INFECTION AND IMMUNITY, Jan. 1980, p. 6-14
0019-9567/80/01-0006/09$02.00/0

Vol. 27, No. 1

Demonstration of Specific Binding Sites for Human Serum Albumin in Group C and G Streptococci

ERLING B. MYHRE* AND GÖRAN KRONVALL

Department of Medical Microbiology, University of Lund, Lund, Sweden

A total of 297 bacterial strains belonging to 27 species was tested for quantitative uptake of radiolabeled human serum albumin. Specific binding sites with high affinity for human serum albumin were found exclusively in group C and G streptococci. The albumin binding was found to be a time-dependent, saturable, and displaceable process which obeyed simple kinetic equations. Scatchard analysis revealed that human serum albumin bound to a homogeneous population of receptors with an affinity in the order of 10^7 liters/mol and that the average bacterial cell carried more than 80,000 binding sites. The albumin receptor is a heat-stable component susceptible to proteolytic digestion. It has a surface localization separate from the receptors for immunoglobulin G, fibrinogen, aggregated β_2-microglobulin, and haptoglobin. In individual strains, albumin reactivity was also detected independently of these other types of interactions with human proteins.

Streptococci are capable of highly specific interactions with human proteins. These interactions are mediated by binding structures on the bacterial surface. At present, four different types of reactivities are known. First, group A, C, and G streptococci can react in a nonimmune way with the Fc portion of immunoglobulin molecules (14, 20-22). Second, most group A, C, and G streptococci can bind aggregated β_2-microglobulin (15, 16). A third type of reactivity is the uptake of fibrinogen by group A, C, and G streptococci (G. Kronvall, C. Schönbeck, and E. Myhre, Acta Pathol. Microbiol. Scand. Sect. B., in press; 7, 29). Finally, strains carrying the T4 antigen can combine with haptoglobin (13). These interactions between mammalian proteins and bacteria are important phenomena for two main reasons. Uptake of host-derived proteins might be of biological significance through modification of the host-parasite relationship. Furthermore, bacteria possessing specific binding structures can be used as laboratory reagents for absorption procedures and for isolation of proteins.

In recent studies of absorption of human serum samples with streptococci, we observed that certain strains removed considerable amounts of serum albumin (17). The present investigation confirms this observation and demonstrates specific binding sites for human serum albumin in group C and G streptococci. The present series of experiments show that these binding sites have a surface localization separate from the receptors for four other human proteins. Thus, a new type of reactivity can be added to the list of bacterial interactions with host proteins.

MATERIALS AND METHODS

HSA. HSA was isolated by a two-step procedure from pooled serum containing serum samples from 58 blood donors. Two-milliliter portions of serum were separated by using preparative block electrophoresis performed in a 0.6% agarose gel with a 0.0075 M Veronal buffer (10). The albumin fraction was further purified by gel filtration on a G-100 Sephadex column (1.5 by 95 cm) eluted at 4°C with phosphate-buffered saline (PBS, 0.12 M NaCl, 0.03 M phosphate, pH 7.2) containing 0.02% sodium azide. The albumin peak was pooled and concentrated by using collodion filters (Satorius membrane filter GmbH, Göttingen, Germany). Immunoelectrophoresis of HSA obtained by the two-step procedure showed a single precipitation arc when tested at a concentration of 2 mg/ml against rabbit anti-HSA and anti-human serum proteins (Dakopatts, Copenhagen, Denmark). Albumin concentrations were measured spectrophotometrically by using an absorbance ($E^{1\%}_{1\,cm}$) value at 280 nm of 5.31 (8). The molecular weight of HSA was taken to be 66,000 (24).

Bacterial strains. A total of 297 strains belonging to 27 different bacterial species were included in the study (Table 1). The following strains were kindly provided by O. Holmberg, National Veterinary Institute, Stockholm, Sweden: *Streptococcus zooepidemicus*, *S. equii*, *S. dysgalactiae*, β-hemolytic bovine group G streptococci, group M, N, P, and U streptococci, *S. uberis*, and *Corynebacterium pyogenes*. Alpha-hemolytic group G streptococci were obtained from R. Gudding, National Veterinary Institute, Oslo, Norway. All other strains were obtained consecutively from clinical specimens sent to the Clinical Microbiology Laboratory, University Hospital, Lund, Sweden. Serogrouping of streptococci was performed with the coagglutination method (4). Streptococci, pneumococci, *C. pyogenes*, and *Brahamella catarrhalis* were grown in Todd-Hewitt broth. Staphylococci, *Esche-*

richia coli, Klebsiella, Proteus, and *Pseudomonas* strains were cultured in tryptone broth. A Todd-Hewitt broth fortified with X and V factors and a broth containing peptone, yeast extract, hemin, starch, and vitamins were used for growing *Haemophilus influenzae* and *Neisseria gonorrhoeae*, respectively.

Radiolabeling. A 200-μl amount of HSA (1 mg/ml in 0.15 M phosphate buffer, pH 7.5) was labeled with 0.2 mCi of ^{125}I (code no. IMS 30, Radiochemical Centre, Amersham, England) by mild electrolysis as described by Rosa et al. (25) and modified for labeling of immunoglobulins (9). Incorporation of ^{125}I into the protein was estimated by paper chromatography (9). Free, noncovalently bound isotope was removed by extensive dialysis against PBS containing 0.02% sodium azide and 0.05% Tween 20 (PBSA-Tween). The labeled preparation was then gel filtered on the G-100 Sephadex column used for purification of HSA. The monomeric albumin fraction was pooled and used in all experiments. The specific activity of the pooled fraction was 0.7 mCi/mg of protein. Labeled HSA was mixed with normal human serum and with unlabeled HSA and analyzed by immunoelectrophoresis as described above. Autoradiographic analysis of the slides showed that the radioactivity was associated only with the albumin precipitate. Commercially available human haptoglobin (Hoechst, Frankfurt, Germany) and human polyclonal immunoglobulin G (IgG), fibrinogen, and aggregated β_2-microglobulin obtained as previously described (15, 16) were also iodinated by mild electrolysis.

HSA-binding assay. Overnight broth cultures of bacterial strains were washed twice and suspended in PBSA-Tween buffer to 10^9 bacteria per ml, as calculated from optical density values at 520 nm on diluted samples in a Beckman CP-1 colorimeter. Binding studies were performed at room temperature in triplicates in polystyrene test tubes (12 by 70 mm; AB Cerbo, Trollhättan, Sweden) by mixing 0.8 μg of radiolabeled HSA with 2×10^8 bacterial organisms in a final volume of 225 μl. After 1 h, 2 ml of cold PBSA-Tween buffer was added, and the bacteria were deposited by centrifugation at 1,800 × *g* for 10 min. The supernatant was sucked off, and the radioactivity of the bacterial pellet was determined in a gamma counter (LKB Rack Gamma 1270, Bioteck, Stockhlom, Sweden). The albumin uptake was expressed as the percentage of total radioactivity added. The reproducibility of the assay system was assessed by testing 10 different broth cultures of a highly reactive strain. The observed binding levels (arithmetic mean of triplicate samples) showed that determinations could be performed with an imprecision of 4.3% (coefficient of variation). The binding to test tubes in the absence of bacteria was less than 1%.

Uptake levels at pH values ranging from 3.0 to 8.0 were determined with bacteria suspended in 0.1 M citrate and phosphate buffers containing 0.05% Tween 20. Binding studies were also performed with 0.8-, 5-, 10-, and 15-, 20- and 25-μg quantities of HSA and heat-killed bacteria (2×10^8 organisms as counted in a Petroff-Hauser Chamber (19) in a final volume of 500 μl. Heat treatment at 80°C for 5 min did not affect the HSA binding capacity. A control sample containing 1 mg of unlabeled HSA was included to estimate the nonspecific uptake, presumed to be the same for all samples. Specific binding was calculated by subtracting from the radioactive uptake the amount that could not be displaced by unlabeled HSA.

Kinetics studies. All experiments were performed in triplicate at room temperature with 0.8 μg of radiolabeled HSA and 2×10^8 heat-killed group G streptococci (G 148). Binding assays were performed with incubation times ranging from 10 s to 90 min. Further uptake of labeled HSA was prevented by addition of 400 μl of ice-cold unlabeled HSA (2 mg/ml in PBSA-Tween buffer). For dissociation studies, bacteria were first reacted with labeled HSA in a final volume of 50 μl. After 1 h the samples were diluted 60 times with PBSA-Tween buffer, and after further 10, 20, 30, 40, 50, and 60 min, the amount of HSA still bound to the pellet was determined. Corrections were made for nonspecific binding. Rate constants were calculated as described by Cuatrecasas and Hollenberg (6). The association rate was computed by using the second order equation $k_1 = 2.303(1/t) \cdot [1/(a - b)] \cdot \log[b(a - x)/a(b - x)]$. The first order equation $k_2 = 2.303\ (1/t) \cdot \log(x/x - a)$ was used for calculation of the dissociation rate. In these equations a is the concentration of albumin, b is the concentration of receptor, and x is the concentration of albumin-receptor complex at the time t. The association constant k_a was calculated from the equation $k_a = k_1/k_2$.

Pepsin and trypsin digestion. Increasing amounts of pepsin and trypsin were added to 10^9 heat-killed bacteria suspended in 1.0 ml of 0.1 M acetate buffer (pH 4.5) and in 1.0 ml of 0.15 M phosphate buffer (pH 7.5), respectively. After 1 h of incubation at 37°C the reaction was stopped by addition of 100 μl of 1 M tris(hydroxymethyl)aminomethane base to the peptic digest and 1 ml of trypsin inhibitor solution (1 mg/ml in PBS) to trypsin-treated bacteria. The bacteria were washed and suspended in PBSA-Tween buffer and then tested for albumin binding. Pepsin, trypsin, and trypsin inhibitor were purchased from Sigma Chemical Co., St. Louis, Mo. (catalog no. P 7012, T 8253, and T 9128).

Inhibition assay. Increasing amounts of human serum albumin, fibrinogen, polyclonal IgG, and normal human serum were mixed with 0.8 μg of labeled HSA, and the uptake of labeled material to 2×10^8 bacteria was determined.

RESULTS

Quantitative binding of HSA. A total of 297 bacterial strains representing 27 different gram-positive and -negative species were tested for the binding of HSA (Table 1). Binding capacities were determined by direct measurement of uptake of 0.8 μg of radiolabeled HSA to 2×10^8 bacterial organisms. Significant binding was observed only with group C and G streptococci (Fig. 1). All four recognized group C streptococcal species were included. Fifteen human *S. equisimilis* strains were all positive with binding levels of 22 to 61%. The majority of 15 nonhuman *S. zooepidemicus* strains showed a low but definite albumin binding. *S. equii* strains demon-

strated the lowest reactivity with only one of seven strains positive, binding 14%. *S. dysgalactiae*, α-hemolytic bovine streptococci, presented a continuous spectrum of uptake levels ranging from 2 to 63%. Group G streptococci of human and bovine origin differed in albumin reactivity. Fifteen β-hemolytic human group G streptococci were reactive with binding levels of 32 to 72%. The highest uptake levels were noted with β-hemolytic bovine group G streptococci. These strains formed a homogeneous group binding ca. 90% of added albumin. In contrast, α-hemolytic bovine group G streptococci were all negative.

Binding studies performed with a group G streptococcus (G 148) suspended in citrate and phosphate buffers with pH values ranging from 3.0 to 8.0 revealed that the albumin uptake was dependent on the hydrogen ion concentration. A maximal uptake of 84% was seen at pH 4.5. Considerably lower levels of binding occurred both at lower and higher pH values. Similar differences were noted when bacteria coated with HSA were resuspended in these buffers. Less than 10% of bound HSA were dissociated at pH 4.5 to 6.5. Resuspension at pH 3.0 and 8.0 produced a 70 and 20% elution of bacteria-associated HSA.

TABLE 1. *Capacity of 27 bacterial species to bind HSA*[a]

Bacterial species	% Albumin bound		No. of strains
	Mean	Range	
Group A streptococci	4	2–8	15
Group B streptococci	1	1–2	10
Group C streptococci			
S. equisimilis	50	22–63	15
S. zooepidemicus	15	5–26	15
S. equii	6	4–15	7
S. dysgalactiae	27	2–62	15
Group D streptococci	1	1–2	17
Group G streptococci			
β-Hemolytic human isolates	50	32–72	15
β-Hemolytic bovine isolates	91	88–92	15
α-Hemolytic bovine isolates	3	2–5	12
Group M streptococci	3	2–3	6
Group N streptococci	2	1–8	11
Group P streptococci	8	3–13	10
Group U streptococci	4	3–8	14
S. uberis	5	4–6	2
Staphylococcus aureus	2	1–2	15
Staphylococcus epidermidis	2	1–3	10
Staphylococcus saprophyticus	2	1–4	10
Diplococcus pneumoniae	1	1–2	10
Corynebacterium pyogenes	3	2–3	3
Brahamella catarrhalis	2	1–4	10
N. gonorrhoeae	1	1–2	10
H. influenzae	2	2–3	10
E. coli	1	1–2	10
Klebsiella spp.	1	1–2	10
Proteus spp.	1	1–2	10
Pseudomonas spp.	2	1–4	10

[a] Binding capacities are expressed as the percent uptake of 0.8 μg of radiolabeled albumin to 2×10^8 bacterial organisms. Arithmetic mean values of uptake levels of individual strains were tested in triplicate.

Binding as a function of HSA concentration. Binding assays performed with 2×10^8 group G streptococci (G 148) showed that the HSA uptake is a saturable process (Fig. 2A). A correlation was observed between the bound and the added albumin fraction at concentrations below 15 μg/500 μl. At higher concentrations a plateau was noted. Thus a suspension of 2×10^8 bacterial organisms (G 148) are capable of binding more than 5 μg of HSA.

Binding as a function of the number of bacteria. Uptake levels were recorded with 10^6 to 10^9 bacteria (G 148) in the assay system (Fig. 2B). A steady increase in the bound fraction was observed over the whole range studied. With 2×10^8 bacteria in a final volume of 300 μl, a 50% binding was obtained, and by increasing the amount of bacteria to 10^9, a nearly 70% uptake was achieved.

Kinetic studies. The kinetics of the albumin binding was explored by mixing small quantities of radiolabeled HSA with sufficient amount of bacteria to ensure an excess of binding sites.

(i) Association kinetics. Early association events could not be resolved merely by separating the components by centrifugation. The binding step was therefore terminated by addition of unlabeled HSA in vast excess. Displacement of bound labeled HSA was minimized by rapid cooling of the mixture before centrifugation. Studies performed with this technique demonstrated a time-dependent process with rapid association of the components. A typical experiment using a human group G streptococcus (G 148) is shown in Fig. 3A. After 1, 2, and 5 min uptake levels of 36, 46, and 61%, respectively, were noted. After 10 min only modest increases in the uptake were seen and after 20 min a state of equilibrium was reached. Regression analysis of these data with an exponential model ($y = ae^{bx}$) showed a correlation with an r^2 value of 0.96. The binding data were used to determine the rate of association. The association rate constant for the strains G 148 was found to be 8.5 $\times 10^3$ M^{-1} s^{-1}.

(ii) Dissociation kinetics. The dissociation process was studied by first reacting radiolabeled HSA with heat-killed group G streptococci (G 148) in a minimal final volume and then diluting

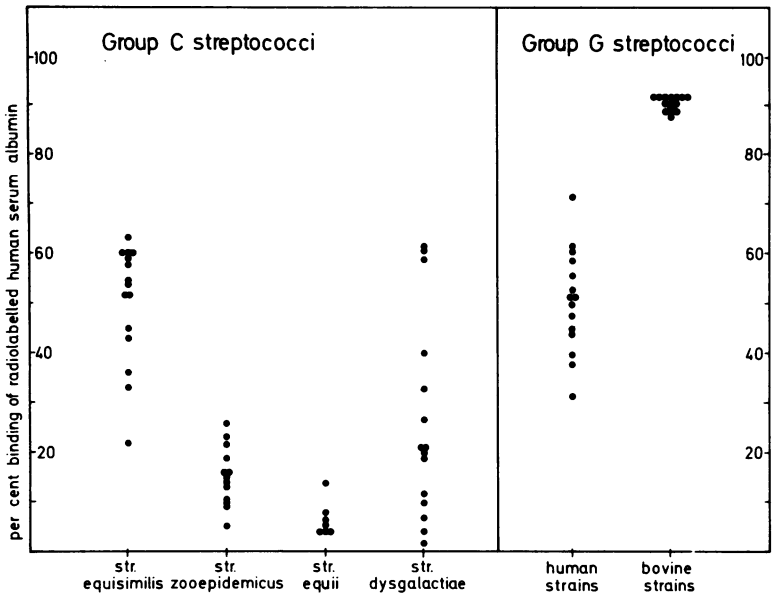

FIG. 1. *Binding of HSA to 52 group C streptococci (streptococcal species equisimilis, zooepidemicus, equii, and dysgalactiae) and to 30 β-hemolytic group G streptococci of human and bovine origin. Radiolabeled HSA (0.8 μg) was added to 2 × 10^8 bacteria, and the uptake is expressed as the percentage of added radioactivity.*

the incubation mixture 60 times with buffer. Complexed HSA will dissociate until a new equilibrium is reached. In our experiments the dissociation was followed for 1 h by determining the amount of HSA still bound to bacteria at selected time intervals (Fig. 3B). Regression analysis of the data showed that a straight line ($r^2 = 0.98$) could be constructed for the semilogarithmic plot. Thus the dissociation process follows first-order kinetics. The dissociation is slow, and the half-time of the albumin-receptor complex determined by this technique is ca. 120 min. This corresponds to a dissociation rate constant of 9.6×10^{-5} s^{-1}.

Digestion with proteolytic enzymes. The susceptibility of the albumin binding component to pepsin and trypsin was determined by incubation of 10^9 heat-killed bacteria with 0.1- to 1,000-μg quantities of enzymes and then testing these suspensions for albumin binding. Several group C and G streptococci were tested with similar results. The data obtained with the human group G strain G 148 is plotted in Fig. 4. The binding component is sensitive both to pepsin and trypsin digestion. Digestion with 10 to 20 μg of enzyme reduced the binding capacity by 50%, and use of 100 μg of enzyme produced an almost complete loss of reactivity. The bacterial suspensions were also tested for binding of radiolabeled human IgG. The immunoglobulin receptor was rather resistant to both pepsin and trypsin treatment. The uptake of human IgG was not affected by enzyme amounts less than 500 μg, and digestion with 1,000 μg of pepsin and trypsin reduced the bound fraction from 85% to only 65 and 40%, respectively.

Relationship to other streptococcal reactivities. In a series of experiments purified human serum proteins with affinity for streptococcal surface structures were tested for their capacity to inhibit the uptake of radiolabeled HSA to a group G streptococcus. Addition of up to 500 μg of IgG and fibrinogen to the test system had no effect on albumin binding (Fig. 5). These amounts were sufficient to saturate the IgG and the fibrinogen receptors on the test organisms. Because no blocking or steric hindrance was observed, it seems justified to conclude that the binding sites for serum albumin are structurally separated from the receptors for IgG and fibrinogen. Addition of 10 μg of unlabeled HSA produced half maximal inhibition, and employment of 100 μg resulted in complete inhibition of the specific uptake of labeled HSA. Dilutions of human serum were tested and found inhibitory at concentrations corresponding to the inhibition obtained with isolated HSA in the absence of other plasma proteins. In a second series of experiments, 60 group C and G streptococcal strains were tested for binding of radiolabeled HSA, human IgG, fibrinogen, haptoglobin, and aggregated β_2-microglobulin. In individual strains, albumin reactivity was detected independently of the capacity to bind any other

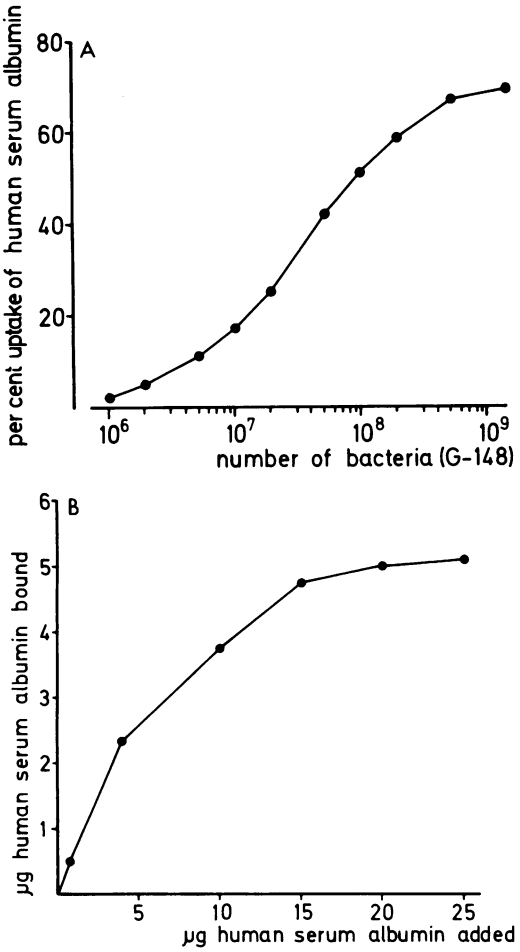

FIG. 2. *(A) Uptake of HSA (micrograms) to 2 × 10^8 organisms of a human group G streptococcus (G 148) tested with 0.8- to 25-μg quantities of labeled HSA in a final volume of 500 μl. (B) Binding of HSA as a function of the number of test organisms. Radiolabeled HSA (0.8 μg) was mixed with bacterial suspensions containing from 10^6 to 10^9 bacteria (G 148).*

protein preparation. In strains binding several of the test proteins, no statistical correlation was found between the uptake of albumin and the levels of the other reactivities.

Determination of number of albumin receptors and affinity constants. A human group G streptococcus (G 148) and a bovine group G strain (DG 6) were tested for binding of variable amounts of radiolabeled HSA. Bound (b) and free (f) fractions were determined, and the data was plotted as described by Scatchard (26), using the relationship $b/f = nK - bK$ (Fig. 6). Linear regression lines were drawn for the plots ($r^2 = 0.99$). The intercept of these lines with the x-axis gives the value n, the amount of albumin bound at saturation. The slope of the lines reflect the effective association constant between the albumin molecules and the receptors. From Fig. 6 it is evident that the two group G streptococci tested have similar binding capacities, but differ in affinity. Based on the assumption that one albumin molecule interacts with one receptor, the average number of binding sites per bacterial cell are 84,000 for G 148 and 82,000 for DG 6. The linearity of the Scatchard analysis suggests that albumin binds to a homogeneous population of receptors. The as-

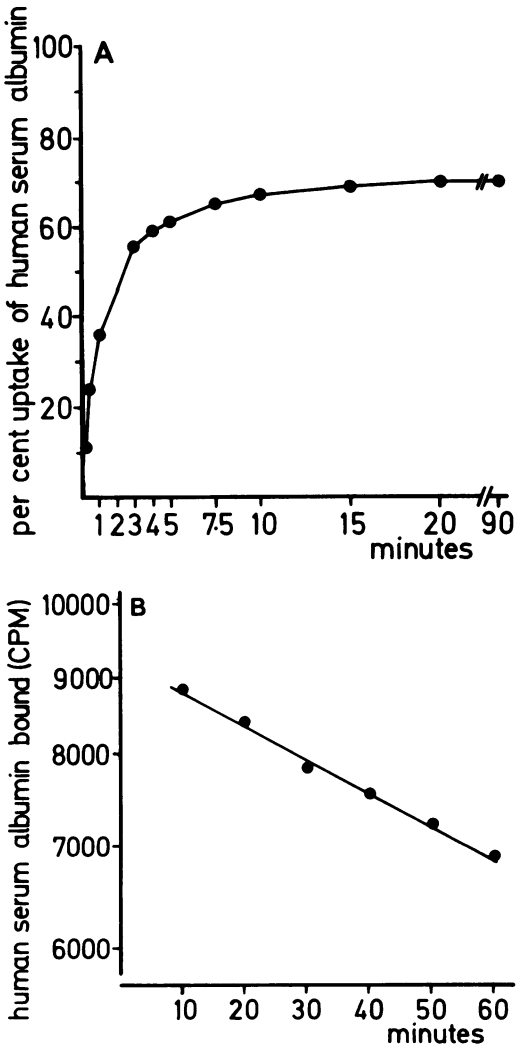

FIG. 3. *(A) Rate of uptake of 0.8 μg of labeled HSA to 2 × 10^8 bacteria (G 148). Uptake is expressed as the percentage of added radioactivity. Ten- and 30-s values are included. (B) Semilogarithmic plot of the dissociation of HSA bound to 2 × 10^8 bacteria (G 148) as a function of time.*

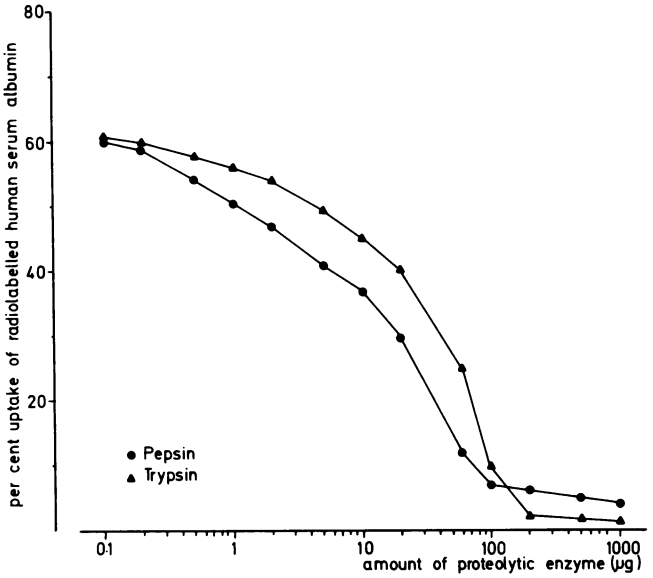

FIG. 4. *Effect of pepsin (●) and trypsin (▲) on heat-killed bacteria (G 148) tested for binding of radiolabeled HSA. Increasing quantities of enzymes were used for digestion of 10^9 bacteria at 37°C for 1 h.*

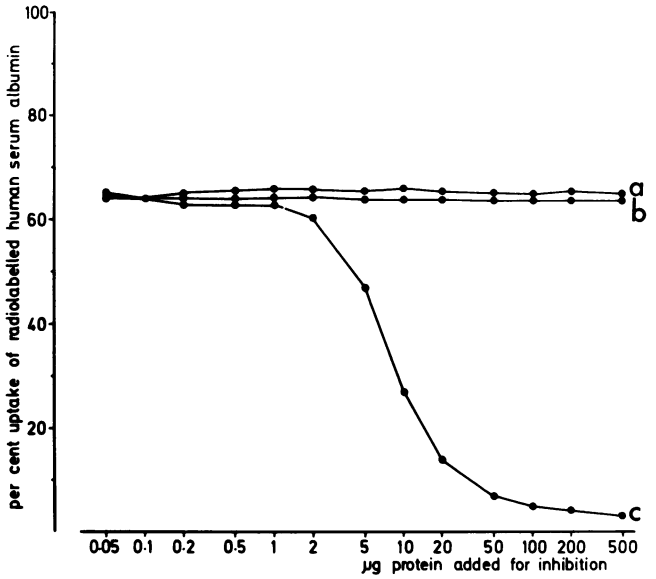

FIG. 5. *Inhibition experiments showing the binding of 0.8 µg of radiolabeled HSA to 2×10^8 bacteria (G 32) with increasing amounts of human fibrinogen (a), polyclonal human IgG (b), and HSA (c) added.*

sociation constants derived from the plots are 5.8×10^6 and 1.7×10^7 liters/mol for G 148 and DG 6, respectively. Association constants can also be calculated from the rate constants of association and dissociation. Based on the kinetic data, the association constant for strain G 148 is 8.9×10^7 liters/mol. This value is about 10 times higher than the value derived from the Scatchard plot. The discrepancy might reflect the differences in test circumstances. The kinetic experiments were performed with an excess of bacterial receptors in contrast to the Scatchard analysis, which was carried out with an excess of albumin molecules. Thus, the effective association constant might depend on the degree of saturation of the receptor. Small quantities of

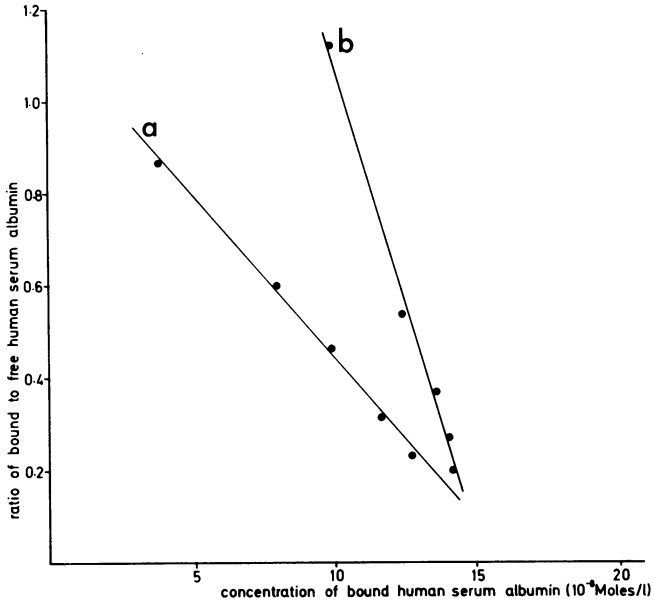

FIG. 6. *Scatchard plots of binding experiments performed with 5 to 25 μg of labeled HSA. A human group G strain (G 148[a]) and a bovine β-hemolytic group G strain (DG 6[b]) were studied.*

ligand will then bind with higher affinities than amounts which saturate the streptococcal binding sites.

DISCUSSION

The present studies describe the presence of binding sites for HSA on the surface of group C and G streptococci. The albumin uptake is a saturable process indicating existence of a limited number of binding sites on the bacterial surface. Rate constants for association and dissociation can be measured, and the kinetic data was found to obey simple rate equations. Thus, this new type of streptococcal reactivity seems indeed to represent a highly specific interaction.

The bacterial structure responsible for the HSA binding was not identified, but some characteristic features were observed. Heat treatment of bacteria at 80°C for 5 min did not affect the reactivity. In contrast, digestion with pepsin at suboptimal pH and with trypsin at pH 7.5 resulted in a complete loss of specific binding activity. Several group C and G streptococci have been subcultured weekly in our laboratory for more than 1 year without loss of albumin reactivity. Thus the albumin receptor appears to be a heat-stable component which is either a protein itself or connected to the bacterial surface by a protein structure. Human IgG and fibrinogen had no inhibitory effect on the uptake of labeled HSA to a streptococcal strain carrying binding sites for all three proteins. Receptors for human IgG and aggregated β_2-microglobulin were found to differ in sensitivity to trypsin digestion. Furthermore, in individual streptococcal strains HSA reactivity was detected independently of the capacity to interact with IgG, fibrinogen, aggregated β_2-microglobulin, and haptoglobin. These observations indicate that the streptococcal surface possesses specific binding sites for HSA and that these binding sites have a molecular localization separated from the receptors for other human proteins.

W. A. Simpson, I. Ofek, and E. H. Beachey have recently demonstrated an interaction between albumin and lipoteichoic acid (LTA) extracted from streptococci (Clin. Res. **27**:357A, 1979). This interaction involves the lipid-containing portion of LTA which combines with the fatty acid binding sites on the albumin molecule. Although LTA is a possible candidate for albumin binding to intact bacterial cells as described in the present paper, we consider this possibility rather unlikely. First of all, LTA is a regular component with a uniform structure found in most gram-positive bacteria, including streptococci (12, 31). The restriction of albumin reactivity to group C and G streptococci is difficult to reconcile with this fact. Second, LTA is clearly amphipathic with a small hydrophobic glycolipid portion associated with the bacterial plasma membrane and a long polar glyceryl phosphate chain extending into the cell wall (12,

31). The lipid portion of LTA which showed affinity for albumin seems to be deeply buried in the bacterial cell wall and hardly accessible at the cell surface. Beachey et al. have, however, presented evidence suggesting a surface localization of the lipid part of LTA in group A streptococci (2, 23). In the present experiments group A streptococci demonstrated only a low background binding seen with nearly all other bacteria tested. In our previous study we noted some absorption of albumin by two group A strains (17). The absorption experiments were performed with about 100 times the number of organisms used in the present test system. The great number of bacteria used favors interactions of low avidity and affinity.

Albumin reactivity was demonstrated in all four group C species and in both human and bovine β-hemolytic group G streptococci (Fig. 1). This observation underlines earlier described similarities between these bacterial species as revealed in immunoglobulin reactivity. Group C and G streptococci carry the same type of Fc binding structures, which differs from that present on group A streptococci (21, 22). Human group C and G streptococci therefore seem to be more closely related to each other than to other streptococcal species. The albumin receptor may have evolved late in the evolution of bacteria and may have then been preserved through the further separation of group C and G streptococci.

In the present investigation estimates were made of the affinity constant for the albumin binding, and the number of binding sites on different bacterial strains was calculated. When new types of interactions are described, attempts should be made to determine these parameters. High-avidity bindings tend to be more specific than low avidity interactions. It is possible that different types of interactions can be related to the degree of avidity. For instance, adherence of bacteria to host cells might require only low-avidity receptors as several adjacent binding sites could be engaged simultaneously resulting in additive effects. Higher avidities may be needed for interactions with single molecules masking determinants on the host cell surface.

Protein A carrying staphylococci have been used in the laboratory for selective absorption of immunoglobulins (1, 18, 28). Albumin binding streptococci might serve similar purposes. Because streptococcal receptors for albumin, immunoglobulins, fibrinogen, and haptoglobin occur independently, strains binding one or more of these plasma proteins can be selected for particular use. Streptococci conserved by heat treatment as described in this study can be stored over several months without loss of albumin binding capacity. The uptake of albumin is a reversible process, and absorbed albumin can be recovered by elution with either acid buffers or with dissociating agents like isothiocyanate. The high capacity and avidity of bovine group G strains make them particularly suitable for specific albumin binding applications.

The biological significance of uptake of human proteins by bacterial surface structures has not been determined. *Schistosoma mansoni*, a mammalian parasite residing in the intestinal blood vessel system of the host, are capable of specific binding of host proteins like blood group substances and histocompatibility antigens to its surface (5, 27). Receptors with specificity for IgG (Fc) and human β_2-microglobulin has recently been identified on the tegumental surface of the parasite (11, 30). Acquisition of host-derived proteins seems to enable the parasite to evade immune response of the host and thus to escape subsequent destruction (3, 30). In analogy, uptake of human serum proteins by streptococci could provide a mechanism to resist normal disposal. Pathogenic bacteria have often several lines of defense, and binding of host proteins might be one of them. The present study adds another type of human protein binding with such potential capacities.

ACKNOWLEDGMENTS

This project was supported by the Swedish Medical Research Council, grant 5210.

We thank Elisabeth Nilsson and Åke Christensson for excellent technical assistance and Eva Carlquist for secreterial help.

LITERATURE CITED

1. **Ankerst, J., P. Christensen, L. Kjellen, and G. Kronvall.** 1974. A routine diagnostic test for IgA and IgM antibodies to rubella virus: absorption of IgG with *Staphylococcus aureus*. J. Infect. Dis. **130**:268–273.
2. **Beachey, E. H., and I. Ofek.** 1976. Epithelial cell binding of group A streptococci by lipoteichoic acid on fimbriae denuded of M protein. J. Exp. Med. **143**:769–771.
3. **Bloom, B. R.** 1979. Games parasites play: How parasites evade immune surveillance. Nature (London) **279**:21–26.
4. **Christensen, P., G. Kahlmeter, S. Jonsson, and G. Kronvall.** 1973. New method for the serological grouping of streptococci with specific antibodies absorbed to protein A-containing staphylococci. Infect. Immun. **7**: 881–885.
5. **Clegg, J. A., S. R. Smithers, and R. J. Terry.** 1971. Acquisition of human antigens by *Schistosoma mansoni* during cultivation in vitro. Nature (London) **232**: 653–655.
6. **Cuatrecasas, P., and M. D. Hollenberg.** 1976. Membrane receptors and hormone action. Adv. Protein Chem. **30**:251–451.
7. **Duthie, E. S.** 1955. The action of fibrinogen on certain pathogenic cocci. J. Gen. Microbiol. **13**:383–393.
8. **Edwards, F. B., R. B. Rombauer, and B. J. Campbell.** 1969. Thiol-disulfide interchange reactions between albumin and disulfides. Biochim. Biophys. Acta **194**:234–245.
9. **Harboe, M., and I. Fölling.** 1974. Recognition of two

distinct groups of human IgM and IgA based on different binding to staphylococci. Scand. J. Immunol. **3**:471-482.

10. **Johansson, B. G.** 1972. Agarose gel electrophoresis. Scand. J. Clin. Lab. Invest. **29**(Suppl. 124):7-19.
11. **Kemp, W. M., S. C. Merrit, M. S. Bogucki, J. C. Rosier, and J. R. Seed.** 1977. Evidence for absorption of heterospecific host immunoglobulin on the tegmentum of *Schistosoma mansoni*. J. Immunol. **119**:1849-1854.
12. **Knox, K. W., and A. J. Wicken.** 1973. Immunological properties of teichoic acids. Bacteriol. Rev. **37**:215-257.
13. **Köhler, W., and O. Prokop.** 1978. Relationship between haptoglobin and *Streptococcus pyogenes* T 4 antigens. Nature (London) **271**:373.
14. **Kronvall, G.** 1973. A surface component of group A, C and G streptococci with non-immune reactivity for immunoglobulin G. J. Immunol **111**:1401-1406.
15. **Kronvall, G., L. Björck, E. Myhre, and L. Wannamaker.** 1979. Binding of aggregated β_2-microglobulin, IgG, IgA and fibrinogen to group A, C and G streptococci with special reference to streptococcal M-protein, p. 74-76. *In* M. T. Parker (ed.), Pathogenic streptococci. Reedbooks Ltd., Surrey, England.
16. **Kronvall, G., E. B. Myhre, L. Björck, and I. Berggård.** 1978. Binding of aggregated human β_2-microglobulin to surface protein structure in group A, C, and G streptococci. Infect. Immun. **22**:136-142.
17. **Kronvall, G., A. Simmons, E. B. Myhre, and S. Jonsson.** 1979. Specific absorption of human serum albumin, immunoglobulin A and immunoglobulin G with selected strains of group A and G streptococci. Infect. Immun. **25**:1-10.
18. **Mallinson, H., C. Roberts, and G. B. Bruce White.** 1976. Staphylococcal protein A; its preparation and an application to rubella serology. J. Clin. Pathol. **29**:999-1002.
19. **Meynell, G. G., and E. Meynell.** 1970. Methods for measuring bacterial mass and concentration, p. 8-34. *In* Theory and practice in experimental bacteriology, 2nd ed. Cambridge University Press, Cambridge, England.
20. **Myhre, E. B., O. Holmberg, and G. Kronvall.** 1979. Immunoglobulin binding structure on bovine group G streptococci different from type III Fc receptors on human group G streptococci. Infect. Immun. **23**:1-7.
21. **Myhre, E. B., and G. Kronvall.** 1977. Heterogeneity of nonimmune immunoglobulin Fc reactivity among gram-positive cocci. Description of three major types of receptors for human immunoglobulin G. Infect. Immun. **17**:475-482.
22. **Myhre, E. B., and G. Kronvall.** 1979. Immunoglobulin binding to group A, C and G streptococci, p. 76-78. *In* M. T. Parker (ed.), Pathogenic streptococci. Reedbooks Ltd., Surrey, England.
23. **Ofek, I., E. H. Beachey, W. Jefferson, and G. L. Campbell.** 1975. Cell membrane-binding properties of group A streptococcal lipoteichoic acid. J. Exp. Med. **141**:990-1003.
24. **Peters, T., Jr.** 1977. Serum albumin, p. 133-181. *In* F. W. Putnam (ed.) The plasma proteins, 2nd ed. Academic Press Inc., New York.
25. **Rosa, U., G. A. Scassellati, F. Rennisi, N. Ricciani, P. Giagnoni, and P. Giordani.** 1964. Labelling of human fibrinogen with ^{131}I by electrolytic iodination. Biochem. Biophys. Acta **86**:519-526.
26. **Scatchard, G.** 1949. The attraction of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. **51**:660-672.
27. **Sher, A., B. F. Hall, and M. A. Vadas.** 1978. Acquisition of murine histocompatibility complex gene products by schistosomula of *Schistosoma mansoni*. J. Exp. Med. **148**:46-57.
28. **Skaug, K., and E. Tjötta.** 1974. Diagnosis of recent Herpes simplex infection. A modified immunofluorescent test for the detection of specific *Herpes simplex* IgM antibodies after staphylococcal absorption of IgG. Acta Pathol. Microbiol. Scand. Sect. B **82**:323-328.
29. **Tillett, W. S., and R. L. Garner.** 1934. The agglutination of hemolytic streptococci by plasma and fibrinogen. A comparison of the phenomenon to serological reactions with the same organisms. Bull. Johns Hopkins Hosp. **54**:145-156.
30. **Torpier, G., A. Capron, and M. A. Quaissi.** 1979. Receptor for IgG (Fc) and human β_2-microglobulin on *S. mansoni* schistosomula. Nature (London) **278**:447-449.
31. **Wicken, A. J., and K. W. Knox.** 1975. Lipoteichoic acids: a new class of bacterial antigen. Science **187**: 1161-1167.