Anchor Structure of Staphylococcal Surface Proteins

A BRANCHED PEPTIDE THAT LINKS THE CARBOXYL TERMINUS OF PROTEINS TO THE CELL WALL

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Surface proteins of Staphylococcus aureus are anchored to the cell wall by a mechanism requiring a COOH-terminal sorting signal. Previous work demonstrated that the sorting signal is cleaved at the conserved LPXTG motif and that the carboxyl of threonine (T) is linked to the staphylococcal cell wall. By employing different cell wall lytic enzymes, surface proteins were released from the staphylococcal peptidoglycan and their COOH-terminal anchor structure was revealed by a combination of mass spectrometry and chemical analysis. The results demonstrate that surface proteins are linked to a branched peptide (NH₂-Ala-γ-Gln-Lys-(NH₂-Gly)₅-Ala-COOH) by an amide bond between the carboxyl of threonine and the amino of the pentaglycine cross-bridge that is attached to the ε-amino of lysyl. This branched anchor peptide is amide-linked to the carboxyl of N-acetylmuramic acid, thereby tethering the COOH-terminal end of surface proteins to the staphylococcal peptidoglycan.

Experimental Procedures

Bacterial Strains and Plasmids—To express the hybrid protein Seb-MLH-Cws, the coding sequence for the protein A cell wall sorting signal harboring the engineered methionine-histidine affinity tag was amplified from the chromosomal DNA of S. aureus strain 8325-4 (12) with the primers Sse-6His (AAGTGACATCATGATCACCACATCATCAGGCTC-AAGCGATCAGAACAAT) and spa-2 (AAGGATCCTTTATCATTTCAATAAAATGTTT). The PCR product was digested with KpnI and BamHI and cloned into the corresponding sites of pSEB-FnBP (7) to generate pHTT4, which was transformed into S. aureus OS2 (5). The coding sequence of the φ11 amidease was amplified from purified phage DNA (12) with the primers (AACATATGCCAACGCAAATTACCTAAATATATGAGATCGTGTTT) and AAGGTCTCTGATGGTGATCTGACGTTGTTCCCGATAACGTGTTTCCCGATAACGTT), digested with Ndel and BamHI and cloned into the corresponding sites of the T7 expression vector pET-9a (13) to yield pHTT2. After transformation of Escherichia coli BL1 (DE3) pLysS (13), the resulting strain was employed for the induced expression of the φ11 amidease.

Purification of Anchor Peptides—S. aureus OS2 harboring pHTT4 was grown overnight in tryptic soy broth supplemented with chloramphenicol (10 μg/ml) and diluted 1:40 into the same medium. Generally 4 liters of culture were grown with shaking at 250 rpm and 37 °C for 5 h. Cells were harvested by centrifugation at 8000 × g for 15 min. Pellets were suspended in 100 ml of water, and the cell wall carbohydrates were extracted by the addition of 100 ml of an ethanol-acetone (1:1) mixture (14) and incubation for 30 min on ice. The cells were collected by centrifugation and washed with 300 ml of ice-cold water. Cell pellets were suspended in 30 ml of 0.1 M Tris-HCl buffer (pH 7.5 for lysostaphin and amidase digests, pH 6.8 for mutanolysin), and the peptidoglycan was digested with either lysostaphin (33 μg/ml, Ambi), amidase (67 μg/ml), or mutanolysin (100 μg/ml) for 5 h. The digest was then filtered through a 0.22 μm filter, and the concentration of peptidoglycan and the extent of digestion were monitored by the phenol-sulfuric acid method and the absorbance of the culture at 260 nm. The eluted material was subsequently subjected to gel filtration on Superdex 200 10/300 GL (Amersham Pharmacia Biotech) in 0.1 M Tris-HCl buffer (pH 8.0) and detected at 254 nm. Control experiments were performed with each enzyme to assess its specificity for peptidoglycan.

The abbreviations used are: ESI-MS, electrospray ionization mass spectrometry; CID, collisionally induced dissociation; Cws, cell wall sorting signal; MALDI-MS, matrix-assisted laser desorption ionization mass spectrometry; MS/MS, tandem mass spectrometry; MurNAc, N-acetylmuramic acid; m/z, mass to charge ratio; PAGE, polyacrylamide gel electrophoresis; PPG, propylene glycol; RP-HPLC, reverse phase high performance liquid chromatography; Seb, staphylococcal enterotoxin B.

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µg/ml), or mutanolysin (333 units/ml, Sigma) and incubated for either 2 (lysozymin) or 16 h (amidase and mutanolysin) at 37 °C. Peptidoglycan-released surface proteins were separated from the acetone-protoplastic (14) by centrifugation at 17,000 × g for 15 min, and the collected supernatant was filtered through a 0.2-µm pore size membrane. Solubilized Seb-MH-Cw was washed by repeated precipitation at 4 °C. The desalted anchor peptides were dried on C-18 matrix prior to MALDI-MS, whereas the other was subjected to RP-HPLC purification. Preparation of anchor peptides from 4 liter of staphylococcal culture (5 × 1013 colony forming units) typically yielded 1 nm (2 µg) of purified compound. Because of the relative small yield of anchor peptides, we chose to analyze our compounds by the more sensitive MALDI-MS and ESI-MS rather than fast atom bombardment mass spectrometry. The latter method has been used extensively in the past for the study of bacterial peptidoglycans (11, 15–17).

An experimental scheme was developed that allowed for the selective purification of COOH-terminal peptides harboring the cell wall anchor structure of surface proteins (Fig. 1). Seb-MH-Cw is a recombinant protein consisting of the NH2-terminal signal (leader) peptide and mature region of staphylococcal enterotoxin B (18) with a COOH-terminal cell wall sorting signal of protein A (5). At the fusion joint between these two sequences, a methionine (M) residue followed by six histidines (H6) was inserted. This engineered insertion serves as an affinity tag for the rapid purification of solubilized surface protein by chromatography on nickel-Sepharose. Purified proteins were cleaved with CNBr at their methionine residues, thereby separating the COOH-terminal anchor peptides from the remainder of the polypeptide chains. The anchor peptides were further purified by a second round of affinity chromatography on nickel-Sepharose and analyzed by MALDI-MS.

Structure of Lysozymin-released Anchor Peptides—Digestion of the staphylococcal cell wall with lysostaphin resulted in the solubilization of Seb-MH-Cw and most of these polypeptides migrated uniformly on SDS-PAGE with a mass of approximately 30,000 Da (Fig. 1). Some of the lysostaphin-released anchor peptides were identified by MALDI-MS with the predicted mass. As judged on Coomassie or silver-stained SDS-PAGE, the amidase preparation was 100% pure. The eluate was dialyzed against 1 liter of 50 mM Tris-HCl, 1 M urea, 0.005% Tween 80, pH 7.5, for 24 h at 4 °C without stirring, followed by a second dialysis against the same buffer without urea for 16 h at 4 °C and slow stirring.

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A recombinant surface protein (Seb-MH6-Cws) was observed in the amidase-solubilized sample; methionyl residues (data not shown). The efficiency (50–60%) as compared with the cleavage at other engineered methionine-histidine sites was reduced with the CnBr cleavage at the threonyl of the LP motif, thereby releasing cell wall residues that COOH-terminal anchor peptides can be liberated from the staphylococcal peptidoglycan by treatment with lysostaphin (11). This result confirmed our previous observation that COOH-terminal anchor peptides harbor two amino-terminal residues, glutamic acid and alanine after the first cleavage cycle (data not shown).

The anchor peptides that eluted from the RP-HPLC at 34–37% CH3CN were ESI-MS measured to be peptides with a calculated average mass of 1665 (m/z 556 and 834) and 1722 (m/z 556 and 834) which is consistent with a peptide of the structure NH2-H6AQALPET-Gly-Gly-(Gly)-COOH and a calculated average mass of 1665 and 1722.

**Structure of Amidase-released Anchor Peptides—Muralytic amidases cleave the bacterial wall peptides at their amide linkage between the amino of l-alanyl and the carboxyl of N-acetylmuramic acid (19, 20). Since no such enzyme was available to us, we cloned, overexpressed, and purified the amidase gene of staphylococcal phage φ11 (21) in E. coli.**

Amidase cleavage of the staphylococcal peptidoglycan solubilized Seb-MH6-Cws as two distinct species, both of which migrated more slowly on SDS-PAGE than the lysostaphin-cleaved, and the COOH-terminal anchor peptides were isolated by affinity chromatography. The sample was desalted over a C18 column and analyzed by MALDI-MS. The numbers indicate the m/z values of the identified ions; B, the same sample as analyzed in A was subjected to HP-HPLC; the absorption peaks were dried under vacuum and subjected to either Edman degradation or ESI-MS. C, ESI-MS spectrum of the lysostaphin-released anchor peptide purified by RP-HPLC (B). The spectrum shows the ion series obtained for the compounds that eluted at 24% CH3CN. Interpretation of the mass signals revealed an average molecular weight of 1665 (m/z 556 and 834) and 1722 (m/z 556 and 834) and 862(m/z 556 and 862) which is consistent with a peptide of the structure NH2-H6AQALPET-Gly-Gly-(Gly)-COOH and a calculated average mass of 1665 and 1722.

**Fig. 2. Characterization of lysostaphin-solubilized anchor peptides.** A, lysostaphin-solubilized surface protein was purified by affinity chromatography and CnBr-cleaved, and the COOH-terminal anchor peptides were isolated by affinity chromatography. The sample was desalted over a C18 column and analyzed by MALDI-MS. The numbers indicate the m/z values of the identified ions; B, the same sample as analyzed in A was subjected to RP-HPLC; the absorption peaks were dried under vacuum and subjected to either Edman degradation or ESI-MS. C, ESI-MS spectrum of the lysostaphin-released anchor peptide purified by RP-HPLC (B). The spectrum shows the ion series obtained for the compounds that eluted at 24% CH3CN. Interpretation of the mass signals revealed an average molecular weight of 1665 (m/z 556 and 834) and 1722 (m/z 556 and 834) and 862(m/z 556 and 862) which is consistent with a peptide of the structure NH2-H6AQALPET-Gly-Gly-(Gly)-COOH and a calculated average mass of 1665 and 1722.
nine, consistent with a branched peptide structure and with the cleavage of the amide bond between L-alanyl and N-acetylmuramic acid by the added amidase (22). After CnBr cleavage and affinity purification, the amidase-released anchor peptides were subjected to MALDI-MS (Fig. 3A). Five major ions with m/z 2235, 2717, 2758, 4335, and 4857 were identified and these compounds were further purified by RP-HPLC (Fig. 3B). An average ESI-MS mass of 2235.05 was observed for the anchor peptide eluting at 26% CH₃CN (Fig. 3C). When subjected to Edman degradation, this compound yielded the sequence NH₂-Ala-HHHHHHAQALPET-Gly-Gly-Gly-Gly-COOH and two phenylthiohydantoin-coupled residues, histidine and alanine, were identified at equimolar concentrations during cycle 1 (Table I). This result is consistent with the structure of a branched peptide consisting of surface protein linked via the pentaglycine cross-bridge to the ε-amino of lysine in the wall peptide and a calculated average mass of 2235.35 as shown in Fig. 4. An NH₂-terminal alanyl of the wall peptide, liberated via amidase cleavage, is likely linked to ε-isoglutamine and presumably no further amino acid was released from the wall peptide due to the nature of its iso-peptide bond (23) (see Fig. 4 for a structural model). The amino acid analysis was consistent with the presented structure (Table II).

To characterize the entire structure of the compound with mass 2235, we performed CID of the triply charged parent ion (m/z 746) in an MS/MS experiment. The resulting daughter ion spectrum was examined for the presence of breakdown products (15, 24, 25) and compared with our structural model (Fig. 4). The structure of the branched peptide was revealed by the presence of the daughter ions at m/z 201, 258, 342, 399, and 982. The ion at m/z 201 was interpreted to be Ala-γ-Gln. The observation that no glutamine was released by Edman degradation during cycle two suggests that the two amino acids are linked by the characteristic iso-peptide bond. The ion at m/z 399 comprises the entire stem peptide (NH₃-Ala-γ-Gln-Lys-Ala-COOH), whereas the ion at m/z 342 represented the loss of Ala from this structure (NH₂-Ala-γ-Gln-Lys-COOH). Evidence for the bond between the fifth glycyl and the ε-amino of lysyl was provided by the fact that Edman degradation during cycles 19 and higher (i.e. after the fifth glycyl) did not release phenylthiohydantoin-coupled amino acids (Table I) and by the presence of the ions 2258 (NH₂-Lys-(NH₂-Gly)-Ala-COOH) and 982 (NH₂-Lys(NH₂-H₂AQALPET-Gly₂)-Ala-COOH), which comprise this bond. A summary of the observed daughter ions and their interpretation is presented in Table III.

The anchor peptide with an observed ESI-MS average mass of 2713.48 eluted at 28% CH₃CN and upon Edman degradation also yielded a sequence of NH₂-HHHHHHAQALPET-Gly-Gly-Gly-Gly-COOH. However, in contrast to the compound with a mass of 2235, no release of phenylthiohydantoin-coupled alanine was observed during the first cycle. These observations are consistent with the linkage of surface protein to a cell wall tetrapeptide that is amide-linked to N-acetylmuramyl-(β1–4)-

### Table I

| Residue | 1722 Da | 2235 Da | 2713 Da |
|---------|---------|---------|---------|
| 1-His (Ala) | 99 | 791 (709) | 2231 (195) |
| 2-His | 138 | 796 (713) | 1893 (64) |
| 3-His | 160 | 775 | 1889 |
| 4-His | 164 | 749 | 1779 |
| 5-His | 183 | 672 | 1350 |
| 6-His | 170 | 351 | 986 |
| 7-Ala | 84 | 212 | 577 |
| 8-Glu | 72 | 196 | 401 |
| 9-Ala | 102 | 207 | 626 |
| 10-Leu | 56 | 163 | 331 |
| 11-Pro | 28 | 66 | 118 |
| 12-Glu | 29 | 67 | 121 |
| 13-Thr | 19 | 54 | 126 |
| 14-Gly | 31 | 73 | 126 |
| 15-Gly | 45 | 116 | 218 |
| 16-Gly | 37 | 146 | 347 |
| 17-Gly | 193 | 462 |
| 18-Gly | 165 | 574 |

*During cycle 1, the 2235-Da amidase-released anchor peptide yielded two phenylthiohydantoin-coupled amino acids (histidine and alanine) at equimolar concentrations, whereas the compounds with mass 1722 Da and 2713 Da did not.
The characteristic ions at m/z 5 revealed by the fragment ions at m/z 1246 and 832, respectively (MurNAc-l-Ala-d-γ-Gln-1-Lys(NH₂-H₄AQLPET-Gly₃)-d-Ala-COOH). The linkage of γ-alanine in the stem peptide to the lactyl moiety of N-acetylglucosamine could be demonstrated with the ions at m/z 1154 and 770 (lactyl-Ala-γ-Gln-Lys(NH₂-H₄AQLPET-Gly₃)-Ala-COOH). Several other ions confirmed the sequence of the stem peptide to be identical to that of the amidase-released anchor peptide. If this sample resulted from the solubilization by a glucosaminidase, its structure would likely be MurNAc-(Ala-γ-Gln-Lys(NH₂-H₄AQLPET-Gly₃)-Ala-COOH)–(β-1-4)-GlcNAc. All daughter ions observed in this MS/MS experiment are listed in Table IV for a comparison of the observed and calculated masses with the proposed structures.

Average compound masses of 4342, 4821, and 4849 were measured by ESI-MS for amidase-released peptides eluting at 36–38% CH₃CN (Fig. 3). Similar to the observations made with the partial CnBr cleavage of lysostaphin-solubilized Seb-MH₆-Cws, these mass measurements are consistent with peptides harboring an additional 16 upstream residues of Seb fused to the amidase-released anchor structures as a result of an uncleaved methionine adjacent to the engineered histidine tag.

Muramidase Release of Surface Proteins—Muramidase leaves the staphylococcal cell wall at the glycan chains (20), i.e., the β-1-4 glucosidic bond between N-acetylglucosamine and N-acetylgalactosamine, and released Seb-MH₆-Cws as a spectrum of species with increasing mass due to linked peptidoglycan (Fig. 1). NH₂-terminal sequencing of the muramidase-solubilized Seb-MH₆-Cws also yielded two phenylthiohydantoin-coupled residues after the first cycle: glutamic acid and alanine. The presence of phenylthiohydantoin-alanine can be explained by the alkali release of ester-linked γ-alanine from cell wall teichoic acid (28, 29) during the sequencing cycle. Because ω-α-amylol-decorated polyribitol teichoic acids are linked to N-acetylglucosamine within the glycan chains, muramidase digestion of the staphylococcal peptidoglycan can solubilize surface proteins with attached teichoic acids (30, 31). Although several attempts were made to purify large quantities of the muramidase-released, CnBr-cleaved anchor species, we failed to obtain sufficient material for a more detailed chemical, ESI-MS, or MS/MS analysis.

**DISCUSSION**

The cell wall anchor structure of surface proteins in *S. aureus* has been revealed by a combination of mass spectrometry and molecular biology techniques. Surface proteins are tethered to the cell wall via an amide bond between the carboxyl of their COOH-terminal threonine and the amino of the pentaglycine cross-bridge, which is attached to the ε-amino of lysyl within a peptidoglycan tetrapeptide. This branched anchor peptide is linked to N-acetylglucosamine within the glycan chains, thereby attaching surface proteins to the glycan chains of the staphylococcal cell wall. We think that surface proteins may be linked to a precursor molecule of peptidoglycan synthesis rather than to the mature, assembled cell wall, because the staphylococcal peptidoglycan is highly cross-linked and very few free pentaglycine amino groups (11, 23, 32–34). During the biosynthesis of bacterial peptidoglycans, a membrane-bound disaccharide-precursor molecule (undecaprenyl-P-O₄-P=C-mMurNAc-(l-Ala-d-γ-Gln-l-Lys-(NH₂-Gly₃)-l-Ala-d-Ala-COOH)–(β-1-4)-GlcNAc) is assembled in the cytoplasm (35, 36) and translocated across the membrane to serve as a substrate for transglycosylation and

N-acetylgalactosamine (19, 26) (calculated average compound mass 2713.85). This was a surprising finding because no N-acetylgalactosaminidase or N-acetylmuramidase had been added that would have released surface proteins with linked disaccharide. One explanation for this result may be that the well-known endogenous N-acetylgalactosaminidase of *S. aureus* (20, 27) had released surface proteins during the prolonged incubation with amidase. Nevertheless, the availability of the compound with mass 2713 provided us with an opportunity to study the linkage of anchor peptides to the glycan strands of the staphylococcal cell wall.

The structure of the 2713-Da anchor fragment was characterized by MS/MS using the parent ion at m/z 905. The resulting daughter ion spectra was compared with our model (Fig. 5). The presence of N-acetylgalactosamines in this structure was revealed by the characteristic ions at m/z 187, 168, and 138
transpeptidation reactions (37, 38) (Fig. 6). Such a precursor molecule could also serve as the peptidoglycan substrate for the cell wall sorting reaction of surface proteins. To characterize a cell wall sorting intermediate consisting of surface protein with linked peptidoglycan precursor, we have begun to purify and combine our mass spectrometric analysis of anchor structures with antibiotic inhibition of peptidoglycan precursor synthesis. In contrast, tapeptide-peptidoglycan precursor molecules may be cleaved by carboxypeptidases, thereby yielding free (unsubstituted) tetrapeptides or cleaved by enzymes that may subsequently be cleaved at its terminal D-alanyl-D-alanine dipeptide (38). Nevertheless, it also seems possible that penicillin-binding proteins could serve as substrates for the sorting reaction of surface proteins. Our current data cannot distinguish between these possibilities, but they may be addressed in the future by combining our mass spectrometric analysis of anchor structures with antibiotic inhibition of peptidoglycan precursor synthesis.

A notable feature of the cell wall anchor structure of surface proteins in S. aureus is its lack of cross-linking with neighbor-
The triply charged parent ion at m/z 905 was subjected to CID generating daughter ions, which were compared with the structural model. B, predicted structure of the anchor peptide with mass 2713, MurNAc-Ala-Gln-Lys-(NH₂-H₆AQALPET-Gly₅)-Ala-COOH. The calculated masses and structures of some daughter ions are indicated in the drawing. See Table IV for a complete listing of observed ions, proposed structures, and calculated masses.

**FIG. 5. Structure of the cell wall anchor of surface proteins in S. aureus.** A, MS/MS analysis of the anchor peptide with mass 2713. The triply charged parent ion at m/z 905 was subjected to CID generating daughter ions, which were compared with the structural model. B, predicted structure of the anchor peptide with mass 2713, MurNAc-Ala-Gln-Lys-(NH₂-H₆AQALPET-Gly₅)-Ala-COOH. The calculated masses and structures of some daughter ions are indicated in the drawing. See Table IV for a complete listing of observed ions, proposed structures, and calculated masses.

**Table IV**

| Observed m/z | Charge state | Calculated m/z | Δobs-calc | Proposed structure |
|--------------|--------------|---------------|-----------|-------------------|
| 84.2         | +3           | 83.4          | +0.8      | HH (a₁)           |
| 128.5        | +1           | 127.1         | -0.6      | Propionyl-Ala     |
| 138.2        | +1           | 138.1         | +0.1      | (2)²             |
| 168.2        | +1           | 168.2         | 0         | GlnNAc            |
| 186.8        | -1           | 186.2         | +0.6      | (1)²             |
| 203.0        | -1           | 204.2         | -1.2      | MurNAc            |
| 274.4        | -1           | 276.3         | -1.9      |                  |
| 302.0        | -1           | 300.4         | +1.6      | Gln-Lys-Ala (~28) |
| 329.6        | -1           | 328.4         | +1.2      | Gln-Lys-Ala       |
| 546.5        | -1           | 547.6         | -1.1      | H₂AQA (b₂)⁶       |
| 603.5        | -2           | 604.1         | -0.6      | H₂AQL (b₃)⁶      |
| 701.9        | +0           | 702.4         | -0.5      | Gln-Lys-(NH₂-H₂AQLPET-Gly)₅-Ala (~43)⁹ |
| 721.7        | -3           | 722.4         | +0.7      | Gln-Lys-(NH₂-H₂AQLPET-Gly)₅-Ala⁸ |
| 770.3        | -3           | 770.8         | -0.5      | Propionyl-Ala-Gln-Lys-(NH₂-H₂AQLPET-Gly)₅-Ala⁹ |
| 832.1        | +3           | 832.1         | 0         | MurNAc-Ala-Gln-Lys-(NH₂-H₂AQLPET-Gly)₅-Ala⁸ |
| 905.0        | +3           | 905.0         | 0         | Parent ion        |
| 1051.7       | +2           | 1053.2        | -1.5      | Gln-Lys-(NH₂-H₂AQLPET-Gly)₅-Ala (~43)⁹ |
| 1082.9       | +2           | 1083.2        | -0.3      | Gln-Lys-(NH₂-H₂AQLPET-Gly)₅-Ala⁸ |
| 1154.3       | +2           | 1155.7        | -1.4      | Propionyl-Ala-Gln-Lys-(NH₂-H₂AQLPET-Gly)₅-Ala⁹ |
| 1207.7       | +1           | 1207.3        | +0.4      | H₂AQL (b₃)⁶      |
| 1246.1       | +2           | 1247.7        | +1.6      | MurNAc-Ala-Gln-Lys-(NH₂-H₂AQLPET-Gly)₅-Ala⁹ |
| 1286.9       | +1           | 1287.3        | +0.4      | MurNAc-Ala-Gln-Lys-(NH₂-PET-Gly)₅-Ala⁹ |

- See Fig. 5A for the relative intensity of daughter ions.
- Calculations were performed as described in Table III.
- The difference between the observed and calculated masses of daughter ions.
- See Fig. 5A for an explanation of the predicted structure.
- Nomenclature refers to NH₂- and COOH-terminal cleavage fragments according to Roepstorff and Fohlman (51) and Biemann (52, 53).
- Calculated as residue mass.
- Calculated as residue mass minus 17 (OH).
- Calculated as residue mass plus 18 (H₂O).
Fig. 6. Model for the sorting of surface proteins to the staphylococcal cell wall. Peptidoglycan precursor molecules are synthesized in the bacterial cytoplasm (1) and translocated across the membrane. Surface proteins are first cleaved (2) between the threonine (T) and the glycine (G) of the LPXTG motif and may subsequently be linked to a peptidoglycan precursor molecule (3). The proposed intermediate of surface protein linked to peptidoglycan precursor may then be incorporated into the cell wall by a transglycosylase reaction (4). The mature anchored peptidoglycan chains are linked to the pentaglycine cross-bridge in the cell wall, which is tethered to the ε-amino side chain of an unsubstituted cell wall tetrapeptide. A carboxypeptidase (5) may cleave the d-Ala-d-Ala bond of pentapeptide structures to yield the final branched anchor peptide in the staphylococcal cell wall.

icillin-binding proteins (50). We tested this prediction and found that neither methicillin nor penicillin G could inhibit surface protein anchoring to the staphylococcal cell wall (data not shown). The sorting reaction may thus represent a novel target for antibiotic therapy if its amide bond exchange reaction proves to be essential for either viability or pathogenesis.

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