Molecular Cloning of a 32-Kilodalton Lipoprotein Component of a Novel Iron-Regulated *Staphylococcus epidermidis* ABC Transporter

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Our previous studies identified two iron-regulated cytoplasmic membrane proteins of 32 and 36 kDa expressed by both *Staphylococcus epidermidis* and *Staphylococcus aureus*. In this study we show by Triton X-114 phase partitioning and tritiated palmitic acid labelling that these proteins are lipoproteins which are anchored into the cytoplasmic membrane by their lipid-modified N termini. In common with some of other Gram-positive bacteria, these highly immunogenic lipoproteins were released from the bacterial cell into the culture supernatants, with release being promoted by growth of the bacteria under iron-restricted conditions. Immunoelectron microscopy with a monospecific rabbit antiserum to the 32-kDa *S. epidermidis* lipoprotein showed that the majority of the antigen was distributed throughout the staphylococcal cell wall. Only minor quantities were detected in the cytoplasmic membrane, and exposure of the lipoprotein on the bacterial surface was minimal. A monoclonal antibody raised to the 32-kDa lipoprotein of *S. aureus* was used in immunoblotting studies to investigate the conservation of this antigen among a variety of staphylococci. The monoclonal antibody reacted with polypeptides of 32 kDa in *S. epidermidis* and *S. aureus* and of 40 kDa in *Staphylococcus hominis*. No reactivity was detected with *Staphylococcus lugdunensis*, *Staphylococcus cohnii*, or *Staphylococcus haemolyticus*. The gene encoding the 32-kDa lipoprotein from *S. epidermidis* has been isolated from a Lambda Zap II genomic DNA library and found to be a component of an iron-regulated operon encoding a novel ABC-type transporter. The operon contains three genes, designated *sitA*, *-B*, and *-C*, encoding an ATPase, a cytoplasmic membrane protein, and the 32-kDa lipoprotein, respectively. SitC shows significant homology both with a number of bacterial adhesins, including FimA of *Streptococcus parasanguis* and ScaA of *Streptococcus gordonii*, and with lipoproteins of a recently described family of ABC transporters with proven or putative metal ion transport functions. Although the solute specificity of this novel transporter has not yet been determined, we speculate that it may be involved in either siderophore- or transferrin-mediated iron uptake in *S. epidermidis*.

Staphylococci respond to iron deprivation in vitro and in vivo by increasing the expression of a number of iron-regulated proteins (24, 28, 36, 41). These include a 42-kDa cell-wall-associated transferrin binding protein and two cytoplasmic membrane proteins of 32 and 36 kDa whose functions are at present unknown (28, 36). The association of the latter proteins with the cytoplasmic membrane is based on cell fractionation data, but the mechanism of anchorage of these proteins to the cytoplasmic membrane is also currently unclear. In other bacteria, some cytoplasmic membrane proteins are integral to the membrane and are held in place by hydrophobic membrane-spanning regions or hydrophobic N- or C-terminal anchors (31). Other membrane-associated proteins have been shown to be lipoproteins, and these are tethered to the outer surface of the membrane by their lipid-modified N termini (17, 38). Functionally, both groups of proteins may be components of ATP binding cassette (ABC)-type transporters involved in solute acquisition (13, 39). These multicomponent transporters have common structural and organizational features, and functions in addition to solute transport have recently been attributed to some ABC transporter lipoproteins. Studies with Gram-positive bacteria show that the lipoproteins SarA and SsaB mediate adherence of *Streptococcus gordonii* and *Streptococcus sanguis*, respectively, to salivary pellicle components (15, 22, 29). FimA from *Streptococcus parasanguis* is involved in attachment of the bacterium to fibrin clots in a rat endocarditis model and to platelet fibrin matrix clots in vitro (14, 40). These functional studies and other antibody binding studies support the surface location of these streptococcal lipoproteins (20, 21, 40). The *Enterococcus faecalis* lipoprotein EfaA also shows sequence homology with streptococcal adhesins, including FimA, SsaB, and ScaA, indicating that EfaA may also be a surface-exposed adhesin (25).

In contrast to the case for other Gram-positive organisms, ABC transporters in the staphylococci have been little studied. Proteins that may function as components of an ABC transporter involved in erythromycin resistance have been detected in *Staphylococcus epidermidis* (32), but this system has not been fully characterized and no homologies with such systems in other bacteria have yet been reported. ABC exporters are implicated in the secretion of the lantibiotics epidermin (13) and Pep5 (26) by *S. epidermidis* and of gallidermin (13) by *Staphylococcus gallidermidis*. DNA sequence analysis has also identified an ABC transporter of unknown function in *Staphy-
Staphylococcal lipoprotein is the β-lactamase of \textit{S. aureus} (2, 30), which is found both in a membrane-bound form and in the bacterial culture supernatant (a common finding for other gram-positive lipoproteins [38]). There is currently no published information on transport systems involved in iron acquisition by the staphylococci.

Our ongoing interest in staphylococcal iron uptake mechanisms has led us to further characterize the role of the staphylococcal 32- and 36-kDa iron-regulated cytoplasmic membrane proteins as possible components of iron transport systems. This paper describes the identification of these iron-regulated proteins as lipoproteins and the molecular cloning of the 32-kDa lipoprotein from \textit{S. epidermidis}, which sequence analysis reveals to be a component of a novel ABC-type transporter. Immunoelectron microscopy has also been used to confirm the cellular location of this lipoprotein in \textit{S. epidermidis}, and immunoblotting has been used to investigate the conservation of these lipoproteins among other staphylococcal species.

**MATERIALS AND METHODS**

**Staphylococcal strains and growth conditions.** \textit{S. aureus}, \textit{S. epidermidis}, \textit{Staphylococcus hominis}, \textit{Staphylococcus cohnii}, \textit{Staphylococcus lugdunensis}, \textit{Staphylococcus warneri}, and \textit{Staphylococcus haemolyticus} clinical isolates were obtained from the University and City Hospital NHS Trusts, Nottingham, United Kingdom. \textit{S. aureus} BB (originally isolated from a case of bovine mastitis [5] and 8325-4) were provided by J. P. Arbutnott. Strains were maintained by regular subculture on horse blood agar.

For broth culture, strains were grown statically for 18 h at 37°C in RPMI 1640 tissue culture medium containing 2 mg of NaHCO\textsubscript{3} per ml. Cultures were incubated in 5% CO\textsubscript{2} in air, and where indicated the medium was supplemented with 0.1% (wt/vol) SDS at room temperature and in 0.5 M Fe\textsubscript{2}(SO\textsubscript{4})\textsubscript{3} to produce iron-rich growth conditions.

**Polyclonal and monoclonal antibody production.** Anti-\textit{S. aureus} BB wall antibody preparations were raised in adult female BALB/c mice. The cell wall extract was prepared by digestion of whole bacterial cells, grown under iron-restricted conditions, with lysostaphin in the presence of 30% (wt/vol) raffinose (36, 41). Mice were bled 2 weeks after the third immunization. Splenocytes recovered from these mice were also used to generate hybridomas by fusion with the myeloma cell line NS0 by standard methods. Hybridomas secreting anti-staphylococcal antibodies were selected by indirect enzyme-linked immunosorbent assay with \textit{S. aureus} BB cell wall extract as the antigen, and antigen specificity was confirmed by immunoblotting.

Rabbit monospecific antisera raised against the \textit{S. epidermidis} 32- and 36-kDa iron-regulated cytoplasmic membrane proteins were available from earlier studies (36, 41).

**Immunoelectron microscopy.** \textit{S. epidermidis} 901 was grown overnight in RPMI 1640. Bacteria were pelleted and fixed by resuspension in 1% (vol/vol) gluteraldehyde in phosphate-buffered saline, pH 7.4 (PBS) at room temperature for 2 h. Bacteria were pelleted and fixed by resuspension in 1% (vol/vol) gluteraldehyde in phosphate-buffered saline, pH 7.4 (PBS) at room temperature for 2 h. Bacteria were pelleted and fixed by resuspension in 1% (vol/vol) gluteraldehyde in phosphate-buffered saline, pH 7.4 (PBS) at room temperature for 2 h.
Triton X-114 phase partitioning experiments with Triton X-114 for growth of staphylococci for the present studies. Consequently, we used RPMI 1640 as a more convenient medium for growth of staphylococci under iron-restricted conditions. Qualitatively, the expression of iron-regulated proteins as lipoproteins was provided by results of labelling studies using [3H]palmitic acid. The 32- and 35-kDa polypeptides of *S. epidermidis* and the 32- and 35-kDa polypeptides detected in detergent extracts of *S. aureus* were radiolabelled when the bacteria were grown in RPMI 1640 under iron-restricted conditions (Fig. 2). Labelled polypeptides with these molecular masses were also detected in Triton X-114 extracts of the respective filtered culture supernatants from both staphylococcal species (Fig. 2).

**[3H]Palmitic acid labelling studies.** Confirmation of the identity of these major Triton X-114-extractable iron-regulated proteins as lipoproteins was provided by results of labelling studies using [3H]palmitic acid. The 32- and 35-kDa polypeptides of *S. epidermidis* and the 32- and 35-kDa polypeptides detected in detergent extracts of *S. aureus* were radiolabelled when the bacteria were grown in RPMI 1640 under iron-restricted conditions (Fig. 2). Labelled polypeptides with these molecular masses were also detected in Triton X-114 extracts of the respective filtered culture supernatants from both staphylococcal species (Fig. 2).

Antigenic relatedness between the staphylococcal lipoproteins. A mouse monoclonal antibody raised against the *S. aureus* BB 32-kDa lipoprotein was used in immunoblotting studies to investigate the conservation of this antigen among a range of staphylococcal species. All bacteria were grown in RPMI 1640 under iron-restricted conditions. A polypeptide of the appropriate size was detected in Triton X-114 extracts of each of four *S. epidermidis* strains and all seven strains of *S. aureus* tested (data not shown). A 40-kDa polypeptide was detected in the single strain of *S. hominis* tested, but no reaction with this antibody was observed by immunoblotting with single strains of *S. lugdunensis*, *S. cohnii*, and *S. haemolyticus* (data not shown).

**Localization of the 32-kDa lipoprotein in *S. epidermidis* by immunoelectron microscopy.** Immunogold labelling of thin sections of iron-restricted bacteria with a rabbit monospecific antibody to the *S. epidermidis* 32-kDa lipoprotein was used to further investigate the distribution of this antigen in the *S. epidermidis* cell. Figure 3A shows that the majority of the 32-kDa lipoprotein is distributed throughout the cell wall of *S. epidermidis*. Smaller quantities of this protein were located in the cytoplasmic membrane, and exposure of the antigen on the cell surface was minimal. Sections incubated with nonimmune rab-

FIG. 1. SDS-PAGE (A) and immunoblots (B) of Triton X-114 extracts prepared from *S. aureus* BB and *S. epidermidis* 901 grown under iron-restricted growth conditions. Lanes: 1, molecular mass markers; 2 and 3, whole-cell polypeptide profiles; 4 and 5, Triton X-114 extracts of intact cells; 6 and 7, Triton X-114 extracts of lysostaphin-digested cells; lanes 8 and 9, Triton X-114 extracts of filtered culture supernatants; 2, 4, 6, and 8, *S. aureus* BB; 3, 5, 7, and 9, *S. epidermidis* 901. The gel in panel A was silver stained, and immunoblots were reacted with a pool of monospecific rabbit antisera to the 32- and 36-kDa iron-regulated cytoplasmic membrane proteins of *S. epidermidis* (36). These polypeptides were not detected in the aqueous phase following detergent partitioning (data not shown). The relationship of these Triton X-114-extractable polypeptides to the previously described staphylococcal iron-regulated cytoplasmic membrane proteins was confirmed by immunoblotting with monospecific rabbit antisera (Fig. 1B). The minor differences between the molecular mass stated for one of the *S. aureus* proteins in the present study and those stated in our previous studies (36, 41) are attributable to minor variations in the electrophoresis technique and the source of molecular mass markers used.

Only trace quantities of these proteins were extractable from lysed bacteria grown under iron-rich growth conditions (data not shown) or from intact iron-restricted cells (Fig. 1A), indicating their regulation by iron availability and the restricted exposure of detergent-soluble forms of these proteins on the surfaces of intact staphylococci.

Triton X-114 extraction of filtered *S. epidermidis* culture supernatants showed that both the 32- and 36-kDa polypeptides were also present in supernatants from iron-restricted cultures but that these polypeptides were not detectable in iron-rich culture supernatants (data not shown). In contrast, small quantities of Triton X-114-extractable polypeptides of 32 and 35 kDa were found in supernatants of iron-rich *S. aureus* cultures (data not shown), although larger amounts of these polypeptides were detected in iron-restricted supernatants (Fig. 1A).

FIG. 2. Autoradiograph showing [3H]palmitate labelling of Triton X-114-extractable lipoproteins of *S. aureus* BB and *S. epidermidis* 901. Lanes: 1 and 2, whole-cell [3H]palmitate-labelled profiles; 3 and 4, Triton X-114 extracts of [3H]palmitate-labelled lysostaphin-digested cells; 5 and 6, Triton X-114 extracts of [3H]palmitate-labelled filtered culture supernatants; 1, 3, and 5, *S. aureus* BB; 2, 4, and 6, *S. epidermidis* 901.
Molecular cloning of the gene encoding the 32-kDa *S. epidermidis* lipoprotein. To further characterize the staphylococcal lipoproteins and investigate their potential functions, we sought to isolate the genes encoding these antigens from staphylococcal genomic DNA libraries. Initial antibody screening of an *S. epidermidis* 901 Lambda Zap II library with monospecific antisera to the 32- and 36-kDa iron-regulated cytoplasmic membrane lipoproteins failed to identify reactive plaques. Since our previous studies had indicated some antigenic cross-reactivity between iron-regulated proteins in different staphylococcal species (36), we rescreened the library with anti-*S. aureus* BB wall antibodies. This antiserum was raised against native staphylococcal antigen, in contrast to the denatured antigen used in the preparation of the monospecific anti-32- and anti-36-kDa

FIG. 3. Electron micrographs of thin sections of *S. epidermidis* 901 cells reacted with monospecific antiserum to the 32-kDa *S. epidermidis* lipoprotein. (A) Sections reacted with monospecific rabbit anti-32-kDa lipoprotein antibody and protein A-gold conjugate; (B) sections reacted with nonimmune rabbit serum and protein A-gold conjugate. Bars, 1 μm.
endonuclease mapping and subcloning of pW32 localized the 32-kDa lipoprotein was designated pW32. The specificity of the 32-kDa lipoprotein was confirmed by immunoblotting with monospecific anti-32-kDa antiserum (Fig. 4B). The plasmid encoding the lipoprotein was designated pW33. The entire DNA fragment was sequenced on both strands following synthesis of appropriate DNA primers, and the plasmid containing this fragment was designated pW32.

Characterization of the cloned DNA fragment. Restriction endonuclease mapping and subcloning of pW32 localized the gene encoding the 32-kDa lipoprotein to a 2.43-kb HpaI-EcoRI fragment. The plasmid containing this fragment was designated pW33. The entire DNA fragment was sequenced on both strands following synthesis of appropriate DNA primers, and three open reading frames encoding putative polypeptides of 28, 35, and 30 kDa were identified. We have designated the three open reading frames encoding putative polypeptides of 28, 35, and 30 kDa as sitA, sitB, and sitC, respectively.”

Southern blotting was used to investigate conservation of this operon among other staphylococcal strains. Probing with the 5.4-kb EcoRI insert from pW32 identified single copies of these genes in genomic DNAs from S. epidermidis 901 and 138; however, no signal was detected when DNA from S. aureus BB or 8325-4, S. lugdunensis, S. cohnii, or S. warneri was similarly probed (data not shown). In addition, although these genes could be amplified by PCR with appropriate sequencing primer pairs and S. epidermidis genomic DNA as the template, no products could be obtained when DNAs from the other staphylococcal isolates were used (data not shown).

The cloned S. epidermidis DNA locus shows significant homology with bacterial ABC transporters. Sequence comparisons indicated significant homologies at the DNA and protein levels between sitA, sitB, and sitC and their products and sequences of previously identified bacterial ABC transporters. Sequence comparisons yielded the highest homologies at the amino acid level are shown in Table 1. SitA shows significant homology with known or theoretical ATP binding proteins in a number of bacterial species, including Streptococcus gordonii and Streptococcus parasanguis. S. epidermidis SitA contains consensus ATP binding motifs, including a P loop (GPNGAGKS) (33). Secondary-structure analysis failed to identify potential membrane-spanning regions in this protein, suggesting a cytoplasmic location for the mature protein. Interestingly, SitA also shows homology with an ATP binding protein, FecE, involved in iron(III) dicitrate transport in the gram-negative pathogen Haemophilus influenzae.

SitB has significant homology with several ABC transporter membrane proteins, including those of Streptococcus gordonii and Streptococcus parasanguis. Other strong homologies include those with MntB, a protein involved in Mn2+ transport in Synechocystis spp., and YfeC, a putative iron transport protein in Yersinia pestis. Secondary-structure analysis identified seven hydrophobic transmembrane-spanning regions in this protein, suggesting a cytoplasmic location for the mature protein. Interestingly, SitB also shows homology with an ABC transport system in the gram-negative pathogen Haemophilus influenzae.

SitC, the 32-kDa S. epidermidis lipoprotein, shows significant homology with other ABC transporter lipoproteins, including EfaA, ScaA, and FimA in Enterococcus faecalis, Streptococcus gordonii, and Streptococcus parasanguis, respectively. These proteins are all thought to play a role in bacterial adherence, and all contain recently described conserved motifs that define the
family of bacterial adhesins designated ADHESINFAMILY or related periplasmic binding proteins (4). The staphylococcal lipoprotein has a consensus prelipoprotein signal peptide cleavage sequence (17) (ILAAACG), with the cysteine residue at position 18, a finding which confirms our biochemical analysis, and also contains an ATP binding motif (GWFEKAL DQAGKSTKDKN) (33).

**DISCUSSION**

The data presented here extend our previous observations on iron-regulated proteins in the staphylococci (36, 41). The iron-regulated cytoplasmic membrane proteins in S. epidermidis and S. aureus have been identified as lipoproteins, and the 32-kDa S. epidermidis lipoprotein has been shown to be a component of a novel, iron-regulated ABC transporter which shows organizational and sequence homologies with previously described transporters in several gram-positive and gram-negative bacterial pathogens.

At present it is unclear which specific solute(s) is bound by the 32-kDa lipoprotein in S. epidermidis. However, the iron-regulated nature of this ABC transporter provides further support for our previous suggestion that this staphylococcal cytoplasmic membrane lipoprotein may be involved in iron transport. This suggestion is supported by recent work by Dintilhac et al. (10, 11), who examined the DNA sequence of the [S. epidermidis](http://www.ncbi.nlm.nih.gov/nuccore/10069738?report=genbank) ABC transporter reported here and found structural similarities with a new family of solute binding proteins (cluster 9) that is distinct from the eight other families previously described by Tam and Saier (39). Members of cluster 9 share the property of proven or hypothetical binding of metallic cations, including Fe, Mn, and Zn. Our own recent unpublished data suggest that the S. epidermidis operon may also be regulated by Mn availability, raising the possibility that the 32-kDa S. epidermidis lipoprotein may be able to bind and transport a variety of metal ions. We are currently attempting to generate S. epidermidis mutants to address this possibility.

The spatial distribution of the 32-kDa lipoprotein within the cell wall of S. epidermidis as shown by immunoelectron microscopy is consistent with those of other solute binding lipoproteins in gram-positive bacteria. These proteins are anchored to the cytoplasmic membrane by a lipid-modified N terminus, with the solute-binding component projecting into or in some cases through the cell wall where they are exposed on the bacterial surface. However, the observation that SitC is located primarily within the cell wall of S. epidermidis initially appears to be at variance with results from our earlier cell fractionation studies which showed that this protein was associated with the staphylococcal cytoplasmic membrane (36, 41). This apparent anomaly can be resolved if, following removal of the cell wall by lysostaphin treatment, SitC remains tethered to the cytoplasmic membrane by its lipid tail. The lipoprotein would then be pelleted with the cytoplasmic membrane fraction of the lysed staphylococcal cells.

This distribution would potentially allow the 32-kDa lipoprotein to function in both siderophore-dependent and -independent iron acquisition pathways. The lipoprotein could act as a ferric siderophore receptor either exposed on the cell surface or within the porous matrix of the cell wall, into which low-molecular-mass molecules such as siderophores readily penetrate. The involvement of lipoproteins in ferrihydroxamate siderophore iron uptake has been previously reported for *Bacillus subtilis* (35), and more recent studies with *Corynebacterium diphtheriae* IRP1 suggest that this iron-regulated lipoprotein may also function as a siderophore receptor (34). Alternatively, the 32-kDa S. epidermidis lipoprotein may be involved in shuttling iron from receptor-bound transferrin or lactoferrin across the cell wall to the cytoplasmic membrane prior to uptake into the cell. Experiments to identify the solute specificity of the 32-kDa lipoprotein in S. epidermidis are under way.

Our immunoblotting data obtained by using a monoclonal antibody specific for the 32-kDa lipoprotein are consistent with our earlier studies which showed that this antigen was conserved among S. epidermidis and S. aureus isolates but was generally not expressed by other staphylococcal species (36, 41). The other staphylococci do, however, produce one or more major lipoproteins in the 30- to 40-kDa size range as shown by

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**TABLE 1. Sequence homologies of the S. epidermidis ABC transporter operon proteins with similar proteins in databases**

<table>
<thead>
<tr>
<th>Protein type (S. epidermidis protein)</th>
<th>Accession no.</th>
<th>% Identity</th>
<th>Organism(s) Protein/function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP binding protein (SitA)</td>
<td>X99127</td>
<td>100</td>
<td>S. epidermidis ATP binding protein/Fe transport?</td>
</tr>
<tr>
<td></td>
<td>L11577</td>
<td>43</td>
<td>Streptococcus gordonii ScaA-associated ATP binding protein?</td>
</tr>
<tr>
<td></td>
<td>M26130</td>
<td>46</td>
<td>Streptococcus parasanguinis FimA-associated ATP binding protein?</td>
</tr>
<tr>
<td></td>
<td>L55214</td>
<td>39</td>
<td>Treponema pallidum TroB?</td>
</tr>
<tr>
<td></td>
<td>U52850</td>
<td>38</td>
<td>Erysipelothrix rhusiopathiae EwIA-associated ATP binding protein?</td>
</tr>
<tr>
<td></td>
<td>L45003</td>
<td>34</td>
<td>H. influenzae FecE/iron(III) dictrate transport</td>
</tr>
<tr>
<td></td>
<td>L34630</td>
<td>33</td>
<td>Synechocystis spp. MmtA/Mn transport</td>
</tr>
<tr>
<td>Membrane protein (SitB)</td>
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<td>100</td>
<td>S. epidermidis Membrane protein/Fe transport?</td>
</tr>
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<td></td>
<td>M26130</td>
<td>48</td>
<td>Streptococcus parasanguinis FimA-associated membrane protein?</td>
</tr>
<tr>
<td></td>
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<td>46</td>
<td>Streptococcus gordonii ScaA-associated membrane protein?</td>
</tr>
<tr>
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<td>50</td>
<td>Streptococcus pneumoniae PapA lipoprotein/adhesion?</td>
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<td>Enterococcus faecalis EfaA/adhesion?</td>
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<td>33</td>
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<tr>
<td></td>
<td>L11577</td>
<td>48</td>
<td>Streptococcus gordonii ScaA/coaggregation adhesion</td>
</tr>
</tbody>
</table>

* A question mark indicates that the function of the protein is presently unknown.
SDS-PAGE analysis of Triton X-114 extracts (37), and we speculate that these lipoproteins may also be components of ABC transporters in these species. Based on these data, it also seems likely that the ~36-kDa and 35-kDa iron-regulated membrane lipoproteins produced by S. epidermidis and S. aureus, respectively, may also be components of other ABC transporters which may also have a role in iron acquisition. We are currently attempting to clone the genes encoding these lipoproteins to confirm this possibility.

Despite the strong antigenic and potential functional homology between the 32-kDa lipoproteins of S. epidermidis and S. aureus, Southern blot and PCR analyses surprisingly failed to detect any homology between the genes encoding these proteins at the DNA level under the experimental conditions used. A previous study has indicated that even within the highly conserved staphylococcal gene encoding HSP60, species-specific variations exist which are sufficient to allow species differentiation by hybridization techniques (16). It is therefore conceivable that such species-specific variation within the staphylococcal ABC transporter gene sequences may also exist and may account for our findings.

Interestingly, the 32-kDa S. epidermidis lipoprotein also shows significant sequence homology with a number of proteins known to be adhesins in other gram-positive bacteria. In these other organisms, the lipoproteins are clearly surface exposed and available for interaction with appropriate tissue or other surface receptors (20, 37). The limited exposure of the 32-kDa antigen on the surface of S. epidermidis as assessed by electron microscopy suggests, however, that it is unlikely that this lipoprotein plays a significant role in the adhesion of S. epidermidis. However, the observation that in both S. epidermidis and S. aureus, significant quantities of membrane lipoproteins are released into culture supernatants under iron-restricted growth conditions in vitro and in vivo (7) may indicate an extracellular function for these lipoproteins. The mechanism of release of the lipoproteins from the staphylococcal surface is presently unclear, but our Triton X-114 phase partitioning and [3H]palmitate labelling studies indicate that the released lipoproteins remain acylated. Similar findings have been reported for extracellular lipoproteins from Streptococcus mutans (37). A possible toxic role for the staphylococcal lipoproteins is supported by recent studies which indicate that membrane proteins of L forms of S. aureus may stimulate cytokine release from mammalian cells in vitro and may be lethal when administered to α-galactosamine-treated mice (1). The relationship between the lipoproteins described in the present studies and the toxic membrane proteins of 30 and 36 kDa isolated by Akashi et al. (1) is presently unclear but merits further investigation, since other bacterial lipoproteins have previously been shown to induce cytokine release from mammalian cells (15).

In addition to their potential toxic activity, the extracellular lipoproteins also induce a significant humoral antibody response in rats with chamber implants inoculated with staphylococci (27), and variable levels of antibodies to these lipoproteins are detectable in human sera from individuals with staphylococcal infection (36). This observation raises the serodiagnostic potential of these lipoproteins for some types of staphylococcal disease.

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REFERENCES


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