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A photosystem 1 *psaFJ-null* mutant of the cyanobacterium *Synechocystis* PCC 6803 expresses the *isiAB* operon under iron replete conditions

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Abstract A *psaFJ-null* mutant of *Synechocystis* sp. strain PCC 6803 was characterised. As opposed to similar mutants in chloroplasts of green algae, electron transfer from plastocyanin to photosystem 1 was not affected. Instead, a restraint in full chain photosynthetic electron transfer was correlated to malfunction of photosystem 1 at its stromal side. Our hypothesis is that absence of PsaF causes oxidative stress, which triggers the induction of the 'iron stress inducible' operon *isiAB*. Products are the IsiA chlorophyll-binding protein (CP43′) and the *isiB* gene product flavodoxin. Supporting evidence was obtained by similar *isiAB* induction in wild type cells artificially exposed to oxidative stress.

Key words: Cyanobacterium; Flavodoxin; *isiAB*; Oxidative stress; Photosystem 1; *psaFJ*; *Synechocystis* PCC 6803

1. Introduction

Cyanobacteria are photoautotrophic prokaryotes with oxygenic photosynthesis. Like in chloroplasts, linear photosynthetic electron transfer makes use of concerted action of photosystems 2 (PS2) and 1 (PS1). The photosynthetic apparatus is framed in thylakoids that are directly immersed in the cytoplasm [1,2].

The integral PS1 subunit PsaF is a well-conserved transmembrane protein of which the N-terminal extremity is located in the thylakoid lumen and the C-terminus in the stroma (cytoplasm) [3]. In chloroplasts of plants and algae, site-directed mutagenesis and functional studies [4–7] as well as cross-linking analyses [8] have supported the hypothesis that the lumenal domain of PsaF contains the docking site for PC (plastocyanin) or its homologue Cyt c6 (cytochrome c553). Through this functional role the presence of PsaF is essential in eukaryotic organisms [7]. However, in cyanobacteria the situation is different, PsaF is not needed for efficient reduction of $P700^+$ either in linear or in cyclic electron transfer [4,9]. This difference has been correlated with the presence (algae and plants) or absence (cyanobacteria) of a region close to the N-terminus, which contains amino acids involved in the binding site for the two soluble PS1 donors PC and Cyt c6 [5,6]. Crystallisation data also indicate that the lumenal extension of PsaF in cyanobacteria is quite distant from the docking site for PC near P700 [10].

The *Synechocystis* PCC 6803 genome contains a single copy of the PsaF encoding gene [11]. The transcriptional organisation of *psaF* is conserved in cyanobacteria, the gene being located upstream in a bi-cistronic operon with *psaJ*, encoding the PsaJ intrinsic subunit of PS1 [3,9]. PsaJ function would be to maintain PsaF in a proper orientation [6,9]. This study focusses on a mutant of *Synechocystis* PCC 6803 carrying a deletion encompassing the *psaFJ* operon [12]. Evidence for functional implication of PsaF at the stromal, rather than at the lumenal side of the PS1 complex, and connected de-repression of the bi-cistronic *isiAB* operon [13] will be presented.

2. Materials and methods

2.1. Strains, mutant construction, culture conditions and estimation of growth rate

Synechocystis sp. strain PCC 6803 wild type (WT) and the *psaFJ-null* mutant were grown photoautotrophically in modified Allen's medium ('standard medium') under continuous illumination (50 μmol m^{-2} s^{-1}) at 34°C [14]. High salt medium refers to the increase of the sodium-ion concentration from 50 mM (standard) to 550 mM (designated as high) by addition of NaCl. All cultures in high salt medium were inoculated from freshly grown pre-cultures in standard medium.

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Abbreviations: Chl, chlorophyll *a*; Cyt c6, cytochrome c553; DCMU, 3-(3,4-dichloro)-1,1-dimethylurea; Fd, ferredoxin; Flv, flavodoxin; isi, iron stress inducible; knt, kilonucleotide; MV, methyl-viologen; PC, plastocyanin; PsaF and PsaJ, integral components of the PS1 complex; PS1, photosystem 1; PS2, photosystem 2; WT, wild type

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Construction of the *psaF* insertion mutant, *psaFJ-null*, was as described in [12]. Complete segregation of the mutant was checked by polymerase chain reaction (PCR). Kanamycin was added at 100 μg ml^{-1} during growth of the *psaFJ-null* mutant. Estimation of growth by measuring turbidity and of Chl content as well as calculation of cell number were done as described in [14].

2.2. Photosynthetic measurements

Overall photosynthetic activity was determined as O_2 evolution in white light at 34°C. Neutral density filters were used to select a range of photon flux densities from 0 to 700 μmol m^{-2} s^{-1}. Photochemical energy storage was measured in vivo using the photoacoustic technique [15]. The electron flux through PS1 was monitored by kinetic spectroscopy at 820 nm [16] with a MKII $P700^+$ kit (Hansatech Instrument Ltd, UK). The P700 content was determined as in [14]. Photosynthetic pigment content was determined by HPLC-assisted separation [15]. Fluorescence emission spectra were recorded at 77 K with an LS50-B luminescence spectrometer (Perkin-Elmer). Excitation was at 440 nm [15].

2.3. Estimation of mRNA abundance by Northern blotting and RT-PCR

Total RNA was obtained as in [13,17]. Aliquots of 10 μg RNA were loaded onto a 1% agarose gel containing formaldehyde. Northern blotting onto nylon membrane filters was followed by hybridisation with a ^{32}P-labelled DNA probe spanning the *isiAB* operon. The full-length transcript signal was detected by phospho-imaging (BAS1000, Fuji). Gel loading was checked by the hybridisation signal from a radio-labelled 16S-rDNA probe applied to the same filters [13]. cDNA synthesis was carried out as in [17] with Superscript one-step RT-PCR from Gibco-BRL (Invitrogen). The following primers were used to amplify internal fragments of the coding sequences of the investigated genes by PCR (*isiA* gene: 5′ primer – GCAAACCTATGGCAACGACACCG, 3′ primer – CCGTTTGGGTGGTGGCGTCGTA; *isiB* gene: 5′ primer – AAGAAATGGGCGGCGATAGTGTGG, 3′ primer – TCCCATTTTTCACCGCTTTTG; *psaA* gene: 5′ primer – AGTCCACCCGAAAGAGAGGC, 3′ primer – CCCGCCAGAGATTAAAACAGG; *psbB* gene: 5′ primer – CCAGACCTTCAGCA ATCCCAGT, 3′ primer – GTCACCAACCTTAGCAAACACACC).

2.4. SDS–PAGE and immunoblotting

Aliquots of total protein extracts (5 μg) were separated in SDS–PAGE (Miniprotean, Bio-Rad) with the Laemmli buffer system. Proteins were blotted semi-dry onto a nitrocellulose membrane (0.2 μm pore size, Schleicher and Schuell). Immuno-decorations were done with polyclonal antibodies raised against PC of *Anabaena* and Flv (flavodoxin) of *Synechocystis* PCC 6803. Binding of the antibodies was monitored with a secondary antibody conjugated to horseradish peroxidase and visualisation with enhanced chemoluminescence detection (ECL-Kit, Amersham Buchler) [18].

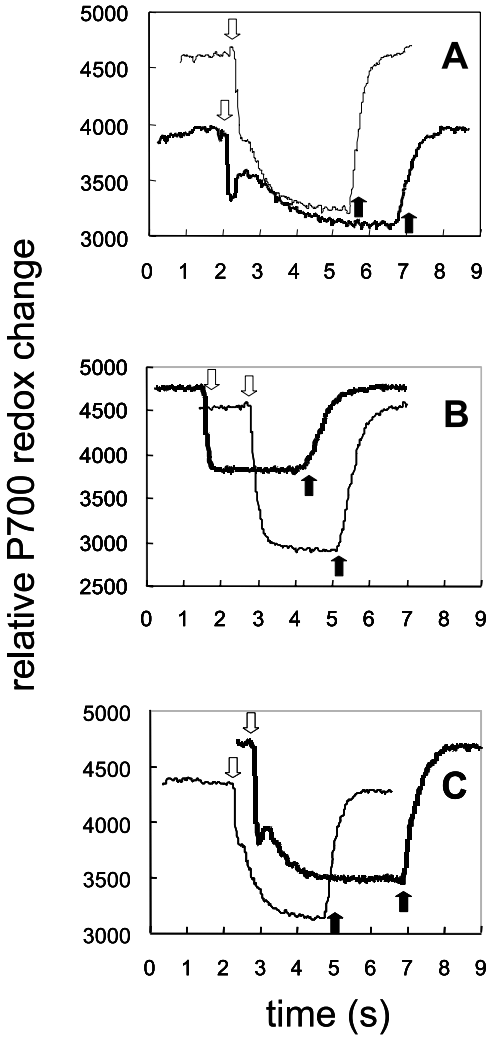

Fig. 1. Kinetic traces of P700 oxidation–reduction in WT and *psaFJ-null* mutant cells, respectively, grown in standard medium for traces A and B or in high salt medium for trace C. White photon flux density was 300 μmol m^{-2} s^{-1} in all cases. Heavy lines are for the *psaFJ-null* mutant, normal lines for the WT. Open arrows indicate light on, filled arrows indicate light off. A: Control cells; B: control cells plus 10 μM DCMU; C: salt-adapted cells.

3. Results and discussion

3.1. Growth and photosynthetic properties of the Synechocystis psaFJ-null mutant

Under standard conditions, global growth properties of WT and the *psaFJ-null* mutant (hereafter referred to as mutant) were the same, like in [4]. However, transfer of exponential-phase cells from standard to high salt medium revealed a difference. Observation of a longer adaptation period ('lag phase') for the mutant than for the WT (30 ± 5 h and 16 ± 3 h, respectively) essentially initiated this study.

The localisation of the PsaF and PsaJ proteins within the PS1 complex prompted us to compare the photosynthetic performance of the mutant and the WT. At low light intensity an about 1.8 times lower oxygen evolution capacity was found for the mutant (Table 1). Higher light intensity during the measurement (Table 1) or culture in high salt medium (not shown) largely abolished this difference in oxygen evolution capacity. A further indication for less efficient light energy transduction in linear photosynthetic electron transfer in the mutant is the reduction of photochemical energy storage in blue light relative to WT (Table 1). Evidence that it concerned impaired PS1 functionality following from suppression of PS1 in the mutant, can be seen from the P700 content estimate and the signal amplitude for maximally oxidised P700 in white light (Table 1). Accordingly, pigment analyses reflected a decrease of typical PS1 carotenoids like β-carotene and stable values for the PS2-related ones (Table 1).

The nature of the problem in the mutant is illustrated in P700 oxidation/reduction kinetics (Fig. 1A). Directly after switching on the white actinic light, P700 oxidation started, after which a rapid transient reduction was observed within about 200 ms, in particular in the mutant. Next to the transient, the subsequent approach to full oxidation was slower and led to a less oxidised state in the mutant than in the WT. The strong intermediate transient in the mutant may find its cause in electron flux from PS2, which is also activated in the white light used. Evidence for such a PS2-related origin for

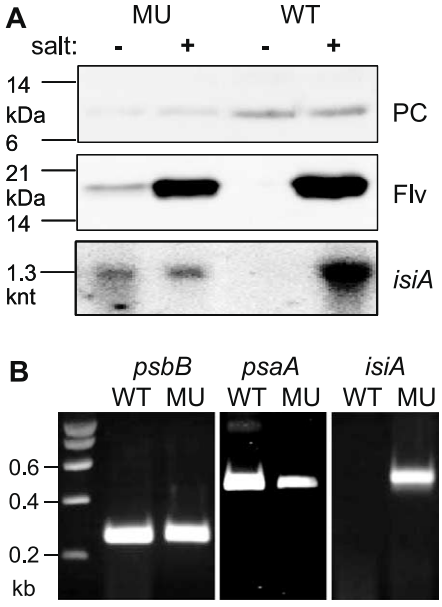

Fig. 2. A: Presence of the soluble electron donor plastocyanin (PC) and ibid acceptor flavodoxin (Flv) shown by Western-blot experiments; Northern-blot experiments for the presence of the chlorophyll-binding protein *isiA* transcript. B: RT-PCR detection of the transcripts of *isiA*, the PS2 core protein *psbB* and the PS1 core protein *psaA*. Total protein or RNA were isolated from cells of the *psaFJ-null* mutant (MU) and WT grown under standard conditions (panel A: salt −; all lanes in panel B) or in high salt medium (panel A: salt +). Molecular mass standards are indicated left of the gels.

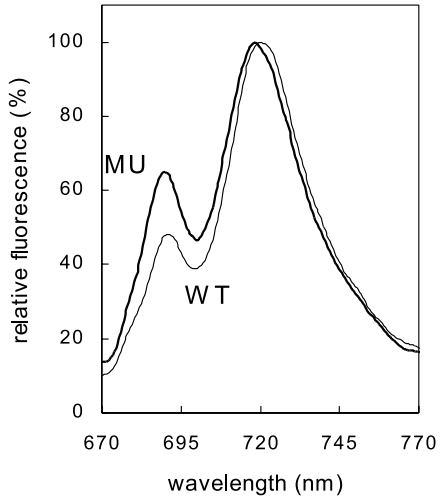

Fig. 3. Chlorophyll fluorescence emission spectra recorded at 77 K from cells of the WT (thin line) or the *psaFJ-null* mutant (MU, thick line). Excitation of Chla was at 440 nm. Both spectra were normalised to 100% at the emission of 720 nm.

the transient follows from its full suppression by 3-(3,4-dichloro)-1,1-dimethylurea (DCMU) (Fig. 1B). The difference between WT and mutant likely demonstrates the consequence of impaired electron flux from the stromal acceptor site of PS1 to the PQ-cytochrome b_6f complex ('cyclic route') in the mutant. Electron influx from the stroma to the complex regulates PS2 function [16]. Altered electron passage from the stroma may install a new redox equilibrium for the complex in culture conditions, which has been advocated as a plausible transcription controlling factor in biosynthesis, in addition to oxygen ion compounds [19]. High salt grown cyanobacterial cells are known to induce more effective and new electron flow pathways in PS1 'cyclic' [14,18,20]. This induction likely changes the redox state of the PQ-cytochrome b_6f complex and suppresses the transient in P700 kinetics in the mutant (Fig. 1C). At the same time, control of electron efflux from PS2 also diminished oxidative stress directly.

3.2. Content of soluble electron carriers involved in electron transfer to and from PSI

The PC content in the mutant was slightly lower than in the WT, corresponding to the reduced level of PS1 (Fig. 2A, top). Surprisingly, a significant amount of the soluble electron acceptor Flv accumulated in the mutant already in standard medium. Another instance in which transcription of the *isiB* gene for Flv occurs, iron limitation in the medium, was ruled out by the total absence of Flv in the WT control (Fig. 2A, middle). Flv induction has been related to functional replacement of ferredoxin (Fd) in iron limitation stress [21] and to increased capacity for PS1-driven cyclic electron flow in salt-adapted *Synechocystis* PCC 6803 [18]. High salt gave rise to accumulation of Flv in the WT in agreement with [18] and additional synthesis in the mutant. The permanent expression

Table 1
Comparison of photosynthetic parameters in cells of the WT and of the *psaFJ-null* mutant grown at standard conditions

Parameter	WT	*psaFJ-null* mutant
Oxygen emission (μmol h^{-1} mg^{-1} Chl)		
White light 15 μmol m^{-2} s^{-1}	13.4 ± 2.0	7.6 ± 1.0
White light 100 μmol m^{-2} s^{-1}	81.0 ± 10.0	67.0 ± 10.0
Max. P700 oxidation in white light (%)	100	77 ± 8
Energy storage, blue light (%)	12.4 ± 0.6	8.2 ± 0.5
Energy storage, far-red light (%)	12.5 ± 0.8	12.3 ± 0.9
P700 centres content in thylakoids (nmol mg^{-1} Chl)	5.5 ± 0.4	4.2 ± 0.2
Pigments (μg 10^{-8} cells)		
Chlorophyll	14.3 ± 0.6	12.3 ± 0.6
Myxoxanthophyll	0.45 ± 0.06	0.48 ± 0.06
Zeaxanthin	0.55 ± 0.06	0.55 ± 0.03
Echinenone	1.06 ± 0.06	0.68 ± 0.06
β-carotene	1.52 ± 0.19	1.10 ± 1.29

Experimental details are presented in Section 2. Standard errors of the mean values from at least three experiments have been indicated. Far-red actinic light was dosed at 35 μmol m^{-2} s^{-1}. For energy storage in blue light 35–40 μmol m^{-2} s^{-1} was used. Saturating white light for determination of the maximal oxidation of P700 was dosed at 700 μmol m^{-2} s^{-1}.

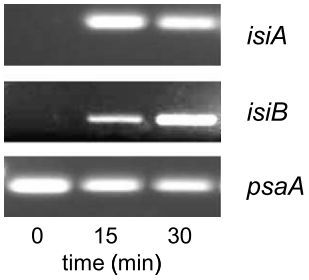

Fig. 4. Transcription of the *isiAB* operon in cells of the WT exposed to artificially created oxidative stress conditions. The latter was realised through incubation of cells with the PS1 electron acceptor MV (0.1 mM final concentration) in white light (60 μmol m^{-2} s^{-1}). This brings about reduction of MV by light-dependent electron transfer via PS1. Reduced MV generates hydrogen peroxide by reduction of molecular oxygen. After this treatment, RNA was isolated at the times indicated and the *isiA*, *psaA* and *isiB* transcript levels were detected by RT-PCR.

of Flv under iron-replete conditions in the *psaFJ-null* mutant of the cyanobacterium *Synechocystis* PCC 6803 is accordingly proposed to be the result of adaptation to stress that is experienced at the stromal side of the thylakoid membrane. Concerted transcription of the *isiA* gene, which is organised with the Flv encoding *isiB* gene in an operon [13], became evident in Northern blot analysis (Fig. 2A, lower frame). Additionally, RT-PCR was used to study the transcript status for *psbB* (PS2), *isiA* and *psaA* (PS1) in WT and mutant (Fig. 2B). It indicates, in agreement with data presented above, that the PS2 transcript level remains constant, that the *isiAB* operon is constitutively transcribed in the mutant and that PS1 transcripts like *psaA* are less abundant. Presence of the chlorophyll *a* (Chl)-binding protein IsiA in the mutant was also suggested from the blue shift in the 77 K fluorescence emission spectrum, and from the increased emission around 690 nm (Fig. 3), which arises from emission of PS2 and probably IsiA. For isolated IsiA an emission at 682 nm has been shown [22], while overexpression of *isiA* in *Synechococcus* sp. PCC 7942 led to increased emission at 685 nm absent in a corresponding mutant [23].

A typical stress that can arise from a decrease in electron flux from the stroma to PQ and may trigger induction of the *isiAB* operon is oxidative stress. Strong evidence for its role in *isiAB* induction was acquired by application of artificial oxidative stress through addition of 100 μM methyl-viologen (MV) to a culture of WT cells in the light. Induced transcription of the *isiAB* operon was shown by RT-PCR (Fig. 4). This observation demonstrated that the induction of *isiAB* expression likely occurred by oxidative stress as a secondary consequence of stresses like iron limitation and high salt or by absence of PsaF/J subunits.

In the mutant the binding pocket for Fd may lack the mechanical support of the little hook that extends from the C-terminal end of the PsaF protein in the PS1 crystal structure [10]. This altered geometry of the binding pocket may cause a less close positioning of Fd and its catalytic partners (PsaC, PsaD, PsaE) for electron acceptance from PS1 in the mutant. Hence, oxidative stress may follow from oxygen acting as acceptor for the very reduced FeS proteins at the 'top' of PS1. The trigger for induction of the *isiAB* gene should accordingly be defined broader; we propose that oxidative stress rather than iron limitation is the actor. For the cyanobacterium *Synechococcus* a better salt tolerance was observed in a catalase-overproducing strain [24]. Catalase is instrumental in breakdown of hydrogen peroxide. In *Escherichia coli* expression of *fldA* encoding Flv is regulated by two transcription factors, *Fur* for iron limitation and *SoxS* for oxidative stress [25]. The operon structure of *isiAB* in *Synechocystis* confers, besides *isiB* expression, also that of *isiA* [13]. The benefit of increased amounts of IsiA, a chlorophyll-binding protein, which has currently attracted much attention after its structural characterisation [26,27], for stressed cyanobacterial cells remains to be defined [22,23]. Our finding that this protein is induced by oxidative stress suggests a role in photoprotection.

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