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Streamlined Chemoenzymatic Synthesis of Cyclic Peptides by Non-ribosomal Peptide Cyclases

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PURPOSE OF THE ABSTRACT
Macrocyclization of peptide improves their pharmaceutical properties such as cell-membrane permeability, target specificity and metabolic stability. However, regio- and chemoselective intramolecular cyclization remain challenging. In the biosynthesis of non-ribosomal cyclic peptides, macrocyclases such as thioesterases (NRPS-TEs) efficiently catalyze the intramolecular peptide cyclization in a regiospecific manner without the use of protecting groups. Thus, NRPS-TEs are promising biocatalysts for the efficient synthesis of macrocyclic peptides. NRPS-TEs generally require the thioester leaving groups on its substrate, which are usually installed by solution-phase coupling reactions during substrate synthesis. However, this step often generates epimerized products which are necessitates the purification of desired peptide from diastereomeric mixture, which is notoriously difficult and time-consuming.

In this study, we have developed a streamlined chemoenzymatic approach to synthesize cyclic peptides that bypasses the need for leaving group installation in solution phase[1]. Linear peptides with diol ester functionalities on C-terminus were synthesized on a solid support. Cleavage of the resin-bound peptides yielded the diol esters with sufficient purity, which could be subjected for the subsequent enzymatic cyclization without further purification. The diol-activated peptides were efficiently cyclized in a head-to-tail manner by SurE, a representative penicillin-binding protein-type thioesterase which we previously discovered in biosynthesis of non-ribosomal peptide, surugamides[2-4]. Therefore, we established a streamlined chemoenzymatic approach for cyclic peptides.

With this method, we synthesized a library of C-terminally-activated peptides. Using these as substrates, we elucidated the broad substrate specificity of SurE on the substrate N-terminus and the chain length. Notably, the introduction of non-peptidic residues (various length of poly-ethylene glycol) in the middle of the substrates was completely tolerated, suggesting that SurE catalysis could be widely applicable for the cyclization of non-peptidic polymers with the appropriate residues at the ring-closing junction. However, SurE had a limited scope for the substrate C-terminus. Specifically, it was able to cyclize substrates with neutral D-amino acids at the C-terminus, but was less effective with substrates containing acidic and basic residues at the C-terminus.

To overcome this limitation, we explored homologous wild type enzymes. SSN analysis of more than 600 homologs as well as the investigating their substrate binding pockets suggested that this peptide cyclase family possess the diverse substrate scope. In particular, WolJ, a homolog of SurE was demonstrated to possess a unique specificity for substrate C-terminus that complements SurE's specificity. Additionally, we were able to manipulate the specificity of SurE through protein engineering. G235L mutation completely changed SurE's specificity on C-terminus from D-aa to Gly. These efforts have broadened the scope of the enzymatic macrolactamization[1]. Our study will potentially accelerate the exploitation of NRPS-TEs as biocatalysts.

FIGURES

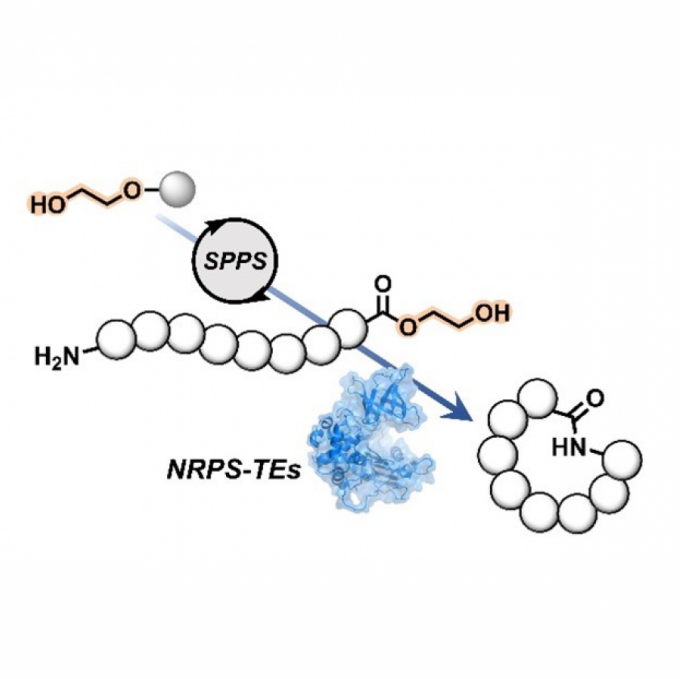

FIGURE 1

Figure 1

Diol-activated peptides were synthesized by SPPS and cyclized by NRP cyclases.

FIGURE 2

KEYWORDS

cyclic peptide | peptide cyclase | chemoenzymatic synthesis | protein engineering

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