Mechanistic Investigation of TfuA-mediated Peptide Thioamidation

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RiPPs (ribosomally synthesized, post-translationally modified peptides) are a class of peptidic natural products whose properties are drastically altered by the modifications. Among the modifications, thioamides are found in thioviridamide, methanobactin, thiostrepton, etc. Recent research from the Mitchell lab have demonstrated that enzymes from the YcaO superfamily are capable of catalyzing this transformation. However, peptide thioamidation catalyzed by the YcaO enzyme alone has low efficiency. Moreover, gene deletion experiments suggest that a protein of unknown function TfuA is also required for this modification. In this study, we first utilized genomic information to identify the protein sulfur carrier that supports thioamidation. We then demonstrated that thioamidation is ~600 times more efficient when this protein sulfur carrier is used instead of inorganic sulfide, and TfuA is critical for the reaction. The TfuA enzyme orchestrates the protein sulfur carrier, YcaO, and the peptide substrate to achieve robust thioamidation. To be specific, TfuA facilitates the binding between the substrate and YcaO, as well as hydrolyzes the thiocarboxylic acid moiety that is used directly as the sulfur donor. In addition, single-residue variants of the TfuA were generated and assayed to reveal the residues important for binding YcaO and catalyzing hydrolysis of the thiocarboxylic acid. Here we propose a model where YcaO, TfuA, and the sulfur carrier protein interact with each other to form the thioamidation machine. Future work includes using protein NMR to characterize the binding surface, conducting kinetics study on TfuA, and exploring its substrate scope.