Poster Presentations

[MS5-P27] Specificity Determinants for Lysine Revealed by the S.aureus MurE Structure

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The third amino acid on the stem of the peptidoglycan chain is a pivotal point in the biosynthesis pathway. It is added by MurE, which adds either L-lysine or meso-diaminopimelic acid (mDAP), depending on whether the enzyme is from a gram positive or gram negative organism. The choice of amino acid can affect downstream processes, such as the PBP dependent steps in biosynthesis, as well as further modifications to the peptidoglycan which can form anchoring points for a variety of cell wall biomolecules via the action of sortase enzymes that are essential for virulence and pathogenesis [1].
In *Staphylococcus aureus*, a gram negative organism, MurE adds L-lysine at the third position of the stem peptide. We have determined the structure to 1.8 Å resolution of this MurE in the presence of the product UDP-Mur*NA*c-L-Ala-γ-D-Glu-L-Lys and ADP. This represents the first lysine specific MurE from a gram positive organism and the first reported structure of MurE in a ternary product complex. Investigating the active site shows that although a consensus sequence was implicated in the selection of the amino acid for the reaction [2], only part of this consensus sequence is involved. In fact there are very few interactions with conserved or other side chains, but instead it is the overall electrostatic contributions within the active site that determined what amino acid is added to the stem. In the case of SaMurE, the active site charge characteristics favour the binding of the positively charged L-Lys substrate side chain as the addition of the D-Carboxyl group, present in the side chain of mDAP, would be electrostatically repelled in the SaMurE active site.
Kinetic analysis of SaMurE reveals a relative poor Km for L-Lys unexpectedly which is explained through analysis of *In vivo* metabolomics data. The intracellular concentration of L-lysine in *Staphylcoccus aureus* rises dramatically during growth and exceeds the concentration of most other amino acids by over one order of magnitude [3]. The high levels of intracellular L-lysine explains the relatively poor kinetic properties of SaMurE, and provide a rationale for mDAP and l-Lysine discrimination in *S. aureus*.

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