



HOKKAIDO UNIVERSITY

Title	A Novel Epimerase Catalyzing Multiple Isomerization of Amino Acid Residues of Ribosomal Peptide [an abstract of dissertation and a summary of dissertation review]
Author(s)	肖, 宛璐
Degree Grantor	北海道大学
Degree Name	博士(工学)
Dissertation Number	甲第15640号
Issue Date	2023-09-25
Doc URL	https://hdl.handle.net/2115/90815
Rights(URL)	https://creativecommons.org/licenses/by/4.0/
Type	doctoral thesis
File Information	XIA0_Wanlu_abstract.pdf, 論文内容の要旨



学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称 博士（工学） 氏名 ショウ エンロ

学 位 論 文 題 名

A Novel Epimerase Catalyzing Multiple Isomerization of Amino Acid Residues of Ribosomal Peptide

(リボソームペプチドの複数アミノ酸残基を異性化する新規エピメラーゼ)

Salinipeptins, grisemycin, and cypemycin are ribosomally synthesized and post-translationally modified peptides (RiPPs). Among these, salinipeptins was reported to comprise 22 amino acid residues with multiple D-amino acids and its biosynthetic gene cluster was identified. However, no genes homologous to known isomerases such as epimerases and racemases existed in the cluster, but a gene, *salL*, which showed no similarities to function known enzymes, located in the cluster, suggesting that *SalL* might be a novel epimerase. Actually, biosynthetic gene clusters of grisemycin and cypemycin also possess orthologs of *salL* although chirality of amino acids composing grisemycin and cypemycin, have not been reported. Therefore, I first examined grisemycin also contains D-amino acid residues. By heterologous expression of grisemycin biosynthetic gene cluster (*grm*) in *Streptomyces lividans*, grisemycin was confirmed to contain multiple D-amino acids, in the similar manner to salinipeptins. The heterologous expression experiments also confirmed the involvement of a novel peptide epimerase in grisemycin biosynthesis. Gene-deletion experiments indicated that *grmL*, an ortholog of *salL*, was indispensable for grisemycin production and that the epimerization preceded decarboxylation and methylation, which are other modifications installed into the precursor peptides of grisemycin (GriA).

To obtain further evidence that GriL encodes the novel epimerase, recombinant precursor peptide (GriA) and GriL were prepared and used for in vitro analysis. However, no isomerase activities were observed under various conditions. Considering that grisemycin contains dehydroamino acid and its biosynthetic gene, *griH*, exists in the gene cluster, the dehydration reaction might occur before isomerization. To examine the possibility, recombinant GriH was prepared and incubated with GriA, but no dehydration activity was detected. Because GriL is the novel enzyme found in *Streptomyces* strain, I consider a possibility that GriL requires a co-factor specifically utilized in *Streptomyces* strain. I therefore again utilized the above-mentioned heterologous expression system. When *griA*, *griH*, and *griL* were co-expressed, a dehydrated and isomerized GriA was produced, but *griA* and either of the two resulted in the production of no modified GriA. The results suggested that an interaction among three enzymes would be essential for dehydration and isomerization of GriA.