Thesis of the PhD dissertation

Investigation of sulfide and arsenic based alternative metabolic pathways in the cyanobacterium *Synechocystis* sp. PCC6803

Csaba István Nagy

Supervisors:

Dr. Imre Vass, Scientific Adviser, director of the Institute of Plant Biology

Dr. Péter Kós, Senior Research Associate, Institute of Plant Biology

Doctoral School of Biology, University of Szeged, Faculty of Science and Informatics

Hungarian Academy of Sciences, Biological Research Centre, Institute of Plant Biology

Szeged

2014

Introduction

Since the beginnings of life on Earth the reduced forms of several chemical elements like arsenic or sulfur were serving as electron source for different prokaryotes to sustain their metabolism and energy need. Those phototrophic organisms which were relying on sulfide to meet their ATP need where using a sulfide-quinone oxidoreductase (SQR), intermediate enzymes and PSI-type photosynthetic system. Other microorganisms, that utilized arsenite as electron donor or arsenate as electron acceptors, where using arsenite oxidase and arsenate reductase enzymes respectively to sustain their growth requirements.

Sulfide-quinone oxidoreductases are specific for several green and purple sulfur and non-sulfur bacteria, thus the discovery by Cohen *et al.* in the 1970s that some cyanobacteria can turn to an ancient bacterial-type photosynthesis using sulfide-quinone oxidoreductases was rather exceptional. Since then it has been shown, that SQR enzymes are spread in most living organisms. The same is true for arsenite oxidases: the finding that several proteobacteria can use arsenic via arsenite oxidases was considered an extraordinary discovery but further investigations where pointing out, that these are actually ancient, pre-LUCA enzymes.

Nevertheless under recent aerobic conditions both sulfide and arsenite are highly toxic compounds for most living organisms, though some evolved efficient resistance and tolerance mechanisms to cope with their toxicity. In the presence of sulfide the PSII reaction center is inhibited in cyanobacteria, but some of them can shift to an alternative photosynthetic pathway by means of a specific SQR to overcome the inhibitory effect of sulfide and to proceed with photosynthesis under anaerobic conditions. Although the biogeochemistry of the two environmentally hazardous compounds arsenic and sulfide has been extensively investigated, the biological interference of these two toxic but potentially energy-rich compounds has only been hypothesized and indirectly proven.

Here we provide direct evidence for the first time that in the photosynthetic model organism *Synechocystis* the two metabolic pathways are linked by co-regulated genes that are involved in arsenic transport, sulfide oxidation and in sulfide-based alternative photosynthesis.

We discovered that in the genome of *Synechocystis* an operon is present that contains several genes, which may be involved in anaerobic photosynthesis/metabolism based on sulfide and/or arsenic as energy source. This operon is localized on the pSYSM plasmid and contains

the following genes according to Cyanobase: sll5035, sll5036, slr5037 and slr5038. Because sll5036 encodes an SQR enzyme we presumed, that the genes are involved in **su**lfide **o**xidation, therefore we designated them as *suoR*, *souS*, *suoC* and *suoT* respectively. The protein product of *suoR* shows high homology with bacterial transcriptional regulatory proteins of the ArsR family that are normally involved in arsenic resistance. *SuoS* is annotated as a sulfide-quinone oxidoreductase that may provide electrons from hydrogen sulfide into the photosynthetic electron transport chain serving as an alternative photosynthetic pathway. The *suoC* gene is a highly conserved gene and the protein encoded by this belongs to the DUF302 superfamily, although without an assigned function. The protein product of the *suoT* gene shows high homology with transmembrane proteins involved in heavy metal export/transport and is annotated as a chromate transporter.

Under normal conditions *Synechocystis* uses water as the electron donor for photosynthesis, so these genes may represent the remnants of an ancient metabolism. The clarification of the role, functionality and interaction of these sulfide- and arsenic based enzymes and metabolic pathways, provides information to the deeper understanding of the genomic plasticity, adaptation and the evolution of cyanobacteria and paves the way towards new perspectives in paleoecological and astrobiological studies as well.

Aims of the study

Because usually cyanobacteria do not thrive in anaerobic sulfidic habitats and do not rely on sulfide or arsenite as energy source for their growth, we aimed to investigate the functionality, role and origin of the *suoRSCT* genes.

Our detailed goals were as follows:

- Since the *suoRSCT* genes presumably encode ancient anaerobic metabolic pathways, we wanted to investigate, whether these genes are active, and to what kind of environmental stimuli do they respond?

- Because SuoS shows high homology with active SQR enzymes we aimed to investigate the activity of the SuoS and whether the cyanobacterium can shift to alternative photosynthetic pathway in sulfidic environment?
- We intended to clarify how the *suoS* gene is regulated since cyanobacteria do not thrive normally in sulfidic habitats and because of the lack of knowledge about how SQR enzymes are regulated.
- We wanted to investigate what is the relation of the *suo* operon with heavy metals and arsenic and to clarify the transporter role of the SuoT protein.
- Because cyanobacteria perform oxygenic phtotosynthesis do not rely usually on anaerobic metabolic processes, therefore we wanted to find out, what could be the origin and the evolutionary advantage of the *suo* operon?

Materials and methods

- Cyanobacterial cell cultures
- Recombinant DNA techniques, cloning procedures, PCR techniques
- Construction of mutant strains
- Heterologous protein expression and –purification by affinity chromatography
- RNA isolation, gene expression measurements by qRT-PCR
- Gas chromatography
- Anaerobic spectrophotometry
- Monitoring of cell growth *via* microplate reader
- DNA-protein interaction studies by electrophoretic mobility shift assays (EMSA)
- Inductively coupled plasma mass spectrometry (ICP-MS)
- Protein 3D modeling (PyMol, YASARA), DNA and protein sequence analysis

Results and discussion

By analyzing the expression of the *suoS* and *suoT* genes, we demonstrated that the *suoRSCT* operon is active, since the genes exhibit an approximately 200 fold induction in the presence of specific inductors. The operon responds only to the reduced forms of sulfur and arsenic not the oxidized forms and neither to a series of heavy metals studied. Both hydrogen sulfide and arsenite are used as electron donors by different prokaryotes.

We proved that SuoS is an active type-I SQR: the oxidative half reaction of SuoS has been determined by gas chromatography while the reductive part of the reaction by anaerobic spectrophotometry. Besides this we showed that SuoS is only functional in the presence of light, thus its function is to provide electrons from sulfide into the photosynthetic electron transport *via* the plastoquinone pool.

The *suo* operon is a negatively controlled inducible operon regulated by the SuoR repressor. However it is an interesting fact that an SQR enzyme is regulated by an ArsR-type protein normally involved in arsenic resistance. We performed an EMSA experiment in order to gain a more direct evidence of the arsenic dependent regulation of the operon. We proved that under normal circumstances the operon is repressed by SuoR and we could also demonstrate the arsenite dependent de-repression of the *suo* genes. Thus we conclude that the product of the *suoR* gene encodes an arsenite dependent repressor of the *suoRSCT* operon. This protein binds to the promoter region, repressing the operon whereas it dissociates in the presence of As(III) allowing the expression of the genes.

Since ArsR, the regulator of the well described arsenic resistance operon (*arsBHC*) of *Synechocystis* and SuoR belong to the same protein family, we tested the affinity of SuoR

towards the promoter sequence of the *arsBHC* operon via an EMSA experiment. We showed that SuoR has no affinity toward the promoter sequence of *arsBHC* but binds solely its specific *suoRSCT* promoter region, hence the *suoRSCT* operon is not playing role in arsenic resistance.

Although SuoT is annotated as a chromate transporter it does not responds to chromate compounds, rather to arsenite. Furthermore according to our ICP-MS measurements SuoT is a transporter protein implicated in arsenite uptake. Although arsenite is not used as an energy source under natural oxygenic photosynthetic conditions, this importer is still functional. This type of arsenic uptake system is characteristic presumably for microorganisms, that thrive in arsenic-rich habitats and which are able to use arsenite as electron donor or arsenate as electron acceptor for their metabolism.

The DNA region of the *suoRSCT* operon shows the characteristic structure of transposable genetic elements of type IS4. We identified a gene encoding a transposase downstream of *suoT* with conserved domain structures characteristic to the DDE superfamily transposases. We also found a pair of 20 bp imperfect inverted repeats delimiting the *suo* operon. Because *Synechocystis* is naturally competent for transformation and conjugation and the presence of 127 putative transposase sequences in *Synechocystis* strongly suggests that this organism has been involved in horizontal gene transfer. Thus we conclude that this operon was acquired *via* horizontal gene transfer originated from microorganisms thriving in sulfide/arsenic-rich habitats.

Synechocystis may have benefits conveyed by these functional genes. Geochemical events like volcanic eruptions or other cataclysms may result in temporal exposure to both arsenic and sulfide. As sulfide inhibits the activity of PSII, the SQR enzyme may provide

advantages, either providing electrons to the photosynthetic electron transport chain or converting sulfide to less toxic substances.

Under normal conditions *Synechocystis* uses water as the electron donor for photosynthesis, so these genes may represent the remnants of an ancient metabolism or a feature acquired from some bacteria with no functional PSII, and they may have retained their activities due to their utility in sulfide and arsenite detoxification. The functionality of the *suo* genes indicates the presence of some selection pressure even in recent times and points to the possibility that when it grows in nature *Synechocystis* can experience environmental conditions under which the function of the *suo* operon is beneficial.

Conclusions

- The *suoRSCT* operon is active; the genes exhibit an approximately 200 fold induction in the presence of both hydrogen sulfide and arsenite that are used as electron donors by different prokaryotes.
- SuoS is an active type-I SQR: the oxidative half reaction of SuoS has been determined by gas chromatography while the reductive part of the reaction by anaerobic spectrophotometry; its function is to provide electrons from sulfide into the photosynthetic electron transport chain *via* the plastoquinone pool.
- The *suo* operon is a negatively controlled inducible operon regulated by the SuoR repressor, a protein belonging to the ArsR family. These regulatory proteins are normally involved in arsenic resistance.

- According to our ICP-MS measurements SuoT is a transporter protein implicated in arsenite uptake. Presumably, this type of arsenic uptake systems are characteristic for microorganisms, that thrive in arsenic-rich habitats and which are able to use arsenite as electron donor or arsenate as electron acceptor for their metabolism.
- The DNA region of the *suoRSCT* operon shows the characteristic structure of transposable genetic elements of type IS4; we conclude that this operon was acquired *via* horizontal gene transfer originated from microorganisms thriving in sulfide/arsenic-rich habitats.

Publications

The thesis was based on the following:

Nagy, Cs. I., Vass, I., Rákhely, G., Vass, I. Z., Tóth, A., Duzs, A., Peca, L., Kruk, J., Kós, P. B., 2014: Co-regulated genes link sulfide:quinone oxidoreductase and arsenic metabolism in *Synechocystis* sp. PCC6803. 196(19):3430-40, *J Bacteriol* IF: 3.298

Loredana Peca, Csaba István Nagy, Attila Ördög, Imre Vass, Péter B. Kós, 2014: Development of a bioluminescent cyanobacterial reporter strain for detection of arsenite, arsenate, and antimonite. *Environmental Engineering and Management Journal*. (Manuscript in press) IF: 1.258

Other publications:

Vass, I. Z., Kós, P. B., Sass, L., Nagy, Cs. I., Vass I., 2013: The Ability of cyanobacterial cells to restore UV-B radiation induced damage to photosystem II is influenced by photolyase dependent DNA repair. 89(2):384-90. *Photochem Photobiol*. IF: 2.684

Nagy, Cs. I., Lupan I., Ferencz B., Popescu O., 2007: Cloning and expression of the gene encoding phosphoketolase in *Pseudomonas aeruginosa* 15442, Annals of West University of Timişoara – Series of Chemistry 16 (3) 73-80.

Nagy, Cs. I., Ferencz B., Lupan I., Popescu O., 2007: Cloning of the gene for phosphoketolase in *Synechocystis* sp. PCC6803. Anal. Soc. Naţ. Biol. Cel. 11.

Oral Presentations

Csaba István Nagy, Péter B. Kós, István Z. Vass, Imre Vass (oral presentation): A novel plasmid-borne arsenite and antimonite responsive operon in *Synechocystis* sp. PCC6803. 14th International Symposium on Phototrophic Prokaryotes, Porto, Portugal, August 5-10, 2012.

Csaba I. Nagy, Imre Vass, Gábor Rákhely, István Zoltán Vass, András Tóth, Ágnes Duzs, Loredana Peca, Péter B. Kós (oral presentation): Co-regulated sulfide- and arsenic responsive genes represent relics of an ancient anaerobic metabolism in *Synechocystis* sp. PCC6803. 15th Biology Days (Conference), Cluj-Napoca, Romania, 2014. April 04-06.

Csaba I. Nagy, Imre Vass, Gábor Rákhely, István Zoltán Vass, András Tóth, Ágnes Duzs, Loredana Peca, Peter B. Kós (oral presentation): Functional link between sulfide- and arsenic metabolism in cyanobacteria. Straub-Days, Biological Research Centre, Szeged, Hungary, May 28-29, 2014.

Csaba I. Nagy, Imre Vass, Gábor Rákhely, István Zoltán Vass, András Tóth, Ágnes Duzs, Péter B. Kós (oral presentation): Co-regulated genes link sulfide:quinone oxidoreductase and arsenic metabolism in *Synechocystis* sp. PCC6803. 9th European Workshop on the Molecular Biology of Cyanobacteria. Texel, The Netherlands, 7-11 September, 2014.

Abstracts and Posters

Nagy, Cs. I., Lupan I., Ferencz B., Popescu O., 2007: Cloning and expression of the gene encoding phosphoketolase in *Pseudomonas aeruginosa* 15442. The Annual International Conference of the Romanian Society of Biochemistry and Molecular Biology, Timişoara, Romania, 2007.

Csaba István Nagy, Péter B. Kós, Loredana Peca, Imre Vass: A novel arsenic responsive operon in the cyanobacterium *Synechocystis* sp. strain PCC 6803. 8th European Workshop on Molecular Biology of Cyanobacteria, Naantali, Finland, August 28 – September 1, 2011.

Csaba István Nagy, Imre Vass, Péter B. Kós: Genetic and functional analysis of a sulfide:quinone oxidoreductase enzyme in *Synechocystis* sp. PCC6803. 4th Central European Forum for Microbiology, Keszthely, Hungary, October 16-18, 2013.