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Broadening the Scope of Sortase-Mediated Ligations using Natural Sortase Homologs

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Abstract
Sortase-mediated ligations have become an attractive option for protein modification chemistry, enabling the synthesis of a wide range of non-natural polypeptide derivatives. In an effort to expand the scope of this methodology, we have been characterizing the in vitro reactivity of a panel of natural sortase homologs. Here we present our studies on the substrate and nucleophile tolerance of sortases from a range of bacterial species. Notable findings include that sortase A from Streptococcus pneumoniae (Spneu) shows a high degree of substrate promiscuity, allowing this enzyme to process a range of substrate variations that deviate from the LPXTG substrate motif typically associated with sortase-mediated methods. In addition, this enzyme has the ability to accept an expanded range of primary amines nucleophiles. To demonstrate the utility of this expanded substrate scope, we have also succeeded in using Spneu to site-specifically modify the N-terminal serine residue of Dermondin (DCD-1L). Overall, these results demonstrate that naturally occurring sortases represent a viable approach for the continued development of sortase-mediated protein modification.

Background
Sortases are ligases found in many gram-positive bacteria that have recently risen to prominence due to their ability to selectively ligate nucleophilic amines to target peptides and proteins both in vitro and in vivo. The most thoroughly characterized example is sortase A from Staphylococcus aureus (SaA) which selectively recognizes the 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 amine, typically glycine, and the enzyme is released. Due to the simple catalytic mechanism by which this occurs, SaA ligations have found use in vitro for the attaching a wide range of non-natural functional groups to polypeptides that contain the LPXTG "sorting motif".

While useful, the LPXTG specificity of SaA typically restricts the technique to substrates that inherently possess the LPXTG motif or those that have been engineered to display this sequence. To address this limitation in substrate scope, we are exploring the reactivity of sortase homologs from alternate bacterial species.

Peptide Substrates for Probing Substrate Tolerance

Substrate Synthesis

![Peptide Substrates](image)

Repeated synthesis / resin cleavage

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Reactivity of sortase A homologs with discretely synthesized peptides as evaluated by RP-HPLC. All reactions incubated for 24-26 hours at RT and included 25 µM sortase, 200 µM peptide substrate, and 10 mM hydroxylamine in Tris buffer pH 7.5 with 10 mM CaCl2. Reactions involving cysteine were supplemented with 100 mM DTT to retain a reductive environment. Conversion percentages were the average of three trials, each of which with a standard deviation < 10%.

Site-Specific Modification of DCD-1L

![Site-Specific Modification](image)

25 µM Spneu, 50 mM Tris (pH 7.5), 150 mM NaCl

Confirmation of Site-Specific Modification of DCD-1L

![Confirmation of Site-Specific Modification](image)

10 µM GluC

Application of Spneu Substrate Tolerance

![Application of Spneu Substrate Tolerance](image)

Spatneu

Conclusion

- Sortase A from S. pneumoniae is capable of recognizing multiple substrate variants other than LPXTG.
- S. pneumoniae sortase A has the potential to allow site-specific modification at a range of N-terminal residues, for example serine (DCD-1L).
- Naturally occurring sortase homologs provide a useful resource for expanding the scope of sortase-mediated protein engineering.

Acknowledgements

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