"It's Not Easy Being Green"

Protein Phosphatase-1 Binds Cyanobacteria Microcystins Causing Hepatotoxicity

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Abstract: The cyanobacteria species *Microcystis aeruginosa* produces microcystin (MC) toxins that can cause liver damage in animals and have resulted in the death of dogs and livestock. MC toxins are cyclic heptapeptides, and they inhibit the enzyme protein phosphatase-1 (PP1), which plays a critical role in reversing the action of protein kinases. Kinases catalyze phosphorylation, which generally promotes cellular activity. In contrast, PP1 catalyzes dephosphorylation, which can slow cellular activity. Balanced phosphorylation and dephosphorylation is critical to liver cell homeostasis, and is disrupted when PP1 is inhibited by MC toxins. MC toxins have two unique amino acid residues: the Mdha and Adda sidechains in addition to standard amino acids. MC binding to PP1 occurs through interactions at four sites on the PP1 enzyme. Two metal atoms of PP1 can coordinate via two water molecules with the glutamic acid residue of MC. The Cys273 residue of PP1 can form a covalent linkage with the Mdha side chain of MC. The hydrophobic Adda side chain of MC fits well in the hydrophobic groove of PP1. Finally, the MeAsp residue of MC hydrogen bonds with Arg96 and Tyr134 residues of PP1. The Valders SMART Team designed a model of PP1 binding MC using 3D printing technology to understand the relationship between MC toxins and PP1.

Introduction:

In August 2014, more than 400,000 residents in Toledo, Ohio and Michigan's Monroe County were without tap water for several days due to excessive cyanobacteria growth in Lake Erie (**Fig.1**). From 2005 to 2009 there were 63 days in which swimming was closed in Lake Monona and Lake Mendota in Madison, Wisconsin due to cyanobacteria blooms - the misnomer blue-green algae is often used when referring to cyanobacteria (**Fig.2**). *Microcystis aeruginosa* is a species of cyanobacteria that can produce the hepatotoxin microcystin. Microcystins (MC) are inhibitors of the enzyme protein phosphatase-1 (PP1). PP1 plays a critical role in maintaining liver cell homeostasis and its inhibition may lead to liver cell damage, intrahepatic bleeding, and the production of tumor promoting signals. Understanding cyanobacteria blooms and MC- caused hepatotoxicity will help protect the health of birds, fish, humans, and other animals.

Fig. 1

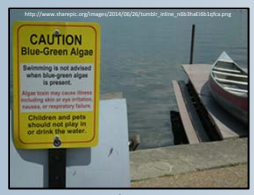


Fig. 2

The Problem Starts with P:

Phosphorus is a necessary element for life (**Fig.3**). However, excess phosphorus in surface water may cause cyanobacteria blooms to develop (**Fig.1**). Cyanobacteria that produce microcystins endanger the health of animals using contaminated water. Phosphorus runoff created by human activities such as excess fertilization and shoreline disturbances set the stage for dangerous blooms to develop. In ecosystems, phosphorus is present in the form known as phosphate, or PO_4. **Fig. 4** shows phosphate levels in four Manitowoc county, Wisconsin creeks 24 and 48 hours after rainfall. The black line* represents the accepted amount of phosphate for surface water. PO_4 levels are continuously above this line illustrating an unacceptable amount of phosphate runoff entering the creeks.

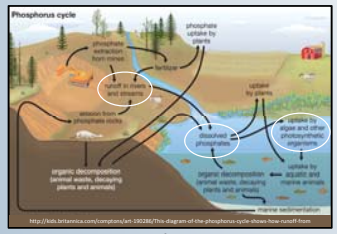


Fig. 3

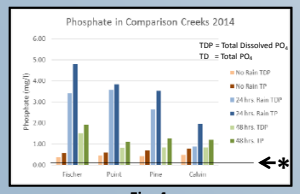
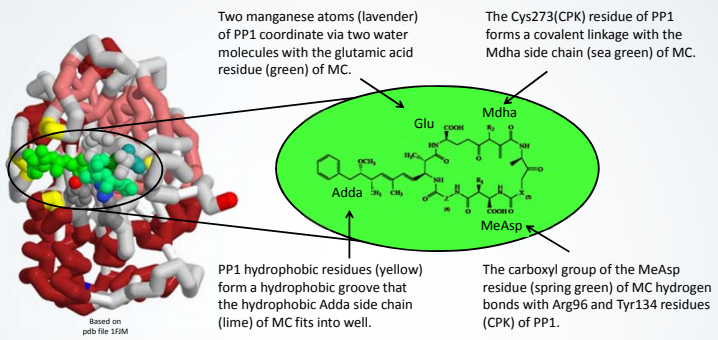


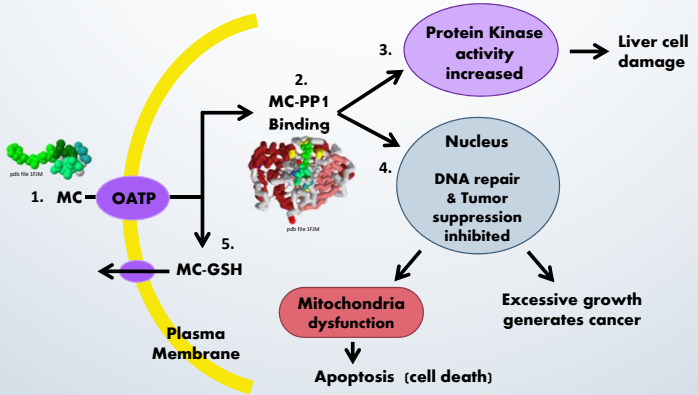
Fig. 4

Protein Phosphatase 1 Binds Microcystin Toxin and Dephosphorylation is Inhibited:

Protein phosphatase-1 (PP1) plays a critical role in reversing the action of kinases. Kinases promote a cellular response by catalyzing phosphorylation. In contrast, PP1 catalyzes dephosphorylation slowing cellular activity, and its inhibition disrupts liver cell homeostasis.



PP1 Inhibition Disrupts Liver Cell Homeostasis:



1. Microcystin (MC) hepatotoxin is transported into liver cells through organic anion-transporting polypeptides (OATP).
2. MC binds and inhibits protein phosphate-1 (PP1).
3. PP1 inhibition prevents dephosphorylation of protein kinases; causing phosphorylation to increase.
4. DNA repair and tumor control is suppressed resulting in cancer and/or apoptosis.
5. Some MC is broken down by the antioxidant glutathione (GSH), and metabolites are excreted.

Environmental Solutions:

Increasing public awareness of water quality issues and bringing together government agencies, researchers, businesses, and water quality advocacy groups will help to reduce phosphorus pollution in surface water. Techniques to reduce cyanobacteria blooms include adding cover crops (**Fig. 5**) and buffer strips to help control soil erosion (**Fig. 6**). Preservation of native ecosystems and protecting wetlands will prevent excess phosphorus from entering water. Land and water restoration projects can also play a crucial role in controlling phosphorus from entering streams and lakes where phosphorus rich sediment has accumulated in mill ponds behind dams constructed since the late 1800's (**Fig. 7**).


Fig. 5


Fig. 6

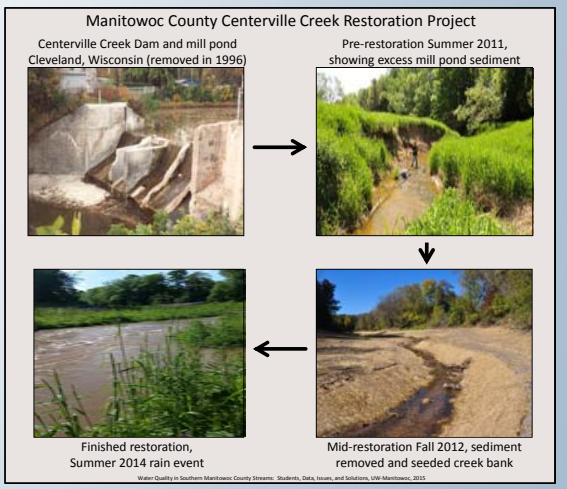


Fig. 7

Conclusion:

All living things depend on phosphorus, but when excess phosphorus enters surface waters, large toxin-producing cyanobacteria blooms may occur. The cyanobacteria species *M. aeruginosa* produce microcystin (MC) toxins that affect the health of animals. Exposure to MC toxin inhibits protein phosphatase-1; an enzyme essential to liver cell homeostasis. This inhibition can cause cell damage, hemorrhaging, and promote tumor growth. Excess phosphorus in runoff can be controlled with conservation practices and help to prevent toxic cyanobacteria blooms, reducing liver intoxication by MC toxin.

References:
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Elke Dittmann, Claudia Wiegand (2006) Cyanobacterial toxins-occurrence, biosynthesis, and impact on human affairs. Mol. Nutr. Food Res. **50:** 7-17
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Fig. 4. Water Quality in Southern Manitowoc County Streams: Students, Data, Issues, and Solutions, UW-Manitowoc, 2015

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