The Site-Specific Fluorescent Labeling of Lanthipeptides

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The modes of action by which many lantibiotics (lanthipeptides with antimicrobial properties) exert their bioactivity are not known and in some cases, the native functions of the lantibiotics have not yet been deduced. Therefore, it would be very beneficial to develop a way to visualize lantibiotics in their natural environment so that their spatial distributions, and ultimately the processes responsible for their bioactivities, may be investigated. Current methods to site-specifically label lanthipeptides with fluorescent probes involve coupling the molecule to the peptides C- or N-termini. However, these labeling strategies would be non-specific for any lanthipeptides containing side chains with carboxylic acids and amines, respectively.

Interestingly, an N-terminal ketone is a naturally occurring post-translational modification in some lanthipeptides. In these peptides, the ketone modification arises after proteolysis of the leader peptide, yielding the core peptide with an N-terminal dehydro amino acid (*3*). In aqueous conditions this N-terminal enamine will spontaneously convert into an α-ketoamide (*6*). We hypothesized that the reactivity of this carbonyl handle would be orthogonal with respect to the twenty canonical amino acids because none of them contain a ketone or aldehyde. We also investigated the installation of an α-ketoamide onto the N-terminus of lanthipeptides that lack this functionality. This ketone was then reacted with an aminooxy-linked fluorophore or, for minimal perturbation, a small alkyne tag, during a bioorthogonal oxime ligation. Once purified, the modified lantibiotic was added to bacteria cells and the distribution of the lantibiotic was visualized by confocal fluorescence microscopy.