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UV-B stress changes the electron flow on photosystem II complex in *Synechococcus* PCC 7002

By

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UV-B stress changes the electron flow on photosystem II complex in *Synechococcus* PCC 7002

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ABSTRACT

Cyanobacterial photosynthesis is a vital process affected by fluctuating environmental parameters. To avoid accumulation of photodamaged photosystem II (PSII), the core D1 protein, undergo quick turnover during high excitation flux. Our study showed how UV-B radiation affects the function of PSII complex and the expression of D1 isoforms in *Synechococcus* PCC 7002. None of the D1 isoforms were sigificantly induced or accumulated under UV-B stress. Further, never before recorded changes in electron flow on the acceptor side of PSII complex resulting from an increased redox potential gap between Q_A and plastoquinone pool were identified under UV-B stress.

KEYWORDS

Cyanobacteria, photosystem, D1, *psbA*, isoforms, chlorophyll, fluorescence, UV-B, stress, qRT-PCR

ABBREVIATIONS

PSI- photosystem I, PSII- photosystem II, Q_A- first quinone acceptor in PSII, Q_B- second quinone acceptor in PSII, PQ-plastoquinone, F_{max}- maximum chlorophyll fluorescence, F_0- minimal chlorophyll fluorescence, TyrZ- tyrosine residue - secondary electron donor of PSII, P_{680}- PS II primary donor, DCMU- (3-(3,4-dichlorophenyl)-1,1-dimethyl urea), UV-B - ultraviolet radiation B, CO_2- carbon dioxide, qRT-PCR- quantitative reverse transcriptase polymerase chain reaction

INTRODUCTION

Cyanobacteria and higher plants have the ability to use water as electron source during the photosynthetic reduction of CO_2 to numerous organic compounds. These photosynthetic organisms take their energy from light and are able to perform oxygenic photosynthesis [1]. It is a highly complex process performed by pigment-protein complex located in thylakoid membranes inside cyanobacteria. The vital protein complex includes Photosystem I (PSI) and Photosystem II (PSII). The photolysis of water, catalyzed by PSII, results in generation of 4 protons and 4 electrons [2]. The electrons from the PSII donor side are transported to the acceptor side via different cofactors, many of them being associated with the core D1 protein [3].

In all known cyanobacteria, the D1 protein is encoded by the *psbA* gene family. *Synechococcus* sp. strain PCC 7002 is a unicellular halotolerant cyanobacterium [4]. It accommodates three *psbA* genes, *a1418* (PsbA1), *a0157* (PsbA2) and *a2164* (PsbA3), which encode two D1 isoforms. *a1418* and *a0157* are almost identical in sequence and encode the same D1 protein, while *a2164* encodes the so-called D1' protein. It exhibits specific changes in the amino acid compositions and the expression of these genes remained ambiguous. Nevertheless, the expression of the orthologous gene in *Synechocystis* PCC 6803 was reported under microoxic growth conditions [5, 6].

Numerous studies on D1 protein carried out so far showed that various environmental factors had a profound influence on its regulation [7,8,9,10,11,12,13,14,15]. Notably, light and UV-B radiations cause significant damages once the received flux of excitation energy overcomes the cells metabolic capacities [16, 17]. In some instances, low intensity visible light protect against UVB damage possibly by promoting the formation of intracellular structures [18]. Nowadays the release of various pollutants resulted in depletion of the ozone layer causing increased solar ultraviolet-B radiation (UV-B, 280–315 nm). Recent studies showed that approximately 1.5–2 Wm^{-2} of UV-B radiations reaches earth surface on a clear sky day [19]. Unfortunately, studies related to UV-B stress effects in *Synechococcus* PCC 7002 is very limited, thus we focused our aim to assess the expression patterns of the *psbA* genes and their relative contribution to overall functionality of PSII complex, under UV-B stress in the cyanobacterium *Synechococcus* sp. PCC 7002.

RESULTS AND DISCUSSIONS

Photosynthesis is an important energy dependent process driven by solar energy. Harvesting of light exposes cyanobacteria simultaneously to harmful doses of UV-B radiation in their natural habitat [20]. The UV-B exposure dramatically reduce the maximal quantum efficiency of PSII, caused due to the degradation of D1 and D2 protein subunits, which forms the heart of the PSII reaction center [21].

We comparatively analyzed the expression levels the two D1 protein isoforms encoded by psbA genes from *Synechococcus* PCC 7002 using real-time qRT-PCR technique. The cells were exposed to naturally relevant UV-B radiation stress for 60 minutes followed by a recovery period in growing light intensity for 60 minutes. Our results show a significant difference between the relative amounts of the two D1 protein isoforms. In all samples D1 was clearly dominant, contributing more than 99.9% percent of the total transcript pool (Table 1). *a2164* gene is slightly induced under UV-B stress -1.8-fold after 60 minutes of UV-B stress, whereas the expression of other pool of D1 proteins demonstrated a 0.5-fold down-regulation (Fig 1). These changes are small and while statistically sustainable, very close to the error threshold of the method. Although the expression levels of various *psbA* genes vary under UV-B stress conditions, in other cyanobacterial strains, [22,12] under our experimental conditions, *Synechococcus* PCC 7002 did not exhibit significant changes on the relative composition and the expression patterns of the D1

isoforms.

The amplitude of the fluorescence signal (F_{max}-F_0) offers a good indication relative to the number of PSII centers active at the time of the actinic flash. Under normal conditions the population of centers into the membrane is heterogeneous with a fraction of the centers in a physiological inactive state at the time of the flash (Fig 2a). Adding DCMU to the cells, the Q_B pocket of PSII complex is blocked, thus bringing all centers into the same physiological state allowing us to measure the total potential active centers (Fig 2b). Previous studies showed that UV-B stress impairs PSII function by damaging donor-side, which is accompanied by an acceleration of the decay curve, originating from Q_A^- recombination with $TyrZ^+$ or even P_{680}^+. [23]. We followed the evolution of the active PSII centers during the UV-B treatment and subsequent recovery period. Both in the presence and absence of DCMU, our results clearly show reduction in the number of active PSII centers down to about 65% of the initial control amount during 60 min of UV-B treatment (Fig 2a and Fig 2b). This inhibition of activity recovers to about 90% of initial state in 60 min after returning the cells to growth light conditions showing that UV-B did not affect the repair capacity of the cells (Fig 2a and Fig 2b).

After the application of the actinic flash that induces charge separation the electrons dislocated from P_{680} reaction center chlorophyll molecules, enter a chain of oxido-reductive reactions with very low lifetime until Q_A^- is formed. Q_A to Q_B transfer occurs in a microsecond to millisecond time scale and can be measured by fluorometer. Under normal conditions reduced Q_A donates sequentially two electrons to the mobile quinone molecule that occupies the Q_B pocket of the protein and the fluorescence level drops fast from the initial maximum value as seen in the control curve (Fig 2c). The following phase of the curve, at slower time range (10 to 100 ms), characterizes the electron transfer from PSII centers that had the Q_B site empty at the time of the flash and take a quinone molecule from the membrane plastoquinone pool (PQ-pool). This portion offers information about the functional status of the PQ-pool. After 60 min of UV-B treatment we observed a slow-down of the fluorescence decay in the middle section of the curve (Fig 2c). This effect is reversed after 60 min of recovery in growth light (Fig 2c). We can conclude that in *Synechococcus* PCC 7002, UV-B specifically and transiently slows-down the electron flow in the acceptor side of PSII due to changes in the redox status of the plastoquinone pool, hence increasing the redox potential gap between Q_A^- and Q_B.

In the presence of DCMU the Q_B site is blocked and Q_A^- is forced into recombination with the donor side of PSII especially with the water-oxidation complex resulting in preservation of a high fluorescence level for a significantly longer time (up to about 100 ms) compared to an untreated sample (Fig 2d). In the presence of UV-B a specific fast phase appears at short time ranges (Fig 2d). This feature has been seen before in other cyanobacteria [9]. In *Synechococcus* PCC 7002, UV-B is also inducing a modification of the donor side of PSII forcing Q_A^- into recombination with closer redox components TyrZ or P_{680}. This modification is quick and fully recovered upon returning the cells to growth light conditions (Fig 2d). Hence, recovery of the number of active PSII centers (Fig 2a and Fig 2b) and the UV-B specific effect on the acceptor and donor side of PSII (Fig 2c and Fig 2d) in *Synechococcus* PCC 7002 while having the same trigger are recovered using specific and possibly independent mechanisms.

CONCLUSIONS

It is important to mention that, it is the first time a direct, functional effect, of UV-B is recorded on the acceptor side of PSII in cyanobacteria. This could be linked to specific mechanisms of UV-B affecting the photosynthetic machinery in *Synechococcus* PCC 7002 or particular characteristic of PSII electron flow in this species that remains to be investigated. The fact that UV-B stress did not significantly induce the expression of either D1 isoforms while it functionally impairs PSII, is also a novel finding in our study.

ACKNOWLEDGEMENTS

The project was partially funded by Romanian National Authority for Scientific Research, CNCS-UEFISCDI, project number: PN-II-IDPCE-2011-3-0765 and the Executive Unit for Financing Higher Education, Research, Development and Innovation (UEFISCDI), Romania, project code PN-II-RU-PD-2011-3-0255.

D. C. was financed by a post-doctoral research fellowship jointly financed by Biological Research Center Jibou and Western University "Vasile Goldis", Arad.

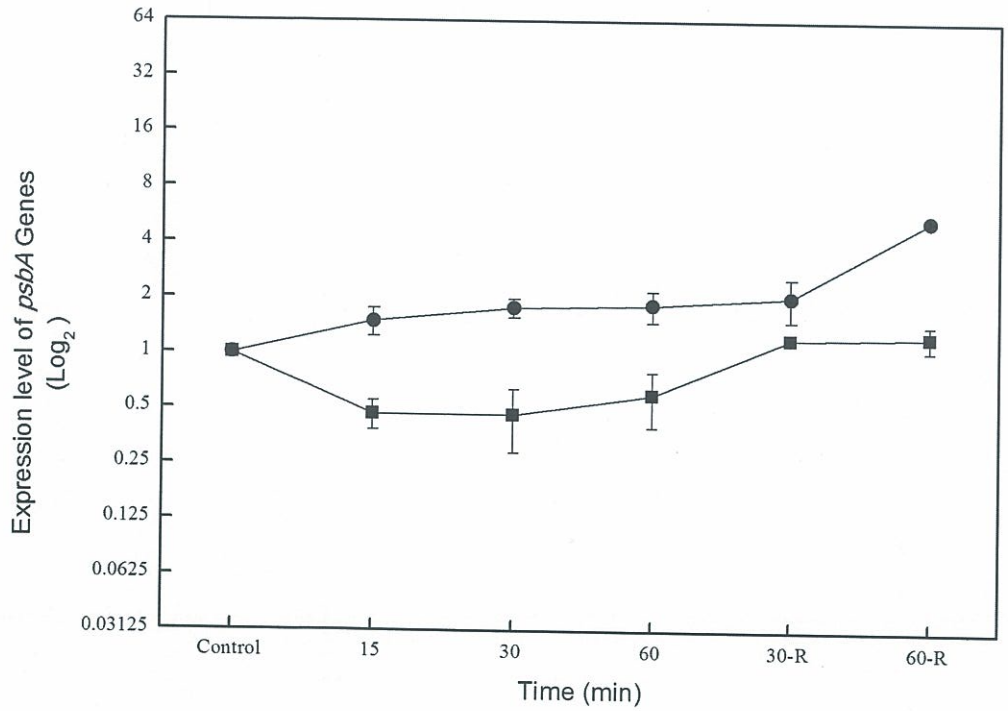
FIGURES

Table 1

psbA isoform Transcripts	UV-B Treatment				Recovery	
	Control	15 min	30 min	60 min	30 min	60 min
D1	99.9854	99.9539	99.9637	99.9649	99.9888	99.9796
D1'	0.0146	0.0461	0.0363	0.0351	0.0112	0.0204

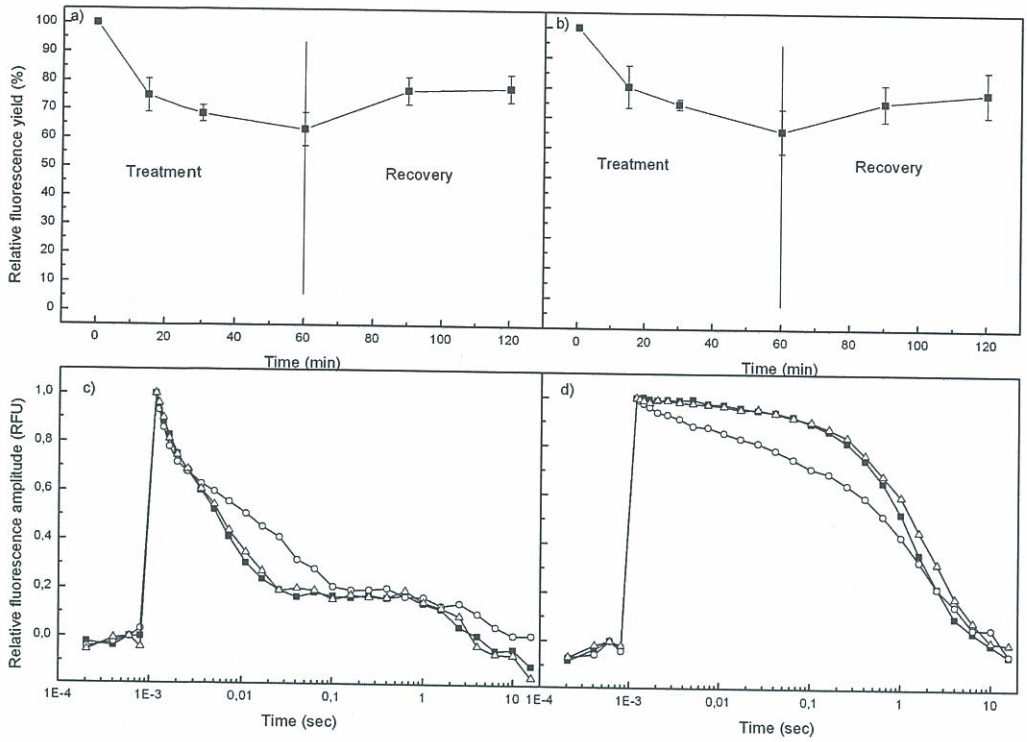
Relative transcripts amount (in percentage) of different D1 isoforms, during control, UVB treatment and recovery of *Synechococcus* PCC 7002.

Figure 1



Real Time Quantitative reverse transcriptase PCR analysis: qRT-PCR expression analysis of *psbA*-D1' (closed square) and other D1 isoforms (closed circle) under UV-B stress (15 min, 30 min, 60 min) followed by recovery under visible light [90 min (30-R) and 120 min (60-R)] in *Synechococcus* PCC 7002 strain. The primers used in the analysis are as follows (D1'- Fwd:

CCACACTGTTGACCGCAACGAT; Rev: GTAGGGGCCACCGTTGTAGAG; other D1 forms-Fwd: TTGCAGCCCACGGCTACTTC; Rev: GTTCAGAATGTCCGCCCAGGT).
Figure 2



Influence of UV-B radiations on the PSII function in *Synechococcus* PCC 7002. Changes in the number of actual active centres (panel a) and potential active centres (panel b) at control, UV-B treatment (15 min, 30 min, 60 min) and subsequent recovery in visible light (90 min and 120 min) in the absence (panel a) and presence of DCMU (panel b). The decay of flash-induced fluorescence was monitored using measuring flashes on a logarithmic time scale in the absence (panel c) and presence of DCMU (panel d) at control (solid black squares), 60 min UV-B treatment (open circles) and after 60 min of subsequent recovery (open up triangles) Changes in the shape of the curves were made evident by normalization of the decay curves to 1, during the treatment

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