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Evaluating Nucleophile and Substrate Specificities of Sortase A Homologs for Orthogonal Reactivity

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Evaluating Nucleophile and Substrate Specificities of Sortase A Homologs for Orthogonal Reactivity

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Abstract

Enzymes have become an attractive option for protein modification chemistry due to the remarkable site-specificity they afford. Of particular interest is sortase A from Staphylococcus aureus (SrtA_{S.a.}), which has garnered attention for its ability to install a variety of non-natural modifications to a conserved oligopeptide substrate. In addition to SrtA_{S.a.}, it has become apparent that sortase A homologs exist in other bacterial strains, each of which is potentially a novel catalyst for protein engineering. Previous work has demonstrated that eight representative sortase A homologs exhibit unique specificities for synthetic peptide substrates, capable of identifying characteristic combinations of amino acids in the “sorting motif.” Presented here is a nucleophile profile of the most promiscuous sortase A homolog investigated, that from S. pneumoniae (SrtA_{S.pnu}). Exhibiting unique specificities, this SrtA variant may enable unique protein modification chemistry.

Background

Sortase A is a ligase found in many gram-positive bacteria that has recently risen to prominence due to the specificity and efficiency with which it transfers nucleophilic amines to target peptides both in vitro and in vivo. The best studied example is that of S. aureus, SrtA_{S.a.}, which selectively recognizes the oligopeptide sequence LPXTG, where X denotes any amino acid. A nucleophilic cysteine in the enzyme active site attacks the carbonyl carbon of the threonine residue, ejecting the C-terminal fragment. This transient acyl-enzyme intermediate is then intercepted by an incoming nucleophilic nucleophile, typically glycine, and the enzyme is released. Due to the simple catalytic mechanism by which this occurs, SrtA_{S.a.} ligations have been found in vitro for the appending of a wide range of non-natural functional groups to polypeptides that contain the “sorting motif” LPXTG.

Site-Specific Modification of CRF

Site-accessible Serine at N-terminus
Possible alternative ligation site at Lysine-36

Conclusion

• Sortase A from S. pneumoniae is capable of recognizing a host of nucleophiles in addition to its substrate promiscuously previously demonstrated
  ○ Includes many amino acid nucleophiles not recognized by SrtA_{S.a.}
  ○ Synthetic primary amines permit less engineering in applications
• Corticotropin-releasing hormone (CRF) used as a nucleophile in Sortase A mediated transpeptidation reactions has allowed for a broader nucleophile application regarding in-vivo conjugates.

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