Binding Activity of *Streptococcus canis* for Albumin and Other Plasma Proteins

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All 24 cultures of *Streptococcus canis* examined bound ¹²⁵I-labelled human albumin, IgG and fibrinogen; but neither IgA nor haptoglobin. Binding of human albumin was time-dependent, saturable and reversible by the addition of unlabelled albumin. The binding of ¹²⁵I-labelled human albumin could be inhibited completely by unlabelled albumin preparations from humans, mice and dogs, and partly by bovine albumin. In contrast, binding of ¹²⁵I-labelled human albumin was not inhibited by unlabelled rabbit albumin, human IgG or human fibrinogen. Data from competition experiments of two *S. canis* cultures with high ¹²⁵I-labelled albumin-binding activities yielded *K_D* values of 10 and 15 nmol l⁻¹, respectively. The estimated number of binding sites per bacterial cell ranged from 30000 to 57000. The binding factor for albumin could be isolated from *S. canis* by boiling the bacteria at pH 2, and it was purified by affinity chromatography on human albumin-Sepharose. The isolated albumin-binding proteins had a molecular mass of approximately 51 kDa and inhibited binding of ¹²⁵I-labelled albumin to *S. canis*. They formed complexes with human albumin that altered its electrophoretic mobility.

**INTRODUCTION**

During the last decade, specific interactions between streptococcal surface structures and plasma proteins of the host have provoked increasing interest. Streptococci of serological groups A, C, G and L interact with the Fc-components of immunoglobulin (Ig) G, showing specific reactivities with IgG subclasses from humans and various animal species (Christensen & Holm, 1976; Grubb et al., 1982; Björck & Kronvall, 1984; Yarnall & Boyle, 1986; Lämmler et al., 1986). Interactions of some streptococci with F(ab)₂-fragments of IgG, IgA and IgD have also been described (Erntell et al., 1983; Russel-Jones et al., 1984; Forsgren & Grubb, 1979). In addition, receptors have been detected for fibrinogen (Hryniewicz et al., 1972; Kronvall et al., 1979a), β₂-microglobulin (Kronvall et al., 1978), α₂-macroglobulin (Müller & Blobel, 1983), fibronectin (Myrhe & Kuusela, 1983), haptoglobin (Köhler & Prokop, 1978) and albumin (Myrhe & Kronvall, 1980). The albumin receptors on streptococci of serological groups A, C and G differ in their binding activities for albumin preparations from humans and various animal species. On this basis five albumin receptor types have been defined (Kronvall et al., 1979b; Widebäck & Kronvall, 1982; Widebäck et al., 1982, 1983). Recently, a protein with albumin-binding activity and a molecular mass of 30 kDa has been isolated from bovine streptococci of serological group G (Widebäck & Kronvall, 1987). Moreover, protein G, the IgG Fc receptor of group C and G streptococci, seems to be responsible for both albumin and IgG binding and these bindings occur at different sites on the protein G molecule (Akerström et al., 1987). These findings led to the present study on binding of plasma proteins to streptococci of serological

**Abbreviation:** ABP, albumin-binding protein.

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group G isolated from dogs (Streptococcus canis) and to the isolation and characterization of surface proteins with albumin-binding activity.

METHODS

Streptococci. A total of 24 streptococcal cultures of serological group G, isolated from dogs, was used. The cultures were characterized biochemically and serologically (Hahn, 1980). Most of them were kindly provided by R. Weiss (Institut für Hygiene und Infektionskrankheiten der Tiere, Giessen, FRG).

Radioiodination of plasma proteins. Human serum albumin (Sigma), human fibrinogen (Deutsche Kabi), human IgG, IgA from human colostrum (Sigma) and pooled human haptoglobin (Behringwerke) were radiolabelled with 125I (Amersham) using the chloramine T method (Hunter & Greenwood, 1962).

Binding assays. These were done in duplicate, with approximately 2 x 10^6 streptococci per assay and 2 x 10^4 to 4 x 10^4 c.p.m. (40 ng) of radiolabelled protein. The variation between duplicate determinations was less than 5%. Binding assays were run for 1 h at 37 °C, if not otherwise stated. The bacteria were then washed in PBS, pH 7.5, containing 0.05% (v/v) Tween 20 (PBST) (Chhatwal et al., 1985) and the radioactivity in the sediment was measured. The amount of radiolabelled protein bound to the bacteria was calculated as a percentage of the total activity used in each binding assay (Chhatwal et al., 1985). The reversibility of albumin binding was determined after 1 h at 22 °C in the presence of 50 μg unlabelled human serum albumin.

Inhibition experiments. Binding of 125I-labelled human albumin was studied in the presence of increasing concentrations of various unlabelled albumin preparations [human, mouse, dog (Sigma) and bovine (Serva)] and in the presence of unlabelled human IgG and human fibrinogen (Chhatwal et al., 1985).

Computer analysis of binding data. Binding data from homologous competition experiments were analysed with the nonlinear least-squares curvefitting program ‘Ligand’ as described by Munson & Rodbard (1980). This program provides bias-free estimation of binding parameters, i.e. dissociation constants (K_D values) and binding-site concentrations based on the solution of the mass-action equations (Feldman, 1972; Feldman et al., 1972). One- or two-binding-site models were assumed and fitted to the data. A decision on the best model was made by means of an F ratio test, which compared the mean square errors in each fit.

Proteolytic enzyme treatments. The streptococci (10^6 bacteria ml^-1) were suspended in 0.05 M-phosphate buffer, pH 7.5, and incubated with increasing concentrations (2-50 μg ml^-1) of trypsin or pronase (Merck) for 1 h at 37 °C. The trypsin reaction was stopped with 50 μg trypsin inhibitor (Bayer). The bacteria were subsequently washed twice in PBST and used in binding assays.

Isolation and detection of albumin binding protein (ABP). Approximately 2 g (wet weight) streptococci were suspended in 10 ml PBS, and the pH of the suspension was adjusted to 7.0 with 5 M-HCl. The suspension was then boiled for 10 min and the pH was adjusted to pH 7.0 (Reis et al., 1984). The extracts were clarified by centrifugation (20 min, 17,300 g) and approximately 10 ml of the supernatant was applied to a column of human albumin (50 mg) coupled to CNBr-activated Sepharose (Pharmacia) previously equilibrated with 0.15 M-NaCl. The column was washed thoroughly with 0.15 M-NaCl and ABP was eluted with 0.1 M-glycine, HCl, pH 7.5. Approximately 200 μg ABP could be obtained from 2 g bacteria (wet weight).

The purified ABP was subjected to SDS-PAGE (Laemmli, 1970) in 7-5% (w/v) polyacrylamide and subsequently transferred electrophoretically onto nitrocellulose for 16 h at 30 V cm^-1 at 7 °C (Burnette, 1981). After treatment with skimed milk to block non-specific binding sites (Lämmler et al., 1986) the nitrocellulose membranes were incubated with 10^6 c.p.m. (1 μg) 125I-labelled albumin for 4 h at 22 °C, washed repeatedly in PBS at pH 7.5, air-dried, and examined by autoradiography. The molecular mass of the protein was estimated by comparing its relative mobility with those of protein standards (Sigma) (Weber & Osborn, 1969).

Immunoelectrophoresis. The purified ABP was preincubated with human albumin (1:1) for 30 min at room temperature. Immunoelectrophoresis was done on microscope slides in 1% (w/v) agarose gel containing 3% (w/v) polyethyleneglycol (Serva) and 0.05% (w/v) NaN_3 (10 V cm^-1, 100 min, 4 °C) with rabbit antiserum raised against human albumin (Behring) (Brückler & Blobel, 1979).

RESULTS

Binding activities of S. canis. All 24 cultures of S. canis bound human albumin, human IgG and human fibrinogen, with mean binding percentages of 76% (range between cultures with highest and lowest binding activity 20-88%), 32% (5-91%) and 47% (6-92%), respectively. No binding activities were observed for human IgA or human haptoglobin [mean binding percentages 6% (2-13%) and 4% (1-17%), respectively].

Specific albumin binding. S. canis strains 3055 and 2875 exhibited high binding activities for human albumin and were therefore selected for further studies. Both strains bound 125I-labelled
Albumin binding to S. canis

Fig. 1. Reversibility of binding of $^{125}$I-labelled human albumin to S. canis strains 3055 (○) and 2875 (●) in the presence of 50 μg unlabelled human albumin.

Table 1. Specificity of binding of $^{125}$I-labelled human albumin to S. canis strains 3055 and 2875

The binding assays were done in the presence of 50 μg of unlabelled competing protein. The results are expressed as percentages of the binding in the absence of competing protein.

<table>
<thead>
<tr>
<th>Competing protein</th>
<th>Strain 3055</th>
<th>Strain 2875</th>
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<tbody>
<tr>
<td>Human albumin</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Mouse albumin</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Dog albumin</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Bovine albumin</td>
<td>74</td>
<td>78</td>
</tr>
<tr>
<td>Rabbit albumin</td>
<td>90</td>
<td>98</td>
</tr>
<tr>
<td>Human IgG</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>Human fibrinogen</td>
<td>97</td>
<td>100</td>
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human albumin in a time-dependent and saturable manner. Binding increased rapidly at 22 °C and reached equilibrium within 10 min. The binding of $^{125}$I-labelled human albumin was reversible in the presence of unlabelled human albumin (Fig. 1). Binding of $^{125}$I-labelled human albumin could be inhibited by unlabelled albumin preparations from humans, dogs and mice; it was weakly inhibited by bovine albumin, but not by rabbit albumin or by IgG and fibrinogen from humans (Table 1).

Data from competition experiments with unlabelled human albumin (Fig. 2) were analysed by a computer program that fitted data to models of multiple binding sites on the basis of nonlinear regression analysis. Best fits were obtained for models of one type of binding site for both S. canis strains. The $K_D$ values for the albumin-binding activities were 10 (SD ± 4.1) nmol l$^{-1}$ for S. canis strain 3055 and 15 (± 2.8) nmol l$^{-1}$ for S. canis strain 2875. The number of albumin-binding sites per bacterial cell was calculated as 30000 (± 3000) and 57000 (± 2400) respectively, on the assumption that one albumin molecule interacted with one receptor molecule. The albumin-binding activity of the streptococci was reduced markedly by pronase and less markedly by trypsin.

Purification. ABP could be readily solubilized by boiling the streptococci at pH 2.0, and was purified by affinity chromatography on albumin-Sepharose. Purified ABP from both S. canis strains inhibited binding of $^{125}$I-labelled albumin to the streptococci (Fig. 3). Both ABP preparations yielded protein bands on SDS-PAGE with almost identical molecular masses of approximately 51 kDa. Upon transfer to nitrocellulose, they displayed identical binding of $^{125}$I-labelled albumin; in addition, low-molecular-mass fragments with $^{125}$I-labelled albumin-binding activity became evident (Fig. 4). Western blots of crude hot acid extracts of five additional S. canis strains yielded almost identical ABPs (Fig. 5). Purified ABP formed complexes with human albumin that had a lower electrophoretic mobility than albumin alone (Fig. 6). Uncomplexed ABP did not react with albumin antiserum.
DISCUSSION

Streptococci of serological group G have long been recognized as a cause of acute human streptococcal infections, of bovine mastitis and of infections of dogs (Ancona et al., 1979; Watts et al., 1984; Biberstein et al., 1980). Group G streptococci from dogs differ from those isolated from humans and have been described as a new species, S. canis (Devriese et al., 1986).

The present studies demonstrated that S. canis exhibits surface structures capable of binding plasma proteins. Most S. canis cultures bound albumin, IgG and fibrinogen from humans. The binding sites of S. canis for fibrinogen are presumed to be similar to the M- or T-protein-like...
Albumin binding to S. canis

Fig. 4. Purified ABP from S. canis strains 3055 (lane A) and 2875 (lane B) stained with Coomassie Blue R. Duplicate gels were blotted and exposed to ¹²⁵I-labelled albumin (strain 3055, lane A'; strain 2875, lane B'). The photograph represents a montage of the Coomassie-stained gels, Western blots and the marker proteins.

Fig. 5. Western blot of crude extracts of S. canis strains 2643 (lane A), 2558 (lane B), 5093 (lane C), 3232 (lane D) and 3247 (lane E) exposed to ¹²⁵I-labelled albumin.

Fig. 6. Precipitin lines of antiserum raised against human albumin with (a) human albumin after prior addition of purified ABP from S. canis strain 3055, and (b) human albumin alone.

Surface structures of group A streptococci (Kantor, 1965; Schmidt & Köhler, 1981). S. canis bound neither human haptoglobin nor IgA. This would indicate a lack of type antigens T4 and M4 (Köhler & Prokop, 1978; Lämmler & Blobel, 1986; Christensen & Oxelius, 1975).

Binding of labelled human albumin to S. canis could be readily inhibited by unlabelled albumin preparations from humans, mice and dogs, and was weakly inhibited by bovine albumin. It was not inhibited by albumin from rabbits, or by IgG and fibrinogen from humans. Comparable inhibition reactions were described for albumin receptors from bovine group G streptococci representing albumin receptor type b (Widebäck et al., 1983). Binding of human albumin to S. canis was time-dependent, saturable and reversible. Trypsin and pronase destroyed the albumin-binding activities of the streptococci, indicating the protein nature of the binding sites. Binding of albumin was apparently not mediated by lipoteichoic acids as proposed by Simpson et al. (1980a, b).
A relationship between albumin binding and streptococcal pathogenicity has not yet been established. However, binding of human albumin to a streptococcal culture of serological group G influenced the surface characteristics of the streptococci as indicated by a change in partition in polymer phase systems (Miörner et al., 1980). Moreover, binding of albumin to streptococci of serological group A enhanced their uptake by polymorphonuclear leucocytes, but not the killing of M-protein-positive cultures (Wagner et al., 1986).

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REFERENCES


