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Fallgren, Corina; Utt, Meeme; Ljungh, Åsa

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

BACTERIAL PATHOGENESIS

Isolation and characterisation of a 17-kDa staphylococcal heparin-binding protein with broad specificity

CORINA FALLGREN, MEEME UTT and ÅSA LJUNGH

Department of Infectious Diseases and Medical Microbiology, University of Lund, Sölvegatan 23, S-223 62 Lund, Sweden

A previous study reported the ability of staphylococci to bind heparin and heparin-dependent host growth factors. The present study isolated and identified heparin- and basic fibroblast growth factor (bFGF)-binding surface components of *S. epidermidis* strain RP12 and *S. haemolyticus* strain SM 131. The staphylococcal heparin-binding component(s) were purified by affinity chromatography on heparin-Sepharose and a major heparin-binding protein, here designated HBP, was identified by immunoblot in these two coagulase-negative staphylococcal (CNS) species. The HBP was shown to be acidic with an approximate pI of 4.6 and a molecular mass around 17 kDa. The binding of heparin to HBP was inhibited by heparin, fucoidan, pentosan polysulphate and various other sulphated polysaccharides, but not by non-sulphated compounds. However, the purified HBP from both *S. epidermidis* and *S. haemolyticus* revealed broad specificity, and also bound bFGF, thrombospondin, von Willebrand factor and, weakly, fibrinogen. The N-terminal sequences of the 17-kDa HBP from *S. epidermidis* and *S. haemolyticus* showed only limited identity. Comparison of the first 15 amino acid residues derived from either strain with known sequences in the protein databases revealed no close similarities. Taken together, these results suggest that the adhesion of at least some CNS to host sulphated glycosaminoglycans may be mediated by a previously uncharacterised group of surface proteins.

Introduction

Staphylococcus aureus is primarily a human pathogen, causing a broad spectrum of diseases ranging from septicaemia to localised skin infections. Both *S. aureus* and coagulase-negative staphylococci (CNS) form part of the normal skin microflora. CNS strains were formerly regarded as opportunist pathogens, inducing systemic infections in immunocompromised patients [1]. However, CNS cause more infections of medical devices than any other group of micro-organisms and have emerged as the most frequently isolated pathogens of nosocomial sepsis [1, 2]. They may also give rise to endocarditis, endophthalmitis, osteomyelitis and urinary tract infections.

Several virulence factors have been identified in *S. aureus* [3, 4] including different toxins and haemoly-

sins, tissue-degrading enzymes, coagulase and fibrinogen-binding proteins, and several surface proteins which mediate adhesion to extracellular matrix (ECM) proteins such as fibronectin (Fn), vitronectin (Vn), collagen (Cn), thrombospondin (TSP), laminin and plasminogen [5].

Current concepts on the pathogenesis of wound and biomaterial-associated infections caused by CNS have focused on various interactions between staphylococci and host ECM proteins or serum components [6]. An earlier study showed that CNS strains bind ECM proteins in immobilised rather than soluble form and often bind immobilised proteins at higher levels than *S. aureus* [5]. This may be particularly relevant in biomaterial-associated infections, as host proteins immediately adsorb to the biomaterial surface and are thus present in immobilised forms [6].

Two surface proteins of *S. aureus* with mol. wts of 66 and 60 kDa, respectively, were earlier identified as binding heparan sulphate (HS) and heparin [7]. Also, CNS may interact with heparin and HS, as shown for

whole staphylococcal cells which bound avidly to heparinised polymer surfaces [8], or to heparin immobilised on latex particles [9]. The ability of both *S. aureus* and CNS strains to bind the heparin-binding growth factors, basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) has also been shown [10].

Heparin and HS constitute a class of glycosaminoglycans (GAGs) with common structural features [11]. The polysaccharide chains in heparin and HS proteoglycans are composed of partially sulphated, alternating hexuronic (L-iduronic or D-glucuronic) acid and D-glucosamine (N-sulphated or N-acetylated) residues. They differ as follows: heparin is highly sulphated and rich in L-iduronic acid, whereas HS is less sulphated and is rich in D-glucuronic acid. HS is located on the surface of most eukaryotic cells and forms part of the ECM [12], whereas heparin is stored intracellularly, mainly in mast cells, and is released upon external signalling. By binding to antithrombin III, which interacts with thrombin, heparin exerts an anticoagulative effect. However, the anticoagulant activity of HS is much lower than that of heparin [13]. Interestingly, a number of growth factors require binding to heparin and cell surface HS upon their activation during tissue repair. During wound healing in human skin, a correlation between expression of basement membrane HS and normal epidermal proliferation was observed [14].

This report describes the isolation of a 17-kDa heparin-binding surface protein from CNS.

Materials and methods

Chemicals

Heparin (mol. wt 4000–6000) was purchased from Fluka (Heidelberg, Germany). A Heparin-HiTrap, column (5 ml) was obtained from Pharmacia Biotech, Uppsala, Sweden. Thrombospondin (TSP) and von Willebrand Factor (vWF) were gifts from Professors J. Lawler and F. Dorner, respectively. Rabbit anti-human TSP, vWF, Fg, Fn and Vn, and peroxidase (HRP)-conjugated goat anti-mouse IgG and HRP-swine anti-rabbit IgG were purchased from Dako (Glostrup, Denmark). Amino-carbazole, 5-[4,6-dichlorotriazin-2-yl]amino fluorescein (DTAF), basic fibroblast growth factor (bFGF) human recombinant, chondroitin 4-sulphate, monoclonal anti-bFGF antibody, long-arm biotin-hydrazide, bovine serum albumin, cholera toxin, dextran sulphate (mol. wt 8000), fucoidan, hyaluronic acid from bovine trachea, pentosan polysulphate, sodium acetate, sodium periodate, HRP-conjugated streptavidin and toluidine blue were obtained from Sigma. Fragmin® (low molecular heparin; mol. wt 5000) was purchased from Kabi AB (Stockholm, Sweden). Suramin (Germanin®) was from Bayer AG (Leverkusen, Germany) and Tween 20 from Merck AG

(Darmstadt, Germany). Blood Agar (BA) base was purchased from Lab M (Salford) and supplemented with horse erythrocytes 4%. Tryptic Soy Broth (TS) was from Difco Laboratories (Detroit, MI, USA). All buffers and chemicals were of analytical grade.

Bacterial strains and growth conditions

S. aureus strain V8, *S. epidermidis* strain RP 12, *S. haemolyticus* strain SM 131 and other strains of different CNS species, isolated from patients with osteomyelitis and biomaterial-associated infections (n = 40) were studied [5]. *S. saprophyticus* strain TW 111, isolated from a patient with urinary tract infection and previously shown by agglutination assay [9] not to bind heparin was included as a negative control. CNS strains isolated from healthy carriers were also included. The test strains were cultured on BA at 37°C for 20–22 h. Bacterial cells were harvested in the late stationary phase by centrifugation (1760 g for 20 min), washed twice with PBS, pH 7.2, and suspended in PBS at a final concentration of 10⁹ cfu/ml.

Biotinylation of staphylococci

Equal volumes of bacteria and biotin (EZ-Link-Sulfo-NHS-LC-biotin, Boule Nordic AB Stockholm, Sweden) solution (0.2 mg/ml in PBS, pH 7.6) were incubated for 2 h at ambient temperature. The staphylococcal cells were then washed three times with PBS and resuspended at the original concentration in PBS containing bovine serum albumin (BSA) 1%.

Cell surface extracts

For extraction of cell-surface components, cells of strain *S. epidermidis* RP 12, *S. haemolyticus* SM 131 and *S. aureus* V8 were washed twice in PBS, resuspended in 2.5 ml of 1.0 M LiCl, supplemented with protease inhibitors (Complete™ Mini, Boehringer Mannheim, Germany) and incubated for 2 h at 37°C with agitation [7]. After centrifugation (8827 g, 30 min) the supernates were dialysed against PBS for 2 h at 4°C. The protein content was determined by a BioRad microassay (BioRad, Richmond, CA, USA), with BSA as a standard. The crude LiCl extracts were stored at –20°C.

Preparation of ¹²⁵I-DTAF-heparin and biotinylated heparin

To enable iodine labelling, DTAF was conjugated to heparin as described previously [15]. DTAF-heparin was dissolved in PBS at a concentration of 1–2 mg/ml. The pellet was lyophilised and stored dry at 4°C. DTAF-heparin was iodinated with Na¹²⁵I (Radiochemical Centre, Amersham) and Iodo-beads (Pierce Chemical Company, Rockford, IL, USA) according to the method of Pascu *et al.* [10]. Heparin was

biotinylated as described by Utt and Wadström [16]. Biotinylated heparin was dissolved in water (1 mg/ml) and used as a stock solution.

Immobilised GAG-binding assay

Nunc-Maxisorp 96-well microtitration plates were covalently coupled with heparin, chondroitin 4-sulphate and dextran sulphate by end-point attachment (EPA) [17]. The presence of GAGs on the microtitration plates was detected by a chromatic method with toluidine blue stain [17]. Briefly, a positive chromatic reaction was detected when the well coated with GAGs was stained violet compared with the colour of uncoated wells which remained uncoloured. The plates were blocked overnight at 4°C with BSA (400 µl of a 1% solution in PBS). Finally, the biotinylated bacterial suspension (100 µl containing 5×10^8 cfu/ml) was added. The plates were incubated on a platform shaker for 2 h at ambient temperature. Unbound bacteria were removed by washing three times with PBS containing Tween 20 0.05% (PBST). HRP-labelled NeutrAvidin™ (Boule Nordic AB) (100 µl of a 1 in 1500 dilution in PBS containing BSA 1%) was added to each well and incubated for 1 h at 37°C. The plates were washed three times with PBS, and 100 µl of ImmunoPure TMB substrate kit (Boule Nordic AB) were added to each well. The colour was allowed to develop for 5 min and the absorbance at 450 nm was measured with a microtitration plate reader (Multiscan, ELISA Reader). The controls used in the ELISA assay were: the heparin-coated wells containing all ingredients except biotinylated bacteria and the heparin-coated wells incubated only with ImmunoPure TMB substrate kit. The OD₄₅₀ values for the controls (background) were 0.270 (SD 0.010) and 0.105 (SD 0.015), respectively. All assays were performed twice and the means and SD were calculated.

Isolation of heparin-binding proteins

The crude LiCl extracts (5–6 ml) of *S. epidermidis* RP 12, *S. haemolyticus* SM 131 and *S. aureus* V8 were applied to a Heparin-HiTrap column equilibrated with PBS containing Tween 20 0.1%, pH 7.2. Material retained by the column was eluted at a flow rate of 1 ml/min with a linear 0–2 M NaCl gradient in the same buffer. The peak fractions at 280-nm absorbance were pooled, dialysed against water (overnight at 4°C), run on SDS-PAGE and silver stained (silver stain kit from BioRad).

SDS-PAGE, immunoblotting and autoradiography

S. epidermidis RP 12, *S. haemolyticus* SM 131 and *S. aureus* V8 cell-surface proteins (LiCl crude preparations or purified fractions) were separated and analysed by SDS-PAGE under reducing conditions in Mini-protean II or Protean II cell slab gel vertical electrophoresis equipment (BioRad) with 12% separating

gels or gradient gels (4–15%). Proteins were transferred to Immobilon® polyvinylidene difluoride membrane (PVDF) (Millipore Intertech, Bedford, MA, USA) by electrotransfer and the membrane was blocked with buffer I for 15 min [18]. Strips of the membrane were incubated with biotinylated heparin or with ¹²⁵I-DTAF-heparin (3×10^5 cpm/ml) diluted in a washing buffer composed of PBS containing BSA 1% and Tween 20 0.1%, overnight at 20°C. The strips probed with biotinylated heparin were then rinsed three times for 5 min in washing buffer and incubated for 2 h at 20°C with streptavidin-HRP (diluted 1 in 3000 in washing buffer) [18]. The controls used in immunoblot assays were the strips incubated only with streptavidin-HRP, except biotinylated heparin. After washing as above the strips were incubated with 50 mM sodium acetate buffer containing 3-amino-9-ethyl carbazole 0.04% and H₂O₂ 0.015%. The blots probed with ¹²⁵I-DTAF-heparin were dried and examined by autoradiography (–70°C, *c.* 48 h) with X-Omat AR film (Eastman, Kodak). The specificity of HBP binding [19] was tested by pre-incubation of the strips with a mixture of heparin or other polysulphated agents (100 µg/ml final concentration) and biotinylated heparin or bFGF.

To ensure that the HBP is a surface protein, bacterial cells were biotinylated before LiCl extraction and the proteins were resolved by SDS-PAGE and Western blotted with streptavidin peroxidase. LiCl extract from non-biotinylated bacterial cells probed with heparin-biotin was used as a positive control.

bFGF immunoblot binding assay

Binding assays with bFGF were performed in PBS containing BSA 1% and Tween 20 0.1% at pH 5 and 6. PVDF strips containing HBP were incubated with bFGF 50 ng/ml overnight at 20°C. The strips were then rinsed three times for 5 min in washing buffer and incubated for 2 h at 20°C with mouse anti-bFGF immunoglobulins. The strips were rinsed as above and incubated with peroxidase-conjugated goat anti-mouse immunoglobulins (diluted 1 in 1000) for 1 h at 20°C. As controls, strips containing HBP incubated only with primary and secondary antibody, except bFGF, were used. After washing as above, the strips were incubated with 50 mM sodium acetate buffer containing 3-amino-9-ethylcarbazole 0.04% and H₂O₂ 0.015%.

Amino-terminal amino acid sequence

Samples of purified heparin-binding proteins from *S. haemolyticus* SM 131 and *S. epidermidis* RP 12 were transferred to PVDF membranes after SDS-PAGE. These membranes were stained with Coomassie Brilliant Blue R-250 0.1% w/v in methanol 50% for 5 min and destained in methanol 50% and acetic acid 10%. Protein bands were cut from the dried PVDF

membranes, and the amino-terminal amino acid sequence was analysed in a peptide sequencer ABI 476A (Foster City, CA, USA).

Iso-electric focusing and two-dimensional electrophoresis

Linear precast Immobilon DryStrips, pH 3–10 (110 × 3 × 0.5 mm) from Amersham Pharmacia Biotech (Uppsala, Sweden), were rehydrated according to the manufacturer's instructions. The crude LiCl extracts of *S. epidermidis* RP 12 (50 µg of protein/strip) were loaded into a sample cup at the cathodic (basic) end of the gel strip submerged in Plusone (Drystrip cover fluid from Amersham Pharmacia Biotech). Iso-electric focusing (IEF) was performed with a programmed voltage gradient for 21 h to a total of 34 kVh with horizontal electrofocusing apparatus (Multiphor II, Amersham Pharmacia Biotech). After IEF the strips were stored at –20°C until required for the second dimension. After equilibration of the strips according to the manufacturer's instructions, the second dimension was performed on SDS polyacrylamide 8–18% gradient slab gels, at a constant voltage of 80 V for 16 h, with Protean II vertical electrophoresis equipment and Laemmli buffer system [19]. Molecular markers in the range 14–94 kDa were used (Amersham Pharmacia Biotech). The completed two-dimensional gels were either silver stained or transferred to PVDF membranes for further immunoblot assay, as described above.

Binding of TSP and vWF by HBP

Wells in a microtitration plate (Nunc MaxiSorp) were coated overnight at 4°C with 100 µl of a 5 µg/ml solution of HBP (*S. epidermidis* RP 12). The wells were then blocked by incubation with BSA 3% for 1 h at 37°C. TSP, vWF, Fg, Fn or Vn (0.1 µg of protein in 100 µl) were added to the wells followed by incubation for 2 h at room temperature. Bound serum proteins were detected by HRP-conjugated rabbit immunoglobulins against TSP, vWF, Fg, Fn or Vn diluted 1 in 1000 in PBST. The plate was washed with PBST after each incubation, developed with ImmunoPure TMB substrate and the colour was read at 450 nm. The controls used in capture ELISA assay comprised the coated wells containing all ingredients except the tested proteins and the coated wells incubated only with ImmunoPure TMB substrate kit. The OD₄₅₀ values for the controls (background) were 0.348 (SD 0.012) and 0.131 (SD 0.015), respectively. All assays were performed twice and the means and SD were calculated.

Results

GAG-binding assay

Binding of immobilised GAGs was common among CNS strains isolated from both biomaterial-associated infections and from healthy individuals. *S. epidermidis*

RP 12 and *S. haemolyticus* SM 131 were selected for characterisation of heparin-binding components in CNS strains (Fig. 1). The binding of heparin was optimal when staphylococci were cultured on BA. Maximal binding was obtained within 30 min; it was most pronounced at pH 5 and was reduced by high ionic strength (data not shown).

Isolation of heparin-binding surface proteins: SDS-PAGE and immunoblot assays

The HPLC profile revealed three (*S. epidermidis* RP 12) and four (*S. haemolyticus* SM 131) major peaks that were eluted at concentrations of 0.5, 0.8, 1.0 and 1.4 M NaCl. The peak materials were subjected to SDS-PAGE and, for *S. epidermidis* RP 12 and *S. haemolyticus* SM 131, four-to-five bands, respectively, were detected after silver staining (three-to-five weak bands in the high mol. wt range, 60–85 kDa) and one strong band of *c.* 17 kDa (Fig. 2a and b). A NaCl concentration of 1.4 M was needed for the elution of this protein from the heparin column, thus demonstrating the binding to be of high affinity. This band was also detected in *S. aureus* V8, but was weaker than the 60- and 66-kDa HS-binding proteins reported previously (Fig. 2c) [7]. In corresponding Western blots, several proteins were found to bind heparin, one with a molecular mass of *c.* 17 kDa being most prominent. This component was designated HBP (heparin-binding protein). Four other proteins bound to a lesser extent.

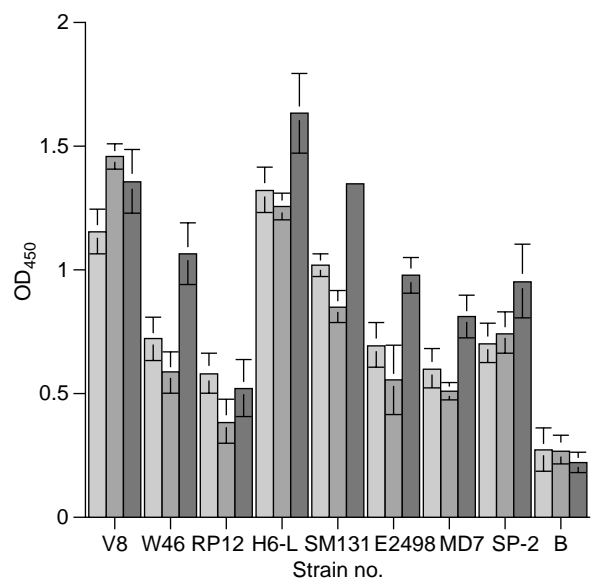


Fig. 1. Binding of staphylococcal strains to GAGs: EPA-immobilised heparin, dextran sulphate and chondroitin 4-sulphate. Biotinylated staphylococcal cells were added to GAG-coated wells and allowed to adhere for 2 h. After removal of non-adherent bacteria, bound bacteria were detected with NeutrAvidin™. Absorbance was recorded in a Multiscan ELISA reader at 450 nm. The results are the mean value of four readings. Each batch of assays also included one or more control strains as well as blank wells (B).

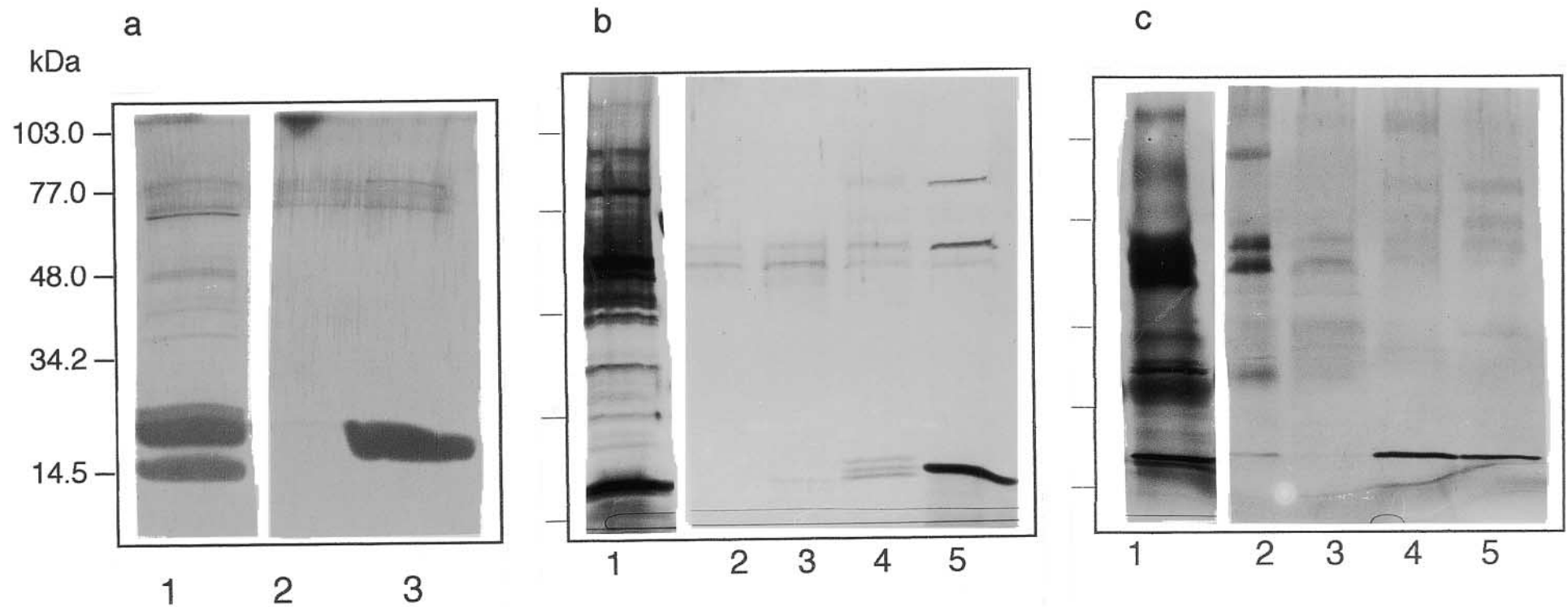


Fig. 2. SDS-PAGE analysis of HBPs purified from three staphylococcal strains. The fraction peaks eluted from the heparin column were dialysed against PBS (overnight at 4°C), separated on SDS-PAGE and silver stained. **(a)** HBPs from *S. epidermidis* RP 12. Lane **1**, LiCl crude extract; **2**, moderate affinity HBPs (eluted with 0.8 and 1 M NaCl, respectively); **3**, 17-kDa HBP from *S. epidermidis* RP 12 (eluted with 1.4 M NaCl). **(b)** HBPs from *S. haemolyticus* SM 131. Lane **1**, LiCl crude extract; **2–4**, low and moderate affinity HBPs (eluted with 0.5, 0.8 and 1 M NaCl, respectively); **5**, 17-kDa HBP from *S. haemolyticus* SM 131 (eluted with 1.4 M NaCl). **(c)** HBPs from *S. aureus* V8. Lane **1**, LiCl extract; **2, 3**, low and moderate affinity HBPs (eluted with 0.5 and 0.8 M NaCl) (the previously described 66- and 60-kDa proteins [7] were detected among these); **4, 5**, 17-kDa HBP from *S. aureus* V8 (eluted with 1.4 M NaCl).

When membranes were probed with biotin-heparin or ^{125}I -DTFA-heparin in PBS containing 1% BSA and Tween 20 0.1%, pH 7.2, only the major fraction with a size of *c.* 17 kDa retained heparin-binding ability (Fig. 3a and b).

Inhibition assays [20] were performed by incubating the strips with biotinylated heparin combined with one of the inhibitors such as heparin, suramin, fucoidan and dextran sulphate (100 $\mu\text{g}/\text{ml}$ final concentration). The heparin binding to HBP was abolished by each of these inhibitors, but not by hyaluronic acid (Fig. 4a).

In SDS-PAGE experiments combined with immunoblot assays, the LiCl-extracted proteins from biotinylated staphylococcal cells included the same 17-kDa HBP as compared with the control, the non-biotinylated staphylococcal cells, thereby demonstrating the surface location of this protein (data not shown).

bFGF immunoblot binding assay

An earlier study demonstrated that CNS may bind bFGF and some other heparin-dependent growth factors [10], therefore the present study tested the possible binding of bFGF to HBP. As shown in Fig. 3a and b, lane 4, bFGF also bound to HBP isolated from *S.*

epidermidis RP 12 and *S. haemolyticus* SM 131. The bFGF binding to HBP was almost completely abolished by heparin (Fig. 4b).

Two-dimensional electrophoresis

By 2-D electrophoresis combined with immunoblot, HBP was shown to be acidic with a pI *c.* 4.6 and a molecular mass of *c.* 17 kDa (Fig. 5a and b).

N-Terminal sequencing

For the 17-kDa proteins purified from *S. epidermidis* RP 12 and *S. haemolyticus* SM 131, the following sequences were obtained: MXTAHSYTXKYNGYTAN (GenBank accession no. tmpseq_AF1167348) and MATQTKGYYSYNGYV (GenBank accession no. tmpseq_1AF169242), respectively. The N-terminal sequences of these two different staphylococcal species showed only limited identity, shown in bold. The sequences were compared in the EMBL Fasta Server Protein database; no homology with known staphylococcal cell-surface proteins or other microbial heparin-binding proteins was found.

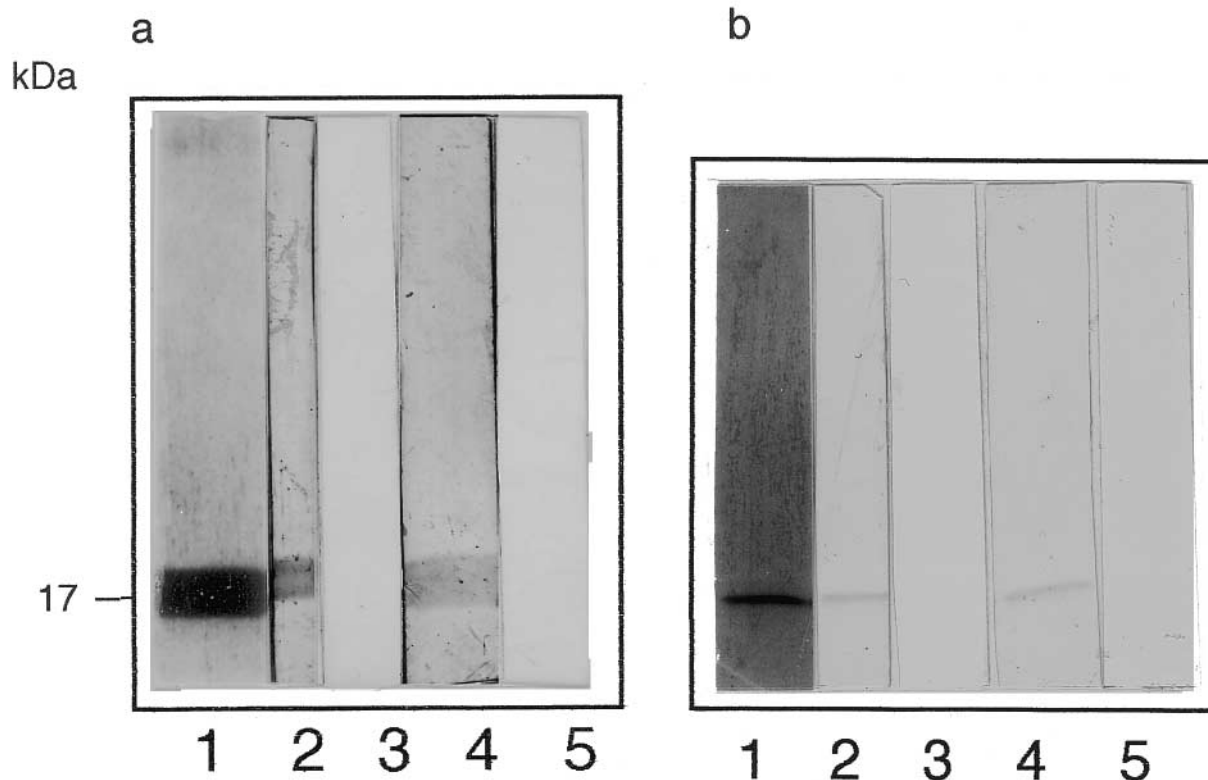


Fig. 3. Binding of heparin and bFGF to purified staphylococcal HBP. (a) HBP from *S. epidermidis* RP 12. (b) HBP from *S. haemolyticus* SM 131. The blots (a and b) were probed with ^{125}I -DTAF-heparin (lane 1), biotinylated heparin (2) and bFGF (4). As controls, strips containing HBP incubated only with streptavidin-HRP (lane 3) or primary/secondary antibodies (5), except biotinylated heparin and bFGF, respectively, were used.

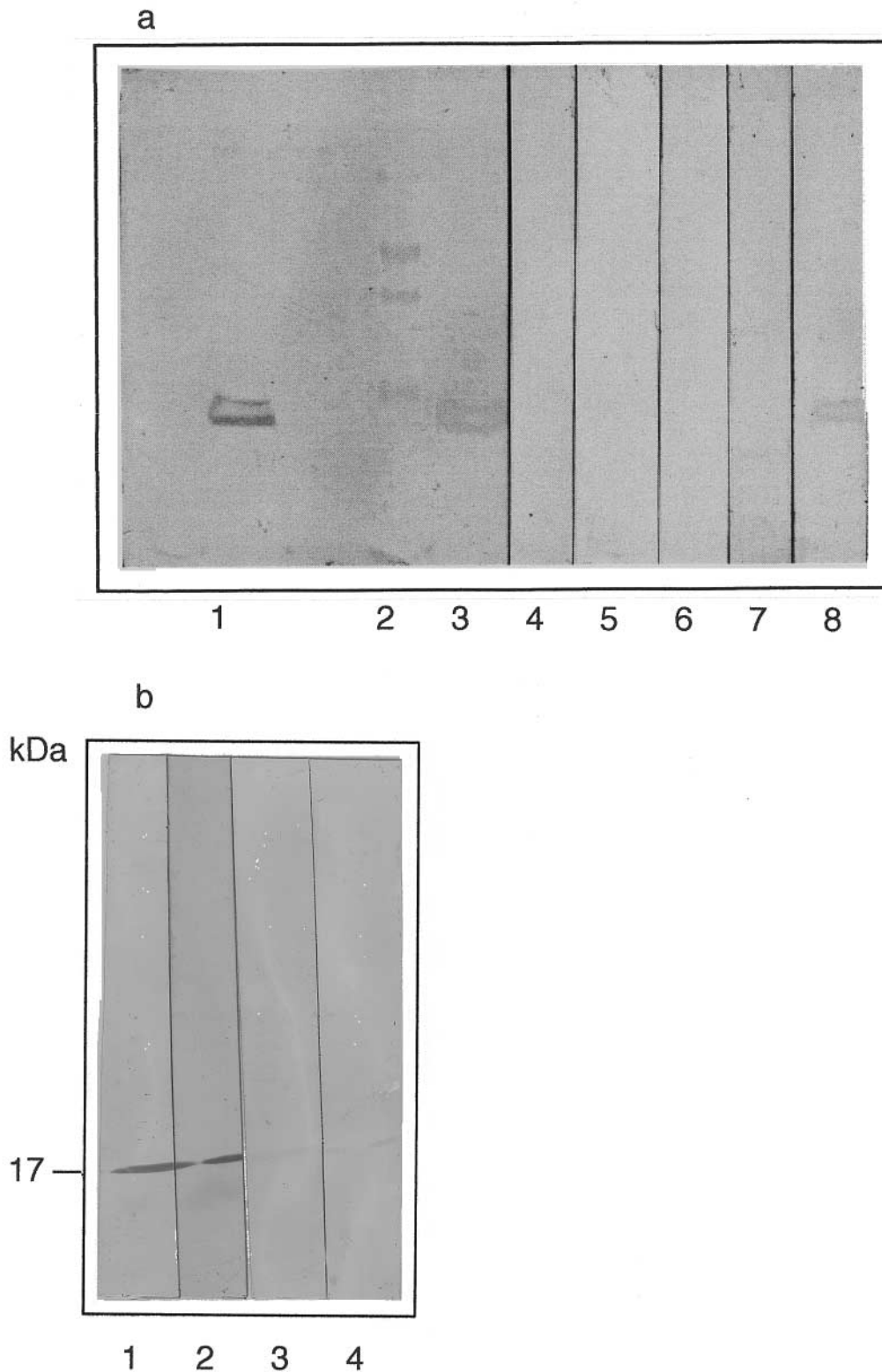


Fig. 4. Western blot competitive inhibition assays of (a) heparin and (b) bFGF binding to purified 17-kDa protein from *S. epidermidis* RP 12. The membranes were probed with biotinylated heparin (a) and bFGF/monoclonal antibody (b), respectively. (a) Lane 1, LiCl crude preparation; 2, molecular mass marker (20–103 kDa); 3, HBP probed with biotinylated heparin; 4–8, HBP incubated with a mixture of biotinylated heparin and one of the inhibitors (100 $\mu\text{g}/\text{ml}$ final concentration), i.e., heparin, fucoidan, dextran sulphate, pentosan polysulphate and hyaluronic acid, respectively. (b) Lane 1, HBP probed with biotinylated heparin; 2, HBP probed with bFGF/anti-bFGF antibody; 3, HBP preincubated with heparin (100 $\mu\text{g}/\text{ml}$ final concentration) before the strip was probed with bFGF/anti-bFGF antibody; 4, HBP incubated with a mixture of heparin and bFGF (50 ng/ml) before the strip was probed with monoclonal bFGF antibody.

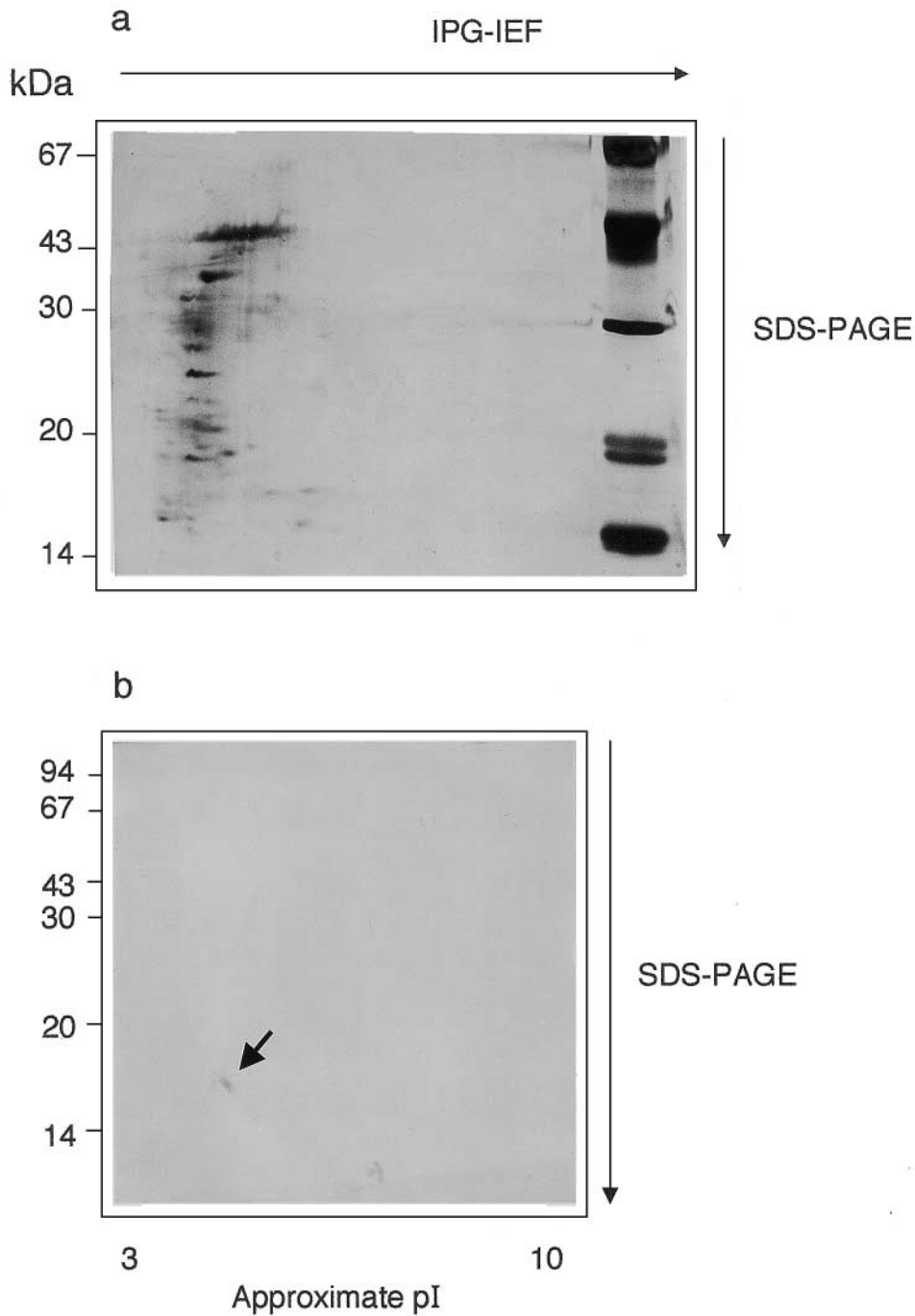


Fig. 5. Two-dimensional gel electrophoresis of a LiCl extract of *S. epidermidis* RP 12 and Western blot after transfer to a PVDF membrane. The first dimension included iso-electric focusing in a pI range 3–10 and was followed by separation on an 8–18% gradient SDS-PAGE, and silver staining. The membrane was probed with biotinylated heparin. (a) Distribution of surface proteins, resolved by 2-D electrophoresis. (b) HBP, at 17 kDa and a pI of 4.6, indicated by arrow.

Binding of TSP and vWF by HBP

The broad specificity in the binding ability of HBP was investigated by a capture enzyme-linked immunosorbent assay (ELISA). As shown in Fig. 6, only TSP and to a lesser extent vWF (1.75-fold less than TSP) could be captured by HBP. Fg bound as well as HBP, but with a very weak affinity, as compared with the controls. Other serum and matrix proteins such as Fn and Vn did

not bind HBP (OD₄₅₀ values similar to the background level).

Discussion

Many pathogenic micro-organisms use cell surface glycolipids and glycoproteins as receptor molecules for attachment or invasion, or both, of eukaryotic cells, as

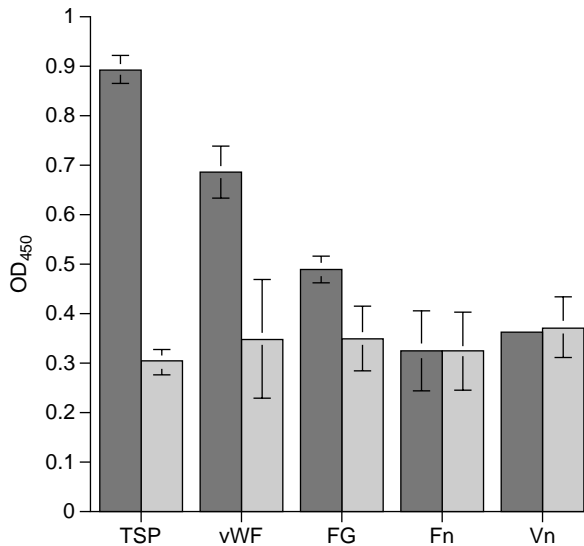


Fig. 6. Capture ELISA of TSP, vWF, Fg, Fn and Vn. Microtitration wells were coated with 100 μ l of a 5 μ g/ml solution of HBP (*S. epidermidis* RP 12). The TSP, vWF, Fg, Fn and Vn (0.1 μ g/100 μ l) were added and binding was measured with HRP-conjugated antibodies against TSP, vWF, Fg, Fn and Vn (1 in 1000), respectively.

well as intercellular migration processes [21]. Various ECM proteins have been implicated in microbial binding, but interactions with GAGs have been poorly studied. Recently, it has become evident that many bacteria, parasites and viruses exploit GAGs as adhesion receptors [22] and that binding to ECM proteins and GAGs may also trigger various tissue reactions, such as thrombus formation and complement activation.

Previous studies have shown that *S. aureus* and *S. epidermidis* strains specifically interact with ECM molecules such as Fn, Vn, Cn, TSP, vWF, lactoferrin and transferrin, all of which contain heparin-binding domains [3–6, 23, 24]. For many of these interactions, not only heparin but also other polysulphated molecules, such as fucoidan, suramin and dextran sulphate, were potent inhibitors, whereas carboxylated or non-sulphated components were not inhibitory [25]. It has also been shown that staphylococci can interact with two heparin-dependent growth factors, bFGF and PDGF [10], which may affect tissue integration of implanted biomaterials. It is likely that these interactions can also stimulate other receptors on eukaryotic cells, which may involve secondary binding to bFGF receptors to trigger cell uptake and intracellular multiplication, as shown for gonococci [26].

For staphylococci, it might be of conceivable benefit to bind the different types of host cell GAGs through suitable adaptor molecules for different GAG motifs. The present study showed that staphylococci, in addition to heparin/HS, may bind other sulphated GAGs, such as chondroitin 4-sulphate and dextran

sulphate (Fig. 1). The fact that staphylococci have the ability to bind to different sulphated polysaccharides with heterogeneous chemical structures may indicate that staphylococci express a promiscuous GAG receptor with variable affinity for different GAGs, which is consistent with other GAG-binding receptors [11].

The study aimed to examine the molecular mechanisms by which heparin/HS and bFGF interact with CNS strains, and a heparin-binding protein. Therefore, a HBP was purified from two different CNS strains by heparin affinity chromatography. The results suggest that HBP is a novel cell-surface protein, with a molecular mass of *c.* 17 kDa and a pI of *c.* 4.6 (Fig. 5b), that has affinity for heparin, for the heparin-binding growth factor, bFGF (Fig. 3a and b), for TSP, for vWF and very weak affinity for Fg (Fig. 6).

A notable finding in the present study was that the N-terminal sequence of the HBP from *S. haemolyticus* SM 131 was almost completely identical to the sequence obtained in this laboratory for a TSP-binding protein (*c.* 16 kDa) isolated from the same strain (M. Stollenwerk, unpublished observations). Furthermore, it was reported that binding to TSP and vWF by staphylococcal cells was totally blocked by heparin [23, 24], suggesting that the heparin-binding domains of these two molecules are involved in bacterial recognition.

Broad specificity binding proteins in *S. aureus* cells have been described previously [27]. By N-terminal analysis, the Vn-binding protein of *S. aureus* V8 showed identity with the HS-binding protein, indicating that the latter 60-kDa surface protein mediates binding of Vn as well [28]. Interestingly, HBP from *S. epidermidis* RP 12 showed binding not only to bFGF, but to TSP, to vWF and very weakly to Fg. TSP and vWF are secreted from activated platelets during wound healing and have been proposed to mediate staphylococcal adhesion to biomaterial surfaces in the blood stream [23, 24, 29]. Recently, it was shown that *S. aureus* can activate platelets and promote their deposition on the ECM. Therefore, the secreted products may also bridge the deposition of *S. aureus* on to the subendothelium [30].

HBP is clearly distinct from the 20-kDa sulphated polysaccharide purified from the extracellular slime layer of *S. epidermidis* [31], which behaves as a 30-kDa band in SDS-PAGE and immunoblotting experiments on crude slime. Moreover, the HBP could not be detected in extracts prepared by sonication from *S. epidermidis* cells grown on tissue-culture dishes in TS (data not shown), as proposed for the isolation of a capsular polysaccharide/adhesin (PS/A, *c.* 28 kDa) [32]. As shown by the N-terminal sequence, *S. epidermidis* HBP is distinct from the previously reported 19-kDa Fg-binding protein of *S. epidermidis*, purified from culture supernates [33]. The relationship of HBP

to other cell-surface components in CNS and its binding specificity merit further investigation.

As binding of heparin was common among CNS strains isolated from both patients and healthy carriers, any role of the expression of HBP for staphylococcal pathogenicity in man is unclear at present. As with binding of ECM molecules, including Fn, Vn, TSP and type IV Cn, GAG binding to staphylococcal cells may allow coating of these bacteria with host molecules, which may prevent attack by phagocytes and the immune system. Heparin binding by microbes was recently highlighted by Duensing *et al.* as a novel strategy in microbial pathogenesis that complements the classical virulence strategy [34]. This mechanism enables different bacterial species that bind heparin/HS and other GAGs to recruit structurally and functionally diverse bioactive host proteins, including adhesive glycoproteins (Fn and Vn), inflammatory and immunomodulatory mediators, and growth factors [34]. Adhesive proteins, such as Fn and Vn, have been implicated as intermediates in bacterial interactions with host cell integrin receptors, resulting in bacterial colonisation and invasion of epithelial cells [4]. From our ongoing studies, we also envisage a possible role for CNS heparin-binding abilities in the initial attachment of staphylococci to endothelial cells expressing both HS and TSP on their surface [35]. The interaction of staphylococci with GAGs may thus be important for efficient infection of eukaryotic cells by staphylococci. Furthermore, the binding of bFGF to staphylococcal cells might interfere with repair processes in the infected tissue.

In summary, the present study has demonstrated that staphylococci express surface components which recognise heparin-related polysaccharides, a heparin-dependent growth factor, bFGF, TSP and vWF. The biological importance of these interactions and their possible role in staphylococcal infections remain to be further investigated. The use and development of implanted artificial biomaterials is hampered by infectious complications. A better understanding of the pathogenesis of these infections might lead to interventions that directly attenuate bacterial virulence or modulate host responses, and ultimately to improved methods of bioimplantation.

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