The serine-aspartate repeat (Sdr) protein family in *Staphylococcus epidermidis*

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*Staphylococcus epidermidis* can express three different cell-surface-associated proteins, designated SdrF, SdrG and SdrH, that contain serine-aspartate dipeptide repeats. Proteins SdrF and SdrG are similar in sequence and structural organization to the Sdr proteins of *Staphylococcus aureus* and comprise unique 625- and 548-residue A regions at their N termini, respectively, followed by 110–119-residue B-repeat regions and SD-repeat regions. The C termini contain LPXTG motifs and hydrophobic amino acid segments characteristic of surface proteins covalently anchored to peptidoglycan. In contrast, SdrH has a short 60-residue A region at its N terminus followed by a SD-repeat region, a unique 277-residue C region and a C-terminal hydrophobic segment. SdrH lacks a LPXTG motif. Recombinant proteins representing the A regions of SdrF, SdrG and SdrH were expressed and purified from *Escherichia coli*. Antisera specific to these proteins were raised in rabbits and used to identify Sdr proteins expressed by *S. epidermidis*. Only SdrF was released from lysostaphin-generated protoplasts of cells grown to late-exponential phase. SdrG and SdrH remained associated with the protoplast fraction and thus appear to be ineffectively sorted along the conventional pathway used for cell-wall-anchored proteins. In Southern hybridization analyses, the *sdrG* and *sdrH* genes were present in all 16 strains tested, whilst *sdrF* was present in 12 strains. Antisera from 16 patients who had recovered from *S. epidermidis* infections contained antibodies that reacted with recombinant A regions of SdrG and SdrH, suggesting that these proteins can be expressed during infection.

**Keywords:** surface protein, adhesin, multigene family

INTRODUCTION

*Staphylococcus epidermidis* is a common inhabitant of human skin and a frequent cause of foreign-body infections. Pathogenesis is facilitated by the ability of the organism to first adhere to, and subsequently form biofilms on, indwelling medical devices such as artificial valves, orthopaedic devices, and intravenous and peritoneal dialysis catheters. Device-related infections jeopardize the success of medical treatment and significantly increase patient morbidity (Kloos & Bannerman, 1994).

Adherence of *S. epidermidis* to synthetic surfaces has been correlated with both surface hydrophobicity and cell-surface proteins (Fleer & Verhoef, 1989; Martin et al., 1989). Protease treatment of *S. epidermidis* has been shown to reduce hydrophobicity and adherence (Pascual et al., 1986), and a monoclonal antibody reactive to a 220 kDa cell-surface protein of *S. epidermidis* was able to partially block bacterial attachment to polystyrene (Timmerman et al., 1991). Polysaccharide expressed by the ica operon is crucial for biofilm formation. One group of researchers has suggested that the polysaccharide adhesin (PS/A) is sufficient for both the adhesion and cell–cell interactions associated with the accumulation phase of biofilm formation (McKenney et

Abbreviations: GST, glutathione S-transferase; Sdr, serine-aspartate (SD) repeat.

The GenBank accession numbers for the sequences determined in this work are AF245041 (*sdrF*), AF245042 (*sdrG*) and AF245043 (*sdrH*).
Like et al. responsible only for the accumulation phase (Heilmann 1996, 1997).

Like S. epidermidis, Staphylococcus aureus can adhere to medical-implant devices but this attachment is predominantly mediated by bacterial adhesins specific for host fibrinogen and fibronectin that coat biomaterial surfaces shortly after implantation (Vaudaux et al., 1995). Even though S. aureus is now known to contain an ica polysaccharide adhesin locus (McKenney et al., 1999), a subfamily of adhesins called MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) have been well characterized and are known to mediate bacterial adherence to fibrinogen (ClfA and ClfB), fibronectin (FnbpA and FnbpB) and collagen (CNA) (reviewed by Foster & Höök, 1998).

Although S. epidermidis may interact with fibrinogen, fibronectin, vitronectin and laminin (Herrmann et al., 1988; Paulsson et al., 1992; Switalski et al., 1983), little is known of specific MSCRAMMs mediating these interactions or about how these interactions influence bacterial adherence to biomaterials coated with host proteins.

The fibrinogen-binding clumping factor protein of S. aureus (ClfA, Fig. 1a) is distinguished by the presence of a serine-aspartate (SD) dipeptide-repeat region (referred to as region-R in previous studies) located between a ligand-binding A region and C-terminal sequences associated with attachment of the protein to the cell wall (McDevitt et al., 1994, 1995). The SD-repeat region is predicted to span the cell wall and extend the ligand-binding region from the surface of the bacteria (Hartford et al., 1997). ClfA is the predecessor of a recently identified SD-repeat-containing protein family (called Sdr) found in S. aureus. Additional members of this family include ClfB (a second fibrinogen-binding clumping factor), SdrC, SdrD and SdrE (Fig. 1a) (Joseffson et al., 1998a; Ni Eidhin et al., 1998). SdrC, SdrD and SdrE proteins contain additional repeats, termed B-repeat regions, located between the A regions and SD-repeat regions. Each B repeat is 110–113 amino acids in length and contains a putative, Ca$^{2+}$-binding EF-hand motif. Ca$^{2+}$ binding has been shown to be required for the structural integrity of the B-repeat regions in SdrD (Joseffson et al., 1998b). The functions of SdrC, SdrD and SdrE are unknown, but the proteins are hypothesized to interact with host molecules via their A regions.

This report describes three Sdr proteins identified in S. epidermidis. The deduced amino acid sequences of SdrF and SdrG have an organization similar to that of the S. aureus Sdr proteins, whilst SdrH is distinct. The genes encoding these proteins are prevalent among S. epidermidis strains. Furthermore, the proteins are expressed among S. epidermidis strains grown in vitro and the presence of Sdr-reactive antibodies in convalescent patient antisera suggest that the proteins are expressed during infection.

**Methods**

**Bacterial strains and growth conditions.** Escherichia coli strains (Stratagene) XL-1 Blue or JM109 were used as recombinant host strains. E. coli strains XL-1 Blue or TOPP 3 (Rif$^{T}$ [F$^{T}$ proAB lac$I^{-}$]ZAM15 TnJ0 [Tet$^{R}$] [Kan$^{R}$]) (Stratagene) were used for protein expression. Bacteria were routinely

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**Fig. 1.** Relationships between the Sdr proteins of S. aureus and S. epidermidis. (a, b) Schematic representation of the previously described S. aureus Sdr proteins and the S. epidermidis SdrF, SdrG and SdrH proteins, showing the relative position and/or size of their signal sequences (S), A regions (A), B-repeat regions (B), SD-repeat regions (SD), C region (C) (SdrH only), and wall/membrane spanning regions (WM). (c) C-terminal amino acid sequences of SdrF, SdrG and SdrH showing the positions of the SD repeats, LPXTG motifs (LVVTG in SdrH) (underlined), hydrophobic membrane-spanning regions (bold) and charged, terminal residues.
grown in Luria broth or agar (Gibco-BRL) supplemented with 100 µg ampicillin ml⁻¹ (USB). *S. epidermidis* strains (Table 1) were grown in tryptic soy broth (TSB) or agar (TSA) (Difco).

**Cloning of the sdr genes.** The *sdrF* gene was cloned from *S. epidermidis* strain 9491. HindIII-digested DNA fragments ranging from 6.5 to 7.5 kb in length were isolated from an agarose gel and ligated into a pBluescript SK(+) cloning vector (Stratagene) digested with HindIII and treated with calf-intestine alkaline phosphatase (CIAP) (Promega). One recombinant plasmid, pC5, was identified by PCR screening with appropriate restriction endonucleases and ligated into various expression vectors to generate recombinant fusion proteins (Erase-a-base System; Promega). Appropriate deletions on both strands (not shown) were identified by PCR screening and restriction mapping.

**Southern hybridizations.** Methods for Southern blot transfers and hybridizations have been described elsewhere (Josefsson *et al.*, 1998a). DNA probes were made from PCR products encoding the SD-repeat region of ClfA or each A region of SdrF, SdrG and SdrH (Table 2). PCR products were generated with *Tag* DNA polymerase (Gibco-BRL), and probes were labelled with digoxigenin (Boehringer Mannheim) or fluoroein (Amersham).

**Genetic constructs used for recombinant proteins.** DNA encoding the recombinant SdrF, SdrG or SdrH A regions was obtained by PCR amplification using genomic template DNA from *S. epidermidis* strains 9491 or K28 and appropriate primers (Table 2). The PCR utilized *Phi* DNA polymerase (Stratagene); specifications have been previously described (Joh *et al.*, 1994). PCR products were digested with appropriate restriction endonucleases and ligated into various expression vectors to generate recombinant fusion proteins containing N-terminal histidine residues (vector pQE30; Qiagen) or glutathione S-transferase (GST) (vectors pGEX-2T or pGEX-KG; Pharmacia).

**Expression and purification of proteins for antisera production.** Proteins were expressed in *E. coli* by growing 41 recombinant organisms to an OD₆₀₀ of 0.5 and inducing with 0.3 mM IPTG (Gibco-BRL) for 2 h. The cells were harvested in PBS (150 mM NaCl, 4.3 mM Na₂HPO₄, 1 mM NaH₂PO₄) and frozen at −80 °C. Cells were then passed through a French press and the supernatants of these lysates were filtered through a 0.45 µm membrane. Soluble histidine fusion proteins, present in the supernatants, were initially purified by metal-chelating chromatography. The supernatants were ap-

### Table 1. *S. epidermidis* strains used in this study

<table>
<thead>
<tr>
<th>Strains</th>
<th>Comments or properties</th>
<th>Source or reference*</th>
</tr>
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<tbody>
<tr>
<td>9491</td>
<td>SdrF and SdrH prototype strain</td>
<td>ATCC strain</td>
</tr>
<tr>
<td>ATCC14990</td>
<td>Reference strain</td>
<td>W. Kloos</td>
</tr>
<tr>
<td>KH11</td>
<td>Clinical isolate</td>
<td>Peters <em>et al.</em> (1982)</td>
</tr>
<tr>
<td>K28</td>
<td>SdrG prototype strain</td>
<td>P. Vaudaux</td>
</tr>
<tr>
<td>RP62a</td>
<td>Slime/biofilm former</td>
<td>Christensen <em>et al.</em> (1982)</td>
</tr>
<tr>
<td>TU3298</td>
<td>Transformable strain</td>
<td>F. Götz</td>
</tr>
<tr>
<td>9142</td>
<td>Biofilm former</td>
<td>Mack <em>et al.</em> (1992)</td>
</tr>
<tr>
<td>1457</td>
<td>Biofilm former</td>
<td>Mack <em>et al.</em> (1992)</td>
</tr>
<tr>
<td>8400</td>
<td>Biofilm former</td>
<td>Mack <em>et al.</em> (1992)</td>
</tr>
<tr>
<td>N910308</td>
<td>Reference strain, Lyon, France</td>
<td>CNRTS</td>
</tr>
<tr>
<td>N910160</td>
<td>Reference strain, Lyon, France</td>
<td>CNRTS</td>
</tr>
<tr>
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<td>Reference strain, Lyon, France</td>
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<td>N910249</td>
<td>Reference strain, Lyon, France</td>
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Table 2. Primers used in PCR amplification of DNA probes and protein expression constructs

<table>
<thead>
<tr>
<th>Regions amplified</th>
<th>Sequence*</th>
<th>Vector destination</th>
<th>Template DNA</th>
</tr>
</thead>
</table>
| clfA SD repeat    | F: GCGCGATCCCAATTCAGAGGATTCA  
R: GGCAAGCCTATTATTAGGACCTGACTC | NA | pCF10† |
| SD repeats        | F: GCGCGATCCCAATTCAGAGGATTCA  
R: GGCAAGCCTATTATTAGGACCTGACTC | NA | sdr clones |
| sdrF region A     | F: CCCGGATCCGGCTGAAGACAATCAATTAG  
R: CCCGAAGCTTAAATTATCCCCCTGTGCTG | pQE30 | Strain 9491 |
| sdrG region A     | F: CCCGGATCCGGCTGAAGACAATCAATTAG  
R: CCCGAAGCTTAAATTATCCCCCTGTGCTG | pQE30 | Strain K28 |
| sdrH full length  | F: CCCGGATCCGGCTGAAGACAATCAATTAG  
R: CCCGAAGCTTAAATTATCCCCCTGTGCTG | pQE30 | Strain 9491 |
| sdrF region A     | F: same as above  
R: CCCGAATTCCTATATTACCCCTGTGCTG | pGEX-2T | Strain K28 |
| sdrG region A     | F: same as above  
R: CCCGAATTCCTATATTACCCCTGTGCTG | pGEX-2T | Strain K28 |
| sdrH region A     | F: GCGCGATCCCAATTCAGAGGATTCA  
R: GGCAAGCCTATTATTAGGACCTGACTC | pGEX-KG | Strain 9491 |

NA. Not applicable.
* Underlined letters indicate restriction endonuclease sites.
† McDevitt et al. (1994).

applied to a 5 ml Ni2+-charged HiTrap chelating column (Pharmacia) and bound proteins were eluted with 200 ml linear gradients of 0–200 mM imidazole in 4 mM Tris/HCl, 100 mM NaCl, pH 7–9, at a flow rate of 5 ml min–1. Fractions were analysed for protein content by determining their absorbance at 280 nm and those containing recombinant proteins were identified by SDS-PAGE (see below), pooled and dialysed against 25 mM Tris–HCl, pH 8.0. Dialysed proteins were concentrated and further purified by ion-exchange chromatography by applying the samples to a 5 ml HiTrap Q column (Pharmacia) and eluting bound proteins with 200 ml linear gradients of 0–0.5 M NaCl in 25 mM Tris/HCl, pH 8.0, at a flow rate of 5 ml min–1. Fractions containing purified recombinant proteins were identified by SDS-PAGE. GST fusion proteins were purified from E. coli lysates obtained as described above. Lysates were passed through 10 ml glutathione-agarose columns under gravity flow and washed with 5 column vols PBS. Proteins were eluted from the columns with freshly prepared 5 mM reduced glutathione (Sigma) in 50 mM Tris/HCl, pH 8.0. Purified proteins were used to raise antisera in New Zealand White rabbits using standard protocols issued by HTI Bioproducts or by the Biological Core Facility at the University of Ireland, Dublin, Ireland.

SDS-PAGE and Western blot transfer. SDS-PAGE utilized tricine gels containing 10% acrylamide (Schägger & von Jagow, 1987). All samples (described above) were heat-denatured under reducing conditions. Separated proteins were transferred to PVDF membrane (Immobilon-P; Millipore) with a semi-dry transfer cell (Bio-Rad Laboratories). Purified proteins (1 µg each) were subjected to SDS-PAGE and stained with Coomassie brilliant blue. E. coli lysates or lysate fractions were obtained as follows. IPTG-induced recombinant E. coli cells were grown to an OD600 of 2.0 (using a Beckman DU-70 spectrophotometer with a quartz cuvette having a light path of 1 cm), washed and resuspended to original volume in PBS, and prepared for SDS-PAGE. Samples (10 µl) of each preparation were loaded into individual wells of acrylamide gels. S. epidermidis strains were grown to early-stationary phase in TSB containing the endoproteinase inhibitor x2–macroglobulin (0.125 U ml–1) (Boehringer Mannheim). The cells were adjusted to an OD600 of 2.0, washed and resuspended in one-half the original volume. Protease inhibitors (4 mM PMSE, 1 mM N-ethylmaleimide and 25 mM aminohexanoic acid) and DNase (10 µg ml–1) were added prior to lysostaphin (100 µg ml–1) and lysozyme (100 µg ml–1). Enzymic digestions were performed for 30 min at 37 °C with shaking. Separation of cell-wall proteins from protoplasts utilized the same conditions in the presence of 30% raffinose. S. epidermidis lysates or lysate fractions were treated as described for E. coli lysates, and 30 µl of sample was placed into wells of acrylamide gels.

Western immunoassays. Membranes containing transferrred proteins were incubated in PBS containing 1% non-fat dry milk for 1 h. The membranes were then incubated with antiserum (diluted in PBS-milk) for 1 h. Monoclonal anti-histidine antibody (Clonetech) was diluted 1:30000; anti-SdrFA antisera (immune, preimmune and antigen-absorbed) were diluted 1:3000; anti-SdrGA antisera were diluted 1:2000 and anti-SdrHA antisera were diluted 1:1000. Potential cross-reactive antibodies present in the anti-SdrFA and -SdrGA antisera were removed with a previously described antiserum absorption method (McCrea et al., 1997). Briefly, anti-SdrFA and anti-SdrGA antisera were extensively absorbed with GST-fused SdrGA and SdrFA proteins, respectively, present in insoluble fractions of induced and sonicated E. coli. Similarly, removal of specific anti-SdrFA, -SdrGA and -SdrHA antibodies was accomplished by absorbing each antiserum with insoluble E. coli fractions containing GST-fused SdrFA, SdrGA and SdrHA, respectively. Following
antiserum incubation, the membranes were washed three times with PBS and incubated with a 1:2000 dilution of goat, anti-rabbit or anti-mouse IgG conjugated to alkaline phosphatase (Bio-Rad Laboratories) for 30 min. The blots were then washed and developed in chromogenic substrate (150 µg 5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt ml⁻¹ and 300 µg p-nitro blue tetrazolium chloride ml⁻¹ in bicarbonate buffer) (Bio-Rad) for 10–15 min.

ELISA. Reactivity of convalescent patient IgG to recombinant proteins has been previously described (Casolini et al., 1998). Antisera from 16 individuals recovering from S. epidermidis infections were collected and IgG was purified using protein A-Sepharose chromatography. Control antibodies included eight antisera from age-matched, healthy adults and pooled antiserum from eight children (2 years of age). ELISA was used to demonstrate reactivity of IgG (2 µg per well) to recombinant proteins (1 µg per well of histidine-fused SdrFA or SdrGA, or GST-fused SdrHA) coated on microtitre plates.

RESULTS
Identification of the sdrf, sdrG and sdrH genes
Initial Southern hybridization analysis of S. epidermidis DNA revealed the presence of several loci hybridizing with DNA encoding the SD repeats of the S. aureus ClfA protein (data not shown). To further define these loci, we cloned three DNA fragments from S. epidermidis strains 9491 and K28. Two clones, pC5 and pC28, were obtained from strain 9491 by direct ligation of HindIII-digested DNA fragments into E. coli plasmid vectors. A third clone, E6-2, was obtained from a λ Gem-11 genomic library made from strain K28. A segment of the E6-2 insert DNA was subcloned into an E. coli plasmid vector to form pE6-2. pC5, pE6-2 and pC28 were found to have 6/8, 6/0 and 2/0 kb DNA inserts, respectively (not shown).

DNA sequence analysis revealed the presence of single ORFs in each plasmid. The ORFs, designated sdrF, sdrG and sdrH, were 5199, 2793 and 1461 bp in length, respectively. A codon encoding leucine rather than methionine is predicted to act as a translational start codon for SdrG. A potential ribosome-binding site (GGAG) was identified 7–12 bp 5’ of each ORF. DNA sequences of 500–1000 bp flanking the sdrF, sdrG and sdrH ORFs were not similar to each other, suggesting that they are not tandemly linked like the sdrC, sdrD and sdrE genes of S. aureus (data not shown).

Deduced amino acid sequences of SdrF, SdrG and SdrH
The primary sequence organization of the deduced S. epidermidis SdrF and SdrG proteins are similar to the S. aureus Sdr proteins and thus have features typical of cell-surface proteins that are covalently anchored to the peptidoglycan of Gram-positive bacteria (Fig. 1b, c). These features include positively charged residues at the extreme C terminus preceded by a hydrophobic, membrane-spanning region and an LPXTG motif. SD-repeat regions of S. aureus Sdr proteins end just N terminal of the LPXTG motifs and are proposed to traverse the cell wall (Hartford et al., 1997; Kehoe, 1994). Similarly, the SD-repeat regions of the S. epidermidis SdrF and SdrG proteins are proximal to the LPXTG motifs (Fig. 1c) and these regions contain 558 and 56 residues, respectively (Fig. 1b). The serine and aspartate dipeptide composition does not diverge in SdrG, whereas twenty-six alanine residues occur within the SD-repeat region of SdrF. At their N termini, SdrF and SdrG contain potential signal sequences (52 and 50 residues, respectively) that are predicted to facilitate secretion of the proteins across the bacterial membrane (von Heijne, 1983). The predicted molecular masses of the mature proteins (after removal of the signal peptides but before processing at the LPXTG motif) are 179 kDa for SdrF and 97.6 kDa for SdrG.

Structurally distinct, known or putative ligand-binding domains, called A regions, are present at the N termini of the S. aureus Sdr proteins (Josefsson et al., 1998a; McDevitt et al., 1995; Ni Eidhin et al., 1998). Similarly, mature SdrF and SdrG of S. epidermidis possess N-terminal A regions of 625 and 548 amino acids, respectively. Pairwise comparisons revealed that the A regions of SdrF and SdrG are 22% identical to each other and 20–35% (mean 23%) identical to the A regions of the S. aureus Sdr proteins. The SdrG protein is almost identical to the fibrinogen-binding protein, Fbe, of S. epidermidis described previously (Nilsson et al., 1998; Pei et al., 1999). We have also shown that the A region of SdrG binds fibrinogen (unpublished results).

Amino acid sequence motifs have been reported in the A regions of S. aureus Sdr proteins; these include a putative, Ca²⁺-binding EF-hand motif in ClfA, a cation-coordinating MIDAS motif in ClfB, and a common Sdr protein motif, TYTFTDYVD, of unknown function (Josefsson et al., 1998a; O’Connell et al., 1998). The A regions of SdrF and SdrG both contain a TYTFTDYVD motif, and the A region of SdrG contains a sequence (DSEYEDVTNDDY) reminiscent of an EF-hand motif.

B-repeat regions are present in variable numbers in three out of five S. aureus Sdr proteins (SdrC, SdrD and SdrE) (Fig. 1a). Each repeat is composed of 110–113 amino acids and contains an EF-hand motif (Josefsson et al., 1998a; b). Likewise, SdrF contains four B-repeat regions (of 119, 110, 111 and 111 residues), and SdrG contains two B-repeat regions (of 113 and 111 residues) (Fig. 1b). Each B-repeat in SdrF and SdrG contains an EF-hand motif with a consensus sequence of DX(N/D)(X/D/N)-GXX(D/N/G)XX(E/D). The B-repeat regions of SdrF and SdrG have 43–85% (mean 55%) identity with each other and 39–73% (mean 54%) identity to the B-repeat regions of the S. aureus Sdr proteins.

The primary sequence organization of SdrH is considerably different from that of SdrF and SdrG. Following a potential 30-residue signal sequence at its N terminus, SdrH has a unique 60-residue stretch, designated as an A region, followed by a 120-residue SD-repeat region and a 277-residue segment, designated the C region. The C region contains a hydrophobic sequence
Fig. 2. Prevalence of the sdr genes in S. epidermidis strains. Southern blots contain S. epidermidis genomic DNA hybridizing to DNA probes encoding the (a) SD-repeat region, (b) SdrH A region, (c) SdrG A region and (d) SdrG and SdrF A regions. Lanes: 1, ATCC 14990; 2, KH11; 3, K28; 4, RP62a; 5, TU3298; 6, 9142; 7, 1457; 8, 8400; 9, N910308; 10, N910160; 11, N910102; 12, N910173; 13, N910191; 14, N910231; 15, N910249. Strain 9491 is not shown. Size markers (kb) are shown at the left of the blots.

at its C terminus but lacks a typical LPXTG motif. Instead, the sequence LGVTG occurs within, rather than precedes, the hydrophobic region. (Fig. 1b, c). SdrH contains no B-repeat regions. The A and C regions of SdrH have no amino acid sequence similarities with other known Sdr proteins or protein sequences from various databases. Furthermore, motifs common to other Sdr proteins were not found. The mature molecular mass of SdrH is predicted to be 50-5 kDa. The unique structural organization of SdrH may imply that the original clone resulted from cloning noncontiguous chromosomal DNA fragments. This event is unlikely since the DNA insert in pC28 was of similar size to that observed on Southern blots (see Fig. 2), there are no internal HindIII sites in the insert DNA of pC28, and sequencing of an independent clone (obtained from S. epidermidis strain 9142) revealed a similar deduced protein structure (unpublished results).
Sdr proteins of S. epidermidis

Fig. 3. Specificity of the recombinant Sdr A region antisera to their respective proteins. (a) Coomassie-stained SDS-PAGE of purified proteins used to raise rabbit polyclonal antisera. Histidine-fused SdrFA and SdrGA, (lanes 1 and 2, respectively) and GST-fused SdrHA (lane 3) are shown. (b) Specificity of the anti-SdrFA and -SdrGA antisera is shown against GST-fused SdrFA (lane 1), SdrGA (lane 2) and SdrHA (lane 3). GST fusions were used to avoid detecting anti-histidine antibodies potentially induced by the original antigens. The left panel shows the reactivity of individual anti-SdrFA, -SdrGA and -SdrHA antisera to E. coli lysates expressing the GST fusions listed above. The middle and right panels show the reactivity of anti-SdrFA and -SdrGA antisera, respectively, to the same proteins. (c) Specificity of the anti-SdrHA antiserum is demonstrated against histidine-fused SdrFA (lane 1), SdrGA (lane 2) and full-length SdrH (lane 3). Histidine fusions were used to avoid detecting reactivity of GST antibodies potentially induced by the original antigen. The left panel shows the positions of the recombinant proteins present in E. coli lysates using an anti-histidine monoclonal antibody. The right panel shows the reactivity of anti-SdrHA antiserum to the same proteins. Size markers (kDa) are shown at the left of the gels/blots.

Fig. 4. Immunoblot analyses of Sdr proteins expressed in S. epidermidis. (a) Reactivity of anti-SdrFA immune antiserum (lane 1), preimmune antiserum (lane 2), or SdrFA-absorbed immune antiserum (lane 3) to a Western blot containing a S. epidermidis 9491 lysate. (b) Reactivity of anti-SdrGA immune (lane 1), preimmune (lane 2) or SdrGA-absorbed immune (lane 3) antisera to a lysate of S. epidermidis strain K28. (c) Reactivity of anti-SdrHA immune (lane 1) and SdrHA-absorbed immune (lane 2) antisera to a lysate of S. epidermidis 9491. Size markers (kDa) are shown to the left of the blots.

Distribution of sdrF, sdrG and sdrH in S. epidermidis strains

In Southern hybridization analysis, a DNA probe representing the encoding region of the ClfA SD-repeats hybridized to several genomic HindIII fragments in sixteen S. epidermidis strains (Fig. 2a). Three hybridizing fragments were observed in most strains, presumably representing the sdrF, sdrG and sdrH genes. To confirm this and determine the frequency of the different genes within these strains, additional analyses were performed with probes specific for DNA encoding each A region. The sdrH probe hybridized to fragments between 1-8 and 6-5 kb in all strains (Fig. 2b). The sdrG probe hybridized to a 16 kb fragment in all strains examined (Fig. 2c), and to 3-4 kb fragments in four of the sixteen strains (KH11, K28, RP62a and N910102). The same 3-4 kb fragments, however, did not hybridize with a probe specific for DNA encoding SD-repeats (Fig. 2a). These results suggest the presence of a gene with similarity to the sdrG A region that lacks a SD-repeat region, or alternatively, a second sdrG gene having an internal HindIII site. DNA encoding the SD-repeats of the second gene would be expected to reside on the 16 kb fragment noted above. Fig. 2(d) shows a Southern blot probed with both sdrG and sdrF A-region DNA. The sdrF probe hybridized to HindIII-digested DNA fragments between 4.5 kb and 10 kb in 12 out of 16 strains (strains K28, RP62a, N910173 and N910191 lacked a hybridizing band). These results suggest that the sdrF, sdrG and sdrH genes are prevalent in S. epidermidis strains.

Together, these results suggest that S. epidermidis has the capacity to express two proteins related to the S. aureus Sdr protein family, as well as a third Sdr protein with a novel structural organization.
Expression of SdrF, SdrG and SdrH in *S. epidermidis*

Immunological methods were used to determine if SdrF, SdrG and SdrH are expressed by *S. epidermidis*. Specific rabbit antisera were raised to recombinant fusion proteins representing the different A regions (designated SdrFA, SdrGA and SdrHA). SdrFA and SdrGA antigens were expressed as polyhistidine (His₉) fusion proteins, and SdrHA was expressed as a GST fusion protein (Fig. 3a). Monospecificity of each antiserum was confirmed against a panel of recombinant proteins present in *E. coli* lysates on Western blots. Specifically, anti-SdrFA and anti-SdrGA antisera did not recognize recombinant SdrGA and SdrFA, respectively, and neither of these antisera recognized recombinant SdrHA (Fig. 3b). Antiserum raised to SdrHA recognized a full-length, His₉–SdrH protein but not SdrFA or SdrGA proteins (Fig. 3c).

The A-region-specific antisera were used to identify native SdrF, SdrG and SdrH in lysates of their cognate *S. epidermidis* strains by Western immunoblotting. The anti-SdrFA antiserum reacted with an approximately 230 kDa band from strain 9491 (Fig. 4a). This band was not present when Western blots were reacted with preimmune antiserum or with anti-SdrFA antiserum that had been absorbed with a recombinant GST–SdrFA fusion protein present in the insoluble fraction of *E. coli* lysates (Fig. 4a). The anti-SdrGA antiserum recognized a 170 kDa band in a lysate of *S. epidermidis* strain K28. This band was not present with preimmune antiserum or with anti-SdrFA antiserum that had been absorbed with a GST–SdrGA fusion protein present in an *E. coli* lysate (Fig. 4b). Antiserum to SdrHA recognized a 75 kDa band in strain 9491, and this reactivity could be removed by absorbing the antiserum with recombinant SdrH present in an *E. coli* lysate (Fig. 4c). The apparent molecular masses of the anti-SdrFA, -SdrGA and -SdrHA immunoreactive bands are larger than the masses predicted from the deduced amino acid sequences (230, 170, and 75 kDa observed vs 179, 97 and 50 kDa predicted, respectively). Decreased migration on SDS-PAGE has been previously noted for two *S. aureus* Sdr proteins, ClfA and ClfB. The acidic nature of the Sdr proteins has been suggested to account for these observations (Ni Eidhin et al., 1998).

### Molecular mass differences of SdrH in *S. epidermidis* strains

Western blot analysis of different strains of *S. epidermidis* revealed that SdrH varied between 60 and 75 kDa (Fig. 5a). Differences in the molecular mass of ClfA among different *S. aureus* strains have been previously correlated with variations in the length of the SD-repeat region (McDevitt & Foster, 1995). PCR
Sdr proteins of \textit{S. epidermidis}

![Graph](https://via.placeholder.com/150)

**Fig. 7.** Reactivity of IgG from healthy controls or patients convalescing from \textit{S. epidermidis} infections to recombinant SdrFA (a), SdrGA (b) and SdrHA (c) coated in ELISA microtitre plates. Error bars show standard deviations obtained from three sample points.

**Analyses of SdrF, SdrG and SdrH in cell-wall extracts and protoplasts**

The presence of a LPXTG motif in both SdrF and SdrG suggests that these proteins are anchored in the cell wall and would therefore be present in cell-wall extracts of lysostaphin-treated \textit{S. epidermidis}. Western blot analyses of \textit{S. epidermidis} strain 9491 grown to early-stationary phase and treated with lysostaphin showed that the anti-SdrFA antiserum recognized the 230 kDa SdrF band in both the whole-cell lysate and the cell-wall extract but not in the protoplast fraction (Fig. 6a). These results suggest that SdrF is processed by a procedure characteristic for cell-wall anchored proteins in Gram-positive bacteria.

In contrast, analysis of the same samples with anti-SdrGA antiserum revealed the presence of intact SdrG (170 kDa) in the lysate and protoplast fractions but not in the cell-wall extract (Fig. 6b). The cell-wall fraction, however, contained smaller immunoreactive components that could represent degradation products of...
SdrG. Similar results were observed with blots containing lysostaphin-treated strain K28 (not shown). Further analysis of the 9491 lysostaphin fractions, used above, with anti-SdrHA antiserum revealed an immunoreactive band in both the lysate and protoplast fractions but not in the cell-wall extract (Fig. 6c). These results suggest that, under these in vitro conditions, SdrF is localized and anchored to the cell wall, and that SdrG (despite its LPXTG motif) and SdrH are either degraded from the cell surface or associated with the cytoplasmic membrane.

**Reactivity of convalescent patient antisera to SdrF, SdrG and SdrH**

Recently, IgG from patients recovering from *S. aureus* infections have been shown to react with the fibronectin-binding protein (FnbpA), suggesting that FnbpA is expressed by *S. aureus* during infection (Casolini et al., 1998). Here, IgG purified from the antisera of 16 patients recovering from various *S. epidermidis* infections were tested by ELISA for reactivity with the recombinant SdrF, SdrG and SdrH A region proteins. Fig. 7 shows that IgG from patients’ antisera had higher reactivity to SdrG and SdrH compared to that of IgG purified from pooled antisera of children or eight antisera obtained from age-matched healthy donors. The patients’ IgG reactivity to SdrFA was negligible. These results suggest that SdrG and SdrH proteins may be expressed during *S. epidermidis* infection in humans.

**DISCUSSION**

*S. epidermidis* is the major cause of infections associated with indwelling medical devices. The organism is an ubiquitous commensal of human skin and has the ability to adhere to unconditioned biomaterial surfaces. Thus, device-related infections may be initiated by *S. epidermidis* adhering to ‘naked’ surfaces prior to implantation. In contrast, *S. aureus* can initiate foreign-body infection following device implantation via adherence to the fibronectin and fibrinogen coating the device surface. The presence of Sdr proteins in *S. epidermidis* is also possible for coagulase-negative staphylococci.

SdrF and SdrG have both organizational and sequence similarity to the Sdr proteins of *S. aureus*, whereas the SdrH protein is very different. SdrG is very similar to a fibrinogen-binding protein, Fbe, recently found in *S. aureus* (Nilsson et al., 1998). B-repeat regions, however, were not described for Fbe; the protein was thought to have a 773 amino acid A region linked directly to the SD-repeat region. In fact, Fbe contains two B-repeats that have 99% amino acid identity to the B-repeat regions of SdrG. The minimum fibrinogen-binding region of Fbe (residues 269–599), described by Nilsson et al. (1998) is now located at the C terminus of the A region, directly adjacent to the first B-repeat region. The A regions of Fbe and SdrG are 93% identical, and the minimum fibrinogen-binding regions are 98% identical. Pei et al. (1999) recently used a recombinant protein representing residues 87–646 of Fbe to show that the protein bound the β chain of fibrinogen.

A cell-wall spanning region of at least 72 residues (including the SD-repeat region) from the end of the A region to the LPXTG motif is required for the expression of functional ClfA (Hartford et al., 1997). In SdrG of *S. epidermidis* strain K28, there are only 73 residues between the end of the B-repeat region and the LPXTG motif, barely enough to permit expression of a functional ligand-binding region on the cell surface. The fibrinogen-binding A region of SdrG, however, is separated from the SD repeats by two B-repeat regions. As shown with SdrD of *S. aureus*, each B-repeat folds independently in a Ca²⁺-dependent fashion to form a rigid structure potentially capable of projecting the A region away from the cell surface (Josefsson et al., 1998a).

Unlike other Sdr proteins, the SD-repeat region of SdrH is very close to the N terminus rather than the C terminus; the SD repeats are separated from a potential sortase recognition sequence (LGVTG) by the 270-residue C region. The SdrH protein is, however, absent from the lysostaphin-generated cell-wall fraction, and this could be due to the lack of a typical LPXTG motif. If we assume that, like other Sdr proteins, the N-terminal A region is surface-exposed and that the SD-repeat region transverses the cell wall, the C region would be positioned between the cell wall and the membrane. In this case, SdrH would certainly have an unorthodox mechanism of anchoring to the cell surface.

The absence of SdrG from the cell-wall fraction and its association with protoplasts may suggest that cell-wall-anchored SdrG is proteolytically cleaved during the assay. Pei et al. (1999), however, recently showed that fibrinogen binding to one *S. epidermidis* strain, strain 19, could be inhibited by recombinant Fbe, the SdrG-homologous protein, or by antiserum raised to Fbe, suggesting that Fbe was expressed and functional on the surface of *S. epidermidis*. In an earlier study, the same authors demonstrated that strains of *S. epidermidis* varied greatly in their ability to bind fibrinogen and, in fact, the *fbe* gene was cloned from a low-fibrinogen-binding strain (strain HB) (Nilsson et al., 1998). Since the 16 strains analysed in the present study carry the *sdrG* gene, it is likely that most *S. epidermidis* strains have the ability to express SdrG and bind fibrinogen on their cell surfaces. Factors regulating the surface expression of SdrG in an *in vivo* or *in vitro* environment have, however, not been identified. An alternative hypothesis for limited SdrG surface expression may include an LPXTG motif that acts as an inefficient substrate for sortase. This would result in a protein that preferentially remains anchored to the cytoplasmic membrane. Analogously, the collagen-binding protein of *S. aureus*, Cna, is also sorted inefficiently and remains partly associated with the protoplast envelope (Hartford et al., 1999; Schneewind et al., 1993). More detailed
analyses of the topological orientation and organization of the Sdr proteins in *S. epidermidis* are clearly warranted.

Southern hybridization showed that the *sdrG* and *sdrH* genes are present in all 16 strains examined whilst *sdrF* is present in 12 of the 16 strains. Thus, the *sdr* genes are ubiquitous in a wide range of *S. epidermidis* isolates from different geographic locations. The presence of A-region-reactive antibodies in the sera of patients recovering from *S. epidermidis* infections suggests that these proteins can also be expressed in the host during infection.

In conclusion, *S. epidermidis* contains members of the Sdr protein family that are both similar and distinct from those of *S. aureus*. Further studies on these Sdr family members may reflect the diversity seen between *S. epidermidis* and *S. aureus* in human colonization and pathogenicity.

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