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Differential regulation of *psbA* and *psbD* gene expression, and the role of the different D1 protein copies in the cyanobacterium *Thermosynechococcus elongatus* BP-1

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Abstract

In *Thermosynechococcus elongatus* BP-1, which is the preferred organism in recent structural studies of PSII, three *psbA* and two *psbD* genes code for three D1 and one D2 protein isoforms, respectively. The regulation and function of these genes and protein products is largely unknown. Therefore, we used quantitative RT-PCR to follow changes in the mRNA level of the respective genes, in combination with biophysical measurements to detect changes in the electron transport activity of Photosystem II under exposure to different visible and UV light, and temperature conditions. In cells which are acclimated to 40 μmol $m^{-2}s^{-1}$ growth light conditions at 40 °C the main populations of the *psbA* and *psbD* transcripts arise from the *psbA1* and *psbD1* genes, respectively. When the temperature is raised to 60 °C *psbA1* becomes the single dominating *psbA* mRNA species. Upon exposure of the cells to 500 μmol $m^{-2}s^{-1}$ intensity visible light *psbA3* replaces *psbA1* as the dominating *psbA* mRNA species, and *psbD2* increases at the expense of *psbD1*. UV-B radiation also increases the abundance of *psbA3*, and *psbD2* at the expense of *psbA1* and *psbD1*, respectively. From the different extent of total D1 protein loss in the absence and presence of lincomycin it was estimated that the PsbA3 protein isoform replaces PsbA1 in about 65% of PSII centers after 2 h of high light acclimation. Under the conditions of different *psbA* transcript distributions chlorophyll fluorescence and thermoluminescence measurements were applied to monitor charge recombination characteristics of the $S_2Q_A^-$ and $S_2Q_B^-$ states. We obtained faster decay of flash-induced chlorophyll fluorescence in the presence of DCMU, as well as lower peak temperature of the Q and B thermoluminescence bands when PsbA3 replaced PsbA1 as the main D1 protein isoform. The relevance of dynamic changes in the abundance of *psbA* and *psbD* transcript levels, as well as D1 protein isoforms in the acclimation of *T. elongatus* to changing environmental conditions is discussed.

Keywords: *psbA* gene; *psbD* gene; D1 protein; Photosystem II; Photo-oxidative stress

1. Introduction

In cyanobacteria the D1 and D2 proteins are encoded by small multigene families of the *psbA* and *psbD* genes, respectively [1]. The *psbA* family contains 1–5 gene copies, which encode 1–3 different D1 protein sequences in the cyanobacteria characterized so far. Whereas, the *psbD* family usually has two copies which encode one D2 protein form. The main physiological role of the different *psbA* and *psbD* genes is most likely related to acclimation to various stress conditions. The best characterized example is *Synechococcus* PCC7942 with three *psbA* gene copies that encode two different D1 protein isoforms (called D1:1 and D1:2) [2–5]. Under environmental stress conditions such as high light [3,6], blue light [7], low temperature [5,8], UV-B [9], or oxygen depletion [10] *psbA* expression is altered to selectively exchange the D1:1 isoform encoded by *psbA1* with the D1:2 isoform, encoded by *psbA2* and *psbA3*. When PSII has the high light isoform of D1 (D1:2) it has 25% higher quantum yield and shows increased tolerance to photoinhibition as compared to PSII centers with the low light isoform of D1 (D1:1) [5]. By expressing the two D1 isoforms of *Synechococcus* PCC7942 in *Synechocystis* PCC6803 background it has been

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PAR, photosynthetically active radiation; PSII, photosystem II

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shown that increased light tolerance is related partly to decreased extent of photodamage of PSII centers containing the D1:2 protein isoform, and partly to its enhanced ability to incorporate into the PSII complex during the repair cycle of D1 [11]. Further examples for light and UV dependent differential *psbA* regulation have been observed in *Anabaena* sp. PCC7120 [12] and *Gloeobacter violaceus* PCC7421 [13] both having five *psbA* genes, which encode 3 different D1 isoforms.

Although cyanobacteria usually contain only two different *psbD* genes, which encode identical D2 polypeptides their expression is also differentially regulated by changing light conditions. This has been demonstrated for *Synechococcus* PCC7942 as well as *Synechocystis* PCC6803. In both organisms the relative contribution of *psbD1* represents the dominating transcript under low light conditions, which is decreased at the expense of *psbD2* when the cells are exposed to high light [14] or UV-B radiation [15].

Thermosynechococcus elongatus BP-1 is a thermophilic cyanobacterium, which has gained importance recently as the source of PSII complexes suitable for crystallization [16–18]. This species has three *psbA* genes, which if all transcribed would encode three different D1 protein isoforms, and two *psbD* genes, which encode identical D2 polypeptides. Under normal growth conditions the dominating *psbA* transcript should arise from the *psbA1* gene, since the crystallized PSII complexes contain the corresponding PsbA1 protein isoform. Based on the analogy with other cyanobacteria having different D1 isoforms it is expected that the *psbA* gene copies and the corresponding D1 protein isoforms are also expressed differentially under different environmental conditions in *T. elongatus* as shown by preliminary results [19,20].

In the present work we studied the expression pattern of the *psbA* and *psbD* genes of *T. elongatus* by quantitative RT-PCR in parallel with measurements of PSII electron transport characteristics. Our results show that the mRNA levels of the *psbA1* and *psbA3*, as well as of the *psbD1* and *psbD2* genes are changing dynamically in response to changes in temperature, light intensity and ultraviolet radiation. These effects are accompanied by changes in PSII charge recombination characteristics, which indicate the role of PsbA1 and PsbA3 protein in acclimation to photo-oxidative stress conditions.

2. Materials and methods

2.1. Culture conditions

T. elongatus BP-1 cells were routinely grown in BG-11 medium in a rotary shaker at 40 °C under CO_2-enriched atmosphere. The intensity of PAR during growth was 40 μmol $m^{-2}s^{-1}$. Cells in the exponential growth phase (OD_{750} of 0.8–1.2) were harvested by centrifugation for 15 min at 4000 ×*g* at room temperature and resuspended at a concentration of 10 μg Chl mL^{-1} in a fresh culture medium.

2.2. Sample treatments

High light illumination experiment was performed in open, square glass containers in which the cell suspension formed a 14 mm high layer, with continuous stirring at 40 °C. An array of 50 W halogen lamps with adjustable light intensities provided the homogenous white light illumination. During light treatment sample aliquots were collected at certain time points, and stored at −80 °C until they were used for various measurements.

UV-B light was provided by a Vilbert–Lourmat lamp, with maximum emission at 312 nm, in combination with 0.1 mm cellulose acetate filter (Clarfoil, Courtalouds Chemicals, UK) yielding an intensity of 12 μmol $m^{-2}s^{-1}$ at the sample surface.

2.3. Gene expression analysis

10 mL of samples were harvested by centrifugation and total RNA was isolated by hot phenol method [21] with minor modifications. The crude RNA was further purified and freed from DNA contamination using NucleoSpinRNA kit (Macherei Nagel, Düren, Germany). 2 μg of the RNA was reverse transcribed using H-MuLV (Fermentas). Aliquots of the resulted cDNA were used in the Q-PCR reaction as template.

Quantitative RT-PCR (RT-PCR) was carried out on an ABI 7000 Sequence Detection System (Applied Biosystems Inc, Foster City, CA, U.S.A.) using SYBR green PCR Master mix of the same manufacturer. Primer pairs for the individual sequences were designed using Primer Express 2.0 program (ABI) as follows. psbaA1-sense: TTTGGACTTATCACGACTATGACCA, psbA1-antisense: CGGTGCTCGTCACCCAGT, psbA2-sense: GTCCCGTTGTTATTTATGGATTCAT, psbA2-antisense: ACGCTCCCACAGATTCGC, psbA3-sense: GGTTTGTAACATTCATTCAT ATTGTTCAA, psbA3-antisense: CAGCCCACATAGAGACGGTTG, psbD1-sense: TCTATATCTGCAAGAGGATTTAATTCCA, psbD1-antisense: ATGTCAAACCAT CCCCGTTC, psbD2-sense: TGCCCTTCGGAGTTGAATTTTA, psbD2-antisense: TCCCCGTTCCGCTGG.

Alignments of the homologous sequences of the *psbA* and *psbD* families, respectively, were carried out for aiding the selection of primers mapping to unique sequence regions of the respective mRNA sequences.

2.4. D1 protein degradation

Changes in the amount of the D1 protein were followed by immunoblotting, as described earlier [22].

2.5. Oxygen evolution measurements

PSII activity was assessed by measuring the light-saturated rate of oxygen evolution from whole cells, in the presence of 0.5 mM 2,5-dimethyl-*p*-benzoquinone as electron acceptor, using a Hansatech DW2 oxygen electrode. Usually, 1 mL of cells at 10 μg Chl mL^{-1} was used in each measurement.

2.6. Fluorescence relaxation kinetics

Flash-induced increase and subsequent decay of chlorophyll fluorescence yield was measured by a double-modulation fluorometer (PSI, Brno), in the 150 μs–100 s time range, in samples which were dark acclimated for 3 min prior to measurements. Analysis of the fluorescence relaxation traces was performed as described earlier [23]. The sample concentration was 10 μg Chl mL^{-1}.

2.7. Thermoluminescence measurements

Thermoluminescence curves were measured with a home built apparatus as described earlier [24]. Cells were harvested by gentle filtration through Whatmann GF/C glass microfibre filters to achieve 50 μg Chl on a filter disc. After 3 min of dark acclimation at 20 °C the samples were excited by a single saturating flash at 5 °C in the absence and at 0 °C in the presence of 10 μM DCMU. This was followed by a fast cooling to −20 °C, from where the slow heating with a rate of 20 °C min^{-1} was initiated, and thermoluminescence was detected.

3. Results

3.1. Effects of external conditions on psbA and psbD transcript levels

In control cells grown at 40 °C and low intensity (40 μmol $m^{-2}s^{-1}$) of photosynthetically active radiation (PAR) 94–95%