Mechanistic Investigation of Enzymatic Thioamide Formation in Methyl-coenzyme M Reductase

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Thioamide is a post-translational modification on peptides where the amide oxygen is replaced by a sulfur. It is present in ribosomally synthesized post-translationally modified peptides (RiPPs) and the enzyme responsible for anaerobic methanogenesis, methyl-coenzyme M reductase (MCR). The resulting chemical and conformational changes render the peptide or protein with new functionalities. We previously reconstituted thioamide installation onto MCR peptide fragments using the modification enzymes YcaO and TfuA, and inorganic sulfur donor. However, the details of this modification, the role of TfuA as well as the identity of the native sulfur donor remained enigmatic. Leveraging the YcaO structure with the peptide substrate bound in the active site, we elucidated the residues involved in substrate recognition and catalysis using a mutational approach. To study the role of TfuA, genome-wide bioinformatic analysis was performed and led to our hypotheses that TfuA desulfurates cysteine to initiate the sulfur relay pathway which eventually delivers the sulfur to YcaO for thioamide formation. Here we report the reconstitution of this reaction and the initial evidence of TfuA being a novel cysteine desulfurase. This study bridged our knowledge gap in YcaO mechanism and explored the novel sulfur assimilation chemistry performed by TfuA.

RiPP Leader Peptide Cleavage by a Lanthipeptide Protease

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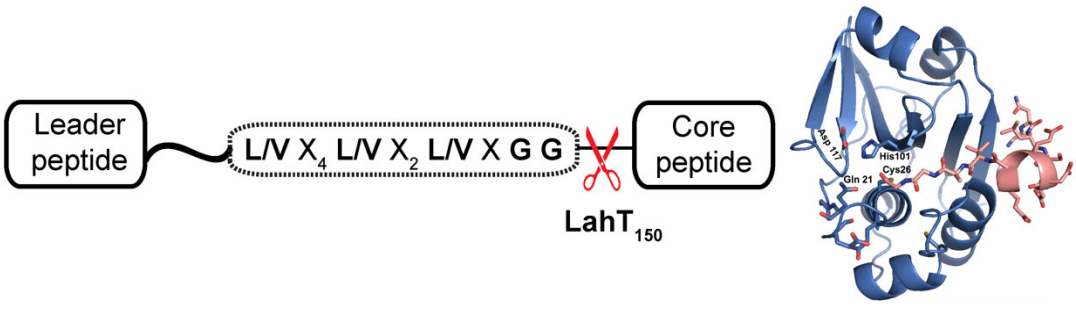
Leader peptide removal at a double glycine motif by a protease (LanT) is the final step in the biosynthesis of class II lanthipeptides. LanTs are comprised of an N-terminal cysteine protease domain, a transmembrane domain and an ATP-binding cassette. A novel lanthipeptide biosynthetic gene cluster was found that encodes multiple precursor peptides with conserved leader regions, but diverse core regions. The LanT associated with this cluster contains a protease domain that can tracelessly remove leader peptides by recognizing a short acidic stretch at the C-terminus of the leader region. This promiscuous enzyme is able to cleave leader peptides from unrelated ribosomally synthesized and post-translationally modified peptides (RiPPs) from Gram-negative or Gram-positive bacteria stemming from different leader peptide families such as Nif11, nitrile hydratase or even glycocins. Substrate residues critical for enzyme activity and the crystal structure with a peptidic aldehyde inhibitor have been elucidated.



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