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A Biosynthetic Gene Cluster for the Acetyl-CoA Carboxylase Inhibitor Andrimid

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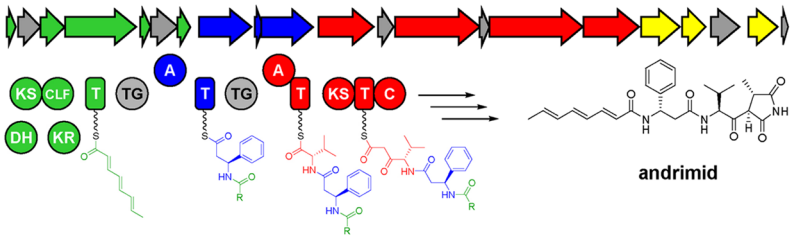
Abstract



Increasing bacterial resistance to antibiotics with conventional targets has focused attention on antibiotics with unconventional targets. One promising candidate, the acetyl-CoA carboxylase (ACC) inhibitor andrimid, is a potent, broad-spectrum antibiotic with high selectivity for prokaryotic ACC. Here, we report the use of a DNA-based approach to clone andrimid biosynthetic gene cluster from *Pantoea agglomerans*, yielding a cosmid that confers robust andrimid production on *Escherichia coli*. This gene cluster encodes a hybrid nonribosomal peptide/polyketide (NRP/PK) synthase with several unusual features, including three enzymes that form and insert beta-phenylalanine, two transglutaminase-like enzymes that likely serve as condensation catalysts, and four densely hybrid modules that form the succinimide precursor. Unlike most type I NRPSs and PKSs, the andrimid gene cluster is a dissociated system comprised of small proteins. Therefore, future efforts can exploit the genetic manipulability of *E. coli* to engineer the andrimid synthase with the goal of producing a diverse set of andrimid analogs for clinical evaluation.

Increasing bacterial resistance to conventional antibiotics has focused attention on antibiotics with unconventional targets. One promising candidate, andrimid (**1**), inhibits the β-subunit of acetyl-CoA carboxylase (ACC).[1] Since ACC converts acetyl-CoA to malonyl-CoA, the substrate for fatty acid biosynthesis, inhibition of ACC prevents cell growth. Accordingly, andrimid is a potent, broad-spectrum antibiotic with high selectivity for prokaryotic ACC. Andrimid biosynthesis is widely distributed in the bacterial realm, which suggests that its potency and broad spectrum confer a selective advantage on its producers.[2] However, until now, the gene cluster associated with andrimid biosynthesis had not been discovered.

As part of an ongoing project to identify genes involved in the production of bacterial secondary metabolites,[3] we have been studying the family of antibiotics produced by *Pantoea agglomerans* (formerly *Erwinia herbicola*). These antibiotics were originally studied because of their potential to control Fire blight,[4] a devastating plant disease caused by *Erwinia*

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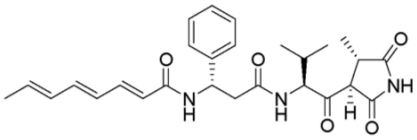
Supporting Information Available: A table of genes and figures showing characterization of cosmid 2194C1 and resistance of *E. coli* BL21-*admQ* to andrimid. This material is available free of charge via the Internet at http://pubs.acs.org.

amylovora, but they have become of more general interest in the study of DNA-based approaches to the discovery of small molecules[5] and the way in which Nature constructs a functional family of antibiotics. *P. agglomerans* strain Eh335 produced a potent antibiotic, and bioassay-guided fractionation led to andrimid. A cosmid library prepared from genomic DNA of Eh335 was screened for andrimid resistance, and resistant clones were screened against *E. coli* in an overlay assay for andrimid production. From several positive clones, we chose a single cosmid (2194C1) that encodes a 21-gene cluster (Figure 1) for further characterization. Cosmid 2194C1 conferred andrimid production on *E. coli* XL1-Blue MR (Figure S1), confirming that this 21-gene cluster is associated with andrimid biosynthesis.

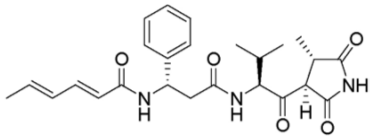
This gene cluster comprises four recognizable sections that play distinct roles (Figure 1b): (i) formation of the polyunsaturated fatty acid by an iterative type II PKS (green), (ii) formation and insertion of β-phenylalanine (blue), (iii) construction of the succinimide precursor from valine, glycine, and C2 units from two equivalents of malonyl-CoA (red), and (iv) host resistance and enzyme priming (yellow). This hybrid nonribosomal peptide synthetase-polyketide synthetase (NRPS-PKS) contains several interesting features: enzymes to form and insert a β-amino acid, transglutaminase-like (TGase-like) enzymes that likely form the central amide bonds, and a densely hybrid NRPS-PKS module to form the precursor to the succinimide moiety.

Formation and insertion of a β-amino acid. (AdmHIJ)

AdmH is homologous to amino acid aminomutases such as SgcC4,[6] which convert α-amino acids to β-amino acids using a 4-methylidene-imidazol-5-one (MIO) cofactor formed by an autocatalytic post-translational modification. AdmH probably converts α-Phe to β-Phe, creating a local pool of this precursor. AdmJ, in turn, is an adenylation (A) domain that would recognize β-Phe specifically, activating it as the adenylate and then installing



andrimid **1**



moiramide B **2**

it covalently with a thioester linkage to the Ppant arm of the AdmI T domain. This strategy of ‘on-site’ transformation of a proteinogenic amino acid into a non-proteinogenic amino acid for subsequent incorporation is conceptually similar to the β-hydroxylation of Tyr during vancomycin assembly, and the oxidation of Pro to pyrrole for clorobiocin biosynthesis.[7]

Transglutaminase-like enzymes as condensation domains? (AdmFS)

Condensation (C) domains are the canonical amide-bond-forming catalysts in NRPSs. However, the only C domain in the synthetase is at the C-terminus of AdmO, where it is predicted to function in a later stage of andrimid assembly. There are no C domains to form the first two amide bonds during assembly. However, AdmF and AdmS both contain a core TGase domain; TGases form isopeptide bonds between the sidechain carboxamide of glutamine and various amine donors.[8] AdmF contains the complete Cys-His-Asp catalytic triad characteristic of TGases, while AdmS is missing the His. We propose that one or both of these enzymes serve as condensation catalysts. The first half-reaction of the TGase catalytic cycle, formation of an acyl-*S*-enzyme intermediate from a Gln carboxamide, would be replaced by an energetically neutral transthiolation of an acyl-*S*-T to an acyl-*S*-enzyme intermediate. The second half-reaction, capture of the acyl-*S*-enzyme intermediate by an amine, would remain the same. These TGase-like enzymes would be similar to KS domains, which—unlike C domains—acylate themselves on an active-site Cys with the acyl donor co-substrate prior to catalyzing condensation.

Densely-hybrid NRPS-PKS elongation modules. (AdmKMOP)

Numerous medicinally important metabolites such as the antitumor agents epothilone and bleomycin and the immunosuppresants rapamycin and FK506 are formed by hybrid NRPS-PKS assembly lines.[9] C and KS domains at the interfaces between NRPS and PKS modules in these synthetases are particularly interesting as these elongation catalysts perform the unusual task of linking peptidyl and ketidyl groups. The latter portion of the cluster is densely hybrid, comprising three hybrid interfaces with just ten domains distributed over four proteins. As such, this assembly line is a promising system in which to study elongation domains at hybrid interfaces. Indeed, while the KS domains of AdmO and AdmM do not have interaction-mediating "linkers", they cluster phylogenetically with peptidyl-accepting KS domains from other NRPS-PKS systems (data not shown).

Post-assembly line succinimide formation

The methyl-succinimide is required for biological activity.[10] Isotope-feeding experiments led Andersen and coworkers to propose that its precursor is a dipeptide of β-keto-γ-amino acids that comes from successive elongations of both Val and Gly with C2 units from malonyl-CoA.[2a] The arrangement of domains among AdmO, AdmP, and AdmM support this biosynthetic proposal. The assembly line product is likely the linear β-keto acid **3**, which would undergo an intramolecular aldol condensation to form tetrahedral adduct **4**. Decarboxylation and dehydration would yield **5**, which would undergo two-electron reduction of the exomethylene and four-electron oxidation of the adjacent carbon to form andrimid. AdmB, AdmL, and AdmN —none of which have recognizable sequence homology to known proteins—are likely to be involved in this the formation of this unusual heterocycle.

A fragmented assembly line

Most NRPS and PKS assembly lines are composed of large, multi-domain proteins. In contrast, the andrimid synthetase contains eleven proteins, none of which have more than three domains. Since none of the proteins have more than one T domain, all chain elongations occur between intermediates tethered to different proteins. As such, andrimid biosynthesis likely involves numerous protein-protein interactions, raising interesting questions about protein organization and scaffolding elements within the synthetase and unidirectionality of small-molecule assembly. The absence of a type II (editing) thioesterase suggests that either aberrant loading/elongation are infrequent, or that this dissociated synthase is not derailed by such mistakes.

Host resistance to andrimid appears to involve a two-fold strategy of antibiotic efflux and target replacement. *admQ* encodes a major facilitator subfamily transporter that confers andrimid resistance on a sensitive *E. coli* strain (Figure S2). *admT* encodes the β-subunit of ACC, and is likely to be an andrimid-resistant form of this enzyme.

Intriguingly, either andrimid or its close relative moiramide (**2**) have been isolated from a wide variety of gram-negative symbiotic bacteria, including marine strains of *Pseudomonas* and *Vibrio* and a terrestrial strain of *Enterobacter*.[2] Such widely-distributed production of a secondary metabolite requires either horizontal gene transfer (HGT) or convergent evolution. HGT is supported by the fact that *admU*—the final gene in the cluster—encodes a small protein that is homologous to transposases associated with mobile genetic elements. It is likely that transposase-mediated transfer of the andrimid cluster among several orders of the class *Gammaproteobacteria* results in the cosmopolitan occurrence of this antibiotic.

Advances in understanding the metabolic logic of NRP and PK assembly lines have enabled efforts to engineer these systems to produce 'unnatural' natural products. Such engineering efforts have yielded analogs of the PK avermectin and the NRP-PK epothilone that are used

industrially.[11] Our identification of a gene cluster that confers metabolite production on *E. coli*, a genetically-manipulable host, will similarly enable efforts to engineer andrimid analogs for clinical evaluation. The dissociated nature of the andrimid synthetase might make it easier to manipulate than a more associated assembly line.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. (a) Freiberg C, Brunner NA, Schiffer G, Lampe T, Pohlmann J, Brands M, Raabe M, Habich D, Ziegelbauer K. J. Biol Chem 2004;279:26066–26073. [PubMed: 15066985] (b) Freiberg C, Fischer HP, Brunner NA. Antimicrob. Agents Chemother 2005;49:749–759. [PubMed: 15673760]
2. (a) Needham J, Kelly MT, Ishige M, Andersen RJ. J. Org. Chem 1994;59:2058–2063. (b) Oclarit JM, Okada H, Ohta S, Kaminura K, Yamaoka Y, Iizuka T, Miyashiro S, Ikegami S. Microbios 1994;78:7–16. [PubMed: 8022309] (c) Fredenhagen A, Tamura SY, Kenny PTM, Komura H, Naya Y, Nakanishi K, Nishiyama K, Sugiura M, Kita H. J. Am. Chem. Soc 1987;109:4409–4411.
3. (a) Brady SF, Chao CJ, Clardy J. J. Am. Chem. Soc 2002;124:9968–9969. [PubMed: 12188643] (b) Brady SF, Clardy J. Angew Chem Int Ed Engl 2005;44:7063–7065. [PubMed: 16206308]
4. Oh CS, Beer SV. FEMS Microbiol. Lett 2005;253:185–192. [PubMed: 16253442]
5. Goodman RM, et al. Appl. Environ. Microbiol 2000;66:2541–2547. [PubMed: 10831436]
6. Christenson SD, Liu W, Toney MD, Shen B. J. Am. Chem. Soc 2003;125:6062–6063. [PubMed: 12785829]
7. (a) Hubbard BK, Walsh CT. Angew. Chem. Int. Ed. Engl 2003;42:730–765. [PubMed: 12596194] (b) Garneau S, Dorrestein PC, Kelleher NL, Walsh CT. Biochemistry 2005;44:2770–2780. [PubMed: 15723521]
8. Walsh, CT. Posttranslational Modification of Proteins: Expanding Nature's Inventory. Roberts and Company: Englewood; 2006. p. 435-460.
9. Fischbach MA, Walsh CT. Chem. Rev. 2006in press
10. Pohlmann J, Lampe T, Shimada M, Nell PG, Pernerstorfer J, Svenstrup N, Brunner NA, Schiffer G, Freiberg C. Bioorg. Med. Chem. Lett 2005;15:1189–1192. [PubMed: 15686939]
11. (a) Krebber A, et al. Metab. Eng 2005;7:27–37. [PubMed: 15721808] (b) Julien B, Shah S. Antimicrob. Agents Chemother 2002;46:2772–2778. [PubMed: 12183227]

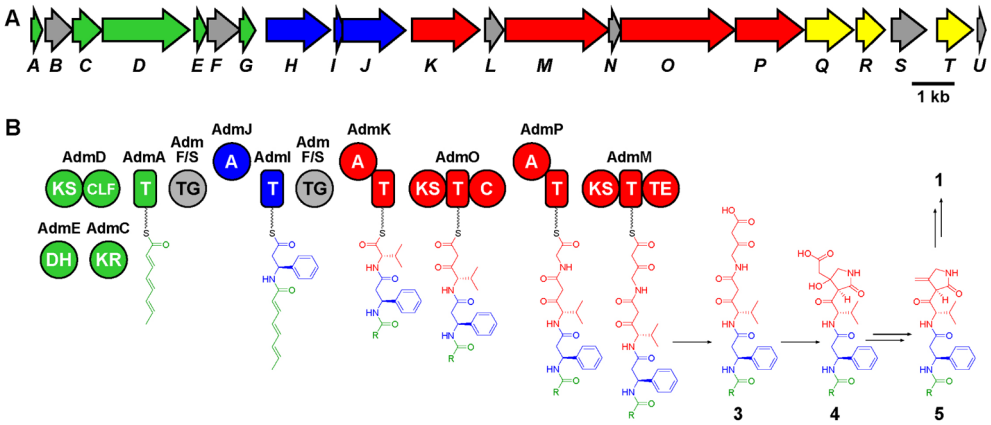

Figure 1.

(A) Schematic of the andrimid gene cluster. (B) Schematic of the andrimid synthetase and proposed biosynthesis of andrimid.