



LUND UNIVERSITY

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

Yanagisawa, Naoko; Li, Dai-Qing; Ljungh, Åsa

Published in:
Journal of Medical Microbiology

2001

[Link to publication](#)

Citation for published version (APA):
Yanagisawa, N., Li, D-Q., & Ljungh, Å. (2001). The N-terminal of thrombospondin-1 is essential for coagulase-negative staphylococcal binding. *Journal of Medical Microbiology*, 50(8), 712-719.
<http://jmm.sgmjournals.org/cgi/content/abstract/50/8/712>

Total number of authors:
3

General rights

Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

MICROBIAL PATHOGENICITY

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

NAOKO YANAGISAWA, DAI-QING LI and ÅSA LJUNGH

Department of Medical Microbiology, Dermatology and Infection, Lund University, Lund, Sweden

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 ¹²⁵I-labelled TSP in a dose-dependent manner. Scatchard plot analysis of the binding of TSP by strain J9P revealed two K_d values of 6.4×10^{-9} M and 2.9×10^{-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].

Received 23 Nov. 2000; revised version accepted 17 Jan. 2001.

Corresponding author: Professor Å. Ljungh (e-mail: asa.ljungh@mmb.lu.se).

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-phenylenediamine was obtained from Abbott Laboratories, Wiesbaden, Germany. Na¹²⁵I 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 NuncTM, 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 CaCl₂ and 0.05 mM MgCl₂ (PBS²⁺), resuspended in the same buffer to 10⁹ cells/ml and immediately used for various binding assays.

Binding of soluble TSP and saturation test

TSP was labelled with Na¹²⁵I according to a modified chloramine-T method with Iodo-beads [21]. The specific activity was 1.2 × 10⁶ cpm/μg of TSP. Radio-labelled proteins of 0.1 μg/ml (c. 30 000 cpm) were incubated with bacterial suspension (10⁸ cells) in PBS²⁺ containing bovine serum albumin (BSA) 0.1% in a final volume of 200 μl. For the saturation studies, bacteria were incubated with increasing amounts of TSP, in a mixture of ¹²⁵I-labelled and unlabelled TSP in a 200-μl volume.

Inhibition assays

Bacteria (10⁸ cells) were incubated with ¹²⁵I-TSP 0.1 μg/ml in the presence of either increasing amounts of unlabelled TSP (maximum 100 μg/ml), competing proteins (10 μg/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 μg/ml or RGD peptide 5 μg/ml. Alternatively, heparin 100 μg/ml or hyaluronic acid 100 μg/ml were pre-incubated with ¹²⁵I-TSP 0.1 μg/ml for 1 h at 22°C. The final reaction volume was 200 μl. 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 PBS²⁺. 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 μg, trypsin 100 μg, chymotrypsin 100 μg 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 PBS²⁺ and were tested for ¹²⁵I-TSP binding.

Saturation study of immobilised TSP

Two-fold dilutions of TSP (0–25 μg/ml) in 100 μl of PBS²⁺ 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 × 10⁸ 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 PBS²⁺ containing BSA 1% at the original concentration.

Immobilised TSP binding assay

TSP (5 µg/ml in PBS²⁺) was immobilised on microtitration plates. The wells were saturated with BSA 1% in PBS and washed three times with PBS. The biotinylated bacterial suspension (10⁸ 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 phenyldiamine 0.4 mg/ml dissolved in 50 mM citric acid buffer, pH 5.0, with H₂O₂ 0.006% and terminated with 1 M H₂SO₄. 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 ¹²⁵I-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 PBS²⁺ 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 ¹²⁵I-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 × 10⁻⁹ M and *K_{d2}* = 2.9 × 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 ¹²⁵I-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 ¹²⁵I-TSP to *S. epidermidis* J9P in a dose-dependent manner, which decreased by 98% when excess unlabelled protein 100 µg/ml was used (data not shown). Fg and Fn inhibited binding of ¹²⁵I-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, ¹²⁵I-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-

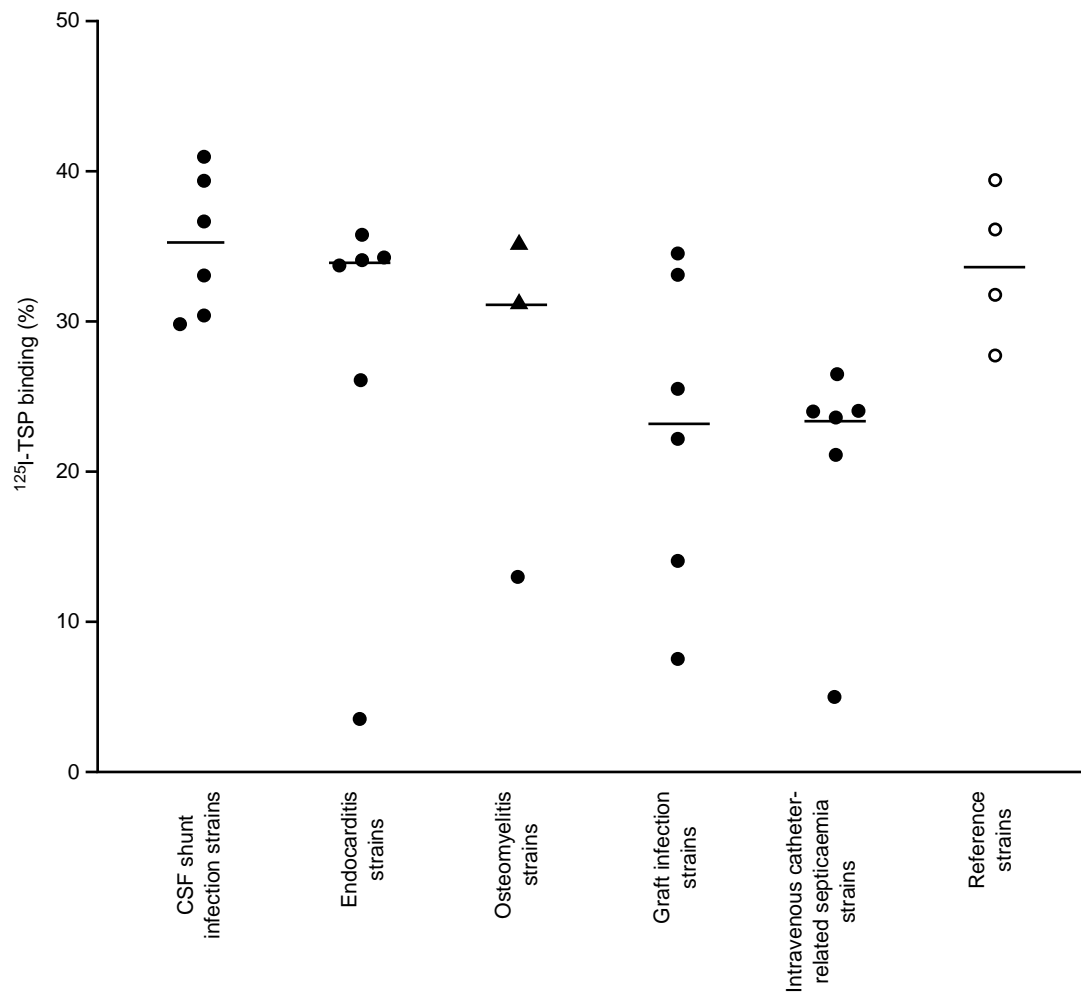


Fig. 1. Binding of ^{125}I -labelled TSP by staphylococcal strains from different origins. The data are presented as mean values. Triplicate samples were tested and repeated twice. SEM = 2.5 ($n = 31$). Horizontal lines denote median values. *S. epidermidis* (●), *S. haemolyticus* (▲), *S. aureus* (○).

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 $\geq 88\%$, but the addition of the same amount of hyaluronic acid 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 $\mu\text{g}/\text{ml}$. A concentration of 5 $\mu\text{g}/\text{ml}$ 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 adsorb 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

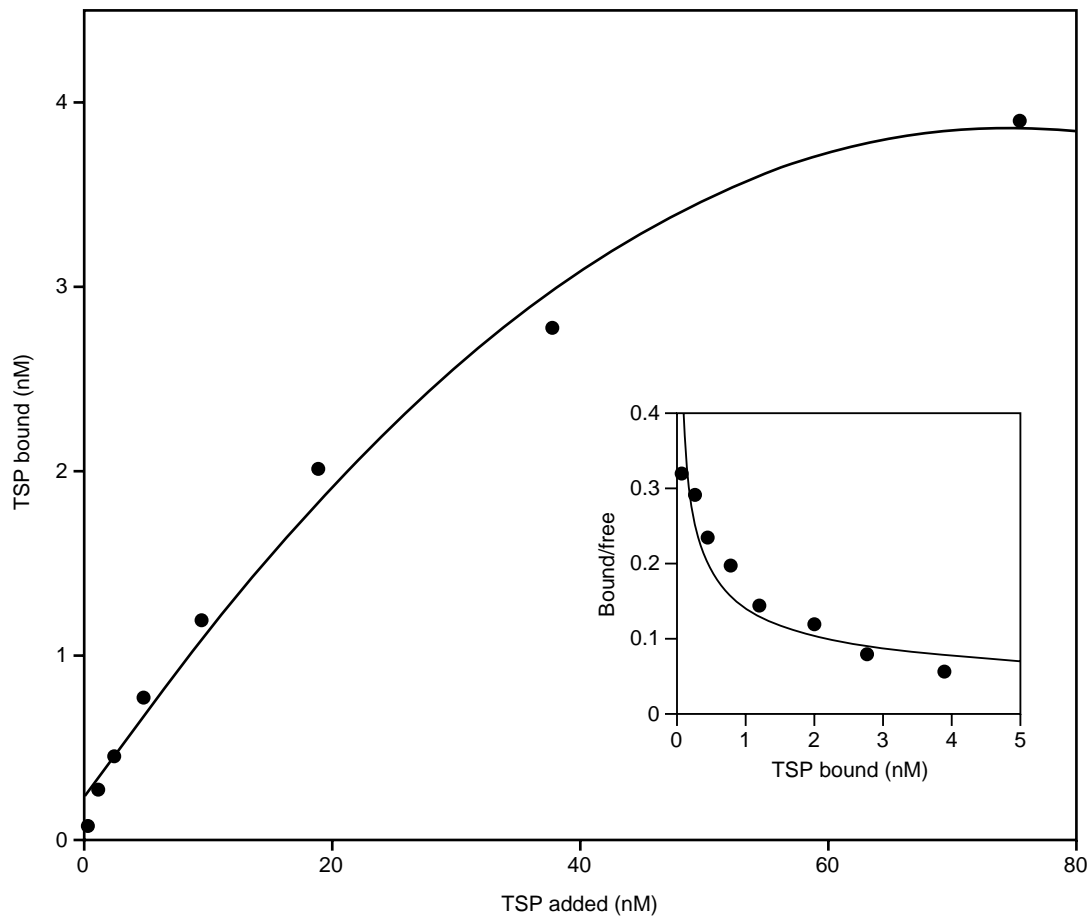


Fig. 2. Saturability of ^{125}I -TSP binding by *S. epidermidis* J9P. Various amounts of TSP were incubated with 1×10^8 cells in a 200- μl volume. Data are presented as mean values of duplicate samples. Inset: Scatchard plot analysis of the data. An upward-curved Scatchard plot represents multiple classes 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

| Treatment | Mean (SEM) relative binding percentage* | |
|---------------------|---|--------------------------|
| | <i>S. epidermidis</i> J9P | <i>S. aureus</i> 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 proteinaceous, 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 ¹²⁵I-TSP to *S. epidermidis* J9P and *S. aureus* Cowan 1

| Inhibitors | Mean (SEM) relative binding percentage* | |
|--|---|-----------------------------|
| | <i>S. epidermidis</i> J9P | <i>S. aureus</i> 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 µg/ml) | 95 (4) | 83 (5) [‡] |
| Fibronectin (10 µg/ml) | 112 (7) | 83 (8) |
| Recombinant von Willebrand factor (10 µg/ml) | 70 (6) [†] | 76 (5) [†] |
| Clusterin (10 µg/ml) | 96 (6) | 101 (3) |
| Vitronectin (10 µg/ml) | 97 (7) | 99 (7) |
| Transferrin (10 µg/ml) | 112 (5) | 101 (4) |
| Immunoglobulin G (10 µg/ml) | 113 (4) | 109 (2) |
| Albumin (10 µg/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 ¹²⁵I-TSP to *S. epidermidis* strains BD5703, BD969, J9P and *S. aureus* Cowan 1

| Inhibitors | Mean (SEM) relative binding percentage* | | | |
|---|---|--------------------------------|------------------------------|-----------------------------|
| | <i>S. epidermidis</i> BD5703 | <i>S. epidermidis</i> BD969 | <i>S. epidermidis</i> J9P | <i>S. aureus</i> Cowan 1 |
| Control (without inhibitor) | 100 (2) | 102 (5) | 100 (0) | 100 (3) |
| TSP (5 µg/ml) [†] | 56 (1) [¶] | 70 (2) [¶] | 57 (3) [¶] | 60 (4) [¶] |
| Heparin (100 µg/ml) [‡] | 6.3 (0.4) [¶] | 12.0 (1.9) [¶] | 7.6 (0.6) [¶] | 9.4 (0.3) [¶] |
| Heparin (100 µg/ml) [§] | NT | NT | 93 (7) | 107 (8) |
| Hyaluronic acid (100 µg/ml) | 93 (2) | 101 (6) | 91 (1) | 93 (2) |
| Type 1 repeats (5 µg/ml) [†] | 115 (13) | 117 (6) | 103 (2) | 106 (8) |
| Type 2 repeats (5 µg/ml) [†] | 120 (9) | 121 (10) | 112 (4) | 112 (9) |
| Type 3 repeats (5 µg/ml) [†] | 120 (4) | 123 (6) | 110 (3) | 114 (8) |
| RGD peptide (5 µg/ml) [†] | 101 (2) | 116 (4) | 98 (2) | 107 (9) |
| C-terminal domain (5 µg/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 ¹²⁵I-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 PBS²⁺ and then ¹²⁵I-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 bio-material 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

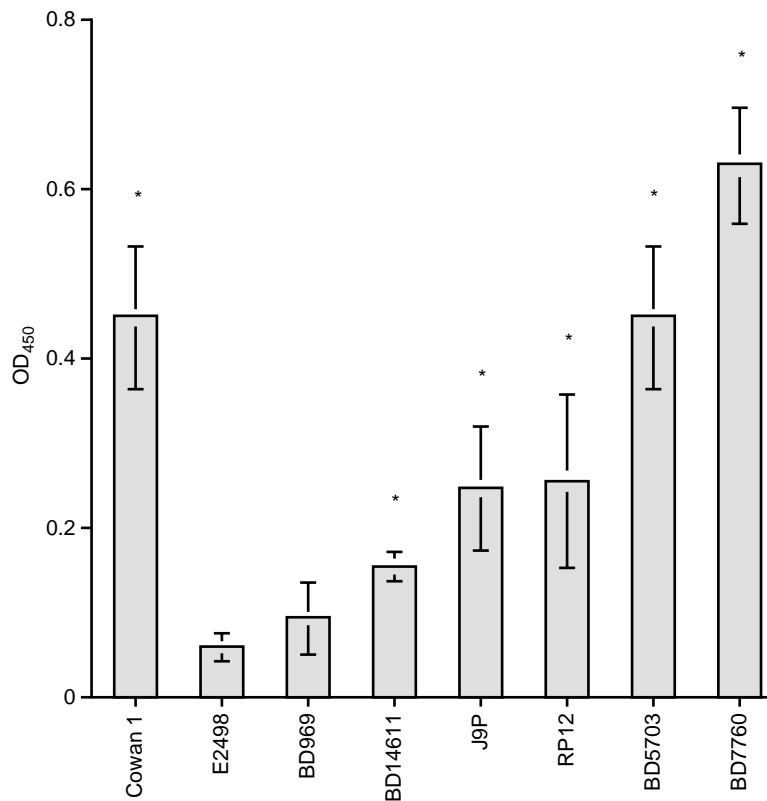


Fig. 3. Adherence of *S. epidermidis* strains to TSP-coated wells (5 µg/ml). **p* < 0.05 compared with negative control *S. haemolyticus* E2489. *S. aureus* Cowan 1 was used as positive control. Data are presented as mean values (SEM). Triplicate samples were tested and repeated twice.

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.

This study was supported by grants from the Swedish Medical Research Council (06×11229), the Board for Technical Development (NUTEK) and the Faculty of Medicine, University of Lund.

References

- Kang JK, Lee IW. Long-term follow-up of shunting therapy. *Childs Nerv Syst* 1999; **15**: 711–717.
- Rubin BG, Santoro SA, Sicard GA. Platelet interactions with the vessel wall and prosthetic grafts. *Ann Vasc Surg* 1993; **7**: 200–207.
- Bandyk DF. Diagnosis and treatment of biomaterial-associated vascular infections. *Infect Dis Clin North Am* 1992; **6**: 719–729.
- Ljungh Å, Wadström T. Interactions of bacterial adhesins with the extracellular matrix. *Adv Exp Med Biol* 1996; **408**: 129–140.
- von Eiff C, Heilmann C, Peters G. New aspects in the molecular basis of polymer-associated infections due to staphylococci. *Eur J Clin Microbiol Infect Dis* 1999; **18**: 843–846.
- Lundberg F, Tegenfeldt JO, Montelius L *et al.* Protein depositions on one hydrocephalus shunt and on fifteen temporary ventricular catheters. *Acta Neurochir* 1997; **139**: 734–742.
- Allignet J, Galdbart J-O, Morvan A *et al.* Tracking adhesion factors in *Staphylococcus caprae* strains responsible for human bone infections following implantation of orthopaedic material. *Microbiology* 1999; **145**: 2033–2042.
- Modun B, Williams P. The staphylococcal transferrin-binding protein is a cell wall glyceraldehyde-3-phosphate dehydrogenase. *Infect Immun* 1999; **67**: 1086–1092.
- Li D-Q, Lundberg F, Ljungh Å. Binding of von Willebrand factor by coagulase-negative staphylococci. *J Med Microbiol* 2000; **49**: 217–225.
- Bornstein P, Sage EH. Thrombospondins. *Methods Enzymol* 1994; **245**: 62–85.

11. Yu H, Tyrrell D, Cashel J *et al.* Specificities of heparin-binding sites from the amino-terminus and type 1 repeats of thrombospondin-1. *Arch Biochem Biophys* 2000; **374**:13–23.
12. Barabino GA, Wise RJ, Woodbury VA *et al.* Inhibition of sickle erythrocyte adhesion to immobilized thrombospondin by von Willebrand factor under dynamic flow conditions. *Blood* 1997; **89**:2560–2567.
13. Mikhailenko I, Krylov D, Argraves KM, Roberts DD, Liao G, Strickland DK. Cellular internalization and degradation of thrombospondin-1 is mediated by the amino-terminal heparin binding domain (HBD). High affinity interaction of dimeric HBD with the low density lipoprotein receptor-related protein. *J Biol Chem* 1997; **272**: 6784–6791.
14. Clezardin P, Lawler J, Amiral J, Quentin G, Delmas P. Identification of cell adhesive active sites in the N-terminal domain of thrombosporin-1. *Biochim J* 1997; **321**:819–827.
15. Shen W, Steinrück H, Ljungh Å. Expression of binding of plasminogen, thrombospondin, vitronectin, and fibrinogen, and adhesive properties by *Escherichia coli* strains isolated from patients with colonic diseases. *Gut* 1995; **36**: 401–406.
16. Zareba TW, Pascu C, Hryniewicz W, Wadström T. Binding of extracellular matrix proteins by enterococci. *Curr Microbiol* 1997; **34**: 6–11.
17. Parrodi F, Wright IG, Kerr JD, Dobson C. In vitro adherence of erythrocytes infected with *Babesia bigemina* and *Babesia rodhaini* to thrombospondin. *Int J Parasitol* 1990; **20**: 899–903.
18. Urban BC, Ferguson DJP, Pain A *et al.* *Plasmodium falciparum*-infected erythrocytes modulate the maturation of dendritic cells. *Nature* 1999; **400**: 73–77.
19. Nakamura T, Amano A, Nakagawa I, Hamada S. Specific interactions between *Porphyromonas gingivalis* fimbriae and human extracellular matrix proteins. *FEMS Microbiol Lett* 1999; **175**: 267–272.
20. Herrmann M, Suchard SJ, Boxer LA, Waldvogel FA, Lew PD. Thrombospondin binds to *Staphylococcus aureus* and promotes staphylococcal adherence to surfaces. *Infect Immun* 1991; **59**: 279–288.
21. Paulsson M, Petersson A-C, Ljungh Å. Serum and tissue protein binding and cell surface properties of *Staphylococcus lugdunensis*. *J Med Microbiol* 1993; **38**: 96–102.
22. Vuento M, Vaheri A. Purification of fibronectin from human plasma by affinity chromatography under non-denaturing conditions. *Biochem J* 1979; **183**: 331–337.
23. Yatohgo T, Izumi M, Kashiwagi H, Hayashi M. Novel purification of vitronectin from human plasma by heparin affinity chromatography. *Cell Struct Funct* 1988; **13**: 281–292.
24. Partridge SR, Baker MS, Walker MJ, Wilson MR. Clusterin, a putative complement regulator, binds to the cell surface of *Staphylococcus aureus* clinical isolates. *Infect Immun* 1996; **64**: 4324–4329.
25. Zardi L, Siri A, Carnemolla B, Cosulich E, Viale G, Santi L. A simplified procedure for the preparation of antibodies to serum fibronectin. *J Immunol Methods* 1980; **34**: 155–165.
26. Liang OD, Ascencio F, Fransson L-Å, Wadström T. Binding of heparan sulfate to *Staphylococcus aureus*. *Infect Immun* 1992; **60**: 899–906.
27. Wisniowski P, Martin WJ. Interaction of vitronectin with *Pneumocystis carinii*: evidence for binding via the heparin binding domain. *J Lab Clin Med* 1995; **125**: 38–45.
28. McGavin MH, Krajewska-Pietrasik D, Rydén C, Höök M. Identification of a *Staphylococcus aureus* extracellular matrix-binding protein with broad specificity. *Infect Immun* 1993; **61**: 2479–2485.
29. Riessen R, Kearney M, Lawler J, Isner JM. Immunolocalization of thrombospondin-1 in human atherosclerotic and restenotic arteries. *Am Heart J* 1998; **135**: 357–364.
30. Reed MJ, Puolakkainen P, Lane TF, Dickerson D, Bornstein P, Sage EH. Differential expression of SPARC and thrombospondin 1 in wound repair: immunolocalization and in situ hybridization. *J Histochem Cytochem* 1993; **41**: 1467–1477.
31. Vuong C, Götz F, Otto M. Construction and characterization of an *agr* deletion mutant of *Staphylococcus epidermidis*. *Infect Immun* 2000; **68**: 1048–1053.
32. Mamo W, Froman G, Wadström T. Interaction of sub-epithelial connective tissue components with *Staphylococcus aureus* and coagulase-negative staphylococci from bovine mastitis. *Vet Microbiol* 1988; **18**: 163–176.
33. Incardona F, Lawler J, Cataldo D *et al.* Heparin-binding domain, type 1 and type 2 repeats of thrombospondin mediate its interaction with human breast cancer cells. *J Cell Biochem* 1996; **62**: 431–442.
34. Guo NH, Krutzsch HC, Negre E, Vogel T, Blake DA, Roberts DD. Heparin- and sulfatide-binding peptides from the type I repeats of human thrombospondin promote melanoma cell adhesion. *Proc Natl Acad Sci USA* 1992; **89**: 3040–3044.
35. Hotchkiss KA, Matthias LJ, Hogg PJ. Exposure of the cryptic Arg-Gly-Asp sequence in thrombospondin-1 by protein disulfide isomerase. *Biochim Biophys Acta* 1998; **1388**: 478–488.
36. Lawler J, Ferro P, Duquette M. Expression and mutagenesis of thrombospondin. *Biochemistry* 1992; **31**: 1173–1180.