Organic LETTERS

Letter

Cite This: Org. Lett. 2018, 20, 7670−7673

pubs.acs.org/OrgLett

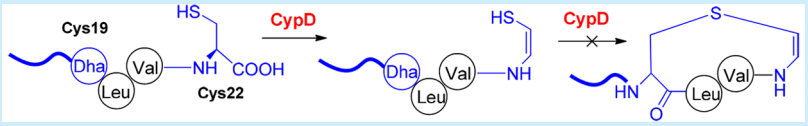
Cypemycin Decarboxylase CypD Is Not Responsible for Aminovinyl−Cysteine (AviCys) Ring Formation

Wei Ding,[†,#] Ning Yuan,[‡,#] Dhanaraju Mandalapu,[†] Tianlu Mo,[†] Suwei Dong,*[,‡] and Qi Zhang*[,†]

[†]Department of Chemistry, Fudan University, Shanghai, 200433, China

[‡]State Key Laboratory of Natural and Biomimetic Drugs, and Department of Chemical Biology, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China

Supporting Information



ABSTRACT: The cypemycin decarboxylase CypD is investigated by using a synthetic oligopeptide, which contains the to-be-cyclized dehydroalanine (Dha) residue. It was shown that CypD efficiently catalyzes the decarboxylation of this Dha-containing peptide, but the expected AviCys ring is not formed in the product, suggesting that CypD alone is not enough to form the AviCys ring. It was also shown that the Dha-containing peptide is a better substrate than two similar peptides with a Ser or a Cys residue, supporting that, in cypemycin biosynthesis, Dha formation is prior to decarboxylation of the C-terminal Cys.

Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a growing class of natural products that exist in all three domains of life and possess diverse biological activities.[1−3] RiPPs are derived from a ribosomally synthesized precursor peptide, which, in most cases, consists of an N-terminal region (leader peptide) that is essential for the recognition by post-translationally modifying enzymes, and a C-terminal region (core peptide) that is finally transformed to the mature product. A unique RiPP structural motif is *S*-[(*Z*)-2-aminovinyl]-*D*-cysteine (AviCys), which has been found in several classes of RiPPs, including lanthipeptides (e.g., epidermin and mutacin),[4−6] lipolanthines (e.g., microvionine),[7] polythioamides (e.g., thioviridamide),[8−12] and linaridins (e.g., cypemycin)[13,14] (see Figure 1A). Biosynthesis of the AviCys moiety involves a flavoprotein (generically termed LanD when it is involved in lanthipeptide biosynthesis), which catalyzes an oxidative decarboxylation of the C-terminal cysteine to form a thioenol. A following Michael-type addition of the resulting thioenol to a dehydroalanine (Dha) or dehydrobutyrine (Dhb) residue generates the AviCys ring (see Figure 1B).[15]

AviCys is structurally similar to lanthionine, a characteristic motif that defines lanthipeptides (lanthionine-containing peptides) (see Figure 1A).[6] In lanthipeptide biosynthesis, a standalone LanC cyclase or a cyclase-containing protein (LanM, LanKC or LanL) is strictly essential, which catalyzes the Michael-type addition of Cys thiols to Dha/Dhb to form lanthionine rings.[16−18] However, neither linaridins nor polythiomides appears to involve a LanC-like enzyme in their biosynthesis. Because thioenols are much more nucleophilic than thiols,[19,20] it appears that AviCys formation does not require a specific cyclase. It was proposed that the AviCys motif may be produced enzymatically by feeding the Dha-containing peptide substrate to the corresponding decarboxylase.[15] Such a strategy appears to be highly appealing, because chemical synthesis of AviCys ring is challenging and was only achieved in poor yields,[21] and AviCys-containing peptides are normally produced with very low yields.[15]

Cypemycin is a prototypical member of the linaridin family, which is defined as linear dehydrated (arid) peptides.[22] Although only three members of linaridin family have been characterized,[22−24] a recent genome mining study showed that this RiPP family is widespread in nature and the members are structurally diverse.[25] In contrast to the intertwined complicated ring structure found in several lanthipeptides, including epidermin, mersacidin, and NAI-107,[6] cypemycin is linear and has only one ring system, providing an ideal system to investigate AviCys formation (see Figure 1A).

The in vitro activity of CypD was reconstituted by Claesen and Bibb, showing that CypD catalyzes an oxidative decarboxylation of the C-terminal Cys of the precursor peptide CypA.[22] By using a series of synthetic oligopeptides as substrates, we recently showed that the minimal sequence for CypD recognition is the C-terminal three residues of the precursor peptide CypA, while most of the N-terminal sequence of CypA is not essential for CypD activity.[26] CypD tolerates various structural variations, allowing for generation of novel cypemycin variants with modified AviCys rings.[26] Such a relaxed substrate specificity of CypD is similar to EpiD involved in epidermine biosynthesis,[27] but is distinct from other LanD enzymes, such as the mersacin decarboxylase MrsD and the NAI-107 decarboxylase MibD, which did not show appreciable substrate tolerance.[28,29]

Received: October 24, 2018
Published: November 19, 2018

DOI: 10.1021/acs.orglett.8b03380
Org. Lett. 2018, 20, 7670−7673

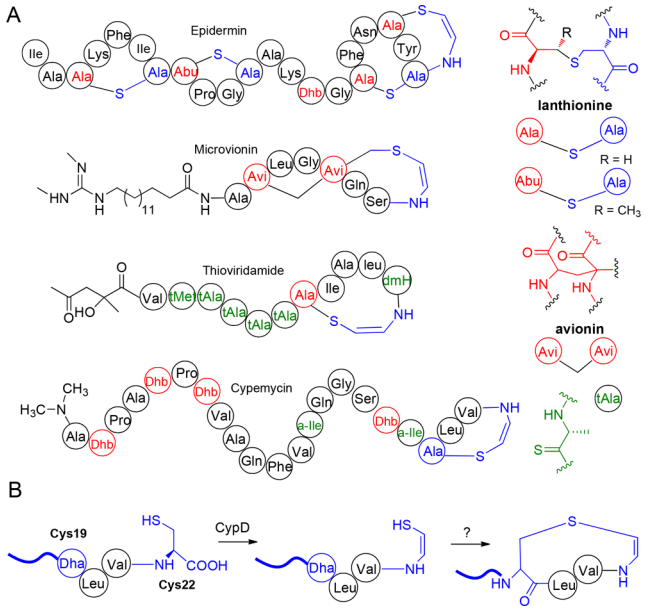
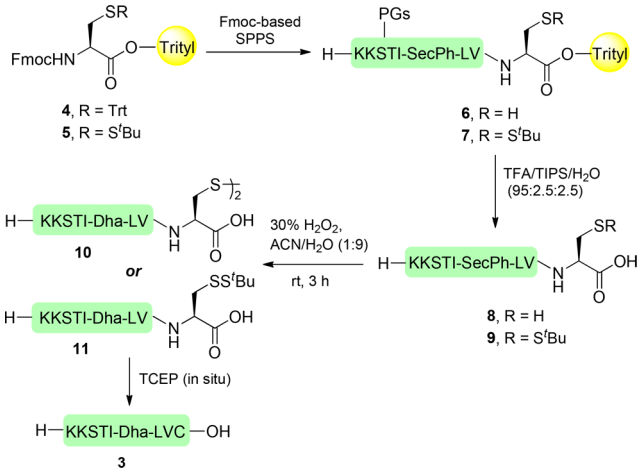


Figure 1. AviCys-containing natural products. (A) Representative examples of different RiPPs that contain AviCys moieties. The Ser/Thr- and Cys-derived residues are shown in red and blue, respectively, and other post-translationally modified residues are shown in green. [Legend: Dha, dehydroalanine; Dhb, dehydrobutyrine; Avi, avionin; tAla, thioalanine; tMet, thiomethionine; dmH, dimethylhistine; and a-Ile, *allo*-isoleucine.] (B) AviCys biosynthesis in cypemycin involves a flavoprotein CypD. Dha is generated from Cys19 via dethiolation. After decarboxylation of the C-terminal Cys (Cys22), a subsequent Michael-type addition of the thioenol to Dha produces the AviCys ring. The blue line represents the N-terminal part of CypA.

Thus far, the exact natural substrate of CypD is unknown, which likely contains the full core peptide of CypA. However, synthesis of such a long peptide with modified residues is challenging. Because only the C-terminal three residues of CypA are essential for CypD recognition, biochemical analyses in this study were performed by using synthetic oligopeptides. Since the C-terminal sequence of CypA is highly hydrophobic, we synthesized peptide **1** (KKSTISLVC) and peptide **2** (KKSTICLVC), which are similar to the CypA C-terminus (see Figure 1B) but contain two Lys residues in the N-termini, to increase aqueous solubility and hence the reaction efficiency. Liquid chromatography coupled with high-resolution mass spectrometry (LC-HR-MS) analysis of each reaction mixture clearly showed that both peptides were decarboxylated by CypD (see Figure S2 in the Supporting Information), suggesting that the two N-terminal Lys residues do not interfere with CypD activity.

To investigate the putative cyclase activity of CypD, we set out to synthesize the Dha-containing peptide **3** (KKSTI**X**LVC, where **X** represents Dha), which contains a Dha residue at the fourth C-terminal position (in contrast to a Ser or a Cys in peptide **1** or **2**) and, hence, could allow AviCys ring formation after decarboxylation. We initially used two synthetic procedures, involving Cys oxidative elimination[30] and phosphoserine elimination,[31] respectively. However, neither of these procedures afforded a sufficient amount of product. We next followed a protocol detailed by Levengood and van der Donk involving oxidative elimination of the phenylselenocysteine (SecPh) residue (see Scheme 1).[32] The desired SecPh-containing peptides were elongated on resin using

Scheme 1. Synthesis of the Peptide Substrate 3[a]



[a]Two synthetic routes with different Cys derivatives were performed, and both led to peptide **3** in decent yields.

typical Fmoc-based SPPS procedures.[32] After cleavage from the resin, followed by purification by preparative HPLC, the obtained nonapeptides **8** and **9** were treated with H_2O_2, affording the Dha-containing peptides **10** and **11** in decent yields (53% for **10**, and 74% for **11**), which were converted to peptide **3** in situ in enzymatic reactions (see Scheme 1, as well as Figures S3−S6 in the Supporting Information for the LC-MS and ^{1}H NMR data of **10** and **11**).

We then performed the reaction by incubation of CypD with peptide **3**, and the result shows that peptide **3** was decarboxylated upon CypD treatment (Figure 2). To

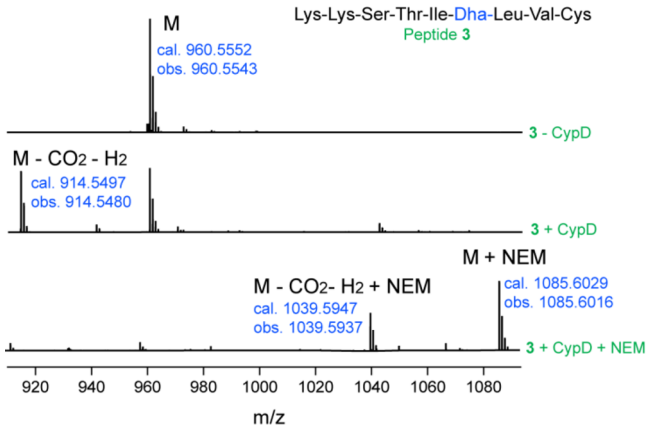


Figure 2. HR-MS analysis of the CypD reaction with peptide **3**, showing the MS spectra of the control reaction, CypD reaction, and CypD reaction further treated with NEM. After decarboxylation, no cyclized (neither enzymatically or nonenzymatically) product could be observed in the analysis.

investigate whether the AviCys ring was formed in the decarboxylated product, we treated the CypD reaction mixture with N-ethylmaleimide (NEM), a reagent that is commonly used to modify thiols. LC-HR-MS analysis of the resulting mixture clearly revealed NEM derivatization of the decarboxylated product of **3** (Figure 2), and the derivatized product was confirmed by HR-MS/MS analysis (Figure S7). We also did NEM reactions with a nonreactive peptide KKSTISLVS and cypemycin, but neither of these two compounds was

DOI: 10.1021/acs.orglett.8b03380
Org. Lett. 2018, 20, 7670−7673

derivatized, excluding the possibility that NEM derivatization can occur on other sites besides thiols. These observations provide strong evidence that CypD alone is unable to form the AviCys ring.

Knowing that the CypD-catalyzed decarboxylation is not coupled with AviCys ring formation, we next asked whether Dha is formed prior to the CypD-catalyzed decarboxylation of Cys22. To this end, we performed a time course analysis of CypD reaction with peptides **1**−**3**. This analysis indicated that peptide **3** appears to be a more preferred substrate than peptide **1** and peptide **2** (Figure 3), suggesting that production of Dha from the CypA Cys19 is likely prior to the CypD-catalyzed decarboxylation of Cys22 (see Figure 1B).

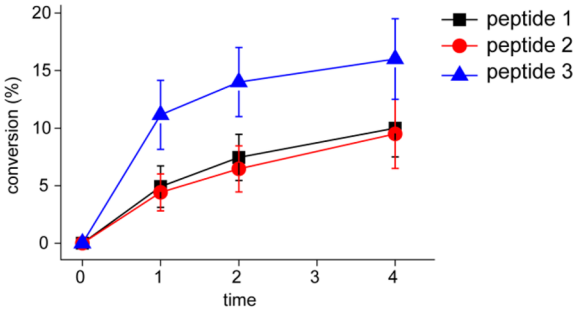


Figure 3. Time-course of the CypD-catalyzed decarboxylation reactions. The conversion yields were estimated according to MS intensities. Assays performed in triplicates the standard deviations (SDs) are shown by the error bars. It is noteworthy that the conversion yields are <50% in all of the assays with varied substrate and enzyme concentrations. It remains to be tested whether this is due, in part, to epimerization of the C-terminal Cys.[33,34]

In summary, we synthesized a Dha-containing oligopeptide **3** and tested the activity of cypemycin decarboxylase CypD, showing that CypD only catalyzes Cys decarboxylation and is not responsible for AviCys ring formation. We also showed that the Dha-containing peptide **3** is a better substrate than the similar peptide **1** and **2**, supporting the belief that the production of Dha by dethiolation of Cys19 likely occurs prior to decarboxylation of the C-terminal Cys. These results answer a long-standing question regarding AviCys biosynthesis and lay the foundation for future biosynthetic investigation of AviCys-containing natural products.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b03380.

Supplementary figures, peptide synthesis, material and methods, and detailed results from calculations (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: dongs@hsc.pku.edu.cn (S. Dong).
*E-mail: qizhang@sioc.ac.cn (Q. Zhang).

ORCID

Suwei Dong: 0000-0002-2648-4344
Qi Zhang: 0000-0002-8135-2221

Author Contributions

#These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Mervyn Bibb (John Innes Centre, UK) for kindly providing *Streptomyces* sp. OH-4156. This work is supported by grants from the National Key Research and Development Program (No. 2016 YF A0501302 to Q.Z.), from the National Natural Science Foundation of China (No. 31600398 to W.D., No. 21672012 to S.D., and Nos. 31500028 and 31670060 to Q.Z.), and from the State Key Laboratory of Microbial Technology Open Projects Fund (No. M2015-01 to W.D.).

■ REFERENCES

(1) Arnison, P. G.; Bibb, M. J.; Bierbaum, G.; Bowers, A. A.; Bugni, T. S.; Bulaj, G.; Camarero, J. A.; Campopiano, D. J.; Challis, G. L.; Clardy, J.; Cotter, P. D.; Craik, D. J.; Dawson, M.; Dittmann, E.; Donadio, S.; Dorrestein, P. C.; Entian, K.-D.; Fischbach, M. A.; Garavelli, J. S.; Göransson, U.; Gruber, C. W.; Haft, D. H.; Hemscheidt, T. K.; Hertweck, C.; Hill, C.; Horswill, A. R.; Jaspars, M.; Kelly, W. L.; Klinman, J. P.; Kuipers, O. P.; Link, A. J.; Liu, W.; Marahiel, M. A.; Mitchell, D. A.; Moll, G. N.; Moore, B. S.; Müller, R.; Nair, S. K.; Nes, I. F.; Norris, G. E.; Olivera, B. M.; Onaka, H.; Patchett, M. L.; Piel, J.; Reaney, M. J. T.; Rebuffat, S.; Ross, R. P.; Sahl, H.-G.; Schmidt, E. W.; Selsted, M. E.; Severinov, K.; Shen, B.; Sivonen, K.; Smith, L.; Stein, T.; Süssmuth, R. E.; Tagg, J. R.; Tang, G.-L.; Truman, A. W.; Vederas, J. C.; Walsh, C. T.; Walton, J. D.; Wenzel, S. C.; Willey, J. M.; van der Donk, W. A. *Nat. Prod. Rep.* **2013**, *30*, 108.
(2) Hetrick, K. J.; van der Donk, W. A. *Curr. Opin. Chem. Biol.* **2017**, *38*, 36.
(3) Hudson, G. A.; Mitchell, D. A. *Curr. Opin. Microbiol.* **2018**, *45*, 61.
(4) Allgaier, H.; Jung, G.; Werner, R. G.; Schneider, U.; Zahner, H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 1051.
(5) Novak, J.; Caufield, P. W.; Miller, E. J. *J. Bacteriol.* **1994**, *176*, 4316.
(6) Willey, J. M.; van der Donk, W. A. *Annu. Rev. Microbiol.* **2007**, *61*, 477.
(7) Wiebach, V.; Mainz, A.; Siegert, M. J.; Jungmann, N. A.; Lesquame, G.; Tirat, S.; Dreux-Zigha, A.; Aszodi, J.; Le Beller, D.; Sussmuth, R. D. *Nat. Chem. Biol.* **2018**, *14*, 652.
(8) Hayakawa, Y.; Sasaki, K.; Nagai, K.; Shin-ya, K.; Furihata, K. *J. Antibiot.* **2006**, *59*, 6.
(9) Izawa, M.; Kawasaki, T.; Hayakawa, Y. *Appl. Environ. Microbiol.* **2013**, *79*, 7110.
(10) Izumikawa, M.; Kozone, I.; Hashimoto, J.; Kagaya, N.; Takagi, M.; Koiwai, H.; Komatsu, M.; Fujie, M.; Satoh, N.; Ikeda, H.; Shin-ya, K. *J. Antibiot.* **2015**, *68*, 533.
(11) Frattaruolo, L.; Lacret, R.; Cappello, A. R.; Truman, A. W. *ACS Chem. Biol.* **2017**, *12*, 2815.
(12) Tang, J.; Lu, J.; Luo, Q.; Wang, H. *Chin. Chem. Lett.* **2018**, *29*, 1022.
(13) Komiyama, K.; Otoguro, K.; Segawa, T.; Shiomi, K.; Yang, H.; Takahashi, Y.; Hayashi, M.; Oxani, T.; Omura, S. *J. Antibiot.* **1993**, *46*, 1666.
(14) Minami, Y.; Yoshida, K.; Azuma, R.; Urakawa, A.; Kawauchi, T.; Otani, T.; Komiyama, K.; Omura, S. *Tetrahedron Lett.* **1994**, *35*, 8001.
(15) Sit, C. S.; Yoganathan, S.; Vederas, J. C. *Acc. Chem. Res.* **2011**, *44*, 261.
(16) Zhang, Q.; Yu, Y.; Velásquez, J. E.; van der Donk, W. A. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 18361.

DOI: 10.1021/acs.orglett.8b03380
Org. Lett. 2018, 20, 7670−7673

(17) Repka, L. M.; Chekan, J. R.; Nair, S. K.; van der Donk, W. A. *Chem. Rev.* **2017**, *117*, 5457.
(18) Montalban-Lopez, M.; van Heel, A. J.; Kuipers, O. P. *FEMS Microbiol Rev.* **2017**, *41*, 5.
(19) Metzner, P. *Synthesis* **1992**, *1992*, 1185.
(20) Metzner, P. *Top. Curr. Chem.* **1999**, *204*, 127.
(21) Carrillo, A. K.; Vannieuwenhze, M. S. *Org. Lett.* **2012**, *14*, 1034.
(22) Claesen, J.; Bibb, M. J. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 16297.
(23) Claesen, J.; Bibb, M. J. *J. Bacteriol.* **2011**, *193*, 2510.
(24) Rateb, M. E.; Zhai, Y.; Ehrner, E.; Rath, C. M.; Wang, X.; Tabudravu, J.; Ebel, R.; Bibb, M.; Kyeremeh, K.; Dorrestein, P. C.; Hong, K.; Jaspars, M.; Deng, H. *Org. Biomol. Chem.* **2015**, *13*, 9585.
(25) Mo, T.; Liu, W. Q.; Ji, W.; Zhao, J.; Chen, T.; Ding, W.; Yu, S.; Zhang, Q. *ACS Chem. Biol.* **2017**, *12*, 1484.
(26) Ding, W.; Mo, T.; Mandalapu, D.; Zhang, Q. *Synth Syst. Biotechnol* **2018**, *3*, 159.
(27) Kupke, T.; Kempter, C.; Jung, G.; Götz, F. *J. Biol. Chem.* **1995**, *270*, 11282.
(28) Majer, F.; Schmid, D. G.; Altena, K.; Bierbaum, G.; Kupke, T. *J. Bacteriol.* **2002**, *184*, 1234.
(29) Ortega, M. A.; Cogan, D. P.; Mukherjee, S.; Garg, N.; Li, B.; Thibodeaux, G. N.; Maffioli, S. I.; Donadio, S.; Sosio, M.; Escano, J.; Smith, L.; Nair, S. K.; van der Donk, W. A. *ACS Chem. Biol.* **2017**, *12*, 548.
(30) Chalker, J. M.; Gunnoo, S. B.; Boutureira, O.; Gerstberger, S. C.; Fernandez-Gonzalez, M.; Bernardes, G. J. L.; Griffin, L.; Hailu, H.; Schofield, C. J.; Davis, B. G. *Chem. Sci.* **2011**, *2*, 1666.
(31) Yang, A.; Ha, S.; Ahn, J.; Kim, R.; Kim, S.; Lee, Y.; Kim, J.; Soll, D.; Lee, H. Y.; Park, H. S. *Science* **2016**, *354*, 623.
(32) Levengood, M. R.; van der Donk, W. A. *Nat. Protoc.* **2007**, *1*, 3001.
(33) Ramos-Tomillero, I.; Rodriguez, H.; Albericio, F. *Org. Lett.* **2015**, *17*, 1680.
(34) Lelievre, D.; Terrier, V. P.; Delmas, A. F.; Aucagne, V. *Org. Lett.* **2016**, *18*, 920.

DOI: 10.1021/acs.orglett.8b03380
Org. Lett. 2018, 20, 7670−7673