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A potpourri of microbes

Staphylococcus aureus sortase A-mediated incorporation of peptides: effect of peptide modification on incorporation

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Background: The endogenous *Staphylococcus aureus* sortase A (SrtA) transpeptidase covalently anchors cell wall-anchored (CWA) proteins equipped with a specific recognition motif (LPXTG) into the peptidoglycan layer of the staphylococcal cell wall. Previous *in situ* experiments have shown that endogenous SrtA is also able to incorporate exogenous, fluorescently labelled, synthetic substrates equipped with the LPXTG motif (K(FITC)LPETG-amide) into the bacterial cell wall, albeit at high concentrations of 500 µM to 1 mM. In the present study, we have evaluated the effect of substrate modification on the incorporation efficiency.

Material/methods: We developed a number of novel synthetic SrtA substrates: (i) variants of the K(FITC)LPETG-amide, which had been elongated with sequences derived from physiological SrtA substrates - sequences containing a hydrophobic membrane spanning domain linked to a stretch of positively charged amino acids; (ii) a substrate (K(FITC)LPMTG-amide) in which glutamate (E) was substituted for methionine (M) and (iii) K(FITC)LPMTG-amide conjugated to vancomycin, designed to potentially improve the targeting to lipid II, the primary acceptor for physiological SrtA substrates in *S. aureus*. *S. aureus* wild type and *srtA* deletion mutant strains were incubated in the presence of various substrate concentrations (up to 1 mM) in Luria-Bertani medium until late stationary phase (24 hrs). After incubation and removal of non-covalently bound and intracellular substrate, the covalent and SrtA-dependent incorporation of these synthetic SrtA substrates was assessed into the staphylococcal cell wall *in situ* by FACS.

Results: This revealed that (i) by elongation of LPETG-amide with a sequence of positively charged amino acids, derived from the C-terminal domain of physiological SrtA substrates, the incorporation efficiency was increased by 20-fold at 10 µM, 100 µM and 250 µM; (ii) Substituting aspartic acid (E) for methionine increased the incorporation of the resulting K(FITC)LPMTG-amide approximately three times at all concentrations tested; (iii) conjugation of the lipid II binding antibiotic vancomycin to K(FITC)LPMTG-amide resulted in the same incorporation levels as K(FITC)LPETG-amide, but much more efficient at an impressive 500-fold lower substrate concentration.

Conclusions: These newly developed synthetic substrates can potentially find broad applications in for example the *in situ* imaging of bacteria; the incorporation of antibody recruiting moieties; the targeted delivery and covalent incorporation of antimicrobial compounds into the bacterial cell wall.