Occurrence of Protein A in Staphylococcal Strains: Quantitative Aspects and Correlation to Antigenic and Bacteriophage Types

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Protein A of Staphylococcus aureus can be detected on cell walls of intact bacteria by use of radioactively labeled myeloma globulin. Of 156 strains of S. aureus, 141 (90%) contained protein A. None of 47 S. epidermidis strains was positive for protein A. The production of protein A was influenced by incubation temperature but not by differences in incubation time or inoculum size. A medium containing a high concentration of NaCl suppressed the production of protein A by 90%. Formalin treatment of protein A-containing strains caused a decrease in the amount detected, but no further decrease was detected after storage at 4°C. No correlation was found between absence or presence of protein A and phage type or phage group. Sixteen S. aureus strains were studied extensively. There was no correlation between protein A and any of the 26 antigenic characteristics which have been previously described in these strains.

Staphylococcal protein A was originally defined by its precipitin reaction with normal human serum (12, 13). Its presence in bacterial strains was determined by agglutination of the organism or by double diffusion in gel with bacterial extracts diffusing against normal human serum. Ninety-seven strains of Staphylococcus aureus were tested by Jensen; 73 of these contained the agglutinogen and 23 gave a protein A precipitin line in agar (12, 13). Jensen also showed that both the agglutinogen and the precipitinogen were absent in S. epidermidis strains. A higher incidence of protein A in S. aureus was noted by Oeding and Haukenes (26). All of the strains in their panel, except Wood 46, produced protein A, as did "nearly all" strains from a diagnostic laboratory. This higher incidence compared with Jensen's results was presumed to be due to increased sensitivity of the method used. An even more sensitive method has been developed in this laboratory by use of isolated IgG myeloma globulins without detectable antibody activity (15, 17, 24). Quantitation of protein A on the surface of bacteria was thus possible by use of radioactively labeled myeloma globulins with protein A combining sites on their Fc parts (15). In the present investigation, this technique was utilized for determining the occurrence of protein A in various bacteria, for quantitation of protein A, and for possible correlation with other microbial characteristics.

MATERIALS AND METHODS

Bacterial strains. Strains of S. aureus were isolated from patients with abscesses and infected wounds (91 strains), patients with osteomyelitis (16 strains), and patients with bacterial endocarditis (15 strains). In all instances, the strains recovered were considered the causative microbial agent of the infections mentioned. In addition, 18 strains of S. aureus used as standard strains in our laboratory were studied, as well as 16 standard strains kindly provided by G. Haukenes, Bergen, Norway, and extensively investigated serologically by Oeding and Haukenes (10, 25). The taxonomic criteria used for bacteria tested followed the Manual of Clinical Microbiology (American Society for Microbiology, Bethesda, Md., 1970). Coagulase tests were performed according to the tube method with the use of coagulase plasma (Difco).

S. epidermidis strains were also included in our study; 11 strains were isolated from the skin of healthy normal persons, and 36 strains were obtained from hospital patients. All S. epidermidis strains were coagulase- and mannoit-negative, and were capable of fermenting glucose.

Representatives of some other microbial species were also studied. These included three strains of Escherichia coli, one each of Pseudomonas, Proteus, Klebsiella pneumoniae, Shigella B, and Shigella D, and five of Serratia marcescens.

Phage typing. A standard set of phages were used.
for phage typing of staphylococci at routine test dilution (RTD) and 1,000 X RTD (1). The phage types of 16 strains (Haukenes strains) were kindly provided by Dr. Haukenes.

**Culture media.** The media used for the bacterial strains tested included Penassay Broth (Difco), Brain Heart Infusion (BHI, Difco), Tryptose Broth (Difco), Staphyloccocus Medium No. 110 (Difco), and Todd-Hewitt broth (Oxoid). Bacteria were incubated at 37 C in a Thelco model 4 incubator (Precision Scientific Co.) unless otherwise stated. In some instances, a water-jacketed CO2 incubator (model 3221, National Applicance Co., Portland, Ore.) was used.

**Formalin treatment of bacteria.** Some strains of S. aureus were treated with Formalin according to the procedures of Lind and Mansa (19) and were subsequently tested for protein A.

**Myeloma globulins.** Protein A precipitating and inhibiting IgG-1 myeloma globulins were used as reagents for protein A (15, 17). The myeloma globulins were isolated by zone electrophoresis on a starch block (18). The purity of the myeloma globulins was determined by immunoelectrophoresis and Gm typing.

**Radioactive labeling.** Isolated myeloma globulins were labeled with iodine-125 by the technique of McConahey and Dixon (20).

**Quantitation of protein A on bacteria.** 125I-labeled myeloma globulins were utilized for the determination of protein A by bacterial organisms as previously described (15). For qualitative assessment of protein A in strains, 5 ug of 125I-labeled myeloma globulin was added to 10^9 bacterial organisms. Uptake of radioactivity was calculated from the decrease in radioactivity of the supernatant. A strain was considered to contain no protein A if the uptake was less than that by a suspension of the protein A-negative Wood 46 strain showing 0 to 1% of the uptake of the Cowan I standard strain. The Cowan I strain was used as a positive control and the Wood 46 strain as a negative control in each experiment. Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer, model 3320.

For quantitation in protein A-containing strains, 20 ug of 125I-labeled myeloma globulin was added to 10^9 bacterial organisms. A standard curve with dilutions of the Cowan I strain was used as a reference, with the Wood 46 strain added to give a total bacterial count of 10^9. The amount present was expressed as a percentage of the Cowan I standard strain giving the same uptake of radioactivity.

The relative number of protein A residues was determined on some S. aureus strains by use of the Scatchard plotting technique (27). To 5 x 10^9 bacterial organisms was added 2.5 to 400 ug of 125I-labeled myeloma globulin in a total volume of 1 ml. The ratio of bound to free myeloma globulin was plotted against the concentration of bound myeloma globulin (15).

**Absorption of serum with staphylococci.** Normal human serum was absorbed twice with equal volumes of packed, washed staphylococci grown overnight in BHI (19). The absorbed serum was then analyzed in immunoelectrophoresis (0.05 M Veronal buffer, pH 8.6) by use of goat anti-whole human serum (IEP, Hyland, Div. Travenol Lab., Inc., Los Angeles, Calif.).

**RESULTS**

**Effect of culture medium, incubation time, and temperature on protein A production.** Two protein A-containing S. aureus strains, Cowan I and Mohr, and two nonproducers of protein A, Wood 46 and Monroe, were grown for 18 hr in BHI, Penassay Broth, and Tryptose Broth; they were then tested for amount of protein A. The Cowan I and Mohr strains produced constant amounts of protein A in all three media; the other two strains remained negative. The Cowan I strain was also cultivated on Staphylococcus Medium No. 110 containing a high sodium chloride concentration. An 18-hr growth on this medium showed a 90% reduction in amount of protein A present as compared with the same strain grown in BHI.

To assess the possible effect of incubation time on the amount of protein A present, the Cowan I strain of S. aureus was grown in BHI for 8, 13, 18, 24, and 42 hr at 37 C. The time of incubation was chosen so that all samples could be tested for amount of protein A on the same day. The protein A content of the Cowan I strain grown for five different lengths of time was the same. The 18-hr incubation time used in the quantitation method therefore did not seem to be critical. The other staphylococcal strains (17A, F21) were also grown for 18 and 42 hr. The amount of protein A for each strain after these two lengths of incubation was the same. A constant amount of protein A in bacteria at different points of the growth curve also indicated that the inoculum size was not critical. This has also been our experience in comparisons of a heavy inoculum with a very small one.

Experiments were also performed to study the effect of increased CO2 tension on protein A production. No difference in amount of protein A present was noted for the Cowan I and 17A strains of S. aureus when grown under increased CO2 tension or in the regular incubator.

S. aureus Cowan I was also grown in BHI at 20, 37, and 41 C for 18 hr. With the 37 C growth as a standard, the culture grown at 20 C showed only about half the amount of protein A, whereas the culture grown at 41 C showed an increase of approximately 20% (Fig. 1).

**Formalin treatment of staphylococci.** S. aureus Cowan I was treated with 0.5% Formalin for 3 hr, 3% Formalin for 30 min, and 5% Formalin in phosphate-buffered saline for 24 hr. The treatment resulted in a decrease in the protein A content to about 70% for treatment with both 0.5 and 3% Formalin and to about 25% with 5%
Formalin. Treated bacteria were also stored in suspension at 4°C for 1, 2, 3, and 6 days. No loss in the uptake of myeloma globulin was noted during storage.

**Protein A in various bacterial strains.** The total number of *S. aureus* strains tested for presence or absence of protein A was 156. Only 15 of these strains did not contain any detectable amount of protein A. Consequently, the frequency of protein A-producing strains in this collection was 90.4%.

When grouped according to the type of infection produced, some differences in frequency of protein A-negative strains were noted. Among the 16 osteomyelitis strains, as many as 3 were negative, whereas no protein A-negative strains were found among 15 strains from patients with staphylococcal endocarditis. *S. aureus* strains from wounds and abscesses dominated the material and showed a percentage of protein A-negative strains of 7.7%. None of the 47 strains of *S. epidermidis* contained any measurable amount of protein A, and no protein A was found in 13 nonstaphylococcal bacterial strains.

**Quantitation of protein A in *S. aureus* strains.** Thirty-seven strains of *S. aureus* were tested for uptake of 100 μg of 125I-labeled myeloma globulin by 5 × 10⁸ bacteria. The amount of protein A present was expressed as a percentage of the reference strain giving the same uptake of labeled myeloma globulin. The experiments showed amounts of protein A ranging from 0 to 100% with no evident separation into different groups.

Since no clear-cut separation into groups was obtained on the basis of the amount of protein A detected, further information was sought by repeated studies on a smaller group of strains. The 16 strains of *S. aureus* extensively characterized antigenically by Oeding and Haukenes (10, 25) were chosen for this study. The results obtained from these experiments in which 20 μg of labeled myeloma globulin was added to 10⁸ bacterial organisms are shown in Table 1. Three of the strains, Wood 46, Cowan II, and 28, were negative both in these tests and in previous qualitative studies. The amount of protein A detected in the

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**Table 1. Quantitation of protein A in 16 strains of Staphylococcus aureus (Haukenes strains)***

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* The amount detected in the strains at the 10 occasions recorded is expressed relative to the amount present in the Cowan I reference strain.
remaining 13 strains showed a wide variation. We have previously found some day to day variation in the Cowan I strain. The uptake of radioactivity by this strain for each experiment showed a mean value of 60.1% with a standard deviation of ± 9.2. An even wider day to day variation was noted for other strains. The 17A strain showed a standard deviation of as much as ± 21.4 from its mean protein A value of 63.6%. No specific factors were found responsible for this seemingly inherent variation of protein A produced in the strains.

Since a decreased uptake of myeloma globulin in the quantitation method might reflect a difference in affinity and not necessarily varying amounts of protein A, Scatchard plots were constructed from experiments in which increasing amounts of myeloma globulin were added to 5 x 10⁹ organisms of strains Cowan I, Cowan III, 17A, and F21. The curves obtained from these experiments were parallel, intersecting the abscissa at different points (Fig. 2). These curves indicated that the variation in uptake of labeled myeloma globulin reflects true differences in protein A content of difference strains.

**Correlation between protein A and phage type.** Of 123 strains of *S. aureus* phage-typed, 41 (33.3%) were nontypable at RTD. Of the 15 protein A-negative *S. aureus* strains, 14 were phage-typed. As many as 11 of the 14 were nontypable at RTD, a frequency of 79%. This difference in frequencies was significant at the 0.01 level. The three strains with phage patterns were Cowan II (3A/3B/3C/55/71), Furua (3A/3C/55/71), and Q-57 (53/83/84). When the amounts of protein A detected in the 16 strains supplied by Dr. Hua-kenes were compared with the phage groups of the strains (1), no apparent correlation was found. The same lack of correlation was true for other protein A-positive strains tested.

**Correlation between protein A and antigenic types.** The absence or presence of protein A in 16 type strains was compared with 26 antigens detected in these strains by Oeding and Haukenes (4-10, 25). No correlation was found for any of these antigenic factors and protein A production.

**Absorption of normal human serum with strains of staphylococci.** To illustrate the effect of the amount of protein A in various strains, normal human serum was absorbed with *S. aureus* strains Cowan I, Cowan II, and Cowan III, as well as Wood 46. As shown in Fig. 3, absorption with Cowan I and Cowan III resulted in almost complete loss or a marked decrease, respectively, of the γG present, whereas Cowan II and Wood 46, containing no protein A, did not change the γG line as compared with a control.

**DISCUSSION**

Protein A is characterized by a unique reactivity with the Fc part of human (2, 14, 17) as well as animal γG globulin (16; G. Kronvall, H. M. Grey, and R. C. Williams, Jr., J. Immunol., in press). These findings have made possible a simple technique for determination of protein A on bacteria by use of isolated human myeloma globulins as reagents (15). This method was utilized in the present investigation. Protein A was found to be present only in *S. aureus* strains, a finding in accordance with previous data obtained by the agar gel precipitation technique (3, 12, 13, 26). We found an incidence of 90% of protein A-producing *S. aureus* strains. All 47 *S. epidermidis* strains tested were protein A-negative. Our results
confirm the prevailing opinion that the presence of protein A is to be regarded as a characteristic of *S. aureus* strains (26). This is further emphasized by the higher incidence of nontypability among protein A-negative strains when tested with routine phages. These latter strains can be regarded as borderline strains by these two criteria (28).

The quantitative studies failed to reveal any pattern among the various strains tested. In addition, most strains tested several times showed some day to day variation of protein A present. This variation was not correlated to certain experiments because strains tested the same day showed both increases and decreases. The variation was not related to the medium used, the inoculum size, or the incubation time or temperature. As judged from Scatchard plots, the difference between strains in protein A content as well as the variations in the same strain were due to real differences in the number of protein A residues. In no case was a difference in affinity noted. James and Brewer have described two strains of *S. aureus* with marked heterogeneity of electrophoretic mobility, reflecting a wide range in the amount of protein associated with the cell walls (11). This heterogeneity was apparent even with cultures derived from single colonies. Their results, as well as our present data, are in line with previous results obtained in our laboratory (24) by use of light microscopic evaluation of protein A content. For most strains, there was a variation in staining for protein A, indicating heterogeneity within individual strains. Our results showing a continuous spectrum for amounts of protein A among strains tested, as well as the day to day variation in individual strains, are thus compatible with a heterogeneity of the cell wall protein production in a given culture. The reason for this variability and lack of full expression of protein A on the cell wall is not yet clear.

Among *S. aureus* strains recovered from osteomyelitic lesions and staphylococcal endocarditis patients, a striking difference in the incidence of protein A-negative strains was apparent. We have previously noted a change in protein A content in a strain after experimental osteomyelitis infection (23). This change in rabbit osteomyelitis, however, was opposite from the trend noted here. It may be that various locations of infection in the body involving different modes of host reaction select out protein A-containing or -noncontaining mutant strains. Further experiments in animals and on fresh cultures from patients with infections are necessary to solve these problems.

In a previous study, we showed a rapid decrease of protein A when the bacteria were stored in the cold (15). For absorption experiments (Kronvall, Grey, and Williams, *in press*), fresh bacteria were therefore used. In the present study, we have found that Formalin-treated staphylococci do not lose their cell wall-associated protein A material. Such bacteria may, therefore, be prepared and stored for absorption experiments. Our results showing a marked reduction of protein A content when staphylococci were grown on high salt medium are also in line with other reports (10, 24). Our more sensitive technique showed that the suppression was not complete.

Cell walls of staphylococci consist of a mucoprotein structure made up of a mucopolysaccharide-tetra-Theichic acid network cross-linked by a cell wall polypeptide (22). To this basic structure are added proteins in an as yet unresolved way. James and Brewer have studied the protein coating of staphylococci by means of electrophoretic mobility measurements (11). Their results indicated a correlation between protein coating and production of protein A. This might be interpreted to mean that only one or a few genetic loci are involved in the production of cell wall-associated protein material. Our inability to correlate the protein A reactivity with phage type or antigenic pattern suggests that all of these factors are structurally different, although they may be located on the same protein molecule. This may in fact also be concluded from other experiments showing inhibition of bacteriophage activity for protein A-reacting human gamma globulin (21). Such inhibition indicates a close arrangement of phage receptors and protein A structure. The variation in protein A coating of bacteria may be due to suppression of the protein A gene or a lack of structures on the cell wall responsible for the synthesis of protein A molecules.

**LITERATURE CITED**


