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Unveiling the mechanism of activation of the *vraSRT* system of *Staphylococcus aureus* by β-lactam antibiotics using photoactive compounds

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Staphylococcus aureus is the leading cause of nosocomial and community-acquired infections. The *vraSRT* system acts as a sentinel that can rapidly sense cell wall peptidoglycan damage and coordinate a response that leads to resistance to β-lactam and glycopeptide antibiotics. VraS is a membrane histidin-kinase and VraR a cytoplasmatic response regulator. However, the rol of VraT, another membrane protein, is yet unknown but essential for the survival of the bacteria. We still do not understand how VraS is activated in response to cell wall-active antibiotics.
The interaction between VraS , VraT and different ampicillin-derived photo-affinity probes was studied. Using a *S. aureus* reporter strain, which has a shuttle vector that allows expression of GFP under the control of the *vraSRT* operator region, we confirmed that the ampicillin photoprobes effectively activate the *vraSRT* system. The photo-affinity probes were used for covalent labeling of VraS and VraT in *E. coli* BL21 Star DE3 spheroplasts. An interaction with VraS was evidenced by a shift in the electrophoretic mobility of the protein. MALDI-TOF/TOF analysis of the purified VraS-photoprobe complexes did not allow the identification of the site of crosslinking. We hypothesized that β-lactams could interact with the extracellular loop of VraS, a peptide not detected by MALDI-TOF/TOF. Hence, we introduced photoactive phenylalanine residues in that loop of VraS and evaluated labeling with the fluorescent penicillin Bocillin-FL. No fluorescent VraS was detected which indicated no direct interaction of the antibiotic with this loop. VraT has an extracellular C-terminal domain, as determined in a Proteinase K susceptibility assay, which does not interact directly with the ampicillin photoprobes.
In conclusion, VraS interacts directly with β-lactams but its extracellular loop is not involved in the recognition. VraT participation in activation of the system is not as a receptor of the antibiotic.

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