Expression of the genes encoding the subunits of the Photosystem II core and the Hox hydrogenase are aiding acclimation of the cyanobacterium *Acaryochloris marina*

Ph.D. thesis

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Introduction

The cyanobacterial hydrogenases are becoming of great importance in the aim of developing new technologies for gaining renewable and clean fuel. One of the biotechnological approaches would use the bidirectional hydrogenase (Hox) that can be directly or indirectly connected to the photosynthetic electron transport. The Hox enzyme by using the reducing power derived from photophosphorylation could convert solar energy into hydrogen that could be used for a wide range of industrial purposes. Herein we studied the expression of the genes encoding the core of the Photosystem II (PSII) and the Hox, the two key enzymes involved in the hydrogen evolution based on water splitting in the marine cyanobacterium, *Acaryochloris marina*.

The regulation of this NiFe-type bidirectional enzyme is well studied in the fresh water cyanobacterium *Synechocystis* PCC 6803. The genes of the bidirectional hydrogenase in this model organism are encoded by the *hoxEFUYH* operon under the regulation of the promoter region upstream of *hoxE*. The arrangement of the *hox* genes in *Acaryochloris marina* is analogous to that in *Synechocystis* PCC 6803, although the *hox* cluster is found on the chromosome in the case of *Synechocystis* PCC 6803, and encoded in plasmid DNA in the case of *Acaryochloris marina*.

The protein surroundings of the Mn_4CaO_5 cluster in the Photosystem II are highly prone to photodamage due to the strongly oxidative chemistry of the water splitting. The D1 subunit of Photosystem II is directly and indirectly involved in the water oxidation with one of its tyrosine residues and by the ligation of the Mn_4CaO_5 cluster, respectively; hence the D1 subunit of the core heterodimer is primarily exposed to photodamage. All of the oxygenic photosynthetic organisms developed a photoprotection mechanism that involves the regular and specific degradation and resynthesis of the D1 subunit

without the further disassembly of the complex.

Cyanobacteria are among the most adaptive photosynthetic organisms on Earth. They developed several methods for protecting their photosynthetic apparatus. One of these mechanisms involves several homologues of *psbA* genes encoding the D1 subunit of the Photosystem II. The principal advantage of these *psbA* gene duplications is to provide an extended transcript pool for the enhanced synthesis of the D1 protein. Some of these *psbA* gene duplications went through mutations, and their products have altered primary structure. Various isoforms of D1 with different redox properties can be found within one cyanobacterial cell. By switching between the different isoforms under varying conditions cyanobacteria can optimize their photosynthesis during acclimation. Some of the strains also contain two *psbD* genes that code for identical D2 proteins of the core heterodimer. The cytochrome b559 is also a key component of the PSII core. It consists of two subunits which has been known to form an α-β heterodimer and are encoded the *psbE* and *psbF* genes. Only one copy of each of the *psbE* and *psbF* genes can be found per genome in the photosynthetic organisms studied up to date.

Acaryochloris marina is a highly adaptive cyanobacterium, which can live under largely different environmental conditions. Its main known habitat is at the underside of ascidians where only far red light penetrates. This cianobacterium is uniquely adapted to the accessible light of long wavelengths by using chlorophyll *d* as a main pigment. However it can be cultured in laboratory environment under visible light, and was shown to perform various responses of chromatic photoacclimation when adapting to different qualities and quantities of light. *Acaryochloris marina* has a considerably large, 8.36 Mb genome containing 8462 genes of which 2129 are located on 9 plasmids. Its extended genome with numerous plasmids was previously proposed to be

connected with its dynamic niche. Its genome project revealed the presence of three *psbA* as well as 3 *psbD* and 2 *psbE* genes on its chromosome. As a unique feature, its three *psbD* genes and 2 *psbE* genes are encoding 2-2 different D2 and PSII-E subunits. respectively. Having several homologues encoding for the PSII core subunits is also likely to be part of its gene arsenal that has been developed during evolution in order to aid adaptation.

Aims of the study

1. Describing the physiological properties of *Acaryochloris marina* during acclimation to various conditions that are similar to the natural environment (extreme low intensities and far red light) concerning mainly the changes in photosynthetic electron transport and Photosystem II functioning.

2. Identifying the changes in the relative transcript levels of the homologous genes encoding the D1/D2/cytochrome b559 core of the Photosystem II reaction centers during acclimation of *Acaryochloris marina* .

3. Identifying those factors that affect the expression of *hox* genes encoding the bidirectional hydrogenase in the unicellular cyanobacteria: *Synechocystis* PCC 6803, *Synechococcus elongatus* PCC 7942 and *Acaryochloris marina*.

Materials and methods

- **Culturing and treatment conditions**

Synechocystis sp. PCC 6803, *Synechococcus elongatus* PCC 7942 and *Acaryochloris marina* cells were propagated under 3% CO_2 enriched atmosphere in BG-11 at 30°C, at 40 μE $m^{-2}s^{-1}$ and in K+ESM medium at 25°C, under 10 μE$m^{-2}s^{-1}$ PAR respectively. Cells in the exponential growth phase (~8 μgmL^{-1} chlorophyll) were used in all experiments.

For high intensity light stress and for extreme low light conditions 100 μmol m^{-2} s^{-1} and 1 μmol m^{-2} s^{-1} visible light were used, respectively. UV-B radiation, with a maximum emission at 312 nm yielding an intensity of 7 μmol m^{-2} s^{-1} was supplemented with 8 μmol m^{-2} s^{-1} intensity visible light. The intensity of the 720 nm wavelength far red light was 0.18 μmol m^{-2} s^{-1} (110 μW). Microaerobic conditions were achieved enzymatically by oxygen scavenging enzyme mix containing 5 mM glucose, 200 U glucose oxidase and 2000 U catalase. For photosynthetic electron transport inhibition at the level of the cytochrome b_6f complex and Photosystem II 20 μM dibromothymoquinone and 10 μM 3-(3,4-Dichlorophenyl)-1, 1-dimethylurea was used, respectively.

- **Determining the chlorophyll content**

For determining the chlorophyll content the absorption of ethanolic pigment extraction of the cultures were measured. For chlorophyll *a* and *d* the (OD_{665}·11.978)-(OD_{696}·2.3238) and the (OD_{696}·12.0995)-(OD_{665}·0.2006) equations were used, respectively.

- **Gene expression assessment**

The gene expression levels were determined by quantitative PCR (Q-PCR) using cDNA prepared from total RNA by the ’hot-phenol’ method. In each gene expression experiment the expression level of *rnpB* that encodes the β subunit of RNase P was determined, as an internal control. For all calculations of the relative expression levels the GED (Gene Expression’s C_T Difference) formula was used.

- **Photosynthetic activity assessment**

Photosynthetic activity was assessed via following oxygen evolution using a Clark-type electrode in the presence of 0.5 mM 2,6-dimethylbenzoquinone and 1 mM potassium ferricyanide as an artificial electron acceptor system.

- **Fluorescence measurements**

The chlorophyll fluorescence (OJIP) transient was measured by a fast fluorimeter in the 10 μs to 1 s time region using a logarithmic time scale. Samples were dark incubated for three minutes before the measurements.

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- ***In silico* protein analyses**

The amino acid sequences were downloaded from the Cyanobase, and the NCBI protein database. The multiple alignments of the amino acid sequences were carried out by the CLUSTALW software. The phylogram was created on the bases of the ‘maximum likelyhood’ method using the online PhyML software. The graphical editing of was done by the Dendroscope online software package.

Results

The *psbA2*, *psbA3*, as well as the *psbD1*, *psbD2* genes are expressed to a relatively high level under control conditions. Therefore, their products are considered as the abundant isoforms of the core heterodimer and named $D1_m$, and $D2_m$ (m=major), respectively. The α subunit (PSII-E) of the cytochrome b559 is also encoded by two homologous genes encoding PSII-E proteins with various amino acid sequences. Those gene products that are insignificantly expressed under control conditions and have different primary structures compared to the abundant copies are indicated with an apostrophe (D1', D2', PSII-E').

1. *Acaryochloris marina* shows intensive dark aerobic respiratory oxidase activity that drives the oxidation of the plastoquinone pool (PQ). Far red light is more accessible for Photosystem I (PSII), than for PSII. Therefore there are stronger oxidizing forces on the PQ towards Photosystem I. The more oxidized PQ under far red light shows the unbalanced excitation of reaction centers that is being compensated by an increased amount of functional Photosystem II complexes, as well as by a decrease in the Photosystem II-attached phycobiliprotein content. Under extreme low light intensities the ratio of the oxidized quinone and reduced quinole molecules also increases in the pool. For acclimation to the poor availability of accessible light cells are using their chlorophyll *d* containing inner Pcb antennas that is shown by a red shift in the absorption peak of chlorophyll *d.*

2. Under control conditions the abundant transcripts are the *psbA2*, *psbA3* and *psbD1*, *psbD2* encoding for the $D1_m$ and $D2_m$ isoforms of the core heterodimer of Photosystem II. High intensity light stress and UV-B radiation induces the *psbA2*, *psbA3* and *psbD2* genes. The relative expression levels of the *psbA1* and

psbD3 genes encoding isoforms with divergent sequences are negligible under these conditions.

3. The *psbD3* gene encoding the divergent D2' isoform is induced by far red light and low light intensities, implying a role of this isoform in the acclimation to these light conditions.

4. Blocking the electron transport at the Q_o site of the cytochrome b_6/f by dibromothymoquinone inhibits the high light induced accumulation of the *psbA2*, *psbA3* and *psbD2* transcripts encoding $D1_m$ and $D2_m$ isoforms. However the relative transcript level of the *psbA1* gene encoding the divergent D1' isoform significantly increases.

5. The genome of *Acaryochloris marina* contains two genes (*psbE1* and *psbE2*) encoding the α subunit of the cytochrome b559. The *psbE1* gene shows two orders of magnitude lower expression compared to the expression level of *psbE2* and also to the abundant transcripts encoding the $D1_m$ and $D2_m$. The β subunit of cytochrome b559 is encoded by the *psbF* gene that is found downstream of *psbE1* and most likely under the transcriptional regulation of the promoter region of the latter gene. As a consequence the relative transcript level of the β subunit of cytochrome b559 is also two orders of magnitude lower compared to the transcript level of the α subunit.

6. The expression of the *hox* operon encoding the bidirectional hydrogenase of *Synechocystis* PCC 6803 is regulated by oxygen and photosynthetic electron transport. The *hox* genes of *Acaryochloris marina* are induced by anoxia, darkness, low light intensities and far red light illumination.

Publications used for the thesis:

É. Kiss, P. B. Kós, I. Vass, (2009) Transcriptional regulation of the bidirectional hydrogenase in the cyanobacterium *Synechocystis* 6803 J Biotechnol 142 (1):31-7

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