How to cite: *Angew. Chem. Int. Ed.* **2024**, *63*, e202402663
doi.org/10.1002/anie.202402663

Polyketides

Mechanism Behind the Programmed Biosynthesis of Heterotrimeric Fungal Depside Thielavin A

Qiaolin Ji, Hao Xiang, Wei-Guang Wang, and Yudai Matsuda**

Abstract: Thielavin A (**1**) is a fungal depside composed of one 3-methylorsellinic acid and two 3,5-dimethylorsellinic acid units. It displays diverse biological activities. However, the mechanism underlying the assembly of the heterotrimeric structure of **1** remains to be clarified. In this study, we identified the polyketide synthase (PKS) involved in the biosynthesis of **1**. This PKS, designated as ThiA, possesses an unusual domain organization with the *C*-methyltransferase (MT) domain situated at the C-terminus following the thioesterase (TE) domain. Our findings indicated that the TE domain is solely responsible for two rounds of ester bond formation, along with subsequent chain hydrolysis. We identified a plausible mechanism for TE-catalyzed reactions and obtained insights into how a single PKS can selectively yield a specific heterotrimeric product. In particular, the tandem acyl carrier protein domains of ThiA are critical for programmed methylation by the MT domain. Overall, this study highlighted the occurrence of highly optimized domain–domain communication within ThiA for the selective synthesis of **1**, which can advance our understanding of the programming rules of fungal PKSs.

Introduction

Depsides, representing ester molecules with two or more units derived from polyphenolic acids, are prevalent in nature. Depside natural products exhibit significant structural diversity and a wide range of biological activities.[1] A large portion of these natural products consist of monomeric units derived from orsellinic acid or its analogues. Differences in the number and modification patterns of these monomeric units contribute to the diversity of this class of natural products. In recent years, several polyketide synthases (PKSs) responsible for depside biosynthesis have been identified in fungi,[2] and the mechanisms underlying ester (or "depside") bond formation during depside biosynthesis have been reported.[3] Three types of biosynthetic mechanisms for fungal depside biosynthesis have been identified (Figure 1). The diaryl ether-forming PKS, AN7909, uses the thioesterase (TE) domain for depside bond formation (Figure 1A),[3a] whereas the starter-unit acyltransferase (SAT) domain performs esterification in DrcA,[3b] resulting in the synthesis of the heterodimeric depside CJ-20,557 (Figure 1B). A similar SAT-catalyzed depside bond synthesis has been observed in the reaction with Preu6 to yield lecanoric acid.[3c] The biosynthesis of nornidulin involves two PKSs, collaborating to afford unguidepside A (Figure 1C).[4] Overall, the biosynthetic mechanisms for dimeric depsides (didepsides) have been comprehensively explored. However, efforts to understand tridepside biosynthesis remain limited. Although PKSs that are putatively responsible for the generation of gyrophoric acid, an orsellinic acid trimer, have been reported,[5] no tridepside-forming PKS has been experimentally characterized, and therefore, the mechanisms for tridepside biosynthesis remain largely enigmatic.

Thielavin A (**1**), originally isolated from the fungus *Thielavia terricola* SANK 10475 as a prostaglandin synthase inhibitor,[6] is a heterotrimeric depside, consisting of one 3-methylorsellinic acid (3-MOA) and two 3,5-dimethylorsellinic acid (DMOA) units. To date, various thielavins have been isolated (Figure 2A), and they exhibit diverse biological activities. For example, thielavins serve as inhibitors of glucose-6-phosphatase,[7] α-glucosidase,[8] indoleamine 2,3-dioxygenase,[9] and telomerase[10] and also exhibit antibacterial,[11] cytotoxic,[12] and antifouling[13] activities. Despite their medicinal importance, the biosynthetic mechanisms of thielavins remain to be clarified. According to recent research on fungal didepsides, **1** may also be synthesized by a single PKS. However, the process by which this PKS achieves two rounds of depside bond formation and selectively affords a specific heterotrimeric product among various possible structures remains unknown. In this context, a biosynthetic study of **1** may help uncover an

[*] Q. Ji, Dr. Y. Matsuda
Department of Chemistry
City University of Hong Kong
Tat Chee Avenue, Kowloon, Hong Kong SAR, China
E-mail: ymatsuda@cityu.edu.hk
H. Xiang, Prof. Dr. W.-G. Wang
Key Laboratory of Natural Products Synthetic Biology of Ethnic Medicinal Endophytes, State Ethnic Affairs Commission; Key Laboratory of Chemistry in Ethnic Medicinal Resources, Ministry of Education
Yunnan Minzu University
Kunming 650031, Yunnan, China
E-mail: wwg@live.cn

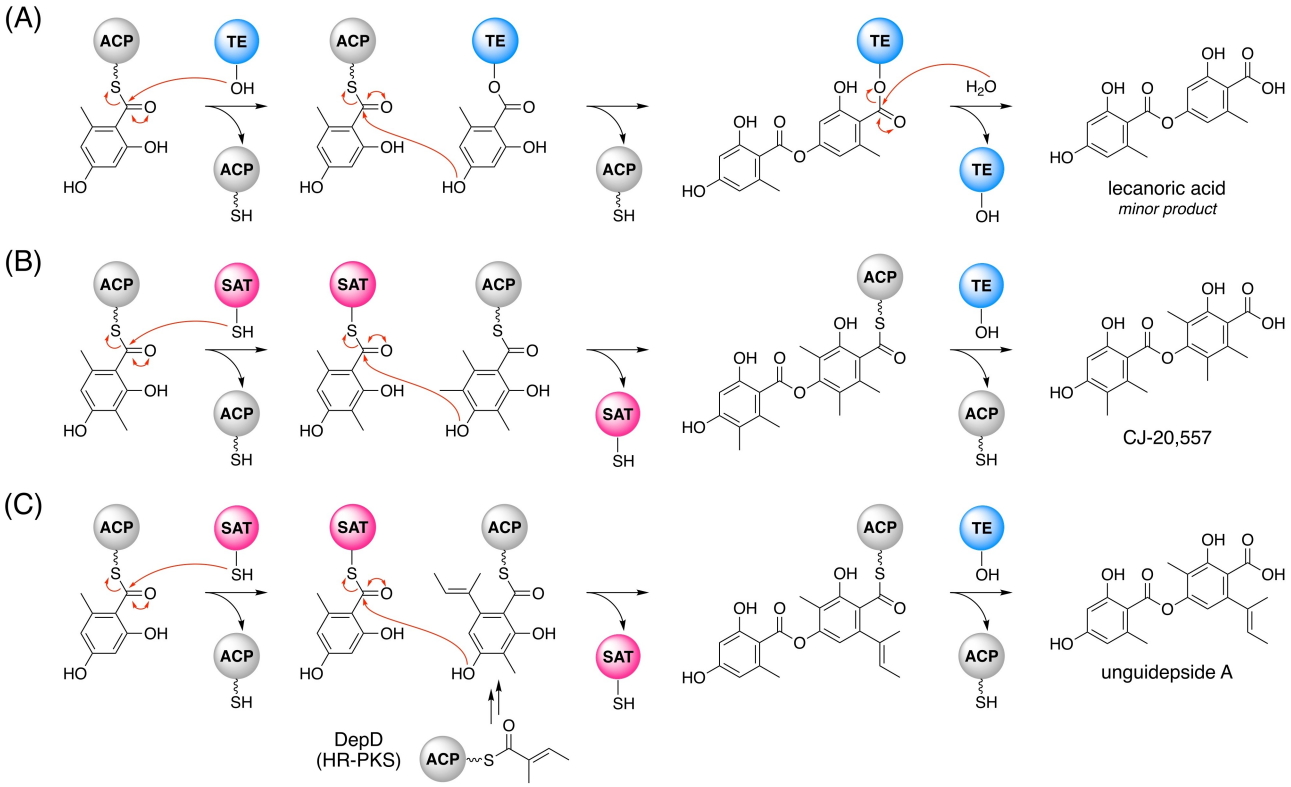

Figure 1. Biosynthetic mechanisms for depside bond formation. Reactions catalyzed by (A) AN7909, (B) DrcA, and (C) DepH. The depside bond is formed by the TE domain in AN7909 and by the SAT domain in DrcA and DepH. HR-PKS: highly reducing PKS.

unprecedented biosynthetic mechanism and provide insights into the programming logic of fungal PKSs.

Therefore, in this study, we identified the PKS responsible for the formation of thielavin A (**1**), designated as ThiA, and revealed that the TE domain of ThiA is responsible for the two rounds of depside bond formation as well as the subsequent hydrolytic polyketide chain release. Furthermore, we present a plausible mechanism for TE-domain-catalyzed depside bond formation, offering insights into how ThiA selectively yields the heterotrimeric depside **1**.

Results and Discussion

Identification and Characterization of the Biosynthetic Gene Cluster of Thielavin B

To elucidate the biosynthetic mechanism of thielavin A (**1**) and its related tridepsides, we performed genome sequencing analysis of the fungus *Chaetomium carinthiacum* ATCC 46463 (CBS 665.82), a known producer of **1** and several other thielavins.[7] Examination of the fungal genome identified the PKS gene *thiA*, whose product shares 42 % amino acid sequence identity with a PKS from *Umbilicaria deusta* that putatively yields the tridepside gyrophoric acid.[5] Although **1** structurally resembles CJ-20,557, a heterodimer of 3-MOA and DMOA, ThiA shares a considerably lower (<30 %) sequence identity with the CJ-20,557-synthesizing PKS DrcA. Intriguingly, ThiA possesses an unprecedented domain organization of SAT-ketosynthase-acyltransferase-product template-(acyl carrier protein)$_2$-TE-*C*-methyltransferase (SAT-KS-AT-PT-ACP-ACP-TE-MT), with the MT domain located at the C-terminus after the TE domain. The flanking regions of *thiA* encode the methyltransferase ThiC and transmembrane DUF3533 protein ThiB, which may serve as a transporter (Figure 2B and Table S1; DDBJ/EMBL/GenBank accession number: LC796268). Considering that thielavin B (**2**), a derivative of **1** with two *O*-methylations, is a major constituent of *C. carinthiacum*,[7] we reasoned that the *thi* cluster is responsible for the biosynthesis of **2**.

To clarify the function of ThiA, we heterologously expressed the PKS gene *thiA* in *Aspergillus oryzae* NSAR1.[14] Analysis of the metabolites derived from the *A. oryzae* transformant revealed a major metabolite (Figure 2C, traces i and ii) with the molecular formula $C_{29}H_{30}O_{10}$. This metabolite was isolated from large-scale cultivation and identified as thielavin A through NMR analysis and a comparison with reported data.[8] This observation indicated that ThiA is solely responsible for the formation of the heterotrimeric depside. To investigate the importance of the tandem ACPs, we expressed ThiA variants with mutations at one of the phosphopantetheine attachment sites in the ACPs (Ser1672 and Ser1783) to alanine. Interestingly, while these mutations did not decrease the overall productivity of the PKS, both variants yielded another major product, **5**, in addition to **1** (Figure 2C, traces iii and iv). Compound **5** was

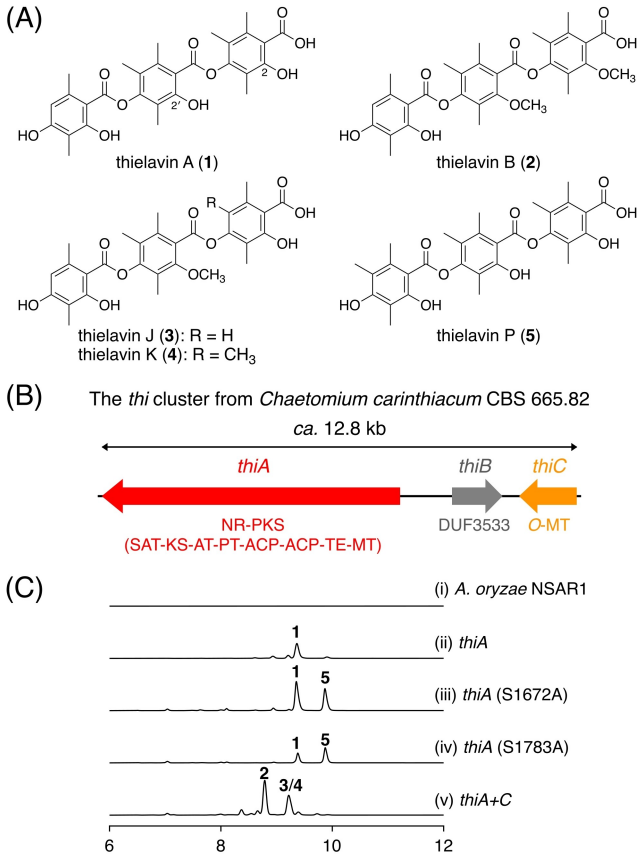

Figure 2. (A) Structures of representative thielavins. (B) Schematic representation of the *thi* cluster. (C) HPLC profiles of metabolites from *Aspergillus oryzae* transformants. The chromatograms were monitored at 254 nm.

then isolated and identified as the DMOA trimer thielavin P.[7] This observation suggests that duplicated ACPs considerably influence product selectivity rather than PKS efficiency. Although the presence of multiple ACPs is not uncommon in PKSs, they typically control the titer of the PKS product instead of the product profile, as observed in the polyunsaturated fatty acid synthase.[15] Similarly, the inactivation of one or two ACPs among the triplet ACPs in DrcA, the PKS that synthesizes the heterodimeric depside CJ-20,557, did not alter the product profile.[3b] It should be noted that some tandem ACPs found in *trans*-acyltransferase modular PKSs are proposed to work "in-series" rather than "in-parallel," meaning that each ACP in such multiple ACPs has a distinct function.[16] Nevertheless, ThiA provides an unusual example of tandem ACPs, in which duplicated ACPs in an iterative PKS affect the methylation pattern of polyketide chains.

Subsequently, we coexpressed *thiA* and the methyltransferase gene *thiC*, which expectedly afforded thielavin B (**2**) as a major product (Figure 2C, trace v). In addition, the transformant also yielded thielavins J (**3**) and K (**4**),[7] both containing only one methoxy group. Thus, ThiC is a multifunctional methyltransferase that can methylate the hydroxy groups at the C-2 and C-2′ positions.

Investigation of the Mechanism of Depside Bond Formation

Next, we focused on the mechanism of ThiA-catalyzed depside bond formation. Previous studies have shown that such esterification reactions can be performed by either the SAT or TE domain.[3] Therefore, we expressed and purified the SAT and TE domains of ThiA using an *Escherichia coli* expression system for in vitro enzymatic reactions. The purified PKS domains were individually reacted with a 1:2 mixture of the *N*-acetylcysteamine (NAC) thioesters of 3-MOA (**6**) and DMOA (**7**), which were synthesized and shown to mimic ACP-bound substrates in our previous study.[3b] Although the reaction with the SAT domain did not yield any detectable product, the TE domain successfully yielded the heterotrimer thielavin A (**1**), along with CJ-20,557[3b] (**8**) and the DMOA dimer (**9**) (Figure 3A, traces i to iv). Interestingly, other possible trimers, such as homotrimers of 3-MOA or DMOA, were not produced, consistent with the predominant detection of **1** in the *A. oryzae* transformant harboring *thiA*. These observations highlighted that the TE domain of ThiA is capable of two rounds of

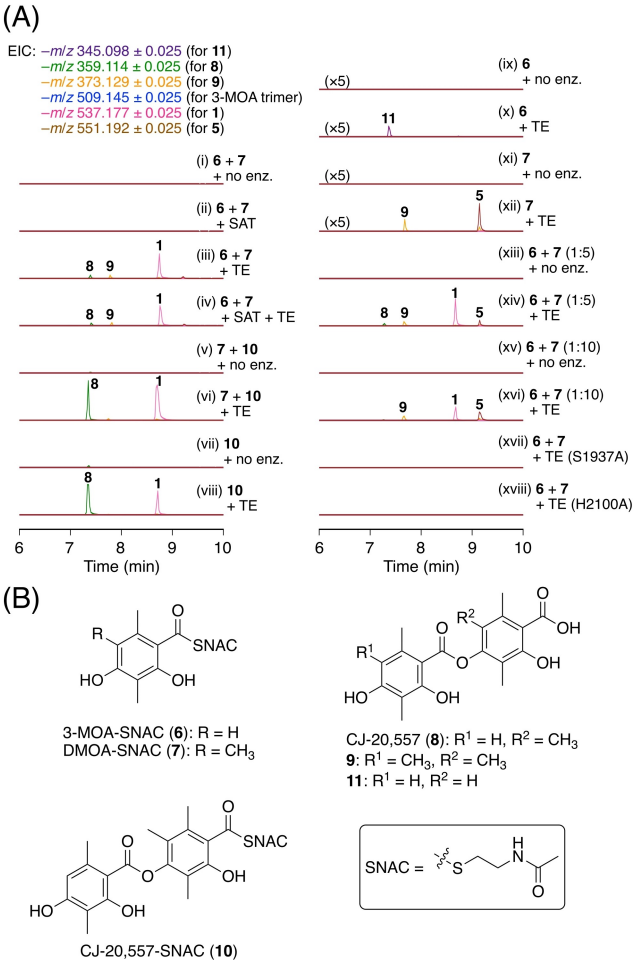

Figure 3. (A) LC-MS analysis of in vitro enzymatic reactions of the SAT and TE domains. Table S2 summarizes the detailed reaction conditions. (B) Structures of substrate analogues and enzymatic products. The structures of **9** and **11** were deduced based on their MS spectra.

esterification and subsequent hydrolysis, displaying high selectivity toward the production of **1**, despite the requirement of two structurally similar substrates.

To obtain further insights into the TE-catalyzed reactions, we reacted CJ-20,557-SNAC[3b] (**10**) and DMOA-SNAC (**7**) with the TE, affording **1** (Figure 3A, traces v and vi). Surprisingly, the reaction with **10** as a sole substrate also yielded **1**, although the productivity was lower than that in the reaction with **7** (Figure 3A, traces vii and viii). This observation suggests that the depside bond of **10** can be hydrolyzed by the TE to generate **7** in situ, which would then react with the TE-loaded CJ-20,557 to afford **1** (Figure S1). Consistently, 3-MOA, resulting from the depside bond hydrolysis, was also detected in the reaction of the TE with **10** (Figure S1). According to these results, during the biosynthesis of **1**, depside bond formation first occurs between 3-MOA and DMOA units, and the resultant didepside-TE then reacts with another DMOA unit.

Next, 3-MOA-SNAC (**6**) was used as a sole substrate. In this case, the 3-MOA dimer (**11**) was detected, without trimer formation. In contrast, DMOA-SNAC (**7**) underwent both dimerization and trimerization reactions to yield **9** and **5**, respectively (Figure 3A, traces ix to xii). Notably, the DMOA trimer **5** was not obviously produced when both 3-MOA-SNAC and DMOA-SNAC (in a 1:2 ratio) were used as substrates. Thus, we performed enzymatic reactions with higher concentrations of **7**. When the ratio was increased to 1:5, the production of **5** became evident, although **1** remained the major product (Figure 3A, traces xiii and xiv). Intriguingly, even when the concentration of **7** was 10 times higher than that of **6**, the production of **1** could be clearly observed (Figure 3A, traces xv and xvi). In summary, the TE domain exhibited a strong preference toward the 3-MOA unit as a priming substrate, whereas the DMOA unit was preferentially used as an extending substrate of the depside chain.

TE domains are known to possess a catalytic triad consisting of Ser-His-Asp residues,[17] in which the serine residue serves as a nucleophile attacking the carbonyl group of the substrate, and the histidine residue functions as a general base deprotonating the serine residue or water. Because these residues are conserved in the TE domain of ThiA, the serine (Ser1937) and histidine (His2100) residues were individually mutated to alanine, and the resultant TE variants were subjected to in vitro enzymatic reactions with 3-MOA-SNAC and DMOA-SNAC. Both variants failed to yield any products (Figure 3A, traces xvii and xviii), demonstrating that Ser1937 and His2100 are the catalytic residues of the TE domain.

Building on these experimental results, we propose the following mechanism for TE-catalyzed depside bond formation resulting in thielavin A (**1**) (Figure 4A). First, a 3-MOA unit formed on the ACP domain is transferred to the catalytic serine residue (Ser1937) of the TE domain. Subsequently, an ACP-bound DMOA unit approaches the active site of TE to undergo depside bond formation. In this process, His2100 deprotonates from the C-4 hydroxy group of the DMOA unit, which then attacks the ester bond formed between the 3-MOA unit and Ser1937. The resulting didepside, bound to the ACP, is then transferred to the TE, followed by the second round of depside bond formation with another DMOA unit. Finally, ester hydrolysis yields **1** as a product.

To investigate the plausibility of the proposed mechanism, we obtained an AlphaFold2[18]-generated structure of the ACP2 (the second ACP)-TE didomain using ColabFold[19] (v1.5.3) and performed covalent-docking and molecular dynamics simulations. In these simulations, the 3-methylorsellinyl group and 3,5-dimethylorsellinyl-phosphopantetheinyl (Ppant) group were attached to Ser1937 and Ser1783, respectively (Figures 4B and S2 to S5). The results showed that the C-4 hydroxy group of the DMOA unit is located close to the carbonyl group of the 3-MOA portion. Similar simulations, with CJ-20,557 ester-bonded to Ser1937, (Figures 4C and S6 to S9), indicated a binding mode consistent with the proposed mechanism. Furthermore, another docking simulation with thielavin A covalently bound to the TE revealed that the TE possesses a sufficient pocket that can be filled by the tridepside unit (Figures 4D and S10 to S11). Overall, the experimental and computational results support the proposed mechanism for tridepside formation. Notably, the TE-catalyzed depside bond formation by AN7909 has been suggested to involve the C-4 hydroxy group of the TE-bound orsellinyl group attacking the thioester of the ACP-bound orsellinyl group (Figure 1A).[3a] However, this mechanism is unlikely for depside bond formation by ThiA, as the direction of the depside chain growth is orthogonal to the path for the Ppant arm in the TE domain. As the detailed mechanism of depside bond formation by AN7909 remains unclear, an esterification strategy similar to that of ThiA may be plausible in this context.

Discovery and Characterization of the PKS for Gyrophoric Acid Biosynthesis

We conducted a search for ThiA-like PKSs in publicly available databases and our in-house genome database. We noted that the fungus *Humicola grisea* CBS 459.76 encodes a PKS that is highly homologous to ThiA (63 % amino acid sequence identity) but lacks an MT domain (Figure 5A and Table S1; DDBJ/EMBL/GenBank accession number: LC796269). Thus, the PKS was expected to yield the orsellinic acid trimer gyrophoric acid and was tentatively named GyrPKS. To characterize the function of GyrPKS, the PKS gene *gyrPKS* was heterologously expressed in *A. oryzae*, and the resulting transformant yielded a major product **12** (Figure 5B, traces i and ii), identified as gyrophoric acid (Figure 5C). Given the high sequence similarity between ThiA and GyrPKS, we hypothesized that their functions could be interchanged through simple domain deletion/addition/swapping experiments. To investigate this hypothesis, we first expressed a truncated variant of ThiA lacking the MT domain in *A. oryzae*, which successfully afforded **12** (Figure 5B, trace iii). In contrast, when the MT domain of ThiA was added to the C-terminus of GyrPKS, the chimeric enzyme (chimera 1) resulted in a complicated

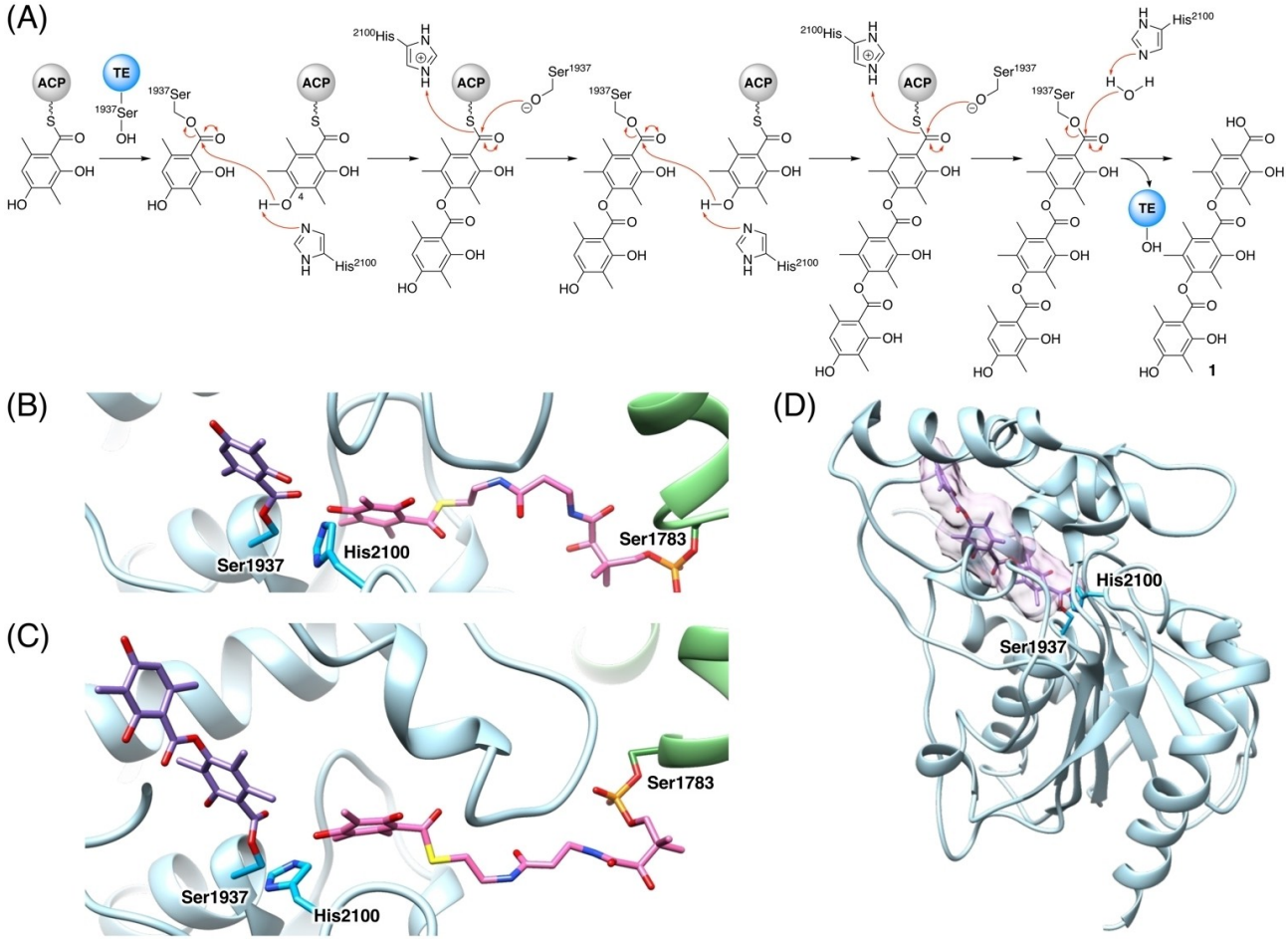

Figure 4. (A) Proposed mechanism for TE-domain-catalyzed depside bond formation. (B–D) Modeled structures from covalent-docking and MD simulations of (B) 3-MOA-TE and DMOA-ACP2, (C) CJ-20,557-TE and DMOA-ACP2, and (D) thielavin A-TE.

metabolic profile with no single major product (Figure 5B, trace iv, and Figure S12). We also created another chimeric PKS (chimera 2) with the TE domain of GyrPKS replaced with the TE-MT didomain of ThiA. However, this PKS also failed to yield major products (Figure 5B, trace v, and Figure S11). Due to the low and complex productivity, we were unable to identify the products from the two chimeric PKSs.

These experiments indicate that ThiA relies on highly optimized domain–domain communications for the synthesis of the heterotrimeric depside **1**. To selectively produce **1**, *C*-methylations by the MT domain must occur at specific timings to afford both 3-MOA and DMOA units in a certain ratio. Additionally, the MT domain competes with the KS domain for the β-ketoacyl substrate.[20] Given that the simple addition of the MT domain of ThiA to GyrPKS did not yield a PKS capable of synthesizing **1**, it appears that the MT domain alone does not govern methylation programming in ThiA. Furthermore, considering the significance of tandem ACPs for the product profile, it might be reasoned that the duplicated ACPs prevent overmethylation by the parallel synthesis of two polyketide chains. This phenomenon limits access to the MT domain and makes the KS domain more competitive toward the β-ketoacyl substrate.

Phylogenetic analysis revealed that the TE domains of ThiA and GyrPKS are distantly related to those of the depside-forming PKSs that use the SAT domain for depside bond formation (Figure S13). The two TE domains are more closely related to those of the other PKSs synthesizing depsides, including AN7909.[3a] Interestingly, the TE domain of GyrPKS exhibits a stronger relationship with that of ThiA than PKS16 from *Umbilicaria deusta* (tentatively termed UdPKS16), although this PKS is also expected to yield gyrophoric acid (**12**).[5] Thus, GyrPKS and UdPKS16 may have acquired the ability to synthesize **12** through different evolutionary paths.

Conclusion

We identified two tridepside-forming PKSs, ThiA and GyrPKS, and attempted to clarify the mechanism underlying heterotrimeric depside synthesis by ThiA through heterologous expression, in vitro enzymatic reactions, and domain deletion/addition/swapping experiments. The TE domain of

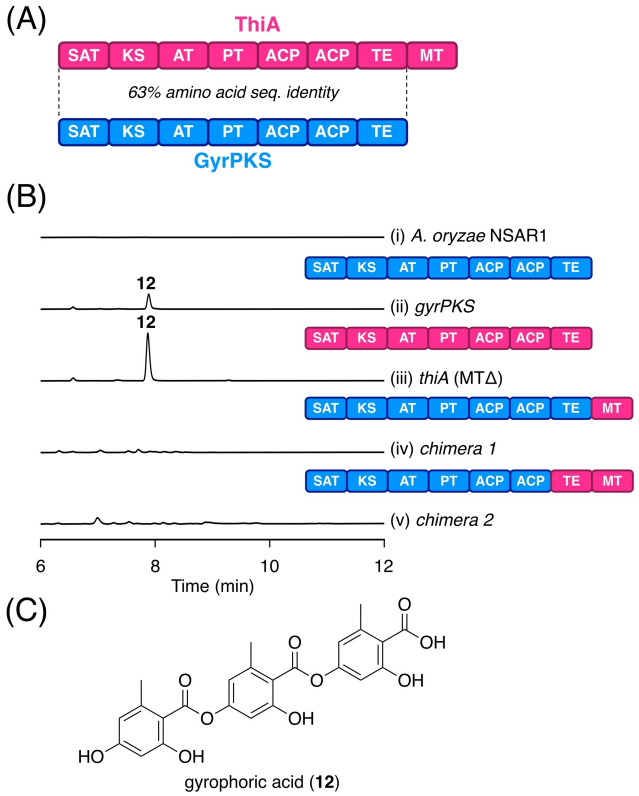

Figure 5. (A) Domain organization of GyrPKS and its comparison with that of ThiA. (B) HPLC profiles of metabolites from *A. oryzae* transformants. The chromatograms were monitored at 254 nm. Figure S12 shows the LC-MS profiles of the products from the chimeric PKSs. (C) Structure of gyrophoric acid (**12**).

ThiA was found to be responsible for conducting two consecutive depside bond formation steps and subsequent chain hydrolysis, revealing the first biosynthetic mechanism for a tridepside. Furthermore, our results highlighted that multiple domains in ThiA are well-tuned to enable the selective synthesis of the heterotrimeric depside, thielavin A (**1**). Further characterization of ThiA and GyrPKS, for example, by additional domain swapping and mutational experiments, can enhance our understanding of the programming rules of fungal PKSs, facilitating their rational engineering for the development of designer PKSs.

Acknowledgements

We are grateful to Prof. Katsuya Gomi (Tohoku University) and Profs. Katsuhiko Kitamoto and Jun-ichi Maruyama (The University of Tokyo) for providing the expression vectors and fungal strain. We thank Dr. Man Kit Tse and Dr. Kwok Chung Law (City University of Hong Kong) for their assistance in the acquisition of NMR spectra. This work was supported by a grant from the City University of Hong Kong (Project No. 7006004), a General Research Fund grant from the Research Grants Council of Hong Kong (Project No. 11301321), a grant from the National Natural Science Foundation of China (Project No. 22377105), and a grant from the Natural Science Foundation of Yunnan Province (Project No. 202401AV070003).

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: Biosynthesis · Depsides · Natural Products · Polyketides · Thioesterases

[1] a) V. Shukla, G. P. Joshi, M. S. M. Rawat, *Phytochem. Rev.* **2010**, *9*, 303–314; b) S. R. M. Ibrahim, A. Sirwi, B. G. Eid, S. G. A. Mohamed, G. A. Mohamed, *Metabolites* **2021**, *11*, 683.
[2] a) D. Armaleo, X. Sun, C. Culberson, *Mycologia* **2011**, *103*, 741–754; b) C. Feng, Q. Wei, C. Hu, Y. Zou, *Org. Lett.* **2019**, *21*, 3114–3118; c) F. Lünne, E.-M. Niehaus, S. Lipinski, J. Kunigkeit, S. A. Kalinina, H.-U. Humpf, *Fungal Genet. Biol.* **2020**, *145*, 103481; d) J. T. Kealey, J. P. Craig, P. J. Barr, *Metab. Eng. Commun.* **2021**, *13*, e00172; e) G. Singh, D. Armaleo, F. Dal Grande, I. Schmitt, *Biomol. Eng.* **2021**, *11*, 1445; f) W. Kim, R. Liu, S. Woo, K. B. Kang, H. Park, Y. H. Yu, H.-H. Ha, S.-Y. Oh, J. H. Yang, H. Kim, S.-H. Yun, J.-S. Hur, B. G. Turgeon, *mBio* **2021**, *12*, e01111–01121; g) K. L. Dunbar, B. Perlatti, N. Liu, A. Cornelius, D. Mummau, Y.-M. Chiang, L. Hon, M. Nimavat, J. Pallas, S. Kordes, H. L. Ng, C. J. B. Harvey, *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2310522120.
[3] a) Q. Wei, Z.-P. Wang, X. Zhang, Y. Zou, *J. Am. Chem. Soc.* **2022**, *144*, 9554–9558; b) L. Chen, X. Wei, Y. Matsuda, *J. Am. Chem. Soc.* **2022**, *144*, 19225–19230; c) Q. Liu, D. Zhang, S. Gao, X. Cai, M. Yao, Y. Xu, Y. Gong, K. Zheng, Y. Mao, L. Yang, D. Yang, I. Molnár, X. Yang, *Angew. Chem. Int. Ed.* **2023**, *62*, e202214379.
[4] J. Yang, Z. Zhou, Y. Chen, Y. Song, J. Ju, *Acta Pharm. Sin. B.* **2023**, *13*, 3919–3929.
[5] G. Singh, A. Calchera, D. Merges, H. Valim, J. Otte, I. Schmitt, F. D. Grande, *Microbiol. Spectr.* **2022**, *10*, e00109–00122.
[6] a) N. Kitahara, A. Endo, K. Furuya, S. Takahashi, *J. Antibiot.* **1981**, *34*, 1562–1568; b) N. Kitahara, H. Haruyama, T. Hata, S. Takahashi, *J. Antibiot.* **1983**, *36*, 599–600.
[7] S. Sakemi, H. Hirai, T. Ichiba, T. Inagaki, Y. Kato, N. Kojima, H. Nishida, J. C. Parker, T. Saito, H. Tonai-Kachi, M. A. VanVolkenburg, N. Yoshikawa, Y. Kojima, *J. Antibiot.* **2002**, *55*, 941–951.
[8] J. Rivera-Chávez, M. González-Andrade, M. d. C. González, A. E. Glenn, R. Mata, *Phytochemistry* **2013**, *94*, 198–205.
[9] J.-P. Jang, J.-H. Jang, M. Oh, S. Son, S. M. Kim, H.-M. Kim, K.-S. Shin, H. Oh, N. K. Soung, Y.-S. Hong, B. Y. Kim, J. S. Ahn, *J. Antibiot.* **2014**, *67*, 331–333.
[10] K.-i. Togashi, H.-R. Ko, J.-S. Ahn, H. Osada, *Biosci. Biotechnol. Biochem.* **2001**, *65*, 651–653.
[11] L. S. de Medeiros, L. M. Abreu, A. Nielsen, H. Ingmer, T. O. Larsen, K. F. Nielsen, E. Rodrigues-Filho, *Phytochemistry* **2015**, *111*, 154–162.
[12] S. Ayers, B. M. Ehrmann, A. F. Adcock, D. J. Kroll, M. C. Wani, C. J. Pearce, N. H. Oberlies, *Tetrahedron Lett.* **2011**, *52*, 5733–5735.

[13] Z. Han, Y.-X. Li, L.-L. Liu, L. Lu, X.-R. Guo, X.-X. Zhang, X.-Y. Zhang, S.-H. Qi, Y. Xu, P.-Y. Qian, *Mar. Drugs* **2017**, *15*, 128.
[14] F. J. Jin, J. Maruyama, P. R. Juvvadi, M. Arioka, K. Kitamoto, *FEMS Microbiol. Lett.* **2004**, *239*, 79–85.
[15] H. Jiang, R. Zirkle, J. G. Metz, L. Braun, L. Richter, S. G. Van Lanen, B. Shen, *J. Am. Chem. Soc.* **2008**, *130*, 6336–6337.
[16] S. Collin, R. J. Cox, C. Paris, C. Jacob, B. Chagot, K. J. Weissman, A. Gruez, *Nat. Commun.* **2023**, *14*, 1327.
[17] M. E. Horsman, T. P. A. Hari, C. N. Boddy, *Nat. Prod. Rep.* **2016**, *33*, 183–202.
[18] J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Žídek, A. Potapenko, A. Bridgland, C. Meyer, S. A. A. Kohl, A. J. Ballard, A. Cowie, B. Romera-Paredes, S. Nikolov, R. Jain, J. Adler, T. Back, S. Petersen, D. Reiman, E. Clancy, M. Zielinski, M. Steinegger, M. Pacholska, T. Berghammer, S. Bodenstein, D. Silver, O. Vinyals, A. W. Senior, K. Kavukcuoglu, P. Kohli, D. Hassabis, *Nature* **2021**, *596*, 583–589.
[19] M. Mirdita, K. Schütze, Y. Moriwaki, L. Heo, S. Ovchinnikov, M. Steinegger, *Nat. Methods* **2022**, *19*, 679–682.
[20] a) M. A. Skiba, A. P. Sikkema, W. D. Fiers, W. H. Gerwick, D. H. Sherman, C. C. Aldrich, J. L. Smith, *ACS Chem. Biol.* **2016**, *11*, 3319–3327; b) P. A. Storm, P. Pal, C. R. Huitt-Roehl, C. A. Townsend, *ACS Chem. Biol.* **2018**, *13*, 3043–3048.

Manuscript received: February 6, 2024
Accepted manuscript online: March 11, 2024
Version of record online: March 27, 2024