Thrombospondin Binds to *Staphylococcus aureus* and Promotes Staphylococcal Adherence to Surfaces

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Bacterial adherence to surfaces is the determining first step in staphylococcal infections. Activated platelets mediate adherence of staphylococci to tissues during inflammation or infection; however, the molecular mechanisms of this interaction are not clearly understood. Thrombospondin, a large multifunctional glycoprotein, is the principal platelet-stored glycoprotein. It is secreted upon platelet activation and either bound to receptors on the platelet surface or released and incorporated into blood clots and extracellular matrices. To characterize thrombospondin binding to staphylococci, we incubated [125I]thrombospondin with *Staphylococcus aureus* Cowan 1 in the presence of albumin and separated bound and free thrombospondin by centrifugation. We found that binding was (i) specific, since it was up to 76% inhibitable and up to 60% reversible in the presence of a 100-fold excess of unlabeled thrombospondin, (ii) saturable, with an apparent dissociation constant (K_d) of 5.6×10^{-9} M and a maximal number of 2,600 binding sites per microorganism, and (iii) Ca^{2+} dependent, since omission of this ion from the medium decreased significantly the binding capacity. The binding reaction was insensitive to previous trypsin treatment of bacteria, but it was strongly inhibited in the presence of heparin. Protein A-negative and -positive strains had similar binding characteristics. To determine the promotion of staphylococcal adherence to surfaces by solid-phase thrombospondin, we incubated ³H-labeled *S. aureus* Cowan 1 and 26 pathogenic staphylococcal isolates with thrombospondin-coated polymethylmethacrylate disks and found that adherence was significantly promoted as a function of adsorbed thrombospondin. These results indicate a role for thrombospondin as an important mediator of staphylococcal adherence to activated platelets, to blood clots, or to extracellular matrices in pyogenic infections.

Specific interaction between *Staphylococcus aureus* and host ligands is a prerequisite for bacterial adherence, the pivotal step in tissue colonization and infection. Bacterial adherence to fibrin-platelet matrices has been extensively studied in nonbacterial thrombotic endocarditis (for a review, see reference 49). Platelets have been shown to play a major role in this interaction, since adherence of streptococci (47) and staphylococci (9) to an immobilized fibrin matrix is largely increased even in the presence of small numbers of platelets in the matrix. Furthermore, platelets interact avidly with staphylococci and streptococci, resulting in platelet activation and aggregation (10, 11). However, the components on platelet membranes responsible for the interaction with staphylococci are not yet well defined.

Thrombospondin, a large multifunctional homotrimeric glycoprotein (M_r, 420,000), is the most prevalent glycoprotein stored in α granules of platelets (26). It is secreted upon stimulation (4) and either bound to the platelet surface (37) through a receptor interaction (1, 3, 54) or released and incorporated into fibrin matrices and blood clots (5, 34). These unique features of thrombospondin in platelet function as well as its sequence homologies with other glycoproteins (25) suggest a role for thrombospondin in the adherence of *S. aureus* to platelets or to extracellular matrices. To test this hypothesis, we carried out the following studies to determine the binding characteristics of staphylococci to purified thrombospondin. These studies demonstrate that

fluid-phase thrombospondin interacts with staphylococci specifically and that thrombospondin adsorbed to a solid phase promotes adherence of staphylococci significantly.

MATERIALS AND METHODS

Chemicals and materials. [³H]thymidine and carrier-free [125I]sodium iodide were purchased from Amersham (Buckinghamshire, United Kingdom). Ca^{2+}-repleted thrombospondin was purified from the supernatant of thrombin-activated platelets, as previously described (12). Examination of the purified protein by using 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (21) revealed the expected band at approximately 180 kDa without any significant contamination. The protein was subsequently iodinated with [125I]NaI by using 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (Iodogen; Pierce Chemical Co., Rockford, Ill.) (13) at a ratio of 100 μCi of [125I]NaI per 300 μg of protein and repurified by affinity chromatography with a Sephadex G25 column (Pharmacia, Uppsala, Sweden). The specific activity obtained was 0.6 μCi/μg of thrombospondin. Fibronectin, fibrinogen, and pooled immunoglobulin G (IgG) (Sandoglobulin), kindly provided by the Central Laboratory of the Swiss Red Cross (Bern, Switzerland), were purified as previously described (41, 51). Purified laminin was purchased from Bioreba Diagnostica (Basel, Switzerland), and purified vitronectin was a generous gift from G. S. Chhatwal, Braunschweig, Federal Republic of Germany. Heparin-Ca^{2+} (specific activity, 171 USP U/mg), EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-
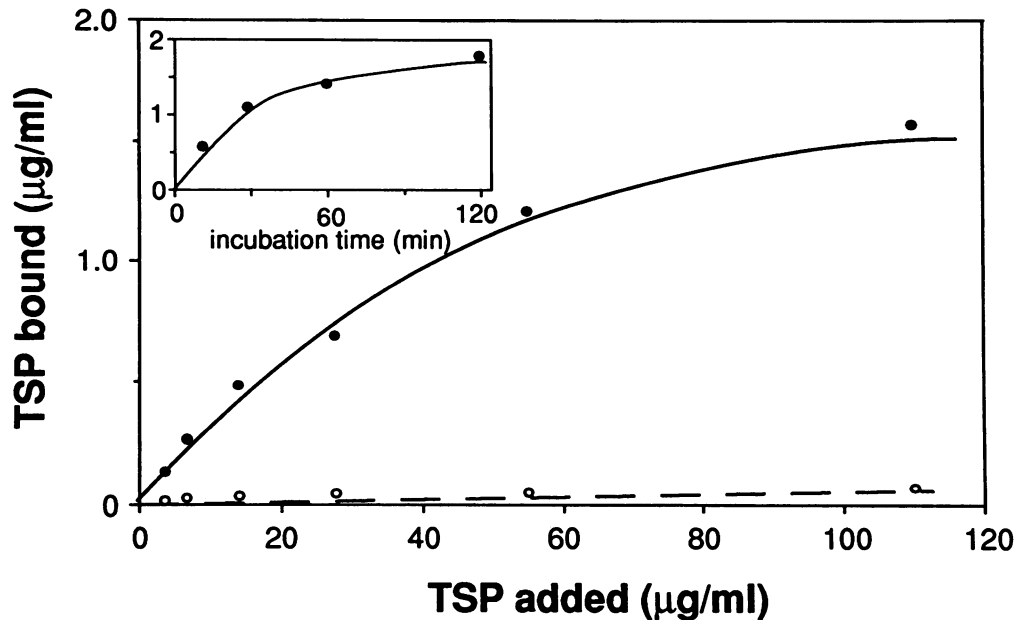
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FIG. 1. Adsorption of [125I]thrombospondin (TSP) to PMMA. PMMA disks were incubated with the indicated concentrations of [125I] thrombospondin for 60 min at 37°C either in the absence (●) or presence (○) of human serum (10%). Subsequently, disks were washed twice and disk-associated radioactivity was determined. Inset, Time course of adsorption of [125I]thrombospondin (25 µg/ml) to PMMA disks.

tetraacetic acid], bovine trypsin, and soybean trypsin inhibitor were purchased from Sigma Chemical Co., St. Louis, Mo. Disks (1 by 1 cm) made of polymethylmethacrylate (PMMA) were cleaned with 100% ethanol and sterilized by heating at 120°C for 30 min. Phosphate-buffered saline (PBS) with 1 mM Ca^{2+} and 0.5 mM Mg^{2+} and without divalent cations (designated as Ca^{2+}/Mg^{2+}-PBS and PBS, respectively) were purchased from GIBCO (Paisley, Scotland).

Bacteria. Experiments were performed with either S. aureus Cowan 1 or a previously described (15) collection of 7 S. aureus and 19 coagulase-negative staphylococcal strains obtained from patients who had bacteremia secondary to intravenous device infection. Bacteria were grown overnight in Mueller-Hinton broth and in some experiments radiolabeled as previously described (52) by incubating 2 × 10⁷ CFU/ml from an overnight culture with [³H]thymidine (50 µCi for 3 h at 37°C).

Binding assay. Binding of thrombospondin to S. aureus was determined by using a modified, previously described (39) assay. Stationary-phase S. aureus Cowan 1 (4 × 10⁸ CFU/ml) was incubated for 120 min at 37°C with various concentrations of [125I]thrombospondin, human serum albumin (0.5%), and Ca^{2+}/Mg^{2+}-PBS (final volume, 0.5 ml). After incubation, bacteria were transferred into Eppendorf tubes and washed twice at 3,000 × g, and the radioactivity in the pellet was determined. In order to determine binding inhibition, bacteria were preincubated (37°C, 30 min) with various concentrations of unlabeled thrombospondin, fibronectin, fibrinogen, or IgG or with heparin. [125I]thrombospondin (2.22 nM) was then added and incubated for 60 min at 37°C. After washing and determination of radioactivity in the pellet, the percentage of binding inhibition was calculated as 100 − (counts per minute [with excess protein]/counts per minute [without excess protein] × 100). Reversibility of binding was determined by incubating Cowan 1 (1 × 10⁹ CFU/ml) with radiolabeled thrombospondin (2.2 nM) for 1 h at 37°C and with subsequent addition of a 100-fold molar

excess of thrombospondin, fibronectin, or fibrinogen or 8 IU of heparin per ml. Aliquots were taken at various time intervals after the addition of competitors, bacteria were washed twice, and bacteria-associated radioactivity was determined. Specificity of the binding was defined according to Limbird (29) as effective inhibition and reversibility of radioligand binding by unlabeled thrombospondin.

Thrombospondin adsorption to PMMA. PBS (100 µl) containing various concentrations of [125I]thrombospondin was deposited on the surface of sterile PMMA disks and was incubated for 60 min at 37°C. Subsequently, disks were washed twice and disk-associated radioactivity was determined. Figure 1 shows the characteristics of [125I]thrombospondin adsorption to PMMA disks. With increasing concentrations of [125I]thrombospondin, higher amounts of the proteins were bound to the surface. Adsorption increased over the whole range of thrombospondin concentrations tested. The addition of 10% human serum blocked adsorption almost completely; similar results were found when purified 0.5% human albumin supplemented the incubation medium (data not shown). Thrombospondin adsorption to the surface was a time-dependent process, with optimal adsorption after 60 min of incubation (Fig. 1, inset).

Staphylococcal adherence to thrombospondin-coated PMMA. We modified a previously described adherence assay (52). One hundred microliters of a solution containing various concentrations of unlabeled thrombospondin was incubated with PMMA disks (1 h, 37°C), and thereafter the disks were washed in PBS. Subsequently, the disks were incubated with 500 µl of a solution containing staphylococci (10⁶ CFU/ml), human serum albumin (0.5%), and Ca^{2+}/Mg^{2+}-PBS in a stirred water bath (60 min, 37°C). Thereafter, fluids were decanted, disks with adherent bacteria were washed twice, and adherent radioactivity was determined. Adherence was calculated as follows: (adherent radioactivity/inoculated radioactivity) × 100. In some instances, the fold increase of adherence promotion by thrombospondin

was calculated as follows: percent thrombospondin adherence/percent albumin adherence (15).

Capsular typing of clinical S. aureus isolates. We modified slightly a previously described method (16). Fresh overnight cultures of S. aureus isolates were inoculated on Columbia medium (Difco Laboratories, Detroit, Mich.) agar supplemented with 2% sodium chloride and grown overnight under 5% CO_2 at 37°C. Cells were suspended with a glass rod in 10 ml of PBS, washed, and resuspended to obtain a concentration of approximately 10^{11} CFU/ml. Purified monoclonal anti-capsular type 5 and type 8 antibodies (kindly provided by CIBA-GEIGY, Basel, Switzerland) and polyclonal pooled human IgG were diluted to obtain a concentration of 20 mg of protein per ml. Subsequently, 30 μl of PBS and 10 μl of either monoclonal antibodies or pooled IgG were added into 96-well microtiter plates (Nunc, Roskilde, Denmark) and stepwise dilutions were prepared. One microliter of the bacterial suspension was subsequently added, and the microtiter plates were incubated at 37°C for 4 h. After incubation, the highest dilution yielding macroscopically visible agglutination was determined for each strain.

Statistics. Two-tailed, unpaired Student's t tests, linear regression analysis, and Mann-Whitney tests for unrelated rankable scores were performed by using a StatView program (Abacus, Berkeley, Calif.).

RESULTS

Characteristics of binding of soluble thrombospondin to S. aureus. Binding assays were performed with S. aureus Cowan 1 and purified ^{125}I-labeled thrombospondin. Binding was increased as a function of thrombospondin concentration in the incubation medium and showed saturable binding characteristics (Fig. 2A). These data were analyzed by using the Scatchard equation (45). The x-axis intercept of the regression line yields the number of thrombospondin molecules bound per bacterium and the slope of the line yields the apparent dissociation constant (K_d). In the presence of Ca^{2+}, Scatchard analysis suggests the presence of two binding sites with different affinities (Fig. 2B). The apparent K_d and the maximal number of binding sites per microorganism, as determined by Scatchard analysis over the linear range at high thrombospondin concentrations, are shown in Table 1. Under Ca^{2+}-free conditions (Fig. 2B and Table 1), the binding capacity of the staphylococcal binding sites was significantly lower when compared with Ca^{2+}-containing conditions ($P < 0.0001$). Trypsin pretreatment of bacteria did influence thrombospondin binding by increasing the K_d approximately twofold when compared with untreated bacteria; the maximal amount of thrombospondin bound was similar and even slightly increased after trypsin treatment (Table 1). The protein A-negative strain, Wood 46, bound thrombospondin with a lower affinity but to a greater extent when compared with Cowan 1 (Table 1).

Binding of thrombospondin to S. aureus increased as a function of time. Binding was half-maximal after 30 min of incubation and reached a plateau at 120 min (Fig. 3A). As the quantity of bacteria was increased, more thrombospondin was bound. A linear relationship between the number of bacteria inoculated and the amount of thrombospondin bound was found with bacterial numbers between 10^7 and 10^9 CFU/ml ($r = 0.98$) (Fig. 3B).

To determine the specificity of the binding reaction, both inhibition and displacement experiments were performed in the presence of unlabeled thrombospondin and other competitors. For inhibition experiments, S. aureus was pretreated

for 30 min with different concentrations of thrombospondin, fibronectin, fibrinogen, or heparin, and subsequently radiolabeled thrombospondin was added for binding. A 100-fold molar excess of unlabeled thrombospondin inhibited the binding to 76.4% ± 11.8% (mean inhibition ± the standard deviation) (Fig. 4A). Furthermore, binding was inhibited up to 56.8% ± 11.0% and 67.2% ± 31.4% by 100-fold molar excesses of fibronectin and fibrinogen, respectively. To determine the relative potency of these various competitors, the EC_{50}, i.e., the concentration of a competitor that reduces by half the specific radioligand binding detected in the absence of competitor, was determined (Fig. 4A). The EC_{50}s in our experiments were <8 nM for thrombospondin, approximately 60 nM for fibrinogen, and >220 nM for fibronectin. This indicates a higher potency of unlabeled thrombospondin when compared with that of fibronectin and fibrinogen in inhibiting binding of the radioligand. The most extensive binding inhibition, however, was observed with heparin: 1.5 μg of heparin per ml (0.26 IU/ml) inhibited 94.1% of the binding of radiolabeled thrombospondin. As a negative control, IgG had no effect on binding, even in 150-fold molar excess. In another approach, we determined the reversibility of the binding reaction. Radiolabeled thrombospondin was allowed to bind to S. aureus Cowan 1, bacteria were washed, and subsequently a 100-fold molar excess of either unlabeled thrombospondin, fibronectin, or fibrinogen or heparin (8 IU/ml) was added. Controls without added competitors were run in parallel. Binding was not reversible in the presence of fibronectin or fibrinogen, whereas unlabeled thrombospondin displaced bound thrombospondin up to approximately 60% and heparin displaced the radioligand to approximately 20% (Fig. 4B).

Promotion of staphylococcal adherence by immobilized thrombospondin. Pretreatment of PMMA disks with soluble thrombospondin promoted adherence of various staphylococcal strains to surfaces. In Fig. 5A, adherence of ^{3}H-labeled S. aureus Cowan 1 is shown as a function of thrombospondin concentration during adsorption of the unlabeled protein to the surfaces. Adherence of S. aureus Cowan 1 to uncoated disks was <0.01% in the presence of 0.5% human serum albumin. In contrast, in the presence of albumin, adherence was significantly promoted by surface-bound thrombospondin as a function of the thrombospondin adsorbed, reaching a plateau when the thrombospondin concentration in the medium was >20 μg/ml. Time courses showed that adherence was optimal after 1 h of incubation (Fig. 5A, inset). At a concentration of 25 μg/ml, thrombospondin promoted adherence of Cowan 1 25-fold when compared with albumin ($P < 0.01$, Mann-Whitney test) (Table 2). The adherence-promoting potency of thrombospondin was comparable to that of laminin, significantly higher when compared with vitronectin, and significantly lower when compared with fibronectin and fibrinogen (Table 2).

In addition to the data obtained with selected laboratory strains, we studied binding characteristics of a variety of clinical staphylococcal strains obtained from the blood of patients with intravenous catheter infections (15). Adherence to uncoated surfaces of S. aureus and coagulase-negative staphylococcal strains was very low (Fig. 5B). In contrast, adherence of all S. aureus strains to thrombospondin-coated disks was significantly increased (median, 60-fold; range, 12- to 91-fold [$P < 0.001$, Mann-Whitney test]). Adherence of a variety of the coagulase-negative strains was also increased but to a lesser extent (median, 14-fold; range, 1 to 55-fold [$P < 0.001$]). The S. aureus strains adhered to a
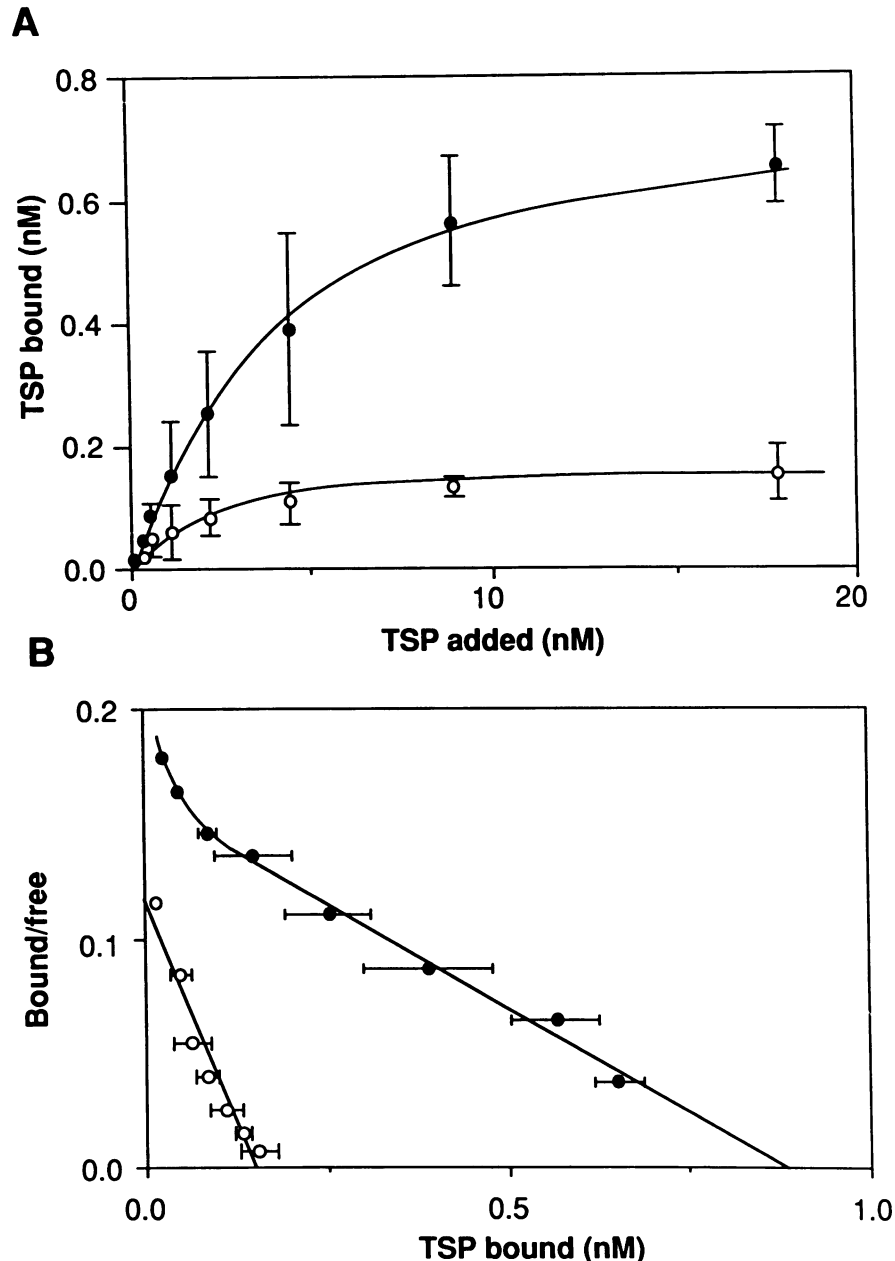
FIG. 2. Binding of [^{125}I]thrombospondin (TSP) to *S. aureus* in the presence and absence of Ca^{2+}. *S. aureus* Cowan 1 (4 × 10^8 CFU/ml) was incubated with various concentrations of ^{125}I-radiolabeled thrombospondin in the presence of albumin either in Ca^{2+}/Mg^{2+}-PBS (●) or in PBS without Ca^{2+} containing 1 mM EGTA (120 min, 37°C) (○) and centrifuged, and radioactivity in the pellet was determined. (A) Quantity of thrombospondin bound as a function of concentration; (B) data plotted by the method of Scatchard. Results are the means of four experiments ± the standard errors of the means.

significantly greater extent to surface-bound thrombospondin when compared with the coagulase-negative strains ($P <$ 0.002, Mann-Whitney test).

Capsular typing of clinical *S. aureus* strains. Since the differences in adherence of the *S. aureus* strains may be due to definable surface characteristics, we determined the presence of two serologically different types of staphylococcal capsules. Table 3 shows the result of a microtiter assay determining agglutination of staphylococci mediated by anti-capsular monoclonal antibodies. All strains tested were not agglutinated in the presence of (control) polyvalent human IgG. Furthermore, the nonencapsulated control strain Wood

46 did not agglutinate in the presence of either monoclonal antibody. In contrast, all the clinical strains were agglutinated by at least one of the monoclonal antibodies, indicating the presence of a microcapsule. However, no significant correlation existed between the presence of one type of the capsule and/or the agglutination titer and the adherence of the strains to thrombospondin-coated disks.

DISCUSSION

S. aureus and coagulase-negative staphylococci are important pathogens in a variety of infections which are

TABLE 1. Effect of calcium ions and trypsin pretreatment on thrombospondin binding to S. aureus Cowan 1 and to the protein A- and capsule-negative strain S. aureus Wood 46

Strain	Condition	Apparent K_d (mean ± SD), nM[a]	No. of molecules/ bacterium (mean ± SD)
Cowan 1	+Ca²⁺	5.55 ± 0.41	2,650 ± 420
	−Ca²⁺, +EGTA	1.30 ± 0.45	450 ± 250
	+Trypsin,[b] +Ca²⁺	9.10	3,780
Wood 46	+Ca²⁺	7.96	4,270

[a] Mean ± standard deviation of four experiments.

[b] Bacteria were pretreated with trypsin (1 mg/ml) for 30 min at 37°C and subsequently with trypsin inhibitor (2 mg/ml) for 15 min and washed. Binding assays were performed as described in Materials and Methods.

frequently associated with a local activation of the clotting system, such as bacterial endocarditis (6, 36) or foreign body infection (17, 53). Staphylococci possess specific binding sites, recognizing a variety of extracellular matrix proteins including fibrinogen (2), fibronectin (19, 39), and laminin (31). A role for these ligands, in particular for fibronectin, in the pathogenesis of staphylococcal infection is now well established in experimental endocarditis (20, 46) and a variety of clinical infections (15, 38).

In contrast to fibrinogen and fibronectin, which are abundant in plasma, plasma concentrations of thrombospondin are very low (7) and the expression in quantity at sites of platelet aggregation is absolutely dependent on platelet activation. However, thrombospondin is not only present on sites of platelet activation but is also a transient component
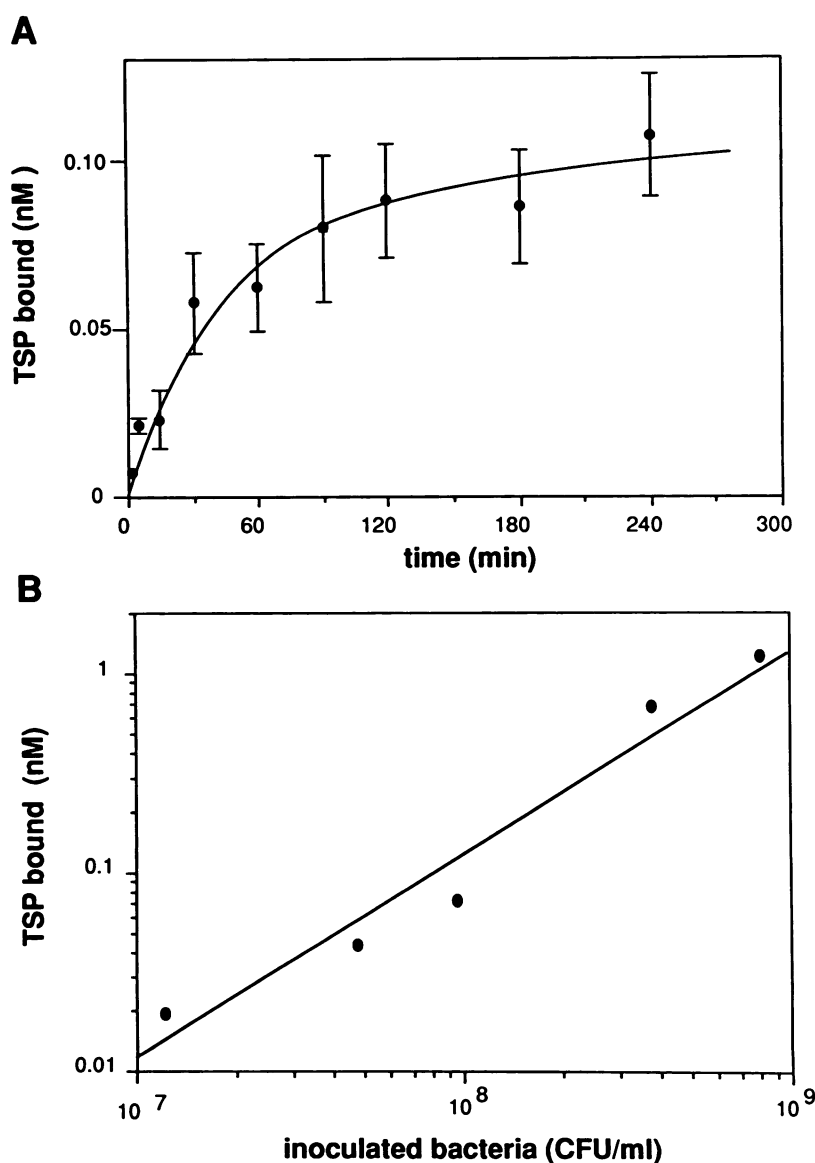

FIG. 3. (A) Time course of [125I]thrombospondin (TSP) binding to S. aureus Cowan 1. Reactions were performed as described in the legend to Fig. 2 at 37°C for various incubation periods with [125I]thrombospondin (1.8 nM), S. aureus Cowan 1 (4 × 10⁸ CFU/ml), and 0.5% albumin in Ca²⁺/Mg²⁺-PBS. Results are means of three experiments ± the standard errors of the means. (B) Relationship between the amount of S. aureus in the reaction mixtures and the total amount of thrombospondin bound. Reaction mixtures containing S. aureus Cowan 1 (10⁷ to 10⁹ CFU/ml) were incubated with [125I]thrombospondin (3.6 nM) in the presence of albumin (120 min, 37°C).
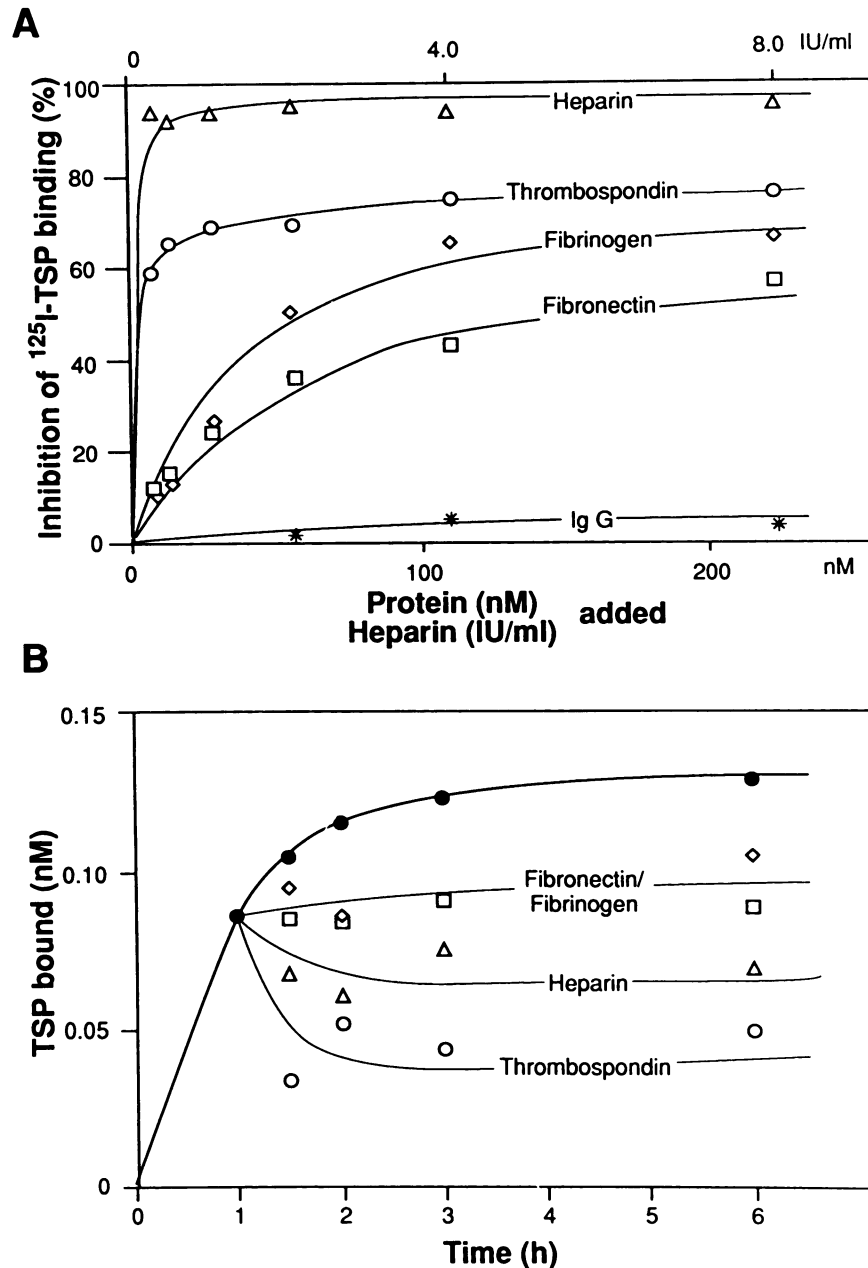
FIG. 4. Inhibition (A) and reversibility (B) of binding of [125I]thrombospondin (TSP) to *S. aureus* Cowan 1 by different proteins and heparin. (A) The indicated concentrations of unlabeled thrombospondin, fibrinogen, fibronectin, IgG, or heparin were incubated with *S. aureus* Cowan 1 in the presence of albumin (30 min, 37°C). Subsequently, [125I]thrombospondin (2.2 nM) was added for 60 min and binding was determined as described in the legend to Fig. 1. Binding inhibition was calculated by comparing the amounts of bound [125I] thrombospondin in the presence and absence of unlabeled protein. Results are means of two experiments. (B) *S. aureus* Cowan 1 was incubated with radiolabeled thrombospondin (2.2 nM) for the indicated time intervals, and the bacterium-associated radioactivity was determined (●). To test reversibility, aliquots were removed after 60 min of incubation, bacteria were washed and subsequently incubated either with unlabeled thrombospondin, fibronectin, or fibrinogen (220 nM) or with heparin (8 IU/ml) for the indicated time periods, and bacterium-associated radioactivity was determined (open symbols).

of the fibrin meshwork in blood clots, synthesized by a large variety of cells, and present in different types of extracellular matrices (for a review, see reference 32). Thus, we performed studies investigating the interaction of staphylococci with thrombospondin both in a soluble phase as well as adsorbed on a solid phase. The binding of *S. aureus* demonstrated the characteristics of a receptor-mediated reaction. Binding was dependent on thrombospondin concentration,

the presence or absence of Ca^{2+}, the incubation time, and the number of microorganisms. Scatchard analysis of the data showed comparable binding affinities at low thrombospondin concentrations both in the presence and in the absence of Ca^{2+}. Ca^{2+} ions exert their principal effect on the binding capacity of the receptor on *S. aureus*: with increasing concentrations of free thrombospondin, increasing amounts of the protein bind to *S. aureus* only in the presence
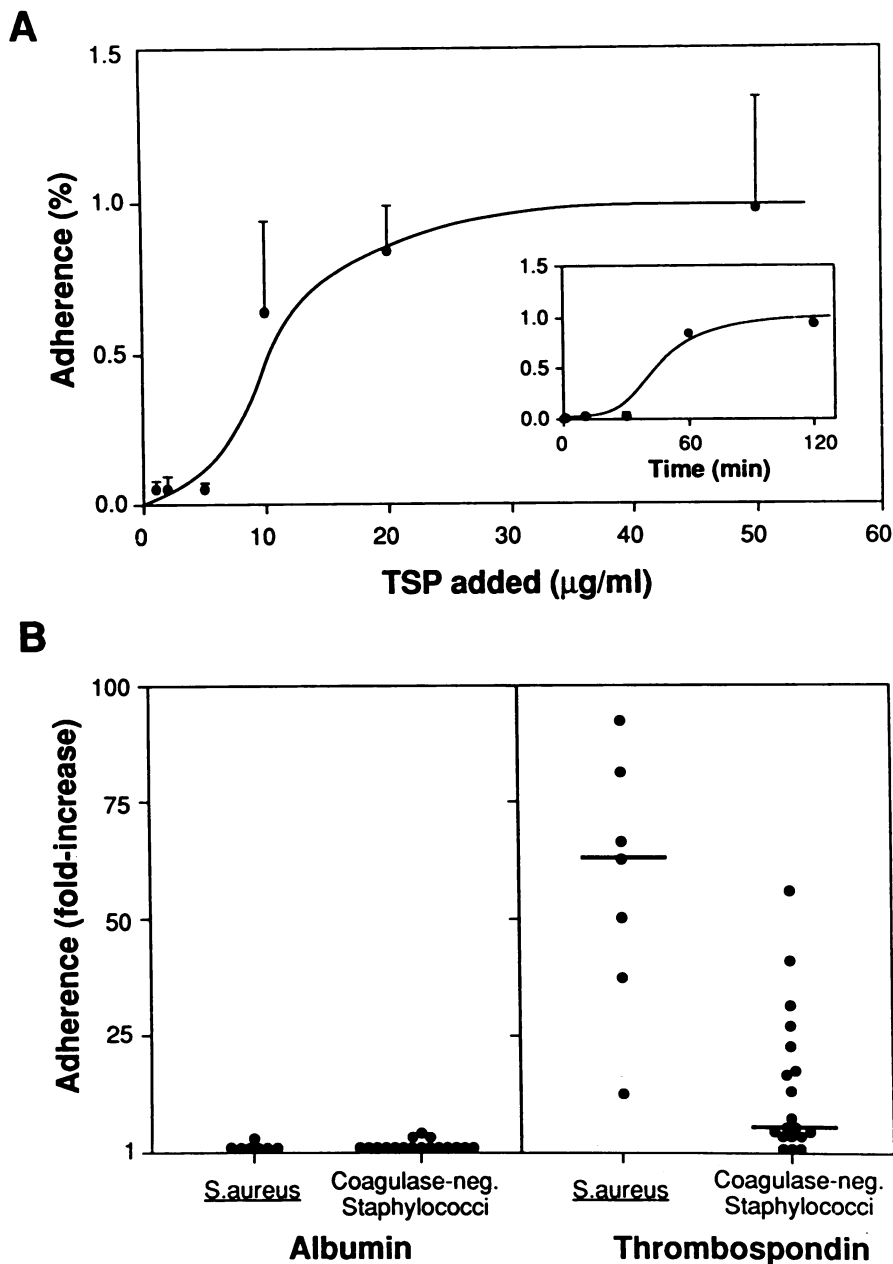
FIG. 5. Adherence of *S. aureus* and coagulase-negative staphylococci to surface-bound thrombospondin. (A) Disks coated with the indicated concentrations of unlabeled thrombospondin (TSP) were incubated with ^{3}H-labeled *S. aureus* Cowan 1 (4 × 10^6 CFU) in the presence of 0.5% albumin (60 min, 37°C) and washed, and adherence was determined as described in Materials and Methods. Results are the means of three experiments performed in triplicate ± the standard errors of the means. Inset, Time course of adherence of *S. aureus* Cowan 1 to thrombospondin-PMMA. Disks were coated with unlabeled thrombospondin (25 μg/ml) and subsequently incubated for the indicated time intervals. (B) Bloodstream isolates of *S. aureus* or coagulase-negative staphylococci (4 × 10^6 CFU/ml) were incubated with either uncoated or thrombospondin-coated (25 μg/ml) disks in the presence of 0.5% albumin (60 min, 37°C), and promotion of adherence was determined as described in Materials and Methods. Results are means of triplicate determinations.

of Ca^{2+}, whereas in the absence of Ca^{2+}, the capacity of the binding sites on staphylococci is significantly lower. This may be due to changes in the thrombospondin molecule or in the receptor on the bacteria or both. Thrombospondin contains a Ca^{2+}-sensitive structure and undergoes conformational changes after the chelating of Ca^{2+} (24). Ca^{2+} has been shown to modulate a variety of thrombospondin functions, including interaction with cells (27) and other proteins

(23). The apparent K_d of 5.6 nM for thrombospondin in the presence of Ca^{2+} was similar when compared with values published for the binding of fibronectin and laminin to *S. aureus* Cowan 1 (31, 39); the number of thrombospondin-binding sites per microorganism was half of the number of fibronectin-binding sites on *S. aureus* Cowan 1 (39) and more than 20-fold larger than the number of laminin-binding sites (31). Time courses showed that binding was maximal after 2

TABLE 2. Promotion of adherence of *S. aureus* Cowan 1
by surface-adsorbed thrombospondin and other
extracellular matrix proteins

Matrix protein	Adherence promotion[a]	
	Fold increase (mean ± SD)	P^b
Thrombospondin	25 ± 15	
Laminin	33 ± 4	NS
Fibrinogen	285 ± 40	<0.0001
Fibronectin	347 ± 29	<0.0001
Vitronectin	6 ± 7	<0.05

[a] Experimental conditions were as described in the legend to Fig. 5B; n = 5. Disks were coated with 25 μg of protein per ml.

[b] Significance level of adherence to thrombospondin compared with adherence to other matrix proteins (Mann-Whitney test).

h of incubation, two- to fourfold longer than reported for fibronectin (39).

Binding of radiolabeled thrombospondin to *S. aureus* was found to be specific, since it was inhibited by unlabeled thrombospondin to a large extent and with a high potency. Similar to results of studies with fibronectin (39) and laminin (31), binding inhibition was not complete even in the presence of high concentrations of unlabeled thrombospondin, probably due to self-association of the competitor molecules (5, 8). Moreover, binding was inhibited by the other glycoproteins, fibronectin and fibrinogen; however, on a molar basis, these proteins were less potent in inhibiting radioligand binding when compared with unlabeled thrombospondin. Since fibronectin and fibrinogen bind to thrombospondin (22, 28), this effect may be due to either direct interaction of these proteins with thrombospondin, competition for the same binding site, or steric hindrance by interaction with different binding sites on staphylococci. Our displacement experiments suggest that the inhibition of thrombospondin binding by fibronectin and fibrinogen is more likely to be due to steric hindrance than to competition, since in contrast to thrombospondin, binding was not reversible after the addition of excess fibronectin and fibrinogen. Heparin binds to the heparin-binding domain of thrombospondin (12, 55), and the binding of thrombospondin to eucaryotic cells is inhibitable as well as displaceable by low doses of heparin (35). The nearly complete inhibition of staphylococcal binding to thrombospondin by heparin may indicate an implication of

the heparin-binding domain in this interaction; however, the interaction of heparin with the thrombospondin-binding site on bacteria cannot be excluded. In contrast to glycoproteins and heparin, IgG failed to inhibit binding. The protein A-negative strain Wood 46 bound thrombospondin to a comparable extent when compared with Cowan 1, and an isogenic protein A-devoid mutant of a protein A-rich *S. aureus* parent strain (kindly provided by Tim Foster, Dublin, Ireland) bound thrombospondin with identical binding patterns as those of the parent strain (data not shown). In contrast to experiments with radiolabeled fibronectin (42) or laminin (31), which showed highly decreased amounts of radioligand bound by staphylococci after trypsin treatment of staphylococci, in our experiments trypsin treatment of bacteria did result in a slightly decreased affinity but did not affect the maximal number of binding sites on *S. aureus*. No clear-cut correlation existed between the presence of a type 5 or type 8 microcapsule, the most prevalent capsular types on clinical *S. aureus* isolates (48), and adherence to thrombospondin-coated coverslips. Moreover, Wood 46, which bound avidly soluble radiolabeled thrombospondin, was shown to be nonencapsulated, as previously described (16). Taken together, these data suggest that the putative binding site for thrombospondin on *S. aureus* is (i) specific for thrombospondin, (ii) different from protein A, (iii) likely to be not of protein nature and (iv) independent of the presence of a microcapsule of the serological type 5 or 8. In addition, our findings suggest that the thrombospondin-binding site is not identical with binding sites previously described for fibronectin (14, 40) and fibrinogen (50). Further studies are necessary to describe in more detail the chemical characterization, function, and localization of this "receptor" on *S. aureus*.

Staphylococci may adhere directly to matrix thrombospondin even in the absence of platelets. Other glycoproteins such as fibronectin bound to surfaces have been shown to undergo conformational changes which may influence their subsequent interactions with cells (30) or with bacteria (52). In our assay, thrombospondin increased adherence of a variety of staphylococcal strains significantly even when adsorbed to solid surfaces, suggesting that the protein is recognizable by bacteria not only in solubilized form but also in its surface-bound conformation. Even if the adherence-promoting potency of surface-bound thrombospondin in vitro is lower when compared with that of fibronectin or fibrinogen, this adhesive protein may play an important role in the promotion of staphylococcal adherence, due to its unique spatial and temporal distribution in blood clots in situ (34).

Our observations allow the following hypothesis. Upon adherence to biological or artificial surfaces, platelets are activated by direct interaction with an artificial surface (44), with fibronectin (43), or with extracellular matrix (18), and they secrete, in addition to other glycoproteins (33), large amounts of thrombospondin. Both *S. aureus* and coagulase-negative staphylococcal strains recognize specifically platelet-bound thrombospondin and adhere at least partly via this ligand to the platelets and/or surrounding surfaces. This hypothesis is supported by preliminary experiments from our laboratory which showed that adherence of *S. aureus* to surface-adherent platelets was significantly inhibited in the presence of soluble thrombospondin and indicated competition of soluble with platelet-bound thrombospondin for binding sites on bacteria (data not shown). The unique role of thrombospondin in platelet function and the presence of this protein in the hemostatic plug suggest a role for thrombo-

TABLE 3. Agglutination reactions between *S. aureus* clinical
strains or Wood 46 and monoclonal anticapsule
antibodies or polyvalent pooled human IgG

Strain	Reciprocal agglutination titer of antibody[a]			Adherence promotion (mean fold increase ± SD)[b]
	Type 5	Type 8	Pooled IgG	
F45	<4	2,048	<4	91 ± 32
G9	512	512	<4	80 ± 25
H5	512	4	<4	64 ± 33
F28	<4	128	<4	60 ± 24
G31	<4	4,096	<4	48 ± 15
I20	<4	1,024	<4	35 ± 12
Wood 46	<4	<4	<4	18 ± 2
E49	2,048	2,048	<4	12 ± 6

[a] Reciprocal of the highest dilution yielding macroscopically visible agglutination.

[b] Determined as described in the legend to Fig. 5.

spondin in the initial adherence step that leads to complex pyogenic processes such as bacterial endocarditis and foreign body infection.

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