2023 The 1st Taiwan-Japan Bilateral Symposium on Natural Products Biosynthesis

Genomics Research Center
Academia Sinica, Taipei, Taiwan
December 25, 2023

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on Natural Products Biosynthesis

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December 25, 2023

December 25 (Monday)

09:00 - **09:10** **Opening Remarks:** ***Tsung-Lin Li 李宗璘***

Chairs: ***Tsung-Lin Li 李宗璘, Hiroyasu Onaka 尾仲宏康***

Session 1

09:10 - 09:35 ***Ikuro Abe 阿部郁朗***
The University of Tokyo
Unusual enzyme reactions in natural product biosynthesis

09:35 - 10:00 ***Hsiao-Ching Lin 林曉青***
Academia Sinica
Exploration of biosynthetic enzymes for the synthesis of isoprenoid natural products

10:00 - 10:25 ***Yasushi Ogasawara 小笠原泰志***
Hokkaido University
Novel enzymes for natural product biosynthesis

10:25 - 10:45 ***Coffee Break & Group Photo***

Session 2

10:45 - 11:10 ***Hiroyasu Onaka 尾仲宏康***
Gakushuin University
Unnatural thiopeptide production using by RIPPs biosynthetic machinery

11:10 - 11:35 ***Chung-Han Chu 朱忠瀚***
National Taiwan University
A boron dependent antibiotic derived from a calcium dependent antibiotic

11:35 - 12:00 ***Taro Shiraishi 白石太郎***
The University of Tokyo
Biosynthetic study on the nucleoside antibiotic amipurimycin

12:00 - 13:30 **Lunch (by invitation)**

Session 3

13:40 - 14:05 ***Hsin-Yang Chang 張欣暘***
National Yang Ming Chiao Tung University
Biosynthesis of vitroprocines by α-oxoamine synthase and oxidoreductase identified from marine *Vibrio* sp. QWI-06

14:05 - 14:30 ***Kenichi Matsuda 松田研一***
Hokkaido University
A new family of peptide cyclases enabled streamlined chemoenzymatic synthesis of cyclic peptides

14:30 - 14:55 ***Wen-Tai Li 李文泰***
National Research Institute of Chinese Medicine
Synthesis of naturally occurring heterotricyclic compounds

14:55 - 15:10 ***Coffee Break***

Session 4

15:10 - 15:35 ***Chitose Maruyama 丸山千登勢***
Fukui Prefectural University
Amide-bond forming enzymes found in the biosynthesis of streptothricin-related compounds

15:35 - 16:00 ***Shotaro Hoshino 星野翔太郎***
Gakushuin University
Actinomycetes expand the diversity of organoarsenic natural products

16:00 - 16:25 ***Chin-Yuan Chang 張晉源***
National Yang Ming Chiao Tung University
FAD-dependent oxidoreductase CpaO involved in the ring formation in cyclopiazonic acid biosynthesis

16:25 - 16:50 ***Tsung-Lin Li 李宗璘***
Academia Sinica
N-formimidoylation/-iminoacetylation modification in aminoglycosides requires FAD-dependent and ligand-protein NOS bridge dual chemistry

Ikuro Abe

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The University of Tokyo
Position Professor
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Research Interests

Natural products biosynthesis; Enzyme engineering

Educational Background

1989	Ph.D.	The University of Tokyo
1984	B.S.	The University of Tokyo

Professional Experiences

2009-present	Professor, Graduate School of Pharmaceutical Sciences, The University of Tokyo
1998-2009	Associate Professor, School of Pharmaceutical Sciences, University of Shizuoka
1996-1998	Research Assistant Professor, Department of Medicinal Chemistry, The University of Utah
1991-1996	Research Assistant Professor, Department of Chemistry, State University of New York at Stony Brook
1989-1991	Boursier du Gouvernement Français. Institut de Chimie des Substances Naturelles (CNRS), & Ecole Nationale Supérieure de Chimie de Mulhouse

Selected Publications

1. Molecular basis for unique carrier protein-dependent amide bond formation in the biosynthesis of lincosamide antibiotics. Mori, T., Kadlcik, S., Lyu, S., Kamenik, Z., Sakurada, K., Mazumdar, A., Janata, J., Abe, I. ***Nature Catalysis***, 6, 531-542 (2023)
2. Molecular insights into the unusually promiscuous and catalytically versatile Fe(II)/α-ketoglutarate-dependent oxygenase SptF. Tao, H., Mori, T., Chen, H., Lyu, S., Nonoyama, A., Lee, S., Abe, I. ***Nature Commun.***, 13, Article number: 95 (2022)
3. β-NAD as a building block in natural product biosynthesis. Barra, L., Awakawa, T., Shirai, K., Hu. Z., Bashiri, G., Abe, I. ***Nature***, 600, 754-758 (2021)
4. Molecular insights into the endoperoxide formation by Fe(II)/α-KG-dependent oxygenase NvfI. Mori, T., Zhai, R., Ushimaru, R., Matsuda, Y., Abe, I. ***Nature Commun.***, 12, Article number: 4417 (2021)
5. Stereodivergent nitrocyclopropane formation during biosynthesis of belactosins and hormaomycins. Shimo, S., Ushimaru, R., Engelbrecht, A., Harada, M., Miyamoto, K., Andreas, K., Uchiyama, M., Kaysser, L., Abe, I. ***J. Am. Chem. Soc.***, 143, 18413-18418 (2021)

Unusual Enzyme Reactions in Natural Product Biosynthesis

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Abstract

β-Nicotinamide adenine dinucleotide (β-NAD) is a pivotal metabolite for all living organisms and functions as a diffusible electron acceptor and carrier in the catabolic arms of metabolism. Furthermore, β-NAD is involved in diverse epigenetic, immunological, and stress-associated processes, where it is known to be sacrificially utilized as an ADP-ribosyl donor for protein and DNA modifications, or the generation of cell-signaling molecules. Here, we report the function of β-NAD in secondary metabolite biosynthetic pathways, in which the nicotinamide dinucleotide framework is heavily decorated and serves as a building block for the assembly of a novel class of natural products. The gatekeeping enzyme of the discovered pathway (SbzP) hereby catalyzes a sophisticated, pyridoxal phosphate (PLP)-dependent (3+2)-annulation reaction between β-NAD and *S*-adenosylmethionine (SAM), generating a 6-azatetrahydroindane scaffold. Members of this novel family of β-NAD-tailoring enzymes are widely distributed in the bacterial kingdom and encoded in diverse biosynthetic gene clusters. The findings of this work set the stage for the discovery and exploitation of β-NAD-derived natural products. [1, 2]

References

[1] Hu, Z., Awakawa, T., Ma, Z. & Abe, I., *Nat. Commun.* **2019**, *10*, 184.
[2] Barra, L., Awakawa, T., Shirai, K., Hu, Z., Bashiri,G. & Abe, I, *Nature* **2021**, *600*, 754.

Hsiao-Ching Lin

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Research Interests

Natural Product Biosynthesis, Biocatalysts, Synthetic Biology

Educational Background

2011 Ph.D. School of Pharmacy, National Taiwan University
2006 M.S. School of Pharmacy, National Taiwan University
2004 B.S. School of Pharmacy, Taipei Medical University

Professional Experiences

2020/12~present, Associate Research Fellow, Institute of Biological Chemistry, Academia Sinica
2015/05~2020/11, Assistant Research Fellow, Institute of Biological Chemistry, Academia Sinica
2012/04~2015/04, Postdoctoral researcher, Department of Chemical and Biomolecular Engineering, University of California, Los Angeles
2011/08-2012/02, Postdoctoral researcher, School of Pharmacy, National Taiwan University

Selected Publications

1. Chen, T. H., Chen, C. T., Lee, C. F., Huang, R. J., Chen, K. L., Lu, Y. C., Liang, S. Y., Pham, M. T., Rao, Y. K., Wu, S. H., Chein, R. J., **Lin, H. C.*** The Biosynthetic Gene Cluster of Mushroom-Derived Antrocin Encodes Two Dual Functional Haloacid Dehalogenase-Like Terpene Cyclases. ***Angew. Chem. Int. Ed. 2023****, 62, e202215566.*
2. Pham, M. T., Chen, S. R., Liang, S. Y., Cheng, Y. B., **Lin H. C.*** Biosynthesis of Piperazine-Derived Diazabicyclic Alkaloids Involves a Nonribosomal Peptide Synthetase and Subsequent Tailoring by a Multifunctional Cytochrome P450 Enzyme. ***Org. Lett.*** **2022**, 24, 22, 4064–4069.
3. Hewage, R. T., Huang, R. J., Lai, S. J., Lien, Y. C., Weng, S. H., Li, D., Chen, Y. J., Wu, S. H., Chein, R. J.*, **Lin H. C.*** An Enzyme-Mediated Aza-Michael Addition Is Involved in the Biosynthesis of an Imidazoyl Hybrid Product of Conidiogenone B. ***Org. Lett.*** **2021**, 23, 5, 1904–1909.
4. Chen, Y. R., Naresh, A., Liang, S. Y., Lin, C. H., Chein, R. J.*, **Lin, H. C.*** Discovery of a Dual Function Cytochrome P450 that Catalyzes Enyne Formation in Cyclohexanoid Terpenoid Biosynthesis. ***Angew. Chem. Int. Ed.*** **2020***, 59,* 13537–13541.
5. Lee, C. F., Chen, L. X., Chiang, C. Y., Lai, C. Y., **Lin, H. C.*** Biosynthesis of norsesquiterpene aculenes requires three cytochrome P450s to catalyze a stepwise demethylation process. ***Angew. Chem. Int. Ed.*** **2019**, *58*, 18414–18418.

Exploration of Biosynthetic Enzymes for the Synthesis of Isoprenoid Natural Products

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Abstract

Isoprenoids represent a diverse family of natural products with significant medicinal and industrial properties. These compounds are synthesized from isoprene units, which are five-carbon building blocks derived from primary metabolisms in living organisms. Isoprenoids undergo a series of chemical reactions, including carbon-carbon bond coupling, cyclization, and oxidation, resulting in their diverse structures and wide range of biological activities. Particularly intriguing are the formation of core skeletons and selective C–H activation for further chemical modification, which have attracted substantial interest from both the synthetic and biological communities. In recent years, our research group has been dedicated to the discovery and elucidation of the biosynthesis of bioactive and structurally complex isoprenoids. Understanding these enzymatic processes not only expands our knowledge of NP biosynthesis but also provides potential insights for the development of new bioactive compounds with pharmacological applications.

References

1. Angew. Chem. Int. Ed. 2023, 62, e202215566.
2. Phil. Trans. R. Soc. B. 2023, B378: 20220033

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Research Interests

Identification and mechanistic study of novel enzymes for natural product biosynthesis

Educational Background

2005 Ph.D. Department of Chemistry, Graduate School of Science and Engineering, Tokyo Institute of Technology, Tokyo, Japan
(Supervisor; Prof. Katsumi Kakinuma and Prof. Tadashi Eguchi)
2000 B.S. Department of Chemistry, Tokyo Institute of Technology, Tokyo, Japan

Professional Experiences

2021-present Associate Professor, Graduate School of Engineering, Hokkaido University
2014-2020 Assistant Professor, Graduate School of Engineering, Hokkaido University
2012-2014 Research Assistant Professor, Department of Chemistry and Chemical Biology, The University of New Mexico (Prof. Charles E. Melancon)
2005-2011 Postdoctoral Fellow, College of Pharmacy, The University of Texas at Austin (Prof. Hung-wen Liu)

Selected Publications

1. Xiaojun Li *et al*. Identification of cyclopropane formation in the biosyntheses of hormaomycins and belactosins: Sequential nitration and cyclopropanation by metalloenzymes. *Angew*. *Chem*. *Int*. *Ed*. **2022**, *61*, e202113189.
2. Zhi Feng *et al*. Identification of the peptide epimerase MslH responsible for D-amino acid introduction at the C-terminus of ribosomal peptides. *Chem*. *Sci*. **2021**, *12*, 2567.
3. Yasushi Ogasawara *et al*. In vitro characterization of MitE and MitB: Formation of *N*-acetylglucosaminyl -3-amino-5-hydroxybenzoyl-MmcB as a key intermediate in the biosynthesis of antitumor antibiotic mitomycins. *Bioorg*. *Med*. *Chem*. *Lett*. **2019**, *29*, 2076.
4. Yasushi Ogasawara *et al*. Involvement of peptide epimerization in poly-γ-glutamic acid biosynthesis. *Org*. *Lett*. **2019**, *21*, 3972.
5. Junpei Kawata *et al*. Biosynthesis of the carbonylmethylene structure found in the ketomemicin class of pseudotripeptides. *Angew*. *Chem*. *Int*. *Ed*. **2017**, *56*, 2026.

Novel enzymes for natural product biosynthesis

Yasushi Ogasawara and Tohru Dairi

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Abstract

This talk will discuss our recent findings of novel enzymes for natural product biosynthesis.

1) Lactacystin (LA) is an irreversible 20S proteasome inhibitor isolated from *Streptomyces lactacystinicus* by Omura et al. in 1991[1,2]. LA possesses a unique structural feature that consists of a cyclic α,α-disubstituted amino acid and *N*-acetylcysteine. Despite its importance for life science researches, the biosynthesis of LA, especially the formation of cyclic α,α-disubstituted amino acid moiety, remains unknown. In the present study, we identified LA biosynthetic gene cluster (*orfA–orfF*) by draft genome analysis of the producer. The functions of these genes were characterized in vivo and in vitro experiments.

2) We discovered ketomemicins, novel pseudotripeptide natural products containing carbonylmethylene structure, by heterologous expression of cryptic biosynthetic gene clusters found in Actinomycetes strains[3]. Our in vitro studies unraveled the pseudo-dipeptide formation, which involves two C–C bond formations by an aldolase (KtmA) and a PLP-dependent glycine-*C*-acetyltransferase (KtmB), a dehydration by dehydratase (KtmC) and a double bond reduction by dehydrogenase (KtmF)[4].

References

1) S. Omura et al. *J. Antibiot*. **1991**, *44*, 113.
2) S. Takahashi et al. *J. Antibiot*. **1995**, *48*, 1015.
3) Y. Ogasawara et al. *ACS Chem. Biol*. **2016**, *11*, 1686.
4) J. Kawata et al. *Angew. Chem. Int. Ed*. **2017**, *56*, 2026.

Hiroyasu Onaka

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Research Interests

Bacterial communication, RiPPs biosynthesis

Educational Background

1993 The University of Tokyo, Tokyo, Japan B.S.

1998 The University of Tokyo, Tokyo, Japan Ph.D.

Professional Experiences

1998-1999 Postdoctoral Fellow at Cold Spring Harbor lab., NY, U.S.A. & Penn State University, PA, U.S.A.

1999-2012 Assistant professor,lecturer, associated professor at the Department of Biotechnology, Toyama Prefectural University, Toyama, Japan.

2012-2023 Specially-appointed professor of Graduate school of Agricultural and Life Sciences, The University of Tokyo

2023- present Professor of Department of Life Science, Faculty of Science, Gakushuin University

Selected Publications

1. Y. Lei, S. Asamizu, T. Ishizuka, H. Onaka. Regulation of Multidrug Efflux Pumps by TetR Family Transcriptional Repressor Negatively Affects Secondary Metabolism in *Streptomyces coelicolor* A3(2). ***Applied and Environmental Microbiology*** 89(3): e0182222 (2023) doi: 10.1128/aem.01822-22.
2. A. A. Vinogradov, Y. Zhang, K. Hamada, J.S. Chang, C. Okada, H. Nishimura, N. Terasaka, Y. Goto, K. Ogata, T. Sengoku, H. Onaka, H. Suga. De Novo Discovery of Thiopeptide Pseudo-natural Products Acting as Potent and Selective TNIK Kinase Inhibitors. ***Journal of the American Chemical Society,*** 144(44):20332-20341 (2022) doi: 10.1021/jacs.2c07937
3. R. Kozakai, T. Ono, S. Hoshino, H. Takahashi, Y. Katsuyama, Y. Sugai, T. Ozaki, K. Teramoto, K.Teramoto, K. Tanaka, I. Abe, S. Asamizu, and H. Onaka*. Acyltransferase that catalyses the condensation of polyketide and peptide moieties of goadvionin hybrid lipopeptides. ***Nature Chemistry,*** 12: 869-877 (2020); doi: 10.1038/s41557-020-0508-2.
4. A. A. Vinogradov, M. Shimomura, Y. Goto, T. Ozaki, S. Asamizu, Y. Sugai, H. Suga*, H. Onaka*. Minimal lactazole scaffold for in vitro thiopeptide bioengineering. ***Nature Communications,*** 2020; 11: 2272. doi: 10.1038/s41467-020-16145-4
5. T. Ozaki, K. Yamashita, Y. Goto, M. Shimomura, S. Hayashi, S. Asamizu, Y. Sugai, H. Ikeda, H. Suga*, and H. Onaka*. Dissection of goadsporin biosynthesis by *in vitro* reconstitution leading to designer analogs expressed *in vivo*. ***Nature communications*** 8, 14207 (2017) doi:10.1038/ncomms14207

Unnatural thiopeptide production using by RIPPs biosynthetic machinery

Hiroyasu Onaka

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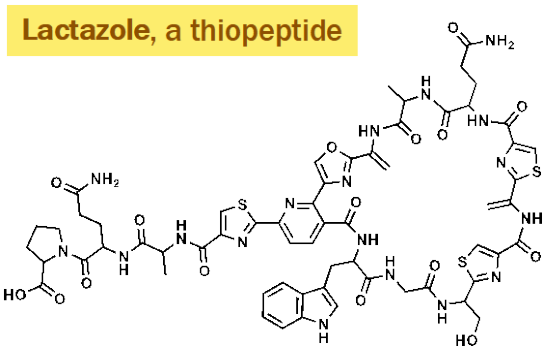
Abstract

Thiopeptides are a group of macrocyclic RiPPs with a pyridine ring as the key and contain internal post-translationally modified structures such as azole rings and dehydroalanines. Since the precursor peptide of RiPPs is encoded by the structure gene, various precursor peptides can be produced by rewriting the DNA sequences. Taking advantage of this feature, the RiPPs biosynthetic machinery is expected to be used to rapidly and easily create a diverse library of RiPP analogs.

Lactazole is a thiopeptide, and the biosynthetic gene cluster from *Streptomyces lactacystinaeus* consists of six genes which are the smallest gene set of thiopeptide biosynthesis. We have succeeded in synthesizing lactazoles in a one-pot reaction by combining a cell-free translation system and a post-translational modification reaction using recombinant enzymes[1]. We have also succeeded in creating various lactazole analogs corresponding to the nucleotide substitutions in the template DNA[2, 3, 4]. Furthermore, by combining mRNA display, we have obtained lactazole analogs that specifically bind to anti-cancer targets[5].

In this presentation, I'm going to talk about the heterologous expression system of lactazole analogs. The drug discovery system using the lactazole biosynthetic machinery consists of a screening system combining a cell-free translation system and mRNA display and fermentation production using actinomycetes, and molecules with high recognition specificity comparable to antibodies can be prepared by the fermentation method at a low cost equivalent to small molecule drugs.



References

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2) ***JACS*** **142, 32, 13886–13897 (2020)**
3) ***JACS*** **142, 48, 20329–20334 (2020)**
4) ***ACS Central Science*** **8, 6, 814-824 (2022)**
5) ***JACS*** **144, 44, 20332-20341 (2022)**

John Chu

Institution Department of Chemistry, National Taiwan University
Position Assistant Professor
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Research Interests

Natural product discovery / Natural product biosynthesis / Mechanism of action studies / Peptide chemistry

Educational Background

2010 Ph.D., The Scripps Research Institute
2004 B.S. National Taiwan University

Professional Experiences

2019-present Assistant Professor
Department of Chemistry, National Taiwan University
2014-2019 Postdoctoral Associate
Laboratory of Genetically Encoded Small Molecules, Rockefeller University
2011-2014 Postdoctoral Fellow
Department of Chemistry, Yale University

Selected Publications

1. Jian, B-S; Chiou, S-L; Hsu, C-C; Ho, J; Wu, Y-W; ***Chu, J**. Bioinformatic analysis reveals both oversampled and underexplored biosynthetic diversity in nonribosomal peptides. *ACS Chem. Biol.* **2023**, *18*, 476 (DOI: 10.1021/acschembio.2c00761)
2. Chen, P-H; Sung, L-K; Hegemann, JD; ***Chu, J**. Disrupting transcription and folate biosynthesis leads to synergistic suppression of *Escherichia coli* growth. *ChemMedChem* **2022**, e202200075 (DOI: 10.1002/cmdc.202200075).
3. Wu, C.-H.; ***Chu, J**. Total synthesis and biological evaluation of pagoamide A. *Front. Chem.* **2021**, 9:741290 (DOI: 10.3389/fchem.2021.741290).
4. **Chu, J**; Koirala, B; Forelli, N; Vila-Farres, X; Ternei, MA; Ali, T; Colosimo, DA; Brady, SF. Synthetic-bioinformatic natural product antibiotics with diverse modes of action. *J. Am. Chem. Soc.* **2020**, *142*, 14158-14168 (doi: 10.1021/jacs.0c04376).
5. **Chu, J**; Vila-Farres, X; Brady, SF. Bioactive synthetic-bioinformatic natural product cyclic peptides inspired by nonribosomal peptide synthetase gene clusters from the human microbiome. *J. Am. Chem. Soc.* **2019**, *141*, 15737-15741 (doi: 10.1021/jacs.9b07317).

A Boron Dependent Antibiotic Derived from a Calcium Dependent Antibiotic

Shao-Lun Chiou, Yi-Ju Chen, Po-Cheng Kuo, Cheng-Chih Hsu, *John Chu

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Abstract

The prevalence of drug-resistant bacterial pathogens foreshadows a healthcare crisis.[1] Calcium-dependent antibiotics (CDAs) have emerged as promising candidates to combat infectious diseases as many of them show mechanisms of action (MOA) orthogonal to widespread resistance mechanisms.[2] The dependence on calcium is nonetheless a major hurdle toward realizing their full potential. Using laspartomycin C (LspC) as a model,[3] we explored the possibility of reducing, or even eliminating, its calcium dependence. We report herein a synthetic LspC analog (**B1**) whose antibacterial activity no longer depends on calcium and is instead induced by phenylboronic acid (PBA). In LspC, Asp1 and Asp7 coordinate to a calcium cation to anchor itself in the active conformation; these residues are replaced by serine in **B1** and condense with PBA to form a boronic ester with the same anchoring effect. Using thin-layer chromatography, mass spectrometry, NMR, and complementation assays, we demonstrated that **B1** inhibits bacterial growth via the same MOA as LspC, *i.e.*, sequestering the cell wall biosynthetic intermediate undecaprenyl phosphate. **B1** is as potent as LspC and is effective against several Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococcus. Our success in engineering a CDA to become a boron-dependent antibiotic opens a new avenue in the design and functional control of drug molecules.

References

[1] V. G. Fowler, A. Jezek, E. S. Spivak, K. Talkington, Urgent, comprehensive federal action needed to stem mortality and medicare costs associated with antimicrobial resistance. *Clin. Infect. Dis.* **2022**, *74*, 1107-1111.

[2] T. M. Wood, N. I. Martin, The calcium-dependent lipopeptide antibiotics: structure, mechanism, and medicinal chemistry. *MedChemComm* **2019**, *10*, 634-646.

[3] L. H. J. Kleijn, H. C. Vlieg, T. M. Wood, J. S. Torano, B. J. C. Janssen, N. I. Martin, A high-resolution crystal structure that reveals molecular details of target recognition by the calcium-dependent lipopeptide antibiotic laspartomycin C. *Angew. Chem. Int. Ed.* **2017**, *56*, 16546-16549.

Taro Shiraishi

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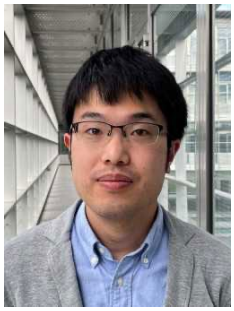

Research Interests

His current research focuses on the biosynthetic study of nucleoside antibiotics and the exploration of unique enzymology in their biosynthesis.

Educational Background

2020 Ph.D. Graduate school of Agricultural and Life Sciences, The University of Tokyo
2010 B.S. The University of Tokyo

Professional Experiences

2022-present Assistant Professor, Graduate school of Agricultural and Life Sciences, The University of Tokyo
2021-2022 Postdoctoral Fellow, Institute of Microbiology, ETH Zürich (Prof. Jörn Piel)
2019-2021 Postdoctoral Fellow, Graduate school of Agricultural and Life Sciences, The University of Tokyo (Prof. Tomohisa Kuzuyama)
2015-2019 Assistant Professor, Biotechnology Research Centre, The University of Tokyo

Selected Publications

1. T. Shiraishi, *et. al.*, "Biosynthesis of the Nucleoside Antibiotic Angustmycins: Identification and Characterization of the Biosynthetic Gene Cluster Reveal Unprecedented Dehydratase Required for *Exo*-glycal Formation," *J. Antibiot.*,**74**, 11, 830–833, 2021.
2. A. J. Romo†, T. Shiraishi†, *et. al.*, "The Amipurimycin and Miharamycin Biosynthetic Gene Clusters: Unraveling the Origins of 2-Aminopurinyl Peptidyl Nucleoside Antibiotics," *J. Am. Chem. Soc.*, **141**, 14152–14159, 2019. †equal contribution
3. T. Shiraishi and T. Kuzuyama*, "Recent Advances in the Biosynthesis of Nucleoside Antibiotics," *J. Antibiot.*, **72**, 913–923, 2019.
4. T. Shiraishi, M. Nishiyama and T. Kuzuyama*, "Biosynthesis of the Uridine-derived Nucleoside Antibiotic A-94964: Identification and Characterization of the Biosynthetic Gene Cluster Provide Insight into the Biosynthetic Pathway," *Org. Biomol. Chem.*, **17**, 3, 461–466, 2018.

Biosynthetic Study on the Nucleoside Antibiotic Amipurimycin

Taro Shiraishi[1,2], Genki Hibi[1], Tatsuki Umemura[1], Hideo Ikeuchi[1], Makoto Nishiyama[1,2] and Tomohisa Kuzuyama[1,2]

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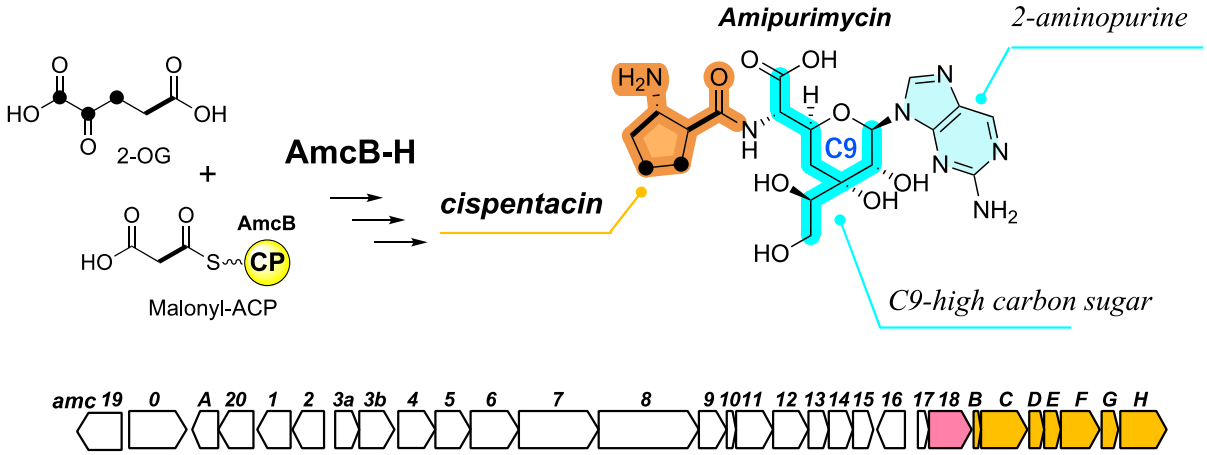
Abstract

Peptidyl nucleoside antibiotics (PNAs) are a diverse class of natural products with promising biomedical activities. These compounds have tripartite structures composed of a core saccharide, a nucleobase, and one or more amino acids. In particular, amipurimycin is a novel 2-aminopurinyl PNA with complex nine-carbon core saccharides and includes the unusual amino acids (–)-cispentacin. Despite their interesting structures and properties, this PNA have heretofore eluded biochemical scrutiny. Herein, we report the identification of the biosynthetic gene cluster for amipurimycin (*amc* clsuter) and the functional characterization of these biosynthetic enzymes. The gene cluster was identified using a comparative genomics approach and heterologous expression of the *amc* gene cluster. Furthermore, *in vitro* analysis of Amc18 established its role as ATP-grasp ligase involved in the attachment of the pendant amino acids (–)-cispentacin.[1)] In addition, analysis of the *amc* cluster and feeding studies also led to the proposal of a biosynthetic pathway for (–)-cispentacin. Finally, a series of *in vitro* reconstitution analyses revealed unprecedented biosynthetic pathway for the noncanonical amino acid cispentacin.[2)]



References

1) A. J. Romo[†], T. Shiraishi[†], *et. al.*, "The Amipurimycin and Miharamycin Biosynthetic Gene Clusters: Unraveling the Origins of 2-Aminopurinyl Peptidyl Nucleoside Antibiotics," *J. Am. Chem. Soc.*, **141**, 14152–14159, 2019. [†]equal contribution

2) G. Hibi, T. Shiraishi, *et. al.*, *Nature Commun.* doi.org/10.1038/s41467-023-43731-z

Hsin-Yang Chang

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Research Interests

1. My lab is interested in how newly synthesized tail-anchored membrane proteins are targeted to, and subsequently inserted into the lipid bilayer during the initial stage of their biosynthesis.
2. We are also interested in the molecular mechanism for the biosynthesis of antibacterial amino-polyketide derivatives from microorganisms.

Educational Background

2010 PhD Department of Biochemistry, University of Illinois at Urbana- Champaign

Professional Experiences

2020-present Associate professor, Department of Life Sciences and Institute of Genome Sciences at National Yang Ming Chiao Tung University

2015-2020 Assistant professor, Department of Marine Biotechnology & Resources at National Sun Yat-sen University

Selected Publications (SCI Journal, 2019-2023)

1. MD Huang, CW Wu, HY Chou, SY Cheng and **HY Chang** (2023) The revealing of a novel lipid transfer protein lineage in green algae. *BMC Plant Biology* 23:21,https://doi.org/10.1186/s12870-023-04040-1
2. CC Liaw, LH Lo, TH Cheng, YT Chan, YR Huang, AHJ Wang, **HY Chang** (2022) Biosynthesis of Vitroprocines by Identified α-oxoamine Synthase and Oxidoreductase from *Vibrio* sp. QWI-06, *Organic Letters*, 24, 17, 3281–3285.
3. **HY Chang**, LH Lo, YH Lan, MX Hong, YT Chan, TP Ko, YR Huang, TH Cheng, CC Liaw (2021) Structural insights into the substrate selectivity of α-oxoamine synthases from marine *Vibrio* sp. QWI-06, *Colloids Surf. B Biointerfaces*, 210, 112224.
4. HC Lin, WH Li, CC Chen, TH Cheng, YH Lan, MD Huang, WM Chen, JS Chang, **HY Chang** (2020) Diverse enzymes with industrial applications in four thraustochytrid genera. Front. Microbiol., https://doi.org/10.3389/fmicb.2020.573907.
5. TW Lin, CC Chen, SM Wu, YC Chang, YC Li, YW Su, CD Hsiao, **HY Chang**. (2019) Structural analysis of chloroplast tail-anchored membrane protein recognition by ArsA1, The Plant Journal, 99:128-143.

Biosynthesis of vitroprocines by α-oxoamine synthase and oxidoreductase identified from marine *Vibrio* sp. QWI-06

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Abstract

Alpha-oxoamine synthases (AOS) are generally believed to be responsible for catalyzing the condensation of amino acids and acyl-CoA thioester substrates. A widely known example is the production of the sphingolipid precursor 3-ketodihydrosphingosine (KDS), generated via condensation of L-serine and palmitoyl-CoA by serine palmitoyltransferase (SPT). Subsequently, KDS reductase (KDSR) catalyzes the reduction of KDS to dihydrosphingosine. Since only few sphingoid derivatives have been found in prokaryotes, the molecular details of these bacterial enzymes with similar biochemical functions for the production of amino-polyketide derivatives are not characterized. We have discovered vitroprocines, L-tyrosine (or L-phenylalanine)-polyketide derivative, a potential new antibiotic identified from marine *Vibrio* sp. QWI-06. It exhibits significant inhibitory activity against the pathogen *Acinetobacter baumannii*, which causes serious nosocomial infections in Taiwan. By using bioinformatics analysis, we identified four putative α-oxoamine synthases (VsAOS) from marine *Vibrio* sp. QWI-06. We have proposed that VsAOS-1 acts strictly in the condensation of L-glycine to C12 and C16 fatty acids ***(Chang et al., Colloids Surf. B: Biointerfaces, 2021)***. In a recent breakthrough, we also identified a brand new α-oxoamine synthase (VsAOS-2) and a human KDSR-like reductase (VsOR), which were responsible for the decarboxylative condensation of L-tyrosine to lauroyl-CoA, following the reduction of ketone group, to form vitroprocines ***(Liaw et al., Organic Letters, 2022)***. Our findings on the biosynthetic enzymes of vitroprocines shed light on the biosynthetic logic for the potential antibiotics, which provide new insights for further understanding in the engineering of bioactive natural products and biocatalyst development.

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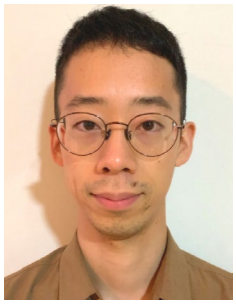

Research Interests

Biosynthesis of nitrogen-nitrogen bond-containing natural products. Characterization and biocatalytic application of peptide modifying enzymes.

Educational Background

2017 Ph.D. Agriculture. Graduate School of Agriculture and Life Science, University of Tokyo (Prof. Makoto Nishiyama)
2012 B.S. Faculty of Agriculture, University of Tokyo (Prof. Makoto Nishiyama)

Professional Experiences

2021-present Lecturer, Faculty of Pharmaceutical Sciences, Hokkaido University
2017-2021 Assistant professor, Faculty of Pharmaceutical Sciences, Hokkaido University
2017-2017 Postdoctoral fellow, Technology Research Association for Next generation natural products chemistry

Selected Publications

1. Arima, K., Akiyama, S., Shin-ya, K., Matsuda, K.*, Wakimoto, T.* Carrier protein mediated formation of the dihydropyridazinone ring in actinopyridazinone biosynthesis. *Angew. Chem., Int. Ed.* **2023**, *62*, e202305155.
2. Kobayashi, M., Fujita, K., Matsuda, K.*, Wakimoto, T.* Streamlined chemoenzymatic synthesis of cyclic peptides by non-ribosomal peptide cyclases. *J. Am. Chem. Soc.* **2023**, *145*, 3270–3275.
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A New Family of Peptide Cyclases Enabled Streamlined Chemoenzymatic Synthesis of Cyclic Peptides

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Abstract

Biosynthetic pathways of natural products are prolific source of biocatalysts which address various synthetic challenges and potentially complement the existing synthetic methods. However, enzymes often require specific recognition moiety on their substrates, thus giving rise to other challenges for their synthetic application. Macrocyclization of peptide improves their pharmaceutical properties such as cell-membrane permeability, target specificity and metabolic stability. However, regio- and chemoselective intramolecular cyclization remain challenging. In the biosynthesis of non-ribosomal cyclic peptides, peptide cyclases such as thioesterases (NRPS-TEs) efficiently catalyze the intramolecular peptide cyclization in a regiospecific manner without the use of protecting groups. Thus, they could potentially be exploited as biocatalysts to tackle the synthetic challenge. However, NRPS-TEs generally require the thioester leaving groups on its substrate, which necessitates labor-intensive solution-phase coupling reactions during substrate synthesis. To overcome this, we installed a diol leaving group at the first step of substrate synthesis. This new method yielded the diol esters with sufficient purity, which could be subjected for the subsequent enzymatic cyclization without further purification[1]. The diol-activated peptides were efficiently cyclized in a head-to-tail manner by penicillin-binding protein-type thioesterases, which is a new family of peptide cyclase involved in the biosynthesis of cyclic non-ribosomal cyclic peptides[2-4].

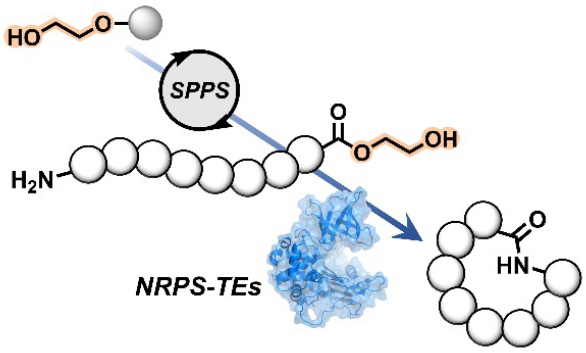

Figure. Diol-activated peptides were synthesized by SPPS and cyclized by NRP cyclases.

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Research Interests

Synthesis of biologically active molecules and natural products derived from Chinese medicine; Development of novel and efficient synthetic methods; Quality control for Chinese medicines

Educational Background

1995-1999 Ph.D. in Chemistry, National Tsing Hua University, Taiwan

Professional Experiences

2011-present Assistant/Associate Research Fellow, National Research Institute of Chinese Medicine, Ministry of Health and Welfare
2021-present Associate Research Fellow (joint appointment), Taipei Medical University

Selected Publications

1. Liang, Y.-E.; Kan, C.-Y.; Barve, B. D.; Chen, Y.-A.; Li, W.-T.* Palladium-catalyzed chemo- and regiocontrolled tandem cyclization/cross-coupling of 2-benzyl-3-alkynyl chromones with aryl iodides for the synthesis of 4*H*-furo[3,2-*c*]chromenes and xanthones. *Org. Lett.* 2022, 24, 37, 6728-6733.
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Synthesis of Naturally Occurring Heterotricyclic Compounds

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Abstract

Oxygen- and nitrogen-containing heterocycles such as chromone, xanthone, and furo[3,2-*c*]quinolone are the key structural units of many natural products and medicinally important molecules. These molecules are ubiquitous as the backbones of traditional Chinese medicines and organic materials. *O*- and *N*-containing heterocyclic motifs have attracted considerable attention due to their preventative and curative effects in a variety of diseases. Therefore, the preparation of *O*- or *N*-containing heterocyclic derivatives exhibiting anti-cancer, anti-inflammatory, and anti-bacterial activities is of interest in synthetic chemistry.

The novel metal-catalyzed chemo- and regiocontrolled tandem cyclization/cross-coupling reaction of 3-alkynyl chromone with aryl iodide or olefin was developed for the synthesis of xanthone, furo[3,2-*c*]chromene, and furo[3,2-*c*]quinoline. The difunctionalization of alkynes involved the sequential nucleophilic domino attack onto an alkyne and cross-coupling with aryl halide or alkene affording the diverse range of structurally interested scaffolds. The one-pot tandem processes directly from gamma-alkynyl-1,3-diketone through multiple cyclizations and cross-coupling reaction were successfully developed.

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Research Interests

Her current research interests include biosynthesis of bioactive microbial metabolites and development of new methods for chemical biology.

Educational Background

2012 Ph.D. Fukui Prefectural University (Prof. Yoshimitsu Hamano)
1999 M.S. Fukui Prefectural University
1997 B.S. Fukui Prefectural University

Professional Experiences

2019-present Associate Professor, Department of Bioscience, Fukui Prefectural University.
2017-2019 Assistant Professor, Department of Bioscience, Fukui Prefectural University.
2012-2017 Postdoctoral Fellow, Department of Bioscience, Fukui Prefectural University.

Selected Publications

1. YL Wang, CY Chang, NS Hsu, IW Lo, KH Lin, CL Chen, CF Chang, ZC Wang, Y. Ogasawara, T. Dairi, C. Maruyama, Y. Hamano, TL Li*, N-Formimidoylation/-iminoacetylation modification in aminoglycosides requires FAD-dependent and ligand-protein NOS bridge dual chemistry, ***Nature Communications***, 14(1), 2528, (2023)
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4. C. Maruyama, H. Niikura, M. Izumikawa, J. Hashimoto, K. Shin-ya, M. Komatsu, H. Ikeda, M. Kuroda, T. Sekizuka, J. Ishikawa, Y. Hamano, tRNA-dependent aminoacylation of an amino-sugar intermediate in the biosynthesis of a streptothricin-related antibiotic, ***Appl. Environ. Microbiol.***, 82, 3640-3648 (2016).
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Amide-bond forming enzymes found in the biosynthesis of streptothricin-related compounds

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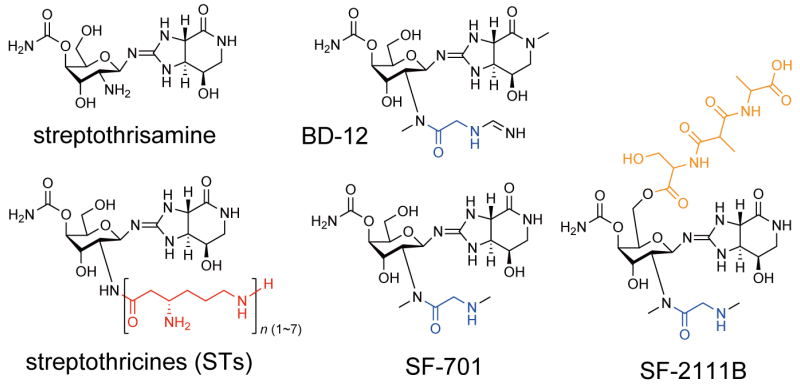
Abstract

Streptothricins (STs) produced by *Streptomyces* strains are broad-spectrum antibiotics and are characterized by a streptothrisamine core structure with the L-β-lysine (β-Lys) residue and its oligomeric side chains [oligo(β-Lys)]. In addition to the STs, *Streptomyces* strains have been known to produce ST-related compounds, BD-12, SF-701, SF-2111B, etc., which possess a glycine-derived side chain rather than the β-Lys residue. Moreover, SF-2111B is an SF-701 derivative with a unique *O*-acylpeptide side chain consisting of serine, methylmalonate, and alanine. It is known that the structural variation of these side chains significantly affects their antibiotic activities. Therefore, the formation mechanisms are of interest in the amino-acid side chains and the *O*-acylpeptide side chain.

To date, we have clarified the formation mechanisms of the oligo(β-Lys) side chain [1] and the glycine-derived side chain [2]. Notably, the tRNA-dependent amide bond-forming enzyme Orf11 catalyzes the condensation reaction between streptothrisamine and glycine in the BD-12 biosynthesis [2], while the streptothricin biosynthesis employs NRPS machinery [1]. In addition, we recently clarified that NRPS and an asparagine synthase-like enzyme catalyze the amide bond formation in the *O*-acylpeptide. In this presentation, we will summarize these amide bond-forming enzymes found in our biosynthetic studies of these ST-related compounds.



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Research Interests

secondary metabolism, organoarsenical, genome mining, natural product discovery

Educational Background

2019 Ph.D. (Pharmaceutical Science, The University of Tokyo)
2016 M.S. (Pharmaceutical Science, The University of Tokyo)
2014 B.S. (Pharmaceutical Science, The University of Tokyo)

Professional Experiences

2023-present: Assistant Professor at Department of Life Science, Faculty of Science, Gakushuin University (PI: Prof. Hiroyasu Onaka)
2022-2023: Specially-appointed Assistant Professor at Graduate School of Agricultural and Life Sciences, The University of Tokyo (PI: Prof. Hiroyasu Onaka)
2019-2022: Postdoctoral Researcher at Department of Chemistry, Princeton University (PI: Prof. Mohammad R. Seyedsayamdost)
2019: Postdoctoral Researcher at Graduate School of Pharmaceutical Sciences, The University of Tokyo (PI: Prof. Ikuro Abe)

Selected Publications

1. Hoshino, S. *et. al.* Insights into arsenic secondary metabolism in actinomycetes from the structure and biosynthesis of Bisenarsan. ***J. Am. Chem. Soc.***, 2023, 145, 17863–17871.
2. Kozakai, R., Ono, T., Hoshino, S. *et. al.* Acyltransferase that catalyses the condensation of polyketide and peptide moieties of goadvionin hybrid lipopeptides. ***Nat. Chem.,*** 2020, 12, 869–877.
3. Hoshino, S. *et. al.* Mycolic acid containing bacterium stimulates tandem cyclization of polyene macrolactam in a lake sediment derived rare actinomycete. ***Org. Lett.,*** 2017, 19, 4992–4995.
4. Hoshino, S. *et. al.* Niizalactams A–C, multicyclic macrolactams isolated from combined culture of *Streptomyces* with mycolic acid-containing bacterium. ***J. Nat. Prod.***, 2015, 78, 3011–3017.
5. Hoshino, S. *et. al.* Chojalactones A–C, cytotoxic butanolides isolated from *Streptomyces* sp. cultivated with mycolic acid containing bacterium. ***Org. Lett.,*** 2015, 17, 1501–1504.

Actinomycetes expand the diversity of organoarsenic natural products

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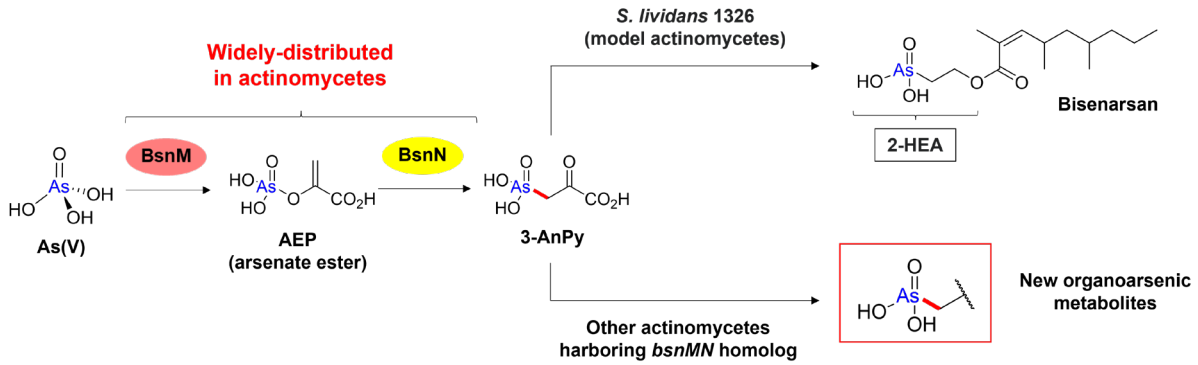
Abstract

Despite unique bioactivities, research on microbial arsenic secondary metabolites is still limited. Here, we focused on the structurally undefined organoarsenic metabolite produced by model actinomycetes (= bisenarsan).[1] Our preliminary spectroscopic analyses predicted that (2-hydroxyethyl)arsonic acid (2-HEA) is a key biosynthetic intermediate of bisenarsan, and we successfully enhanced the bisenarsan production by feeding of 2-HEA, which provided us with sufficient yield for structural elucidation. Finally, bisenarsan was the novel *O*-acyl derivative of 2-HEA, and the first actinomycete-derived arsenic secondary metabolite.[2]

Our genetic studies have identified the genes responsible for the bisenarsan biosynthesis. In particular, the putative EPSP synthase (= BsnM) and phosphoglycerate mutase (= BsnN) appear to catalyze C–As bond formation via arsenate ester generation and subsequent intramolecular rearrangement of a pentavalent arsenate ester. The proposed C–As bond formation by BsnN is quite different from the conventional C–As bond formation reported in the biosynthesis of other organoarsenic natural products. Homologs of BsnM and BsnN are widely distributed in the genome of actinomycetes along with related genes, suggesting the existence of diverse arsenic secondary metabolism in actinomycetes.



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[2] Hoshino, S. *et. al.* ***J. Am. Chem. Soc.***, 2023, 145, 17863–17871.

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Research Interests

Natural product biosynthesis, Enzyme chemistry, Structural biology

Educational Background

2011 Ph.D. Biological Science and Technology, National Yang Ming Chiao Tung University
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Professional Experiences

2022~present Associate Professor, Department of Biological Science and Technology, National Yang Ming Chiao Tung University
2018~2022 Assistant Professor, Department of Biological Science and Technology, National Yang Ming Chiao Tung University
2015~2018 Department of Chemistry, Scripps
2011~2015 Genomics Research Center, Academia Sinica

Selected Publications

1. Chen IH, Cheng T, Wang YL, Huang SJ, Hsiao YH, Lai YT, Toh SI, Chu J, Rudolf JD, **Chang CY***. Characterization and structural determination of CmnG-A, the adenylation domain that activates the nonproteinogenic amino acid capreomycidine in capreomycin biosynthesis. *ChemBioChem*, 23, e202200563, ***2022***.
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FAD-Dependent Oxidoreductase CpaO Involved in the Ring Formation in Cyclopiazonic Acid Biosynthesis

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Abstract

Cyclopiazonic acid (CPA) is a pentacyclic indole natural product discovered from the *Aspergillus* and *Penicillium* species. CPA is a nanomolar inhibitor of sarcoplasmic/endoplasmic reticulum calcium-adenosine triphosphatase (SERCA), which regulates the Ca ion concentration and affects the muscle contraction and relaxation cycle. Furthermore, CPA has been reported to have antibacterial and antiviral activities. CpaO is a FAD-dependent oxidoreductase and was proposed to catalyze dehydrogenation of β-CPA to form a pentacyclic ring system; however, the enzyme catalytic mechanism about the unusual intramolecular ring closure is still a mystery. Here, we used a combination of biochemical characterization, mutagenesis analysis, and structural biology approaches to unravel the catalytic mechanism of CpaO. The crystal structures of CapO in apo form and in complex with the substrate β-CPA and the product α-CPA, respectively, shed light on the substrate binding site and the catalytic mechanism. This study also provides new insights into the ring formation for the L-Trp-based natural products.

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Research Interests

His current research focuses on chemoenzymatic synthesis of unnatural natural glycopeptide and aminoglycoside antibiotics, for which structure-based biocatalysts are developed and exploited.

Educational Background

2003 Ph.D., Chemistry, The University of Cambridge

Professional Experiences

2019-present Professor, Genomics Research Center, Academia Sinica
2011-2019 Associate Professor, Genomics Research Center, Academia Sinica
2004-2011 Assistant Professor, National Taiwan Ocean University/Genomics Research Center, Academia Sinica

Selected Publications

1. Y. L. Wang, C. Y. Chang, N. S. Hsu, I. W. Lo, K. H. Lin, C. L. Chen, C. F. Chang, Z. C. Wang, Y. Ogasawara, T. Dairi, C. Maruyama, Y. Hamano*, T. L. Li*, 2023, "N-Formimidoylation/-iminoacetylation modification in aminoglycosides requires FAD-dependent and ligand-protein NOS bridge dual chemistry", *Nat Commun*, 14:2528, 1-14.
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3. S. Y. Lyu, Y. C. Liu, C. Y. Chang, C. J. Huang, Y. H. Chiu, C. M. Huang, N. S. Hsu, K. H. Lin, C. J. Wu, M. D. Tsai, T. L. Li*, 2014, "Multiple complexes of long aliphatic N-acyltransferases lead to synthesis of 2,6-diacylated/2-acyl-substituted glycopeptide antibiotics effectively killing vancomycin-resistant Enterococcus", *J Am Chem Soc*, 136(31), 10989-10995.
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N-Formimidoylation/-iminoacetylation modification in aminoglycosides requires FAD-dependent and ligand-protein NOS bridge dual chemistry

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Abstract

Oxidized cysteine residues are highly reactive and can form functional covalent conjugates, of which the allosteric redox switch formed by the lysine-cysteine NOS bridge is an example. Here, we report a noncanonical FAD-dependent enzyme Orf1 that adds a glycine-derived *N*-formimidoyl group to glycinothricin to form the antibiotic BD-12. X-ray crystallography was used to investigate this complex enzymatic process, which showed Orf1 has two substrate-binding sites that sit 13.5 Å apart unlike canonical FAD-dependent oxidoreductases. One site could accommodate glycine and the other glycinothricin or glycylthricin. Moreover, an intermediate-enzyme adduct with a NOS-covalent linkage was observed in the later site, where it acts as a two-scissile-bond linkage facilitating nucleophilic addition and cofactor-free decarboxylation. The chain length of nucleophilic acceptors vies with bond cleavage sites at either N–O or O–S accounting for *N*-formimidoylation or *N*-iminoacetylation. The resultant product is no longer sensitive to aminoglycoside-modifying enzymes, a strategy that antibiotic-producing species employ to counter drug resistance in competing species.

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