The Regulatory Mechanisms underlying the Action of Cyanotoxin β-N-Methylamino-L-Alanine (BMAA) on the Key Cellular Processes in Diazotrophic Cyanobacteria

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HIGHLIGHTS

- A remarkable regulatory effect of β-N-methylamino-L-alanine (BMAA) on *Anabaena* (*Nostoc) sp*. PCC 7120 proteome expression was detected during the heterocyst formation stage under nitrogen starvation conditions.
- The action of BMAA in nitrogen-fixing filamentous cyanobacteria leads to changes in nitrogen-carbon balance regulation that differs under nitrogen starvation and in nitrogen-replete conditions.
- BMAA downregulates PII, a key nitrogen regulatory protein, and downshifts glutamyl-tRNA synthetase (gltX) and proteins that are involved in nitrogen metabolism and heterocyst formation.
- BMAA disturbs carbon fixation, carbon dioxide concentrating mechanism, photosynthesis, and amino acid metabolism.
- Stress response proteins and DNA repair enzymes are upregulated in the presence of BMAA, clearly indicating severe intracellular stress.

Keywords: *Anabaena*; BMAA; cyanobacteria; cyanotoxin; nitrogen metabolism; heterocyst differentiation; amino acid metabolism; protein PII (GlnB); nodM; glutamate metabolism; gltX; NtcA; protein synthesis; carbon fixation; photosystems; oxidative stress; DNA repair; RecA; SecY

Abstract. In order to supply all cells in cyanobacterial filaments with nitrogen in nitrogen-free growth condition cyanobacteria use mature heterocysts (Figure 1) that are known as specialized nitrogen fixing cells that convert atmospheric nitrogen into bioavailable nitrogen and transport it into neighbor vegetative cells; while vegetative cells provide heterocysts with carbon and reductants, which are essentially required for the energy consuming heterocyst functioning process (Figure 2) [1].

Non-proteinogenic neurotoxic amino acid β-N-methylamino-L-alanine (BMAA) (Figure 3) is a bioactive molecule synthesized by various phytoplankton species, such as: cyanobacteria, diatoms and dinoflagellates, and is known to be a causative agent of human neurodegeneration diseases. The ability of different microalgae to synthesize BMAA may be an indicator of the importance of this molecule in phytoplankton organisms' interactions in nature. We were interested in the question: what kind of mechanisms underlie BMAA's action in cyanobacterial cells under different nitrogen supply conditions. To answer this question we have performed molecular studies using a model cyanobacterial strain *Nostoc* (*Anabaena*) sp. PCC 7120. The action of BMAA on nitrogen-fixing filamentous cyanobacteria leads to changes in nitrogen-carbon balance regulation that differs under nitrogen starvation and in nitrogen-replete conditions. The primary main targets of BMAA's action in cyanobacteria cells are, apparently, metabolic processes, such as nitrogen fixation [2-5], photosynthesis, carbon fixation and different biosynthetic processes, the regulation of which involves 2-oxyglutarate and glutamate and proteins, whose gene transcription is under NtcA regulation (Figure 4).

Our proteomic studies have demonstrated that under BMAA-treatment the most significant difference lies in the expression change of a key nitrogen regulatory protein PII (Figure 5). This protein is downregulated in nitrogen-starving conditions and it is upregulated in nitrogen-replete conditions in the presence of BMAA [5,6]. This could be the main reason behind a specific regulatory effect on heterocyst formation and an abnormal heterocyst- and nitrogenase-related gene expression that is caused by this amino acid in *Nostoc* sp. PCC 7120 [3,4]. Due to the fact that all metabolic processes are interconnected and well balanced in cyanobacteria cells, the disturbance in nitrogen metabolism leads to changes in carbon metabolism and photosynthesis. This could explain the severe changes in the CO_2 fixation protein expression and photosystem reaction centre protein expression that were found in our proteomics studies. BMAA addition leads to disorder in both amino acid synthesis and in purine synthesis, as well as disturbs DNA transcription and protein translation. Finally, many enzymes of oxidative stress, chaperones and SOS-response proteins were noticed to be upregulated under such metabolic stress conditions. Therefore we can conclude that the noticed disorder in many metabolic pathways leads to severe intracellular stress that induces the upregulation of stress-activated proteins, such as: starvation-inducible DNA-binding protein, stress-response enzymes, proteases and SOS-response and DNA repair enzymes. It can be hypothesized that BMAA can be used by phytoplankton representatives (cyanobacteria, diatom, dinoflagelates) as a possible allelopatic tool to control cyanobacteria cell populations during their competition for nitrogen and other resources.

CONCLUSION: BMAA addition affects the regulation of the key nitrogen regulatory protein PII, as well as some of its protein partners, which are involved in nitrogen and carbon metabolisms in cyanobacterial cells.

Filamentous cyanobacterium Anabaena (*Nostoc*) *sp. PCC 7120*

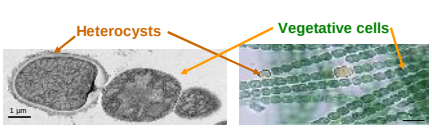

Fig.1. Two types of cells in cyanobacterial filaments: heterocysts and vegetative cells.

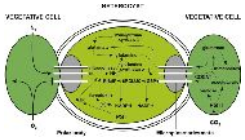

Fig.2. Heterocysts are specialized cells for nitrogen fixation [1].

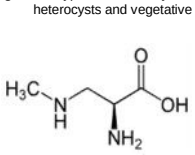

Fig.3. Non-proteinogenic neurotoxic amino acid β-N-methylamino-L-alanine (BMAA).

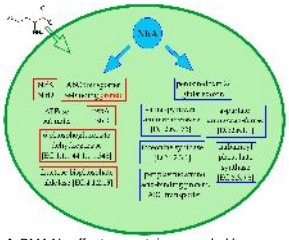

Fig.4. BMAA's effect on proteins encoded by genes that are under transcription control of the NtcA global regulator. Downregulated proteins are highlighted with a red frame and upregulated proteins are highlighted with a blue frame.

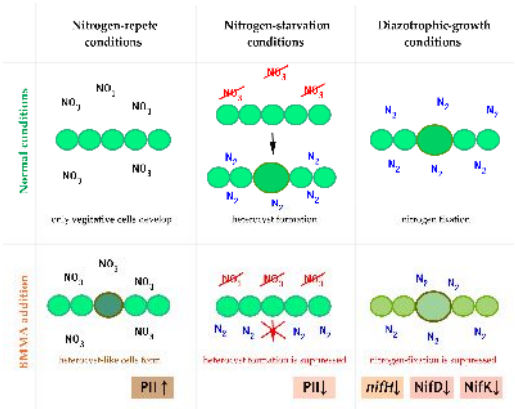

Fig. 5 . Nitrogen-fixing filamentous cyanobacteria *Nostoc* sp. in its normal state and after BMAA treatment under different nitrogen conditions.

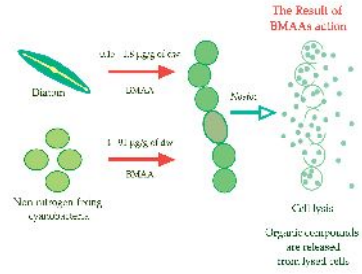

Fig.6. BMAA may be used by phytoplankton species as a possible allelopatic tool for control diazotrophic cyanobacteria cell populations during their strong competition for nitrogen and other nutrients. *Nostoc* cells undergo lysis under the presence of exogenous BMAA (in relatively high amounts) and as a result: dissolved organic compounds that are necessary for the algae community nutrition are released.

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