Effect of Protein A on Adsorption of Bacteriophages to *Staphylococcus aureus*

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Experiments were performed to determine if protein A influenced the association of bacteriophages with *Staphylococcus aureus*. Bacteriophage adsorption was compared in a *S. aureus* strain rich in protein A and mutants of this strain with very little protein A, in a strain with little protein A, and in mutants of this strain with increased protein A. In addition, the effect of growth in mannitol-salt broth and trypsin digestion (known to reduce protein A) on bacteriophage adsorption was measured. There was an inverse relationship between protein A content of strains and the quantity of bacteriophage adsorbed. However, no inhibition of staphylococcal phages was obtained with purified soluble protein A. Protein A as a surface component rendered the bacteria more resistant to adsorption of staphylococcal typing phages presumably by masking the phage receptor sites. When protein A-deficient mutants were incubated with bacteriophages, there was survival of staphylococci with increased protein A content probably due to a selective action.

The cell wall of *Staphylococcus aureus* consists of mucopeptide to which ribitol teichoic acid is covalently bound. Several investigators have reported that teichoic acid, and especially the *N*-acetyl-glucosamine component of ribitol teichoic acid, is required for bacteriophage adsorption by *S. aureus* (4, 5). In addition, it has been shown that the capacity of staphylococcal cell walls to inactivate phage can be destroyed by muralytic enzymes and that the *O*-acetyl-groups of the mucopeptide are required for phage attachment (17). Murein therefore appeared to be a component of the phage receptor on *S. aureus*.

Several reports indicate that protein A is located in the outermost part of the staphylococcal cell wall, (11, 13, 15, 23) and that it can be liberated with cell wall lytic enzymes, (7) and is covalently bound to the mucopeptide (20).

The investigation described in this report demonstrates an inhibiting effect of cell-bound protein A on adsorption of bacteriophages to *S. aureus* by masking the phage receptor sites.

MATERIALS AND METHODS

Bacterial strains. *S. aureus* strains Cowan I and 8325 N (originally obtained from M. H. Richmond) were used. Mutants of Cowan I with a very low content (traces detected with hemagglutination) of protein A (NG 274 Class I_{NG} and NG 297 Class I_{NG} and NG 316, Class I_{NG}) were used. Mutants with a

combined loss of protein A, nuclease, coagulase, alpha-hemolysin, fibrinolysin, utilization of mannitol, and phage-pattern changed to nontypable (EMS 252 Class VI_{EMS} and EMS 258 Class VI_{EMS}) also used in this report have been described earlier (8). Protein A mutants of 8325 N were obtained by treatment with nitrosoguanidine. (1, 2). Protein A negative and positive mutants were isolated by spreading the mutagenized bacteria on nutrient agar containing anti-protein A serum as earlier described (8). The protein A content of the mutants were then determined by a hemagglutination technique for quantitation of protein A as earlier described (8). The wild-type 8325 N has a low content of protein A (hemagglutination titer 1:1 to 1:2), mutant NG 11 had a high content of protein A (hemagglutination titer 1:64), and mutant NG 143 lacked protein A (undetectable by hemagglutination). The propagation strain for phage 88 from the standard typing set (3) was also used in some experiments. The bacteria were grown in trypticase soy broth (TSB; Difco) and in mannitol-salt broth (MSB, 7.5% NaCl; Difco).

Phages. Three staphylococcal bacteriophages were used in this study. Phage 52 and 80 from the standard typing set (3) were obtained from the National Bacteriological Laboratory, Stockholm, Sweden. Phage 80 α was obtained from R. P. Novick (16). High-titer phage stocks were prepared by the soft agar overlay method (22). Nutrient agar plates with an overlay of 2.5 ml of soft (0.7%) trypticase soy agar (TSA, Difco) were used. The stock suspensions were sterilized by filtration (0.45 μ m pore size; Millipore Corp.), and stored at 4 C in TSB. The bacterial strain Cowan I and the propagation strains for phage 80 and 88 were used as indicator strains for titration of

phages 52 and 80, and strain 8325 N for titration of phage 80 α . In each experiment one indicator strain was used for all determinations of phagetiter.

Adsorption of phages. The bacteria were grown in TSB and harvested at the beginning of the log phase and concentrated by centrifugation to 10^9 to 2×10^9 colony forming units (CFU)/ml. Bacterial growth was arrested by addition of 0.004 M potassium cyanide and after 10 min an equal volume of phage suspension was added to give multiplicities of infection (MOI) of 0.1 to 5.0. The mixtures of phages and bacteria containing 0.004 M CaCl₂ were incubated at 37 C with shaking. At various times, samples were withdrawn and unattached phages were titrated after centrifugation by the soft agar layer method (22).

Selection experiments. In some experiments phages 52 and 80 were propagated on *S. aureus* strain Cowan I or its protein A-deficient mutants NG274, (Class I_{NG}) and NG297, (Class I_{NG}) until lysis occurred. Samples of the mixture were spread on nutrient agar (NA) plates containing anti-protein A serum (8) for detection of protein A production.

Trypsin treatment of bacteria. For elimination of protein A (11, 15) *S. aureus*, strain Cowan I, was treated with trypsin in a concentration of 0.5 mg/ml in phosphate-buffered saline (PBS), pH 7.4, and at 37 C. At various times samples were withdrawn and the digestion process was interrupted by centrifugation, washing twice in PBS, and finally suspending in TSB. The enzymatically digested bacteria were used for phage adsorption experiments.

Purified protein A. Protein A was prepared as previously described (10) by extraction at 100 C in phosphate buffer and with subsequent purification by chromatography on DEAE-Sephadex and Sephadex G-100. In addition, protein A was isolated from *S. aureus* Cowan I by digestion with lysostaphin and purified as described by Sjöquist et al. (19).

RESULTS

Adsorption of phages to *S. aureus*, Cowan I and protein A-deficient mutants. Cowan I-derived mutant NG 274 with very low protein A adsorbed 99.7% of bacteriophage 80 at a MOI of 0.1 after incubation for 10 min. In contrast, Cowan I adsorbed only 23% of the phages. As seen in Fig. 1, there is more than 100-fold difference between the wild type and the mutant in number of unattached phages after adsorption. A similar difference was also obtained when other protein A-deficient mutants of strain Cowan I (NG 297 and NG 316) were studied. The same differences in adsorption efficiency between the wild type and the protein A-deficient mutants were also obtained when phage 80 propagated on a different bacterial strain with a very low protein A content (the propagation strain for phage 88 in the standard typing set was used). In addition, when a different phage, phage 52, propagated on Cowan I was used there was a similar difference in adsorption

FIG. 1. Inactivation of phage 80 by Cowan I and by a protein A-deficient mutant, NG 274. MOI is 0.1.

of phage between protein A deficient mutants and the wild-type Cowan I.

When adsorption experiments were performed with pleiotropic mutants of Cowan I (Class VI_{EMS}) with a combined loss of protein A, nuclease, coagulase and mannitol fermentation, and loss of phage type, no adsorption of phages (80 and 52) was detected.

Adsorption of phages to *S. aureus*, 8325 N, and protein A-rich mutants. Since there was increased adsorption of phage to protein A-deficient mutants of a protein A-rich strain, investigations were done to determine if there might be decreased phage adsorption to protein A-rich mutants of a protein A-poor strain. Adsorption of phages were compared by using *S. aureus* 8325 N with a low protein A content and a derived mutant NG 11 with a high protein A production, and NG 143 without detectable protein A. As shown in Fig. 2, there was prompt adsorption of phage 80 α during 10 min of incubation with the wild-type 8325 N. The mutant NG 11 with a high protein A content was about 100 times less efficient in adsorption of phage (Fig. 2). The adsorption of phage to the protein A-negative mutant NG 143 was approximately the same as to the wild-type 8325 N.

Adsorption of phages to *S. aureus* treated with trypsin or cultured in mannitol-salt broth. *S. aureus*, strain Cowan I, was treated

FIG. 2. Inactivation of phage 80 α by 8325 N and by a protein A-producing mutant, NG 11. MOI is 0.1.

with trypsin and phage 80 was incubated with the bacteria for 10 min. As shown in Fig. 3, there was increased adsorption of phage 80 to Cowan I treated with trypsin. The longer bacteria were incubated with trypsin (0.5 mg/ml) the more efficient they became in phage adsorption (Fig. 3). Protein A-deficient mutant NG 274 of Cowan I adsorbed phage with a similar efficiency as the wild-type Cowan I trypsin digested for 10 min.

S. aureus Cowan I was grown in MSB, in attempts to eliminate a surface layer of protein A, and then used for phage adsorption experiments (Fig. 4). The adsorption rate of phage 80 to strain Cowan I grown in MSB was higher than that of Cowan I grown in TSB. As seen in Fig. 4, there is a 10-fold difference in number of unattached phages.

Effect of soluble protein A on phages. Adsorption experiments were performed with phage 80 and protein A-deficient mutants of Cowan I in the presence of soluble purified protein A. Twofold dilutions of protein A in TSB were used. Even at the highest concentration of protein A (10 mg of protein A per ml), there was no inactivation of bacteriophage adsorption. These results show that protein A only inhibits attachment of phage to receptor sites when located on the bacterial surface.

Selection experiments. Suspensions of protein A-deficient mutants NG 274 (Class I_{NG}) and NG 297 (Class I_{NG}) were incubated with

FIG. 3. Inactivation of phage 80 after 10 min of incubation with strain Cowan I treated with trypsin (0.5 mg/ml) in PBS, pH 7.4, for 1 to 30 min. MOI is 0.1.

FIG. 4. Inactivation of phage 80 by Cowan I grown in TSB and MSB. MOI is 0.1.

TABLE 1. Percent protein A producing colonies after incubation of protein A-deficient mutants (NG 274 and NG 297) with phage 52 and 80 for about 3 h at multiplicities of infection of 0.1 to 1.0

Phage	Propagation strain	No. of exp	Mutant NG 274 (Class I _{NG})	Mutant NG 297 (Class I _{NG})
80	CI	5	100	100
52	CI	1	97	97
52	88	5	70-100	50-100

phage 52 and 80 at MOI of 0.1 to 1.0 until lysis occurred. The protein A-positive wild-type Cowan I did not go to lysis under these conditions. After lysis, the bacteria were spread on nutrient agar containing anti-protein A serum for detection of protein A production. Table 1 shows that when the protein A-negative mutants were incubated with phage 52 or 80 almost all colonies developed from surviving bacteria produced protein A. The same result was obtained when these phages were propagated on either protein A-rich Cowan I or protein A-poor

strain 88. No differences in reversion could be detected when phages were added at MOI of 0.1 to 1.0. The frequency of spontaneous reversion when no phages were added to protein A-deficient mutants were followed in all experiments. It was always less than 0.1%. Fifty to 100 colonies which survived phage lysis were quantitatively tested for protein A production by a hemagglutination technique. All tested colonies had a protein A content similar to the wild-type Cowan I and none of the tested colonies were lysogenic for phage 52 and 80.

DISCUSSION

Three different types of experiments demonstrated that protein A located on the cell surface inhibits the adsorption of bacteriophages to *S. aureus*. Mutants with a reduced production of protein A showed an increased ability to adsorb phages and mutants with an increased production of protein A adsorbed less phages. To further exclude the possibility that the protein A mutants are pleiotropic for phage-binding trypsin digestion known to remove protein A was performed. After trypsin digestion the *S. aureus* bacteria had an increased capacity to adsorb phages. Finally, when the production of protein A was reduced by growing the bacteria in MSB, there was also a significant increase in phage adsorption. However, bacteria grown in MSB were in some experiments slightly less efficient in phage adsorption than protein A-deficient mutants, indicating the possibility that the phage receptors had been slightly affected by these growth conditions. These experiments suggest that protein A located on the cell surface can mask phage receptor sites. As the mucopeptide and especially the *O*-acetyl groups of the mucopeptide are required for phage adsorption and protein A has been demonstrated to be covalently bound to the peptidoglycan subunit of the cell wall it seems likely that protein A has the ability to cover the phage receptors. On the other hand, protein A in soluble form did not affect phage infectivity. This was shown both with heat-extracted protein A and more intact protein A obtained by digestion with lysostaphin. Of interest is also the fact that no adsorption of phages could be detected to mutants of Cowan I with a combined loss of protein A, nuclease, coagulase, alpha-hemolysin, fibrinolysin, utilization of mannitol, and changed phage-type pattern.

A mutant of *S. aureus* H with an additional cell surface antigen composed of two amino sugars, *N*-acetyl-D-fucosamine and *N*-acetyl-D-mannosamine uronic acid has been isolated by Wu and Park (24). It is of great interest that the

presence of this additional surface component rendered the cells resistant to staphylococcal typing phages, presumably by masking the phage receptor sites in a similar fashion as protein A.

In the experiments reported in Table 1 where phages 52 and 80 were propagated on protein A-deficient mutants until lysis occurred almost all survivors were rich in protein A. This very high frequency of bacteria regaining protein A production is probably not due to transduction, but to differences in adsorption rate of bacteriophages between bacteria with high and low protein A production. This is further supported by the observations that when phages propagated on a protein A-poor *S. aureus* strain were incubated with protein A-deficient mutants all survivors are protein A rich. In addition, protein A-negative mutants were lysed at an MOI of 0.1 to 1.0, whereas the protein A-positive wild-type Cowan I required a more than 10 times higher concentration of phages to be lysed. All of these experiments support the concept that there is selection of protein A-rich bacteria during phage lysis. It is possible that a selection of protein A-rich staphylococci also can be caused by bacteriophages in human beings and animals. As protein A has been shown to be an anticomplementary (21) and antiphagocytic substance (6, 9) and to cause hypersensitivity reactions (18) and platelet injury (12), the effect of phages on *S. aureus* may contribute to the pathogenicity of these organisms.

In work with *Streptococcus pyogenes*, Maxted (14) reported that, by using selective action of bacteriophages on cultures of susceptible streptococci, it was possible to enhance the production of hyaluronic acid capsule and M-protein. In addition, it was shown that trypsin treated cells which no longer possessed M-protein were more susceptible to phage lysis than the untreated cells. With this background, it is of interest to notice that in the absence of protein A on the bacterial surface, the adsorption of phages was more efficient and that protein A production is increased in bacteria which survive phage action with what apparently seems to be a selective process.

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