Surface proteins of *Staphylococcus aureus* are covalently linked to the bacterial cell wall by a mechanism requiring a COOH-terminal sorting signal with a conserved LPXTG motif. Cleavage between the threonine and the glycine of the LPXTG motif liberates the carboxyl of threonine to form an amide bond with the pentaglycyl cross-bridge in the staphylococcal peptidoglycan. Here, we asked whether altered peptidoglycan cross-bridges interfere with the sorting reaction and investigated surface protein anchoring in staphylococcal *fem* mutants. *S. aureus* strains carrying mutations in the *fema*, *femb*, *femAB*, or the *femX* genes synthesize altered cross-bridges, and each of these strains displayed decreased sorting activity. Characterization of cell wall anchor structures purified from the *fem* mutants revealed that surface proteins were linked to cross-bridges containing one, three, or five glycyl residues, but not to the ε-amino of lysyl in muropeptides without glycine. When tested in a *femAB* strain synthesizing cross-bridges with mono-, tri-, and pentaglycyl as well as tetracyglcal-monoseryl, surface proteins were found anchored mostly to the five-residue cross-bridges (pentaglycyl or tetracyglcal-monoseryl). Thus, although wild-type peptidoglycan appears to be the preferred substrate for the sorting reaction, altered cell wall cross-bridges can be linked to the COOH-terminal end of surface proteins.

Gram-positive bacteria display proteins on their surface as a means to interact with host tissues and to establish human infections (1, 2). The mechanism of surface protein anchoring to the bacterial cell wall has recently been established for protein A of *Staphylococcus aureus*. After synthesis in the cytoplasm, protein A is first initiated into the secretory pathway by an NH2-terminal signal (leader) peptide (3). A 35-residue COOH-terminal sorting signal is necessary and sufficient for the anchoring of protein A and functions to first retain the polypeptide within the secretory pathway (4). This allows proteolytic cleavage between the threonine and the glycine of the conserved LPXTG motif (5). The liberated carboxyl of threonine is amide linked to the amino of the pentaglycyl cross-bridge of the staphylococcal peptidoglycan, thereby tethering the COOH-terminal end of protein A to the bacterial cell wall (6, 7). This amide bond exchange mechanism displays striking similarity to the penicillin-sensitive transpeptidation reaction (8), during which the cell wall pentapeptide precursor is cleaved at the peptide bond between ε-alanylated alanine, while the liberated carboxyl of ε-alanylated alanine is amide linked to the free amino of the peptidoglycan cross-bridge (9), pentaglycyl in *S. aureus* (10). Elements involved in transpeptidation and the sorting reaction are conserved in Gram-positive bacteria (6, 11). Thus, it seems likely that cell wall sorting is a universal mechanism for the anchoring of surface proteins (6). If so, sortase, the enzymatic activity that is thought to catalyze this reaction, might also be found conserved in many different bacterial species and could provide a target for an antibacterial therapy that interferes with surface protein anchoring (6).

Although the presence of free amino groups is a common feature in the cross-bridges of bacterial peptidoglycans, the overall chemical nature of this structure varies between different Gram-positive bacteria (11). In staphylococci, the cross-bridge is composed of five glycyl, whereas in some streptococci and listerial species it consists of two alanyl and meso-diaminopimelic acid, respectively (11). Genetic analysis of staphylococcal methicillin resistance has provided insights into the synthesis of peptidoglycan cross-bridges. Staphylococcal strains expressing the penicillin binding protein PBP2a (PBP2) are resistant to most β-lactam antibiotics including methicillin (12–16). Genetic screens designed to identify elements that are also necessary for methicillin resistance yielded mutations in approximately ten different *fem* (auxes) genes (17–21). Some of these genes are involved in the synthesis of the pentaglycyl cross-bridge (22–26) or the amidation of ε-iso-glutamyl within the wall peptide (27, 28). The precise biochemical defect of other *fem* mutations is still unknown (29). Presently available staphylococcal strains harboring mutations in the *fema*, *femb*, and *femX* genes synthesize altered cell wall cross-bridges with either three glycyl (*femb*), one glycyl (*fema*), or a combination of no or one glycyl (29). The latter phenotype has been reported for a mutant with a combination of a *fema* mutation and a second one leading to a partial non-functional FemX protein (hereafter called *femX*) (29).

Biochemical studies on the synthesis of staphylococcal peptidoglycan revealed that the pentaglycyl cross bridge is synthesized via modification of the lipid II precursor (undeca-prenylphosphatase-MurNAC(1-1-Ala-d-1Gln-1-Lys-d-Ala-d-Ala)-[(1-4)-GlcNAC] (30–32). Three glycyl tRNA species are dedicated to this biosynthetic pathway (33–36). After being charged with amino acids, these tRNAs are thought to serve as substrate in a sequence of reactions that successively add glycine either directly to the ε-amino of lysyl or to the amino of another glycyl (31, 34). It seems likely that the *fema*, *femb*, and...
**Surface Protein Anchoring in S. aureus fem Mutants**

fem genes specify enzymatic activities that catalyze these reactions (29). In this model, one enzyme, presumably FemX, adds the first glycine to the -e-amino side chain of lysyl within lipid II (29). FemA and FemB are thought to each add two additional glycines, thereby synthesizing cross-bridges with three and five glycyl, respectively (29). It is conceivable that each Fem factor might recognize one of the three glycyl tRNA species (35, 36). If so, one would predict that mutations in any one of the three glycylic tRNA genes should cause the same phenotype as the corresponding fem mutants.

Here, we asked whether the sorting reaction of surface proteins can proceed in fem mutant staphylococci. As compared with wild-type cells, the half-life of surface protein precursors molecules was increased in fem mutant strains, suggesting that the sorting reaction is partially hindered. Characterization of anchor structures revealed that surface proteins were linked to tegidoglycan with cross-bridges harboring mono-, tri-, or pentaglycyl as well as tetracyglycyl-monoseryl. No surface protein was found attached to the -e-amino of lysyl, suggesting that the sorting reaction discriminates between certain peptidoglycan cross-bridges.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Plasmids—**Plasmids pHTR4 (7) and pSeb-Cws-BlaZ were introduced into strains harboring pHTT4 or pSeb-Cws-BlaZ, respectively (29), UT34–2 (femB25), BB270 (femAB) (18), and UK31 (femAX) strains (29). Briefly, strains were grown in CY broth containing glycerol phosphate overnight (per liter CY: 10 g of casamino acids, 10 g of yeast extract, 5 g of glucose, 5.9 g of NaCl, 40 ml of sterile filtered 1.5 M NaCl, and a glycerol phosphate were added after autoclaving) (37). Staphylococci (107 colony-forming units) were sedimented by centrifugation at 7000 g for 3 min and suspended in 200 ml of phage buffer (1 mM MgSO4, 4 mM CaCl2, 50 mM Tris-HCl, 100 mM NaCl, pH 7.8, and 1 g of gelatin added per liter). Phage lysate, 200 ml generated from S. aureus OS2 carrying either pHTR4 or pSeb-Cws-BlaZ, was added for 10 min, and cells were plated on tryptic soy agar containing 10 mg/ml chloramphenicol, Escherichia coli BL21 (DE3) pLYS8, pHTR2 was used to purify staphylococcal ϕ11 lysate as described previously (7).

**Pulse-Chase Analysis of Seb-Cws-BlaZ Processing—**Staphylococcal strains harboring pSeb-Cws-BlaZ were grown overnight in tryptic soy broth supplemented with chloramphenicol (10 µg/ml), diluted 1:20 into fresh medium, and grown with shaking at 37 °C until A600 0.6. Cells from 1 ml of culture were sedimented by centrifugation at 8000 x g for 2 min and washed with 1 ml of prewarmed minimal medium (3). The cells were suspended in 1 ml of minimal medium, and newly synthesized polypeptide was labeled with 100 µCi of [35S]Promix (Amersham) for 1 min. Labeling was quenched by the addition of an excess non-radioactive amino acid (50-µl chase (100 mg/ml casamino acids, 20 mg/ml methionine)). At timed intervals after the chase of the chase, 0, 2, 5, and 10 min, 250-µl aliquots were removed, and protein was precipitated by the addition of 250 µl of 10% trichloroacetic acid. The precipitate was sedimented by centrifugation 15,000 x g for 10 min, washed with 1 ml of acetone, and dried. Samples were suspended in 1 ml of 0.5 M Tris-HCl, pH 6.8, and staphylococcal peptidoglycan was digested by adding 50 µl of mutanolysin (5000 units/ml, Sigma) and incubating 4 h at 37 °C. Proteins were again precipitated with trichloroacetic acid, washed with acetone, and subjected to immunoprecipitation with a-Blaz (38) followed by SDS-PAGE and PhosphorImager analysis.

**Purification of Anchor Peptides—**Staphylococcal strains harboring pHTR4 were grown overnight in tryptic soy broth supplemented with 10 µg/ml chloramphenicol, diluted 1:40 into 4 liters of fresh medium, and grown with 250 rpm shaking for 5 h at 37 °C. Cells were sedimented by centrifugation at 8000 x g for 15 min. Pellets were suspended in 100 ml of water, extracted with 100 ml of ethanol-acetone (1:1), and incubated for 30 min on ice. The cells were collected by centrifugation, washed with 300 ml of ice-cold water, and suspended in 30 ml of 0.1 x Tris-HCl, pH 7.5. The peptidoglycan was digested by adding 210 µl of purified ϕ11 hydrolase for 16 h at 37 °C. Seb-MH6-Cws was purified as described previously (7). Briefly, the digested cell wall was centrifuged at 17,000 x g for 15 min to remove insoluble material, and the supernatant was subjected to affinity chromatography. 2 ml of Ni-NTA Sepharose (Qiagen) were washed with equilibration buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.5, loaded with the supernatant of the ϕ11 hydrolase digest, washed first with 30 ml of wash buffer (50 mM Tris-HCl, 150 mM NaCl, 10% glycerol, pH 7.5) and then with 30 ml of equilibration buffer. Bound protein was eluted with 0.5 M imidazole in wash buffer. Seb-MH6-Cws was precipitated with 7% trisfluoroacetic acid (v/v), washed with acetone, dried under vacuum, and dissolved in 600 µl of 70% acetonitrile. A crystal of ϕ11 lysozyme was added, and the cleavage reaction was incubated for 16 h at room temperature. The reaction mixture was dried under vacuum, washed with water, and dissolved in 1 ml of buffer A (6 M guanidine hydrochloride, 0.1 M NaH2PO4, 0.01 M Tris-HCl, pH 8.0). The sample was subjected to 1 ml Ni-NTA Sepharose column pre-equilibrated with buffer A, washed with 10 ml of buffer A, 10 ml of buffer B (6 M urea, 0.1 M NaH2PO4, 0.01 M Tris-HCl, pH 8.0), and 10 ml of buffer C (same as buffer B, but pH 6.3). Anchor peptides were eluted with 2 ml of 0.5 M acetic acid, desalted over C18 cartridge (Altech), and subjected to MALDI-MS.

MALDI-MS—MALDI-MS spectra were obtained on a reflectron time-of-flight instrument (PerSeptive Biosystems Voyager RP) in the linear mode. Samples (0.5–1.0 µl) were co-spotted with 1.0 µl of matrix (α-cyano-4-hydroxycinnamic acid) at 1 mg/100 µl CH3CN/water-trisfluoroacetic acid (70:30:0.1) and mass measured using an external calibration with bovine insulin (7).

**RESULTS**

**Sorting of Surface Protein Precursor in Staphylococcal fem Mutants—**Previous work characterized the processing of Seb-Cws-BlaZ, harboring a central sorting signal flanked by NH2-terminal Seb and COOH-terminal BlaZ domains (5). Seb-Cws-BlaZ is exported from the cytoplasm by an NH2-terminal signal peptide and cleaved between the threonine and the glycine of its LPXTG motif (5). The NH2-terminal Seb fragment is linked to the staphylococcal peptidoglycan, whereas the COOH-terminal BlaZ domain remains in the bacterial cytosol (5). All surface protein that is cleaved at the LPXTG motif is also anchored to the cell wall (5). Hence, the rate of cleavage at the LPXTG motif of pulse-labeled Seb-Cws-BlaZ is a measure for the rate of surface protein anchoring in various staphylococcal fem mutant strains.

Staphylococci were pulse labeled with [35S]methionine for 1 min. At timed intervals during chase, culture aliquots were precipitated with trichloroacetic acid and washed in acetone. The staphylococcal peptidoglycan was digested with mutanolysin, and all protein was again precipitated with trichloroacetic acid prior to immunoprecipitation with a-Blaz and SDS-PAGE. The amount of Seb-Cws-BlaZ precursor and BlaZ cleavage fragment were determined by PhosphorImager analysis. Fig. 1B shows the autoradiogram of a typical experiment that measured the processing of Seb-Cws-BlaZ in S. aureus BB308 (femAB). In wild-type cells the half-life of the precursor was 0.53 min for S. aureus OS2 (spa, ert) and 1.4 min for BB270 (Mc). All S. aureus fem strains displayed an increased half-life of the Seb-Cws-BlaZ precursor: 2.12 min for OS2 (spa, ert), 2.25 min for UK17 (femA of BB270), 2.58 min (UT34–2 femB: Tn551 of BB270), 2.58 min (BB308 femA: Tn551 of BB270), and 2.25 min (UK31 femA of BB270). Together, these results suggested that the sorting reaction of surface proteins is significantly slowed in the fem mutant staphylococci.

**Purification of Surface Proteins from ϕ11 Hydrolase-digested Peptidoglycan—**To characterize the anchoring structure of surface protein in fem mutant staphylococci, we employed another hybrid protein. Seb-MH6-Cws is composed of enterotoxin B carrying an NH2-terminal signal peptide and a COOH-terminally fused sorting signal of protein A. At the fusion joint between Seb and the sorting signal, a methionine followed by six histidines is inserted. When expressed in staphylococci, this protein is exported and linked to the bacterial cell wall. ϕ11
hydrolysis cleaves the staphylococcal peptidoglycan at the peptide bonds between N-acetylglucosamine-L-alanine (amidase) and D-alanyl-glycyl. After peptidoglycan solubilization with the \( \phi11 \) hydrolase, Seb-MH\(_p\)-Cws was affinity purified on nickel Sepharose, cleaved at methionyl with CnBr, and COOH-terminal anchor peptides were purified by another round of chromatography on Ni-NTA Sepharose. Fig. 2 shows that purified Seb-MH\(_p\)-Cws migrated as two distinct species on SDS-PAGE. The faster migrating species has L-Ala-D-iGln-L-Lys(Gly3)-D-Ala amide linked to the carboxyl of its COOH-terminal threonine, whereas the slower migrating species carries MurNAc-L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-1–4)-GlcNAc and MurNAc-L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-1–4)-GlcNAc, respectively. The ion with \( m/z \) 2642 was explained as anchor peptide linked to \( \alpha \)-6-acetylmuramoyl-N-acetyl-L-glutamic acid linked to the COOH-terminal threonine by an L-Lys(Gly3)-D-Ala-D-Ala amide bond, whereas the ion with \( m/z \) 2601 and 2670 represented anchor peptides with linked MurNAc-L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-1–4)-GlcNAc and MurNAc-L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-D-Ala-1–4)-GlcNAc, respectively. The ion with \( m/z \) 2009 was consistent with an anchor peptide and linked L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-D-Ala. The compound with \( m/z \) 2601 and 2670 represented anchor peptides with linked MurNAc-L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-1–4)-GlcNAc and MurNAc-L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-D-Ala-1–4)-GlcNAc, respectively. The ion with \( m/z \) 2642 was explained as anchor peptide linked to O-6-acetylmuramoyl-N-acetyl-L-glutamic acid linked to the COOH-terminal threonine by an L-Lys(Gly3)-D-Ala-D-Ala amide bond, whereas the ion with \( m/z \) 2601 and 2670 represented anchor peptides with linked MurNAc-L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-1–4)-GlcNAc and MurNAc-L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-D-Ala-1–4)-GlcNAc, respectively. The ion with \( m/z \) 2009 was consistent with an anchor peptide and linked L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-D-Ala. The compound with \( m/z \) 2601 and 2670 represented anchor peptides with linked MurNAc-L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-1–4)-GlcNAc and MurNAc-L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-D-Ala-1–4)-GlcNAc, respectively. The ion with \( m/z \) 2009 was consistent with an anchor peptide and linked L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-D-Ala. The compound with \( m/z \) 2601 and 2670 represented anchor peptides with linked MurNAc-L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-1–4)-GlcNAc and MurNAc-L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-D-Ala-1–4)-GlcNAc, respectively. The ion with \( m/z \) 2009 was consistent with an anchor peptide and linked L-Ala-D-iGln-L-Lys(Gly3)-D-Ala-D-Ala.

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terminal threonine of surface proteins. Compounds with m/z 2486 and 2557 represented the disaccharide-linked species of the aforementioned anchor peptides, whereas the observation of ions at m/z 2528 and 2599 suggested N,O-6 diacetylation of muramoyl in similar structures. Thus, the data revealed that all anchor peptides were linked to the peptidoglycan of the femA mutant strain UK17 via monoglycyl cross-bridges.

**Cell Wall Anchor Structure of Surface Proteins in femAB Mutant Staphylococci**—Strain BB308 carries a transposon insertion in the femAB operon, a mutation which is known to affect the expression of the fem operon (18–20). In contrast to the femA and femB mutants analyzed above, strain BB308 synthesizes cross-bridges containing pentaglycyl, tetraglycyl-monoseryl (Gly$_2$ Ser$_1$), monoglycyl, and small amounts of triglycyl (26, 29). When subjected to MALDI-MS, anchor peptides purified from $\phi$11 hydrolyase-digested peptidoglycan of strain BB308 yielded four main ion clusters. For better analysis of the signals at m/z 2000–3000, the spectrum was drawn on an expanded scale in Fig. 5B. Observation of ions with m/z 2286 and 2745 was consistent with the calculated mass of anchor peptides harboring l-Ala-d-iGln-L-Lys (Gly$_2$ Ser$_1$)-d-Ala and MurNAc-[l-Ala-d-iGln-l-Lys (Gly$_2$ Ser$_1$)-d-Ala]-d-Ala in the COOH-terminal peptide sequence. The ion at m/z 2281 represented anchor peptide linked to the transglycosylation intermediate. The ion at m/z 2337 (l-Ala-d-iGln-l-Lys (Gly$_2$ Ser$_1$)-d-Ala) was identified as harboring either wild-type pentaglycyl (m/z 2237, 2309, 2715, 2757, 2787, 2829) or monoglycyl cross-bridges (m/z 2007, 2080, 2529, 2601). Compounds with m/z 2470 and 4887 were due to incomplete CnBr cleavage of Seb-MH$_6$-Cws, and their anchor structures were similar to those described for ions with m/z 2266 and 2787.

**Cell Wall Anchor Structure of Surface Proteins in femAX Mutant Staphylococci**—The femAX mutant strain UK31 has been generated by chemical mutagenesis of strain UK14 (femA) (29). The peptidoglycan of S. aureus UK31 displayed reduced peptidoglycan cross-linking as compared with its UK14 parent (63% as compared with 67% for UK14 and 76% for wild-type strain BB270) (29). In contrast to the cell wall of strain UK14, in which all cross-bridges consist of monoglycyl, about half of all peptidoglycan monomer isolated from S. aureus UK31 contains cross-bridges without lysyl, in which the ε-amino of lysyl is directly linked to the ε-alanyl at position four of a neighboring cell wall subunit. The remaining 50% is composed of monoglycyl cross-bridges, similar to the UK17 parent (29). MALDI-MS analysis of anchor peptides released from the peptidoglycan of UK31 with $\phi$11 hydrolyase treatment revealed prominent ions with m/z 1454, 2010, 2081, 2488, 2530, 2559, 3557, and 4184 (Fig. 6). The compound with m/z 1454 is likely a COOH-terminal degradation product of surface protein; its mass is consistent with the peptide sequence NH$_2$-HHHHHH-HAQALPE (calculated mass of 1450 Da). The ion at m/z 3557 can be explained as a COOH-terminal peptide with a structure similar to that of m/z 1454 and an additional 16 upstream residues due to incomplete CnBr cleavage. The observed compound with m/z 2010 represented anchor peptide linked to the monoglycyl cross-bridge of a cell wall tetrapeptide (l-Ala-d-iGln-l-Lys (Gly$_1$)-d-Ala). Ions with m/z 2081, 2488, 2530, 2559, and 2601 were explained as anchor peptide linked to monoglycyl cross-bridges with additional peptidoglycan structures: 2081 (l-Ala-d-iGln-l-Lys (Gly$_1$)-d-Ala-d-Ala), 2488 (MurNAc-l-Ala-d-iGln-l-Lys (Gly$_1$)-d-Ala-d-Ala), 2530 (6-OAc-MurNAc-l-Ala-d-iGln-l-Lys (Gly$_1$)-d-Ala-d-Ala), 2559 (MurNAc-l-Ala-d-iGln-l-Lys (Gly$_1$)-d-Ala-d-Ala) and 2601 (6-OAc-MurNAc-l-Ala-d-iGln-l-Lys (Gly$_1$)-d-Ala-d-Ala) (β1-4)-GlcNAc). None of the observed signals were consistent with anchor peptide structures in which the carboxyl of threonine was linked directly to the ε-amino of lysine.

**DISCUSSION**

All bacterial peptidoglycan is synthesized from lipid-linked precursor molecules (10, 11). Penicillin binding proteins have evolved to cleave these precursors at the amide bond of β-alanyl-d-alanine (9). Even when compared with penicillin binding protein homologs from distantly related species, these enzymes share significant sequence and structural similarity (9). In contrast to the conservation of β-alanyl-d-alanine, the chemical nature of cross-bridges within bacterial peptidoglycans is highly variable. Nevertheless, the presence of a free amino group is a common feature (11), and this element functions as a nucleophile to attack the acyl intermediate formed between the carboxyl of β-alanyl and the active site serine residue of PBPs. The nucleophilic attack results in the formation of a peptide bond between β-alanyl and the peptidoglycan cross-bridge as well as the regeneration of the hydroxyl at the active site serine (9). In vitro, when tested with purified pentapeptide substrate and enzyme, several different nucleophiles can substitute for the free amino of the peptidoglycan cross-bridge, including hydroxylamine, alanine, glycine, and others (40). However, in vivo the transpeptidation reaction is known to proceed with great specificity, cross-linking only neighboring wall peptides (41). These observations suggest that the fidelity of the transpeptidation reaction may depend at least in part on...
a unique environment in which the free amino of the cross-bridge is the only available nucleophile.

The sorting reaction of Gram-positive bacteria displays similarity to the penicillin-sensitive transpeptidation reaction (6). Here, the substrate for proteolytic cleavage is the LPX\(\text{TG}\) motif of surface proteins, which is cleaved between the threonine (T) and the glycine (G) (5). The nucleophilic amino group donor of the sorting reaction is identical to that of the transpeptidation reaction, i.e., the peptidoglycan cross-bridge. Because the LPX\(\text{TG}\) motif is found in sorting signals of many different surface proteins (42), it seems likely that sortase, the enzyme proposed to catalyze this reaction, is structurally conserved between different Gram-positive bacteria. Here, we asked whether mutationally altered cross-bridges of the staphylococcal peptidoglycan can serve as substrates for the sorting reaction. The rate of surface protein precursor cleavage at the LPX\(\text{TG}\) motif was employed to measure this reaction.

\(\text{S. aureus}\) strains carrying mutations in the \(\text{fem}\) genes displayed a decreased rate of precursor cleavage as compared with the wild-type strains, suggesting that the altered cross-bridges slowed the anchoring of surface proteins.

We also revealed here the anchor structures of surface proteins expressed in \(\text{S. aureus}\) strains that carry mutations in various \(\text{fem}\) genes. The results showed that surface protein was linked to tri- and monoglycyl cross-bridges of peptidoglycan isolated from \(\text{femB}\) and \(\text{femA}\) mutant staphylococci, respectively. However, no surface protein was found linked directly to the \(\epsilon\)-amino of lysyl within the cell wall of the femAX strain UK31, indicating that not all cross-bridges serve as a substrate for the sorting reaction. \(\text{S. aureus}\) BB308 carries a transposon insertion in the promoter of the \(\text{femAB}\) operon (18). Peptidoglycan analysis of this strain revealed the presence of pentaglycyl, tetraglycyl-monoseryl, and monoglycyl as well as small amounts of triglycyl cross-bridges. Analysis of anchor peptides purified from the peptidoglycan of \(\text{S. aureus}\) BB308 showed that surface proteins were mostly linked to tetraglycyl-monoseryl as well as pentaglycyl. Although monoglycyl-containing murein is known to be the most abundant species in strain BB308, we observed little surface protein anchoring to this cross-bridge, suggesting that the sortase activity of \(\text{S. aureus}\) preferred cross-bridges containing five residues. BB308 also contains small amounts of peptidoglycan with triglycyl cross-bridges; however, we could not identify surface protein linked to this species. This is likely due to the low abundance of triglycyl murein subunits in the peptidoglycan BB308 since the results from strain UT34–2 (\(\text{femB}\)) indicate that surface proteins can be linked to triglycyl amino donors.

The loss of \(\beta\)-lactam resistance in \(\text{fem}\) mutants of strain BB270 suggests that the \(\text{PBP2}\) enzyme cannot efficiently recognize the altered peptidoglycan cross-bridges (20). This is
corroborated by electron microscopic studies of fem strains that revealed gross defects in morphology as well as cell lysis (29). Furthermore, all fem strains contain reduced amounts of cross-linked peptidoglycan (29). Introduction of the femA or femB mutation into strains that do not express the PBP2’ enzyme also caused significant changes in the amount of peptidoglycan cross-linking as well as cell wall physiology. Thus, similar to the sorting reaction, staphylococcal PBPs can employ different
amino group donors for transpeptidation, although these cross-
bridges cannot fully substitute for the wild-type pentaglycyl
substrate.

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