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MICROBIAL PHYSIOLOGY AND BIOCHEMISTRY

Heterologous Production and Structure Determination of a New Lanthipeptide Sinosporapeptin Using a Cryptic Gene Cluster in an Actinobacterium *Sinosporangium siamense*

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Abstract
Lipolanthine is a subclass of lanthipeptide that has the modification of lipid moiety at the *N*-terminus. A cryptic biosynthetic gene cluster comprising four genes (*sinA*, *sinKC*, *sinD*, and *sinE*) involved in the biosynthesis of lipolanthine was identified in the genome of an actinobacterium *Sinosporangium siamense*. Heterologous coexpression of a precursor peptide coding gene *sinA* and lanthipeptide synthetase coding gene *sinKC* in the host *Escherichia coli* strain BL21(DE3) resulted in the synthesis of a new lanthipeptide, sinosporapeptin. It contained unusual amino acids, including one labionin and two dehydrobutyrine residues, as determined using NMR and MS analyses. Another coexpression experiment with two additional genes of decarboxylase (*sinD*) and *N*-acetyl transferase (*sinE*) resulted in the production of a lipolanthine-like modified sinosporapeptin.

Keywords Heterologous expression · Lanthipeptide · Lipolanthine · Biosynthesis

Introduction

Lanthipeptides are natural peptides that contain unusual amino acids, including lanthionine (Lan)/methyllanthionine (MeLan), and are classified into ribosomally synthesized and post-translationally modified peptides (RiPPs) based on the biosynthetic system (Hegemann & Sussmuth, 2020; Li et al., 2021; Montalbán-Lopez et al., 2021; Repka et al., 2017; van der Donk & Nair, 2014; Zhang et al., 2012). During the biosynthesis of lanthipeptide, a linear precursor peptide is initially ribosomally synthesized from its gene, and it has an *N*-terminal leader peptide and a *C*-terminal core peptide which normally has multiple Cys and Ser/Thr residues. The leader peptide is necessary for the recognition of processing enzymes that are involved in the modification and extracellular transport. In the core peptide, modification occurs by following two steps: (1) dehydration of Ser/Thr residues to give dehydroalanine (Dha)/dehydrobutyrine (Dhb) in the core peptide, (2) formation of Lan/MeLan rings by thioether bridging between Cys residues and dehydrated amino acids (Dha/Dhb). So far, the lanthipeptides are categorized into five classes (class I–V) based on their biosynthetic systems (Lee & van der Donk, 2022; Li et al., 2021).

Actinobacteria are Gram-positive bacteria that produce a wide variety of secondary metabolites based on genetic diversity. For decades, they have served as the major bioresources that aid in the discovery of novel bioactive compounds including lanthipeptides. A class I lanthipeptide microbisporicin (NAI-107) was found in the actinobacterium *Microbispora corallina* (Carrano et al., 2015; Castiglione et al., 2008; Fernández-Martínez et al., 2015; Foulston & Bibb, 2010, 2011; Maffioli et al., 2014; Vasile et al., 2012). Microbisporicin contains chlorinated tryptophan and dihydroxyproline residues in the molecule and exhibits potent antibacterial activity against *Staphylococcus aureus* through

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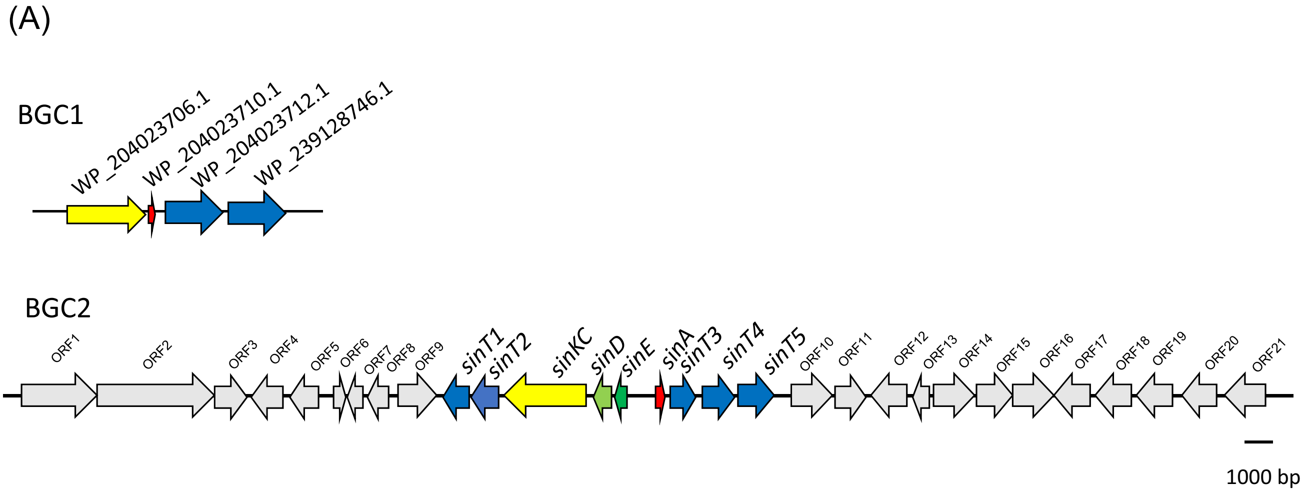
the blocking of peptidoglycan biosynthesis. Cinnamycin is a class II lanthipeptide that has 19 amino acids and is produced by the actinobacterium *Streptomyces cinnamoneus* (Ambrose, 1954; Benedict et al., 1952; Dvonch et al., 1954; Hosoda et al., 1996). Cinnamycin binds with high affinity to phosphatidylethanolamine of the bacterial cell membrane in a ratio of 1:1, resulting in potent antibacterial activity (Zhao, 2011). The morphogenetic peptide SapB was isolated from the model actinobacterium *Streptomyces coelicolor* (Willey et al., 1991). The structure of SapB was determined to be lanthipeptide which contained two lanthionine rings (Kodani et al., 2004; Willey et al., 2006). The lanthipeptide SapB was classified into class III lanthipeptide according to the biosynthetic gene cluster. The class III lanthipeptides labyrinthopeptins A1, A2, and A3 were isolated from the actinobacterium *Actinomadura namibiensis* (Férir et al., 2013, Meindl et al., 2010; Müller et al., 2010, 2011; Oeyen et al., 2021; Sambeth and Sussmuth, 2011). Labyrinthopeptins contain two units of a quaternary α,α-disubstituted amino acid named labionin (Lab) that is formed by bridging among one unit of Cys and two units of Dha with the function of the modification enzyme LabKC. In the biosynthesis of class III lanthipeptide, one modification enzyme (LanKC) accomplishes dehydration and cyclization on the precursor peptide. Based on structure, class III lanthipeptides are classified into three types: Lan/MeLan containing type (SapB [Kodani et al., 2004], AmfS [Takano et al., 2017; Ueda et al., 2002, 2005], curvopeptin [Jungmann et al., 2014; Krawczyk et al., 2012]), Lab containing type (labrynthopeptin [Férir et al., 2013; Meindl et al., 2010; Müller et al., 2010, 2011; Oeyen et al., 2021; Sambeth and Sussmuth, 2011], catenulipeptin [Wang & van der Donk, 2012], flavipeptin [Völler et al., 2013], stackepeptins [Jungmann et al., 2016], NAI-112 [Sheng et al., 2020; Tocchetti et al., 2021]), and Lan/MeLan/Lab-mix type [erythreopeptin (Völler et al., 2012)]. Lipolanthines are a recently advocated subclass of lanthipeptide. The first reported lipolanthine microvionin (Wiebach et al., 2018, 2020) contains an unusual amino acid avionin derived from labionin and triamino-dicarboxylic acid moiety is attached to amide of *N*-terminus amino acid. The lipolanthine goadvionin was isolated from *Streptomyces* sp. TP-A0584 (Kozakai et al., 2020). By analysis of the biosynthetic genes, goadvionin was indicated to be polyketide/RiPP hybrid lipopeptide. New fatty-acylated lanthipeptide solabiomycin A and B were isolated from *Streptomyces lydicus* (Asamizu et al., 2022). Previously reported antibiotics albopeptins (Isono et al., 1986) were identified as fatty-acylated lanthipeptides containing Lab (Oikawa et al., 2022).

In this study, we found a new cryptic biosynthetic gene cluster of lipolanthine in the genome of a rare actinobacterium *Sinosporangium siamense* (Suriyachadkun et al., 2014). Coexpression of genes coding precursor peptide and modification enzyme in *Escherichia coli* strain BL21 (DE3) resulted in the production of a new lanthipeptide, sinosporapeptin. Here we describe the heterologous production and structure determination of a new lanthipeptide sinosporapeptin.

Results

Assignment of a Biosynthetic Gene Cluster of Sinosporapeptin

Previously, one BGC for class III lanthipeptide BGC (BGC1, Fig. 1A) was indicated in the genome of *Sinosporangium siamense* (Walker et al., 2020). The BGC1 includes genes for precursor peptide (accession number: WP_204023710.1), lanthipeptide modification enzyme *lanKC* (WP_204023706.1), and transporter (WP_204023712.1 and WP_239128746.1). The amino acid sequence of the precursor coding gene (WP_204023710.1) had a high similarity with SapB/AmfS precursor coding gene (WP_223734132.1) of *Streptomyces purpurogeneiscleroticus* with an identity of 64%. In the core peptide, the positions of amino acids involved in lanthionine or labionin formation (Ser and Cys) were conserved (red and blue letters in Fig. 1B). Interestingly, we found another gene coding lanthipeptide modification enzyme *lanKC* (WP_204021205.1, *sinKC* in BGC2 of Fig. 1A) in the genome sequence of *S. siamense*. However, the precursor peptide coding gene was not annotated. Thus, we searched the precursor peptide coding gene in the region close to the *lanKC* gene (WP_204021205.1, named to be *sinKC*, Fig. 1A) from the raw DNA sequence. As a result, we found a precursor peptide coding gene (*sinA*) on the upstream region of *sinKC* with an opposite direction oriented (red arrow in Fig. 1A). Since the amino acid sequence of *sinA* contains the sequence of SXXSXXXC (Fig. 1C), the lanthipeptide derived from *sinA* was expected to have one lanthionine or labionin. The amino acid sequence of the precursor peptide coding gene *sinA* did not show a similarity with those of the other lipolanthine precursor peptides (Fig. 1C). The leader peptide region in *sinA* is ambiguous. In microvionin biosynthesis, α-helix structure at *N*-terminus leader peptide was indicated for recognition of lanthipeptide synthetase *micKC* (Wiebach et al., 2020). We subjected the amino acid sequence of *sinA* to the secondary structure analysis program CRNPRED (Kinjo & Nishikawa, 2006). As a result, the sequence ILELQE was indicated to form α-helix structure for possible recognition sequence of the lanthipeptide modification enzyme. Two ABC-transporter coding genes (*sinT1* and *sinT2*) existed on the downstream region of gene *sinKC* (Fig. 1A). Three putative ABC-transporter coding genes (*sinT3*, *sinT4*, and *sinT5*, in Fig. 1A) were



(B)

Sinosporangium siamense	`MAL`**`LDLQGME`**`NETPG`**`G`**`G-P`**`RS`**`TA`**`S`**`RH`**`C`**`G`**`GG`**`A`**`S`**`W`**`LS`**`L`**`L`**`L`**`C`**
Sinosporangium album	`MAL`**`LDLQGME`**`SEAPG`**`G`**`G--`**`RS`**`HY`**`S`**`RG`**`C`**`-`**`GG`**`G`**`S`**`W`**`LS`**`L`**`L`**`I`**`C`**
Streptomyces sp. WAC05374	`MTI`**`LDLQGME`**`TRHH-`**`G`**`KPR`**`RS`**`GA`**`S`**`KY`**`C`**`G`**`GG`**`A`**`S`**`R`**`LS`**`L`**`L`**`L`**`C`**
Streptomyces purpurogeneiscleroticus	`MTI`**`LDLQGME`**`TAHHG`**`G`**`KPR`**`RS`**`GA`**`S`**`KY`**`C`**`-`**`GG`**`T`**`S`**`Q`**`LS`**`L`**`L`**`L`**`C`**
Streptomyces ochraceiscleroticus	`MTI`**`LDLQGME`**`TAHHG`**`G`**`RPR`**`RS`**`GA`**`S`**`KY`**`C`**`-`**`GG`**`T`**`S`**`Q`**`LS`**`L`**`L`**`L`**`C`**
Streptomyces sp. CB03238	`MTI`**`LDLQGME`**`TSHH-`**`G`**`KPR`**`RS`**`GA`**`S`**`KY`**`C`**`-`**`GG`**`A`**`S`**`R`**`LS`**`V`**`L`**`L`**`C`**
Streptomyces violens	`MTI`**`LDLQGME`**`TAHH-`**`G`**`RPR`**`RS`**`GA`**`S`**`KY`**`C`**`-`**`GG`**`T`**`S`**`Q`**`LS`**`L`**`L`**`L`**`C`**

(C)

sinA	`MDLSNILELQELEPAPEYAELPG`**`S`**`NN`**`S`**`TIT`**`C`**
microvionin	`MESMTLEQLEALDASSEAAEMAA`**`S`**`LG`**`S`**`-QS`**`C`**
goadvionin	`MESVLDLQELETSEEES-ALMAA`**`S`**`TI`**`S`**`-QN`**`C`**
albopeptin	`MSIMDLQGLEVPGERD--AQALG`**`S`**`TV`**`S`**`-TG`**`C`**

Fig. 1 **A** Biosynthetic gene clusters (BGCs) of lanthipeptide in the genome of *Sinosporangium siamense*, colored genes are following: red, precursor peptide; yellow, lanthipeptide synthase *lanKC*; blue, transporter; green, decarboxylase; light green, GNAT family *N*-acetyltransferase. **B** Alignments of amino acid sequences of lanthipeptide precursor coding genes similar to that of the precursor in BGC1, **C** alignments of amino acid sequence of precursor coding genes similar to that of the precursor (*sinA*) in BGC2, conserved amino acid: bold letter, Ser and Thr in core peptide: Blue, Cys in core peptide: red

located downstream of *sinA* gene. The decarboxylase coding gene (*sinD*, light green in Fig. 1A) and GNAT family *N*-acetyltransferase coding gene (*sinE*, green in Fig. 1A) were located on the upstream region of gene *sinKC*. The two genes seemed to get involved in modification of the lanthipeptide to give lipolanthine. Lipolanthine biosynthesis normally has PKS biosynthetic genes for synthesis of a lipid moiety. We investigated the PKS biosynthetic genes in/near the BGC2 (ORF1-ORF21, Fig. 1A and Table S1). Although ORF21 (Fig. 1A and Table S1) encodes fatty acyl-AMP ligase which has low similarity (coverage 22%, similarity 32%, in amino acid sequence) with Acyl-CoA ligase gene of microvionin (*micE*), no PKS biosynthetic gene was present in/near the BGC. We investigated the MeOH extract of the cells of the actinobacterium *S. siamense* to check the production of the expected peptides biosynthesized from the BGC by HPLC and ESI–MS. However, the expected peptide was not detected (data not shown). Thus, we firstly planned to express the minimum gene set of *sinA* and *sinKC* to produce lanthipeptide.

Heterologous Production of Sinosporapeptin

The expression plasmid pET29-109515-AKC (Fig. 2A, Figs. S1 and S3) was constructed using chemically synthetic genes based on the vector pET29b (+). The vector pET29-109515-AKC was transformed into the cells of *Escherichia coli* BL21 (DE3). The transformant *E. coli* BL21 (DE3) harboring pET29-109515-AKC was cultured with induction of gene expression by Isopropyl-β-D-thiogalactopyranoside (IPTG) using a modified basal agar medium at 23 °C for 24 h. The cells were harvested from the surface of the agar medium, followed by MeOH extraction. As a result of analysis using HPLC and ESI–MS, the expected peptide sinosporapeptin was detected in the MeOH extract (Fig. 2C and Fig. S4). However, the production yield of sinosporapeptin

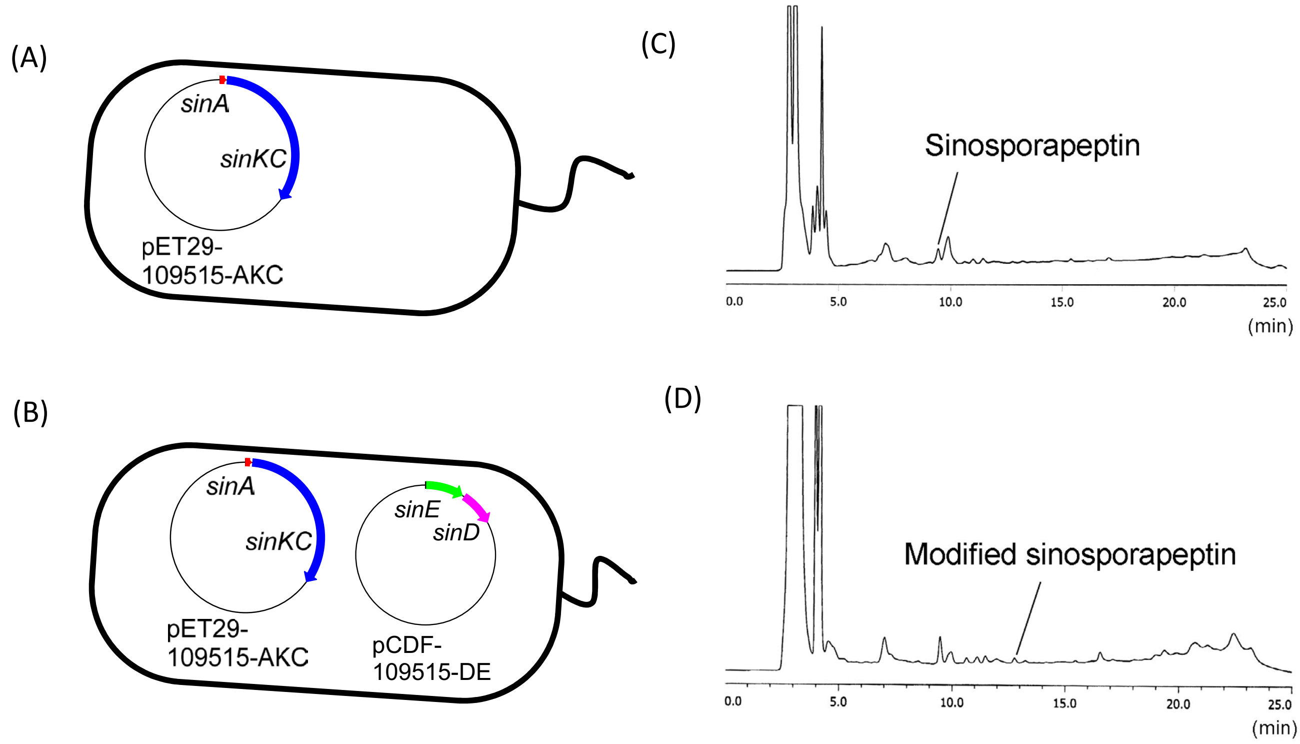


Fig. 2 **A** Scheme for *Escherichia coli* BL21 (DE3) harboring pET29-109515-AKC, **B** scheme for *E. coli* BL21 (DE3) harboringg pET29-109515-AKC and pCDF-109515-DE, **C** HPLC chromatogram of extract of *Escherichia coli* BL21 (DE3) harboring pET29-109515-AKC, **D** HPLC chromatogram of extract of *E. coli* BL21 (DE3) harboring pET29-109515-AKC and pCDF-109515-DE

was low. Thus, we performed optimization of the production medium. As a result, the production yield increased when a modified basal agar medium supplemented with amino acids was used for culture. To obtain enough amount of the peptide, we cultured the transformant *E. coli* BL21 (DE3) harboring pET29-109515-AKC using the modified basal agar medium supplemented with amino acids. The harvested cells were extracted with MeOH, followed by condensation using a rotary evaporator. The extract was subjected to open-column chromatography using hydrophobic resin CHP-20P with stepwise elution of aqueous MeOH. The 60% MeOH fraction was repeatedly subjected to HPLC separation using an ODS column to obtain sinosporapeptin (yield: 1.0 mg/the harvested cells from 1 L culture).

Structure Determination of Sinosporapeptin

The molecular formula of sinosporapeptin was determined to be $C_{37}H_{54}N_{12}O_{11}S$ (Fig. S5), since the ion peak was observed at *m/z* 921.3870 (theoretical value: *m/z* 921.3861) by high-resolution mass spectrometry. To determine the structure, measurement of NMR spectra including ^{1}H, ^{13}C, DEPT-135, DQF-COSY, TOCSY, NOESY, ROESY, HSQC, and HMBC (Fig. S6–S60) was accomplished on sinosporapeptin using DMSO-d_6 as a solvent (Fig. 3A, Table S2). Data of DQF-COSY and TOCSY were used for the identification of amino acid spin systems of usual amino acids (Pro, Gly, Ile, Asn × 2) in the molecule. Regarding Dhb, TOCSY correlation between methyl protons and β-proton was observed in Dhb7 and Dhb9. In Dhb9, HMBC correlations from methyl protons to β-carbon and β- carbon were observed. A long-range correlation was observed from methyl protons to carbonyl carbon in Dhb9. In Dhb7, HMBC correlation was observed from methyl protons to β-carbon. Although HMBC correlations from methyl protons to β-carbon and carbonyl carbon in Dhb7 were not observed, the presence of Dhb7 was indicated by the characteristic chemical shift values in γ-position and β-position. NOESY correlation between amide proton and methyl protons in Dhb7 and Dhb9 was observed, which indicated that the stereochemistry of Dhb7 and Dhb9 was *Z*-form. In the labionin moiety (partial structures: Lab3, Lab6, Lab10), the proton spin systems (amide proton/α-proton/β-protons) in Lab3 and Lab10 moieties were indicated by TOCSY spectrum. In Lab6, HMBC correlations were observed from β-protons to α-carbon and carbonyl carbon. In Lab6, amide proton was not observed. The connection between Lab6 and Lab10 was established by HMBC correlation from β-proton of Lab6 to α-carbon of Lab10. The connection

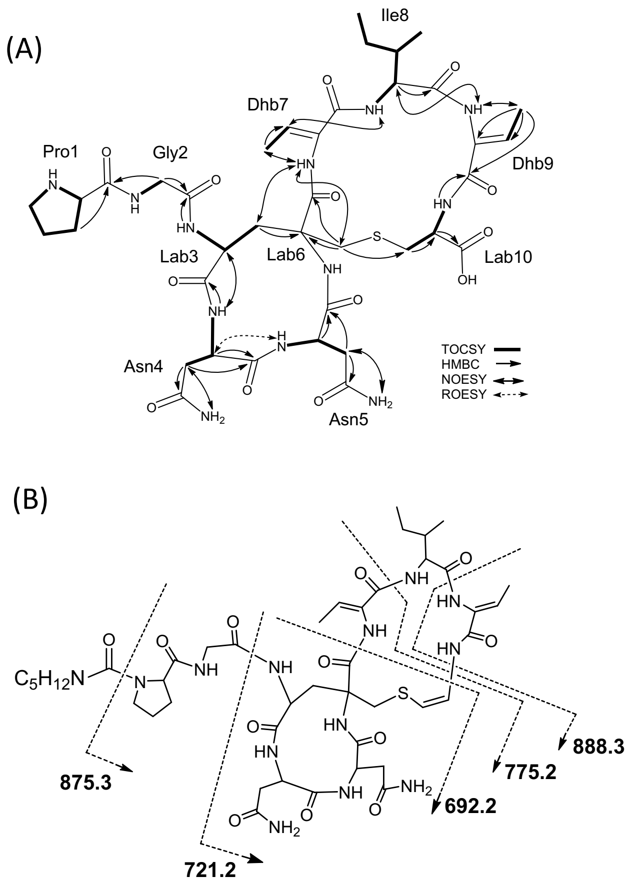


Fig. 3 **A** Key correlations of 2D NMR in sinosporapeptin, **B** Fragmentation ions in MS/MS experiment of the modified sinosporapeptin

between Lab3 and Lab6 was indicated by HMBC correlation from β-protons of Lab3 to α-carbon of Lab6. Regarding amino acid sequence, 2D NMR spectra including HMBC, NOESY, and ROESY were used to construct the two partial sequences (Pro1-Gly2-Lab3-Asn4-Asn5, Lab6-Dhb7-Ile8-Dhb9-Lab10). The connection between Asn5 and Lab6 could not be confirmed due to the lack of correlation in HMBC, NOESY, and ROESY spectra. However, we concluded that the structure of sinosporapeptin was Lab-containing lanthipeptide depicted as shown in Fig. 3A, considering the biosynthesis.

Antibacterial Activity Test on Sinosporapeptin

We performed an antibacterial test of sinosporapeptin using five bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Micrococcus luteus, Staphylococcus aureus*). As a result, sinosorapeptin did not show antibacterial activity to all testing bacteria at the concentration of 64 μg/ml.

Co-expression of *sinA* and *sinKC* with Additional Modification Genes *sinD* and *sinE* in *E. coli*

In the BGC, we found additional genes (*sinD*: decarboxylase and *sinE*: *N*-acetyltransferase coding genes) located close to *sinA* and *sinKC* (Fig. 1A). To perform the co-expression experiment, the genes (*sinD* and *sinE*) were chemically synthesized and the expression vector pCDF-109515-DE was constructed by integrating the genes into pCDF-Duet vector (Fig. 2B, Fig S2 and S3). The vector (pCDF-109515-DE) was transformed into *E. coli* BL21 (DE3) possessing pET29-109515-AKC. The transformant that had two vectors (pET29-109515-AKC and pCDF-109515-DE, Fig. 2B) was cultured for co-expression of the four genes (*sinA*, *sinKC*, *sinD*, and *sinE*) in the same condition as production of sinosporapeptin. As a result, we detected the modified sinosporapeptin by HPLC (Fig. 2D and Fig. S61). High-resolution mass spectrometry analysis indicated that the molecular formula of the modified sinosporapeptin was $C_{43}H_{65}N_{13}O_{12}S$ (Fig. S62), since the ion peak was observed at *m/z* 988.4670 (theoretical value: *m/z* 988.4674). The production amount was too low to perform NMR analysis for the determination of the structure. Thus, we performed MS/MS analysis on the modified sinosporapeptin (Fig. 3B, and Figs. S63–S65). The *N*-terminus modification was indicated by the fragmentations (*m/z* 875.3 and 721.2). Considering the biosynthesis of *N*-acetyl transferase SinE, we proposed that aminoacyl residue which had the molecular formula of $C_6H_{12}NO$ attach to *N*-terminus Pro. Considering the function of decarboxylase SinD, the presence of avionin was proposed, and fragmentation analysis of MS/MS supported the proposed structure (Fig. 3B). The production yield of the modified sinosporapeptin was quite low, so it was difficult to perform an antibacterial activity test.

Chemical Investigation to Detect Sinosporapeptin and the Modified Sinosporapeptin in *S. siamense*

To investigate the presence of sinosporapeptin and the modified sinosporapeptin in the original strain *S. siamense*, *S. siamense* was cultured on yeast-starch agar medium with a cellophane filter at 30 °C for 7 days. The cells were harvested and extracted with MeOH. The MeOH extract was analyzed by HPLC and ESI–MS (Figs. S66 and S67). On HPLC analysis several peaks were detected. However, ESI–MS analysis indicated that these peaks did not relate to sinosporapeptin.

mRNA Expression Profiles of Genes involved in Sinosporapeptin Biosynthesis in *S. siamense*

To investigate whether the production of sinosporapeptin by *S. siamense* below detectable levels was due to poor expression of its biosynthetic genes, mRNA expression profiles of *sinA*, *sinKC*, *sinD*, and *sinE* were analyzed by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). Although mRNA expression of these four genes could be detected, their expression levels were considerably low (Fig. S68). The expression levels of each gene peaked on days 3–5 of culture and then decreased markedly on day 7 of culture.

Discussion

In the biosynthesis of the lipolanthine microvionin, the lanthipeptide modification enzyme MicKC performs dehydration on Ser in the core peptide sequence to give Dha (Wiebach et al., 2018). Subsequently, the formation of labionin occurs by bridge formation among one unit of Cys and two units of Dha. The enzyme MicD decarboxylate *C*-terminus Cys along with dehydrogenation results in the formation of avionin. In the studies of biosynthesis of other lipolanthines, such as microvionin (Wiebach et al., 2020) and goadvionin (Kozakai et al., 2020), LanD-catalyzed decarboxylation was indicated to precede LanKC-catalyzed cyclization by in vitro experiments. In the biosynthesis of lipolanthine microvionin, protease (MicP) cleaves the leader peptide. Then, the lipid moiety bismethylated guanidino fatty acid (MGFA) was attached to *N*-terminus Ala. The moiety of MGFA is biosynthesized by a series of single-standing PKS enzymes. The condensation is proposed to occur by the function of *N*-acetyltransferase MicB. Recently the homologous *N*-actyltransfarase GvdG was indicated to catalyze the condensation reaction between lipid and peptide moieties in the biosynthesis of lipolanthine goadvionin (Kozakai et al., 2020). Based on genome-mining, the BGCs of lipolanthine were classified into subtype I-IV and unique PKS/NRPS hybrid type according to PKS biosynthetic genes and the presence of decarboxylase gene (Wiebach et al., 2020). In the biosynthesis of sinosporapeptin, PKS biosynthetic genes are not present in/near the biosynthetic gene cluster (*sinA*, *sinKC*, *sinD*, *sinE* in Fig. 1A). The proposed biosynthetic pathway of sinosporapepton in *E. coli* is shown in Fig. 4. The precursor peptide SinA is modified by SinKC to form two Dhb and one labionin (Fig. 4). By the function of SinD, avionin residue is formed through decarboxylation along with dehydrogenation. In this study, we performed in vivo biosynthesis by co-expression of *sinKC* and *sinD*. Thus, the order of reaction of dehydration, cyclization, and decarboxylation was not clear in the biosynthesis of the modified sinosporapeptin. However, we proposed a similar biosynthetic system with other lipolanthines, microvionin (Wiebach et al., 2020) and goadvionin (Kozakai et al., 2020), as shown Fig. 4. Judging by the result of MS/MS analysis, *N*-terminus Pro seems to be modified with the moiety of the molecular formula of $C_6H_{12}NO$, possibly synthesized by the function of SinE (Fig. 4). Since the biosynthesis takes place in the cell of *E. coli*, an endogenous protease cleaves *N*-terminus sequence including leader peptide (Fig. 4). For acylation by *N*-acetyltransferase SinE, endogenous aminocarboxylic acid may be incorporated into the modified sinosporapeptin (Fig. 4). In the chemical investigation, sinosporapeptin related peptides were not detected in the extract of the original strain, *S. siamense*. In addition, the gene expression analysis indicated that the biosynthetic genes (*sinA*, *sinKC*, *sinD*, and *sinE*) were hardly expressed in the original strain. Thus, the substrate for *N*-acetyl transferase SinE in the original strain *S. siamense* is unclear. Further study of activatation of the BGC in the original strain, *Sinosporangium siamense* to produce lipolanthine is needed to clarify the true substrate.

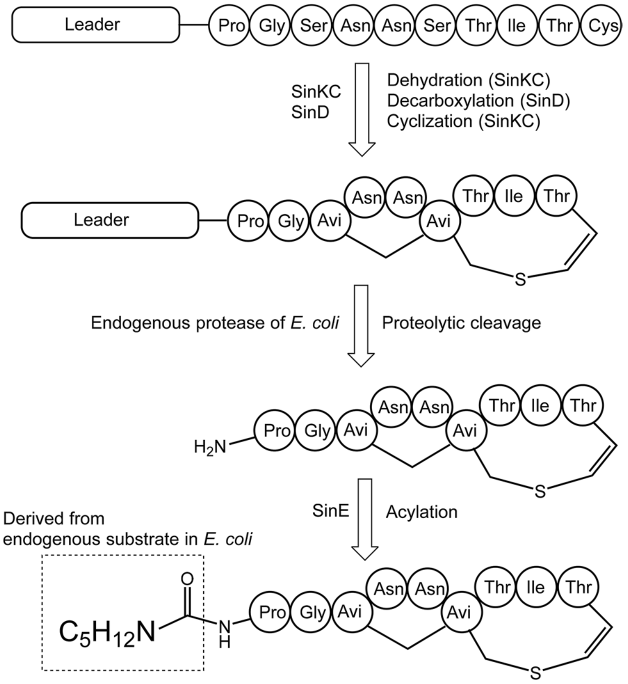


Fig. 4 Proposed biosynthetic pathway of the modified sinosporapeptin by heterologous expression in the cell of *E. coli*

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12275-023-00059-z.

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Author Contributions Conceptualization: S.K. Supervision: S.K., T. H. Methodology: K. S., K. M., I. K., H. N., T. H. Writing – original draft preparation: S.K. Writing – review and editing: S.K. All authors have read and agreed to the published version of the manuscript.

Data Availability All data needed to evaluate the conclusions in the paper are present in the paper and supplemental information.

Declarations

Conflict of Interest The authors declare no conflict of interest regarding this research.

Ethical Approval This study does not contain any experiments with human participants or animal.

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