INFECTION AND IMMUNITY, Sept. 1981, p. 683-689
0019-9567/81/090683-07$02.00/0

Vol. 33, No. 3

Fibronectin Binding to Protein A-Containing Staphylococci

JAN E. DORAN[1]* AND ROBERT H. RAYNOR[2]

Medical Research Service, Veterans Administration Medical Center, Augusta, Georgia 30910,[1] and Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, Georgia 30912[2]

Received 2 February 1981/Accepted 26 May 1981

Fibronectin (Fn) was found to bind to protein A-containing isolates of *Staphylococcus aureus*, but not to mutant strains devoid of this protein nor to clinical isolates of *S. epidermidis*. Fn was purified from human plasma by affinity chromatography on gelatin-Sepharose. After elution with 4 M urea, sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified material detected no immunoglobulin contamination. This purified Fn was radiolabeled with ^{125}I and used in binding assays. Quantitatively, Fn binding was directly correlated with the cellular protein A content of the various strains tested. Mannitol salt broth preculture of organisms resulted in a reduction of their cellular protein A and a decrease in Fn binding by these cells. However, soluble protein A maximally inhibited the binding of radiolabeled Fn to protein A-positive strains of staphylococci by only 50%, indicating the possibility of multiple Fn binding sites. Fn's binding to protein A-containing *S. aureus* strains may play a role in the pathogenicity of these organisms by promoting their attachment to and subsequent invasion of host tissues.

Fibronectin (Fn) is a high-molecular-weight (440,000) glycoprotein important in many biological systems. Within recent years, many of Fn's properties have been elucidated, and its physiological role in host homeostasis has been recognized. Fn has been shown to bind to a number of different ligands, including native and denatured collagens (gelatin) (4), fibrin (21), and heparin (12). In vivo, soluble Fn is found in body fluids and, in its cell-associated form, on connective tissues and basement membranes (13, 16), as well as on platelets (17) and fibroblasts (22). It functions in vitro to modulate cell-to-cell adhesion and promotes cell-to-substratum adherence (1). Soluble Fn can act to enhance the phagocytosis of target particles, including some bacterial species, by both macrophages (3, 11) and polymorphonuclear leukocytes (R. A. Proctor, E. Prendergast, and D. F. Mosher, Clin. Res. **27**:650a, 1979; M. E. Lanser, and T. M. Saba, RES J. Reticuloendothel. Soc. **28**:21a, 1980; J. E. Doran, R. H. Raynor, A. C. Reese, and H. T. Edmondson, Fed. Proc. **40**:755, 1981). Clinically, the knowledge of Fn's opsonic activity has led to the treatment of trauma-associated bacterial sepsis by the therapeutic administration of cryoprecipitate (a fraction of human plasma, rich in Fn) (19, 23, 24).

Fn does not appear to bind all bacterial species uniformly, as demonstrated by Kuusela (9), who showed that Fn binds to *Staphylococcus aureus* but not to *Mycobacterium butyricum*. Kuusela's results also suggested that the binding site for *S. aureus* is distinct from the gelatin-binding site on the Fn molecule. Mosher and Proctor (14) have confirmed and extended these findings by demonstrating that the *S. aureus*-binding site of Fn is located on a 27-kilodalton fragment of Fn which is distinct from the gelatin-binding region. They further showed that either intact Fn or the 27-Kilodalton fragment of Fn could be cross-linked to *S. aureus* by coagulation factor XIIIa.

Although the binding site on the Fn molecule for *S. aureus* has been elucidated, the site or sites of binding of Fn on the *S. aureus* cell wall have not been determined. We now report that Fn binding to staphylococci is related to the protein A content of these organisms.

MATERIALS AND METHODS

Bacterial strains. The protein A-rich strain *S. aureus* Cowan I was obtained from the American Type Culture Collection. Protein A-deficient *S. aureus* strains Wood 46 and EMS were a gift from P. K. Peterson. The remainder of *S. aureus* strains studied were recently obtained clinical isolates, as were the 10 strains of *S. epidermidis*. All clinical isolates designated *S. aureus* were determined to produce coagulase (8), ferment mannitol (8), and have demonstrable surface protein A by slide agglutination (27). *S. aureus* strains Cowan and Wood 46 were verified to be coagulase and mannitol positive, whereas EMS lacked both of these properties. Furthermore, EMS and Wood 46 were both surface protein A negative, as expected. *S. epidermidis* strains were coagulase and protein A neg-

ative, as expected; however, one isolate was able to ferment mannitol. All cultures were maintained on tryptic soy agar (Difco Laboratories) slants.

Protein A. Commercially available purified protein A (Sigma Chemical Co.; P-8143) was used as the source of protein A in inhibition experiments. This material was prepared by Sigma, using a modification of the ion-exchange and gel chromatography procedures outlined by Sjöquist et al. (25). Such procedures are reported to yield homogeneous preparations as determined by disc gel electrophoresis.

Purification and radiolabeling of Fn. Fn was purified from citrated human plasma by affinity chromatography on gelatin-Sepharose by a modification (3) of the basic procedure of Ruoslahti and Engvall (20). Briefly, citrated human plasma was batch incubated with gelatin-Sepharose (coupled by the cyanogen bromide procedure of Porath et al. [18]) for 2 h at room temperature. The slurry was poured into a column and washed thoroughly with phosphate-buffered saline, pH 7.4 (PBS), followed by additional washing with 1 M urea in 0.05 M tris(hydroxymethyl)-aminomethane (Tris) buffer, pH 7.5. The purified Fn was eluted with 4 M urea in the same Tris buffer and concentrated by vacuum dialysis against 0.05 M Tris buffer, pH 7.8. Fn purified in this manner was shown to be free of low-molecular-weight contaminants by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis stained with Coomassie brilliant blue (Fig. 1).

Purified Fn was radiolabeled with ^{125}I by the oxidative iodine monochloride method of Doran and Spar (2) or by using Iodogen (Pierce Chemical Co.) according to the procedure of Markwell and Fox (10). The labeled material was stored in the presence of 0.1% bovine serum albumin (Sigma).

Fn binding assays. Overnight cultures of bacteria were grown in tryptic soy broth (TSB) or mannitol salt broth (MSB) as indicated for each experiment. The bacterial cells were harvested by centrifugation, washed three times with PBS, and adjusted spectrophotometrically to an absorbance of 1.2 at 540 nm in PBS (for organisms grown in TSB, this represents approximately 6×10^8 colony-forming units/ml). Various numbers of organisms, as indicated for each experiment, were placed in polystyrene culture tubes (12 by 75 mm; Sardstet), adjusted to a 1.0-ml volume, and centrifuged at 2,000 × *g* for 15 min. The supernatant liquids were discarded, and the binding reaction mixture consisting of 300 μl of bovine serum albumin (35 mg/ml), 50 μl of inhibitor or buffer control, and 20 μl of [^{125}I]Fn (60 μg of Fn/ml) was added to the pellet. The tubes were placed on a platform shaker and rocked at 37°C for 90 min, after which time the bacteria were harvested by centrifugation and washed three times with PBS containing 1% Tween 20 (Sigma). Radioactivity that remained associated with the bacterial cell pellet was determined by using a Nuclear-Chicago gamma spectrometer.

Protein A assay. The cell-associated protein A content of each *S. aureus* strain was quantified by a modification of the procedure described by Winblad and Ericson (27). Briefly, sheep erythrocytes (SRBC) were sensitized with anti-sheep hemolysin (GIBCO Laboratories) by incubation of a 4% suspension of SRBC in PBS with an equal volume of hemolysin (1:100 dilution in PBS) for 45 min at room temperature. This dilution of hemolysin was shown in preliminary trials to be sufficient for sensitization without causing agglutination. The SRBC were then washed twice with PBS and resuspended to effect a 2% suspension.

Overnight cultures of each *S. aureus* strain were adjusted spectrophotometrically to an absorbance of 1.2 at 540 nm. One-milliliter portions were removed and centrifuged at 2,000 × *g* for 10 min. The resulting pellets were incubated for 2 h at 37°C with 1 ml of PBS containing 25 U of lysostaphin (Sigma). At the end of the incubation period, nonlysed cells and debris were removed by centrifugation, and the supernatant fluids were decanted and diluted in PBS before the addition of the sensitized SRBC. To 200 μl of each protein A supernatant dilution was added 100 μl of a 2% suspension of sensitized SRBC. This mixture was incubated for 2 h at 37°C and then kept at 4°C overnight. The protein A agglutination titer is defined as the inverse of the highest dilution which was found to agglutinate the sensitized SRBC.

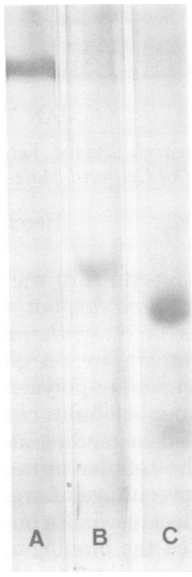

FIG. 1. *Polyacrylamide gel electrophoresis of purified Fn. Purified human Fn (25 μg) (A), human serum albumin (25 μg; Sigma) (B), and Cohn fraction II human immunoglobulin (50 μg; Difco) (C) were reduced in 2-mercaptoethanol at 99°C and electrophoresed in the presence of 0.1% SDS on 5% polyacrylamide gels. The gels were stained with Coomassie brilliant blue.*

RESULTS

In preliminary experiments, we observed staphylococcal aggregation when Fn was incubated with a clinical isolate of *S. aureus* designated Williams, but not when incubated with the protein A-deficient strain Wood 46. Since protein A is known to bind to human immuno-

globulin G (8), our Fn preparations were checked for immunoglobulin contamination. Figure 1 shows the results of SDS-polyacrylamide gel electrophoresis of Fn, human serum albumin (Sigma), and Cohn fraction II human immunoglobulin (Difco). Visual examination of these gels indicated no low-molecular-weight contaminants in our Fn preparation. The sensitivity of such gels when stained with Coomassie brilliant blue is 0.5 μg of protein per cm^2 (5). Hence, with 25 μg of protein loaded on the gel initially, undetectable contaminants would therefore constitute a maximum of 2% of the Fn preparation. The extent of immunoglobulin G contamination in two different Fn preparations determined by laser nephelometry (Calbiochem-Behring) was 1.1 and 1.5%.

In further experiments, we quantified the actual binding of Fn to *S. aureus* strains Cowan I, Williams, and EMS and to an *S. epidermidis* strain designated SE1. Radiolabeled Fn was incubated with bacterial cells ranging from 5×10^6 to 3×10^8 cells per assay, and binding was assessed (Fig. 2). The background control in these experiments was radiolabeled Fn incubated in reaction tubes in the absence of bacteria. Protein A-positive strains Williams and Cowan demonstrated statistically significant amounts of Fn bound above background with as little as 5×10^6 organisms. This binding continued to increase as cell concentrations were raised and began to show ligand depletion at a concentration of 10^8 cells per vial. The maximal binding achieved under these experimental conditions was 42% of labeled material offered. Preculture of the organisms in MSB reduced the amount of Fn bound at all concentrations of cells tested and increased the concentration of cells neces-

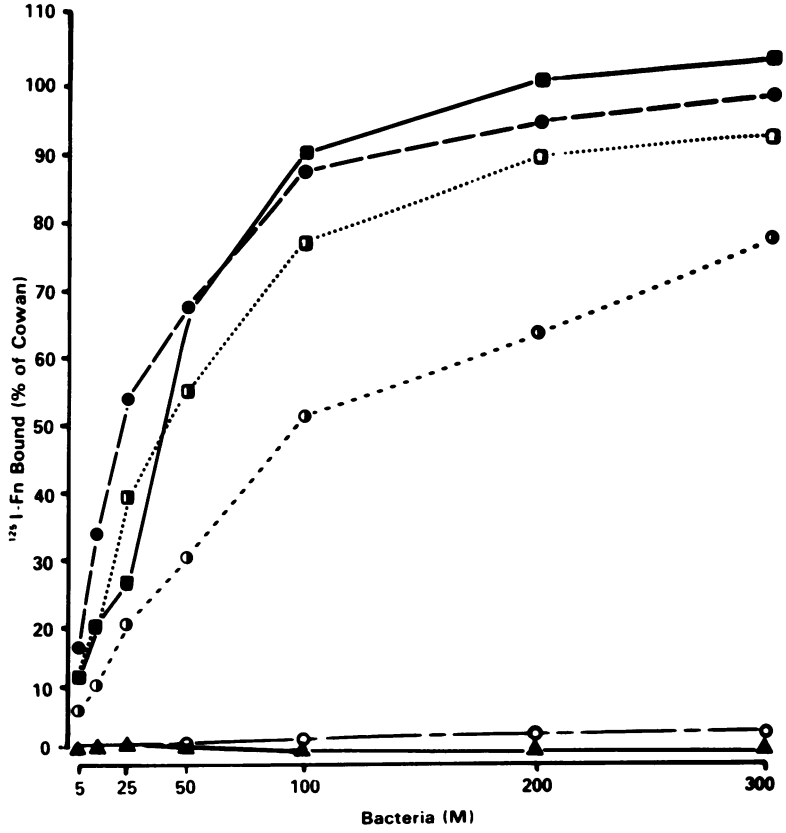

FIG. 2. *Binding of Fn to different concentrations of staphylococci. Various concentrations (expressed in millions of organisms per reaction mixture) of several strains of staphylococci were tested for their ability to bind Fn. The organisms were incubated with Fn (4.33 × 10^5 cpm) for 90 min at 37°C, after which the unbound material was removed and cell-associated Fn was determined (blank control retained 4.6 × 10^3 cpm). Each point represents the mean of duplicate determinations expressed as percentage of the amount of Fn that bound to S. aureus Cowan (1.72 × 10^5 cpm). Fn binding by each strain is represented by a different symbol: S. aureus Williams grown in TSB (■) or MSB (◨), S. aureus Cowan grown in TSB (●) or MSB (◑), S. aureus EMS grown in TSB (▲), and S. epidermidis SE1 grown in TSB (○).*

sary for the Fn binding plateau to be attained. Fn binding appeared to be restricted to protein A-positive *S. aureus* strain s.

Additional binding experiments were performed to determine the generality of this observation. To compare the relative Fn binding between strains, we examined the binding of Fn to 10^8 organisms of each isolate of *S. aureus* and *S. epidermidis* (Fig. 3). This number of organisms was chosen for use since it was the minimal number of organisms that showed maximal differences in Fn binding as compared with the control. This number of cells also formed a fairly compact, visible pellet upon centrifugation, making aspirations simpler to perform. Although the percentage of labeled Fn bound to each bacterial isolate varied between experiments, the rank order of isolates remained constant between experiments, e.g., Cowan $\simeq$ Williams > Pascal > Wood > EMS. A significant difference ($P < 0.001$ by the Mann-Whitney U test) in Fn-bacteria binding was observed between the *S. epidermidis* and *S. aureus* protein A-positive strains. Only minimal binding was seen in protein A-deficient strains Wood or EMS. Organisms precultured in MSB bound between 28 and 41% less Fn than did cells of the same strain cultured in TSB.

Since it appeared that Fn bound preferentially to protein A-positive staphylococcal isolates, the correlation between the protein A content of these organisms and Fn binding was studied. Figure 4 shows a scattergram of the results of concurrent protein A and Fn binding determinations. Fn binding to the cell surface was found to correlate with cell-associated protein A with a Spearman rank correlation coefficient of $r = 0.62$ ($P < 0.005$, df = 16). Organisms grown in MSB showed reduced Fn binding and protein A titer as compared with their TSB-cultured counterparts (Fig. 4).

Finally, purified protein A was tested for its ability to inhibit Fn binding to protein A-positive *S. aureus* strains. Figure 5 illustrates the effect of increasing amounts of protein A (Sigma P-8143) on the binding of [^{125}I]Fn to *S. aureus* Williams cultured in TSB or MSB. Soluble protein A at concentrations of 50 μg per assay and above maximally inhibited the binding of Fn to *S. aureus* Williams by approximately 50%. The binding of Fn to MSB-grown *S. aureus* Williams was only weakly inhibited by soluble protein A to the same plateau as TSB-cultured organisms.

DISCUSSION

Previous investigations have demonstrated that Fn binds to cells of *S. aureus* (9, 14); however, the site of this binding on the cell wall of these organisms has not as yet been elucidated. This report examines the binding of Fn to a number of strains of both *S. aureus* and *S. epidermidis* and the relationship of this binding to the presence of staphylococcal protein A.

Although the purification procedure for Fn is well established, it was important for this study to demonstrate minimal human immunoglobulin contamination. The SDS-polyacrylamide electrophoresis gels pictured in Fig. 1 confirm the lack of significant low-molecular-weight contaminants in our Fn preparation. Furthermore, nephelometric measurements verified less than 2% immunoglobulin G contamination in rou-

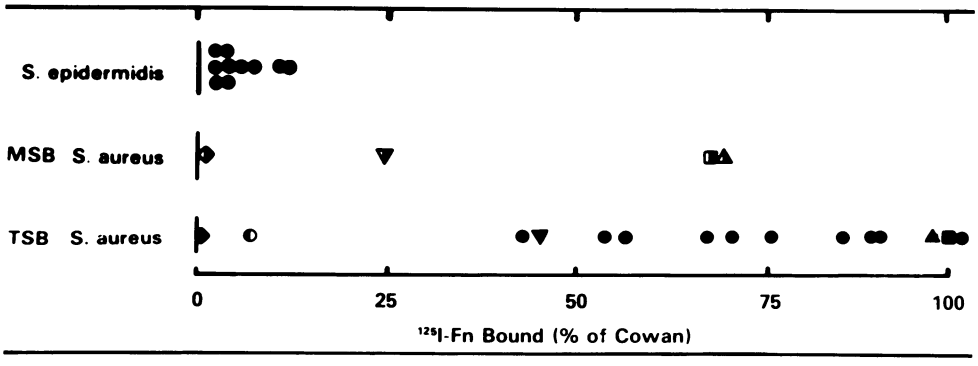

FIG. 3. *Fn binding to staphylococcal strains. [^{125}I]Fn binding (4.62×10^5 cpm offered) to 10^8 organisms of a number of strains of S. aureus was determined. Four S. aureus strains were grown in the presence of TSB and MSB, and the results for both culture conditions are included. Presented are the means of Fn binding of quadruplicate samples expressed as a percentage of Fn bound by the protein A-enriched strain S. aureus Cowan (■; 1.76×10^5 cpm). Blank control retained 6.3×10^3 cpm. Closed circles (●) represent Fn binding for various clinical isolates of S. aureus or S. epidermidis cultured in TSB. S. aureus strains Cowan (■), Williams (▲), Pascal (▼), Wood (◐) and EMS (◆) were cultured in TSB as well as in MSB, [Cowan (◨) Williams (◭), Pascal (▽) and EMS (◇)].*

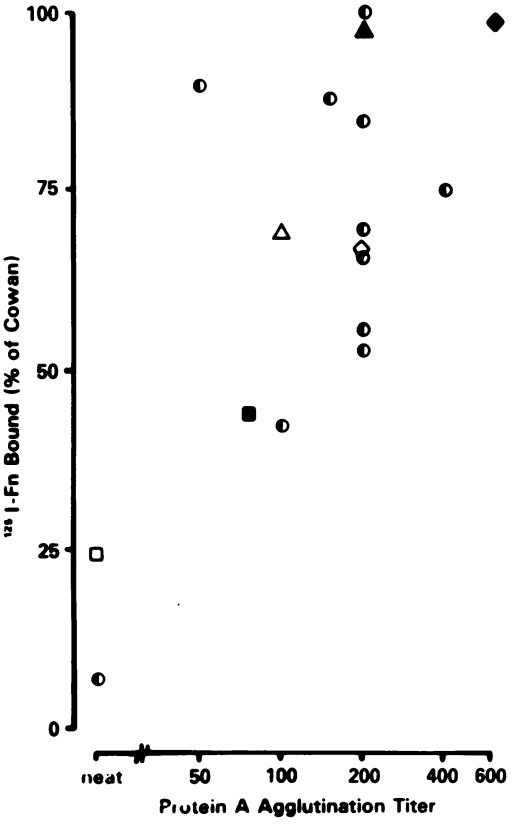

FIG. 4. *Correlation of protein A content with Fn binding to S. aureus strains. The binding of [^{125}I]Fn to various strains of S. aureus was determined concurrently with measurements of cellular protein A content of these organisms. Lysostaphin digests of each strain were diluted and tested for their ability to agglutinate immunoglobulin-sensitized SRBC. The protein A titer is expressed as the reciprocal of the highest dilution which caused the visible agglutination of the SRBC. Fn binding by each strain is expressed as the percentage of the labeled material that bound to the protein A-enriched strain S. aureus Cowan. Determinations of Fn binding and protein A content were performed on S. aureus strains Cowan (◆), Williams (▲), and Pascal (■) grown in TSB as well as in MSB (◇, △, □, respectively). All other isolates are shown by ◐. [^{125}I]Fn offered, present in blank control, and associated with Cowan as in Fig. 3.*

tinely prepared Fn samples.

The data in Fig. 2 and 3 indicate that Fn is able to bind to most strains of *S. aureus* within the 90-min incubation time, whereas it appears unable to bind significantly to strains of *S. epidermidis*. Interestingly, little or no Fn bound to the protein A-deficient *S. aureus* strains EMS and Wood. This binding was seen with as few as 5×10^6 cells, was dependent on bacterial cell concentration, and reached a plateau (indicating Fn depletion) with approximately 10^8 organisms (Fig. 2). The extent to which Fn was bound (40%) was far greater than expected if the binding was due to immunoglobulin contamination alone. Nonspecific adsorption or trapping of labeled Fn did not appear to occur, as evidenced by the lack of cell-associated Fn with *S. aureus* EMS or the *S. epidermidis* strain SE1 (Fig. 2). Thus, variations in Fn binding between strains appear to reflect differences in binding site densities on the bacteria themselves, differential affinities for Fn, or both.

Further experiments demonstrated that cell-associated protein A was significantly correlated with the amount of Fn bound to the staphylococci; however, a one-to-one correspondence of protein A content and Fn binding was not observed. This is probably because the protein A assay used in these experiments measures total cellular protein A in the organisms rather than the cell surface materials available to react with Fn. The fact that Fn binding to protein A-positive staphylococci can be inhibited by soluble protein A (Fig. 5) is consistent with Fn binding to the protein A on the surface of the staphylococci. Experiments using organisms cultured in MSB, in which bacteria synthesize less protein A (7, 15), clearly show less Fn binding than TSB-precultured cells (Fig. 2–4). Although these experiments do not in themselves confirm Fn's binding to protein A, they are certainly consistent with this hypothesis. The possibility of a contaminant in Sigma's protein A preparation which represents the actual Fn binding site cannot be excluded. The contaminant, if present, is highly associated with protein A, since its presence is correlated with the protein A content of cells grown in vitro, and this association is preserved throughout two purification procedures. One possibility under investigation is that Fn's binding site is the point at which protein A is covalently linked to the staphylococcal cell wall (26).

These data appear to contradict those obtained by Kuusela (9), who reported that 100 μg of protein A per ml caused no inhibition of Fn binding to Formalin-fixed *S. aureus* cells. However, Kuusela's assays do not appear to be as sensitive as the one used in these experiments, since she observed half-maximal inhibition with 3 to 4 μg of Fn per ml, whereas our system reached similar inhibition with 1 μg of Fn (data not shown). In addition, we show that protein A only partly inhibits the binding of Fn to *S. aureus* Williams (Fig. 5). This phenomenon was also demonstrated with *S. aureus* Cowan and other clinical isolates of *S. aureus* (data not

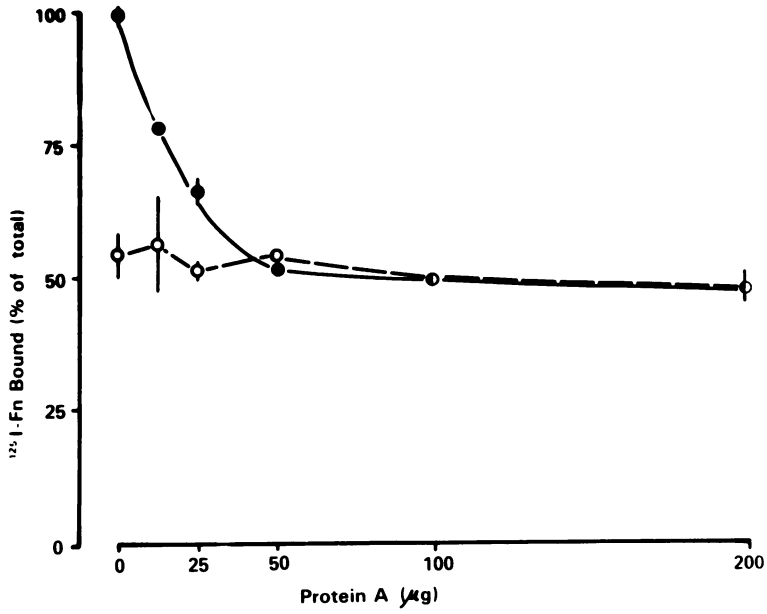

FIG. 5. *Protein A inhibition of Fn binding to S. aureus Williams. Different amounts of protein A were added to reaction mixtures that contained organisms of S. aureus Williams cultured in TSB (●) or MSB (○), and [^{125}I]Fn (2.77 × 10^5 cpm offered) binding to the organisms was determined. Results are expressed as a percentage of the amount of Fn bound by TSB-cultured S. aureus Williams in the absence of any competing protein A (7.1 × 10^4 cpm). Shown are the means ± standard deviation of quadruplicate determinations. Blank control retained 4.1 × 10^3 cpm.*

shown). These data suggest that Fn binds readily to protein A (or a closely associated material that copurifies with protein A) as well as to some other unidentified component(s) of the protein A-positive staphylococcal cell wall. Since Fn bound to neither EMS nor Wood 46 to any great extent during the incubation period, Fn's unknown ligand must be absent or very much reduced in these *S. aureus* strains or have a comparably low affinity for Fn. This view is supported by binding experiments now in progress which determine the number of Fn binding sites per Cowan organism. Mathematical analysis of preliminary binding data produced a nonlinear Scatchard plot consistent with a heterogeneity of Fn receptors on the Cowan organism.

The ability of Fn to bind to protein A-containing strains of *S. aureus* leads to speculation on the relationship of this phenomenon to the pathogenicity of this organism. Diagnostically, coagulase production has been the accepted criterion for differentiating the "pathogenic" species of staphylococci, *S. aureus*, from "nonpathogenic" *S. epidermidis* (8). Protein A production by staphylococci is highly correlated with coagulase production, being present in approximately 99% of coagulase-positive staphylococcal strains (6). Since protein A is covalently bound to the peptidoglycan structures of the bacterial cell wall (26), it would be clearly exposed to the host environment. Fn, present on basement membranes and connective tissues (13, 16), may therefore facilitate the attachment, growth, and dissemination of pathogenic staphylococci through its binding to the protein A on these organisms.

ACKNOWLEDGMENTS

We thank Madeline Crouch for technical assistance and Andy C. Reese, Gary K. Best, and David F. Scott for their helpful comments on the manuscript. We acknowledge J. Peter Rissing for his help in obtaining *S. aureus* EMS.

This research was supported by a grant from the Veterans Administration Medical Research Service.

LITERATURE CITED

1. **Culp, L. A., B. A. Murray, and B. J. Rollins.** 1979. Fibronectin and proteoglycans as determinants of cell-substratum adhesion. J. Supramol. Struct. **11**:401–427.
2. **Doran, D. M., and I. L. Spar.** 1980. Oxidative iodine monochloride iodination technique. J. Immunol. Methods **39**:155–163.
3. **Doran, J. E., A. R. Mansberger, and A. C. Reese.** 1980. Cold insoluble globulin-enhanced phagocytosis of gelatinized targets by macrophage monolayers: a model system. RES J. Reticuloendothel. Soc. **27**:471–483.
4. **Engvall, E., and E. Ruoslahti.** 1977. Binding of soluble form of fibroblast surface protein, fibronectin, to collagen. Int. J. Cancer **20**:1–5.
5. **Fairbanks, G., T. L. Steck, and D. F. H. Wallach.** 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry **10**: 2606–2617.

6. **Forsgren, A.** 1970. Significance of protein A production by staphylococci. Infect. Immun. **2**:672–673.
7. **Haukenes, G.** 1967. Serological typing of *Staphylococcus aureus*. Acta Pathol. Microbiol. Scand. **70**:590–600.
8. **Ivler, D.** 1974. *Staphylococcus*, p. 91–95. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
9. **Kuusela, P.** 1978. Fibronectin binds to *Staphylococcus aureus*. Nature (London) **276**:718–720.
10. **Markwell, M. A. K., and C. F. Fox.** 1978. Surface-specific iodination of membrane proteins of viruses and eucaryotic cells using 1,3,4,6-tetrachloro-3,6-diphenyl glycoluril. Biochemistry **17**:4807–4817.
11. **Molnar, J., S. McLain, C. Allen, A. Gara, and F. Gelder.** 1977. The role of an α2-macroglobulin of rat serum in the phagocytosis of colloidal particles. Biochim. Biophys. Acta **493**:37–54.
12. **Mosesson, M. W.** 1978. Structure of human plasma cold-insoluble globulin and the mechanism of its precipitation in the cold with heparin or fibrin-fibrinogen complexes. Ann. N.Y. Acad. Sci. **312**:11–30.
13. **Mosesson, M. W., and D. L. Amrani.** 1980. The structure and biologic activities of plasma fibronectin. Blood **56**:145–158.
14. **Mosher, D. F., and R. A. Proctor.** 1980. Binding and Factor XIIIa mediated cross-linking of a 27 kilodalton fragment of fibronectin to *Staphylococcus aureus*. Science **209**:927–929.
15. **Nickerson, D. S., J. G. White, G. Kronvall, R. C. Williams, Jr., and P. G. Quie.** 1970. Indirect visualization of *Staphylococcus aureus* protein A. J. Exp. Med. **131**:1039–1047.
16. **Pearlstein, E., L. I. Gold, and A. Garcia-Pardo.** 1980. Fibronectin: a review of its structure and biologic activity. Mol. Cell. Biochem. **29**:103–128.
17. **Plow, E. F., C. Birdwell, and M. H. Ginsberg.** 1979. Identification and quantitation of platelet-associated fibronectin antigen. J. Clin. Invest. **63**:540–543.
18. **Porath, J., K. Aspberg, H. Drevin, and R. Axen.** 1973. Preparation of cyanogen bromide-activated agarose gels. J. Chromatogr. **86**:53–56.
19. **Robbins, A. B., J. E. Doran, A. C. Reese, and A. R. Mansberger.** 1980. Cold insoluble globulin levels in operative trauma: serum depletion, wound sequestration and biological activity: an experimental and clinical study. Am. Surg. **46**:663–672.
20. **Ruoslahti, E., and E. Engvall.** 1978. Immunochemical and collagen-binding properties of fibronectin. Ann. N.Y. Acad. Sci. **312**:178–191.
21. **Ruoslahti, E., and A. Vaheri.** 1975. Interaction of soluble fibroblast surface antigen with fibrinogen and fibrin. Identity with cold insoluble globulin of human plasma. J. Exp. Med. **141**:497–501.
22. **Ruoslahti, E., A. Vaheri, P. Kuusela, and E. Linder.** 1973. Fibroblast surface antigen: a new serum protein. Biochim. Biophys. Acta **322**:352–358.
23. **Saba, T. M., F. A. Blumenstock, W. A. Scovill, and H. Bernard.** 1978. Cryoprecipitate reversal of opsonic alpha-2-surface binding glycoprotein deficiencies in septic surgical and trauma patients. Science **201**:622–624.
24. **Scovill, W. A., T. M. Saba, F. A. Blumenstock, H. Bernard, and S. R. Powers, Jr.** 1978. Opsonic α2 surface binding glycoprotein therapy during sepsis. Ann. Surg. **188**:521–529.
25. **Sjöquist, J., B. Meloun, and H. Hjelm.** 1972. Protein A isolated from *Staphylococcus aureus* after digestion with lysostaphin. Eur. J. Biochem. **29**:572–578.
26. **Sjöquist, J., J. Movitz, I. B. Johansson, and H. Hjelm.** 1972. Localization of protein A in the bacteria. Eur. J. Biochem. **30**:190–194.
27. **Winblad, S., and C. Ericson.** 1973. Sensitized sheep red cells as a reactant for *Staphylococcus aureus* protein A. Acta Pathol. Microbiol. Scand. Sect. B **81**:150–156.