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Characterization of Binding of Human α_2-Macroglobulin to Group G Streptococci

GURSHARAN S. CHHATWAL, HANS P. MÜLLER, AND HANS BLOBEL*

Institut für Bakteriologie und Immunologie, der Justus-Liebig-Universität, D-6300 Giessen, Federal Republic of Germany

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An interaction was observed between human α_2-macroglobulin (α_2M) and streptococci belonging to group A, C, and G. Of 27 group C and 19 group G streptococcal cultures, 13 and 14, respectively, bound ^{125}I-labeled α_2M. Some group A streptococci also interacted with α_2M. A number of other bacterial species tested did not react with α_2M. The binding of ^{125}I-labeled α_2M to group G streptococci was time dependent, saturable, and could be inhibited by unlabeled α_2M. Inhibition experiments indicated that the streptococcal binding site for α_2M differed from the receptors for immunoglobulin G, fibrinogen, aggregated β_2-microglobulin, albumin, and fibronectin. The α_2M binding activity was remarkably sensitive to trypsin and heat treatment indicating its protein nature. Kinetic analysis indicated a homogenous population of binding sites. The number of binding sites per bacterial cell was estimated to be approximately 20,000.

Several interactions of serum proteins with some pathogenic streptococci have been described. These interactions are mediated by binding sites on the bacterial surface. Streptococci of group A, C, and G are capable of reacting in a nonimmune way with immunoglobulin G (IgG) (4, 11, 15). Interaction of some streptococci with IgA and IgD has also been reported (5, 7). Besides immunoglobulins, the streptococci also have binding sites for fibrinogen (9), aggregated β_2-microglobulin (2, 12), human serum albumin (16), haptoglobulin (17), and fibronectin (18, 19). Binding of host proteins might play an important role in host-parasite relationships.

Recent studies on the absorption of human serum with streptococci indicated that some strains bound considerable amounts of α_2-macroglobulin (α_2M) (14a). α_2M, with a molecular weight of 725,000, has the unique capacity to bind essentially all active endopeptidases (1, 13). There are indications that α_2M may contribute to the body's defenses against invasive pathogens (1, 14). Thus, this novel interaction of α_2M with streptococci could be important not only in host-parasite relationships, but might also be useful in elucidating the exact role of α_2M. The present paper describes the characterization of α_2M binding to streptococci of serological group G.

MATERIALS AND METHODS

Bacteria. Of the streptococcal cultures, 17 belonged to serological group A, 23 to group B, 27 to group C, and 20 to group G. Some of these cultures were obtained through the courtesy of the Bundesanstalt für Milchforschung, Kiel, Federal Republic of Germany. The streptococci were grown in Todd-Hewitt broth (GIBCO Laboratories, Europe GmbH, Karlsruhe, Federal Republic of Germany) supplemented per 1 liter of medium with 3 g of yeast extract (Merck, Darmstadt, Federal Republic of Germany) and 10 ml of a vitamin solution. The vitamin solution, sterilized by filtration, contained 30 mg of folic acid, 30 mg of nicotinamide, 30 mg of panthothenic acid, 5 mg of riboflavine, 30 mg of pyridoxal, 30 mg of thiamine HCl, 7.5 mg of biotin, 30 mg of *p*-hydroxybenzoic acid, and 30 mg of *m*-inositol in 100 ml of distilled water. Respectively, 100 ml of the medium was distributed in 1-liter Erlenmeyer flasks and inoculated with 1 ml of the streptococcal cultures, previously incubated for 5 h in supplemented Todd-Hewitt broth. After incubation for 16 to 18 h at 37°C and 60 rpm, the streptococci were removed by centrifugation (20 min, 15,000 × *g*).

In addition, 30 cultures of *Staphylococcus aureus*, 5 of *Staphylococcus epidermidis*, 9 of *Staphylococcus intermedius*, 7 of *Escherichia coli*, 6 of *Pasteurella multocida*, 5 of *Klebsiella pneumoniae*, 6 of *Proteus vulgaris*, 5 of *Enterobacter aerogenes*, 5 of *Salmonella gallinarum*, and 8 of *Pseudomonas aeruginosa* were cultured in brain heart infusion broth (Merck, Darmstadt, Federal Republic of Germany) under the conditions described for the cultivation of the streptococci.

Radioiodination of proteins. α_2M (Behringwerke AG, Marburg, Federal Republic of Germany) was radiolabeled with ^{125}I by using the Chloramin T method (8). Of the added radioactivity, 65% was incorporated into α_2M as determined after precipitation with trichloroacetic acid. The specific activity was 0.81 mCi/mg. Human IgG and human fibronectin radiola-

TABLE 1. Binding of ^{125}I-labeled α_2M to various bacterial species

Bacterial species	No. of cultures		% ^{125}I-labeled α_2M bound[a]	
	Total	No. positive for α_2M binding[b]	Mean	Range
Group A streptococci	17	4	22	14–42
Group B streptococci	23	1	12	12
Group C streptococci	27	13	43.8	23–78
Group G streptococci	20	14	57	39–75
Staphylococcus aureus	30	0	1.5	0.5–3
Staphylococcus intermedius	9	0	1	0.8–2
Staphylococcus epidermidis	5	0	0.9	0.8–1
Escherichia coli	7	0	0.9	0.8–1
Pasteurella multocida	6	0	1.6	1–3
Klebsiella pneumoniae	5	0	1.2	1–3
Proteus vulgaris	6	0	1.2	1–2
Enterobacter aerogenes	5	0	0.9	0.5–1.5
Salmonella gallinarum	5	0	0.7	0.5–2
Pseudomonas aeruginosa	8	0	0.8	0.5–1.5

[a] The binding is expressed as the percent uptake of 0.2 μg of ^{125}I-labeled α_2M by 2×10^8 bacteria. The values are the means of the duplicates for each culture. The mean and range for streptococci is only from the positive cultures.

[b] The binding of more than 5% was considered positive.

beled in the same way had specific activities of 1 and 0.65 mCi/mg, respectively.

Binding assay. Essentially the method of Kronvall et al. (12) was used for the binding assay. The bacteria were washed twice in phosphate-buffered saline (PBS) and suspended in PBS containing 0.05% Tween 20 and 0.1% human serum albumin (PBSTA). If not otherwise stated, the suspension contained approximately 10^9 bacteria per ml (percent transmission of 10 at 620 nm, Spectronic 20, Bausch & Lomb, Inc., Rochester, N.Y.). A total of 0.2 ml of the bacterial suspension was added to 20 μl (10 μg/ml) of ^{125}I-labeled α_2M. The reaction mixture was incubated for 1 h at room temperature in Eppendorf tubes. The bacteria were then centrifuged at 10,000 × *g* and washed with ice cold PBSTA. The radioactivity in the sediment was measured in a γ-spectrometer (Packard Instrument Co., Inc., Rockville, Md.). The uptake of radiolabeled protein was expressed as the percent of the total activity remaining in the pellet.

Trypsin treatment. The bacterial suspension (10^9 bacteria per ml) in 0.25 M phosphate buffer, pH 7.5, was incubated for 30 min at 37°C with increasing amounts (2.5 to 250 μg) of trypsin (Merck). The reaction was stopped by an excess of pancreatic trypsin inhibitor (Bayer, Leverkusen, Federal Republic of Germany) and subsequent washing with PBSTA. Resuspended bacteria were then tested for binding.

Heat treatment. One milliliter of bacterial suspension (10^9 bacteria per ml) was preincubated at different temperatures and for the time periods indicated in Fig. 6. The suspension was then cooled to room temperature and tested for binding of labeled α_2M as described above.

Determination of dissociation constants and density of binding sites. To determine the density of binding sites and the equilibrium binding (k_d) of α_2M, saturation isotherm was constructed with 0.5 μg of ^{125}I-labeled α_2M diluted with increasing concentrations of unlabeled α_2M. The number of bacteria in each assay tube was 4×10^7. Nonspecific binding was subtracted, and the data were fitted to the general dose response equation by using the BMDP 77 (6) computer program. The equation used was:

$$Y = \frac{-a}{1 + \frac{[x]^b}{k_d}} + a$$

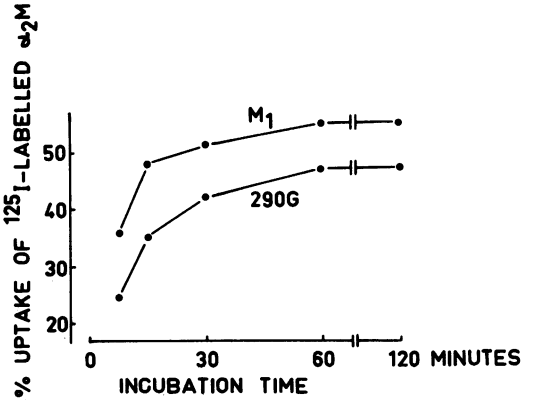

FIG. 1. Effect of incubation time on the binding of α_2M to group G streptococci (strains M_1 and 290G). The incubation of 0.2 μg of ^{125}I-labeled α_2M and 2×10^8 streptococci was carried out at room temperature for the given time periods. Each point represents the mean of duplicates. Uptake is expressed as the percentage of added radioactivity.

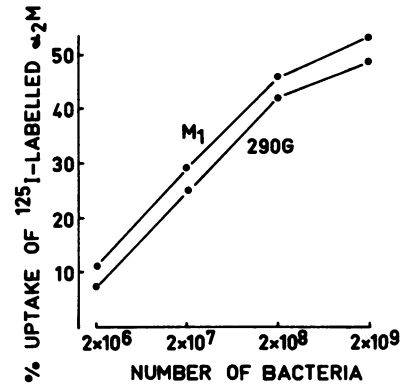

FIG. 2. Binding of α_2M as a function of the number of test organisms. ^{125}I-labeled α_2M (0.2 μg) was mixed with a 0.2-ml suspension containing 2×10^6 to 2×10^9 group G streptococci (strain M_1 and 290G). Each point represents the mean of duplicates.

where Y is the response, a is the maximum response, b is the slope factor, $[x]$ is the concentration, and k_d is the dissociation constant.

RESULTS

Binding of α_2M. Of the 27 streptococcal cultures belonging to serological group C, 13 bound ^{125}I-labeled α_2M, and of 20 group G cultures, 14 were α_2M positive. The binding activity of these cultures was relatively strong, reaching values of up to 78% (Table 1). A somewhat weaker α_2M reactivity was observed with 4 of 17 streptococcal cultures of group A. The group B streptococci and all other bacterial species tested did not bind the labeled α_2M.

α_2M binding of two representative group G strains was time dependent reaching saturation within 60 min (Fig. 1). It was also related to the number of streptococci, with a maximum binding of 0.2 μg of labeled α_2M being observed with 2×10^8 streptococci (Fig. 2).

Inhibition experiments. Inhibition experiments were conducted to evaluate the inhibition of labeled α_2M binding with high concentrations of unlabeled protein. Furthermore, they served to evaluate a possible steric interference by IgG, fibrinogen, and fibronectin, all of which could also be bound to streptococci. Thus, in the binding assay, unlabeled α_2M, IgG, fibrinogen, or fibronectin was mixed, respectively, with 0.2 μg of ^{125}I-labeled α_2M before addition of the streptococcal suspension. With two representative strains of group G streptococci, the binding of labeled α_2M could be completely inhibited by the addition of increasing amounts of unlabeled α_2M (Fig. 3). On the other hand, addition of IgG or fibrinogen did not affect the binding of labeled α_2M. Human fibronectin partially inhibited the α_2M binding only at higher concentrations (Fig. 3). The binding of ^{125}I-labeled fibronectin to group G streptococci, however, was not influenced by unlabeled α_2M, even up to a concentration of 500 μg (Fig. 4), indicating that fibronectin and α_2M have different binding sites on streptococci.

Trypsin sensitivity of α_2M binding sites. Uptake of ^{125}I-labeled α_2M by the two group G streptococcal strains tested was abolished by treatment with trypsin. A rapid decrease in the binding activity already had occurred with 2.5 μg of trypsin (Fig. 5). With higher concentrations of trypsin, the binding was completely lost.

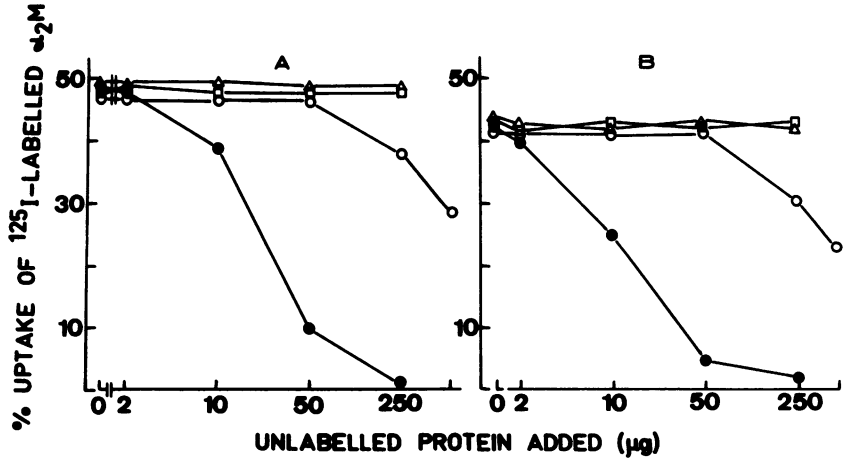

FIG. 3. Inhibition experiments showing the binding of 0.2 μg of ^{125}I-labeled α_2M to 2×10^8 group G streptococci. Strain M_1 (A) and 290G (B) with increasing amounts of unlabeled α_2M (●), human fibrinogen (△), human IgG (□), and human fibronectin (○). Each point is the mean of duplicates.

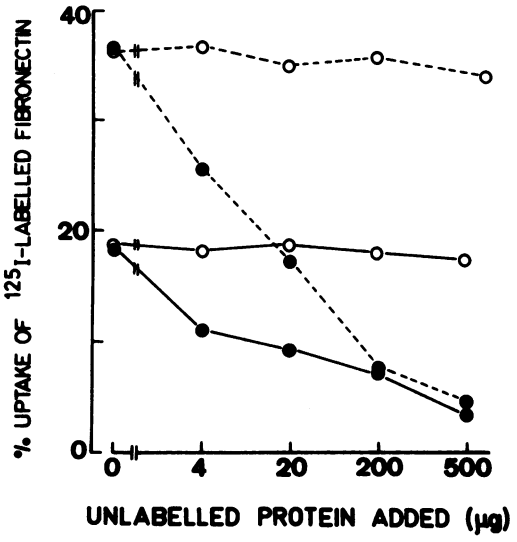

FIG. 4. Inhibition experiments showing the binding of 0.2 μg of ^{125}I-labeled human fibronectin to 2 × 10^8 group G streptococci. Strain M_1 (——) and 290G (-----) with increasing amounts of unlabeled fibronectin (●) and human α_2M (○). Each point is the mean of duplicates.

In contrast, binding of ^{125}I-labeled IgG remained almost unchanged over the entire range of trypsin concentrations (Fig. 5).

Heat sensitivity of α_2M binding. Binding of α_2M was markedly sensitive to heat. Exposure of the streptococci to 95°C for more than 30 min resulted in a significant loss of α_2M binding, with only negligible activity remaining after 2 h (Fig. 6). Also, at lower temperatures, in the range of 37 to 80°C, a considerable loss of binding activity was observed.

Dissociation constants and number of α_2M binding sites. The presence of high affinity α_2M binding sites was indicated by the ability of unlabeled α_2M to effectively inhibit the binding of tracer α_2M.

For two group G strains, the dissociation constant was 17.9 ± 0.16 nM for strain M_1 and 34.17 ± 3.03 nm for strain 290G (Fig. 7). The number of binding sites, as calculated from the maximum slope factor, were 20,317 for M_1 and 19,745 for 290G. This was based on the assumption that one molecule of α_2M is bound to one site. Thus, it became evident that both strains had similar binding capacities but differed in their affinity to α_2M by a factor of two. The slope factor of the saturation isotherms was close to unity, indicating that α_2M binds to a homogenous, noninteracting population of binding sites in these two strains of bacteria.

DISCUSSION

Of the various bacterial species tested, only streptococci of group A, C, and G bound α_2M. The α_2M binding sites on group G streptococci

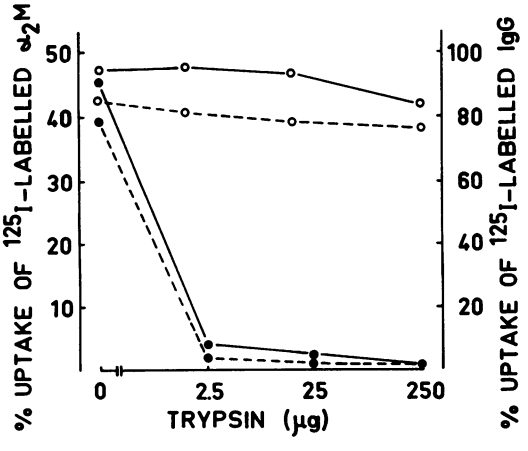

FIG. 5. Effect of trypsin on the binding of 0.2 μg ^{125}I-labeled α_2M (●) and 0.2 μg of ^{125}I-labeled IgG (○) to 2 × 10^8 streptococci strain M_1 (——) and 290G (-----). The bacteria were incubated with increasing concentrations of trypsin at 37°C for 30 min. Each point represents the mean of duplicates.

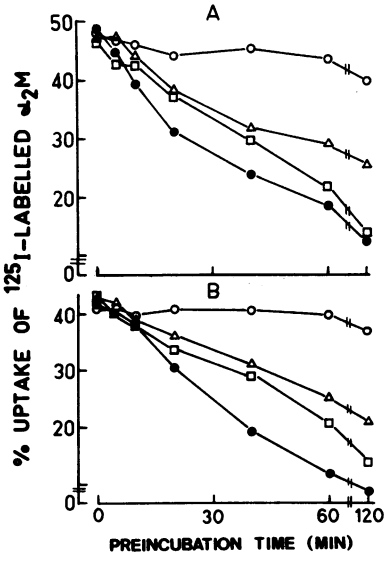

FIG. 6. Heat sensitivity of α_2M binding to group G streptococci strain M_1 (A) and 290G (B). The bacterial suspensions (10^9/ml) were preincubated at 95°C (●), 80°C (□), 60°C (△), and 37°C (○). The binding of 0.2 μg of ^{125}I-labeled α_2M to 2 × 10^8 streptococci was measured. Each point represents the mean of duplicates.

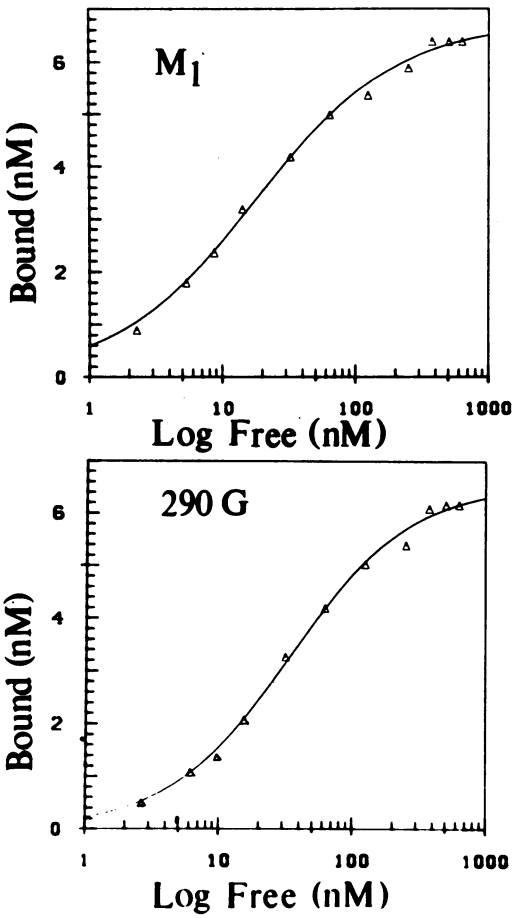

FIG. 7. Saturation isotherm of α_2M binding to group G streptococci strain M_1 and 290G plotted as $\log_{10}$ free α_2M in nanomolar versus specifically bound α_2M in nanomolar. Corrections were made for nonspecific binding before the data were fitted in the general dose response equation by using the BMDP 77 computer program run on a Cyber 170 computer. The data points were fitted according to the error sum of squares principle, and the following parameter estimates gave the best fit (mean ± a symptotic standard deviation). Strain M_1: maximum slope factor, 6.75 ± 0.15 nM (20,317 ± 451 binding sites per bacterium); dissociation constant, 17.9 ± 0.16 nM with a slope factor of 0.82 ± 0.05. Strain 290G: maximum slope factor, B_{max} 6.56 ± 0.16 nM (19,745 ± 481 sites per bacterium); dissociation constant, 34.17 ± 3.03 nM with a slope factor of 0.949 ± 0.06. When the same data were transformed into Scatchard plots, almost identical parameter estimates were calculated (data not shown).

were different from those of IgG, fibrinogen, aggregated β_2-microglobulin, and albumin. In inhibition experiments, only unlabeled α_2M could completely inhibit ^{125}I-labeled α_2M binding. Both IgG and fibrinogen showed no interference with α_2M uptake. Extreme trypsin sensitivity also differentiated α_2M binding sites from those of IgG and fibrinogen. Unlabeled albumin, which was always included in binding assays, also did not affect α_2M uptake by streptococci. The binding of albumin, as well as of aggregated β_2-microglobulin of streptococci, was heat resistant (3, 16), whereas that of α_2M was heat sensitive. Unlike α_2M binding, the aggregated β_2-microglobulin binding could be completely inhibited by fibrinogen (2). Partial inhibition of α_2M binding at a higher concentration of fibronectin could not be due to the same binding site because of the inability of α_2M to interfere with fibronectin binding. For determination of dissociation constants and density of binding sites, the saturation isotherm was plotted as $\log_{10}$-free α_2M versus specifically bound α_2M as suggested recently by Klotz (10). The data indicate high affinity and saturability of binding sites. When the same data were transformed into Scatchard plots, almost identical parameter estimates were calculated. The slope factor of close to unity indicated the homogeneity of binding sites. Additional studies on the molecular mechanism of α_2M binding and identification of the binding sites might be helpful to elucidate the possible role of α_2M in streptococcal infections. These aspects are currently under investigation.

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