Point of VIEW

ACS chemical biology

To Cyclize or Not To Cyclize: Catching Enzyme Evolution in the Act

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ABSTRACT If you look at the biggest genes in soil and marine bacteria, you tend to see the chemical blueprints for making natural products such as peptides and polyketides. Over the past decade, collective efforts of enzymologists working with synthetic and analytical chemists have been catching up with the data dump from microbial genome sequencing. Following this story line, we now understand how cyanobacteria construct scaffolds for the related natural products curacin and jamaicamide using subtle tweaks to nonstandard biosynthetic machinery.

The great diversity of natural products and their biosynthetic pathways continues to come into view as more microbial genomes are sequenced. In 2004, the sequences of the curacin and jamaicamide gene clusters became available, and those in the field marveled at the nonstandard nature of the enzymes involved (*1, 2*). This paralleled an explosion of such cases in this subfield of enzymology that deals with the "assembly line" biosynthesis of polyketides (PK) and non-ribosomally produced peptides (NRP). For standard systems in this field, there is a strong correlation between the order of enzymes in the genome and the structure of the natural product made. This is confounded by strange types of organizational logic in the enzymes for such systems as curacin and jamaicamide. Five years after sequencing, Professors Gerwick and Sherman pulled together a team to reveal how relatively small changes to just a few enzymes can result in drastic changes to the final product, for example, incorporation of a chlorine atom *versus* a cyclopropane ring (Figure 1, far right) (*3*).

Covalent Chemistry. Almost all NRP and PK biosyntheses proceed via molecular "conveyor belt"-type systems, also called "assembly lines" by those in the field (*4*). This type of biosynthetic pathway has advantages for both the host organism and the researchers investigating them. By virtue of covalent enzyme-intermediates, the organism shuttles substrates along with high energy content but without leakage of precious or toxic compounds to the cytosol. To the enzymologist, this mode of biosynthesis allows interrogation of the chemistry and mechanism at specific active sites along the assembly line. Gu, Sherman, and colleagues take advantage of this mode of biosynthesis to investigate not only the chemistry occurring in the pathways of curacin and jamaicamide but also the evolution of these divergent pathways from a common ancestral pathway.

Some Amazing Covalent Chemistry. Key among the findings presented in their recent letter to *Nature* were studies focused on a cassette of unusual polyketide synthase machinery, likely recruited into a primordial biosynthetic pathway that subsequently diverged to yield a chlorine atom or a cyclopropane ring in the analogous portions of curacin and jamaicamide (Figure 1, **7** and **9**). As setup, some chemistry borrowed from primary metabolism in hydroxymethyl-glutaryl-CoA biosynthesis (worked out previously by the Walsh and Sherman laboratories (*5, 6*)) was found to occur in secondary metabolism on a covalent cofactor of acyl carrier proteins (ACPs) (Figure 1, **1** → **2**). Recruitment of a non-heme Fe(II), α-ketoglutarate-dependent enzyme *into* the assembly has different outcomes for the two different pathways explored due to the divergence of the downstream dehydratases and decarboxylases. Diversification at the decarboxylase (Figure 1, **4** → **5** and **8**) is responsible for

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Published online July 17, 2009

10.1021/cb900154b CCC: $40.75

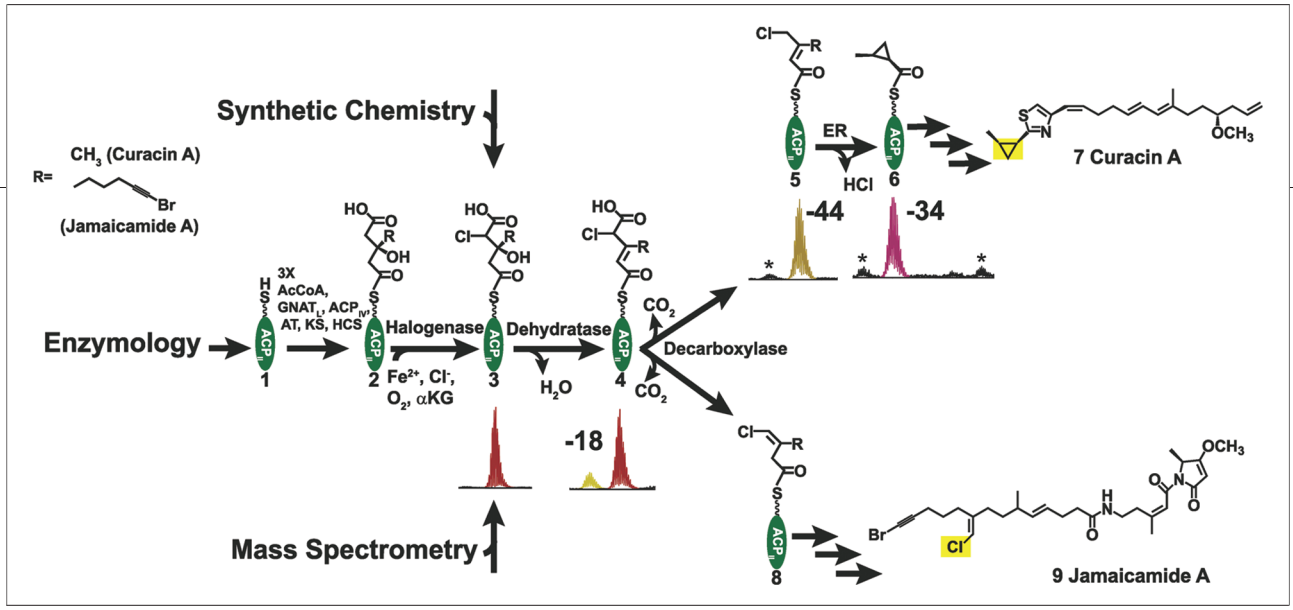

Figure 1. Homology and divergence of the Cur and Jam pathways. Earlier this decade, the plethora of new microbial genome sequences started to show some bizarre domain architectures. Warp ahead a few years and many mysteries have been solved by teams of enzymologists, synthetic chemists, and analytical chemists. Just such a team reported an exemplary study of curacin and jamaicamide biosynthesis that occurs in the marine cyanobacterium *Lyngbya majuscula*. The jamaicamide and curacin pathways diverge at the decarboxylation step; the rest of the pathway is >85% identical, whereas the decarboxylases are 59% identical, tipping researchers off to their importance. Cur, curacin; Jam, jamaicamide; AcCoA, acetyl coenzyme A; ACP, acyl-carrier protein; GNAT, GCN5 related *N*-acetyl transferase; KS, ketosynthase; HCS, hydroxymethyl-glutarate coenzyme A synthase; αKG, α-keto glutarate; ER, enoyl reductase.

the switch from a canonical αβ unsaturation outcome to a unique βγ unsaturation outcome. Finally, diversification at the enoyl reductase results in chloride elimination coupled to cyclopropane formation (Figure 1, **5** → **6**) or a vestigial enzyme with no or unknown function, perhaps in the process of evolving.

Role of Multiple ACPs. Another curious feature of jamaicamide and curacin biosynthesis is the role of the three ACP domains on the largest enzymes in the pathways. Most polyketide systems only have one ACP domain. There is mounting evidence in the field from polyketide and polyunsaturated fatty acid biosynthesis that such tandemly repeated carrier proteins are present to increase the overall flux of the assembly line for enhanced production of secondary metabolites (Figure 2) (*7*, *8*).

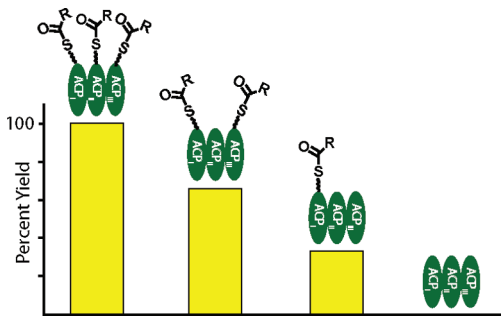

Figure 2. Removing functional ACPs decreases overall yield. A simple model for how redundant carrier proteins may affect overall yield in a secondary metabolic pathway. As the number of functional ACPs decrease (by point mutation or deletion), the amount of product accumulated is decreased, although the pathway is still competent for production (*7*, *8*). ACP, acyl-carrier protein.

Mass Spectrometry. A prominent analytical approach utilized in the work of Gu *et al.* is Fourier transform mass spectrometry (FTMS). This method is especially suited to analysis of thiotemplate systems due to its ultrahigh resolution and the ability to selectively eject covalently bound intermediates from assembly line active sites (*9*, *10*). High resolution allows for detection of relatively small mass shifts on the enzyme(s) at high mass and in the context of complex mixtures. The analytical arm of the team was led by Kristina Håkansson. The team performed a rigorous set of experiments demonstrating the identity of enzyme reaction products and the relative catalytic efficiencies of the Cur and Jam enoyl reductases using the FTMS-assay based on selective cofactor ejection during tandem MS (MS/MS).

General Relevance. Marine natural products offer fascinating chemical structures that can be potent toxins or useful medicines. Most have unknown function in the native producers. Peptide and polyketide marine products offer a unique glimpse into their evolution due to their co-linear biosynthesis and use of conserved domains whose order dictates the final structure. Comparisons of sequences and domain order allow us to peer back in time to trace the origins of this diverse collection of metabolites. For the jamaicamide and curacin biosynthetic pathways, two *Lyngbya majuscula* samples collected from opposite ends of the earth. Focusing on the enzymes with only ~60% amino acid identity, instead of the ~90% for the rest of two pathways, these researchers have illuminated how relatively small changes in amino acid sequence effects the final natural products. Elucidation of the evolution of these pathways requires a talent set as diverse as the natural products themselves, including marine biology, microbiology, enzymology, and synthetic and analytical chemistry.

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