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Binding of Human Fibronectin to Group A, C, and G Streptococci

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A total of 387 bacterial strains belonging to 35 species were tested in direct binding experiments for the uptake of purified radiolabeled human fibronectin. Positive binding was found in group A, C, and G streptococci and in *Staphylococcus aureus*. The group C streptococcal species *Streptococcus equisimilis*, *Streptococcus zooepidemicus*, *Streptococcus equi*, and *Streptococcus dysgalactiae* were uniformly reactive with fibronectin. Beta-hemolytic bovine group G streptococci showed the same degree of reactivity as human group G strains. In contrast, only 4 out of 15 alpha-hemolytic bovine group G strains were able to bind fibronectin. The uptake of fibronectin measured at room temperature with a human group G streptococcus was a slow, time-dependent process with maximum binding after approximately 1 h. Human polyclonal immunoglobulin G and serum albumin tested in inhibition experiments did not affect the fibronectin binding. Fibronectin seems, therefore, to interact with a surface component that is different from the specific binding sites previously described for human immunoglobulin G and serum albumin.

Fibronectin is a high-molecular-weight glycoprotein found in a soluble form in plasma and many other body fluids and in an insoluble form in connective tissue and basement membranes. Characteristic of fibronectin is its ability to interact with a variety of ligands, such as collagen, fibrinogen, fibrin, actin, and glycosaminoglycans (see recent reviews 25, 27, 35).

The binding of fibronectin to microorganisms was first reported by Kuusela, who described an interaction with protein A-carrying staphylococci (13). Recently, Beachey and Simpson have presented evidence for a similar interaction with group A streptococci (1, 28–30). Studies by other investigators have indicated that fibronectin might be involved in the attachment of *Candida albicans* to human host cells (E. Segal, K. S. Kerk, R. Calderone, and W. M. Scheld, Clin. Res. **30**:378A, 1982).

The possible significance of fibronectin reactivity as a modulator of the host-parasite relation initiated the present investigation, which was designed to explore systematically bacterial species for fibronectin binding capacity. Direct binding experiments, performed with purified isotope-labeled human fibronectin, showed that group C and G streptococci can bind soluble fibronectin as effectively as group A streptococci do.

MATERIALS AND METHODS

Bacterial strains. A total of 387 bacterial strains belonging to 35 species were included in the investigation (Table 1). The streptococcal strains studied have been described in earlier reports (21, 22, 24). All of the other strains were clinical isolates from human specimens cultured at the Clinical Microbiology Laboratory, University Hospital, Lund, Sweden. Bacterial strains were stored at −80°C suspended in fetal calf serum. Some of the streptococcal strains were also maintained on sealed blood agar plates and transferred every 4 to 6 weeks. Streptococci were serogrouped by the coagglutination method (2). Group A streptococci were T typed by slide agglutination with commercially available antisera (Institute of Sera and Vaccines, Prague, Czechoslovakia).

For binding studies, streptococci and pneumococci were grown in Todd-Hewitt broth (Oxoid Ltd., Basingstoke, United Kingdom). *Haemophilus influenzae* strains were cultured in Todd-Hewitt broth fortified with X and V factors. A broth containing peptone, yeast extract, hemin, starch, and vitamins was used for growing *Neisseria gonorrhoeae* (31). All other strains were grown in Tryptone broth (Difco Laboratories, Detroit, Mich.).

Purification of human fibronectin. Fibronectin was isolated from fresh human plasma by affinity chromatography on immobilized gelatin (6). The purification procedure was identical to that described earlier, except that bound fibronectin was eluted with 2.0 M arginine in 0.04 M Tris-hydrochloride buffer (pH 7.5) instead of urea (14). Fibronectin concentration was

measured spectrophotometrically by using an absorbance ($E = {}^{1}_{1}{}^{cm}_{mg/ml}$) value at 280 nm of 1.28 (19). Possible aggregates were removed by centrifugation at 10,000 × *g* for 15 min. Purified material was analyzed by 6% sodium dodecyl sulfate, polyacrylamide gel electrophoresis (15) in the presence and absence of β-mercaptoethanol. Major polypeptide bands corresponding to molecular weights of 440,000 and 220,000 were seen when tested under nonreducing and reducing conditions, respectively.

Radiolabeling. Purified human fibronectin (0.4 mg/ml in 0.15 M phosphate buffer, pH 7.5) was labeled with ^{125}I (Radiochemical Centre, Amersham, United Kingdom) by a modified lactoperoxidase method (17). Briefly, 20 μg of fibronectin was mixed with 0.2 mCi of isotope and 5 μg of lactoperoxidase (Sigma Chemical Co., St. Louis, Mo.) in a final volume of 50 μl of phosphate buffer. The reaction was initiated by adding 2 μl of 8.8 mM hydrogen peroxide and was allowed to proceed at room temperature under vigorous mixing for 40 s. The sample was diluted with 2 ml of phosphate-buffered saline (0.12 M NaCl, 0.03 M phosphate, pH 7.4) containing 0.02% sodium azide and 0.05% Tween 20 (PBSA-Tween). Incorporation of isotope into the protein was determined by paper chromatography (10). Free unreacted isotope was removed by extensive dialysis against PBSA-Tween buffer. Labeled fibronectin (specific activity, 5 mCi/mg of protein) was stored at 4°C and was used within 1 month. Proteolytic fragmentation was minimized by adding the plasmin inhibitor epsilon-amino-caproic acid (Epsikapron; AB Kabi, Stockholm, Sweden) to a final concentration of 0.025 M. Approximately 90% of the radioactivity of the labeled material could be precipitated with specific rabbit anti-fibronectin antibodies.

Binding assay. Bacteria grown overnight in appropriate liquid culture medium were harvested by centrifugation (1,800 × *g* for 10 min), washed twice in PBSA-Tween buffer, and then suspended to a concentration of 10^9 organisms per ml, as calculated from the optical density at 520 nm on diluted samples in a Beckman CP-1 colorimeter (18). Selected bacterial organisms were stabilized by heating the bacterial suspension to 80°C for 5 min.

Binding experiments were performed in duplicate in disposable polystyrene tubes (70 by 11 mm; A/S Nunc, Roskilde, Denmark) by mixing 0.1 μg of labeled fibronectin with 2×10^8 bacterial organisms in a final volume of 225 μl of PBSA-Tween buffer. After 1 h at room temperature, 2 ml of PBSA-Tween buffer was added, the bacteria were deposited by centrifugation, and the radioactivity of the pellet was determined. The fibronectin bound was expressed as a percentage of the total labeled protein which was added to 2×10^8 cells. Binding to test tubes in the absence of bacteria was less than 5%.

Binding experiments were also performed with incubation times ranging from 2 to 120 min. Further uptake of labeled fibronectin was prevented by adding 500 μl of ice-cold normal human serum diluted 1:10 in PBSA-Tween buffer.

Dissociation studies. Radiolabeled fibronectin (0.2 μg) was allowed to bind to 5×10^8 bacterial organisms. The radioactivity of the bacterial pellet was determined, and pellets were suspended in 1.0-ml portions of KSCN solutions with molarities ranging from 0.25 to 3.0. After 20 min at room temperature, the bacteria were again deposited by centrifugation, and the radioactivity of the second pellet was measured. The amount of fibronectin still bound to the pellet was expressed as the percentage of radioactivity of the first pellet.

Inhibition experiments. Increasing amounts of normal human serum (containing 0.2 mg of fibronectin per ml), purified polyclonal human immunoglobulin G (IgG; AB Kabi), and human serum albumin (Sigma Chemical Co.) were mixed with 0.1 μg of radiolabeled fibronectin, and the binding of radioactivity to 2×10^8 bacterial organisms was determined.

RESULTS

Fibronectin reactivity in different bacterial species. A total of 387 bacterial strains belonging to 35 species were tested for binding of purified isotope-labeled human fibronectin (Table 1). Fibronectin reactivity, defined by a definite uptake to freshly grown bacterial organisms, was found in group A, C, and G streptococci as well as in *Staphylococcus aureus* (Fig. 1). Binding levels below 15% were considered negative. Of 17 group A streptococcal strains tested, 15 were reactive, with binding levels ranging from 18 to 53%; 2 strains were consistently negative. There was no correlation between binding capacity and T type. All 53 group C streptococci, representing the species *Streptococcus equisimilis* (human group C strains), *Streptococcus zooepidemicus*, *Streptococcus equi*, and *Streptococcus dysgalactiae*, showed binding of fibronectin with uptake levels between 30 and 68%. *S. zooepidemicus* and *S. dysgalactiae* strains were more reactive than *S. equisimilis* and *S. equi* isolates (Fig. 1).

Beta-hemolytic group G streptococci of human and bovine origin were uniformly reactive. In contrast, only 4 out of 15 alpha-hemolytic bovine group G streptococci showed positive binding (Fig. 1). All *S. aureus* strains showed a definite but variable degree of reactivity. A total of 14 *Staphylococcus epidermidis* and 11 *Staphylococcus saprophyticus* isolates were negative in the fibronectin binding test (Fig. 1). Two *Escherichia coli* strains exhibited a weak reactivity with an uptake of approximately 20%. All other strains were negative (Table 1).

The effect on heat treatment was determined in direct binding experiments with heat-treated organisms. Studies on 10 streptococcal strains capable of binding fibronectin showed that heat-treated bacteria were as reactive as freshly grown organisms.

A total of 20 human group A, C, and G streptococcal strains which had been maintained in the laboratory for 4 to 5 years on blood agar plates were compared with the original isolates stored at −80°C. No difference in fibronectin binding capacity was found (data not shown).

A number of experiments were performed

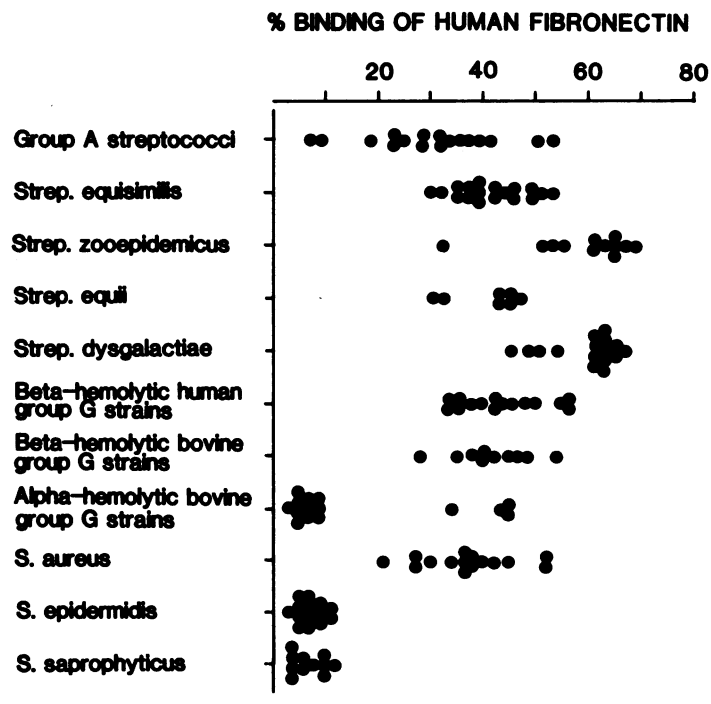

FIG. 1. Binding of human fibronectin to 150 bacterial strains representing defined streptococcal and staphylococcal species. Reactivity is expressed as the capacity of 2×10^8 bacterial cells to bind 0.1 μg of purified isotope-labeled fibronectin.

with bacteria suspended in phosphate-buffered saline containing 0.1% bovine serum albumin instead of PBSA-Tween buffer. No difference was observed when 10 fibronectin-binding and 10 nonreactive strains were tested for binding (data not shown).

Binding as a function of time. The rate of fibronectin uptake was determined in experiments performed with variable incubation times. Further binding of labeled material was prevented by adding normal human serum, which provided an excess of unlabeled fibronectin competing for binding. Displacement of bound fibronectin was minimized by rapid cooling of the mixture before separation of the components by centrifugation. A human group G streptococcus (strain G-148) and an *S. aureus* strain (Cowan I) presented similar pictures, with a rather slow uptake detectable after 5 to 10 min of incubation (Fig. 2A). Maximum binding was obtained with an incubation time of 60 to 120 minutes.

Binding as a function of bacterial cell concentration. Uptake levels were recorded with 10^6 to 10^9 bacterial organisms in the test system. The total number of organisms was kept constant by adding *E. coli* organisms which were unable to bind fibronectin (Fig. 2B). A steady increase in binding levels was observed, with a maximum uptake of 50 to 60%. The group G streptococcus strain G-148 was capable of binding slightly more fibronectin than *S. aureus* Cowan I did.

Elution of bacteria-bound fibronectin. Radiolabeled fibronectin was dissociated from a group G streptococcus and from a *S. aureus* strain by using KSCN solutions of different molarities (Fig. 3). Fibronectin bound to staphylococci was eluted at lower molarities than fibronectin attached to the streptococcal cell surface. For 50% detachment, KSCN concentrations of 0.3 and 1.0 M were needed for staphylococci and streptococci, respectively, indicating a more stable binding between fibronectin and streptococci.

Inhibition experiments. Normal human serum showed a dose-dependent inhibition of the fibronectin binding to group G streptococci (strain G-148). Adding 2 μl of serum reduced the uptake of labeled fibronectin by 50%, and the use of 10 μl of serum resulted in complete inhibition (data not shown). Human IgG and serum albumin reduced the fibronectin uptake by less than 10%, even at a concentration of 0.5 mg/ml (data not shown). This concentration was sufficient to saturate the IgG and albumin receptors exposed on the surface of the test organisms.

DISCUSSION

The present systematic investigation revealed that fibronectin reactivity, defined by in vitro binding of purified isotope-labeled human fi-

bronectin, is found in certain staphylococcal and streptococcal species. Our data confirm earlier studies by other investigators reporting that *S. aureus* strains (4, 7, 13, 20, 34) and group A streptococci (1, 30) can interact with human fibronectin, but the fibronectin reactivity demonstrated in group C and G streptococci has not been described before. Segal and co-workers have reported that fibronectin-coated microtiter dishes are capable of binding *C. albicans* yeast cells (Clin. Res. **30**:378A, 1982). Six different *C. albicans* strains were tested in the present study, but none of them showed any reactivity. However, a low-avidity binding, requiring multipoint attachment provided by a fibronectin-coated surface, cannot be totally excluded by our binding experiments performed with fibronectin in a soluble phase.

TABLE 1. Capacity of different bacterial species to bind human fibronectin[a]

Bacteria	% Fibronectin binding		No. of strains tested
	Mean	Range	
Group A streptococci	30	7–53	17
Group B streptococci	5	3–9	16
Group C streptococci			
S. equisimilis	41	30–49	18
S. zooepidemicus	59	32–68	12
S. equii	41	30–47	7
S. dysgalactiae	60	45–66	16
Group D streptococci	4	3–5	18
Group G streptococci			
Beta-hemolytic human strains	42	34–56	15
Beta-hemolytic bovine strains	41	28–54	10
Alpha-hemolytic bovine strains	17	5–44	15
Group M streptococci	4	2–5	7
Group N streptococci	6	3–11	11
Group P streptococci	9	4–15	11
Group U streptococci	5	3–8	15
Streptococcus uberis	10	5–22	2
Streptococcus pneumoniae	7	4–12	10
Staphylococcus aureus	37	21–52	15
Staphylococcus epidermidis	6	3–12	14
Staphylococcus saprophyticus	6	3–10	11
Neisseria meningitidis	8	6–10	8
Neisseria gonorrhoeae	7	3–13	21
Haemophilus influenzae	8	6–12	10
Escherichia coli	6	3–20	18
Other gram-negative species[b]		<10	90
Candida albicans	6	5–7	6

[a] Binding levels expressed as the percentage of uptake of 0.1 μg of radiolabeled fibronectin to 2×10^8 bacterial cells.

[b] Species of the genera *Klebsiella, Proteus, Pseudomonas, Enterobacter, Acinetobacter, Citrobacter, Alcaligenes, Providencia, Salmonella, Shigella,* and *Yersinia.*

Fibronectin binding was detected in human group C streptococci (*S. equisimilis*) as well as in *S. zooepidemicus, S. equi,* and *S. dysgalactiae,* species of bovine and equine origin (3, 5). Binding was also obtained with human and bovine group G streptococci, including the alpha-hemolytic bovine isolates described by Fodstad (9). Thus, fibronectin reactivity is apparently a basic property of group C and G streptococci. This observation suggests a relationship with fibronectin-binding group A streptococci, perhaps through a common evolutionary pathway. Group A, C, and G streptococci have many other common characteristics, including a ca-

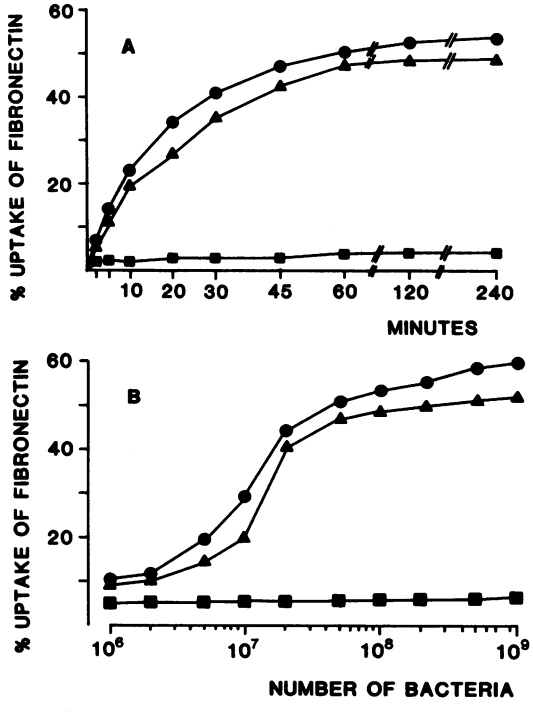

FIG. 2. Fibronectin uptake as a function of time (A) and bacterial cell concentration (B). Symbols: ●, group G streptococcus strain G-148; ▲, *S. aureus* Cowan I; ■, *E. coli* 315.

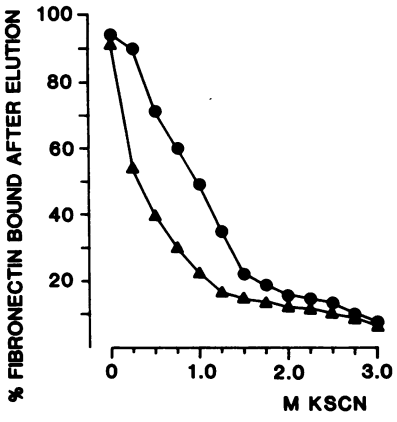

FIG. 3. Elution of isotope-labeled fibronectin bound to bacterial organisms. The amount of radioactivity remaining on the bacteria after elution is plotted as a percentage of radioactivity initially bound to the bacterial pellet. Symbols: ●, group G streptococcus strain G-148; ▲, *S. aureus* Cowan I.

pacity to interact with human proteins such as IgG (21, 22, 24), fibrinogen (12), and β_2-microglobulin (11). Most group A, C, and G streptococcal strains can, therefore, interact through separate binding sites with more than one human protein. This fact should be kept in mind when studies are performed to determine the in vivo significance of these host-parasite interactions between fibronectin and streptococci.

Although specific attempts were not made to identify the fibronectin-reactive component on the bacterial cell surface, some characteristic features were observed. A limited number of streptococcal strains were heated 80°C for 5 min without any obvious loss in fibronectin-binding capacity, implying that the receptor is a relatively heat-stable structure. Streptococcal strains maintained on blood agar plates for several years showed reactivity, suggesting that the fibronectin-binding structure is a genetically stable component. The fact that polyclonal IgG and serum albumin did not inhibit the fibronectin uptake indicates that the fibronectin molecule interacts with a surface component different from the specific binding sites previously described for these serum proteins (22, 23). On the basis of these results, it is not possible to determine whether the binding sites for fibronectin on staphylococci and various streptococci are similar, but our elution studies suggest that there are at least two different binding sites. Studies by Simpson and Beachey have suggested that the fibronectin binding to group A streptococci is mediated by lipoteichoic acid exposed on the cell surface (28, 29). Similar studies by Verbrugh and co-workers have indicated that the fibronectin binding site on staphylococci is a minor protein component distinct from lipoteichoic acid (34). In fact, a fibronectin-binding staphylococcal protein has recently been isolated and characterized by Espersen and Clemmensen (8).

Fibronectin is widely distributed in the human body (25, 27, 32), and it is reasonable to think that colonizing and invading bacteria which bind fibronectin in vitro may interact also in vivo with either soluble or cell-associated fibronectin. Purified plasma fibronectin has been shown to enhance the phagocytosis of gelatin-coated latex particles (33), but in the case of *S. aureus*, fibronectin seems to mediate the attachment of bacteria to effector cells and to optimize the phagocytosis (16, 26, 34). Firbronectin is also present on oropharyngeal epithelial cells (1, 36). Recent studies by Simpson and Beachey have suggested that cell-associated fibronectin mediates the adherence of group A streptococci to the mucosal surface (28, 29). The present demonstration of fibronectin-binding capacity in group C and G streptococci indicates that these species may also be capable of similar host interactions.

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