【Grant-in-Aid for Scientific Research (S)】

Dissecting biosynthetic machineries of natural peptides leading to structural and functional diversities.

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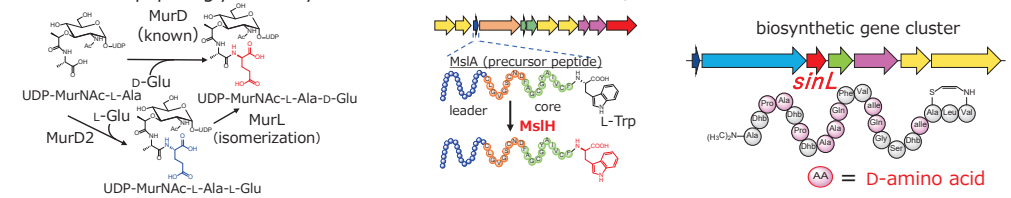
Purpose and Background of the Research

●Outline of the Research

Recently, peptide compounds with medium molecule weight are attracting attentions because they can be prepared with low cost but have structural and functional diversities. Focusing on natural peptides, their basic skeletons are biosynthesized by ribosome or non ribosomal peptide synthetase (NRPS). Since peptides composed of only L-amino acids are easily degraded by proteases, some peptides contain D-amino acids. Although some NRPSs introduce D-amino acids into elongation peptide by epimerase domains, only limited examples were reported about epimerization of ribosomal peptides. We recently identified four novel epimerases, which have no similarities with other known enzymes. In this study, we try to clarify the detailed reaction mechanisms of the enzymes.

Many natural pseudopeptides with structural diversities are also isolated but most of their biosynthetic mechanisms remain unknown. Therefore, we select 4 compounds to investigate their biosynthetic details.

Fig. 1 Studies on the four novel epimerases【1】~【4】



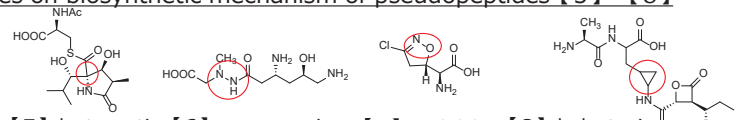
【1】During peptidoglycan biosynthesis, D-Glu is directly attached to UDP-MurNAc-L-Ala, an intermediate substrate. However, some bacteria firstly attach L-Glu to the intermediate and then MurL epimerizes the terminal L-Glu. In this study, the reaction mechanism is investigated.

【2】MS-271 is a ribosomal peptide but has D-Trp at C-terminus. MslH was revealed to catalyze the epimerization of C-terminus L-Trp of precursor peptide (MslA). In this study, the reaction mechanism is investigated.

【3】Salinipeptin is a ribosomal peptide but contain nine D-amino acid residues. However, no epimerase genes were involved in the biosynthetic gene cluster. *sinL*, the sole function-unknown gene, perhaps encodes a new epimerase and the possibility is examined.

【4】D-Glu in polyglutamate (PG) is believed to be introduced into elongating PG by direct attaching of D-Glu. However, we confirmed that L-Glu is firstly attached to the elongating intermediate and then epimerized. PgsA was suggested to catalyze the epimerization and the possibility is examined.

Fig. 2 Studies on biosynthetic mechanism of pseudopeptides【5】~【8】



【5】lactacystin【6】negamycin【7】acivicin【8】belactosin

【5】Lactacystin possesses a α,α-disubstituted amino acid structure and was suggested to be synthesized from L-Leu, L-Cys, and methylmalonic acid by tracer experiments. In this study, the biosynthetic mechanism is investigated.

【6】The biosynthetic mechanism of hydrazide structure (N-N-C=O) is investigated.

【7】The biosynthetic mechanism of isoxazoline structure (N-O) is investigated.

【8】The biosynthetic mechanism of cyclopropane structure found in belactosin was recently clarified (*Angew. Chem. Int. Ed*. **61**, e202113189 (2022)). The functions of orthologs found in genome database are investigated.

Expected Research Achievements

【1】MurL requires ATP and activates the substrate by adenylation and catalyzes unidirectional epimerization of the terminal L-Glu of UDP-MurNAc-L-Ala-L-Glu. To elucidate the reaction mechanism, a structure of co-crystal of MurL and an adenylated mimic is essential. Therefore, synthesis of the mimic compound (Fig. 3) is now in progress. Finally, reaction mechanism is investigated with mutant enzymes.

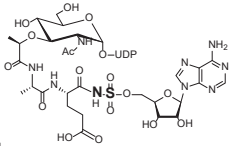


Fig. 3, Adenylated mimic compound

【2】To elucidate the reaction mechanism of MslH, a structure of co-crystal of MslH and a substrate mimic, MslAΔW, was solved. Since some important residues for the reaction were identified (Fig. 4), a probable reaction mechanism is examined with mutant enzymes.

【3】Salinipeptin is a ribosomal peptide but contains 9 D-amino acid residues. We confirmed that an epimerase gene was involved in the biosynthetic gene cluster. *salL* gene is a sole gene with function-unknown and perhaps a novel epimerase. The possibility is examined by in vitro analysis.

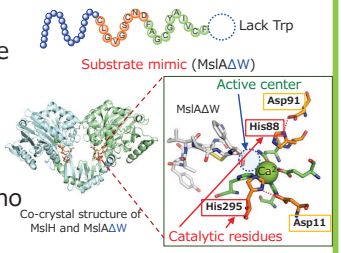


Fig. 4, Co-crystal of MslH and a substrate mimic

【4】When *pgsA,* a PG biosynthetic gene, was replaced with another *pgsA* from producers of PG containing different D-Glu contents, D-Glu ratio varied depending on the used *pgsA*, suggesting that PgsA is an epimerase. Since PgsA is partly similar to MslH, the reaction mechanism is investigated based on PgsA structure simulated with MslH.

【5】~【7】Lactacystin, negamycin, and acivicin possess amido bond, N-N bond, and Cl group, respectively. Genes responsible for the formation of these structures are firstly isolated by genome mining and then biosynthetic gene clusters will be identified by heterologous expression and/or gene-knockout. Finally, the detailed biosynthetic mechanisms will be investigated by in vitro analysis.

【8】Since the biosynthetic mechanism for cyclopropane formation by BelK and BelL was recently clarified (Fig. 5), the functions of their orthologs found in genome database is investigated.

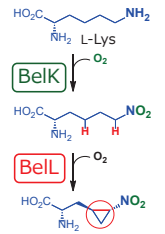


Fig. 5, Mechanism of cyclopropane formation

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