Manipulation of Cellular Machinery to Produce Anti-cancer Drugs

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Abstract

The nocardioazine natural products are uniquely prenylated and methylated indole alkaloid diketopiperazines (DKPs) that reverse drug resistance of cancer cell lines. We unveiled the nocardioazine biosynthetic pathway from a marine actinomycete, demonstrating that a cyclodipeptide synthase catalyzes *cyclo*(L-Trp-L-Trp) DKP precursor formation followed by tailoring of this DKP via a novel racemase, prenyltransferase, and methyltransferase to yield nocardioazine B. These results highlight the aptitude of bacteria for chemical synthesis and offer new enzymatic tools for crafting complex organic molecules.

Introduction

- Actinomycetes have been a key resource in natural product discovery since the 1950's[1]. Diketopiperazines (DKPs) are one class of products produced by these bacteria.
- Molecules with a DKP scaffold are structurally diverse owing to the multiple amino acids that can be incorporated into the scaffold as well as tailoring of the scaffold[2,3]. This diversity and demonstrated array of biological activities[4] makes these especially interesting compounds.
- Nocardioazines (Fig. 1a) are one DKP example and have been shown to inhibit drug resistance in cancer cell lines[5]. We hypothesized that a cluster of genes encoding three enzymes (Fig. 1b) works in conjunction with the products of either of two cyclodipeptide synthases (CDPS) present in a *Nocardiopsis sp. a*ctinomycete to yield the nocardioazines[3].

Fig. 1a

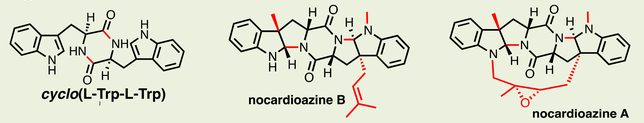


Fig. 1b

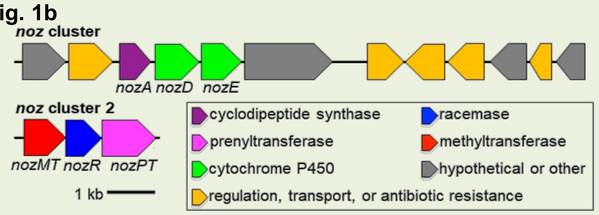


Fig. 1. DKP Structures and genes. (a) *Cyclo*(L-Trp-L-Trp) (cWW) [DKP assembled by the NozA CDPS from noz cluster 1[3]], nocardioazine B, and nocardioazine A structures. (b) Hypothesized nocardioazine gene clusters from *Nocardiopsis sp.*

Methods

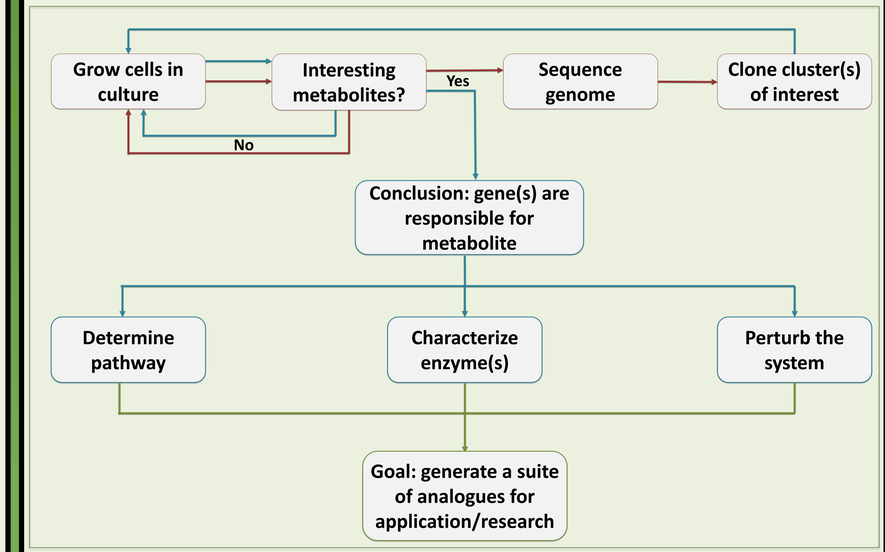


Fig. 2. General research flow. This outline shows the overall method our lab uses to search out and attempt to understand natural products.

Conclusions

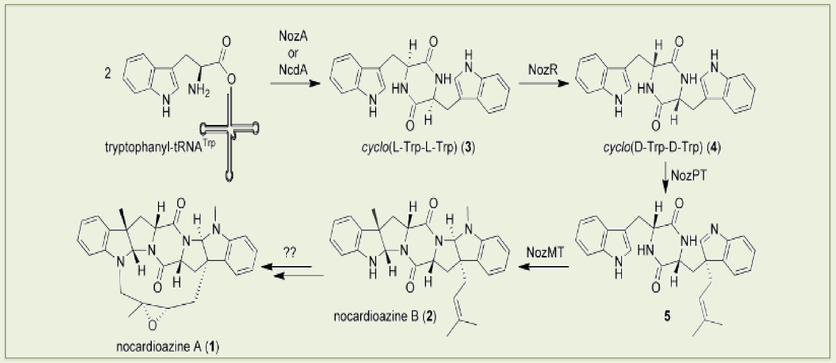


Fig. 8. Proposed Nocardioazine Pathway. This marks the first report of a MT catalyzing both N- and C-methylation of any natural product.

- **This work establishes that *noz* 1 + 2 gene clusters encode nocardioazine assembly (Fig. 3). Most bacterial natural products are assembled by one gene cluster, making the nocardioazine pathway unusual.**
- **MT activity assays (Fig. 6) show that MT acts as the final enzyme in the pathway.**
- **These results set the stage for biocatalytic application of Noz enzymes to yield new DKPs with cost effective, green methods.**
  - **Traditional DKP synthesis methods require a complex system of reactions in excess of 20 steps, specialized equipment/techniques, and generate large amounts of hazardous waste in the form of solvents[6].**
  - **Biosynthesis uses fewer harmful solvents, often requires only several steps, and after initial research investment can produce complex molecules with little more than high school lab tools.**

Results

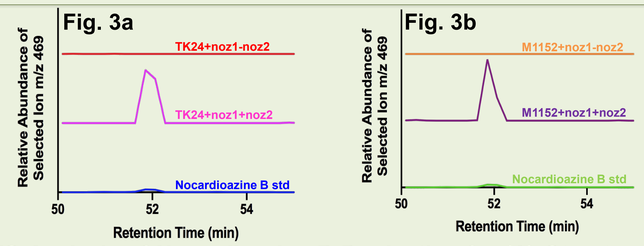
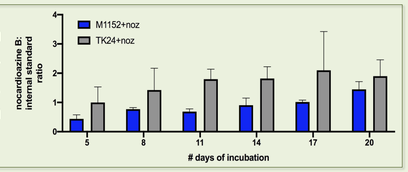


Fig. 3. LC-MS Analysis of Nocardioazine B Production for (A) *Streptomyces* M1152 and (B) *Streptomyces* TK24 carrying either *noz1* cluster or both *noz1+2* clusters. No nocardioazine B was produced with only one gene cluster (+noz1 -noz2). Nocardioazine B was only produced when both gene clusters were present (+noz1+noz2), as indicated by comparison with nocardioazine B standard at the retention time and molecular ion *m/z* 469. This links both of the two *noz* gene clusters (Fig. 1b) to nocardioazine B assembly.

Fig. 4. Timeline of Nocardioazine B Production by *Streptomyces* TK24 and M1152 strains. TK24 was shows to yield more nocardiozine than M1152. The error bars represent +/- 1 standard deviation for n=2.



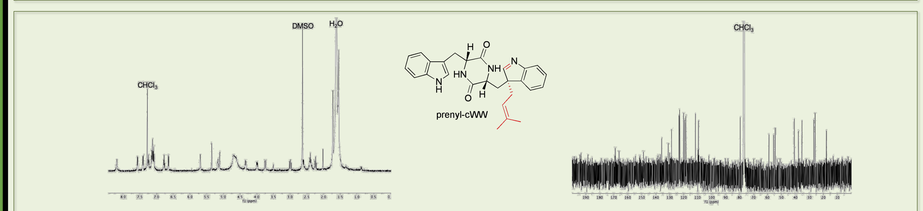


Fig. 5. ^{1}H (Left) and ^{13}C NMR (Right) Spectra for Prenyl-cWW. These ^{1}H and ^{13}C NMR spectra of isolated prenyl-cWW allows us to propose the molecular structure. Observation of this intermediate also supports that prenylation comes prior to methylation in the nocardioazine metabolic pathway.

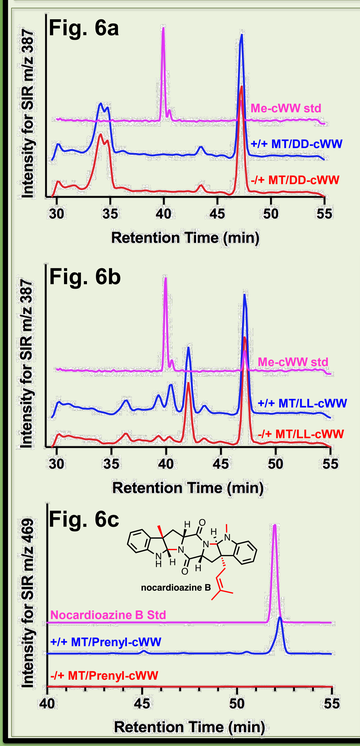


Fig. 6. LC-MS Analysis of MT Enzyme Activity Assays.
(a) No methylation evident with DD-cWW as substrate.
(b) No methylation evident with LL-cWW as substrate.
(c) Presence of nocardioazine B indicates double methylation of prenyl-cWW. Observed methylation of prenyl-cWW but not of DD/LL-cWW supports that prenylation of cWW occurs prior to methylation during nocardioazine assembly.

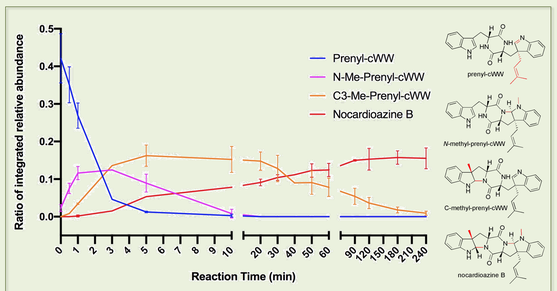


Fig. 7. LC-MS Analysis of MT Time-Course Experiment. Rapid drop-off in prenyl-cWW in conjunction with rise in mono-methylated intermediates followed by rise in dual-methylated nocardioazine B indicates the sufficiency of these components in the nocardioazine B pathway. Error bars indicate ±1σ of n=2 replicates.

Acknowledgements

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