学 位 論 文 内 容 の 要 旨

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Biosynthetic studies on a D-tryptophan-containing lasso peptide, MS-271
(D-トリプトファン含有ラッソペプチド天然物 MS-271 の生合成研究)

MS-271, originally isolated from *Streptomyces* sp. M-271 as a potent inhibitor of calmodulin-activated myosin light-chain kinase, is a lasso peptide natural product comprising 21 amino acids (aa) with a D-tryptophan (Trp) at its C-terminus. Lasso peptides are peptide natural products that have a characteristic isopeptide-bonded slipknot structure. In terms of their biosynthesis, lasso peptides belong to a group of ribosomally synthesized and post-translationally modified peptides (RiPPs). Thus, the biosynthesis of MS-271, especially the mechanism of D-Trp introduction, is of great interest. In this study, I investigated the biosynthesis of MS-271.

In chapter 2, I first identified MS-271 biosynthetic gene cluster (*msl*) spanning a ca. 11-kbp region from *mslR1* to *mslH* by draft genome sequencing followed by searching for DNA region encoding the amino acid sequence of MS-271. Sequence analysis revealed that precursor peptide gene (*mslA*) encoded 42-residue peptide with a leader peptide at its N-terminus, and most importantly, the C-terminal core region contained all 21 amino acid residues of MS-271 including the C-terminal Trp. This suggested that the D-Trp residue is introduced via epimerization into a ribosomal peptide as a post-translational modification. The cluster also contained genes for modification enzymes such as a macrolactam synthetase (*mslC*), precursor peptide recognition element (*mslB1*), cysteine protease (*mslB2*), disulfide oxidoreductases (*mslE*, *mslF*). Although obvious epimerase genes were absent in the cluster, the cluster encoded a protein of unknown function (*mslH*). Hence, I next carried out heterologous expression of the *msl* cluster in *Streptomyces lividans*. As the results, the production of MS-271 was confirmed by LC-MS and chiral amino acid analysis, indicating that the cluster contains all the necessary genes for MS-271 production including a novel peptide epimerase gene. I also showed that MslB1, B2, C and H were indispensable for MS-271 production by gene knockout experiments. Overall, these results suggested that MslH is responsible for the epimerization of the C-terminal Trp.

In chapter 3, I characterized the function of MslH *in vivo* and *in vitro*. Considering that many modification enzymes involved in RiPP biosynthesis require leader peptides for their substrate recognition, I speculated that the epimerization occurs on the nascent full-length MslA in MS-271 biosynthesis. As expected, *in vivo* experiments revealed the formation of epi-MslA when *mslA* was expressed with *mslH* in *Escherichia coli*. Additional coexpression of precursor peptide recognition element (*mslB1*) enhanced the formation of epi-MslA. Furthermore, *in vitro* experiments revealed that MslH catalyzed epimerization of

C-terminal Trp of MslA in metal- and cofactor-independent manner and that the leader peptide in MslA is indispensable for the substrate recognition by MslH. I also examine substrate specificity of MslH by heterologous expression of the *msl* cluster to produce MS-271 derivatives by altering the core peptide sequences of the *mslA* gene, and demonstrated that MslH exhibited broad substrate specificities toward the N-terminal region of core peptides while the C-terminal "CFW" sequence is important for substrate recognition. Overall, I fully characterized MslH as a novel peptide epimerase. This is the first example epimerase that catalyzes epimerization at the C_{α} center adjacent to a carboxylic acid in a cofactor-independent manner.

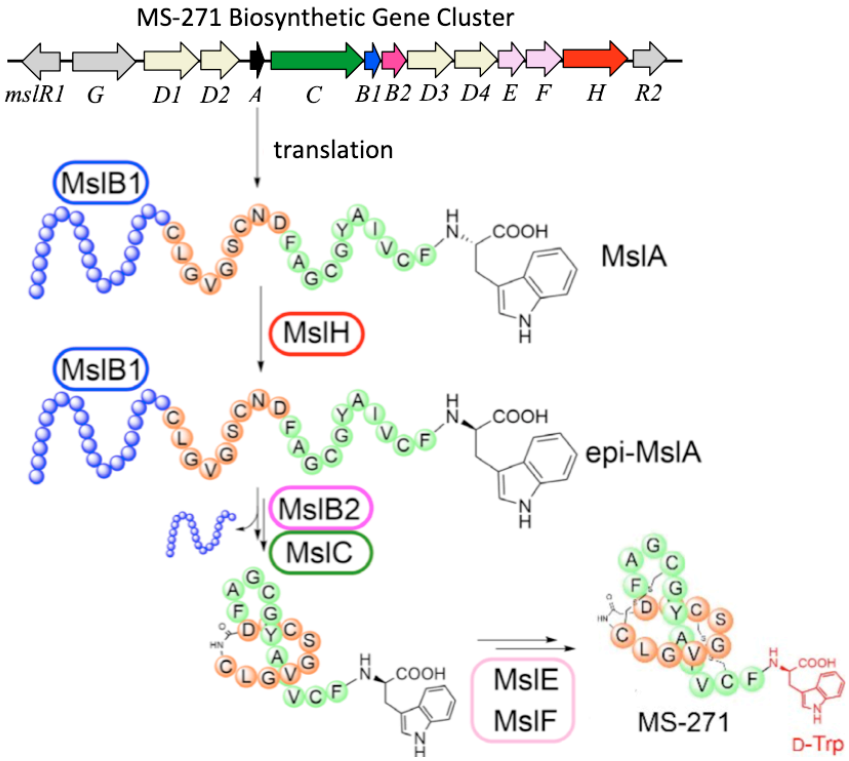

Figure 1. Proposed biosynthesis of MS-271.

Taken together, I identified MslH, previously annotated as a hypothetical protein, as a novel epimerase involved in the post-translational epimerization of the C-terminal Trp residue of the precursor peptide MslA. I also demonstrated that MslH exhibited broad substrate specificity toward the N-terminal region of the core peptide, showing that MslH-type epimerases offer opportunities in peptide bioengineering.