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Rapid Identification of Fibronectin, Vitronectin, Laminin, and Collagen Cell Surface Binding Proteins on Coagulase-Negative Staphylococci by Particle Agglutination Assays

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Seventeen strains of ten different species of coagulase-negative staphylococci were shown to interact with collagen, laminin, fibronectin, and vitronectin immobilized on latex beads. Different species of coagulase-negative staphylococci have different capacities to agglutinate proteins. Cells of 18 strains of *Staphylococcus haemolyticus* reacted more strongly than did cells of 18 *Staphylococcus epidermidis* strains with proteincoated latex beads, although no significant difference in cell surface hydrophobicity or charge could be shown. The cell surface receptors of *S. haemolyticus* were more heat and protease resistant than were *Staphylococcus aureus* receptors. Strains of *Staphylococcus saprophyticus* isolated from urinary tract infections showed a high capacity to adhere to laminin. The ability to agglutinate fibronectin and collagen was common among coagulase-negative staphylococci isolated from other infections; 55% (31 of 56) and 63% (35 of 56) agglutinated fibronectin and/or collagen. *S. haemolyticus* and *S. epidermidis* bound to both N-terminal (29-kDa) and C-terminal (120-kDa) fragments of fibronectin.

Coagulase-negative staphylococci (CNS) are opportunistic pathogens causing infections associated with foreign bodies, such as intravascular catheters, and vascular grafts; these infections usually persist until the biomaterial is removed (33). The CNS group is heterogenous, comprising more than 30 different species (13). *Staphylococcus epidermidis* is the species most commonly isolated from human infections, but *Staphylococcus haemolyticus* is commonly isolated from severe vascular graft infections (16) and *Staphylococcus saprophyticus* is a urinary tract pathogen (10). The virulence factors involved in the pathogenesis of CNS infections are not well understood. Cell surface hydrophobicity and slime production have been proposed to be involved in the adhesion and colonization of CNS to biomaterials (8).

For many pathogens, binding to various components of the extracellular matrix in open wounds and damaged tissues is most likely the first step in tissue colonization and establishment of an infection (7, 31). Several studies have shown the binding of collagen (9, 29), laminin (7, 16, 26), fibronectin (14, 25), and vitronectin to *Staphylococcus aureus* (4, 6, 23). We previously showed a synergistic binding of vitronectin and collagen on the staphylococcal surface (23). However, how CNS interact with such host-tissue proteins has not been much investigated. Strains of various species bind fibronectin and collagen, although often to a much lesser extent than do strains of *S. aureus* (27).

We developed a rapid, simple latex particle agglutination assay (PAA) for screening *S. aureus* cell surface structures interacting with collagen and fibronectin (22). This test was evaluated for studies on fibronectin, vitronectin, collagen, and laminin binding properties of CNS strains of different species isolated from various infections. The influence of hydrophobicity and charge on the cell surface properties, as measured by partitioning in polymer two-phase systems, was also studied to evaluate the possible role of nonspecific binding.

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MATERIALS AND METHODS

Chemicals. Fibronectin was purified from human plasma on gelatin-Sepharose (30). The 29-kDa N-terminal and 150-kDa fragments of bovine fibronectin were kind gifts from Marcus Back, Medicarb AB, Stockholm, Sweden. The 120- and 105-kDa fragments of human fibronectin were kind gifts from Staffan Johansson, Biomedicum, Uppsala, Sweden. The 120-kDa fragment was obtained by cleavage of fibronectin with chymotrypsin, whereas the 150-kDa fragment was obtained by cleavage of fibronectin with thermolysine. Vitrogen 100 collagen I (containing 95% type I collagen and 5% type III collagen) was purchased from Collagen Corp., Palo Alto, Calif. Collagen type II was a kind gift from K. Rubin, Biomedicum, Uppsala, Sweden. Highly purified human serum fibrinogen and immunoglobulin G were supplied by Kabi, Stockholm, Sweden. Laminin, isolated from an Engelbreth-Holm-Swarm transplantable mouse tumor (28), was kindly supplied by Kaija Valkonen, University of Oulu, Oulu, Finland. Vitronectin was purified from human plasma on heparin-Sepharose (32). D-Glucose was purchased from BDH, Poole, England. A fibronectin-binding protein–protein A (SpA) fusion protein, ZZFR, was obtained from Kabigen, Stockholm, Sweden. D-Galactose, L-fucose, *N*-acetylgalactoseamine, D-mannose, lactoferrin, transferrin, ovalbumin, collagen IV from human placenta (C-7521), poly-L-proline (P-2254), poly-L-aspartic acid (P-6762), poly-L-lysine (P-6516), chymotrypsin, pepsin, proteinase K, trypsin tunicamycin (T-7765), and gelatin were purchased from Sigma Chemical Co., St. Louis, Mo. Poly-L-lysine (33220) was from Serva, Heidelberg, Germany. Latex bead (0.8-μm diameter) suspensions and Todd-Hewitt broth were purchased from Difco Laboratories, Detroit, Mich. Merthiolate was from Kebo lab AB, Stockholm, Sweden, and heparin was from Lövens Läkemedel, Malmö, Sweden. Staphaurex (a latex suspension detecting surface receptors of *S. aureus* interacting with fibrinogen and immunoglobulin G) was purchased from Wellcome Diagnostics, Dartford, England. Blood agar base from Lab M, Salford, England, was supplemented with 4% horse blood. Mannitol salt, staphylococcus

medium 110, and proteose peptone agar were purchased from Oxoid Ltd., Basingstoke, England.

Bacterial strains. Seventeen laboratory reference strains were used in the first evaluation of the PAA. Clinical isolates of CNS were from various clinical human infections: 7 from osteomyelitis, 9 from endocarditis, 28 from serious graft infections, 14 from urinary tract infections, and 26 from catheter-related sepsis. In addition, 15 CNS strains from the skin and nasal flora of healthy persons were also studied. *S. haemolyticus* E 2498, used in previous study (23), was an osteomyelitis isolate, and *S. lugdunensis* 2342 was an endocarditis isolate. Strains were grown on blood agar for 24 h at 37°C for all binding assays. Bacterial colonies were suspended and washed twice in 0.02 M potassium phosphate (Pp) buffer (pH 6.8). Bacterial cells were resuspended in the same buffer to approximately 10^{10} CFU/ml and immediately used in various binding assays. The same cell suspensions were used for the two-phase system tests (see below). *S. aureus* Cowan 1 and Wood 46 were included in all experiments as positive and negative controls, respectively.

Preparation of latex reagents. Standard latex reagents were prepared and used as previously described (1, 22). Briefly, 1-ml samples of latex particle suspensions were mixed with 3.0 ml of 0.17 M glycine-NaOH buffer (pH 8.2), 100-μg samples of highly purified protein or protein fragments were added, and the mixtures were kept at 30°C for 12 h on a horizontal shaker at 50 rpm. The mixtures were centrifuged (9,200 × *g*, 5 min, 10°C), and the pellets were suspended in 2.0 ml of 0.17 M glycine-NaOH buffer containing 0.01% ovalbumin and 0.01% merthiolate and kept at 4°C for 12 h. Gelatin was dissolved by heating at 45°C for 10 min in a water bath and then added to the latex beads. Protein-coated latex bead suspensions were diluted 1:5 in Pp buffer (pH 6.8) and then tested in two-phase system tests.

PAA. The PAA was performed as previously described (1, 22). Briefly, latex reagents (20 μl) were placed on glass slides, and equal volumes of bacterial cell suspensions were added. The two drops were gently mixed, and the agglutination reaction was read after 2 min. The reactions were scored (PAA value) from strongly positive (score of 3) to weakly positive (score of 1) or negative (score of 0) as previously described (22). The strains were tested for autoaggregation by mixing one drop of bacterial cell suspension with one drop of Pp buffer.

Particle agglutination inhibition assay. PAA reactivity was blocked with 0.1 M D-galactose, D-glucose, L-fucose, *N*-acetylgalactoseamine, or D-mannose or 0.1% fibrinogen, immunoglobulin G, lactoferrin, transferrin, collagen I, fibronectin, the 29-kDa fragment of fibronectin, gelatin, or heparin. The inhibitor (100 μl) was preincubated with an equal volume of a bacterial cell suspension for 30 min at 20°C and then mixed with the PAA reagents. Pp buffer was used as a control.

For blocking of PAA reactivity with polypeptides, poly-L-proline, poly-L-asparagine, or poly-L-lysine was dissolved in Pp buffer, and 100 μl (containing 100 μg) of the solution was mixed with 100 μl of bacterial cell suspension. After 30 min of incubation at 20°C, cells were tested for reactivity to collagens I and II. Test reactions were scored as in the standard PAA.

Protease treatment. Bacterial cells (10^9 cells in 100 μl) were suspended in appropriate buffers and incubated with 100 μg of various proteases at 37°C for 1 h. The cells were washed once with 2 ml of Pp buffer (pH 6.8) (15 min, 1,760 × *g*) and tested in the standard binding assay. The buffers for the various proteases were 0.01 M phosphate-buffered saline (PBS) with 0.5 mM $CaCl_2$ (pH 7.2) for chymotrypsin and proteinase K, 0.07 M PBS (pH 4.0) for pepsin, and 0.07 M PBS (pH 7.2) for trypsin.

Tunicamycin. *S. haemolyticus* E 2498 and SM 131 cells were grown at 37°C for 24 h with continuous shaking in Todd-Hewitt broth with tunicamycin (0, 3.1, 6.3, and 12.5 μg/ml) (24). The cells were washed, suspended in Pp buffer, and tested in the PAA as described above.

Partitioning in aqueous two-phase system. An aqueous polymer two-phase system containing polyethylene glycol (PEG 6000; Kebo, Stockholm, Sweden) (7.13%, wt/wt) and dextran (molecular weight, 48,000; Sigma) (8.75% [wt/wt] in 0.015 M NaCl [pH 6.8]) was prepared as a phase system (1, 12). To determine particle charge, negatively charged dextran-sulfate (molecular weight, 500.000; Pharmacia, Uppsala, Sweden) at a concentration of 0.40% (wt/wt) was included in the phase system, replacing an equivalent amount of dextran. Similarily, positively charged DEAE-dextran at a concentration of 0.40% was included, replacing an equal amount of dextran in the phase system.

Hydrophobic affinity partitioning was performed by including monosubstituted PEG-palmitate and dextran-palmitate, replacing an equal amount of PEG (for the PEG-palmitate) or dextran (for the dextran-palmitate) in the phase system. Partitioning was performed by adding 100 μl of a bacterial cell suspension (approximately 5×10^9 CFU/ml) or 100 μl of coated latex particles to 0.9 ml of the phase systems (previously homogenized by stirring), mixing by gently shaking, and allowing phase separation at 20°C for 1 h. The concentration of latex particles in the PEG-rich top phase and the dextran-rich bottom phase was estimated turbidimetrically at 540 nm. The amount of cells (or beads) recovered in the bottom phase was expressed as a percentage of the original concentration of added cells (or beads). Differences in the hydrophobic and charge properties of the various bacterial strains (or coated latex beads) are expressed as delta log *G*, which is defined by the equation log (*G* value of dextran-sulfate or PEG-palmitate system)/(*G* value of the PEG-dextran system), where *G* is the percentage of cells in the bottom phase divided by the percentage of cells in the rest of the system. Dextran-sulfate was used for the delta log *G* charge, and PEG-palmitate was used for delta log *G* hydrophobicity.

RESULTS

Influence of culture media. Reference strains were grown on blood agar, mannitol-salt agar, brain heart infusion agar, proteose agar, or staphylococcus medium 110 and tested for reaction with latex particles coated with proteins, i.e., agglutinated fibronectin, collagen I, gelatin, and Staphaurex. Blood agar was the optimal medium for CNS strains because few strains autoaggregated. The numbers of strains reacting in the PAA with fibronectin, collagen I, and gelatin were highest when the strains were grown on blood agar (data not shown). None of the CNS strains agglutinated Staphaurex.

Studies on reference strains and clinical isolates. Seventeen reference CNS strains were examined with different proteins immobilized on latex beads (Table 1). Ten of the strains showed strong reactions with collagen and gelatin (denatured collagen), and seven bound fibronectin. Agglutination with laminin or vitronectin was less common. Eighty-four CNS strains isolated from different human infections and 15 CNS strains from healthy persons were examined in the PAA with fibronectin, vitronectin, laminin, collagen I, gelatin, and Staphaurex (Fig. 1). The numbers of autoaggregating strains were 1 of 7 osteomyelitis strains, 2 of 9 endocarditis

TABLE 1. Interaction of CNS reference strains with serum and connective tissue proteins in a PAA

Reference strain	PAA reaction[a] with:					
	Fibronectin	Vitronectin	Laminin	Collagen I	Gelatin	Staphaurex
S. capitis LK 499 (ATCC 27840)	0	0	0	3	0	0
S. capitis MCS A11 (ATCC 27842)	0	0	1	1	3	0
S. cohnii GH 137	3	3	0	3	2	0
S. cohnii DM 224	2	2	2	3	3	0
S. epidermidis RP 12	2	1	0	2	1	0
S. epidermidis AW 269	1	1	0	1	1	0
S. haemolyticus SM 131	3	3	3	3	3	0
S. hominis SP2	3	3	3	3	3	0
S. hominis KL 243 (ATCC 27846)	2	3	2	3	3	0
S. hyicus VA 401	0	0	0	2	0	0
S. hyicus NCTC 10350	0	0	0	0	2	0
S. saprophyticus TW 111	0	0	0	0	0	0
S. saprophyticus KL 20	0	0	0	1	2	0
S. simulans ATCC 27849	0	0	0	0	0	0
S. simulans MK 148 (ATCC 27848)	0	0	0	1	2	0
S. xylosus DM 37	0	0	0	1	0	0
S. xylosus KL 162	3	3	3	3	3	0
S. warneri AW 25 (ATCC 27836)	0	0	1	2	3	0
S. warneri NT 163	1	0	0	3	2	0

[a] Reactions were scored from strongly positive (scored as 3) to weakly positive (scored as 1) or negative (scored as 0).

strains, 3 of 28 graft infection strains, 4 of 14 urinary tract infection strains, 8 of 26 catheter-related sepsis strains, and 8 of 15 strains from healthy individuals. Among the clinical strains isolated from blood (n = 56), PAA agglutination was common to fibronectin (n = 31) and collagen I (n = 35) (Fig. 1). Laminin binding was extremely common (9 of 10 strains) in strains isolated from urinary tract infections, i.e., *S. saprophyticus*. Only one of the seven strains isolated from healthy individuals adhered to fibronectin, vitronectin, laminin, and gelatin, and only three strains showed a positive PAA reaction to collagen I.

Protease treatment. The *S. aureus*, *Staphylococcus lugdunensis*, and *S. haemolyticus* cell surface structures that bound various proteins were sensitive to protease treatment (Table 2). Cell surface structures of *S. aureus* Cowan 1 and *S. lugdunensis* 2342 were sensitive to all proteases used, whereas those of *S. haemolyticus* SM 131 were more protease resistant (Table 2). Only chymotrypsin treatment of cells of *S. haemolyticus* totally abolished the agglutination of all of the proteins tested.

Tunicamycin. Although tunicamycin affected the growth of *S. haemolyticus* SM 131 and E 2498 at the concentrations

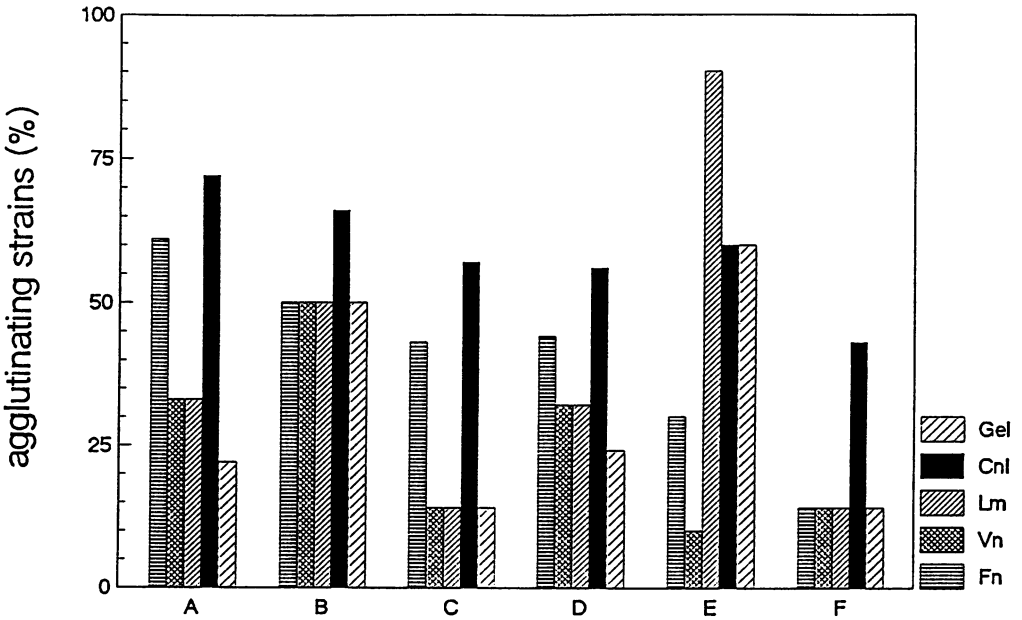

FIG. 1. Binding of clinical CNS isolates (n = 84) to serum and connective tissue proteins in the PAA. Isolates from healthy individuals (n = 15) were tested in the same way. Agglutinating strains were those scored as 2 or 3 in the PAA. Isolates were from following: A, catheter-related sepsis; B, osteomyelitis; C, endocarditis; D, graft infections; E, urinary tract infections; F, skin of healthy individuals. Gel, gelatin; CnI, collagen I; Lm, laminin; Vn, vitronectin; Fn, fibronectin.

TABLE 2. Reaction in the PAA after protease treatment (chymotrypsin, pepsin, proteinase K, and trypsin) of CNS cells

Strain and treatment	PAA reaction[a] with:				
	Collagen I	Fibronectin	Laminin	Vitronectin	Staphaurex
S. aureus Cowan 1					
Chymotrypsin	0	0	0	NT	1
Pepsin	0	0	1	NT	3
Proteinase K	0	0	0	NT	0
Trypsin	0	0	0	NT	1
Control (none)	3	2	2	0	3
S. lugdunensis 2342					
Chymotrypsin	0	0	0	0	NT
Pepsin	0	0	0	NT	NT
Proteinase K	0	0	0	NT	NT
Trypsin	1	0	1	NT	NT
Control (none)	3	1	2	1	0
S. haemolyticus SM 131					
Chymotrypsin	0	0	0	0	NT
Pepsin	3	2	0	2	NT
Proteinase K	3	2	0	0	NT
Trypsin	3	3	0	3	NT
Control (none)	3	3	1	3	0

[a] See footnote *a* of Table 1. NT, not tested.

mentioned above, no effect was observed in the agglutinating ability the coated latex beads; all of the proteins agglutinated like the non-antibiotic-treated control (data not shown).

Inhibition of PAA. Preincubation of fibronectin-coated beads with fusion protein ZZFR at 20°C for 30 min completely blocked the clumping of *S. haemolyticus* SM 131 and *S. aureus* Cowan 1, confirming the receptor specificity of the assay (data not shown). Fibronectin-particle agglutination was completely inhibited by preincubating cells of Cowan 1 with soluble fibronectin, the 29-kDa fibronectin fragment, or lactoferrin, whereas no inhibition could be seen after preincubation of *S. haemolyticus* cells with the same proteins (Table 3). The other glycoproteins, carbohydrates, and heparin did not inhibit cells of either Cowan 1 or SM 131. Heat treatment of *S. aureus* Cowan 1 (88°C, 20 min) almost abolished the PAA reaction to fibronectin and collagen, whereas the same treatment of *S. haemolyticus* SM 131 did not significantly affect PAA binding to fibronectin and collagen.

TABLE 3. Binding of CNS to protein-coated latex beads after preincubation of the cells with carbohydrates, proteins, or peptides

Strain and treatment	PAA reaction[a] with:		
	Collagen I	Fibronectin	Staphaurex
S. aureus Cowan 1			
Control (none)	3	2	3
Fibronectin	3	0	3
29-kDa fibronectin fragment	2	0	3
Gelatin	AA	AA	AA
Lactoferrin	2	0	3
Poly-L-proline	1	NT	NT
S. haemolyticus SM 131			
Control (none)	3	3	0
Fibronectin	3	3	
29-kDa fibronectin fragment	3	3	
Gelatin	0	0	
Lactoferrin	3	3	
Poly-L-proline	3	NT	

[a] See footnote *a* of Table 1. AA, autoaggregating; NT, not tested.

TABLE 4. Ability of staphylococci to react with immobilized human fibronectin and fibronectin fragments of 29 and 150 kDa (bovine) and 105 and 120 kDa (human)

Strain	PAA reaction with:			
	Fibronectin	Fibronectin fragment		
		29 kDa	150 kDa	120 kDa
S. aureus				
Cowan 1	2	2	0	1
Wood 46	0	0	0	0
S. haemolyticus				
MD-7	3	3	0	3
MD-20	3	2	0	1
MD-76a	3	3	0	2
MD-143	2	3	0	2
MD-249a	3	3	0	2
MD-303	3	3	0	3
DSM 20264	3	3	0	3
DSM 20265	2	2	0	1
DSM 20263	3	3	0	2
J9-007	2	3	0	2
B3-107	2	3	0	2
E6-D	1	3	0	1
E2498	2	3	0	3
SM 131	3	3	0	3
S. epidermidis				
E 3380	0	0	0	0
H9-E	1	1	0	1
B2-101	0	0	0	1
H6-L	2	2	0	2
I4-N	2	2	0	2
J9-P	0	0	0	1
H1-P	2	2	0	2
B2-H	0	0	0	1
J8-202	3	3	0	2
C3-104	0	0	0	0
H2-W	2	2	0	2

[a] See footnote *a* of Table 1.

Preincubation of *S. aureus* Cowan 1 cells with poly-L-proline abolished the ability to agglutinate collagen I- and II-coated latex beads, whereas the same incubation of cells of *S. haemolyticus* SM 131 did not affect the PAA reaction with collagen I or II (Table 3). Preincubation of *S. haemolyticus* cells with gelatin (denatured collagen) totally abolished agglutination to all of the proteins tested.

Fibronectin fragments. Strains of *S. haemolyticus* and *S. epidermidis* adhered to the 29- and 120-kDa fibronectin fragments, but none of the strains tested agglutinated with the 150-kDa fragment of fibronectin (Table 4).

***S. haemolyticus* versus *S. epidermidis*.** Generally, cell suspensions of *S. haemolyticus* strains agglutinated in the PAA faster and stronger than did suspensions of *S. epidermidis* (Fig. 2). The cell surface hydrophobicity and charge properties of *S. haemolyticus* and *S. epidermidis* were studied (Fig. 3). There was no significant difference between the strains of the two different species in partitioning in the two-phase system, although strains of each species tended to form two clusters.

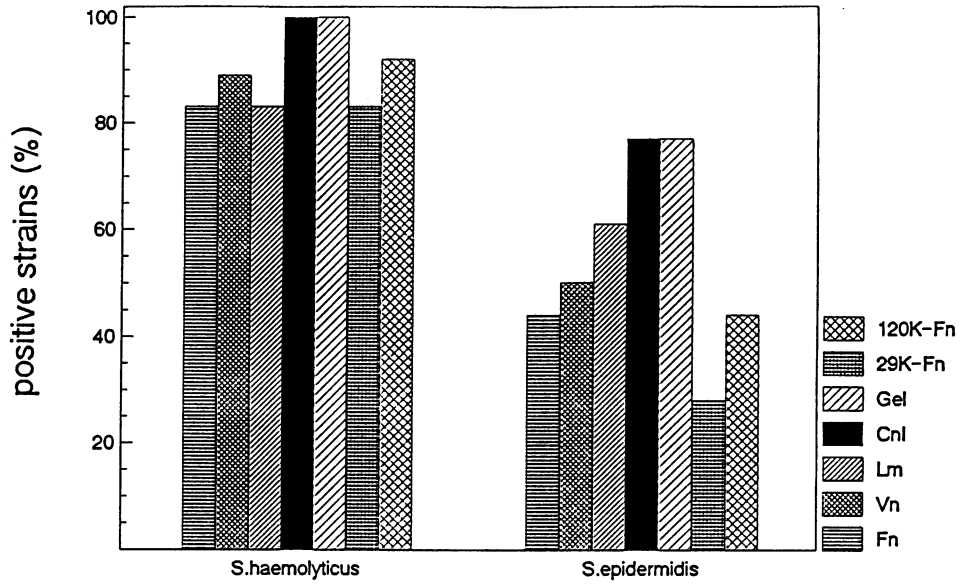

FIG. 2. Percentage of *S. haemolyticus* (n = 18) and *S. epidermidis* (n = 18) strains reacting in the PAA. Agglutinating stains were those scored as 2 or 3 in the PAA. Abbreviations are as in Fig. 1.

Charge and hydrophobicity of coated beads. Uncoated latex beads showed a strong negative charge (−1.3) and a high surface hydrophobicity (−1.3); these characteristics changed when the beads were coated with the different proteins (Table 5).

DISCUSSION

Immobilizing various proteins on latex beads has become a standard procedure for the study of fibrinogen-immunoglobulin binding cell surface structures for rapid diagnosis of *S. aureus* infection (2, 5). We developed a PAA for the detection of fibronectin, fibrinogen, and collagen receptors on *S. aureus* (22). The increasing problem with biomaterial-associated infections caused by CNS encouraged us to develop similar tests to study whether these organisms react with various host proteins. Connective tissue proteins such as collagen and laminin and serum-derived fibronectin and vitronectin may all be involved in a tissue colonization process in surgical and other infections. The CNS are heterogenous, comprising more than 30 species isolated from human skin (13). We have shown that strains of different species of CNS have different capacities to bind to proteins (Table 1). Different culture media and growth conditions influence the expression of cell surface proteins of *S. aureus* (3). For our PAA, culturing on blood agar was optimal, with a low number of autoaggregating strains, for both *S. aureus* and CNS. Autoaggregating strains were commonly isolated from healthy persons (8 of 15 strains). Among the clinical isolates, many strains showed binding to collagen I and fibronectin, whereas laminin commonly bound to *S. saprophyticus* cells. This finding is particularly

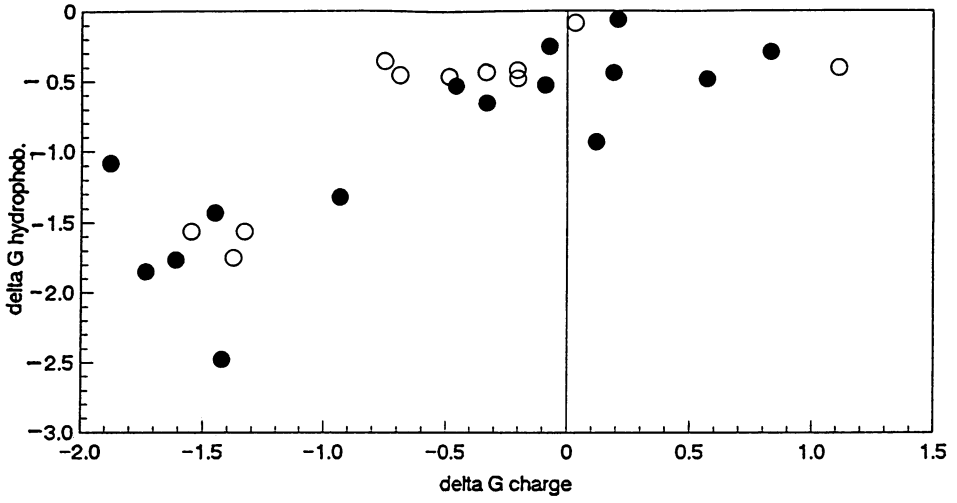

FIG. 3. Partitioning of *S. haemolyticus* (●) and *S. epidermidis* (○) in an aqueous two-phase system. Negative values denote negatively charged cell surfaces or high surface hydrophobicities.

TABLE 5. Partitioning behavior of latex beads coated with different proteins in an aqueous two-phase system of PEG-dextran

Latex beads coated with:	delta log G:	
	Charge[a]	Hydrophobicity[b]
Collagen I	0.0	−0.1
Fibronectin	0.0	−0.9
Vitronectin	−0.1	−0.1
Laminin	−0.2	0.4
Gelatin	−1.0	−0.1
Without protein	−1.3	−1.3
Staphaurex	−0.5	−0.4

[a] Negative value indicates negative charge.
[b] Negative value indicates cell surface hydrophobicity.

interesting, since *S. saprophyticus* is a urinary tract pathogen and laminin is the major basal membrane protein in the kidney (19).

Fibronectin binding. *S. aureus* binds to the NH_2-terminal 29-kDa fragment of fibronectin (21). This fragment contains no sugar, in contrast to other fragments that are highly glycosylated (11). A second binding site for *S. aureus* cells in the C-terminal region has also been reported (15). We show here that cells of various CNS strains commonly bind to immobilized N- and C-terminal fragments of fibronectin. Our observation that the 29-kDa fragment of fibronectin did not inhibit the fibronectin PAA reaction is consistent with the findings that strains of *S. haemolyticus* did not react with the fibronectin binding protein gene probe of *S. aureus* (16a). That a protein A–fibronectin-binding protein fusion protein inhibited the binding of fibronectin to both *S. aureus* and *S. haemolyticus* in the PAA suggests some sequence homology of the fibronectin-binding proteins of these two species. Different binding was previously reported for group A, C, and G streptococci (18) and *S. aureus* (15, 20); this difference is probably due to different conformations of the protein molecules when they are immobilized or in solution. Using ^{125}I-labeled soluble fibronectin and the 29-kDa N-terminal fragment, we could not demonstrate binding to strains of *S. haemolyticus* (2.8 ± 0.7% binding of ^{125}I-labeled fibronectin [n = 29]; 6.8 ± 2.0% binding of ^{125}I-labeled 29-kDa fragment [n = 16]). It was reported previously that fibronectin PAA values do not correlate well with the amount of ^{125}I-labeled protein binding (1). The difference between the protein configuration in solution and that of surface-immobilized proteins could explain this and why preincubation of cells with homologous soluble protein did not inhibit the PAA reactions.

Collagen binding. Soluble poly-L-proline did inhibit the binding of *S. aureus*, but not that of *S. haemolyticus*, to immobilized collagens, thus lending strength to the hypothesis that *S. haemolyticus* and *S. aureus* have different receptors.

Subinhibitory concentrations of tunicamycin, an antibiotic that interferes with protein glycosylation (24), did not affect the binding of *S. haemolyticus* to coated beads. This suggests that it is unlikely that surface carbohydrates are involved in binding.

Why strains of *S. haemolyticus* agglutinated more strongly and more quickly than strains of *S. epidermidis* will be investigated further. These differences cannot simply be explained by differences in cell surface hydrophobicity and charge.

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