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PROBING PEPTIDE SUBSTRATE RECOGNITION OF RADICAL S-ADENOSYLMETHIONINE ENZYME TTE1186 VIA ASSAY OF SYNTHESIZED PEPTIDE SUBSTRATE MUTANTS AND LIQUID CHROMATOGRAPHY/MASS SPECTROSCOPY ANALYSIS

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Abstract

Ribosomally synthesized and post-translationally modified peptide Tte1186a and several point mutation variants are synthesized using solid phase peptide synthesis with FMOC amino acids. Substrate recognition and thioether linkage formation from the radical S-adenosyl methionine Tte1186 maturase is determined by analyzing assays with liquid chromatography/mass spectroscopy. Results indicate a very high tolerance to mutation in the region of original thioether linkage and surrounding residues, allowing 24 mutations throughout the peptide with only 3 mutations on the crosslinking site not tolerated. Results did not elucidate a clear pattern of recognition due to high tolerance for point mutations throughout the peptide substrate.

Introduction

Many natural products such as Polyketides (PK), non-ribosomal peptides (NRP), and hybrids of the two are well sequenced and studied, including well known antibiotics including penicillin G, vancomycin, and tetracycline.[1] These products consist of covalently linked monomers polymerized by coordinated enzymes that loosely resemble an assembly line.[1] There is rising interest in a lesser known group of natural products called ribosomally-synthesized and post translationally modified peptides (RiPPs). Unlike PKs and NRPs, RiPPs are synthesized ribosomally, but are processed after translation by enzymes that have a wide variety of functions.[2] Similarly to many PKs and NRPs, genes encoding the maturases for the peptide tend to be colocalized, suggesting convergent regulation.[2,3] Although many different classifications of RiPPs have been identified, they typically originate as a translated structural peptide that contains more residues than the final product, and is typically 20-100 residues.[2] Leader sequences or peptides are most often appended to the N-terminus of the core peptide to be used for recognition of modification enzymes and export, and are removed via proteolysis after modification of the core peptide.[2] This aspect of RiPPs is promising because the core peptide can vary widely in composition and still be recognized by the maturases, allowing for extensive mutation and possibly incorporation of novel structures.[2]

One of the more well studied RiPPs is the lanthipeptide antimicrobial peptide nisin. Lanthipeptides formation is initiated when enzymes (recognizing the leader sequence) catalyze the dehydration of Ser and Thr residues to form dehydroalanine (Dha) and dehydrobutyrine (Dhb) residues on the peptide substrate.[4] A separate enzyme catalyzes the Michael addition of Cys thiols targeting the carbon double bond of the dehydrated residues to form methyllanthionine bridges in the peptide.[4] Lanthipeptides can have multiple bridges and form large cyclic structures, follow slightly variational mechanistic pathways, and have been shown to tolerate many mutations to the core peptide.[2,4]

While lanthipeptides are the most studied RiPPs, sactipeptides are one of the least studied subdivisions. Sactipeptides are unique in that they create thioether crosslinks between cys and the alpha carbon of a variety of unmodified residues.[5] These reactions are catalyzed by enzymes in the radical SAM superfamily, and are carried out with a two-step radical mediated reaction.[5] These enzymes usually contain the CxxxCxxC motif, in which the Cys residues coordinate iron atoms of 4Fe-4S clusters.[5] These clusters generate dado radicals from SAM when in reducing conditions, and the dado radicals abstract a H atom from the substrate thiol, initiating a radical mediated mechanism forming the thioether crosslink.[5] A general sactipeptide peptide and maturase pair Tte1886 has been identified and its reaction has been replicated in-vitro.[5] Although the function of the product peptideTte1186 is unknown, it is has the potential to be manipulated in an experimental probe of mutant peptide substrates to determine maturase selectivity.

References

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