JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
pubs.acs.org/JACS
Communication

Gatekeeping Ketosynthases Dictate Initiation of Assembly Line Biosynthesis of Pyrrolic Polyketides

Dongqi Yi, Atanu Acharya, James C. Gumbart, Will R. Gutekunst, and Vinayak Agarwal*

Cite This: J. Am. Chem. Soc. 2021, 143, 7617−7622

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Assembly line biosynthesis of polyketide natural products involves checkpoints where identities of thiotemplated intermediates are verified before polyketide extension reactions are allowed to proceed. Determining what these checkpoints are and how they operate is critical for reprogramming polyketide assembly lines. Here we demonstrate that ketosynthase (KS) domains can perform this gatekeeping role. By comparing the substrate specificities for polyketide synthases that extend pyrrolyl and halogenated pyrrolyl substrates, we find that KS domains that need to differentiate between these two substrates exercise high selectivity. We additionally find that amino acid residues in the KS active site facilitate this selectivity and that these residues are amenable to rational engineering. On the other hand, KS domains that do not need to make selectivity decisions in their native physiological context are substrate-promiscuous. We also provide evidence that delivery of substrates to polyketide synthases by non-native carrier proteins is accompanied by reduced biosynthetic efficiency.

Downloaded via UNIV OF SASKATCHEWAN on August 12, 2021 at 13:29:30 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

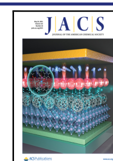
Type I polyketide synthases (PKSs) are multimodular enzymes that construct polyketide natural products.[1] The assembly line biosynthesis of polyketides involves repetitive steps: choice of an extender unit by the acyltransferase (AT) domain, decarboxylative Claisen condensation of this extender unit to the polyketide by the ketosynthase (KS) domain, and reductive tailoring of the β-carbonyl by ketoreductase, dehydratase, and enoyl reductase domains. Substrates for these PKS domains are thioesterified to carrier proteins (CPs). The ATs and the reductive tailoring domains were traditionally thought to determine the diversity of polyketide natural products.[2] The role of KSs in determining the polyketide diversity is relatively less well studied. Intermediary KSs in collinear PKS assembly lines are evolutionarily linked to the CP and the tailoring domains that precede them,[1,3,4] and as polyketide extension progresses, the selectivity of these intermediary KSs can constrain the extension of noncognate substrates.[5−8] Here we demonstrate that the strict selectivity of the very first KS in a collinear PKS assembly line can perform a gatekeeping function by precluding the initiation of polyketide extension of noncognate substrates and that the selectivity and promiscuity of initiating KSs are modulated in tune with the physiological natural product biosynthetic scheme.

The biosynthesis of several pyrrole-containing natural products involves polyketide extensions. Thioesterified pyrroles are delivered by a type II nonribosomal peptide synthetase (NRPS) adenylation−oxidation cascade that oxidizes the L-proline pyrrolidine to pyrrole (Figure 1A).[9−11] Thiotemplated pyrroles can be modified, as exemplified by dichlorination in the biosynthesis of pyoluteorin (**1**) (Figure 1B).[12] The thiotemplated dichloropyrrole is then handed off, without intermediary offloading, from the type II NRPS donor CP PltL to the type I PKS PltB. This handoff occurs by transthioesterification of the molecular cargo from the donor CP to the active-site cysteine thiol of the KS. Malonylation of the PltB CP by the AT enables the KS to catalyze the decarboxylative chain extension. Additional polyketide extensions followed by aromatization afford **1** (Figure S1). The precursor to 4,5-dichloropyrrolyl-*S*-PltL, pyrrolyl-*S*-PltL,[12] is also available *in situ* to the PltB PKS module (Figure 1B). However, deschloro-**1** is not produced (Figure S2). This is in contrast to the biosynthetic scheme for calcimycin (**2**) (Figure 1C), in which pyrrole (pyrrolyl-*S*-CalN3) acts as the physiological substrate to initiate polyketide extension by CalA.[13]

Why is pyrrolyl-*S*-PltL rejected as a substrate by PltB while pyrrolyl-*S*-CalN3 is accepted by CalA? To query the specificity determinants for PltB and CalA activities, we adopted an approach comparing the intermolecular recognition events that dictate polyketide extension of pyrrolic molecular cargoes. First, we determined the KS−AT−CP module boundaries for the bimodular PltB and CalA proteins by alignment with other KS−AT and CP crystal structures.[14−16] The tridomain sequences were expressed in *Escherichia coli*, and the PltB and CalA KS−AT−CP modules were purified (Figure S3). The CPs PltL and CalN3 were purified in their apo forms.

A library of pyrrolyl-*S*-pantetheines were synthesized (Scheme 1). The previously described synthesis of *S*-acyl pantetheines involved thioesterification of the acyl groups to give acetonide-protected pantetheine and subsequent acid deprotection to unmask the 1,3-diol.[17,18] However, we found the halogenated pyrrole derivatives to be labile toward acidic

Received: March 2, 2021
Published: May 14, 2021



ACS Publications
© 2021 American Chemical Society

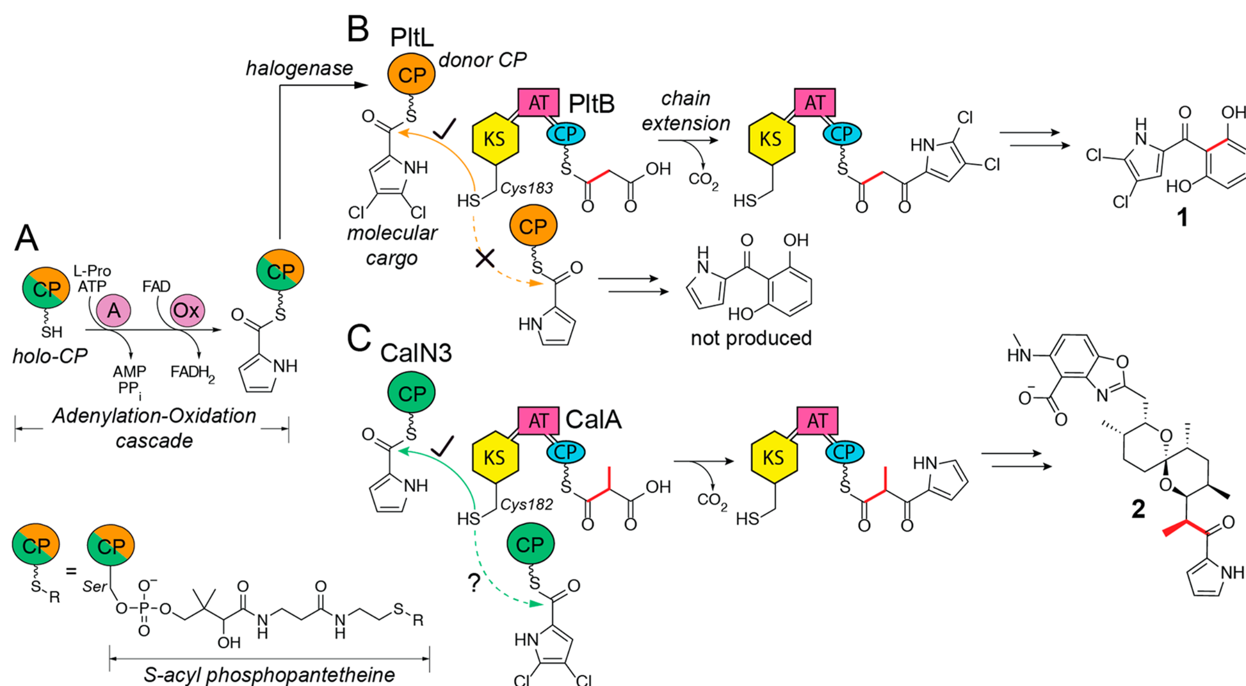
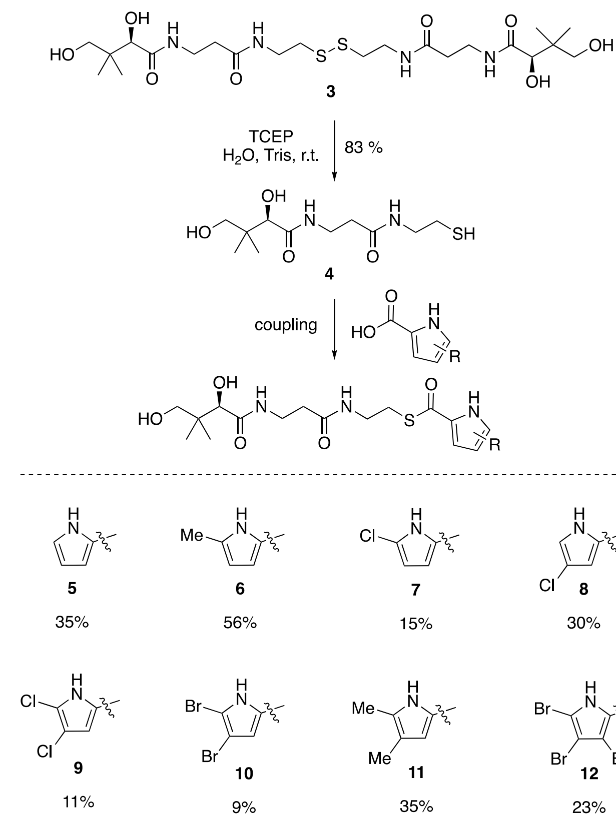


Figure 1. Biosynthesis of pyrrolic polyketides. (A) A type II NRPS furnishes the pyrrolyl-S-CPs. (B) Halogenation of pyrrolyl-S-PltL and polyketide elongation by PKS PltB (Cys183 is the KS active-site residue). (C) Polyketide extension by CalA.

Scheme 1. Synthesis of S-Acyl Pantetheines 5−12



deprotection. Instead, D-pantetheine (**4**) was accessed by disulfide reduction of D-pantethine (**3**) and used directly for ligation to pyrrolyl-2-carboxylic acids to furnish **5**−**11** (Figures S4−S17). Molecule **12** was accessed by tribromination of the pyrrolyl methyl ester followed by conversion to an acyl chloride, which was thioesterified to **4**.[19] Compounds **5**−**12** were extended to the corresponding S-acyl CoAs, followed by enzymatic transfer of the S-acyl phosphopantetheines to the donor CPs (Figures S18−S34).[17,18,20]

With the substrates in hand, we compared the abilities of the PltB and CalA KS−AT−CP modules to extend the pyrrolic cargoes. Malonyl-CoA required for the malonylation of the PltB CP by the AT was exogenously provided. For CalA, methylmalonyl-CoA was generated *in situ* by the CoA ligase MatB (Figure S35).[21] PltB Cys183Ala and CalA Cys182Ala were used to establish negative controls.[22] Upon incubation of PltB with a 2-fold molar excess of dichloropyrrolyl-S-PltL and excess malonyl-CoA, we detected the formation of a diketide product; this diketide was not observed with the PltB Cys183Ala enzyme (Figure S36). Dichloropyrrolyl-S-PltL was completely consumed; no consumption of the substrate was observed for the PltB Cys183Ala enzyme (Figure S37).

The abundance of the diketide detected was not reproducible. Thus, we relied on quantifying the abundance of the leftover S-acyl donor CP as a proxy for the extent of the reaction. Compared with complete consumption of dichloropyrrolyl-S-PltL, the consumption of pyrrolyl-S-PltL was negligible (Figure 2A). Physiologically, this implies that despite its presence in the molecular milieu within the producer of **1**, pyrrolyl-S-PltL is not accepted as a substrate by the PltB KS, thus precluding the production of deschloro-**1**.

We tested the activity of PltB for other pyrrolyl derivatives. Both 4- and 5-chloropyrrolyl-S-PltL were accepted as substrates, but with a reduced preference relative to 4,5-dichloropyrrole (Figure 2A). Bromine could replace chlorine; 4,5-dibromopyrrolyl-S-PltL was accepted well. However, the activity was diminished for the 3,4,5-tribromo derivative. Methyls could not replace halides. Methylpyrroles are observed in aminocoumarin natural products,[23] where 5-methylpyrrole does not undergo polyketide extension but is instead esterified to afford glycosyl moieties. We observed a marked decrease in preference for 5-methylpyrrole. An even further reduction in

https://doi.org/10.1021/jacs.1c02371
J. Am. Chem. Soc. 2021, 143, 7617−7622

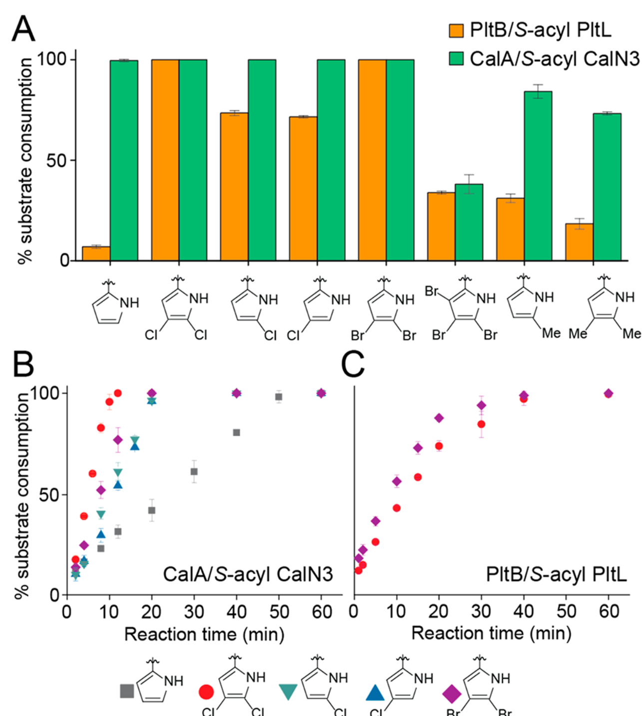


Figure 2. KS specificities. (A) Depletion of the end-point substrate S-acyl CP by PltB and CalA. (B, C) Time-dependent depletion of all substrates that demonstrated full consumption in the end-point assay by (B) CalA and (C) PltB.

preference was observed for 4,5-dimethylpyrrolyl-S-PltL (Figure 2A). Overall, we posit that substitutions on the pyrrole ring have a strong influence on the PltB KS activity.

Compared with PltB, the CalA KS demonstrated relaxed substrate selectivity. Complete consumption of pyrrolyl- and dichloropyrrolyl-S-CalN3 substrates along with other halogenated derivatives was observed (Figure 2A). As before, a reduction in substrate consumption was observed when the 3-position was brominated. Methyl substituents were less preferred than the halides; however, the distinction was less pronounced compared with PltB (Figure 2A). Querying time-dependent substrate consumption for all of the substrates that were fully depleted by CalA revealed that the rate of consumption of the physiological substrate pyrrole-S-CalN3 was lower than that of dichloropyrrolyl-S-CalN3 (Figure 2B). The faster consumption of dichloropyrrolyl-S-CalN3 was not due to substrate degradation (Figure S37). These data allow us to posit that the PltB KS exercises substrate selectivity, as it needs to differentiate between thiotemplated cargoes within the biosynthetic milieu. On the other hand, the CalA KS, which does not encounter differentially modified pyrrolic substrates, has relaxed substrate selectivity. Without an evolutionary pressure to maintain fidelity, relaxation in substrate selectivity is observed for primary and secondary metabolic enzymes.[24] Dichloro- and dibromopyrrole substrates were consumed by CalA and PltB at similar rates (Figure 2B,C).

Sequence alignment identified a methionine residue that was conserved for all KSs that extend dichloropyrroles (PltB Met222; Figure 3A). Homology structure of the PltB KS-AT didomain places the Met222 side chain within the KS active site (Figure 3B). The methionine thioether is a validated halogen-bonding partner.[32,33] Electrostatic potential (ESP)

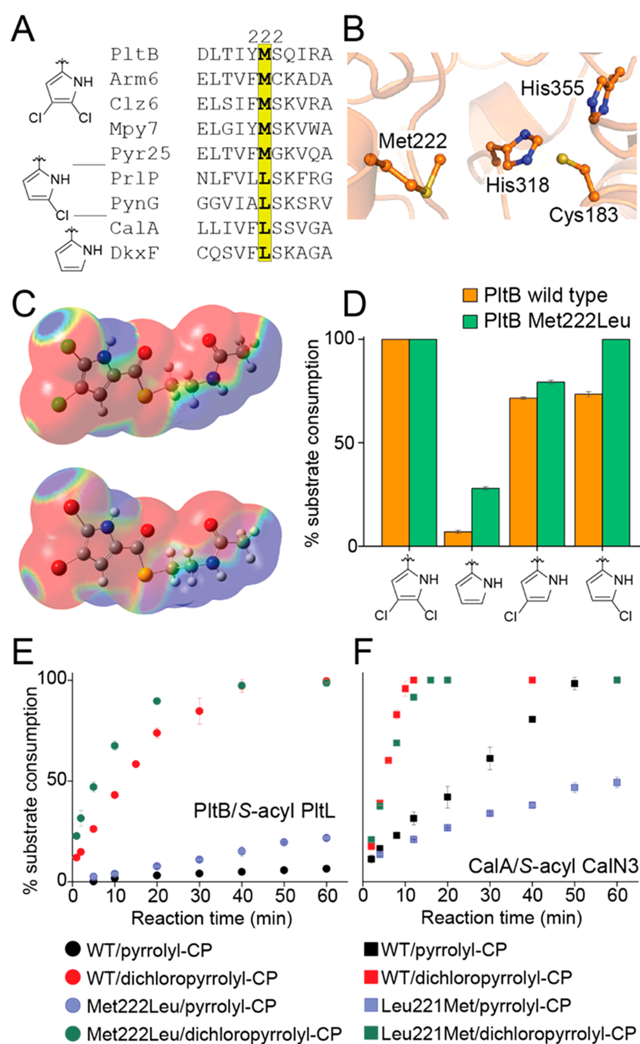


Figure 3. KS active site. (A) KS sequence alignment. Sequences for (from top to bottom) **1**, armeniaspirol,[25] chlorizidine,[26] marinopyrrole,[27] pyrrolomycin,[28] pyralomycin,[29] pyrronazol B,[30] **2**, and DKxanthene[31] KSs are illustrated. (B) The PltB KS active site. The Cys183-His318-His355 catalytic triad[22] side chains are illustrated. (C) ESPs mapped onto the electron densities (isovalue = 0.0004) for (top) dichloropyrrolyl- and (bottom) dibromopyrrolyl-S-N-acetylcysteamines. Regions of negative potential and positive potential (>0.01 au) are displayed in red and blue, respectively. ESPs between 0.00 and 0.01 are displayed with a color gradient from red to blue. (D) Substrate specificity of the PltB Met222Leu mutant compared with the wild type. (E, F) Time-dependent consumption of pyrrolyl- and dichloropyrrolyl substrates by wild-type and mutant PltB (Met222Leu) and CalA (Leu221Met).

maps for dichloro- and dibromopyrrole-N-acetylcysteamines computed at the wb97X-D/aug-cc-pVTZ level of theory demonstrated the presence of complementary halogen-bonding σ holes on the halogen substituents on the pyrrole rings (Figures 3C and S38). To probe the role of Met222 in determining the KS specificity, the substrate selectivity of the PltB Met222Leu enzyme was queried. The PltB Met222Leu mutant demonstrated a 4-fold increase in ability to deplete the pyrrolyl-S-PltL substrate while maintaining activity for dichloropyrrolyl-S-PltL (Figure 3D,E). Consumption of 4- and 5-chloropyrrolyl-S-PltL was also enhanced, mirroring the activity of CalA. Introducing the complementary mutation Leu221Met in CalA led to a reduction in depletion of the

https://doi.org/10.1021/jacs.1c02371
J. Am. Chem. Soc. 2021, 143, 7617−7622

pyrrolyl-*S*-CalN3 substrate while maintaining reactivity for the dichlorinated substrate (Figure 3F). Thus, the Met222/Leu221 side chains participate in the selection for the correct derivatization state of the pyrrolyl molecular cargoes in KS active sites. Active-site residues also tailor the substrate specificities of intermediary KS domains in collinear PKS pathways.[5,6] Overall, our data demonstrate that the strict substrate selectivity of the very first KS active site in collinear PKS assembly lines can perform a gatekeeping role to determine whether polyketide extension can be initiated.

Intermolecular protein−protein interactions underlie polyketide assembly.[34] CPs sequester their molecular cargoes; the structure of pyrrolyl-*S*-PltL demonstrates that the phosphopantetheine arm folds in such a way that the pyrrole ring binds at a hydrophobic patch on the PltL surface.[35] Hence, the exclusion of pyrrolyl-*S*-PltL by PltB but acceptance of pyrrolyl-*S*-CalN3 by CalA could depend on how PltL and CalN3 differentially present the pyrrolyl substrates to the KS. To query this hypothesis, we tested pyrrolyl- and dichloropyrrolyl-*S*-CalN3 as substrates for PltB. Compared with dichloropyrrolyl-*S*-PltL, the preference for dichloropyrrolyl-*S*-CalN3 was reduced. However, pyrrolyl-*S*-CalN3, just like pyrrolyl-*S*-PltL, was not depleted by PltB at all (Figure 4A). This result

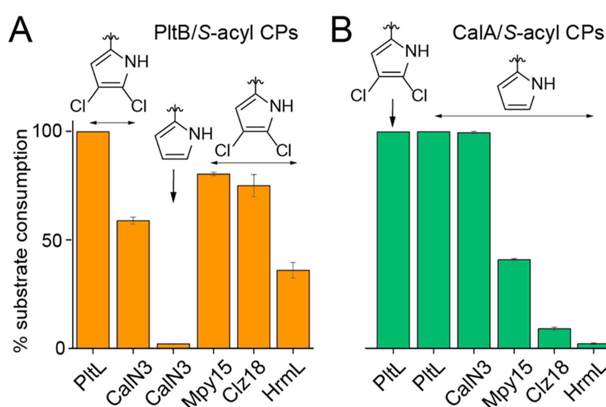


Figure 4. KS activity for noncognate donor CPs. (A) Relative depletion of dichloropyrrolyl-*S*-CPs by PltB. Depletion of pyrrolyl-*S*-CalN3, the cognate substrate for CalA, is marked by the arrow. (B) Relative depletion of pyrrolyl-*S*-CPs by CalA. Depletion of dichloropyrrolyl-*S*-PltL, the cognate substrate for PltB, is marked by the arrow.

demonstrates that while a penalty is indeed paid when the donor CP for PltB is changed from PltL to CalN3, the principal determinant for catalysis is the recognition of the molecular cargo by the KS. CalA depleted both pyrrolyl-*S*-PltL and dichloropyrrolyl-*S*-PltL (Figure 4B). The extents and rates of depletion of pyrrolyl-*S*-CalN3 and pyrrolyl-*S*-PltL by CalA were similar (Figures 4B and S39).

We also tested the ability of the type II NRPS CPs Mpy15, Clz18, and HrmL to pair with PltB and CalA. Mpy15 and Clz18 deliver thiotemplated 4,5-dichloropyrrole to PKS modules in the respective production of marinopyrroles[27] and chlorizidine,[26] and HrmL delivers 5-chloropyrrole to an NRPS module for the production of hormaomycin.[36] The activities of PltB and CalA were tested for their cognate thiotemplated substrates acylated to these different donor CPs (Figure S40−45). We observed that PltB was more accepting of different donor CPs than CalA. A marked decrease in acceptance for Mpy15 and Clz18 was observed for CalA compared with PltB (Figure 3A,B). Both PltB and CalA demonstrated the least preference for HrmL. Phylogenetically, HrmL is more distant to PltL and CalN3 than any of the other type II NRPS CPs tested (Figure S46).

Here we have demonstrated a gatekeeper role for KSs in initiating polyketide extension. Combinatorial expansion of the chemical space explored by pyrrolyl natural products will require an understanding of the intermolecular interactions that occur between the molecular cargo, the donor CP, and the initiating PKS modules. While structural models have guided modulation of KS substrate selectivities,[5,6,37,38] this study provides evidence that certain KSs, such as CalA, are already substrate-promiscuous and should be prioritized for further exploration for diversity-generating efforts.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.1c02371.

Materials and methods for protein expression and purification, chemical synthesis and spectroscopic characterization data, assay and data analysis procedures, and computational details (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Vinayak Agarwal − *School of Chemistry and Biochemistry and School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia 30332, United States;* orcid.org/0000-0002-2517-589X; Email: vagarwal@gatech.edu

Authors

Dongqi Yi − *School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332, United States*

Atanu Acharya − *School of Physics, Georgia Institute of Technology, Atlanta, Georgia 30332, United States;* orcid.org/0000-0002-6960-7789

James C. Gumbart − *School of Chemistry and Biochemistry and School of Physics, Georgia Institute of Technology, Atlanta, Georgia 30332, United States;* orcid.org/0000-0002-1510-7842

Will R. Gutekunst − *School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.1c02371

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors acknowledge support from the National Science Foundation (NSF) (CHE-2004030 to J.C.G. and V.A.), N. Garg for access to mass spectrometers, and A. Keatinge-Clay for the gift of MatB. Computational resources were provided by the Extreme Science and Engineering Discovery Environment (XSEDE Allocation TG-MCB130173, NSF), and the Partnership for an Advanced Computing Environment (PACE) at Georgia Institute of Technology.

https://doi.org/10.1021/jacs.1c02371
J. Am. Chem. Soc. 2021, 143, 7617−7622

REFERENCES

(1) Nivina, A.; Yuet, K. P.; Hsu, J.; Khosla, C. Evolution and diversity of assembly-line polyketide synthases. *Chem. Rev.* **2019**, *119* (24), 12524−12547.

(2) Klaus, M.; Grininger, M. Engineering strategies for rational polyketide synthase design. *Nat. Prod. Rep.* **2018**, *35* (10), 1070−1081.

(3) Zhang, L.; Hashimoto, T.; Qin, B.; Hashimoto, J.; Kozone, I.; Kawahara, T.; Okada, M.; Awakawa, T.; Ito, T.; Asakawa, Y.; Ueki, M.; Takahashi, S.; Osada, H.; Wakimoto, T.; Ikeda, H.; Shin-ya, K.; Abe, I. Characterization of giant modular PKSs provides insight into genetic mechanism for structural diversification of aminopolyol polyketides. *Angew. Chem., Int. Ed.* **2017**, *56* (7), 1740−1745.

(4) Keatinge-Clay, A. T. Polyketide synthase modules redefined. *Angew. Chem., Int. Ed.* **2017**, *56* (17), 4658−4660.

(5) Murphy, A. C.; Hong, H.; Vance, S.; Broadhurst, R. W.; Leadlay, P. F. Broadening substrate specificity of a chain-extending ketosynthase through a single active-site mutation. *Chem. Commun.* **2016**, *52* (54), 8373−8376.

(6) Klaus, M.; Buyachuihan, L.; Grininger, M. Ketosynthase domain constrains the design of polyketide synthases. *ACS Chem. Biol.* **2020**, *15* (9), 2422−2432.

(7) Wu, J.; Kinoshita, K.; Khosla, C.; Cane, D. E. Biochemical analysis of the substrate specificity of the *β*-ketoacyl-acyl carrier protein synthase domain of module 2 of the erythromycin polyketide synthase. *Biochemistry* **2004**, *43* (51), 16301−16310.

(8) Busch, B.; Ueberschaar, N.; Behnken, S.; Sugimoto, Y.; Werneburg, M.; Traitcheva, N.; He, J.; Hertweck, C. Multifactorial control of iteration events in a modular polyketide assembly line. *Angew. Chem., Int. Ed.* **2013**, *52* (20), 5285−5289.

(9) Jaremko, M. J.; Davis, T. D.; Corpuz, J. C.; Burkart, M. D. Type II non-ribosomal peptide synthetase proteins: structure, mechanism, and protein−protein interactions. *Nat. Prod. Rep.* **2020**, *37* (3), 355−379.

(10) Corpuz, J. C.; Podust, L. M.; Davis, T. D.; Jaremko, M. J.; Burkart, M. D. Dynamic visualization of type II peptidyl carrier protein recognition in pyoluteorin biosynthesis. *RSC Chem. Biol.* **2020**, *1* (1), 8−12.

(11) Thapa, H. R.; Robbins, J. M.; Moore, B. S.; Agarwal, V. Insights into thiotemplated pyrrole biosynthesis gained from the crystal structure of flavin-dependent oxidase in complex with carrier protein. *Biochemistry* **2019**, *58* (7), 918−929.

(12) Dorrestein, P. C.; Yeh, E.; Garneau-Tsodikova, S.; Kelleher, N. L.; Walsh, C. T. Dichlorination of a pyrrolyl-S-carrier protein by FADH2-dependent halogenase PltA during pyoluteorin biosynthesis. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102* (39), 13843−13848.

(13) Wu, Q.; Liang, J.; Lin, S.; Zhou, X.; Bai, L.; Deng, Z.; Wang, Z. Characterization of the biosynthesis gene cluster for the pyrrole polyether antibiotic calcimycin (A23187) in *Streptomyces chartreusis* NRRL 3882. *Antimicrob. Agents Chemother.* **2011**, *55* (3), 974−982.

(14) Tang, Y.; Kim, C.-Y.; Mathews, I. I.; Cane, D. E.; Khosla, C. The 2.7-Å crystal structure of a 194-kDa homodimeric fragment of the 6-deoxyerythronolide B synthase. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (30), 11124−11129.

(15) Whicher, J. R.; Smaga, S. S.; Hansen, D. A.; Brown, W. C.; Gerwick, W. H.; Sherman, D. H.; Smith, J. L. Cyanobacterial polyketide synthase docking domains: a tool for engineering natural product biosynthesis. *Chem. Biol.* **2013**, *20* (11), 1340−1351.

(16) Herbst, D. A.; Jakob, R. P.; Zähringer, F.; Maier, T. Mycocerosic acid synthase exemplifies the architecture of reducing polyketide synthases. *Nature* **2016**, *531* (7595), 533−537.

(17) Agarwal, V.; Diethelm, S.; Ray, L.; Garg, N.; Awakawa, T.; Dorrestein, P. C.; Moore, B. S. Chemoenzymatic synthesis of acyl coenzyme A substrates enables *in situ* labeling of small molecules and proteins. *Org. Lett.* **2015**, *17* (18), 4452−4455.

(18) Worthington, A. S.; Burkart, M. D. One-pot chemo-enzymatic synthesis of reporter-modified proteins. *Org. Biomol. Chem.* **2006**, *4* (1), 44−46.

(19) El Gamal, A.; Agarwal, V.; Diethelm, S.; Rahman, I.; Schorn, M. A.; Sneed, J. M.; Louie, G. V.; Whalen, K. E.; Mincer, T. J.; Noel, J. P.; Paul, V. J.; Moore, B. S. Biosynthesis of coral settlement cue tetrabromopyrrole in marine bacteria by a uniquely adapted brominase-thioesterase enzyme pair. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (14), 3797−3802.

(20) Dorrestein, P. C.; Bumpus, S. B.; Calderone, C. T.; Garneau-Tsodikova, S.; Aron, Z. D.; Straight, P. D.; Kolter, R.; Walsh, C. T.; Kelleher, N. L. Facile detection of acyl and peptidyl intermediates on thiotemplate carrier domains via phosphopantetheinyl elimination reactions during tandem mass spectrometry. *Biochemistry* **2006**, *45* (42), 12756−12766.

(21) Hughes, A. J.; Keatinge-Clay, A. Enzymatic extender unit generation for in vitro polyketide synthase reactions: structural and functional showcasing of *Streptomyces coelicolor* MatB. *Chem. Biol.* **2011**, *18* (2), 165−176.

(22) Robbins, T.; Kapilivsky, J.; Cane, D. E.; Khosla, C. Roles of conserved active site residues in the ketosynthase domain of an assembly line polyketide synthase. *Biochemistry* **2016**, *55* (32), 4476−4484.

(23) Heide, L. The aminocoumarins: biosynthesis and biology. *Nat. Prod. Rep.* **2009**, *26* (10), 1241−1250.

(24) Copley, S. D. An evolutionary biochemist's perspective on promiscuity. *Trends Biochem. Sci.* **2015**, *40* (2), 72−78.

(25) Fu, C.; Xie, F.; Hoffmann, J.; Wang, Q.; Bauer, A.; Brönstrup, M.; Mahmud, T.; Müller, R. Armeniaspirol antibiotic biosynthesis: chlorination and oxidative dechlorination steps affording spiro[4.4]-non-8-ene. *ChemBioChem* **2019**, *20* (6), 764−769.

(26) Mantovani, S. M.; Moore, B. S. Flavin-linked oxidase catalyzes pyrrolizine formation of dichloropyrrole-containing polyketide extender unit in chlorizidine A. *J. Am. Chem. Soc.* **2013**, *135* (48), 18032−18035.

(27) Yamanaka, K.; Ryan, K. S.; Gulder, T. A.; Hughes, C. C.; Moore, B. S. Flavoenzyme-catalyzed atropo-selective N,C-bipyrrole homocoupling in marinopyrrole biosynthesis. *J. Am. Chem. Soc.* **2012**, *134* (30), 12434−12437.

(28) Zhang, X.; Parry, R. J. Cloning and characterization of the pyrrolomycin biosynthetic gene clusters from *Actinosporangium vitaminophilum* ATCC 31673 and *Streptomyces* sp. strain UC 11065. *Antimicrob. Agents Chemother.* **2007**, *51* (3), 946−957.

(29) Flatt, P. M.; Wu, X.; Perry, S.; Mahmud, T. Genetic insights into pyralomicin biosynthesis in *Nonomuraea spiralis* IMC A-0156. *J. Nat. Prod.* **2013**, *76* (5), 939−946.

(30) Witte, S. N. R.; Hug, J. J.; Géraldy, M. N. E.; Müller, R.; Kalesse, M. Biosynthesis and total synthesis of pyrronazol B: a secondary metabolite from *Nannocystis pusilla*. *Chem. - Eur. J.* **2017**, *23* (63), 15917−15921.

(31) Meiser, P.; Weissman, K. J.; Bode, H. B.; Krug, D.; Dickschat, J. S.; Sandmann, A.; Muller, R. DKxanthene biosynthesis–understanding the basis for diversity-oriented synthesis in myxobacterial secondary metabolism. *Chem. Biol.* **2008**, *15* (8), 771−781.

(32) Wilcken, R.; Zimmermann, M. O.; Lange, A.; Joerger, A. C.; Boeckler, F. M. Principles and applications of halogen bonding in medicinal chemistry and chemical biology. *J. Med. Chem.* **2013**, *56* (4), 1363−1388.

(33) Parisini, E.; Metrangolo, P.; Pilati, T.; Resnati, G.; Terraneo, G. Halogen bonding in halocarbon−protein complexes: a structural survey. *Chem. Soc. Rev.* **2011**, *40* (5), 2267−2278.

(34) Khosla, C.; Herschlag, D.; Cane, D. E.; Walsh, C. T. Assembly line polyketide synthases: mechanistic insights and unsolved problems. *Biochemistry* **2014**, *53* (18), 2875−2883.

(35) Jaremko, M. J.; Lee, D. J.; Opella, S. J.; Burkart, M. D. Structure and substrate sequestration in the pyoluteorin type II peptidyl carrier protein PltL. *J. Am. Chem. Soc.* **2015**, *137* (36), 11546−11549.

(36) Höfer, I.; Crüsemann, M.; Radzom, M.; Geers, B.; Flachshaar, D.; Cai, X.; Zeeck, A.; Piel, J. Insights into the biosynthesis of hormaomycin, an exceptionally complex bacterial signaling metabolite. *Chem. Biol.* **2011**, *18* (3), 381−391.

https://doi.org/10.1021/jacs.1c02371
J. Am. Chem. Soc. 2021, 143, 7617−7622

(37) Jenner, M.; Frank, S.; Kampa, A.; Kohlhaas, C.; Pöplau, P.; Briggs, G. S.; Piel, J.; Oldham, N. J. Substrate specificity in ketosynthase domains from trans-AT polyketide synthases. *Angew. Chem., Int. Ed.* **2013**, *52* (4), 1143−1147.
(38) Gay, D. C.; Gay, G.; Axelrod, A. J.; Jenner, M.; Kohlhaas, C.; Kampa, A.; Oldham, N. J.; Piel, J.; Keatinge-Clay, A. T. A close look at a ketosynthase from a trans-acyltransferase modular polyketide synthase. *Structure* **2014**, *22* (3), 444−451.