Structural And Biochemical Investigation of a Novel Natural Product Amination Domain

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Bacteria manufacture a diverse range of natural products with pharmaceutical value as potent antibiotics and chemotherapeutic agents, many of which are FDA-approved. Two well-characterized biosynthetic systems, the modular polyketide synthases (PKS) and non- ribosomal peptide synthetases (NRPS), are major sources of pharmaceutical natural products (1). These systems are organized into assembly lines of multidomain modules, where each module extends an intermediate by an acyl unit (PKS) or amino acid (NRPS).

During each step of biosynthesis, the growing intermediate is tethered to a carrier protein (CP) via its phosphopantetheine (Ppant) cofactor. PKS and NRPS are generally encoded by gene clusters and synthesize natural products that can be predicted from the gene sequence alone. This makes them very attractive targets for engineering to create new or purposefully altered products.

Release of the product from the CP of the final module is typically performed by a thioesterase (TE) domain to yield a carboxylic acid or cyclized product. In contrast, marine cyanobacterial lipopetides carmabin A (2), vatiamides E-F (3), and hectoramide B (unpublished) contain a terminal amide instead of the canonical carboxyl group. These three pathways encode an unknown 400-residue domain at the C-terminus in lieu of the expected TE. We hypothesize that this domain is responsible for both thioester offloading and nitrogen incorporation, we refer to the domain as a terminal amination domain (TAD).

We present a 1.75Å crystal structure of the carmabin A TAD bound to putative cofactor NADH. A structural homology search reveals that the TAD fold is very similar to that of *S. elongatus* acyl-ACP reductase (AAR) (4), despite low sequence identity. Using AAR as a guide, we propose a mechanism where the TAD uses free ammonia to perform aminolysis on the Ppant-bound thioester. By synthesizing peptide analogs of each natural product and loading them onto their respective CP, we will use a mass spectrometry approach to determine the requirements for TAD activity.

This uncharacterized NRPS domain has biocatalytic potential to convert CP-tethered thioesters to amides. Complete understanding of the structure and chemistry will facilitate the construction of natural product analogs with alternative termini and new properties.

Funded by NIH R01 DK042303 to JLS and F31 CA265082 and T32 GM8353-29 to MRR.

References

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