibronectin. Purity of fibronectin prepared from spent cell culture medium was assessed by SDS-PAGE with silver staining. Only one band at approximately 220 kDa was seen (Fig. 1). This indicates sufficient purity, as the staining method will detect <5 ng of protein per band (8). When a sample buffer that did not contain 2-mercaptoethanol was used, only one band with a larger apparent molecular size was seen (Fig. 1). In Western blots, the polypeptide reacted with an antiserum to human fibronectin.

Hemagglutinating strains of *S. saprophyticus* bind to immobilized fibronectin. We analyzed binding of three hemagglutinating (CCM883, 7108, and 9520) and three nonhemagglutinating strains (9325, 11635, and 1711) to fibronectin-coated microtiter wells. Wells coated with BSA served as controls.

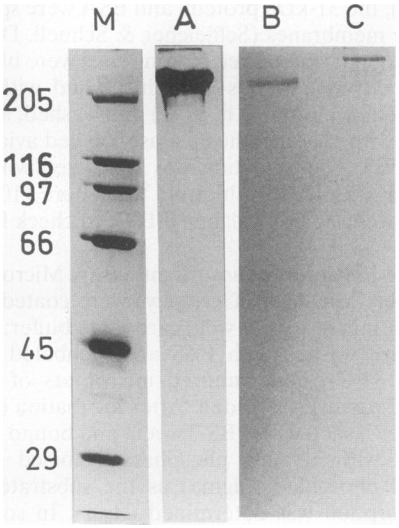

FIG. 1. Characterization of fibronectin prepared from fibroblasts. Fibronectin was purified from culture supernatants of human fibroblasts by affinity chromatography of gelatin-agarose and separated by SDS-PAGE. (A) Purified fibronectin at a concentration of 25 μg per lane; (B) purified fibronectin electrophoresed after being reduced by mercaptoethanol; (C) fibronectin run under nonreducing conditions. M, molecular size markers (Sigma; sizes in kilodaltons shown on the left).

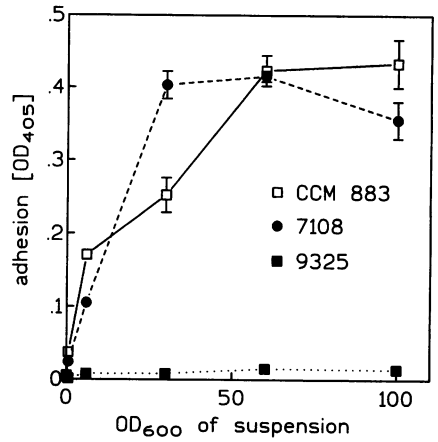

FIG. 3. Binding of bacteria to immobilized fibronectin is saturable. Bacterial suspensions of strains CCM883, 7108, and 9325 were adjusted to various densities between 0.3 and 100 (OD_{600}) and incubated with fibronectin immobilized to microtiter wells, and the absorbances of the adherent bacterial films were recorded. Cells of strain 9325 did not bind to fibronectin, and binding of strains 7108 and CCM883 was saturable by increasing the number of bacteria applied to the wells. Means of three determinations and standard errors of the means are given.

Hemagglutinating strains bound much more strongly to wells coated with fibronectin, the absorptions being more than 10 times that of the BSA controls (Fig. 2). Nonhemagglutinating strains bound less intensely to fibronectin-coated wells, whereas the absorptions of the BSA-coated controls were similar to those of the hemagglutinating strains. Binding of strain 1711, which is a an Ssp-negative derivative of strain 9325, did not differ from that of the parent strain. *S. aureus* Cowan I, known to express a fibronectin-binding protein, bound more strongly than CCM883.

It could be demonstrated by varying the density of the bacterial suspensions that binding of whole bacteria to immobilized fibronectin was saturable (Fig. 3). Saturation was reached at a density of 30 to 60 (OD_{600}) for strains 7108 and CCM883, whereas no binding was observed with strain 9325.

Addition of BSA (5 mg/ml) to the bacterial suspensions did not interfere with binding (data not shown). Similarly, antibody to Ssp did not inhibit binding, whereas antibody to the hemagglutinin reduced attachment (Fig. 4).

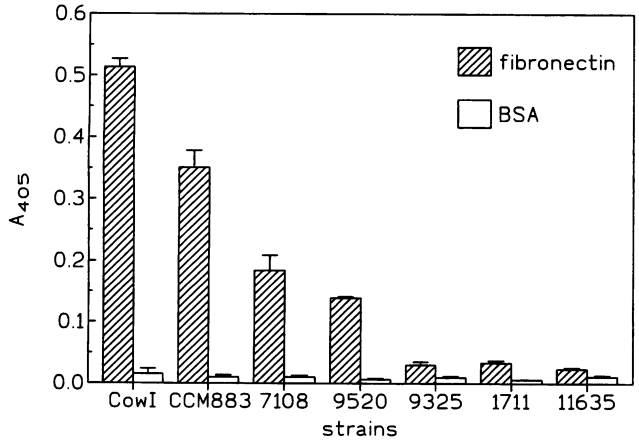

FIG. 2. Binding of *S. saprophyticus* to immobilized fibronectin. Suspensions of hemagglutinating (CCM883, 7108, and 9520) and nonhemagglutinating (9325, 1711, and 11635) strains of *S. saprophyticus* were incubated (3 h, 37°C) with microtiter wells that had been coated with fibronectin or BSA. The absorptions of the adherent bacterial films were then determined. *S. aureus* Cowan I (CowI) served as a control. Means of three determinations and standard errors of the means are given.

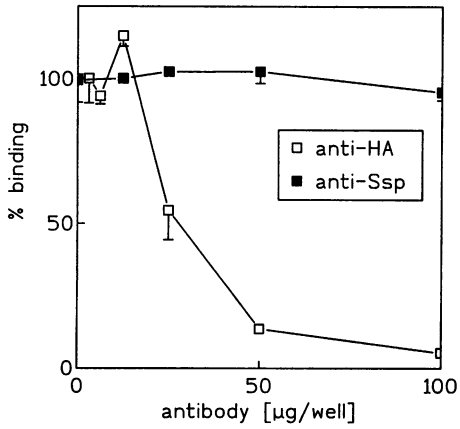

FIG. 4. Inhibition of bacterial binding by antibody to the hemagglutinin. Suspensions of *S. saprophyticus* CCM883 containing various concentrations of antibody to Ssp (anti-Ssp) or to the hemagglutinin (anti-HA) were incubated with fibronectin-coated microtiter wells (3 h, 37°C). Absorptions of the bacterial films were recorded. Binding was inhibited by antibody to the hemagglutinin but not by antibody to the Ssp. Means of three determinations and standard errors of the means are given. Error bars are not shown if they were smaller than the symbols.

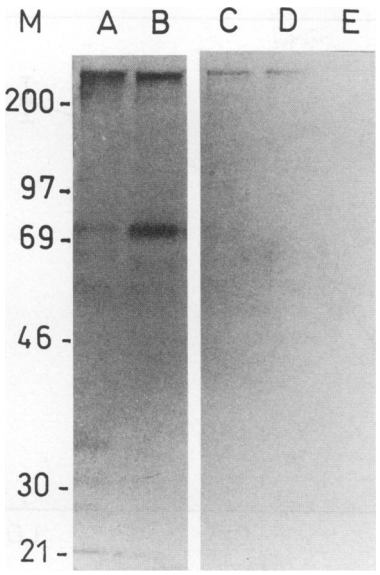

FIG. 5. Whole bacteria bind fibronectin. Whole bacterial cells of strain CCM883 were incubated with biotin-labeled fibronectin, and after the cells were washed, the surface proteins were solubilized by heating in the gel sample buffer. Proteins were separated by SDS-PAGE and blotted onto nitrocellulose. Biotinylated proteins were detected with alkaline-phosphatase-labeled avidin. (A) Biotin-labeled fibronectin purified from fibroblasts; (B) commercial fibronectin labeled with biotin; (C) bacteria incubated with labeled commercial fibronectin; (D) bacteria incubated with labeled fibronectin purified from fibroblasts; (E) bacteria incubated with unlabeled commercial fibronectin. M, positions of molecular size markers (rainbow markers [in kilodaltons]; Amersham, Braunschweig, Germany).

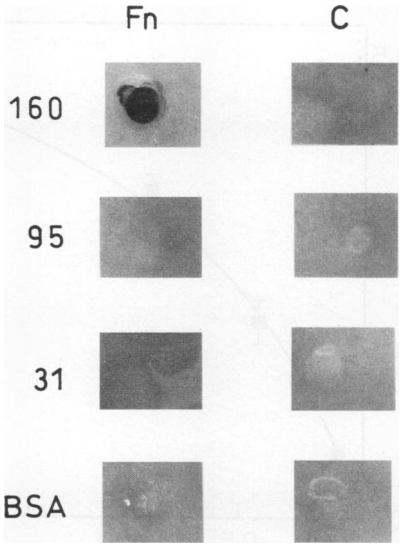

FIG. 6. The hemagglutinin binds fibronectin. The purified hemagglutinin (160 kDa), Ssp (95 kDa), a 31-kDa staphylococcal protein, and BSA were spotted onto nitrocellulose membranes (1 to 3 μg per spot) and incubated with biotin-labeled fibronectin (Fn). Bound fibronectin was detected with alkaline phosphatase-labeled avidin. Only the hemagglutinin bound labeled fibronectin. C, incubation with alkaline phosphatase-labeled avidin only.

Whole bacterial cells bind fibronectin. To determine whether whole bacterial cells bind fibronectin, suspensions of bacteria were incubated with the labeled protein. Proteins were eluted from the bacteria, separated by SDS-PAGE, and blotted onto nitrocellulose, and biotin-labeled proteins were detected with phosphatase-labeled avidin (Fig. 5). Although the labeled fibronectin preparations, especially the commercial one, contained some contaminating protein (lanes A and B), only one biotinylated polypeptide of the size of fibronectin was detected in the material eluted from the bacterial cells (Fig. 5, lanes C and D). No biotinylated polypeptides were detected when bacteria were incubated with unlabeled fibronectin (lane E).

The 160-kDa protein binds fibronectin. When the purified 160-kDa, 95-kDa, and 31-kDa proteins and BSA were spotted onto nitrocellulose membranes and incubated with biotin-labeled fibronectin, reactions were observed with the 160-kDa protein only (Fig. 6). No reactions were seen with phosphatase-labeled avidin nor with biotin-labeled BSA (data not shown).

Fibronectin binding is receptor mediated. If fibronectin binding is mediated by a structure on the 160-kDa protein that acts as a receptor, the signal should be saturable and should be inhibited by unlabeled fibronectin. To allow semiquantitative assessment of the reaction, microtiter wells were coated with a constant concentration of the 160-kDa protein (1 μg per well), and various concentrations of biotin-labeled fibronectin were applied. Figure 7A shows that binding was saturable by increasing the fibronectin concentration, whereas Fig. 7B shows that the signal depended on the concentration of the 160-kDa protein used for coating.

Fibronectin binding was inhibited by the simultaneous addition of soluble 160-kDa protein (Fig. 8A), antibody to the 160-kDa polypeptide (Fig. 8B), or unlabeled fibronectin (Fig. 9). Presence of the 95-kDa protein or of antibody towards it had no effect (data not shown). Concentrations of the 160-kDa protein needed for inhibition of fibronectin binding were much lower (2.4 μg per well for an 80% inhibition) than those of unlabeled fibronectin (17 μg per well).

RGDS or the D3 peptide do not inhibit binding. Because these results indicated a specific, receptor-mediated binding, the following experiments were designed to locate the attachment site for the 160-kDa protein on the fibronectin molecule. Known inhibitors of fibronectin binding were applied. No inhibition was seen with the D3 peptide (at 40 μM), representing the sequence needed for fibronectin binding of *S. aureus*. With the RGDS and GRGDSE peptides, analogs of the cell attachment site of fibronectin, inhibition of up to 85% was observed when very high concentrations (0.01 to 0.1 M) were applied. At those concentrations, however, unrelated peptides (RGDE and VGSE) also inhibited the interaction to a similar extent. In addition, heparin did not reduce binding (at concentrations of 0.02 to 20 μg per well).

The hemagglutinin does not bind to glycosylation sites of fibronectin. Various carbohydrates (glucose, mannose, *N*-acetylglucosamine, methyl-α-D-mannopyranoside, fucose, and galactose) did not significantly inhibit fibronectin binding at concentrations of up to 0.1 M (data not shown). Similarly, deglycosylation of fibronectin by mild periodate treatment did not reduce binding to the 160-kDa peptide. When binding of the native fibronectin was set to 100%, the values for deglycosylated fibronectin were 99% (90 to 107%), with a fibronectin concentration of 2 μg/ml, and 88% (77 to 99%), with a fibronectin concentration of 3.5 μg/ml (means of four determinations and 95% confidence intervals are given). Neither of these differences was statistically significant ($P > 0.1$). Moreover, periodate treatment of immobilized fibronectin did not

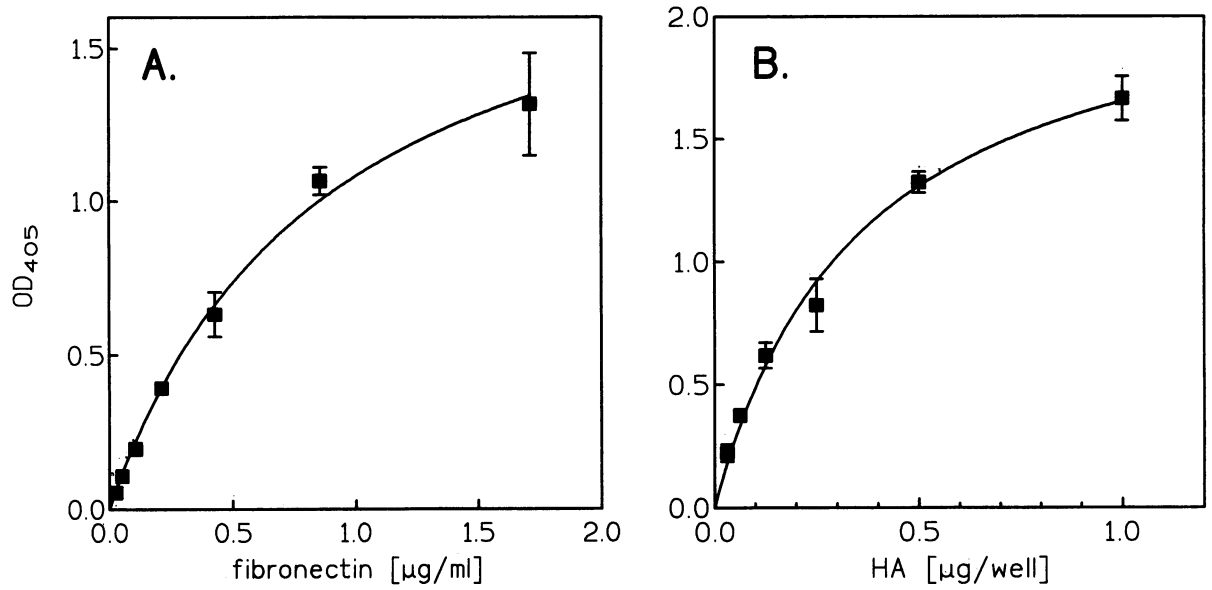

FIG. 7. Binding of fibronectin to the purified hemagglutinin is saturable. (A) When the amount of hemagglutinin bound was kept constant (1 µg per well), the reaction was saturable by increasing the concentration of labeled fibronectin. (B) Similarly, when the amount of labeled fibronectin was constant (1.7 µg/ml), the reaction was saturable by increasing the amount of hemagglutinin (HA) used for coating. Means ± standard errors of the means of at least three determinations are given. Error bars are not shown if they were smaller than the symbols.

reduce binding of whole bacteria. The absorbancy (OD_{405}) of CCM883 incubated with mock-treated fibronectin was 0.197 (0.139 to 0.254); that with periodate-treated fibronectin was 0.213 (0.152 to 0.273) (means of four determinations and 95% confidence intervals are given; $P > 0.1$).

The 160-kDa surface protein is bifunctional. Inhibition of hemagglutination by fibronectin was then assessed. BSA and antibody to the hemagglutinin were used as controls. Hemagglutination titers of the three hemagglutinating strains were not reduced by fibronectin (reduction by two or fewer dilution steps); however, antibody to the 160-kDa protein inhibited hemagglutination (titers reduced by three or more steps) (Table 1).

DISCUSSION

Binding to extracellular matrix proteins is an important step during pathogenesis of many microbial infections. Binding of

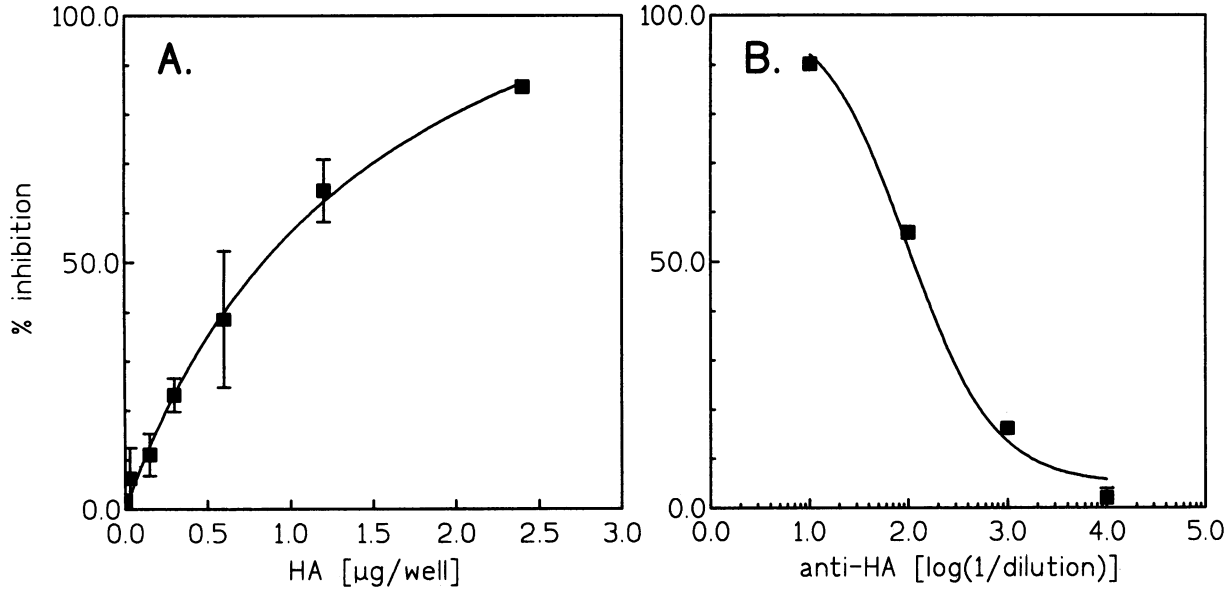

FIG. 8. Binding can be inhibited by the hemagglutinin or antibody towards it. When soluble hemagglutinin (A) or antibody to the hemagglutinin (B) was present during the incubation with labeled fibronectin, binding was inhibited. Means ± standard errors of the means of at least three determinations are given. Error bars are not shown if they were smaller than the symbols.

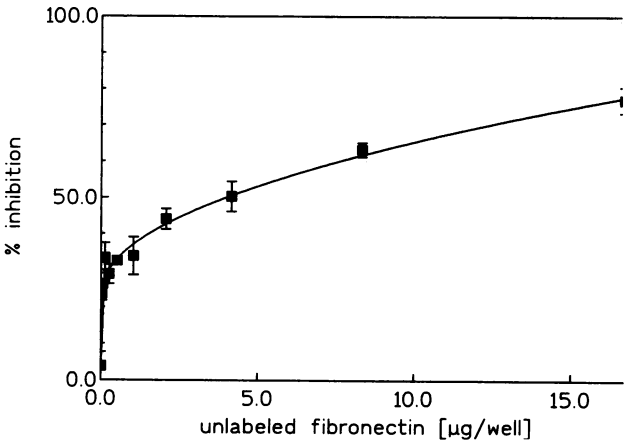

FIG. 9. Binding of labeled fibronectin can be inhibited by the unlabeled protein. When unlabeled fibronectin was present during the incubation, binding of the labeled protein could be inhibited. Very high concentrations of the unlabeled protein, exceeding that of the labeled fibronectin by 200, were needed for substantial inhibition. Means ± standard errors of the mean of at least three determinations are given. Error bars are not shown if they were smaller than the symbols.

S. aureus to collagen is a prerequisite for the development of septic arthritis in experimental infections (16), and attachment to fibronectin plays a role in the pathogenesis of endocarditis (33). Although it is possible that fibronectin-covered cells are more easily ingested by macrophages (6), surface-bound host proteins may also inhibit recognition by the immune system (11, 28, 43).

Bacterial receptors bind to matrix proteins by protein-protein interactions (24) as well as protein-carbohydrate (20) or protein-lipoteichoic acid (1) interactions. Fibronectin binding has been studied most extensively in *S. aureus* and is associated with invasiveness of the organism (30, 31). Binding has also been described for various coagulase-negative staphylococci (39), including *S. saprophyticus*. Most studies focused on the analysis of adherence or hemagglutination caused by this organism. The recently described hemagglutinin (10) apparently binds to a carbohydrate structure on the erythrocyte (12), but the identity of the carbohydrate is still controversial (2, 12). Most *S. saprophyticus* isolates hemagglutinate sheep erythrocytes (17), and some also react with horse or guinea pig cells (2), but agglutination of human erythrocytes has been described only rarely (17). Although the protein is thought to play a role in clinical infections, a human target has not been identified.

TABLE 1. Fibronectin does not inhibit hemagglutination

Strain	Reciprocal hemagglutination titer with protein added[a]			
	BSA	anti-Ssp[b]	anti-160[c]	FN[d]
CCM883	64	64	8	64
9520	32	32	4	16
7108	32	16	2	8
9325	N[e]	N	N	N

[a] All proteins were added at 290 μg/ml (final concentration).
[b] Antibody (purified IgG) to the Ssp.
[c] Antibody (purified IgG) to the hemagglutinin.
[d] Fibronectin purified from fibroblasts.
[e] N, no hemagglutination observed.

In the present study, we reassessed fibronectin binding by *S. saprophyticus* and found that hemagglutinating strains bound to fibronectin whereas nonhemagglutinating strains did not. Binding was found to be saturable by increasing the density of the bacterial suspension. Since strain CCM883, which does not produce Ssp, strongly attached to fibronectin-covered microtiter wells, we wanted to exclude that the ability to express Ssp reduces fibronectin binding. To this end, we grew the bacteria in liquid culture, which is known to hinder expression of the Ssp, and used a Ssp-negative mutant derived from strain 9325. Adhesion of the mutant, strain 1711, was not enhanced. Moreover, other Ssp-positive, hemagglutinating strains (7108 and 9520) attached to immobilized fibronectin. We therefore conclude that the Ssp is not involved in the interaction of *S. saprophyticus* with fibronectin.

An antiserum to the hemagglutinin but not one directed against the Ssp inhibited binding of whole bacterial cells to immobilized fibronectin. Since strain CCM883 does not express Ssp, our result shows that binding is not inhibited by the mere presence of IgG and indicates that the binding site on the hemagglutinin responsible for binding of fibronectin does not bind IgG by a site different from the antigen-binding domain.

We then assessed whether hemagglutination is causally related to or merely associated with fibronectin binding. In dot blot assays, we found that labeled fibronectin interacted with the 160-kDa surface protein, i.e., the hemagglutinin, of *S. saprophyticus* but not with the Ssp or another 31-kDa protein of the organism. In addition to this qualitative test, we found that the interaction was saturable by increasing the amount of 160-kDa surface antigen or the concentration of labeled fibronectin. As expected, binding could be inhibited by the 160-kDa antigen or antibody towards it and by unlabeled fibronectin. To achieve significant inhibition, very high concentrations of unlabeled fibronectin had to be used (1/200 labeled fibronectin/unlabeled fibronectin ratio); this, however, is commonly observed with bacterial fibronectin receptors (22) and may be attributed to multiple fibronectin-fibronectin interactions (29).

Several bacteria bind to the NH_2-terminal part of the fibronectin molecule (26, 29); however, *T. pallidum* binds to its RGDS sequence (40), i.e., the cell attachment site (29), and the binding site for *Y. enterocolitica* has not yet been identified (35). The D3 peptide, an analog of the sequence of the *S. aureus* fibronectin receptor needed for binding (24), did not inhibit the interaction between fibronectin and the 160-kDa peptide of *S. saprophyticus*. We used concentrations of up to 40 μM, which are well above those needed for inhibiting the interaction of the *S. aureus* receptor with fibronectin (24). In addition, heparin, applied at concentrations known to interfere with binding of fibronectin to *S. aureus* (41), did not inhibit binding. Heparin interacts with the N-terminal sequence and with a C-terminal domain of fibronectin (25). To test whether *S. saprophyticus* uses the same attachment site as *T. pallidum*, inhibition by RGDS was also tested. Very high concentrations were needed for inhibition; however, at these concentrations, other peptides, which served as controls, also inhibited binding. We therefore conclude that *S. saprophyticus* does not attach to fibronectin via a sequence similar to that of *S. aureus* and probably does not use the RGDS sequence of fibronectin as its primary target.

Escherichia coli and *Salmonella enterica* bind to laminin through protein-carbohydrate interactions involving oligomannoside chains of the matrix protein (20). Since fibronectin is a glycoprotein (24), we tested whether binding to the 160-kDa protein could be inhibited by those carbohydrates that are present in the glycosylating side chains of fibronectin prepared

from human fibroblasts (18) or that are known to inhibit binding of other bacteria to matrix proteins (20). Of the six sugars tested, none interfered with this interaction. In addition, binding of fibronectin to the hemagglutinin or of bacteria to fibronectin was not impeded by oxidizing the carbohydrate moieties of the matrix protein with a procedure (44) that has been recommended for this purpose (26). The part of the fibronectin molecule acting as a target for binding of the 160-kDa molecule therefore remains unknown.

In a recent publication, we reported that antibody to the 160-kDa surface protein inhibited hemagglutination and hence that the molecule is the hemagglutinin of *S. saprophyticus* (10). If hemagglutination and fibronectin binding are mediated by the same domain of the 160-kDa protein, hemagglutination should be inhibited by fibronectin. This, however, was not the case, whereas, expectedly, antibody to the 160-kDa polypeptide inhibited hemagglutination.

Our data thus indicate that the 160-kDa surface polypeptide of *S. saprophyticus* is an at-least-bifunctional molecule, acting as the hemagglutinin and mediating binding of fibronectin. Multiple specificities have been found for other surface proteins, e.g., the M protein of streptococci (34) or the YadA protein of *Y. enterocolitica* (34, 35). Recently, a surface protein of *S. aureus* that binds a variety of matrix proteins has been described (23). Receptors with broad specificity may be advantageous for microorganisms that exist in different microenvironments. *S. saprophyticus* has been isolated from domestic animals (14) and is thought to cause infection after being transferred to a susceptible human host. The hemagglutinin may thus serve as an adhesin in both animal and human hosts.

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