Specific Binding of the Human S Protein (Vitronectin) to Staphylococcus aureus, and Escherichia coli

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Specific binding of the 125I-labeled human S protein (vitronectin) which has been shown to be identical with serum-spreading factor, was observed with group A, C, and G streptococci as well as with Staphylococcus aureus and Escherichia coli. The specific binding of S protein to group A, C, and G streptococci was high, whereas the binding to S. aureus and E. coli cultures was moderate. In contrast, group B streptococci and a number of other bacterial species tested did not interact with S protein. The binding of S protein to bacteria was saturable and could be inhibited only by unlabeled S protein but not by albumin. Trypsinization and heat treatment of bacteria destroyed the S-protein binding capacity for group G streptococci, S. aureus, and E. coli but not for group A and C streptococci. Likewise, unlabeled human fibronectin and heparin inhibited the binding of labeled S protein to group G streptococci, S. aureus, and E. coli, but did not influence the binding to group A and C streptococci. Double-reciprocal plots of S-protein binding to group G streptococci indicated that fibronectin inhibited the binding in a competitive manner, while heparin acts in a noncompetitive manner. Moreover, the binding of S protein to G streptococci could be partially inhibited by the synthetic peptide Gly-Arg-Gly-Asp-Ser, which contains the cell attachment site of S protein. Trypsin-treated S protein had similar binding activity as untreated S protein for group G streptococci, S. aureus, and E. coli, but showed reduced binding to group A and C streptococci. The present data are indicative of two different types of bacterial binding sites in S protein. The binding to group G streptococci, S. aureus, and E. coli is mediated in part through a domain in the S protein containing the sequence Arg-Gly-Asp, whereas a different site is responsible for the binding to group A and C streptococci.

Human S protein exists in its native form as a 4.6S single-chain polypeptide with a molecular weight of about 78,000 and in a proteolyzed form with a molecular weight of about 65,000 (10, 33, 38). The normal concentration of S protein in plasma is between 4 and 6 μM (38). S protein was initially described as an inhibitor of complement-mediated cell lysis (19). As such it blocks bystander cell lysis and membrane insertion of precursor complexes of the membrane attack complex (30–32). Thus the final assembly of the membrane attack complex, which is based on the polymerization of the terminal complement component C9 (49), is blocked by S protein (34), resulting in macromolecular structures lacking the tubular morphology of poly C-9 (37). S protein is also able to bind to enzyme-inhibitor complexes in the terminal stage of the coagulation pathway (17, 29, 39). In addition, it interferes with the thrombin-antithrombin III reaction by counteracting the accelerative effect of heparin in this inhibition, thereby protecting thrombin against fast inactivation (29, 36).

Besides its role in complement-dependent cell lysis and the coagulation system, S protein has been recognized as a promoter of cellular adhesion and spreading of cells (2, 14). It shows a high degree of similarity with respect to structural and functional properties with the serum-spreading factor vitronectin (35, 47), and the amino acid sequences of both proteins share more than 98% homology (18, 45). The effects of S protein (vitronectin) on cell adhesion are similar to those of fibronectin (16, 40, 41), another adhesive protein present in plasma. The two proteins are, however, different by biochemical, immunological, and structural criteria (2, 13, 44). Fibronectin has specific binding sites on staphylococci and streptococci (23, 28, 46) and serves as receptor in the adherence of bacteria to host cells (1, 8, 42, 49, 50). Fibronectin thus plays an important role in the inhibition of bacterial infection. Since the cell attachment properties of fibronectin are comparable to those of S protein, it was of interest to see whether S protein also interacts with bacteria. In the present communication we report the specific binding of S protein to streptococci, Staphylococcus aureus, and Escherichia coli.

MATERIALS AND METHODS

Bacteria. A total of 240 bacterial cultures were included in the present study. Of the streptococcal cultures, 30 belonged to serogroup A, 30 belonged to serogroup B, 45 belonged to serogroup C, and 48 belonged to serogroup G. Other bacterial species were 25 cultures of S. aureus, 10 cultures of Staphylococcus intermedius, 8 cultures of Staphylococcus hyicus, 3 cultures of Staphylococcus epidermidis, 5 cultures of Actinomyces pyogenes, 5 cultures of E. coli, 5 cultures of Pasteurella multocida, 5 cultures of Klebsiella pneumoniae, 4 cultures of Proteus vulgaris, 4 cultures of Enterobacter aerogenes, 5 cultures of Salmonella gallinarum, and 8 cultures of Pseudomonas aeruginosa. All cultures were obtained from the collection of the Institute for Bacteriology, University of Giessen, Giessen, Federal Republic of Germany, and had been identified. The culture medium was Todd-Hewitt broth (GIBCO Europe, Karlsruhe, Federal Republic of Germany) for streptococci and brain heart infusion broth (E. Merck, AG, Darmstadt, Federal Republic of Germany) for other bacteria. The cultures were inoculated in 100 ml of medium in 1-liter Erlenmeyer flasks, and after
incubation on a rotary shaker for 16 to 18 h at 37°C and 60 rpm, the bacteria were sedimented by centrifugation for 20 min at 15,000 × g.

**Purification of S protein.** S protein was purified from platelet-poor human plasma as previously described (38). Briefly, the method consisted of barium citrate adsorption, ammonium sulfate precipitation, chromatography on DEAE-Sephalac and Blue Sepharose, and gel filtration on Sephacryl S-300 (Pharmacia, Upsala Sweden). The yield was approximately 5%, and the purified protein was homogeneous as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (24) and Western blot analysis (48). Antibodies against human S protein were raised in rabbits, and the immunoglobulin G fraction was prepared by standard methods. The rabbit immunoglobulin G fraction against human fibronectin was obtained from Dakopatts, Hamburg, Federal Republic of Germany.

**Radioiodination.** S protein was radioiodinated with ¹²⁵I (carrier free) (New England Nuclear Corp., Dreieich, Federal Republic of Germany) by using the chloramine T method (15). Of the added radioactivity, 78% was incorporated into S protein as determined after precipitation with trichloroacetic acid; the specific activity was 1.4 mCi/mg. Radiolabeled S protein was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (24), and the distribution of radiolabel was detected by autoradiography.

**Binding assay.** The method of Kronvall et al. (21) for the binding assay was used essentially as previously described (9). The bacteria were washed twice in 0.15 M phosphate-buffered saline (pH 7.5) (PBS) and suspended in PBS containing 0.05% Tween 20 (PBS-Tween). The test suspension was adjusted photometrically to 620 nm (Spectronic 20; Bausch and Lomb, Inc. Rochester, N.Y.) to contain approximately 10⁸ bacteria per ml. A 20-μl sample of labeled S protein (2 μg/ml) was added to 0.2 ml of the bacterial suspension, and after incubation for 1 h at room temperature, the bacteria were centrifuged at 10,000 × g and washed with ice-cold PBS-Tween. The radioactivity in the sediment was measured in a y-spectrometer (Packard Instrument Co., Inc., Rockville, Md.). The uptake of radioactivity was expressed as the percentage of the total activity remaining in the pellet. In inhibition experiments, labeled S protein was preincubated with unlabeled S protein, fibronectin (purified by the method of Miekka et al. [25]), serum albumin (Behringwerke, Marburg, Federal Republic of Germany), heparin (Sigma Chemical Co., Munich, Federal Republic of Germany), or synthetic peptide Gly-Arg-Gly-Asp-Ser (Bachem, Basel, Switzerland) before bacterial suspension was added.

**Proteolysis of bacteria.** A 1-ml sample of bacterial suspension (10⁹ bacteria per ml in 0.25 M phosphate buffer (pH 7.5) was incubated for 30 min at 37°C with increasing amounts (2 to 250 μg) of trypsin (Merck). The reaction was stopped by the addition of pancreatic trypsin inhibitor (Bayer, Leverkusen, Federal Republic of Germany) and subsequent washing with PBS-Tween (6). For pronase treatment, 1 ml of bacterial suspension in 0.07 M phosphate buffer (pH 7.4) was incubated for 10 min at 40°C with increasing amounts (2 to 250 μg) of pronase E (Boehringer GmbH, Mannheim, Federal Republic of Germany) and washed three times in 0.07 M phosphate buffer (pH 7.4) (7).

**Heat treatment.** Samples of the bacterial suspension (10⁹ bacteria per ml in PBS) were heated for different periods of between S and 80 min at 80°C. The suspensions were then cooled to room temperature and used for the binding assay.

**Trypsinization of S protein.** Equal volumes of ¹²⁵I-labeled S protein and trypsin-Sepharose were incubated at room temperature for 30 min. Immobilized trypsin was then removed by centrifugation. Under these conditions, the major protein band with Mr 78,000 totally disappeared and a protein band with Mr 35,000, together with additional low-Mr peptides, appeared. This digest was used in binding assays without further separation of peptides.

**TABLE 1. Binding of ¹²⁵I-labeled S protein to various strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of cultures</th>
<th>% Labeled S protein bound*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Positive for S-protein binding</td>
<td>Mean</td>
</tr>
<tr>
<td>Group A streptococci</td>
<td>30</td>
<td>26</td>
</tr>
<tr>
<td>Group B streptococci</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Group C streptococci</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. dysgalactiae</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>S. equisilis</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>S. equi</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Group G streptococci</td>
<td>48</td>
<td>29</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>Staphylococcus intermedius</td>
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<td>Staphylococcus hyicus</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
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<td>0</td>
</tr>
<tr>
<td>Actinomyces pyogenes</td>
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<td>2</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>5</td>
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</tr>
<tr>
<td>Pasteurella multocida</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
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<tr>
<td>Enterobacter aerogenes</td>
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</tr>
<tr>
<td>Salmonella gallinarum</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

* Binding is expressed as the percent uptake of 100 ng of ¹²⁵I-labeled S protein (62,000 cpm) by 2 × 10⁸ bacteria. The values are the means of duplicates for each culture. The mean and range are given only for positive cultures.

* Binding of more than 5% was considered positive.
RESULTS

Characterization of purified S protein. The purified S protein migrated as a single band with an apparent $M_r$ of 74,000 under nonreducing conditions and as a doublet of $M_r$ 78,000 and 65,000 upon reduction (Fig. 1). S protein showed the characteristic doublet when analyzed by immunoblotting with anti-S protein antibodies but did not cross-react with antibodies against fibronectin. The functional properties of S protein were demonstrated by its ability to inhibit complement-dependent lysis, to neutralize the anticoagulant activity of heparin in the thrombin-antithrombin III reaction (38), and to promote attachment of fibroblasts (35).

Binding of S protein to bacteria. Streptococci belonging to serogroups A, C, and G showed a strong binding for $^{125}$I-labeled S protein, with a mean of 42, 46, and 52% of added ligand bound, respectively (Table 1). $S$. aureus and $E$. coli cultures interacted moderately with S protein, whereas only weak binding was observed with some cultures of $A$. pyogenes. Group B streptococcal cultures, as well as other bacterial species, did not interact with labeled S protein (Table 1). The bacterial binding sites for S protein on Streptococcus pyogenes A8189, Streptococcus dysgalactiae Sc1, $S$. aureus H.Mi4, and $E$. coli K-S could be saturated by increasing the concentration of labeled S protein. The binding was reversible, reaching equilibrium within 40 min at 25°C. Increasing the incubation temperature to 37°C did not influence the binding of S protein to bacterial cultures.

Proteolytic and heat sensitivity of binding sites. Two representative cultures each from group A, C, and G streptococci, as well as $S$. aureus and $E$. coli, with a high level of S-protein binding were selected for further characterization of binding. S-protein binding sites on all tested bacteria were sensitive to pronase treatment. Following trypsin treatment of bacteria, however, two types of binding sites were recognized. Binding sites on group A and C streptococci were relatively insensitive to trypsin, whereas those on group G streptococci, $S$. aureus, and $E$. coli were highly sensitive, showing about a 50% loss of binding on addition of 2 µg trypsin and almost complete loss of binding following incubation with

FIG. 2. Effect of trypsinization of bacteria on S-protein binding to group A (○), C (●), and G (□) streptococci, $S$. aureus (△), and $E$. coli (■).

FIG. 3. Effects of heat treatment on S-protein-binding activities of group A (○), C (●), and G (□) streptococci, $S$. aureus (△), and $E$. coli (■).

FIG. 4. Binding of labeled S protein in the presence of unlabeled S protein (●), albumin (△), fibronectin (○), or heparin (□). The values represent means for two cultures of each species. (A) Group A streptococci; (B) group C streptococci; (C) group G streptococci; (D) $E$. coli.

FIG. 5. Double-reciprocal plots of S-protein binding to group G streptococci in the absence (●) or presence of 100 nM fibronectin (○) or heparin (□).
higher trypsin concentrations (Fig. 2). Similar differences were observed with heat-treated bacteria. Heating the bacteria at 80°C for 40 min led to about a 75% loss in the binding of S protein to group G streptococci, *S. aureus*, and *E. coli* cultures. The group A and C streptococcal cultures, however, did not lose their S-protein binding capacity even after prolonged heating at 80°C (Fig. 3).

**Specificity of binding.** Inhibition experiments were conducted to evaluate the specificity of S-protein binding to bacterial cultures. In all cultures to which 100 ng of ¹²⁵I-labeled S protein was added, the binding of radiolabeled S protein was almost completely inhibited by 2 μg of unlabeled S protein, whereas 50 μg of unlabeled albumin was ineffective in displacing labeled S protein (Fig. 4). Likewise, at the same concentration as albumin, other plasma proteins such as immunoglobulin G, α₂-macroglobulin, haptoglobin, β₂-microglobulin, or transferrin did not significantly inhibit S-protein binding.

Binding of labeled S protein in the presence of unlabeled fibronectin and heparin further revealed the existence of two different bacterial binding sites for S protein. Fibronectin, as well as heparin, inhibited the binding of labeled S protein in group G streptococci and *E. coli* cultures but not in group A and C streptococci (Fig. 4). Likewise, fibronectin and heparin moderately inhibited the binding of S protein to *S. aureus* cultures (data not shown). Double-reciprocal plots of ¹²⁵I-labeled S-protein binding to group G streptococcal cultures indicated competitive inhibition by fibronectin, whereas the inhibition due to heparin was noncompetitive (Fig. 5). The binding of S protein to group G streptococci was also partially inhibited by synthetic peptide Gly-Arg-Gly-Asp-Ser. This peptide had no influence on the binding of S protein to group A streptococci (Fig. 6).

**Binding of proteolyzed S protein.** Since S protein could also exist in a proteolyzed form in vivo, we determined the binding of trypsinized ¹²⁵I-labeled S protein to selected strains of different bacterial species. For group A and C streptococcal cultures, the binding of trypsinized S protein was considerably less than that of untreated S protein. For group G streptococci, *S. aureus*, and *E. coli* cultures, however, no such difference was observed (Fig. 7).

**DISCUSSION**

Several interactions of host plasma proteins with certain pathogenic bacteria have been described previously (3, 5, 8, 20, 22, 27). There is evidence that the binding of host proteins to bacteria plays a role in the pathogenicity of the bacteria (3, 51). Fibrinogen binding to streptococci, for example, inhibits the fixation of the third component of human complement (4, 6), the fibronectin has been identified as a mediator for streptococcal adherence to epithelial cells and thus plays a role in the initiation of infection (43). Since S protein plays an important role as an inhibitor of complement-mediated cells lysis (19, 31, 34), as a regulator in the terminal phase of the coagulation system (17, 29, 38, 39), and as a promoter of cellular adhesion (13, 35, 45), its interaction with bacteria might influence the process of infection as well.

The interaction of S protein with bacteria was more pronounced with gram-positive cocci, as was described for other plasma proteins (4, 8). In addition, prominent binding of S protein to *S. aureus*, which has been reported recently (12), and to *E. coli* was found. Since binding of other plasma proteins to *E. coli* is rare, the present findings indicate an interesting feature of our binding studies with S protein. The binding sites for S protein seem to be proteinaceous in nature, as revealed by their pronase sensitivity. The specificity of the binding was established in various inhibition experiments. The present data indicate at least two different bacterial binding sites specific for S protein. The binding sites on group A and C streptococci were insensitive to trypsin and heat treatment and could not be inhibited by fibronectin or heparin. In contrast, those on group G streptococci, *S. aureus*, and *E. coli* were sensitive to trypsin.

![FIG. 6. Binding of labeled S protein to group A (●) and group G (○) streptococci in the presence of the synthetic peptide Gly-Arg-Gly-Asp-Ser (GRGDS).](http://iai.asm.org/)

![FIG. 7. Binding of native □ or trypsinized □ S protein to bacterial species as indicated.](http://iai.asm.org/)
and heat treatment, and the binding of S protein to those cultures could be inhibited in part by fibronectin or heparin. However, the inhibition of S-protein binding by fibronectin was competitive, whereas heparin appeared to partly mask the binding domain of S protein such that binding was prevented in a noncompetitive fashion. A possible binding site in S protein for this type of interaction is the recently identified heparin-binding domain in the carboxy-terminal portion of the protein molecule (44). Support for this interpretation stems from the fact that fibronectin appeared to competitively inhibit S-protein binding and that fibronectin has also been shown to bind to S. aureus (26) and E. coli (11) through its amino-terminal portion, which contains a heparin-binding site. The adherence of S. aureus to immobilized fibronectin, which is not inhibited by S protein (12), seems to be mediated by a different mechanism.

Interestingly, the binding of S protein to group G but not to group A streptococci was partially suppressed by the synthetic peptide Gly-Arg-Gly-Asp-Ser, indicating the involvement of the cell attachment domain of S protein in this interaction. In contrast to the situation with group A streptococci, no loss in binding to group G streptococci, S. aureus, or E. coli was found with proteolyzed S protein in which the cell attachment domain remained intact (unpublished observations).

Our findings are indicative of the expression of at least two binding regions on the S-protein molecule for interaction with certain types of bacteria. One is the cell attachment domain in S protein, which may in part mediate the binding to group G streptococci, S. aureus, and E. coli and thereby facilitate the adherence of bacteria to host cells as recently suggested (12). The other binding region, which is involved in the binding to group A and C streptococci, is different from the former site and has not yet been identified on the S-protein molecule. The present data indicate that in addition to its role as complement inhibitor, S protein may also influence the phagocytic processes during inflammatory reactions. The property of S protein as a promoter of cellular adhesion could bring about the ability of this protein, like that of fibronectin, to act as a mediator between bacteria and the host cells, thus ultimately influencing the colonization of pathogenic bacteria.

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LITERATURE CITED