ADAPTATION OF CYANOBACTERIAL PHOTOSYSTEM II FUNCTION TO LIGHT AND DARK PERIODS

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Summary: Due to strongly oxidative chemistry of photosystem II (PSII) water splitting, the D1 protein is prone to constant photodamage requiring its replacement in order to regain PSII function. Cyanobacteria developed multiple strategies to cope with this function loss including the development of a small gene family encoding the D1 protein subunit. There is ample studies dealing with the adaptation of PSII function to different light regimes but not much is known about the ability of these photosynthetic organisms to deal with the lack of photosynthetically active radiation. The main objective of our study was to investigate the changes photosystem II donor and acceptor side function during a 12/12 hours light and dark cycles in an effort to understand the intrinsic mechanisms of adaptation to these conditions. We used primarily the measurement of flash-induced chlorophyll fluorescence decay to investigate the function of cyanobacterial PSII. Using specific electron transport inhibitors we can measure the function of both donor and acceptor side of PSII. Our investigation showed significant differences in PSII function between dark and light periods, both on donor and acceptor side of the system. These differences are also dependent on the cyanobacterial species studied and relate probably to the type of habitat there organisms are adapted to. Our conclusion is that specific cyanobacteria use modification of PSII function during light and dark periods of time as a way to adapt to specific environmental conditions.

Introduction

The *psbA* gene family in *Cyanothece* sp. ATCC 51142 contains 5 *psbA* genes differentially expressed according to environmental conditions. From these 5 genes, it has been proved that one encodes a D1 sentinel (rogue) protein.The *psbA* gene family in *Synechocystis sp.* PCC 6803 contains 3 *psbA* genes. It has been shown that *psbA3* gene is UV-B responsive and is induced by oxidative stress. *PsbA2* is responsible for supplying D1 under normal growth, and the *psbA1* gene is induced by microaerobic, low oxygen conditions. *Anabaena Variabilis* ATCC 29413 has 6 psbA genes differentially expressed in different environmental conditions, while *Cyanothece* sp. PCC 8801 has 4 genes expressed differentially according to the environmental cues. Both *Anabaena Variabilis* ATCC 29413 and *Cyanothece* sp. PCC 8801 have a potential D1 sentinel (rogue) protein, which blocks the PSII during the night and allows fixation of nitrogen.

Materials and Methods

Cyanothece sp. ATCC 51142 and *Anabaena Variabilis* ATCC 29413 were obtained from Biological Research Institute from Cluj Napoca, Romania. *Synechocystis sp*. PCC6803 and *Cyanothece* sp. PCC 8801 were obtained from Pasteur Culture Collection.

The cyanobacterial strains were grown at 30ºC and 50µmol photons $m^{-2}s^{-1}$ light, until at a chlorophyll concentration of 6µg chl ml^{-1} .

In order to investigate the changes in PS II function during 12 h light/12 h dark cycles, we designed the following experiments:

We performed flash fluorescence measurements every 60 minutes during 26h, in absence and presence of DCMU (3-(3,4-Dichlorophenyl)-1,1-dimethylurea The fluorescence measurements were made with an FL3500 fluorometer from Photon Systems Instruments, we used a Q_A recombination protocol, than the results were processed in the Origin.8 program and for each sample was applied a Joliot Correction before graphics.

Results

Cyanothece sp. ATCC 51142

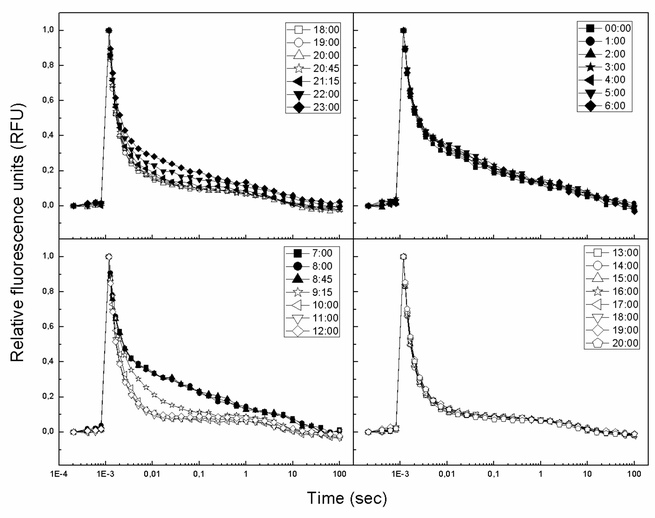

Figure 1: Changes of acceptor side of PS II function during a 12h Light/12h Dark cycle in *Cyanothece* sp. ATCC51142

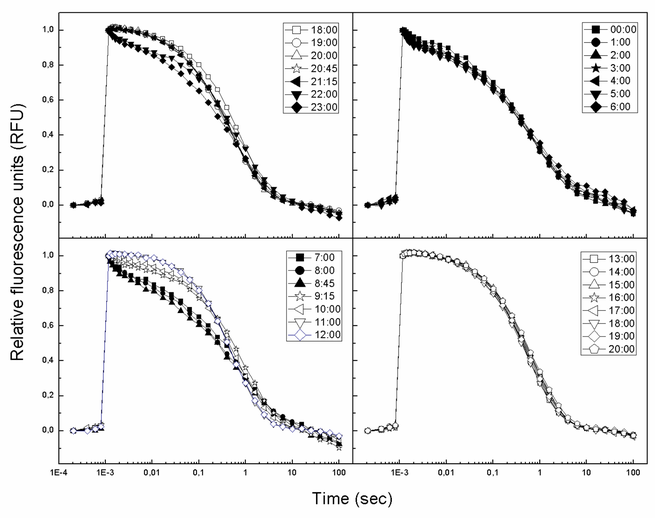

Figure 2: Changes of donor side of PS II function during a 12h Light/12h Dark cycle in *Cyanothece* sp. ATCC 51142

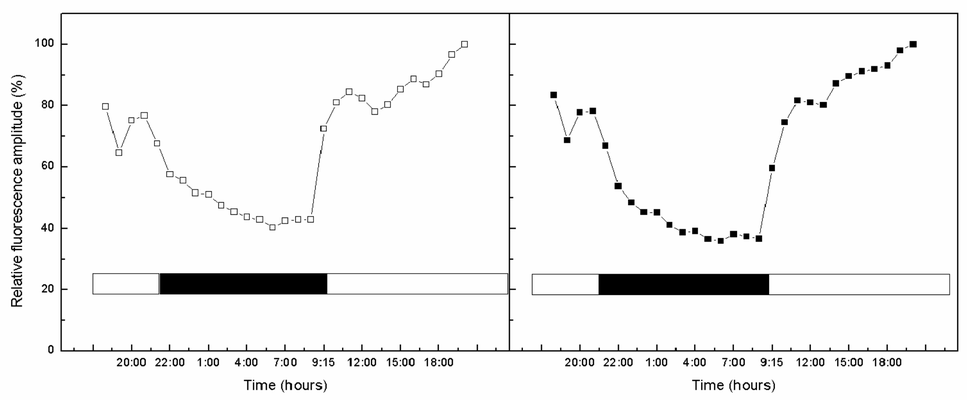

Figure 3: Changes in the number of PS II active centers during a 12h Light/12h Dark cycle in *Cyanothece* sp. ATCC51142 in absence (open square) and presence (solid square) of DCMU

Synechocystis sp. PCC 6803

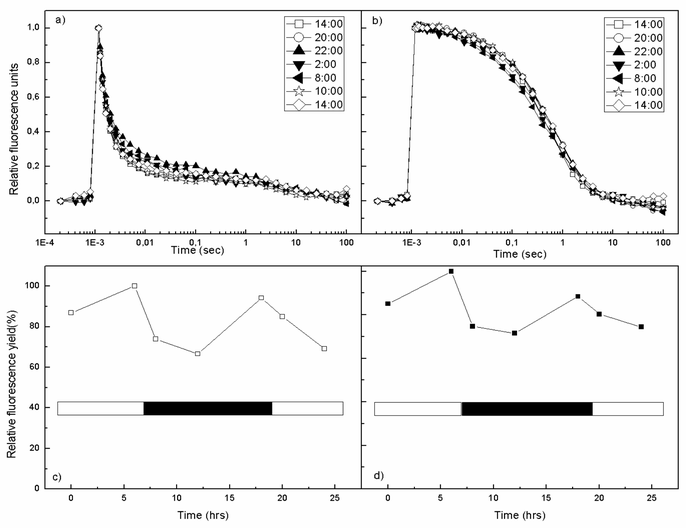

Figure 4: Changes of acceptor side of PS II function (panel a) and of the donor side of PS II function (panel b) during a 12h Light/12h Dark cycle in *Synechocystis* sp. PCC6803. Changes in the number of PS II active centers during a 12h Light/12h Dark cycle in *Synechocystis* sp. PCC6803 in absence (panel c) or presence (panel d) of DCMU.

Anabaena variabilis ATCC 29413

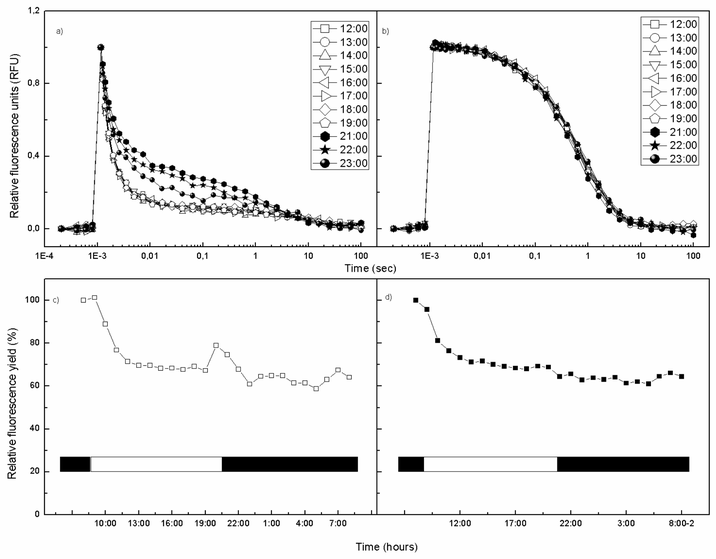

Figure 5: Changes of acceptor side of PS II function (panel a) and of the donor side of PS II function (panel b) during a 12h Light/12h Dark cycle in *Anabaena Variabilis* ATCC 29413 . Changes in the number of PS II active centers during a 12h Light/12h Dark cycle in *Anabaena Variabilis* ATCC 29413 in absence (panel c) or presence (panel d) of DCMU.

Cyanothece sp. PCC 8801

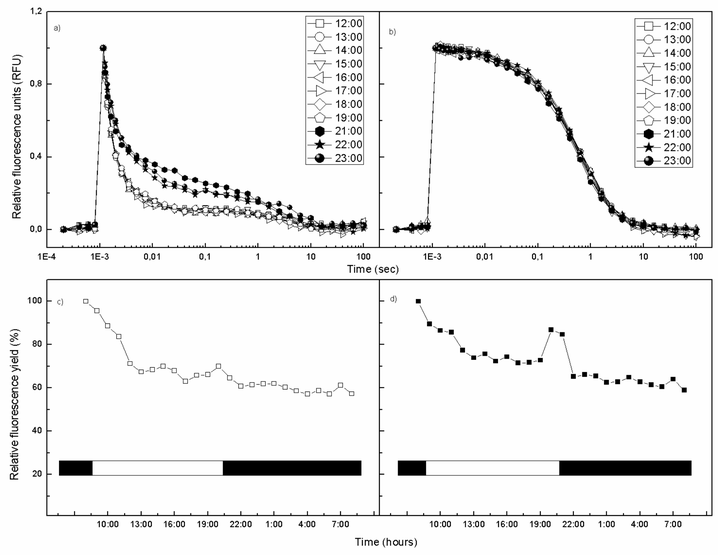

Figure 6: Changes of acceptor side of PS II function (panel a) and of the donor side of PS II function (panel b) during a 12h Light/12h Dark cycle in *Cyanothece* sp. PCC 8801 . Changes in the number of PS II active centers during a 12h Light/12h Dark cycle in *Cyanothece* sp. PCC 8801 in absence (panel c) or presence (panel d) of DCMU.

Results and Discussions

- ***Cyanothece* sp. ATCC 51142, Anabaena variabilis ATCC 29413 and Cyanothece sp. PCC 8801, species that have a potential D1 sentinel form show during the dark period the acceptor side of PS II modified by a slowdown of Q_A to Q_B transfers, that is most evident in centers that have an empty Q_B pocket at the time of the flash. This change is reversed fast, in about one hour after the light is turned on.**
- **The ON-set of a fast phase on the flash fluorescence decay curve in presence of DCMU during dark period suggests an inhibition of Water Oxidation Complex that is rapidly reversed during the light phase. This modification is only present in *Cyanothece* sp. ATCC 51142 and is not present in Anabaena variabilis ATCC 29413 and *Cyanothece* sp. PCC8801.**
- **During the dark period, in all D1 sentinel containing species, the amplitude of the flash fluorescence curve (F_M-F_0), proportional with number of PS II active centers is decreased. This suggests a temporary inhibition, during the night, of the active PS II centers. This effect is significantly reduced in Anabaena variabilis ATCC 29413 and *Cyanothece* sp. PCC8801.**
- ***Synechocystis* sp. PCC 6803, a strain that does not contain a D1 sentinel form, does not show significant modifications of the PSII donor and acceptor side function while the total number of active centers fluctuates around control values during the light and dark cycles.**

Conclusions

- **The strains of cyanobacteria studied show a variety of responses regarding PSII function in a dark and light cycle growth.**
- **All three species containing a putative D1 sentinel form, show drastic modification of the acceptor side of PSII, particularly in the middle part of the curve, representing the Q_A^- recombination in centers with an empty Q_B site at the time of the flash. This signifies a substantial inpairement of the PSII function durind the dark part of the cycle.**
- **While *Cyanothece* sp. ATCC 51142 displays modifications of the donor side of PSII as well the other two species Anabaena variabilis ATCC 29413 and *Cyanothece* sp. PCC 8801 are not showing this type of modification and we can conclude that there is a functional difference between these strains possible involving a particularly different form of D1 that requires further investigations.**
- ***Synechocystis* sp. PCC 6803 does not present any modification of PSII function both in the donor or acceptor side maintaining a fully functional PSII during the dark period.**

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