Chapter 19: Toxins Workgroup Poster Abstracts

Microginin peptides from *Microcystis aeruginosa*

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Introduction

Freshwater cyanobacteria have been shown to produce several classes of unique peptide metabolites. *Microcystis aeruginosa* in particular has been a rich source of many interesting peptides, most notably the heptapeptide microcystins. In addition to the cyclic hepatotoxic microcystins, *M. aeruginosa* also produces microginins, a family of linear peptides composed of 3–6 amino acid residues. Previously characterized microginins show angiotensin converting enzyme (ACE) inhibition as well as leucine aminopeptidase M inhibition. Consequently, these compounds are of interest as lead compounds in the discovery of novel antihypertensive agents as well as treatments for congestive heart failure.

Hypothesis

Cyanobacteria have been extensively studied for toxins that they produce. In addition to these toxins, other unusual cyanopeptides are continually found. This suggests the presence of other complex biosynthetic pathways capable of producing a range of novel peptide metabolites with exotic chemistries and important bioactivities. Thus, we hypothesize that *M. aeruginosa* is likely to be a source of new bioactive secondary metabolites.

Methods

Microcystis aeruginosa cells (UTEX 2385) were grown in B3N media in a 14/10 light–dark cycle. Cells were collected by vacuum filtration and extracted with 80% methanol. The organic extract was dried and applied to a reversed phase column followed by elution with a step gradient of aqueous methanol. The fractions of interest were combined and further purified by size exclusion chromatography. Appropriate fractions were then combined and further subjected to reversed–phase HPLC, yielding microginin 527 (compound **2**, rt = 16.4 min) and microginin 690 (compound **1**, rt = 17.9 min) in addition to several more minor congeners.

Results

Compound 1 was isolated as a white powder. The HRMS and NMR data revealed a molecular formula of $C_{37}H_{46}N_4O_9$ (m/z = 691.334305). The UV data (λ_{max} 224, 276 nm) indicated the presence of an aromatic amino acid. Indeed, the ESI–MS data revealed a strong fragment ion at [M+H –180] and m/z 180 consistent with a C–terminus tyrosine moiety. Another fragment ion at m/z 343 revealed a second tyrosine residue adjacent to the C–terminal tyrosine. The fragment ion at m/z 128 suggested an N–terminus 3–amino–2–hydroxy decanoic acid (Ahda). A moiety corresponding to 161 Da remained unaccounted for. 2D NMR analysis suggested that this unknown component was a fourth amino acid, perhaps a modified tyrosine. Compound **2** was also isolated as a white powder. This compound had the same UV absorbance as compound 1 and shared fragment ions of m/z 128 and m/z 180 but had a molecular weight of 528. It was deduced that this compound shared the N–terminus Ahda residue and a single C–terminus tyrosine.

Conclusion

Cyanobacteria that produce toxins often have the ability to produce other secondary metabolites. We have isolated two previously undiscovered peptides from *Microcystis aeruginosa* as well as some minor derivatives. These compounds may belong to a new family of microginins containing an unknown residue that we believe to be a modified tyrosine. Further NMR analysis is required to fully characterize the nature of this amino acid derivative.

Inactivation of an ABC transporter, *mcyH*, results in loss of microcystin production in the cyanobacterium *Microcystis aeruginosa* PCC 7806

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Introduction

The cyanobacterium *Microcystis aeruginosa* is widely known for its production of the potent hepatotoxin microcystin. Microcystin is synthesized nonribosomally by the thiotemplate function of a large, modular enzyme complex encoded within the 55 kb microcystin synthetase (*mcy*) gene cluster. Also encoded within the *mcy* gene cluster is a putative ATP binding cassette (ABC) transporter, McyH. This study details the bioinformatic and mutational analyses of McyH and offers functional predictions for the hypothetical protein.

Hypotheses

It is hypothesized that *mcyH* encodes an ABC–transporter that is responsible for the biosynthesis and/or transport of Microcystin.

Methods

The *mcyH* gene has been characterized bioinformatically via structural, functional and phylogenetic analyses. In addition, an *mcyH* null mutant has been engineered and characterized with respect to its ability to produce and export microcystin. The McyH enzyme has been heterologously expressed in *E. coli,* purified and used to raise anti–McyH antibodies. These antibodies have been used in immunoblotting experiments to investigate McyH expression in mutant and wild–type strains of *M. aeruginosa.*

Results

The McyH transporter is putatively comprised of 2 homodimers, each with an N–terminal hydrophobic domain and a C–terminal ATPase. Phylogenetically, McyH was found to cluster with members of the ABC–A_1 subgroup of ABC ATPases, suggesting an export function for the protein. The m*cyH* null mutant strains were unable to produce microcystin. Whilst the *mcyH* deletion had no apparent effect on the transcription of other *mcy* genes, the complete microcystin biosynthesis enzyme complex could not be detected in *mcyH* mutant strains. Expression of McyH was reduced in *mcyA* and *mcyB* mutants and completely absent in the *mcyH* mutant.

Conclusion

By virtue of its association with the *mcy* gene cluster and the bioinformatic and experimental data presented in this study, we predict McyH functions as a microcystin exporter and is, in addition, intimately associated with the microcystin biosynthesis pathway.