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Biosynthesis of the Class III Lantipeptide Catenulipeptin

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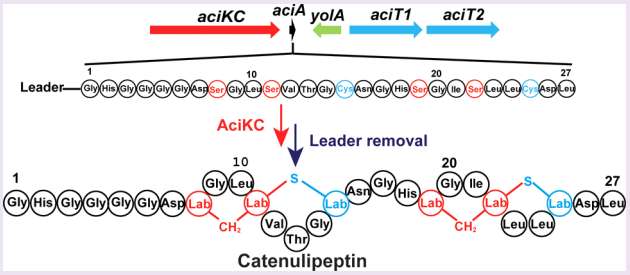
Supporting Information

ABSTRACT: Lantipeptides are ribosomally synthesized and posttranslationally modified peptides containing lanthionine and/or labionin structures. In this study, a novel class III lantipeptide termed catenulipeptin was discovered from *Catenulispora acidiphila* DSM 44928, and its biosynthesis was reconstituted *in vitro*. The multifunctional enzyme AciKC catalyzes both dehydration and cyclization of its peptide substrate AciA and installs two labionin structures in catenulipeptin. AciKC shows promiscuity with respect to cosubstrate and accepts all four NTPs. The C-terminal domain of AciKC is responsible for the labionin formation in catenulipeptin. The cyclase activity of AciKC requires the leader peptide of AciA substrate but does not require ATP or Zn^{2+}. Mutagenesis studies suggest that the labionin cyclization may proceed in a C-to-N-terminal direction. Catenulipeptin partially restores aerial hyphae growth when applied to surfactin-treated *Streptomyces coelicolor*.



Lantipeptides are a family of ribosomally synthesized peptides produced by a wide variety of bacteria that are generated by extensive posttranslational modifications.[1,2] Their common structural features are thioether cross-links that are formed between Ser/Thr and Cys residues. The Ser/Thr are first dehydrated to dehydroalanine/dehydrobutyrine (Dha/Dhb), followed by Michael-type addition of Cys residues onto the dehydroamino acids. The resulting thioether structures are called lanthionine (from Ser; Figure 1A) and methyllanthionine (from Thr). Many members of this family of compounds have antimicrobial activities and are therefore named lantibiotics,[3] but some lantipeptides exhibit other biological functions. For example, SapB produced by *Streptomyces coelicolor* (Figure 1B) is a morphogenetic peptide that functions as a biological surfactant and is essential for aerial hyphae formation.[4]

Lantipeptides have been divided into four classes based on their biosynthetic pathways.[5] In compounds from class I, II, and IV, the Michael-type addition is catalyzed by enzymes that contain a catalytic zinc in the active site that is believed to activate the Cys nucleophiles.[2,6−8] A domain with sequence homology to these Zn^{2+}-dependent cyclases is also believed to be involved in cyclization of the class III lantipeptides, but this domain does not contain the Zn ligands.[4] RamC, the enzyme that generates the lanthionines in SapB by posttranslational modification of the RamS peptide, was the first class III synthetase identified.[9] Recently, a novel class of lantibiotics was discovered that contain carbocyclic structures called labionins (Figure 1A).[10] These structures are formed from two Ser residues and one Cys residue by synthetases that are homologous to RamC. These enzymes contain a central kinase and C-terminal cyclase domain and are therefore called LanKC.[10] The kinase domain phosphorylates the Ser and Thr residues to be dehydrated,[2,11] and a third N-terminal domain acts as a lyase that eliminates the phosphate from phosphoSer and phosphoThr to generate the Dha and Dhb residues.[2,12]

The reaction catalyzed by the putative cyclase domain is less well understood. Although RamC and LabKC have 43% sequence identity and their substrate peptides have identical $Ser(Xxx)_2Ser(Xxx)_3Cys$ motifs, their products, SapB and labyrinthopeptin A2, respectively, are quite different (Figure 1B). LabKC installs two labionin motifs in labyrinthopeptin by the addition of a Cys thiol to Dha to generate an enolate intermediate that then adds to a second Dha to form the labionin structure (Figure 1A).[11] RamC does not catalyze a tandem addition process, and the initially formed enolates are protonated to generate two typical lanthionine structures (Figure 1B). Intrigued by the novel structures and activities of class III lantipeptides, we decided to investigate one of the other homologues of RamC/LabKC that are encoded in the bacterial genome sequences. In this report, we present a new class III lantipeptide that we termed catenulipeptin and characterize its processing enzyme AciKC.

RESULTS AND DISCUSSION

Class III Lantipeptide from *C. acidiphila* DSM 44928. Our study focused on a gene cluster from the actinomycete *Catenulispora acidiphila* DSM 44928.[13,14] Based on sequence homology, this gene cluster encodes a class III lantipeptide synthetase AciKC, a substrate peptide AciA, two ABC transporters, and one hypothetical protein with unknown

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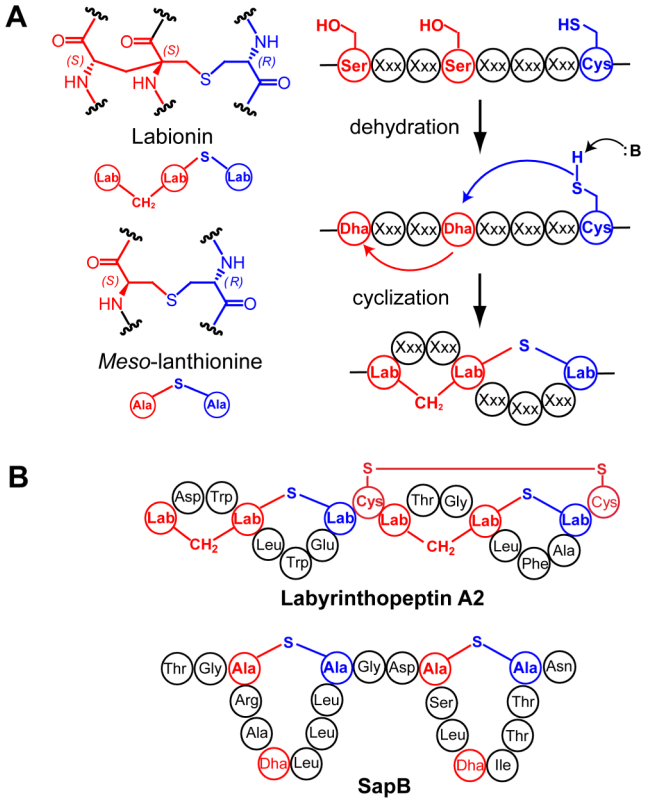

Figure 1. (A) Structures of labionin and *meso*-lanthionine and the proposed mechanism of labionin formation by a tandem Michael addition. Shown below the chemical structures are the shorthand notations used throughout this work. (B) Structures of labyrinthopeptin A2 and SapB.

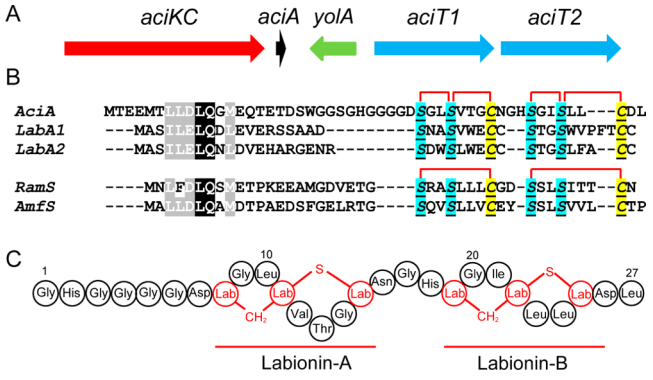

Figure 2. (A) Catenulipeptin biosynthetic gene cluster. (B) Sequence alignment of class III lantipeptide precursor peptides. Red lines indicate the proposed lanthionine/labionin rings formed after enzymatic modification. RamS is the precursor to SapB. (C) Proposed structure of catenulipeptin.

function (Figure 2A). Like all other lantipeptide precursor peptides, AciA contains an N-terminal leader peptide that is not modified and a C-terminal core peptide where the posttranslational modifications take place (*vide infra*).[15] A protease for leader peptide removal was not found in the gene cluster nor in neighboring gene clusters. The leader sequence of the peptide substrate AciA contains a Leu-Leu-Asp-Leu-Gln motif (Figure 2B), which is highly conserved in class III lantipeptide precursor peptides and has been shown to be essential for enzymatic processing.[16] The core peptide of AciA contains two Ser-Xxx-Xxx-Ser-Xxx-Xxx-(Xxx)-Cys motifs that could be precursors to either lanthionines as in SapB or to labionins as in labyrinthopeptins or both (Figure 2B). Like LabKC and RamC, AciKC consists of an N-terminal lyase domain, a central Ser/Thr kinase domain, and a putative C-terminal cyclase domain based on sequence homology.

We first examined culture extracts of *C. acidiphila* DSM 44928 by electrospray ionization (ESI) mass spectrometry (MS) and detected a putative triply charged peptide with a mass of 800.0283 Da (Supplementary Figure S1a). Subsequent tandem MS analysis showed the peptide to be 4-fold dehydrated AciA after removal of a 24 amino acid leader peptide (Supplementary Figure S1b). The fragmentation pattern suggested the formation of two labionins (Figure 2C and Supplementary Figure S1b). Different from SapB and labyrinthopeptins, but similar to other recently described class III lantipeptides,[17] catenulipeptin possesses a stretch of unmodified amino acids at its N-terminus. No catenulipeptin derivatives with overhangs of different length were detected by ESI-MS.

***In Vitro* Activity of AciKC.** To investigate the biosynthetic pathway, the substrate peptide AciA was expressed in *Escherichia coli* with an N-terminal hexahistidine tag and three additional lysine residues immediately following the His tag to increase its solubility (His_6-K_3-AciA). The putative lantipeptide synthetase AciKC was also expressed in *E. coli* with an N-terminal hexahistidine tag (His_6-AciKC). After purification (see Supporting Information), His_6-K_3-AciA was incubated with His_6-AciKC in the presence of Mg^{2+} and ATP. After 4 h, the AciA peptide was dehydrated five times as determined by matrix-assisted laser desorption ionization (MALDI) MS (Figure 3), which confirmed the dehydratase activity of

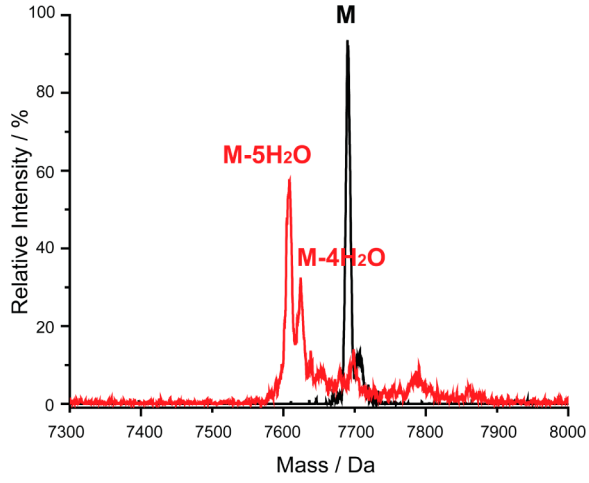

Figure 3. MALDI-TOF mass spectrum illustrating the *in vitro* dehydration activity of AciKC. AciA before incubation with AciKC is shown in black, and AciA after incubation with AciKC is shown in red.

AciKC. Interestingly, AciKC shows promiscuity with respect to its cosubstrate because use of ATP, GTP, CTP, or TTP all resulted in 5-fold dehydrated AciA peptide (Supplementary Figure S2a–d). However, when NTP was omitted from the assay, no dehydration was observed. The cyclase activity of AciKC was confirmed by incubating the AciKC-modified AciA peptide with 5 mM iodoacetamide (IAA), a thiol selective reagent, at pH 8.5. This assay did not result in any change in

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mass, as determined by MALDI-TOF MS, suggesting that the two Cys residues had been converted to nonreactive thioethers (Supplementary Figure S3). This conclusion was further supported by tandem mass spectrometry. AciKC-modified AciA was digested by endoprotease Glu-C to partially remove the leader peptide, and the product was subjected to ESI-MS−MS analysis. A similar fragmentation pattern was observed as that of the mature catenulipeptin from culture extract (Supplementary Figure S4a). These results show that AciKC is a multifunctional enzyme that catalyzes both dehydration and cyclization reactions.

Structure and Bioactivity of Catenulipeptin. In order to confirm the formation of labionins during the *in vitro* assay, AciKC-modified AciA peptide was hydrolyzed in acid, and the resulting amino acids were derivatized to the corresponding *N*-trifluoroacetamido ethyl esters by a previously reported procedure.[18] The resulting material was analyzed by gas chromatography (GC) coupled to positive chemical ionization (PCI) MS, resulting in two peaks with retention times of 11.24 and 11.32 min (Supplementary Figure S5a). The molecular ions associated with these peaks are consistent with those reported previously for derivatized labionin.[18] Furthermore, the fragment ions match those previously reported (Supplementary Figure S5b).[18] The observation of two peaks is the result of epimerization of labionin during hydrolysis as previously reported.[18] Unlike a very recent report on new class III lantipeptides from actinomycetes that contained both labionin and lanthionine,[17] catenulipeptin produced *in vitro* did not contain lanthionine derivatives as determined by GC−MS.[19,20] Combined with the ESI-MS−MS data, we conclude that like LabKC but unlike RamC, AciKC installs labionins in its substrate peptide. Labionin B in catenulipeptin is somewhat unusual in that it has only two residues between Cys25 and Ser22 rather than the three or five residues found thus far in other class III lantipeptides that contain labionins (eg LabA1 and LabA2, Figure 2B).[10,17]

The observation of a 5-fold dehydrated AciA peptide in the *in vitro* assay indicated an extra dehydration site in addition to the four Dha residues required for labionin formation. β-Mercaptoethanol (βME) was employed to determine the location of the additional Dha residue. After incubating with 5 mM βME at pH 8.5, AciKC-modified AciA peptide was fully converted to a product containing a single βME adduct, indicating only one dehydroalanine residue was present (Supplementary Figure S6). This result also further confirms the proposed catenulipeptin structure (Figure 2C) because it precludes the possibility of lanthionine formation, which would have resulted in more Dha residues and hence more βME adducts. Subsequent ESI-MS−MS analysis confirmed that the addition of βME occurred at position −1, indicating that Ser−1 was the fifth dehydration site (Supplementary Figure S7). A Dha residue in the leader peptide was also recently reported in another class III lantipeptide erythreapeptin when expressed heterologously.[17]

The dehydroalanine at the −1 position of modified AciA offers a convenient strategy to generate mature catenulipeptin *in vitro*. By reacting with cysteamine under mild alkaline conditions, the Dha−1 was converted into its cysteamine adduct, which is a lysine mimic (Figure 4, Supplementary Figure S8a). Subsequent proteolytic digestion by trypsin resulted in removal of the leader peptide and yielded the mature catenulipeptin *in vitro* (Figure 4, Supplementary Figure S8b).

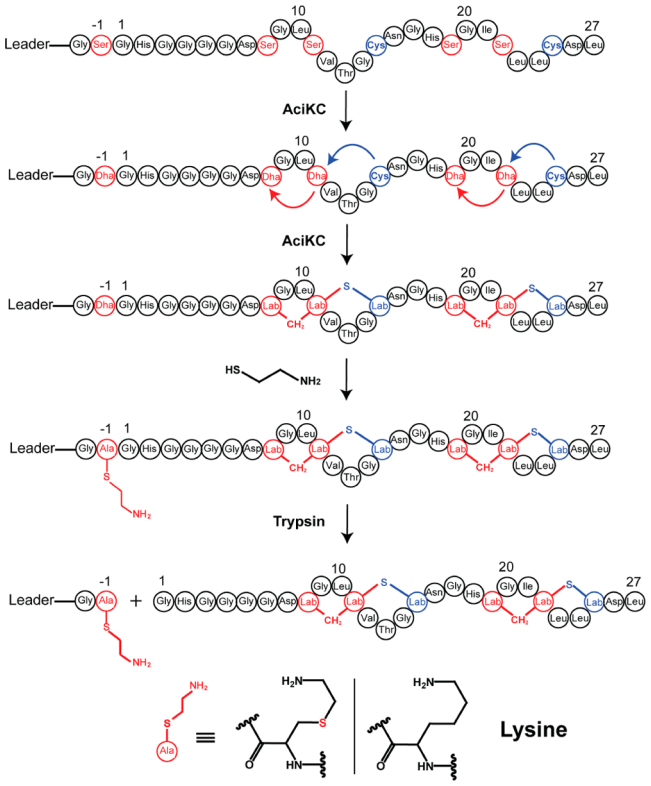

Figure 4. Generation of catenulipeptin *in vitro* by AciKC-catalyzed dehydration and cyclization, followed by addition of cysteamine to Dha−1, and treatment with trypsin.

With catenulipeptin generated *in vitro*, we were able to evaluate its bioactivity. The peptide was tested against a number of Gram-positive and Gram-negative bacteria including *Escherichia coli* DH5α, *Bacillus subtilis* ATCC6633, *Lactococcus lactis* HP, and *Streptomyces coelicolor*; no growth inhibitory activity was observed. Further investigation then focused on possible biological functions of catenulipeptin other than antimicrobial activities. SapB functions as a biological surfactant and facilitates the aerial mycelium formation in its producing strain *S. coelicolor*.[4] The lipopeptide surfactin has been shown to inhibit aerial hyphal formation in *S. coelicolor*, generating a halo of bald cells that do not have hyphae (Supplementary Figure S9A, right panel).[21] SapB can partially restore the aerial growth when applied to surfactin-treated cells. Similarly, when catenulipeptin was applied to wild type *S. coelicolor* together with surfactin, the diameter of the halo of bald cells was significantly reduced (Supplementary Figure S9A, left panel). Although a direct comparison with literature data is difficult, it appears that SapB is more potent than catenulipeptin in this assay (Supplementary Figure S9B). It has also been shown that SapB can trigger early onset of aerial hyphae formation of *S. coelicolor*.[21] When catenulipeptin was applied to plates seeded with a lawn of spores of *S. coelicolor* A3(2), no acceleration of aerial hyphae formation was observed compared to a control. Furthermore, unlike SapB, which can complement a range of bald mutants (in the *bld* genes) that cannot generate an aerial mycelium,[22] catenulipeptin could not restore aerial hyphae formation in *S. coelicolor* M600 Δ*bldA*.[23] Thus, whereas catenulipeptin can counteract the balding effect of surfactin, it cannot fully replicate the functionality of SapB on *S. coelicolor*. Since the molecular mechanism underlying the surfactin-mediated inhibition of aerial hyphae formation is not well

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understood, the role of catenulipeptin in aerial hyphae restoration is not clear and requires further studies. It may potentially be involved in aerial mycelium formation in its producing organism since only one class III lantipeptide gene cluster is present in the genome of *C. acidiphila* DSM44928[13] and this genus has been reported to form aerial hyphae.[14]

***In trans* Activity of Core and Leader Peptides.** The leader sequences of lantibiotic precursor peptides are important for enzymatic processing but are not strictly required in some examples, such as LctM.[24] The possibility that AciKC might also be able to process the AciA core peptide in the absence of the leader sequence was examined by incubation of the core peptide $AciA_{25-51}$ (1 μM) with AciKC (5 μM). Subsequent MALDI-TOF analysis revealed that no modifications occurred under such conditions. However, when the leader peptide $AciA_{1-24}$ was supplied *in trans* (2 μM), AciKC converted the AciA core peptide into a mixture of phosphorylated and dehydrated products (Supplementary Figure S10). These results clearly show that the leader peptide of AciA, either covalently attached to or supplied *in trans* with the core peptide, is essential for enzymatic processing of AciKC. Similar to the system of LctA and LctM,[25,26] the AciA leader sequence likely activates AciKC by introducing a conformational change.

Separation of Dehydration and Cyclization Activity. Intrigued by the mechanism of formation of the unusual labionin structure, we further investigated the cyclization activity of AciKC. A truncated protein, AciKC-ΔC, lacking the C-terminal putative cyclase domain (C-terminal 340 amino acids truncated), retained its dehydration activity (Figure 5A). However, tandem MS−MS analysis revealed that the formation of labionin rings was greatly impaired (Figure 5B, Supplementary Figure S11), which confirms the essential role of the C-terminal domain of AciKC for labionin formation. As mentioned previously, the zinc binding amino acid residues are missing in AciKC. To investigate whether perhaps another metal binding site might be present, AciKC was subjected to ICP-MS analysis and PAR assay analysis after removal of loosely bound metal ions by dialysis. Less than 0.1 equiv of zinc was observed by both methods. Furthermore, no significant amounts of other metals, such as Co, Fe, and Mg, were detected by ICP-MS.

One challenge to investigating multifunctional enzymes is that several reactions take place sequentially in a very short time scale and therefore analysis of one specific reaction is usually difficult. To further probe the cyclase activity of AciKC, dehydrated peptide substrate was desired. Fortuitously, the dehydratase activity of the AciKC protein tolerates protection of the Cys residues in AciA peptide as an intramolecular disulfide. The two cysteine residues in AciA peptide were protected by incubation with oxidized glutathione (Figure 5C). The resulting peptide was then incubated with AciKC, Mg^{2+}, and ATP. Although the disulfide bond introduced a cyclic structure into the linear substrate, AciKC dehydrated the peptide with the same efficiency as linear AciA.

With dehydrated AciA peptide in hand, we were able to investigate whether ATP is required for the cyclase activity of AciKC. Dehydrated AciA peptide (5 μM) was incubated with AciKC (5 μM) in the presence or absence of ATP (5 mM). TCEP (0.1 mM) was added to reduce the disulfide bond and release free the Cys thiol groups for cyclization. The samples were incubated for 1 h at room temperature (RT), digested by protease Glu-C, and subsequently analyzed by ESI-MS−MS (Supplementary Figure S12a). The data showed that labionins

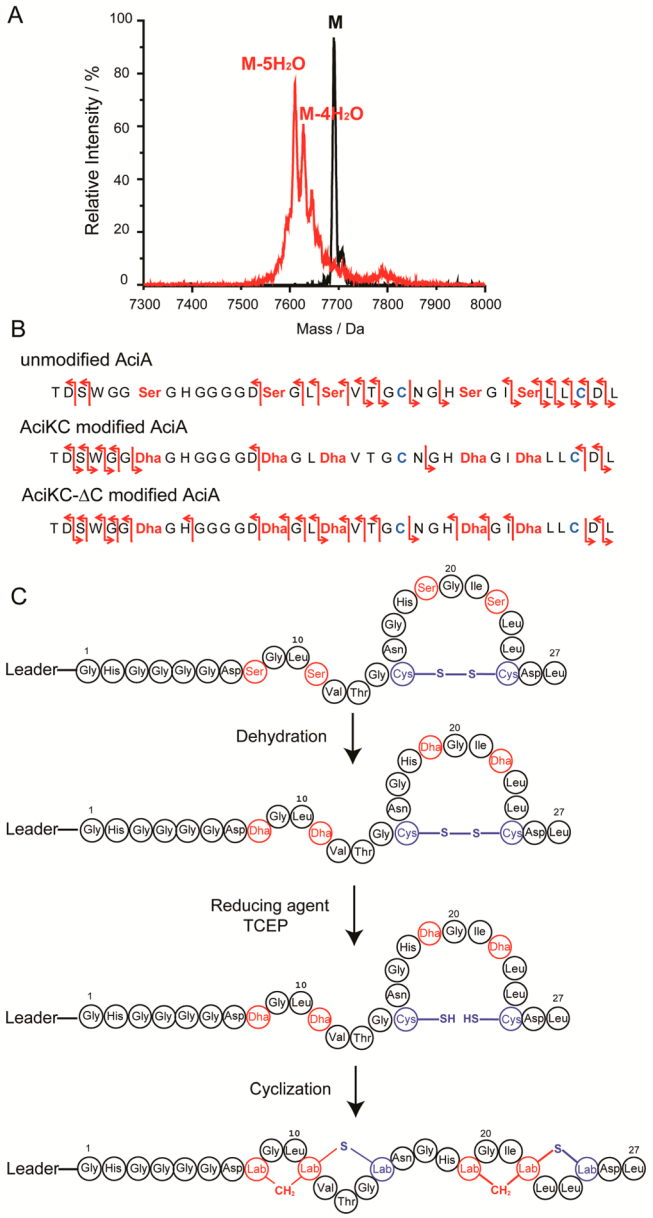

Figure 5. (A) MALDI-TOF spectrum illustrating the *in vitro* dehydration activity of AciKC-ΔC. (B) MS−MS fragmentation patterns of unmodified AciA peptide, AciKC-modified AciA, and AciKC-ΔC-modified AciA peptide. (C) Generation of dehydrated AciA peptide with an intramolecular disulfide bond and enzymatic cyclization by AciKC *in vitro*.

were formed correctly with or without ATP under such conditions. As a control, AciKC was omitted from the assay and no labionin was formed after incubation of dehydrated AciA and TCEP, as determined by ESI-MS−MS analysis (Supplementary Figure S12b). Therefore, ATP is not required for the cyclase activity of AciKC, and nonenzymatic cyclization does not result in labionin formation.

As described previously, the leader peptide of AciA is essential for the dehydratase activity of AciKC. Similarly, the leader peptide of LabA2, especially the highly conserved Leu-Leu-Glu-Leu-Gln motif, has been shown to be essential for the dehydratase activity of LabKC.[16] However, the role of the leader peptide for the cyclase activity of class III lantipeptide synthetases has not been evaluated yet. Therefore, a truncated

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dehydrated AciA peptide (N-terminal 17 amino acids removed) was generated. AciA was first oxidized to the corresponding disulfide, then treated with AciKC, and digested with Glu-C, which removed most of the leader peptide. This truncated peptide was then purified, reduced with TCEP, and incubated with AciKC for 1 h at RT. Subsequent ESI-MS–MS analysis showed that no labionin was formed in the truncated AciA peptide (Supplementary Figure S13). Thus, the leader peptide is essential for both dehydration and cyclization activity of class III lantipeptide synthetases.

The formation of labionin is proposed to involve two tandem Michael addition reactions initiated by a C-terminal cysteine thiol.[11] It is not yet known whether this process takes place in a stepwise manner with a lanthionine intermediate or whether both cross-links are made without any intermediates that are released from the enzyme. To further understand the cyclase activity of AciKC, a series of Ala mutations were introduced into the AciA peptide at positions that are precursors to labionin, and these mutant peptides were expressed heterologously in *E. coli* (Figure 6). The purified mutant peptides

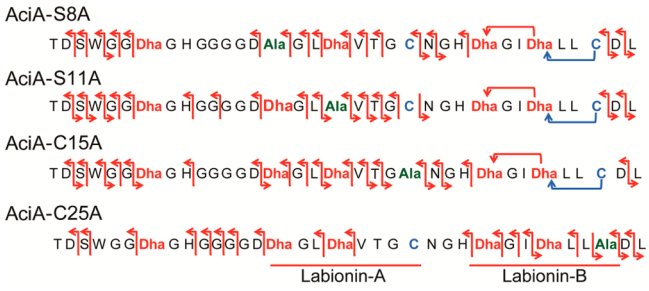

Figure 6. Fragmentation patterns of AciA mutant peptides modified by AciKC. All assays were performed in the presence of 5 mM NTP mixture, 10 mM $MgCl_2$, and 0.1 mM TCEP. Modified peptides were digested by endoprotease Glu-C before further tandem MS analysis.

were incubated with AciKC, ATP, and Mg^{2+}, digested with endoprotease Glu-C, and subjected to ESI-MS–MS for ring structure analysis. As expected, when Ser11 was mutated to Ala, the formation of labionin A (generated from Dha8-Dha11-Cys15) was completely abolished (Figure 6, Supplementary Figure S14b). Similarly, incubation of AciA-S8A not only disrupted the formation of the carbon–carbon bond between residues 8 and 11 but also greatly impaired the formation of the thioether between Dha11 and Cys15 (Supplementary Figure S14a). This result suggests either a concerted mechanism for formation of both cross-links in a labionin, or that no active site acid is available to protonate the enolate intermediate to generate a lanthionine when the second Dha is not available. The formation of labionin B (Dha19-Dha22-Cys25) was not impacted by the loss of labionin A, as determined by tandem MS analysis.

The effect of mutation of Cys15 and Cys25 was remarkably different (Figure 6, Supplementary Figure S14c,d). The formation of labionin A in the AciA-C15A peptide was disrupted as expected, whereas labionin B remained intact. Surprisingly, when Cys25 was mutated to Ala, it affected efficient formation of both labionins (Figure 6, Supplementary Figure S14d). These results suggest that formation of the C-terminal labionin B is important for the cyclization of labionin A but not *vice versa*. In turn, this suggests that cyclization may proceed in a C-to-N-terminal direction. This directionality would be opposite that observed for the class II lantibiotic synthetases HalM2 and LctM,[27] but it is similar to the recently reported directionality of the dehydration reaction of the class III lantipeptide synthetase LabKC,[28] and that of a cyclodehydratase involved in the biosynthesis of another posttranslationally modified peptide containing thiazoles and oxazoles.[29]

Conclusions. In summary, by genome mining we discovered a novel class III lantipeptide catenulipeptin from *Catenulispora acidiphila* DSM 44928 to add to the growing number of class III lantipeptides. Like LabKC, the multifunctional enzyme AciKC catalyzes both dehydration and cyclization *in vitro* to form the labionin structures in catenulipeptin. AciKC shows promiscuity toward its NTP cosubstrate and can partially modify the AciA core peptide when the leader sequence is supplied *in trans*. The cyclase activity of AciKC resides in its C-terminal domain and does not require ATP or Zn ions. However, the leader of AciA peptide is required for cyclization. Furthermore, mutagenesis studies indicate that the formation of labionin A in catenulipeptin is dependent on the formation of the C-terminal labionin B. Thus far, catenulipeptin has not shown antimicrobial activity, but the compound can partially restore aerial hyphae growth of surfactin-treated *S. coelicolor*. It cannot restore hyphae formation in a *bldA* mutant. Whether its function in its producer strain is similar to that of SapB in *S. coelicolor* remains to be determined. Certainly, structurally very dissimilar compounds have been shown to be important in morphogenesis in different actinomycetes such as SapT[30] and goadsporin.[31,32] It has been suggested that these types of molecules play a strictly mechanical role that requires a surfactant activity, possibly by enforcing an amphipathic structure *via* the posttranslational modifications.[33]

METHODS

Materials. All oligonucleotides were purchased from Integrated DNA Technologies. Restriction endonucleases, DNA polymerases, and T4 DNA ligase were purchased from New England Biolabs or Invitrogen. Media components for bacterial cultures were purchased from Difco laboratories. Chemicals were purchased from Fisher Sciencific or from Roche Biosciences. *E. coli* DH5α was used as host for cloning and plasmid propagation, and *E. coli* BL21 (DE3) was used as a host for expression of proteins and peptides.

Cloning, Protein, and Peptide Expression. Detailed procedures are described in the Supporting Information. Primer sequences for peptide mutants are included in Supplementary Table S1. The protein sequence of AciKC is available in the National Center for Biotechnology Information (NCBI) database under accession number YP_003114944.

Production of Catenulipeptin from *C. acidiphila*. *Catenulispora acidiphila* DSM 44928 was cultivated in trypticase soy broth liquid medium, pH 5.5–6.0, at 30 °C for 5–7 days. The supernatant of the culture was lyophilized to dryness, and the lantipeptide was extracted with acetonitrile.

Enzyme Activity Assay. In a typical experiment, AciA substrate (30 μM) was incubated with AciKC (3 μM) in the presence of TCEP (0.1 mM), $MgCl_2$ (10 mM), and NTP (5 mM) at RT in 50 mM Tris buffer (pH 8.0) for 4 h. The reaction was quenched by addition of TFA and analyzed by MALDI-TOF MS.

Detection of Labionin by GC–MS. The protocol was adopted and modified from ref 18. Lyophilized AciKC-modified AciA (0.8 mg) was dissolved in 6 M hydrochloric acid (2 mL) and heated at 110 °C in a high-pressure, sealed vessel for 20 h. The reaction was cooled to RT, and the hydrochloric acid was removed under a gentle stream of nitrogen. The dry hydrolysate was then dissolved in 500 μL of 2 M ethanolic HCl, prepared from acetylchloride in ethanol (1:4, v:v). The sample was heated at 110 °C for 15 min, and reagents were again removed under a gentle stream of nitrogen. To the resulting sample

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were added dichloromethane (200 μL) and trifluoroacetic anhydride (50 μL), and the mixture was heated for 10 min at 110 °C. The reaction was allowed to cool and was dried under a stream of nitrogen. The residue was dissolved in methanol and subjected to GC−MS. Experimental parameters for GC−MS analysis are included in Supplementary Table S2.

***In vitro* Generation of Catenulipeptin.** AciKC-modified AciA peptide (30 μM) was incubated with 2 mM cysteamine in 20 mM Tris buffer, pH 8.2, at RT for 12 h. The formation of peptide-cysteamine adduct was monitored by MALDI-TOF MS. Trypsin (20 μg mL^{-1}) was then added into the mixture, and the generation of catenulipeptin was monitored by ESI-MS. The mixture was further purified by reverse-phase high-performance liquid chromatography (RP-HPLC). HPLC factions containing catenulipeptin were combined and dried by lyophilization.

Bioactivity of Catenulipeptin. Wild type *S. coelicolor* spores were spread on a YEME agar plate, pH 7. A filter paper containing only surfactin (50 μg) or a filter paper containing both surfactin (50 μg) and catenulipeptin (25 μg) was placed in the center of the plate. The plate was incubated for 4 days at 30 °C until aerial hyphae were visible outside a zone of bald halo caused by the presence of surfactin. Detailed experimental procedures are described in the legend of Supplementary Figure S9.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Description of all molecular biology procedures, protein purifications, and supporting figures. This information is available free of charge *via* the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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