INFECTION AND IMMUNITY, Aug. 1989, p. 2358–2363
0019-9567/89/082358-06$02.00/0

Vol. 57, No. 8

Fibrinogen-Binding Protein/Clumping Factor from *Staphylococcus aureus*

MARIA K. BODÉN* AND JAN-INGMAR FLOCK

Center for Biotechnology, Karolinska Institute, Huddinge University Hospital, S-141 86 Huddinge, Sweden

Received 21 February 1989/Accepted 27 April 1989

The binding of staphylococcal components to fibrinogen was studied. Fibrinogen-binding material from lysed staphylococcal cells or culture supernatants was affinity purified on fibrinogen-Sepharose and analyzed on Western (immuno-) blots by the use of fibrinogen and antifibrinogen antibodies. Two main bands of 87 and 19 kilodaltons (kDa) and a weaker band of 35 kDa bound specifically to fibrinogen. A monoclonal antibody bound to all three bands, indicating that these were of the same origin. The yield of these components was much higher in the culture supernatant than on washed cells, suggesting that these molecules are essentially extracellular products. In a plasma coagulase test, the 87-kDa band, but not the 19-kDa band, clotted rabbit plasma, demonstrating that the 87-kDa molecule is coagulase. This was further confirmed by the fact that the 87-kDa band binds specifically to prothrombin. It was shown that the 87- and the 19-kDa molecules were present on the cell surface by surface labeling the cells with ^{125}I. In addition, the fact that killed and washed cells could induce plasma clotting demonstrates that staphylococci have coagulase exposed on the surface. It was concluded that cell-bound coagulase has affinity for fibrinogen also in the absence of prothrombin and thus is responsible for the clumping of staphylococci in fibrinogen.

The fact that staphylococci clump in plasma has been known for more than 80 years (27). The great interest in clumping has been due to its suggested role as a staphylococcal virulence factor (10, 18, 20, 28). It was discovered early that staphylococcal cultures could induce coagulation of plasma. The causative agent of this clotting activity was found to be an extracellular product, coagulase, which was shown to induce polymerization of fibrinogen into fibrin. It has been suggested that coagulase exerts its action by binding to prothrombin, thereby converting the prothrombin into an active form. The coagulase-prothrombin complex causes the release of fibrinopeptides from fibrinogen in a manner similar to that described for thrombin in physiological blood clotting (17). When it was found that washed staphylococci free of culture medium aggregated in plasma, it was suggested that staphylococci produce both free and cell-bound coagulase and that the free coagulase is responsible for the coagulase reaction and the bound coagulase is responsible for the clumping reaction. In the 1950s, Duthie showed that there are additional factors apart from coagulase that contribute to the clumping effect (10). The term clumping factor was introduced, and it was assumed that clumping factor as well as coagulase acted on fibrinogen. Other investigators have claimed that coagulase and clumping factor are identical or very closely related (16). The identity of clumping factor still remains to be solved.

Several factors may be involved in the clumping or aggregation of staphylococci. This has made the task of identifying clumping factor difficult. Staphylococci can aggregate in the presence of specific immunoglobulins directed against staphylococcal antigens, and most normal human sera contain such antibodies (13). Lysozyme in the concentration found in tears can aggregate staphylococci (25). Extracellular matrix proteins in a soluble state, e.g., collagen type IV and laminin, have been found to aggregate staphylococci in vitro (37), and in the same manner clumping can be caused by fibronectin at a concentration found in sera (28, 37). Autoaggregation in isotonic buffers of staphylococcal cells grown in vitro has been described previously (19). Staphylococci can also be incorporated unspecifically into a polymerizing fibrin matrix (9). Fibrin formation can be initiated by coagulase, which is normally extracellular, but which to some extent can be found on the surface of staphylococci (1, 11). An additional possibility is that clumping can be due to the paracoagulation phenomenon, which is caused by electrostatic or hydrophobic forces (6, 22).

In this investigation, we have attempted to find out whether there is a specific nonenzymatic fibrinogen-binding protein on the surface of staphylococcal cells that can contribute to clumping or whether the clumping seen by Much (27), Duthie (10), and others is due only to the presence of protein A, fibronectin-binding protein, and coagulase.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The bacterial strains used are listed in Table 1. Staphylococci were grown as described by Miller et al. (26). Cells that were not used immediately were washed in 70% ethanol and freeze-dried. Bacterial cell walls were solubilized by the method of Fröman et al. (14), with some slight modifications.

Affinity chromatography. Fibrinogen-Sepharose was prepared by coupling 70 mg of fibrinogen (IMCO, Stockholm, Sweden) to 3.5 g of CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) by the procedure recommended by the manufacturer. Since IMCO fibrinogen is delivered freeze-dried in a Tris buffer, the buffer was changed by gel filtration on a Sephadex G-25 (Pharmacia) column. This was done to avoid exposing the highly sensitive fibrinogen to overnight dialysis. A column with fibrinogen-Sepharose was equilibrated with phosphate-buffered saline (PBS) (0.145 M NaCl, 10 mM phosphate; pH 7.4) containing 0.02% NaN_3 and 0.05% Nonidet P-40. The lysed staphylococcal cells or culture supernatants were applied, and the column was subsequently washed with PBS supplemented with 0.355 M NaCl, 0.02% NaN_3, and 0.05% Nonidet P-40. The absorbed

* Corresponding author.

TABLE 1. Bacterial strains

Strain	Characteristic			Source
	Clumping factor	Coagulase	Protein A	
S. aureus Newman	+	+	+	M. Lindberg[a]
S. aureus Newman D_2C	+	−	+	Sigma
S. aureus U320	+	+	−	M. Lindberg
S. epidermidis 247	−	−	−	M. Lindberg

[a] Department of Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

material was eluted with 0.7% acetic acid containing 0.05% Nonidet P-40. The eluted material (eluate) was precipitated with 5% PBS and 80% acetone, left for 5 min at room temperature, and centrifuged at 11,000 × *g* for 15 min.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western (immuno-) blotting. SDS-PAGE was performed with the Phast system (Pharmacia) on 8 to 25% gradient gels supplied by the manufacturer. Gels were run for 100 V · h and were subsequently blotted over to nitrocellulose filters at 65 to 70°C (diffusion blotting), as recommended by Pharmacia. Gels were stained with Coomassie blue in the developer unit, according to the user's manual. The amount of protein was estimated on Coomassie blue-stained gels in a dual-wavelength thin-layer chromatography scanner CS-930 (Shimazdu Corp., Kyoto, Japan). After the protein bands were transferred to nitrocellulose filters, the remaining binding sites were blocked with 0.05% Tween 20 in PBS for 15 min at room temperature. The filters were incubated for 1 h at room temperature with fibrinogen (IMCO), fibronectin (Sigma Chemical Co., St. Louis, Mo.), or human gamma globulin (HGG) (Pharmacia) at concentrations of 10 μg/ml in PBS supplemented with 0.05% Tween 20 and 0.02% NaN_3, or the filters were incubated with prothrombin (10 μg/ml) in PBS containing 0.05% Tween 20. Primary antibodies (goat antifibrinogen [Sigma], goat antifibronectin [Sigma], rabbit anti-HGG, and rabbit antiprothrombin [Dakopatts, Glostrup, Denmark]) were diluted 1:500. Monoclonal antibody 23, which was made by immunizing a mouse with fibrinogen-binding components, affinity purified from lysed *Staphylococcus aureus* Newman cells, was a kind gift from Lech Switalski, University of Alabama, Birmingham. Secondary antibodies conjugated with alkaline phosphatase (ALP) were rabbit anti-goat immunoglobulin G (IgG) (Sigma), goat anti-rabbit IgG (Sigma), and goat anti-mouse IgG (Jackson Immuno Research Laboratories, West Grove, Pa.). The ALP reaction was developed in 100 mM Tris hydrochloride (pH 8.0) containing 10 mM $MgCl_2$, 0.02 mg of α-naphthylphosphate per ml (E. Merck AG, Darmstadt, Federal Republic of Germany), and 0.02 mg of fast blue (Merck) per ml for 10 to 20 min.

^{125}I labeling of whole staphylococcal cells. Staphylococcal cells were surface labeled with ^{125}I by the glucose oxidase-lactoperoxidase method (31).

Coagulase test. Staphylococcal cells from overnight cultures were sterilized as described elsewhere (26). Cells were spread on L-broth agar plates and incubated at 37°C overnight to ensure that there were no live cells in the preparation. Culture supernatants were filtered through 0.22-μm filters (Millipore Corp., Bedford, Mass.) before use. Coagulase tests were performed in coagulase plasma (Difco Laboratories, Detroit, Mich.) with or without the addition of the protease inhibitors *N*-ethylmaleimide (2 mM), EDTA (6 mM) (15), aprotinin (Sigma; 200 U/ml), heparin (Sigma; 40 U/ml) (39), and phenylmethylsulfonyl fluoride (1 mM).

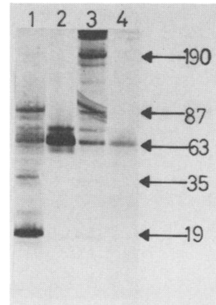

FIG. 1. Western blot analysis of fibrinogen-binding components affinity purified from lysed *S. aureus* Newman cells (300× concentration). Blots were visualized by ALP-conjugated secondary antibody. Arrows indicate molecular masses (in kilodaltons). Lanes: 1, fibrinogen and antifibrinogen antibody; 2, antifibrinogen antibody; 3, fibronectin and antifibronectin antibody; 4, HGG and anti-HGG antibody.

RESULTS

SDS-PAGE and Western blot analysis of whole staphylococcal cells, lysed cells, culture supernatants, and purified material from affinity chromatography. When cells of *S. aureus* Newman (a protein A-, coagulase-, and clumping factor-positive strain) were run on SDS-PAGE and transferred to nitrocellulose filters, five main bands could be detected by probing the filters with fibrinogen and antifibrinogen antibodies. Two of these bands, of approximately 75 and 63 kilodaltons (kDa), were strongly positive in the control with preimmune antisera, antifibrinogen antibodies, or ALP-conjugated secondary antibodies only and could consequently be identified as protein A or protein A derivatives. The remaining three bands of 87, 35, and 19 kDa bound fibrinogen. In *Staphylococcus epidermidis* 247, which is protein A, coagulase, and clumping factor negative, no protein A or 19- and 87-kDa bands could be detected in this assay, but a band of 35 kDa (which is not necessarily identical to the 35-kDa band of strain Newman) was seemingly specific in the fibrinogen-binding assay (data not shown). *S. aureus* U320, which is protein A negative and coagulase positive (but not as strongly positive as *S. aureus* Newman), gave a strong 35-kDa band with fibrinogen but a very weak 87-kDa band and no detectable 19-kDa band. *S. aureus* U320 also gave weak immunoglobulin-binding bands, presumably protein A (data not shown).

To isolate fibrinogen-binding components, *S. aureus* Newman cells were lysed with lysostaphin. The soluble fraction was run over fibrinogen-Sepharose, and eluted material was analyzed in the fibrinogen-binding assay (Fig. 1). In addition to protein A, with a main band of 63 kDa and two additional bands of approximately 190 and 170 kDa, the same three fibrinogen-specific bands as those detected in lysates from whole cells could also be found in the affinity-purified material. After the filters were incubated with fibronectin and antifibronectin antibodies, the high-molecular-mass bands were subsequently identified as fibronectin-binding protein.

When fibrinogen-binding material from culture supernatant of *S. aureus* Newman was affinity purified on fibrinogen-Sepharose and analyzed in the fibrinogen-binding assay, the same bands as from cell lysates could be detected (Fig. 2). These bands were also visible when the filters were incubated with fibronectin and antifibronectin antibodies. This was probably due to contaminating fibrinogen in the fi-

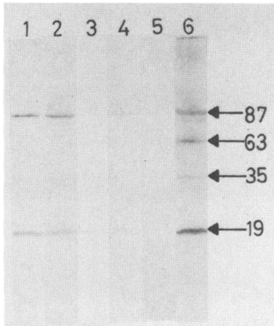

FIG. 2. Western blot analysis of eluate from affinity purification on fibrinogen-Sepharose of fibrinogen-binding components from culture supernatant of *S. aureus* Newman. Blots were visualized by ALP-conjugated secondary antibody. Arrows indicate molecular masses (in kilodaltons). Lanes: 1, Coomassie blue staining of eluate (100× concentration); 2, fibrinogen and antifibrinogen antibody (eluate, 2.5× concentration); 3, antifibrinogen antibody (eluate, 2.5× concentration); 4, fibronectin and antifibronectin antibody (eluate, 2.5× concentration); 5, HGG and anti-HGG antibody (eluate, 2.5× concentration); 6, monoclonal antibody 23 (eluate, 100× concentration).

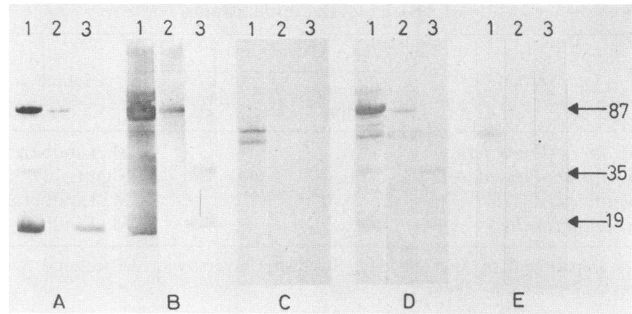

FIG. 3. Western blot analysis of 87- and 19-kDa bands further purified from eluate from affinity purification of fibrinogen-binding components from culture supernatant of *S. aureus* Newman. Blots were visualized by ALP-conjugated secondary antibody. Arrows indicate molecular masses (in kilodaltons). (A) Coomassie blue. (B) Fibrinogen and antifibrinogen antibody. (C) Antifibrinogen antibody. (D) Prothrombin and antiprothrombin antibody. (E) Antiprothrombin antibody. Lanes: 1, eluate from supernatant (800× concentration); 2, purified 87-kDa band; 3, purified 19-kDa band.

bronectin preparation. One major difference between the preparations from lysed cells and culture supernatants was that the 87- and 19-kDa bands were about 100-fold more abundant in the culture supernatant preparations, as determined by multiple dilutions of preparations and scanning of Coomassie blue-stained gels. This suggests that the 87- and 19-kDa bands are essentially extracellular products. Monoclonal antibody 23 bound to protein A and to the 87-, 35-, and 19-kDa bands. This strongly suggests that these three bands are of the same origin.

When culture supernatant from *S. aureus* Newman D_2C, a clumping factor-positive and coagulase-negative mutant of strain Newman, was used in these fibrinogen-binding tests, the protein A bands dominated, even though weak 87-, 35-, and 19-kDa bands could be detected in the Western blot. The cell lysate from the *S. aureus* Newman D_2C strain contained about 10 times less of the 87- and 19-kDa bands compared with cell lysates from the wild-type *S. aureus* Newman strain. In contrast, *S. aureus* Newman D_2C secreted only trace amounts (0.1% compared with the wild type) of these molecules into the culture medium (data not shown).

The 87- and 19-kDa bands from the *S. aureus* Newman culture supernatant preparation were separately cut out from Coomassie blue-stained SDS gels and eluted. These bands were rerun on SDS-PAGE and probed with fibrinogen and antifibrinogen antibodies as well as prothrombin and antiprothrombin antibodies (Fig. 3). As shown in the Coomassie blue staining and even more strongly in the fibrinogen-binding assay, the purified 19-kDa band gave rise to bands of higher molecular mass, one of them being a 35-kDa band, and the 87-kDa band gave rise to a smear of material with both an increased and a decreased migration pattern. Both the 87- and the 19-kDa bands were detected with fibrinogen, but only the 87-kDa band gave a strong signal with prothrombin. An attempt to reduce the 35-kDa portion in the 19-kDa preparation by boiling with 5% β-mercaptoethanol failed (Fig. 4).

Analysis of fibrinogen-Sepharose eluate from surface-labeled cells. To elucidate whether the 19- and the 87-kDa bands from lysed *S. aureus* Newman cells originated from the surface of the cells, intact staphylococcal cells were labeled with ^{125}I. The labeled cells were washed and lysed, and fibrinogen-binding components were affinity purified on fibrinogen-Sepharose. The fibrinogen-binding bands were identified in the fibrinogen-binding assay before the same nitrocellulose blot was subjected to autoradiography (Fig. 5). The 87- and the 19-kDa bands, which were stained on the immunoblot, were also visible on the autoradiograph, indicating that both these bands are exposed on the surface of the staphylococci.

Plasma coagulase test. Coagulase tests were performed on killed and washed cells as well as on cell-free culture supernatant from overnight cultures in brain heart infusion medium (Table 2). Culture supernatant from *S. aureus* Newman contained more coagulase than did washed *S. aureus* Newman cells. *S. aureus* Newman D_2C and *S. epidermidis* 247 were both negative in all coagulase tests, whereas *S. aureus* U320 was positive but the reaction was not as rapid as for *S. aureus* Newman. When eluted material from fibrinogen-Sepharose and purified 87- and 19-kDa bands were used in the coagulase test, the fibrinogen-binding molecules from *S. aureus* Newman culture supernatant and

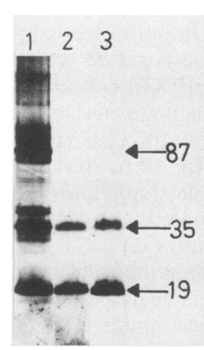

FIG. 4. Western blot analysis of reduced and unreduced purified 19-kDa band from eluate of affinity purification of fibrinogen-binding components from culture supernatant of *S. aureus* Newman, probed with fibrinogen and antifibrinogen antibody. Blots were visualized by ALP-conjugated secondary antibody. Arrows indicate molecular masses (in kilodaltons). Lanes: 1, eluate from supernatant (100× concentration); 2, purified 19-kDa band, unreduced; 3, purified 19-kDa band, reduced.

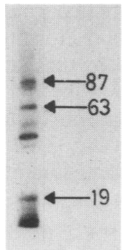

FIG. 5. Autoradiography of eluted material from affinity purification on fibrinogen-Sepharose of fibrinogen-binding components from surface-labeled and lysed *S. aureus* Newman cells. Arrows indicate molecular masses (in kilodaltons).

the purified 87-kDa band were positive (Table 2). This clearly demonstrates that this preparation contained coagulase activity and that this coagulase is the 87-kDa molecule. Fibrinogen-binding material from *S. aureus* Newman lysed cells and *S. aureus* Newman D_2C lysed cells and culture supernatant were all negative in these tests. Possibly the amounts of coagulase from these preparations were not enough to cause a positive coagulase test. The 19-kDa band was also negative in this test.

DISCUSSION

This study was undertaken to investigate the mechanism behind the staphylococcal clumping reaction. It has been suggested previously that the clumping of staphylococci in plasma is caused by a proteinaceous molecule situated on the staphylococcal cell surface and that this molecule binds specifically and nonenzymatically to fibrinogen (17). This molecule, the so-called clumping factor, is supposed to be distinct from coagulase (10) and, since it acts only on fibrinogen, also distinct from fibronectin-binding protein (28) and protein A (4).

Many attempts have been made to isolate clumping factor, but all of these have been unsuccessful, possibly because of insensitive detection assays. These purifications have generated molecules with various molecular masses, ranging from 14.3 (8) to 420 (12) kDa. The most commonly used assays are clumping and inhibition of clumping, using whole bacterial cells (5, 10, 12, 21) or coated particles (5, 32, 35, 36), e.g., sheep erythrocytes or latex particles. Clumping of whole bacterial cells is, for reasons mentioned above, not a reliable assay. Uncoated sheep erythrocytes can clump spontaneously in fibrinogen (30) or in protamine sulfate or chondroitin sulfate (32).

TABLE 2. Coagulase test

S. aureus strain	Preparation[a]	Clotting at: 0.5 h	1 h	1.5 h	24 h
Newman	Cells	−	+	+	+
D_2C	Cells	−	−	−	−
U320	Cells	−	−	−	+
Newman	Supernatant	+	+	+	+
D_2C	Supernatant	−	−	−	−
U320	Supernatant	−	−	+	+
Newman	Eluate from cells	−	−	−	−
Newman	Eluate from supernatant	+	+	+	+
Newman	87-kDa band	+	+	+	+
Newman	19-kDa band	−	−	−	−

[a] Preparations tested were killed and washed cells, cell-free supernatants from staphylococcal cultures, affinity-puriifed material (eluate) from fibrinogen-Sepharose, and purified 87- and 19-kDa bands.

Our strategy has been to use purification methods and detection assays in which fibrinogen is involved in direct binding to staphylococcal products, i.e., fibrinogen-Sepharose and fibrinogen used in Western blots. It is important in this context to keep in mind that there are no perfectly pure fibrinogen preparations commercially available. Therefore, the fibrinogen will contain significant amounts of other blood protein, e.g., fibronectin and immunoglobulins, and consequently other serum protein preparations will also contain contaminations of fibrinogen, for example. Commercial antibodies against fibrinogen also contain antibodies against these contaminants, since equally impure preparations are used for immunization.

In this study, numerous controls were used to eliminate the risk of false-positive signals caused by contaminating ligands. Staphylococcal cells and culture supernatants were analyzed on SDS-PAGE and Western blots both before and after being purified on fibrinogen-Sepharose. Bands were detected by the incubation of nitrocellulose filters with fibrinogen, fibronectin, HGGs, and antibodies against these proteins. As a control, some filters were incubated with preimmune sera or with antifibrinogen antibodies only. It was evident from the controls with fibronectin, HGGs, and antifibrinogen antibodies that the 190-kDa band was a fibronectin-binding protein and that the 75- and 63-kDa bands originated from protein A (Fig. 1). The 87-, 35-, and 19-kDa bands that were seemingly specific for fibrinogen all bound monoclonal antibody 23 (Fig. 2, lane 6).

The fibrinogen-binding bands could be obtained from cells grown in defined media (RPMI 1640 and Parker), which confirms that the 87-, 35-, and 19-kDa molecules originated from the staphylococci and were not derived from the brain heart infusion medium (data not shown). Periodate oxidation by the method of Woodward et al. (41) showed that carbohydrates are most likely not involved in the binding of fibrinogen to the 87-, 35-, and 19-kDa bands. The periodate treatment of the fibrinogen-binding molecules on nitrocellulose filters or of the fibrinogen used in Western blots did not abolish this binding (data not shown).

The amounts of 87- and 19-kDa molecules that could be purified on fibrinogen-Sepharose differed markedly between preparations from the various strains, as judged by serial dilutions before detection on gels and Western blots and by scanning of Coomassie blue-stained gels. In this study, culture supernatants from *S. aureus* Newman were the richest sources of these bands. The large yields from culture supernatants suggest that these products are essentially extracellular. In contrast to the wild-type *S. aureus* Newman, the *S. aureus* Newman D_2C strain produced more cell-bound than extracellular 87- and 19-kDa molecules. The amounts found on *S. aureus* U320 cells were intermediate between the amounts found on the *S. aureus* Newman and *S. aureus* Newman D_2C strains.

The amounts of 87- and 19-kDa molecules found in the different strains and preparations correlate well with the results from the coagulase test (Table 2). *S. aureus* Newman produced large amounts of coagulase and was immediately positive, while *S. aureus* U320 took a longer time for a positive reaction. *S. aureus* Newman D_2C did not produce enough coagulase for a positive clotting reaction. Protease inhibitors were added to these tests to verify that the recorded results were true coagulase reactions and not caused by pseudocoagulase activity. The true identity of the 87-kDa molecule as coagulase was conclusively determined by subjecting a very pure preparation of this molecule to a coagulase test (Table 2). The 87-kDa molecule had an affinity for prothrombin (Fig. 3), which further indicates that this molecule is coagulase. Interestingly, the 87-kDa molecule was able to induce fibrin formation in a fibrinogen solution without the addition of prothrombin. This fibrin gel was of rather poor quality, and no extensive network was formed. The 19-kDa molecule could not induce fibrin formation when added either to plasma or fibrinogen, but a precipitate was formed (data not shown).

When rerun on SDS-PAGE (Fig. 3), the 87-kDa band re-formed the smear seen in the original preparation. Similarly, the 19-kDa band gave rise to bands of higher molecular masses. One of these was a 35-kDa band, which was probably a dimer of the 19-kDa band. The 35-kDa band could not be reduced by β-mercaptoethanol (Fig. 4). This makes it probable that the main mechanism behind this dimerization is not the formation of disulfide bonds.

The identity between free and bound coagulase has been shown previously (16), although later investigators have not given these findings enough credit. If we assume that coagulase is responsible for clumping, there are two possibilities for its mode of action. One possibility is that the coagulase-induced fibrin loosely surrounds each cell until a gel network that entraps all the staphylococci into one big aggregate is formed. The second alternative is that fibrin fibers bind to cell-bound coagulase. The latter explanation is confirmed by the electron micrographs by Umeda et al. (34), in which it is clearly seen that clumped staphylococci are held together by thick fibrin fibers originating from the cell surface. It might be surprising that the affinity is so strong as to retain the fibrin polymers bound to the surface, but here the effect of numerous binding sites must be considered. Briefly, this effect can be explained by the following example. Binding of antibodies to an antigen having two epitopes available for the antibody, each representing an affinity of 10^5 liters/mol, will give a combined effect that is not 2×10^5 but rather $10^5 \times 10^5$, i.e., 10^{10} liters/mol. This effect is due to the increased local concentration of epitopes and paratopes (3, 29). This second alternative is further substantiated by the result of the analysis of surface-labeled cells, in which the coagulase from cell lysates is shown to be accessible on the cell surface (Fig. 5). The same conclusion can be drawn from the coagulase test, in which killed and washed cells induced a positive coagulase reaction (Table 2). The *S. aureus* Newman D_2C strain produced some coagulase, even if most of this was cell bound and did not give a positive coagulase test (Table 2). Furthermore, the possibility exists that this cell-bound coagulase on the D_2C strain is unable to induce coagulation.

It has been suggested that clumping of staphylococci in plasma plays an important role in the virulence of this pathogen. Johanovsky (18) and Kapral (20) argued that clumping factor-positive staphylococci, when injected into the peritoneal cavities of mice, are more virulent than clumping factor-negative staphylococci. This has not been confirmed by other investigators. Instead, it has been shown that the capsule-containing, nonclumping staphylococcal strains survive longer than the clumping factor-positive staphylococcal strains in the mouse peritoneum. This is because the capsule renders the staphylococci resistant to phagocytosis (40). Dunn and Simmons (9) claim that clumping is not at all a virulence factor but rather a nonspecific host defense against bacteria, in which particles of a certain size are unspecifically incorporated into the fibrin matrix during the process of blood clotting.

It has also been suggested that staphylococcal aggregation in plasma and binding of staphylococci to molecules of the extracellular matrix are important for virulence of wound pathogens, for example (28, 38). Indeed, it has been found that staphylococci bind to fibronectin (28, 37), collagen (37), laminin (24), and vitronectin (7), in addition to interacting with fibrinogen. However, it must be kept in mind that staphylococci and their hosts have evolved together and are mutually adapted to one another. Hence, bacterial binding to host cell structures, e.g., fibrinogen, may not necessarily be beneficial to the bacteria. Toy et al. suggested that the binding of staphylococci to fibronectin in fibrin thrombi mediates the adherence of *S. aureus* to fibrin in wounds (33). It could be questioned, however, whether this binding is beneficial to the bacteria, because in wounds in which inflammatory fluid is present, soluble fibronectin may inhibit the binding of *S. aureus* to solid fibronectin in the thrombi. This means that the staphylococci would be confined to external wounds without inflammatory fluid and would be prevented from further penetrating the wounded tissue. Furthermore, fibronectin is an efficient opsonin for staphylococci (23).

There are some thoroughly studied and well-documented examples in which adhesion to host cell structures is necessary for virulence. The best-studied examples are the pili adhesins of *Escherichia coli*, but also bacterial lectins from other gram-negative bacteria and lipoteichoic acids from streptococci and staphylococci have been found to be important in this aspect. These binding structures have been found on bacteria infecting mucosal surfaces. On these surfaces, bacteria must possess adhesins to protect themselves against the clearing activities of mucosal secretions, saliva and urine flow, etc. After the bacteria invade deeper tissues, these adhesins are of no benefit to the bacteria, since the adhesins can facilitate the attachment to phagocytic cells. The adhesins must therefore be shed or masked by capsules (2).

The complexity of staphylococcal infection in vivo makes it difficult to evaluate the contributions of individual factors to the overall virulence of staphylococci. However, it is important to increase our knowledge of all these binding structures and proposed virulence factors in order to completely understand the mechanisms of staphylococcal virulence and pathogenicity. In this report, we have shown that coagulase, in addition to binding and activating prothrombin, also binds fibrinogen in the absence of prothrombin and that this property is the basis for the clumping reaction of staphylococci in fibrinogen. These new findings might have implications for our view of the mechanism of coagulase and will hopefully give more information on the role of extracellular and cell-bound coagulase in the virulence of staphylococci.

LITERATURE CITED

1. **Altenbern, R. A.** 1966. On the nature of albumin-promoted coagulase release by *Staphylococcus aureus*. J. Infect. Dis. **116**:593–600.
2. **Beachey, E. H.** 1981. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Infect. Dis. **143**:325–345.
3. **Bell, G. I.** 1978. Models for the specific adhesion of cells to cells. Science **200**:618–627.
4. **Blackstock, R., and F. C. Kelly.** 1968. Comparison of staphylococcal clumping factor and protein A. J. Bacteriol. **96**:855–856.
5. **Brückler, J., W. Schaeg, and H. Blobel.** 1974. Isolation of

''clumping factor'' of *Staphylococcus aureus*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **228**:465–473.

6. **Carr, M. E., D. A. Gabriel, J. C. Herion, and H. R. Roberts.** 1986. Granulocyte lysosomal cationic protein alters fibrin assembly: a possible mechanism for granulocyte control of clot structure. J. Lab. Clin. Med. **107**:199–203.
7. **Chhatwal, G. S., K. T. Preissner, G. Müller-Berghaus, and H. Blobel.** 1987. Specific binding of the human S protein (vitronectin) to streptococci, *Staphylococcus aureus*, and *Escherichia coli*. Infect. Immun. **55**:1878–1883.
8. **Davison, V. E., and B. A. Sanford.** 1982. Factors influencing adherence of *Staphylococcus aureus* to influenza A virus-infected cell cultures. Infect. Immun. **37**:946–955.
9. **Dunn, D. L., and R. L. Simmons.** 1982. Fibrin in peritonitis. III. The mechanism of bacterial trapping by polymerizing fibrin. Surgery **92**:513–519.
10. **Duthie, E. S.** 1954. Evidence for two forms of staphylococcal coagulase. J. Gen. Microbiol. **10**:427–436.
11. **Engels, W., and M. A. F. Kamps.** 1981. Secretion of staphylocoagulase by *Staphylococcus aureus*: the role of a cell-bound intermediate. Antonie van Leeuwenhoek J. Microbiol. **47**:509–524.
12. **Espersen, F., I. Clemmensen, and V. Barkholt.** 1985. Isolation of *Staphylococcus aureus* clumping factor. Infect. Immun. **49**:700–708.
13. **Espersen, F., and P. O. Schiøtz.** 1981. Normally-occurring precipitating antibodies against *Staphylococcus aureus* in human serum and colostrum. Acta Pathol. Microbiol. Scand. Sect. C **89**:93–98.
14. **Fröman, G., L. M. Switalski, P. Speziale, and M. Höök.** 1987. Isolation and characterization of a fibronectin receptor from *Staphylococcus aureus*. J. Biol. Chem. **262**:6564–6571.
15. **Heczko, P. B., Z. Wegrzynowicz, M. Bulanda, J. Jeljaszewicz, and G. Pulverer.** 1981. Taxonomic implications of the pseudocoagulase activity of staphylococci, p. 43–47. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections, suppl. 10. Gustav Fischer Verlag, Stuttgart, Federal Republic of Germany.
16. **Jacherts, D.** 1956. Experimentelle Untersuchungen über die Identität freier und gebundener Coagulase. Z. Hyg. Infektionskr. **142**:502–509.
17. **Jeljaszewicz, J., L. M. Switalski, and C. Adlam.** 1983. Staphylocoagulase and clumping factor, p. 525–557. *In* C. S. F. Easmon and C. Adlam (ed.), Staphylococci and staphylococcal infections, vol. 2. Academic Press, Inc. (London), Ltd., London.
18. **Johanovsky, J.** 1957. The significance of the clumping factor for pathogenicity of staphylococci. Folia Biol. (Prague) **3**:338–342.
19. **Jonsson, P., and T. Wadström.** 1984. Cell surface hydrophobicity of *Staphylococcus aureus* measured by the salt aggregation test (SAT). Curr. Microbiol. **10**:203–210.
20. **Kapral, F. A.** 1965. Factors involved in experimental staphylococcal peritonitis. Ann. N.Y. Acad. Sci. **128**:259–273.
21. **Kato, Y., and G. Omori.** 1959. Extraction of bound coagulase from staphylococcal cells. Biken J. **2**:321–332.
22. **Kopec, M., Z. Wegrzynowicz, and Z. S. Latallo.** 1970. Precipitation of soluble fibrin monomer complexes SFMC by cellular basic proteins, and the antagonistic effect of sulfonated mucopolysaccharides. Proc. Soc. Exp. Biol. Med. **135**:675–679.
23. **Lanser, M. E., and T. M. Saba.** 1981. Fibronectin as a co-factor necessary for optimal granulocyte phagocytosis of *Staphylococcus aureus*. RES J. Reticuloendothel. Soc. **30**:415–424.
24. **Lopes, J. D., M. Dos Reis, and R. R. Brentani.** 1985. Presence of laminin receptors in *Staphylococcus aureus*. Science **229**:275–277.
25. **Millar, M. R., and T. Inglis.** 1987. Influence of lysozyme on aggregation of *Staphylococcus aureus*. J. Clin. Microbiol. **25**:1587–1590.
26. **Miller, K. D., D. L. Hetrick, and D. J. Bielefeldt.** 1977. Production and properties of *Staphylococcus aureus* (strain Newman D_2C) with uniform clumping factor activity. Thromb. Res. **10**:203–211.
27. **Much, H.** 1908. Über eine Vorstufe des Fibrinfermentes in Kulturen von *Staphylokokkus aureus*. Biochem. Z. **14**:143–155.
28. **Proctor, R. A., G. Christman, and D. F. Mosher.** 1984. Fibronectin-induced agglutination of *Staphylococcus aureus* correlates with invasiveness. J. Lab. Clin. Med. **104**:455–469.
29. **Roitt, I.** 1988. Essential immunology, 6th ed., p. 61–63. Blackwell Scientific Publications, Ltd., London.
30. **Rotter, J., and F. C. Kelly.** 1966. Serological reactions associated with the clumping factor of *Staphylococcus aureus*. J. Bacteriol. **91**:588–594.
31. **Schenkein, I., M. Levy, and J. M. Uhr.** 1972. The use of glucose oxidase as a generator of H_2O_2 in the enzymatic radioiodination of components of cell surfaces. Cell. Immunol. **5**:490–493.
32. **Switalski, L. M.** 1976. Isolation and purification of staphylococcal clumping factor, p. 413–425. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustav Fischer Verlag, Stuttgart, Federal Republic of Germany.
33. **Toy, P. T. C. Y., L.-W. Lai, T. A. Drake, and M. A. Sande.** 1985. Effect of fibronectin on adherence of *Staphylococcus aureus* to fibrin thrombi in vitro. Infect. Immun. **48**:83–86.
34. **Umeda, A., T. Ikebuchi, and K. Amako.** 1980. Localization of bacteriophage receptor, clumping factor, and protein A on the cell surface of *Staphylococcus aureus*. J. Bacteriol. **141**:838–844.
35. **Usui, Y.** 1986. Biochemical properties of fibrinogen binding protein (clumping factor) of the staphylococcal cell surface. Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A **262**:287–297.
36. **Usui, Y., T. Ohtomo, and K. Yoshida.** 1985. Biochemical characterization of clumping factor extracted from a strain of *Staphylococcus aureus*, p. 197–205. *In* J. Jeljaszewicz (ed.), The staphylococci, suppl. 14. Gustav Fischer Verlag, Stuttgart, Federal Republic of Germany.
37. **Vercellotti, G. M., J. B McCarthy, P. Lindholm, P. K. Peterson, H. S. Jacob, and L. T. Furcht.** 1985. Extracellular matrix proteins (fibronectin, laminin, and type IV collagen) bind and aggregate bacteria. Am. J. Pathol. **120**:13–21.
38. **Wadström, T., L. Switalski, P. Speziale, K. Rubin, C. Rydén, G. Fröman, A. Faris, M. Lindberg, and M. Höök.** 1985. Binding of microbial pathogens to connective tissue fibronectin: an early step in localized and invasive infections, p. 193–207. *In* G. G. Jackson and H. Thomas (ed.), The pathogenesis of bacterial infections. Bayer Symposium 8. Springer-Verlag, Berlin.
39. **Wegrzynowicz, Z., P. B. Heczko, J. Jeljaszewicz, M. Neugebauer, and G. Pulverer.** 1979. Pseudocoagulase activity of staphylococci. J. Clin. Microbiol. **9**:15–19.
40. **Wilkinson, B. J.** 1983. Staphylococcal capsules and slime, p. 481–523. *In* C. S. F. Easmon and C. Adlam (ed.), Staphylococci and staphylococcal infections, vol. 2. Academic Press, Inc. (London), Ltd., London.
41. **Woodward, M. P., W. W. Young, Jr., and R. A. Bloodgood.** 1985. Detection of monoclonal antibodies specific for carbohydrate epitopes using periodate oxidation. J. Immunol. Methods **78**:143–153.