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MICROBIAL PATHOGENICITY

# The N-terminal of thrombospondin-1 is essential for coagulase-negative staphylococcal binding

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**Bacterial binding was studied to determine whether thrombospondin-1 (TSP) acts as a ligand in attachment of coagulase-negative staphylococci (CNS). Twenty-five of 27 CNS strains bound soluble TSP. *Staphylococcus epidermidis* J9P bound $^{125}$I-labelled TSP in a dose-dependent manner. Scatchard plot analysis of the binding of TSP by strain J9P revealed two $K_d$ values of $6.4 \times 10^{-9}$ M and $2.9 \times 10^{-8}$ M. The binding structures of strain J9P were sensitive to protease and were resistant to heat treatment. Unlabelled TSP and recombinant von Willebrand factor inhibited binding of TSP by strain J9P, but other proteins or monosaccharides did not. Heparin inhibited binding of TSP to strain J9P and two other *S. epidermidis* strains, BD5703 and BD969. Fusion proteins of the type 1 repeats, type 2 repeats, type 3 repeats and C-terminal domain of TSP or the synthetic Arg-Gly-Asp peptide did not inhibit binding of TSP to bacteria. TSP promoted adhesion of *S. epidermidis* strains when it was immobilised on polymer surfaces. These results indicate that the specific interaction between CNS and TSP may contribute to bacterial adhesion on biomaterial surfaces. The N-terminal heparin-binding domain of TSP appears to be the major region for recognition by CNS.**

## Introduction

Biomaterials such as intravascular catheters, cerebrospinal fluid (CSF) shunts, aortic grafts and prosthetic heart valves have been used increasingly in modern medicine. Complications can arise from these implants, including obstruction of the intraluminal cavity, thrombus formation or embolism within the cardiovascular system, and bacterial infection [1, 2]. Epidemiological studies have revealed that the majority of these biomaterial-associated infections are caused by coagulase-negative staphylococci (CNS), especially *Staphylococcus epidermidis* [3–5]. CNS are microbes of the normal skin flora, and thus have long been regarded as contaminants. They are now recognised as major pathogens of nosocomial septic infections subsequent to implantation of biomaterials or surgical processes which allow penetration of the microbes into the host substrate tissue. Moments after insertion of the device, the biomaterial surface becomes coated with host components. Amongst the components, extracellular matrix and serum proteins have been shown to adsorb to polymeric surfaces [6]. Strains of staphylococci commonly express binding of these proteins, which has been recognised to be important in the establishment of biomaterial infections. CNS have been demonstrated to bind extracellular matrix proteins such as vitronectin (Vn), fibronectin (Fn), transferrin (Tf) and von Willebrand factor [7–9].

Thrombospondin-1 (TSP), a glycoprotein with a molecular mass of *c*. 420 kDa, is found in connective tissues, plasma and in alpha granules of platelets. It is a modular protein composed of three identical subunits, which are covalently linked by interchain disulphide bonds. TSP interacts with several extracellular matrix proteins, glycosaminoglycans, integrins and cell surface receptors including CD36, by which it regulates attachment, proliferation, migration and differentiation of various cell types [10–14]. It has also been shown that bacteria and protozoa such as *Escherichia coli*, enterococci, babesia, *Plasmodium falciparum* and *Porphyromonas gingivalis* bind TSP [15–19]. *S. aureus* has also been shown to bind TSP [20]. There have been a few reports on the binding of TSP by CNS but the mechanism of the interaction has not been demonstrated clearly [9, 20, 21].

This study investigated the binding of TSP in both fluid and solid phases by clinical CNS isolates. To characterise interactions, inhibition by different host proteins, glycosaminoglycans, four fusion proteins corresponding to the different structural domains of the TSP molecule (type 1 repeats, type 2 repeats, type 3 repeats and C-terminal domain) and synthetic Arg-Gly-Asp (RGD) peptide were determined.

## Material and methods

### Chemicals

Human platelet TSP, type 1 repeats, type 2 repeats, type 3 repeats and C-terminal domain were gifts from Professor J. Lawler, Boston, MA, USA. Human recombinant von Willebrand factor (rvWF) and synthetic RGD peptide were gifts from Professor F. Dorner, Vienna, Austria and Dr A. Jonczyk, Merck AG, Darmstadt, Germany, respectively. Fn, Vn and clusterin (Clu) were purified from human plasma as described previously [22–24]. Rabbit antibodies to human TSP were raised as described previously [25]. All common chemicals were purchased from Kebo, Spånga, Sweden, and were of analytical grade. Human fibrinogen (Fg) was purchased from Imco AB, Stockholm, Sweden. Human immunoglobulin G (IgG), Tf, serum albumin, D-mannose, D-fructose, D-galactose, proteinase K, trypsin, chymotrypsin, sodium periodate and hyaluronic acid were from Sigma. Horseradish peroxidase (HRP)-conjugated streptavidin was from Dakopatts AB, Denmark. Heparin sodium salt was from Fluka Chemie AG Neu-Ulm, Buchs, Switzerland. Colour reagent 1,2-phenylediamine was obtained from Abbott Laboratories, Wiesbaden, Germany. $Na^{125}I$ was purchased from Amersham, Little Chalfont, Buckinghamshire. Iodo-beads and EZ-Link-Sulfo-NHS-LC-biotin were from Pierce Chemicals, Rockford, IL, USA. Enzyme-linked immunosorbent assay (ELISA) plates (MaxiSorp F96) were from Nunc[TM], Roskilde, Denmark. Todd-Hewitt (TH) Broth, Tryptic Soy (TS) Broth and Brain Heart Infusion (BHI) Broth were purchased from Difco Laboratories, Detroit, MI, USA. Agar base was purchased from LabM, Salford, and was supplemented with horse erythrocytes 4%.

### Bacterial strains and culture conditions

A total of 31 strains was tested for binding of soluble TSP. Clinical isolates of CNS were from various infections: six from CSF shunt infections, six from endocarditis, three from osteomyelitis, six from graft infections and six from intravenous catheter-related septicaemia. Four *S. aureus* reference strains (Cowan 1, Wood 46, V8 and ISP 546) were analysed [9, 20, 26]. Bacterial cells were grown in TH broth at 37°C for 20–22 h on a gyratory shaker with vigorous agitation, except when different culture conditions were compared. Bacterial cells were washed twice in 0.07 M phosphate-buffered saline (PBS), pH

7.2, containing 0.1 mM $CaCl_2$ and 0.05 mM $MgCl_2$ ($PBS^{2+}$), resuspended in the same buffer to $10^9$ cells/ml and immediately used for various binding assays.

### Binding of soluble TSP and saturation test

TSP was labelled with $Na^{125}I$ according to a modified chloramine-T method with Iodo-beads [21]. The specific activity was $1.2 \times 10^6$ cpm/$\mu$g of TSP. Radiolabelled proteins of 0.1 $\mu$g/ml (c. 30 000 cpm) were incubated with bacterial suspension ($10^8$ cells) in $PBS^{2+}$ containing bovine serum albumin (BSA) 0.1% in a final volume of 200 $\mu$l. For the saturation studies, bacteria were incubated with increasing amounts of TSP, in a mixture of $^{125}I$-labelled and unlabelled TSP in a 200-$\mu$l volume.

### Inhibition assays

Bacteria ($10^8$ cells) were incubated with $^{125}I$-TSP 0.1 $\mu$g/ml in the presence of either increasing amounts of unlabelled TSP (maximum 100 $\mu$g/ml), competing proteins (10 $\mu$g/ml) including Fg, Fn, rvWF, Clu, Vn, Tf, IgG, albumin, 0.1 M monosaccharides (D-mannose, D-fructose, D-galactose), TSP fractions (type 1 repeats, type 2 repeats, type 3 repeats, C-terminal domain) 5 $\mu$g/ml or RGD peptide 5 $\mu$g/ml. Alternatively, heparin 100 $\mu$g/ml or hyaluronic acid 100 $\mu$g/ml were pre-incubated with $^{125}I$-TSP 0.1 $\mu$g/ml for 1 h at 22°C. The final reaction volume was 200 $\mu$l. The binding assay was performed as described above. In a separate experiment, bacteria were incubated with heparin for 30 min at 22°C, and washed twice with $PBS^{2+}$. These treated bacterial cells were subsequently introduced to the binding assay as described above.

### Protease, heat and periodate treatment

Bacteria were incubated with proteinase K 40 $\mu$g, trypsin 100 $\mu$g, chymotrypsin 100 $\mu$g or 100 mM sodium periodate, as described previously [9, 27]. Suspensions of bacterial cells were heated at 100°C for 30 min and rapidly ice-cooled. After treatment, bacteria were washed twice in $PBS^{2+}$ and were tested for $^{125}I$-TSP binding.

### Saturation study of immobilised TSP

Two-fold dilutions of TSP (0–25 $\mu$g/ml) in 100 $\mu$l of $PBS^{2+}$ were added to the wells of ELISA plates and held at 4°C overnight. The ELISA was performed as described previously [9].

### Biotinylation of bacteria

Equal volumes of bacteria ($1 \times 10^8$ cells/ml) and biotin solution (0.2 mg/ml in PBS, pH 7.6) were incubated for 2 h at 22°C. The bacterial cells were then

washed three times and resuspended in $PBS^{2+}$ containing BSA 1% at the original concentration.

## Immobilised TSP binding assay

TSP (5 $\mu$g/ml in $PBS^{2+}$) was immobilised on microtitration plates. The wells were saturated with BSA 1% in PBS and washed three times with PBS. The biotinylated bacterial suspension ($10^8$ cells) was added and the plates were incubated on a platform shaker for 2 h at 22°C. After washing three times with PBS, HRP-conjugated streptavidin diluted 1 in 5000 in PBS was added, incubated for 2 h and then washed three times. The reaction was developed in the dark with phenylediamine 0.4 mg/ml dissolved in 50 mM citric acid buffer, pH 5.0, with $H_2O_2$ 0.006% and terminated with 1 M $H_2SO_4$. The absorbency values at 450 nm were measured in a spectrophotometer (Labsystem Multiskan PLUS, Labsystem OY, Finland). Wells coated with BSA alone served as background. The values of the latter were subtracted from the values of other wells in the experiment before the percentages were calculated.

## Statistical analyses

Two-tailed Student's $t$ test was used; $p < 0.05$ was considered to represent a significant difference.

## Results

### Comparison of culture media and cultivation time

Optimal culture conditions for CNS strains for the expression of TSP binding were determined. *S. epidermidis* H9E, *S. haemolyticus* E2498 and *S. aureus* Cowan 1 were grown on or in blood agar, TH broth, TS broth or BHI broth, and were cultured for 6–48 h. The binding extent of $^{125}$I-TSP of each strain varied according to different culture media and length of cultivation. CNS presented high binding extents when they were cultured for 12–24 h in TH broth or TS broth. *S. haemolyticus* E2498 had the highest binding extent (17.5%) when it was cultured for 24 h in TH broth. *S. epidermidis* H9E reached the highest binding extent (13.5%) when it was grown for 12 h in TH broth, although the difference in the binding extents compared with when it was cultured for 24 h was only 2%. Hence, 20–22 h in TH broth was chosen for the culture conditions for the further binding tests with CNS. The presence or absence of calcium in the incubation buffer (PBS) did not influence the binding of TSP to CNS, although the binding extents were slightly enhanced when $PBS^{2+}$ was used (data not shown).

### Binding of soluble TSP and saturation test

All four strains of *S. aureus* and 25 of 27 CNS strains expressed binding of $^{125}$I-TSP at different percentages from 7 to 40%. Two *S. epidermidis* strains, isolated from endocarditis and intravenous catheter-related sep-

ticaemia, respectively, showed binding of TSP <5%, which is considered negative [16]. Comparison of the TSP binding capacities between CNS strains from different clinical sources revealed that those isolated from CSF shunt infections bound better than those from the endocarditis, osteomyelitis, graft infection and septicaemia groups (Fig. 1).

The specificity of the interaction between CNS and the TSP molecule was determined by the saturability of the binding and the value of the dissociation constant ($K_d$). Binding of *S. epidermidis* J9P, a graft infection isolate, was saturated by addition of TSP within the concentration range tested (Fig. 2). Scatchard plot analysis of the binding data exhibited an upward/biphasic-curve, and two affinity values were obtained ($K_{d1} = 6.4 \times 10^{-9}$ M and $K_{d2} = 2.9 \times 10^{-8}$ M) (Fig. 2, inset).
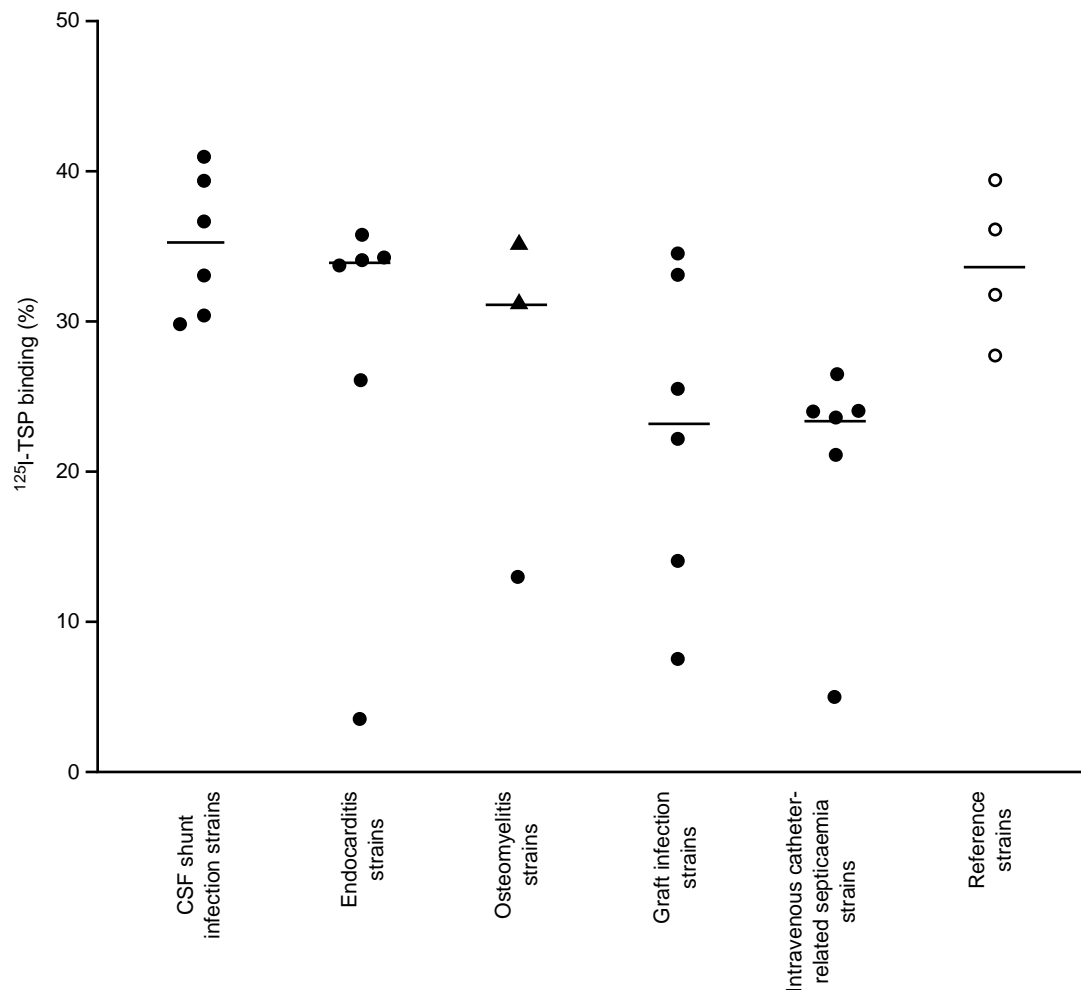
### Protease, heat and periodate treatment

To determine whether protein- or carbohydrate-mediated interaction is involved in the binding of $^{125}$I-TSP, *S. epidermidis* J9P and *S. aureus* Cowan 1 were selected for testing the susceptibility of the binding to protease, heat and periodate treatment. Protease treatment of the cells significantly decreased the binding of TSP by both strains. Heat treatment slightly increased the binding of TSP by strain J9P, and reduced that by strain Cowan 1. Sodium periodate treatment increased the binding of TSP by both strains (Table 1).

### Inhibition experiments

Bacterial receptors capable of binding a broad spectrum of matrix glycoproteins have been reported [28]. To determine the specificity of the binding of TSP to CNS, inhibition experiments were performed in the presence of unlabelled TSP or other putative inhibitors. Unlabelled TSP inhibited binding of $^{125}$I-TSP to *S. epidermidis* J9P in a dose-dependent manner, which decreased by 98% when excess unlabelled protein 100 $\mu$g/ml was used (data not shown). Fg and Fn inhibited binding of $^{125}$I-TSP to *S. aureus* Cowan 1 by 17%, but did not inhibit that to *S. epidermidis* J9P. Human rvWF decreased the binding of TSP to both *S. epidermidis* J9P and *S. aureus* Cowan 1. Clu, Vn, Tf, IgG and albumin did not reduce the binding of TSP to either *S. epidermidis* J9P or *S. aureus* Cowan 1. Fructose and galactose inhibited binding of TSP to *S. aureus* Cowan 1, but mannose did not. The inhibitory effect of the monosaccharides to the binding of TSP to *S. epidermidis* J9P was not significant (Table 2).

To define the domain of TSP that binds to CNS, $^{125}$I-TSP binding was performed in the presence of excess proteins corresponding to the different TSP structural domains, or with heparin, with three *S. epidermidis* strains: BD5703 from CSF shunt infection, BD969 from endocarditis and J9P. The fusion proteins encoding for either the type 1, type 2, type 3 repeats, C-

**Fig. 1.** Binding of ^{125}I-labelled TSP by staphylococcal strains from different origins. The data are presented as mean values. Triplicate samples were tested and repeated twice. SEM = 2.5 (n = 31). Horizontal lines denote median values. *S. epidermidis* (●), *S. haemolyticus* (▲), *S. aureus* (○).

terminal domain, or the synthetic peptide RGD had no inhibitory effect on the bacterial binding of ^{125}I-TSP. Heparin, when incubated with ^{125}I-TSP before the addition of bacteria, reduced the relative binding by ≥88%, but the addition of the same amount of hyaluronic did not influence the binding. However, pre-incubation of heparin with the cells did not affect binding of TSP to either *S. epidermidis* J9P or *S. aureus* Cowan 1 (Table 3).

### Saturation study of TSP to polystyrene (ELISA plate)

The adsorption of TSP to the wells of ELISA plates increased in a dose-dependent manner and reached saturation between 3.125 and 6.25 μg/ml. A concentration of 5 μg/ml in each well was selected for coating.
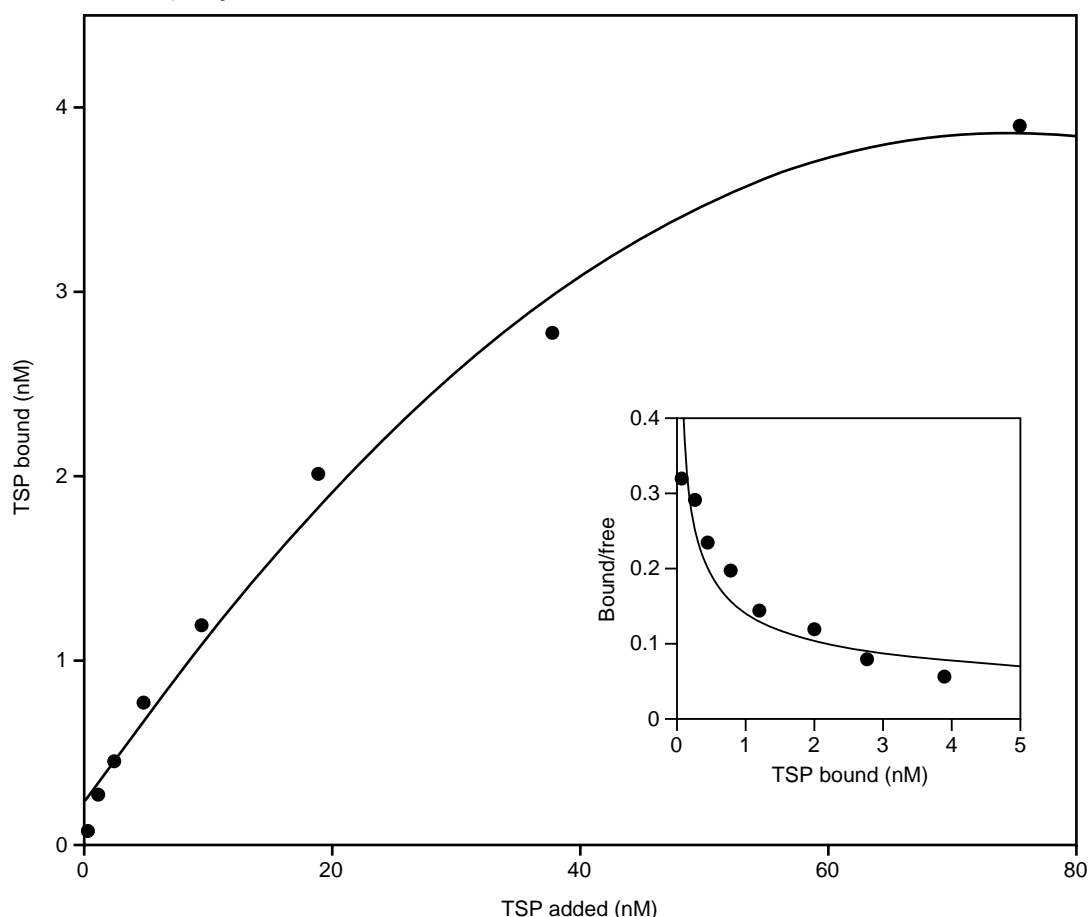
### Adherence to immobilised TSP

The adherence of CNS to surface-bound TSP was determined. Five of six *S. epidermidis* strains expressed binding of immobilised TSP to a greater extent than

that of the negative control *S. haemolyticus* E2498 (Fig. 3).

## Discussion

Although TSP is found only at low concentrations in the plasma, it has been shown to absorb and to accumulate on polymeric surfaces *in vivo*, and *in vitro* subsequent to CSF perfusion [6, 10]. It can also be found locally in increasing amounts upon stimulation of various connective tissue cells and platelets, at sites such as fibrin thrombi, atherosclerotic plaques and open wounds, where infection of CNS may commonly occur [29, 30]. Therefore, it was reasonable to hypothesise that TSP may be a candidate for mediating attachment of CNS to surfaces of prosthetic devices and to damaged host tissues.

Many exoproteins and surface proteins are controlled by the accessory gene regulator system present in both *S. aureus* and *S. epidermidis*, and its expression is affected by the growth phase [31]. The growth conditions also contributed to the optimal expression

**Fig. 2.** Saturability of ^{125}I-TSP binding by *S. epidermidis* J9P. Various amounts of TSP were incubated with $1 \times 10^8$ cells in a 200-$\mu$l volume. Data are presented as mean values of duplicate samples. Inset: Scatchard plot analysis of the data. An upward-curved Scatchard plot represents multiple classes of independent binding sites. Affinity constants are obtained from the data ($Y_1 = -0.157X_1 + 0.324$, $r_1 = 0.986$, $K_{d1} = 6.4 \times 10^{-9}$ M; $Y_2 = -0.034X_2 + 0.185$, $r_2 = 0.987$, $K_{d2} = 2.9 \times 10^{-8}$ M).

**Table 1.** Effect of protease, heat and periodate treatment of *S. epidermidis* J9P and *S. aureus* Cowan 1 on binding of ^{125}I-TSP

| | Mean (SEM) relative binding percentage* | |
|---|---|---|
| Treatment | *S. epidermidis* J9P | *S. aureus* Cowan 1 |
| Non-treated control | 100 (1) | 100 (1) |
| Proteinase K | 23 (5)[‡] | 19 (1)[‡] |
| Trypsin | 33 (2)[‡] | 48 (8)[‡] |
| Chymotrypsin | 50 (7)[‡] | 34 (3)[‡] |
| Heat[†] | 107 (6) | 71 (1)[‡] |
| Sodium periodate | 184 (3)[‡] | 174 (14)[‡] |

*Triplicate samples were tested and repeated twice.
[†]Bacterial cells were heated at 100°C for 30 min and rapidly cooled in an ice bath.
[‡]p <0.01 compared with non-treated control.

of TSP binding of bacteria; >90% of the clinically isolated CNS strains expressed binding of ^{125}I-TSP. Binding of TSP by *S. epidermidis* J9P was dose-dependent, as was inhibition by unlabelled TSP, suggesting a specific adhesion–receptor interaction. In comparison with one previous study, in which *S. aureus* Cowan 1 showed one apparent affinity site in the presence of calcium ions [20], *S. epidermidis* J9P showed at least two affinity binding sites (Scatchard plot analysis), and the binding was not influenced by calcium. The affinity levels of the binding sites indicate a specific interaction between *S. epidermidis* and the TSP molecule. Additional affinity sites may be obtained by saturation with higher concentration of TSP, but these concentrations are difficult to evaluate due to multimeric formation of the TSP molecule. The findings suggest that binding structures of both *S. aureus* Cowan 1 and *S. epidermidis* J9P are protein-aceous, and that those of *S. epidermidis* J9P, but not of *S. aureus* Cowan 1, are heat resistant. The degree of TSP binding by both strains increased after sodium periodate digestion of cell surface glycosylated motifs, suggesting an unmasking of the binding sites [27, 32].

Binding of TSP to *S. epidermidis* J9P and *S. aureus* Cowan 1 decreased in the presence of rvWF. This finding is consistent with the previous observation that the binding of rvWF and TSP is mediated through similar cell surface structures in both CNS and *S. aureus*, and suggests that the inhibitory effect of rvWF may be the result of competition for the same surface receptor [9]. Glycoproteins including Clu, Vn, Tf and

**Table 2.** Influence of different inhibitors on binding of $^{125}$I-TSP to *S. epidermidis* J9P and *S. aureus* Cowan 1

| | Mean (SEM) relative binding percentage* | |
|---|---|---|
| Inhibitors | *S. epidermidis* J9P | *S. aureus* Cowan 1 |
| Control (without inhibitor) | 100 (5) | 100 (4) |
| D-Mannose (0.1 M) | 110 (4) | 97 (7) |
| D-Fructose (0.1 M) | 84 (9) | 74 (3)[†] |
| D-Galactose (0.1 M) | 85 (5) | 70 (2)[†] |
| Fibrinogen (10 $\mu$g/ml) | 95 (4) | 83 (5)[‡] |
| Fibronectin (10 $\mu$g/ml) | 112 (7) | 83 (8) |
| Recombinant von Willebrand factor (10 $\mu$g/ml) | 70 (6)[†] | 76 (5)[†] |
| Clusterin (10 $\mu$g/ml) | 96 (6) | 101 (3) |
| Vitronectin (10 $\mu$g/ml) | 97 (7) | 99 (7) |
| Transferrin (10 $\mu$g/ml) | 112 (5) | 101 (4) |
| Immunoglobulin G (10 $\mu$g/ml) | 113 (4) | 109 (2) |
| Albumin (10 $\mu$g/ml) | 109 (4) | 102 (3) |

*The components were added at the same time as bacteria. Triplicate samples were tested and repeated twice.
[†]$p < 0.01$ compared with control.
[‡]$p < 0.05$ compared with control.

**Table 3.** Inhibitory effect of fusion proteins, synthetic peptide and glycosaminoglycans on binding of $^{125}$I-TSP to *S. epidermidis* strains BD5703, BD969, J9P and *S. aureus* Cowan 1

| | Mean (SEM) relative binding percentage* | | | |
|---|---|---|---|---|
| Inhibitors | *S. epidermidis* BD5703 | *S. epidermidis* BD969 | *S. epidermidis* J9P | *S. aureus* Cowan 1 |
| Control (without inhibitor) | 100 (2) | 102 (5) | 100 (0) | 100 (3) |
| TSP (5 $\mu$g/ml)[†] | 56 (1)[¶] | 70 (2)[¶] | 57 (3)[¶] | 60 (4)[¶] |
| Heparin (100 $\mu$g/ml)[‡] | 6.3 (0.4)[¶] | 12.0 (1.9)[¶] | 7.6 (0.6)[¶] | 9.4 (0.3)[¶] |
| Heparin (100 $\mu$g/ml)[§] | NT | NT | 93 (7) | 107 (8) |
| Hyaluronic acid (100 $\mu$g/ml)[‡][‖] | 93 (2) | 101 (6) | 91 (1) | 93 (2) |
| Type 1 repeats (5 $\mu$g/ml)[†] | 115 (13) | 117 (6) | 103 (2) | 106 (8) |
| Type 2 repeats (5 $\mu$g/ml)[†] | 120 (9) | 121 (10) | 112 (4) | 112 (9) |
| Type 3 repeats (5 $\mu$g/ml)[†] | 120 (4) | 123 (6) | 110 (3) | 114 (8) |
| RGD peptide (5 $\mu$g/ml)[†] | 101 (2) | 116 (4) | 98 (2) | 107 (9) |
| C-terminal domain (5 $\mu$g/ml)[†] | 113 (14) | 127 (5) | 110 (3) | 137 (2) |

*Triplicate samples were tested and repeated twice.
[†]The components were added at the same time as bacteria.
[‡]The components were pre-incubated with $^{125}$I-TSP for 1 h at 22°C, and then bacteria were added.
[§]The component was incubated with bacteria for 30 min at 22°C, washed twice with PBS$^{2+}$ and then $^{125}$I-TSP was added.
[‖]Hyaluronic acid was used as a negatively charged control to heparin.
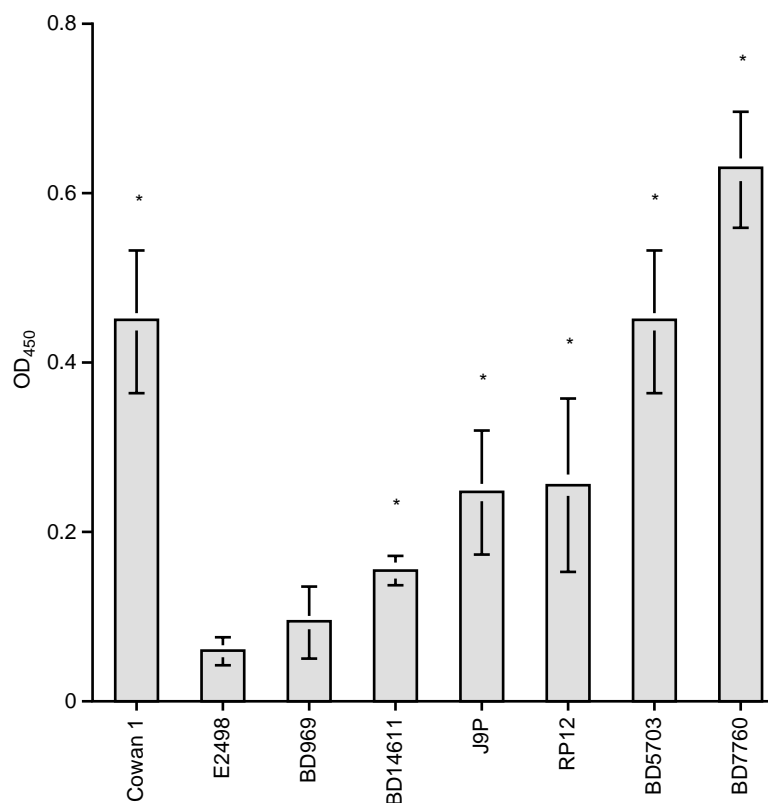[¶]$p < 0.05$ compared with control.
NT, not tested.

IgG did not inhibit binding of TSP by either *S. epidermidis* J9P or *S. aureus* Cowan 1, indicating that the binding molecules on the bacteria for TSP are not identical to those for these glycoproteins [4, 8, 24]. In agreement with the previous observation, Fg and Fn decreased binding of TSP by *S. aureus* Cowan 1 [20] but not that by *S. epidermidis* J9P. This may indicate that the TSP binding structures differ between the two strains. The fact that, except for rvWF, no other tested proteins inhibited binding of TSP to the *S. epidermidis* strain suggests the presence of a specific receptor without broad specificity as previously known for *S. aureus* [20, 28]. Binding of TSP by *S. aureus* Cowan 1 was partially inhibited by fructose and galactose, but not by mannose, similar to the previous studies of TSP binding by enterococci [16]. Despite the fact that the monosaccharides did not inhibit the binding of TSP to *S. epidermidis* J9P, carbohydrate-mediated interaction cannot be excluded, as the biological behaviour of the

carbohydrates may depend on their chain length [11]. *S. epidermidis* strains bound to TSP-coated microtitration wells to varying extents, which suggests that TSP is recognised by bacteria not only in soluble forms but also in its immobilised conformation [33–35]. A 1.4-fold higher binding of TSP in the solid phase to an endocarditis strain BD7760 compared with that to the positive control *S. aureus* Cowan 1 may indicate the expression of a hindered cryptic site by immobilisation of the TSP molecule, which may be relevant in biomaterial infections where host proteins adsorb to the surface.

This study has indicated that the heparin-binding domain of TSP is crucial for the binding to both *S. epidermidis* and *S. aureus* strains. This was shown by the fact that heparin reduced the binding of TSP to staphylococci by almost 90%. The same amount of hyaluronic acid did not inhibit the binding, which

**Fig. 3.** Adherence of *S. epidermidis* strains to TSP-coated wells (5 $\mu$g/ml). *p <0.05 compared with negative control *S. haemolyticus* E2489. *S. aureus* Cowan 1 was used as positive control. Data are presented as mean values (SEM). Triplicate samples were tested and repeated twice.

indicated that the interaction was not inhibited by the negative charge alone [11]. The possibility that heparin interfered with the binding molecules on the bacteria may be less likely, because TSP binding by bacteria was not reduced when bacteria were treated with heparin before the addition of TSP, which may be explained by the relatively low affinity of glycosaminoglycans for staphylococci reported previously [26]. The TSP molecule contains multiple glycosaminoglycan-binding sequences in the N-terminal domain and the type 1 repeats [34, 36]. As the fusion proteins reflecting the type 1, type 2, type 3 repeats and C-terminal domain of the TSP molecule and the RGD peptide did not inhibit binding of radiolabelled TSP to bacteria, these domains are less likely to be involved. The results indicate that the N-terminal heparin-binding domain is essential for staphylococcal binding.

The present results suggest that the recognition determinants for both bacteria and for glycosaminoglycans reside within the same N-terminal domain. By attachment to the glycosaminoglycans in the extracellular matrices, the N-terminal of TSP may contribute to a co-operative role in mediating incorporation of the bacteria into the subendothelium of damaged tissues and on biomaterial surfaces. Further studies to specify the sequences that mimic the binding capacity will presumably give insight into the bacterial interactions with TSP.

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