Wall Teichoic Acid Protects Staphylococcus aureus against Antimicrobial Fatty Acids from Human Skin

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Skin-colonizing gram-positive bacteria produce wall teichoic acids (WTAs) or related glycopolymers for unclear reasons. Using a WTA-deficient Staphylococcus aureus mutant, we demonstrated that WTA confers resistance to antimicrobial fatty acids from human sebaceous glands by preventing fatty acid binding. Thus, WTA is probably important for bacterial skin colonization.

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Bacterial life on mammalian skin depends on efficient adaptation strategies to cope with high salt concentrations and dryness. In addition, skin is protected by a variety of antibacterial molecules, such as antimicrobial peptides (16), bacteriolytic enzymes (14), and antibacterial fatty acids (AFAs) (6, 11, 23). The main source of free fatty acids is the sebum, produced by sebaceous glands, and differentiating keratinocytes of the stratum corneum, the outermost layer of the epidermis, which is composed of dead, keratin-filled cells. Sebaceous glands are found in nearly all mammals, and the composition of the sebum is remarkably species specific (12). Up to 47% of human sebum consists of free fatty acids with palmitoleic acid isomer (C_{16:1\alpha}) as the predominant monoenic AFA. Lauric acid (C_{12:0}) is the most potent saturated AFA (23). Palmitic acid (C_{16:0}), stearic acid (C_{18:0}), oleic acid (C_{18:1\beta}), and linoleic acid (C_{18:2\Delta9,12}) are the main fatty acids in the stratum corneum (9, 23).

While most skin-colonizing bacteria are harmless commensals, Staphylococcus aureus frequently causes endogenous infections, ranging from cutaneous infections to life-threatening sepsis and endocarditis (10). S. aureus has developed efficient strategies to survive in its natural niches, the human anterior nares and skin, and to evade the immune system (4, 8). However, only a few studies have previously addressed the molecular basis of staphylococcal resistance to AFA. The major surface protein expressed by S. aureus under iron-limited conditions, IsdA, has recently been shown to confer AFA resistance because it increases the bacterial surface hydrophilicity (2). In addition to proteins, cell wall glycopolymers such as the teichoic acids are thought to govern bacterial surface hydrophobicity. Such polymers are found in most gram-positive bacteria, forming a highly charged mesh within the cell wall (21). They often consist of alternating glycerolphosphate or ribitol-phosphate units, which are partially substituted by ε-amino and various glycosyl residues (13, 21). Teichoic acids are anchored in the cytoplasmic membrane via a glycolipid (lipoteichoic acid) or in the peptidoglycan via a phosphodiester link-age (wall teichoic acid [WTA]). A variety of roles in bacterial cell envelope processes and integrity have been assigned to WTA but the major functions of WTA have still remained elusive (21). Our group has recently generated a WTA-deficient S. aureus mutant and demonstrated that WTA is crucial for S. aureus nasal colonization and endovascular infection (19, 20, 22). The tagO gene disrupted in this mutant encodes an N-acetylglucosamine-phosphate transferase catalyzing the first step of WTA biosynthesis (24). The tagO mutant shows a total loss of WTA but seems to be unaffected in growth behavior and susceptibility to different antimicrobial peptides (19). However, the mutant exhibits increased resistance to human beta-defensin 3 (7).

In order to study the contribution of WTA to the surface hydrophobicity of S. aureus SA113, a frequently used laboratory strain (5, 19, 22), the affinities of the wild type and the tagO mutant for the hydrophobic solvent dodecan were compared by the microbial adhesion to hydrocarbon test (15). In fact, the hydrophilicity of the WTA-deficient mutant was considerably decreased compared to those of the parental and complemented mutant strains (Fig. 1), confirming the crucial impact of WTA on the physicochemical surface properties of S. aureus SA113.

FIG. 1. The WTA-deficient ΔtagO mutant has decreased surface hydrophilicity compared to the wild type and the complemented (compl.) mutant strain, as assessed by the microbial adhesion to hydrocarbon test. The percentages of bacteria associated with the hydrophilic phase are shown. Data represent means ± standard errors of the means from three independent experiments. *** P < 0.001; ns, not significant (in comparison to the wild-type value).
S. aureus. Subsequently, the MICs of a variety of saturated and unsaturated fatty acids occurring in human sebum and stratum corneum were determined (Table 1). Twenty-four-well plates with 50%-concentrated Müller-Hinton broth (Sigma) containing increasing concentrations of AFAs were inoculated with the bacterial strains, and the optical density was measured after 48 h of growth at 37°C. The tagO mutant showed a profound increase in susceptibility to all tested AFAs compared to the parental strain and the complemented mutant. The strongest MIC reductions were found for palmitoleic acid (sixfold) and linoleic acid (26-fold). In order to compare potential differences in susceptibility to the bactericidal activities of AFAs, bacteria grown overnight in 50%-concentrated Müller-Hinton broth were resuspended in phosphate-buffered saline (PBS) at an optical density of 0.5 at 578 nm, and 1 ml of each suspension was shaken with increasing concentrations of AFAs at 37°C. Incubation was stopped at different time points by dilution with PBS, and numbers of surviving bacteria were determined by counting CFU. Palmitoleic acid exhibited dose-dependent bactericidal activity to SA113, with the tagO mutant having 26-fold reduced survival compared to that of the wild type at 1.25 mM after 10 min of incubation (Fig. 2A). When different incubation times were used for a given concentration, the tagO mutant was much more rapidly killed than the parental strain, thereby confirming the crucial role of WTA in AFA resistance (Fig. 2B).

We assumed that the decreased surface hydrophilicity of the tagO mutant leads to loss of AFA resistance because the hydrophobic fatty acids can better penetrate the cell wall and bind more efficiently to the cytoplasmic membrane where the antimicrobial activity is exerted. In order to test this hypothesis, we resuspended bacteria grown overnight in PBS at optical densities of 0.05 at 578 nm as described above. For each of the suspensions, 100 μl was incubated with 0.5 μl (1 μg/μl) of fluorescently labeled palmitic acid (Invitrogen) for 5 min at 4°C, and bacterial fluorescence was measured in a flow cytometer at 530 nm (25,000 bacteria per experiment) (FACSCalibur; Becton Dickinson). The fluorescence data shown in Fig. 3 indicate the mean fluorescence levels from three independent experiments. In fact, the tagO-deficient mutant showed a strong increase in palmitic acid binding compared to the wild-type strain and the complemented mutant strain (Fig. 3).

Taken together, our study demonstrates that WTA protects S. aureus against skin AFAs. Notably, the susceptibility of the tagO mutant seems to increase with AFA length, suggesting that the level of WTA-mediated AFA resistance increases with AFA hydrophobicity. In concert with IsdA (2) and further AFA resistance mechanisms, such as the fatty acid-modifying

TABLE 1. WTA-deficient Sa113 tagO mutant is more susceptible to growth-inhibiting activity of AFAs than wild-type and complemented mutant strains

<table>
<thead>
<tr>
<th>Antimicrobial fatty acid</th>
<th>MIC (mM) against S. aureus</th>
<th>Wild type</th>
<th>tagO mutant</th>
<th>Complemented tagO mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric acid (dodecanoic acid)</td>
<td>1.71 ± 0.032</td>
<td>0.88 ± 0.062</td>
<td>1.15 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>cis-6-Hexadecenoic acid</td>
<td>0.56 ± 0.061</td>
<td>0.16 ± 0.017</td>
<td>0.57 ± 0.078</td>
<td></td>
</tr>
<tr>
<td>Palmitoleic acid (hexadecenoic acid)</td>
<td>0.44 ± 0.003</td>
<td>0.076 ± 0.003</td>
<td>0.32 ± 0.027</td>
<td></td>
</tr>
<tr>
<td>Linoleic acid (octadecadienoic acid)</td>
<td>1.08 ± 0.064</td>
<td>0.042 ± 0.0076</td>
<td>0.94 ± 0.11</td>
<td></td>
</tr>
</tbody>
</table>

* Purchased from Sigma.
* Purchased from Matreya LTT.
* Data represent means ± standard errors of the means from at least three independent experiments.

FIG. 2. The WTA-deficient tagO mutant is more susceptible to the bactericidal activity of AFAs than the wild-type strain. (A) Bacteria were exposed to the indicated concentrations of palmitoleic acid for 10 min. **, P < 0.005; ***, P < 0.001. (B) The wild type (■) and the tagO mutant (▲) were exposed to lauric acid (5 mM), cis-6-hexadecenoic acid (5 mM), and palmitoleic acid (1.25 mM) for the indicated times. Data represent means ± standard errors of the means from at least three independent experiments.
enzyme activity described for certain staphylococcal strains (1), WTA may enable S. aureus to survive on skin. Of note, most skin-colonizing bacteria, including corynebacteria, propionibacteria, micrococci, streptococci, and staphylococci, are gram positive and produce teichoic acids or related cell wall glycopolymers (17, 21). Thus, WTA may be a general strategy of gram-positive bacteria to evade killing by AFAs or other highly lipophilic antimicrobial molecules. The skin represents a complex ecosystem with a highly dynamic biodiversity, which can be altered by subtle changes in host defense molecule amounts (3). Accordingly, reduced levels of cis-6-hexadecenoic acid in atopic dermatitis patients have been associated with increased S. aureus skin colonization and, as a consequence, eczema exacerbation (18). Conversely, topical application of cis-6-hexadecenoic acid on skin leads to a decrease in S. aureus colonization (2). Hence, AFAs may become helpful drugs for treatment of skin infections. Moreover, inhibitors targeting highly conserved steps of WTA biosynthesis, such as the TagO enzyme, may render a large variety of bacteria susceptible to AFAs and other innate host defenses.

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