

Mechanistic Studies on the Substrate-tolerant Lanthipeptide Synthetase ProcM

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Lanthipeptides are a class of post-translationally modified peptides with characteristic lanthionine (Lan) and methyllanthionine (MeLan) thioethers. These cyclic thioether crosslinks confer stability and endow lanthipeptides with various biological activities. The mechanism of catalysis of ProcM, a highly substrate-tolerant lanthipeptide synthetase, was investigated. We found that ProcM dehydrates the precursor peptides with C-to-N directionality. Cyclization by ProcM was found to occur in an ordered fashion with certain thioether crosslinks forming faster than the others. A hybrid ligation strategy was developed to prepare a series of semi-synthetic substrate analogs to further probe enzyme catalysis. Through assays using substrates bearing orthogonal cysteine side-chain protection, spontaneous non-enzymatic cyclization events were ruled out. The final ring topology of the mature prochlorosins depended on the order of crosslink formation. Finally, synthetically generated intermediates with non-native ring topologies did not undergo ring opening to interconvert to native ring topologies. Thus, cyclization by ProcM appears to be kinetically driven. Such insights are expected to aid future efforts in lanthipeptide engineering.

