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RESEARCH ARTICLE

The PsbZ subunit of Photosystem II in *Synechocystis* sp. PCC 6803 modulates electron flow through the photosynthetic electron transfer chain

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Abstract The *psbZ* gene of *Synechocystis* sp. PCC 6803 encodes the ~6.6 kDa photosystem II (PSII) subunit. We here report biophysical, biochemical and in vivo characterization of *Synechocystis* sp. PCC 6803 mutants lacking *psbZ*. We show that these mutants are able to perform wild-type levels of light-harvesting, energy transfer, PSII oxygen evolution, state transitions and non-photochemical quenching (NPQ) under standard growth conditions. The mutants grow photoautotrophically; however, their growth rate is clearly retarded under low-light conditions and they are not capable of photomixotrophic growth. Further differences exist in the electron transfer properties between the mutants and wild type. In the absence of PsbZ, electron flow potentially increased through photosystem I (PSI) without a change in the maximum electron transfer capacity of PSII. Further, rereduction of $P700^+$ is much faster, suggesting faster cyclic electron flow around PSI. This implies a role for PsbZ in the regulation of electron transfer, with implication for photoprotection.

Keywords Non-photochemical quenching · Photosynthesis · Photosystem II · PsbZ · *Synechocystis* sp. PCC 6803

Abbreviations

CP1	Major chlorophyll *a*-protein of PSI
cyt b_6f	Cytochrome b_6f
DCBQ	2,6-Dichloro-*p*-benzoquinone
EPR	Electron paramagnetic resonance
LHC	Light-harvesting complex
NPQ	Non-photochemical quenching
orf	Open reading frame
P700	Reaction centre chlorophyll of photosystem I
$P700^+$	Oxidized P700

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Introduction

The chloroplast-encoded *psbZ* gene (formerly *ycf9, orf62*) encodes a ~6.6 kDa, highly conserved, hydrophobic protein containing two putative membrane-spanning segments. *psbZ* is present in all chloroplast genomes sequenced so far (Rochaix 1997; Douglas and Penny 1999; Sato et al. 1999; Turmel et al. 1999; Hupfer et al. 2000; Lemieux et al. 2000) except for the peridinin-containing dinoflagellates (Koumandou et al. 2004). In tobacco, the *psbZ* gene is located downstream of the *psbD* and *psbC* genes and is part of the transcription unit formed by these three genes

(Yao et al. 1989). In the alga, *Chlamydomonas reinhardtii*, *psbZ* is found close to the *psbM* gene of photosystem II (PSII).

Several groups have studied tobacco *psbZ* knockout mutants, resulting in ambiguous results. Since *psbZ* knockout leads to reduced amounts of the minor antenna protein, CP26, it was first suggested (Ruf et al. 2000) that PsbZ plays a role in the stable integration of CP26 into the PSII antenna complex. However, PsbZ was later shown to be a PSII component in tobacco and *C. reinhardtii* (Swiatek et al. 2001) and more recently, its peripheral location in PSII were confirmed (Ferreira et al. 2004; Arteni et al. 2005; Rokka et al. 2005). Proteomic analysis of a *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis* PCC 6803) PSII preparation also revealed the presence of PsbZ in cyanobacterial PSII (Kashino et al. 2002). Further, the presence or absence of CP26 in *psbZ* knockout plants was shown to be due to growth conditions (Baena-Gonzalez et al. 2001). Thus, it has also been concluded that, rather than a role in light-harvesting, PsbZ functions in regulating the mode of electron transfer in plant chloroplasts (Baena-Gonzalez et al. 2001). Although the PSII core complex of cyanobacteria highly resembles its plant counterparts, the light-harvesting system differs greatly (MacColl 1998), with a membrane-peripheral phycobilisome complex rather than the integral membrane complex. Importantly, CP26 is not found in the cyanobacterial thylakoids. In cyanobacteria, both oxygenic photosynthesis and cell respiration take place in the same cell compartment: photosynthetic electron transfer exclusively in the thylakoid membranes, while the respiratory electron transfer components can be found both in the thylakoid and cytoplasmic membranes (Molitor and Peschek 1986). Some common components like plastoquinone, cytochrome b_6f (cyt b_6f), soluble electron transfer components, and NADH dehydrogenase, are shared between the photosynthetic and respiratory electron transfer (Mi et al. 1995). However, the respective roles of photosynthesis and respiration in cyanobacterial metabolism are currently poorly understood.

To investigate the role of the *psbZ* gene in *Synechocystis* PCC 6803 disruption and deletion mutants of *psbZ* were generated. Examination of the *Synechocystis* PCC 6803 genome (Kaneko et al. 1996) confirms that there are no other photosynthetic genes in the proximity of the *psbZ* gene. Thus, phenotypes arriving from mutations in the *psbZ orf* of *Synechocystis* PCC 6803 can be examined without the potential for an indirect effect. Biophysical, biochemical and in vivo characterization of the cyanobacterial *psbZ* mutants reveal no obvious phenotype under standard growth conditions: the mutants are able to perform light-harvesting, photosynthesis, state transitions and non-photochemical quenching (NPQ). However, their growth is impaired under low-light intensity; the mutants have lost the glucose tolerance phenotype of the wild-type *Synechocystis* sp. PCC 6803 strain used in this study; and differences exist in the electron transfer properties of the mutant when compared to the wild type.

Materials and methods

Cell material, growth conditions and determination of growth rates

Synechocystis PCC 6803 cells were grown in BG-11 medium (Stanier et al. 1971) under low light (7 μmol photons m^{-2} s^{-1}; LL) or growth light (40 μmol photons m^{-2} s^{-1}; HL) conditions at 32°C and used during the logarithmic growth phase. Spectrophotometric determination of growth rates was performed according to Mäenpää et al. (1998). Selection and maintenance of the *psbZ* mutants was performed in the presence of 100 μg ml^{-1} kanamycin. Where indicated, BG-11 was also supplemented with 5 mM glucose. Spot tests were carried out on agar plates: a 10 μl aliquot of the appropriate culture in log phase was spotted onto a plate, and then incubated under low light at 32°C.

Plasmid construction, cell transformation and selection of mutant lines

The *psbZ* orf, or *orf* sll1281, together with 452 bp 5′ and 245 bp 3′-flanking sequences was amplified by polymerase chain reaction (PCR) of genomic DNA isolated from wild-type *Synechocystis* PCC 6803 using primers 5′-GCCAGGGAAATTGATCGTTGGAGTG-3′ and 5′-CACATCAATGCCATGACGTTTGAGGC-3′. The 886 bp PCR product was then cloned into the *Hinc*II site of the plasmid vector pUC19 generating pSpsbZ. Present within the PCR product were two *Bsm*FI sites at positions 429 (23 bp upstream of the *psbZ* ATG) and 564 (within the *psbZ* orf). The 1,291 bp *Hinc*II fragment from pUC4K (Amersham Pharmacia Biotech) containing the kanamycin-resistance cassette was legated into the *Bsm*FI sites, producing two different constructs. The first, pSpsbZ2, was the result of the insertion of the kanamycin-resistance cassette at position 429. This was used to generate the mutant Kn2 in which the transcription of *psbZ* is disrupted by the insertion of the cassette. In the second, pSpsbZ5, the kanamycin-resistance cassette replaced the 429–564 *Bsm*FI fragment and was used to generate the mutant Kn5 in which most of the *psbZ* coding region is deleted. DNA sequence analysis confirmed the integrity of the plasmids together with insert orientations. The kanamycin-resistance cassette was present in the opposite orientation to *psbZ* orf

in both plasmids. Wild-type *Synechocystis* PCC 6803 was transformed with pSpsbZ2 and pSpsbZ5, and transformants selected on the basis of kanamycin resistance. Transformant homoplasmicity was confirmed using Southern analysis. The wild type used in this study was the glucose-tolerant strain developed by Williams (1998).

Isolation of thylakoid membranes, gel electrophoresis, immunoblotting and chlorophyll determination

Cell membranes were isolated at 4°C as described (Tyystjärvi et al. 1994). The chlorophyll *a* content of the cell suspensions and isolated membranes was determined according to the method of Porra (1989). The proteins were separated by using 15% PAGE containing 6 M urea (Laemmli 1970), with samples loaded on an equal chlorophyll basis. For detection of PsbZ, thylakoid polypeptides were separated on Tricine-SDS PAGE (Schägger and von Jagow 1987) and stained with silver nitrate. Polyclonal antibodies against the D1 and D2 polypeptides were purchased (Research Genetics, Inc.). Other antibodies were gifted as follows: CP1 (Dr Hundal), CP 43, α-Rieske and Photosystem I (PSI) (Dr Barbato), Cyt b_6f (Dr Wollman), ndhH (Dr Peltier) and ndhK (Dr Appel). Immunoblotting was performed using standard methods.

Measurements of oxygen evolution in vivo

Oxygen evolution at 32°C was measured with a Clark-type oxygen electrode under saturating red light. For each measurement, 1 ml of cell suspension corresponding to 10 μg chl ml^{-1} was re-suspended in 1 ml of BG-11. 0.5 mM 2,6-dichloro-*p*-benzoquinone (DCBQ) in the presence of 0.25 mM ferricyanide was used as electron acceptor for measurements of PSII activity. Oxygen evolution was also measured using 0.6 mM bicarbonate as the electron acceptor for measurements of photosynthetic capacity of the cells.

Dark re-reduction of P700

The redox state of the reaction centre chlorophyll of PSI (P700) in isolated thylakoids was determined from the absorbance of the oxidised form of P700 ($P700^+$) at 810 nm, using 860 nm as a reference. Absorbance changes were monitored using an ED-P700DW unit attached to the PAM 101 fluorometer. Measurements were done under anaerobic conditions in a temperature-regulated cuvette (25°C) containing 0.5 ml of buffer (50 mM Tricine (pH 7.5), 5 mM $MgCl_2$, 6 mM glucose, 2 mM NH_4Cl, 400 U ml^{-1} catalase, 50 μM ferredoxin) (Scheller 1996). The mixture was flushed with nitrogen, and 2 U of glucose oxidase and thylakoids (50 μg chl) were added. An initial illumination period (1,000 μmol photons m^{-2} s^{-1}) of 30 s was applied to reduce ferredoxin. After that, the samples were kept in darkness for 10 s. Thereafter, 30 cycles of actinic light (1.2 s) and darkness (8.8 s) were applied and the average post-illumination change in the $P700^+$ signal of the 30 repetitions was resolved in the sum of two exponentials. The results reported considers only the fast component of the signal.

Electron paramagnetic resonance (EPR) analysis

EPR analysis was performed as in Bishop et al. (2003) on whole cell samples concentrated from 200 ml of exponentially growing culture. The pellet was re-suspended in TES buffer (5 mM Tris (pH 8.5), 50 mM NaCl, 5 mM EDTA). Samples were dark-adapted then frozen, and analysed using a Jeol REIX spectrometer with an Oxford Instruments liquid helium cryostat. The EPR conditions were microwave power 10^{-3} mW, modulation amplitude 0.2 mT and temperature 10 K.

Analysis of state transition by 77 K fluorescence emission spectroscopy

This measurement enables energy transfer to and between PSI and PSII to be determined. Seventy-seven Kelvin fluorescence emission spectra were taken using a Perkin Elmer LS50 luminescence spectrometer with excitation and emission slit widths of 5 and 10 nm, respectively. Measurements were taken following excitation at 435 nm (chlorophyll *a*) and 600 nm (allophycocyanin). Results were analysed using Sigma Plot for Windows Version 5.0.

Analysis of state transition

Synechocystis PCC 6803 cells were grown photoautotrophically under low-light conditions and whole cell samples at 5 μM chlorophyll prepared. Cells were dark-adapted at room temperature for a minimum of 5 min (state 2). Transition from state 2 to state 1 was initiated by excitation at 600 nm (phycocyanin absorption) after 60 s. Cells were illuminated for a further 140 s (transition to state 1), and then allowed to return to state 2. Fluorescence emission was measured at 680 nm (PSII chlorophyll *a*).

Results

Verification and phenotype of the mutant strains

Two different *Synechocystis* PCC 6803 transformant lines were generated in which *psbZ* was inactivated. In the Kn2 transformants, the transcription of *psbZ* was disrupted by

insertion of the kanamycin-resistance cassette immediately upstream of the *psbZ* translational start. In the Kn5 transformants, 112 bp of *psbZ* were deleted by insertion of the cassette within the gene. Transformant homoplasmicity was confirmed using Southern analysis (data not shown).

Preliminary growth comparisons of the transformants Kn2 and Kn5 with wild type were performed using spot tests. Four types of plates were used: BG-11, BG-11 with 5 mM glucose, BG-11 with 100 μg ml^{-1} kanamycin or BG-11 with glucose and kanamycin. Wild type *Synechocystis* PCC 6803 cells were capable of growth on BG-11 plates and BG-11 plates with glucose, but did not grow in the presence of kanamycin, as expected (Fig. 1A). The mutants Kn2 and Kn5 were able to grow on BG-11 plates and BG-11 plates with kanamycin, showing that they were capable of photoautotrophic growth and that the antibiotic-resistance cassette was functional. Unexpectedly, however, the mutants did not grow in the presence of glucose.

Under standard growth conditions (40 μmol photons m^{-2} s^{-1}) the *psbZ* mutants grew slightly slower than wild-type cells (Fig. 1B). Growth under low-light conditions (7 μmol photons m^{-2} s^{-1}) did not affect the wild-type growth rate. In contrast, the mutants were further impaired at this lower light intensity—wild-type cells had a doubling time of 2.8 days under both growth and low-light conditions, whilst the doubling time for the mutant under growth light was 3.4 days. This was further reduced to 4.3 days under low-light conditions.

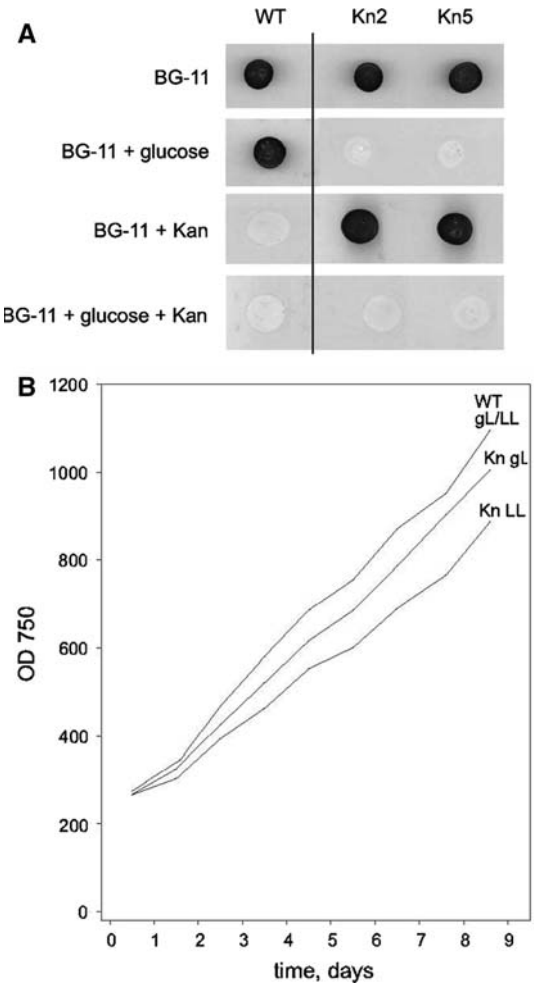

Fig. 1 Growth analysis of wild-type *Synechocystis* PCC 6803 and the *psbZ* mutants. (**A**) Spot tests for growth on BG-11, BG-11 supplemented with glucose (5 mM), BG-11 supplemented with kanamycin (100 μg ml^{-1}) and BG-11 supplemented with glucose and kanamycin. An aliquot of each culture was spotted onto the corresponding plate, then grown at 30°C and 7 μmol photons m^{-2} s^{-1}. (**B**) Median growth rates of wild type and Kn2 mutant strains in liquid BG-11 under growth light (gl) and low light (LL) conditions. *Note*: both Kn2 and Kn5 showed the same growth response (data not shown)

Thylakoid protein composition and oxygen evolution properties in vivo

Silver staining of polyacrylamide gels containing separated thylakoid polypeptides confirmed the absence of PsbZ in the mutant lines Kn2 and Kn5 (data not shown). Immunoblot analysis did not reveal significant changes in the amounts of specific subunits of PSI, PSII, cyt b_6f complex, ndhH polypeptide and the ATP synthase following loss of PsbZ (Fig. 2). There is no molecular reason to suppose that the Kn2 and Kn5 mutants would not have the same phenotype.

Electron transfer from H_2O to either DCBQ (PSII oxygen evolution) or to bicarbonate (photosynthetic capacity)

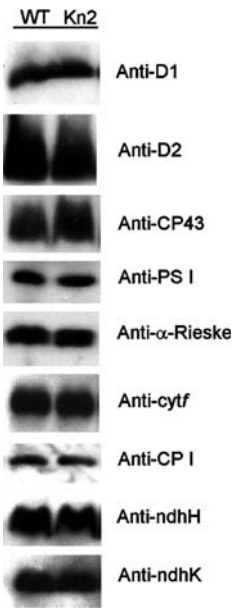

Fig. 2 Western blot analysis of isolated thylakoid membranes from wild-type *Synechocystis* PCC 6803 and the *psbZ* mutants. (1 μg chlorophyll/lane, except for ndhH, 15 μg chlorophyll/lane)

was measured in the *psbZ* mutants and wild-type cells. The light-saturated PSII activities of wild type and the mutants did not differ being (218 ± 20 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1}). However, the oxygen evolution activity obtained from the photosynthetic capacity measurement of the mutant strains was significantly increased (129 ± 8 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1}) when compared to the wild-type value (89 ± 9 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1}).

Re-reduction of $P700^+$ in vitro

Post-illumination re-reduction rates of $P700^+$ were determined from isolated thylakoid membranes. Figure 3 demonstrates that the reduction of $P700^+$ occurred at a much faster rate in both *psbZ* mutants strains when compared to wild-type *Synechocystis* PCC 6803, suggesting an increased flow of electrons to $P700^+$, referring to activated cyclic electron flow around PSI.

EPR analysis of the *psbZ* mutants

Next, EPR spectra were obtained for dark-adapted wild-type cells together with the *psbZ* mutants Kn2 and Kn5 and a PSII-deficient mutant generated by the deletion of *psbH* (O'Connor et al. 1998; Fig. 4A). A typical $Y_D^{\cdot}$ spectrum was produced by the wild-type cells, showing that the PSII core complex was assembled and active. For both the *psbZ* mutants similar spectra were observed, indicating that the PSII core complex was not compromised in these cells. The detection of the $Y_D^{\cdot}$ signal also confirmed that PSII was able to oxidise Y_D via P680. As expected, the control PSII-deficient cells lacked the $Y_D^{\cdot}$ signal.

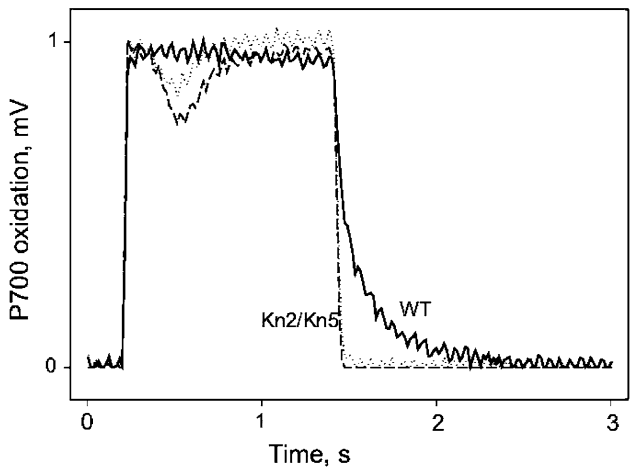

Fig. 3 Dark re-reduction of $P700^+$ in isolated thylakoids from wild-type *Synechocystis* PCC 6803 and the *psbZ* mutants. Post-illumination reduction of $P700^+$ was measured by monitoring changes in A_{810} in the dark after a light-induction period. Each curve is an average of 30 repetitions

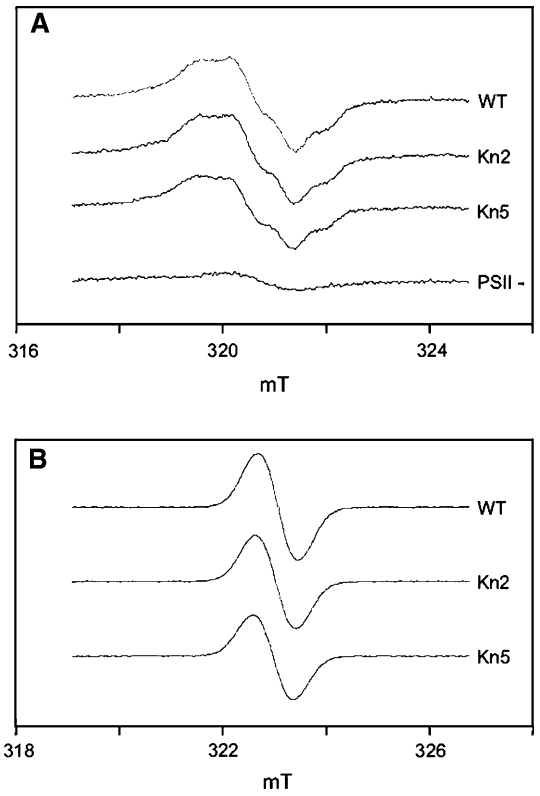

Fig. 4 EPR analysis of wild-type *Synechocystis* PCC 6803, the *psbZ* mutants and a PSII$^-$ mutant. (**A**) EPR spectra of the Y_D in wild-type *Synechocystis* PCC 6803, Kn2, Kn5 and PSII$^-$ dark-adapted cells. (**B**) EPR analysis of $P700^+$ in wild-type *Synechocystis* PCC 6803, Kn2 and Kn5 cells. The spectra are the illuminated spectrum (at 10 K) minus the dark spectrum. See Materials and methods for further details

Figure 4B shows the $P700^+$ photoinduced EPR spectra for wild type and the *psbZ* mutants. The spectra confirm that PSI was also functional in all three cell types. Comparative quantitative analysis was achieved by normalising the EPR signals to the same chlorophyll concentration. This showed that the levels of PSII (measured by the $Y_D^{\cdot}$ signal) relative to PSI (measured by the $P700^+$ signal) were slightly lower in the Kn2 and Kn5 mutants, with respect to wild type. This reduction, although not striking, was consistently observed when measurements were compared for several independent wild type and mutant samples (data not shown).

State transition assay of the *psbZ* mutants

There is a high degree of similarity in the PSII of oxygenic photosynthetic organisms. However, one of the major differences is the light-harvesting system that is present. In cyanobacteria, the phycobilisomes are responsible for capturing light, whilst in higher plants and green algae, chlorophyll containing LHC performs this function. Con-

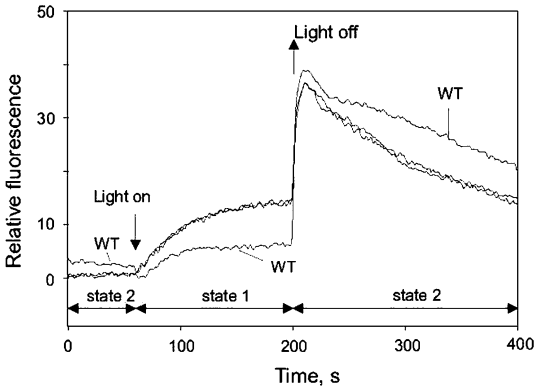

Fig. 5 Room temperature fluorescence time course for dark-adapted (state 2) wild-type *Synechocystis* PCC 6803 and the *psbZ* mutants. Excitation was at 600 nm (phycocyanin absorption) with fluorescence measured at 680 nm (PSII chlorophyll *a*). The samples were illuminated at 60 s to induce a transition to state 1, then the light switched off at 200 s. The spectra from the two Kn mutants are very similar. The distinct wild-type spectrum is labelled

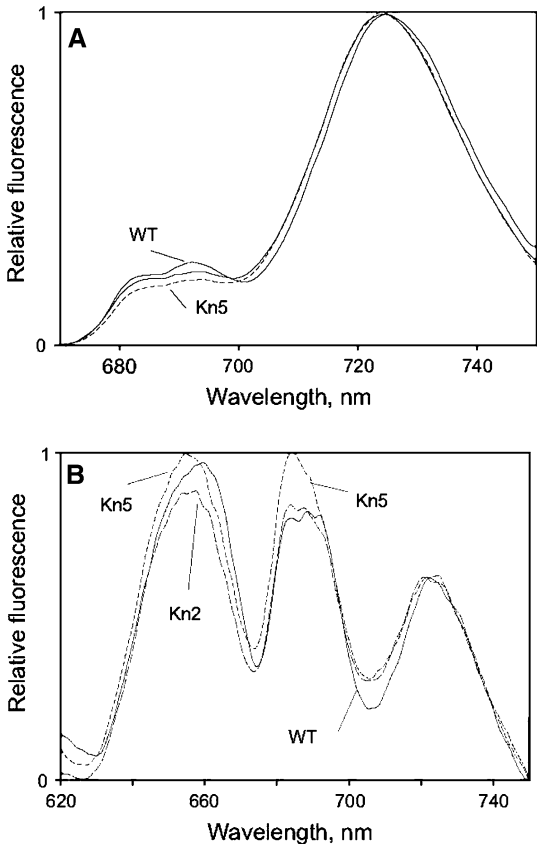

Fig. 6 Seventy-seven Kelvin fluorescence emission spectroscopy of dark-adapted (state 2) wild-type *Synechocystis* PCC 6803 (*solid line*) and the *psbZ* mutants Kn2 (*thick dashed line*) and Kn5 (*thin dashed line*). (**A**) Excitation at 435 nm or (**B**) at 600 nm. Both spectra are normalised to the PSI maximum at ~725 nm

sequently, the interaction of antenna systems with the PSII core complex will be different in prokaryotes and eukaryotes. Swiatek et al. (2001) proposed that PsbZ might play a role in the interaction between the LHC and PSII. If this is the case, it is plausible that state transitions in the *psbZ* mutants may give a difference in light-harvesting between eukaryotes and prokaryotes. Therefore, we examined whether the loss of PsbZ in *Synechocystis* PCC 6803 had any effect on cyanobacterial state transitions (Fig. 5).

Dark-adapted wild-type cells (state 2—PBS associated with PSI) produced a stable level of relative fluorescence. Following illumination of the cells for 60 s there was a slight, but temporary, decrease in the relative fluorescence, resulting from the rapid oxidation of the plastoquinone pool. This was followed by a rise in fluorescence as the PBS moves from PSI to PSII (transition to state 1), equilibrating at 200 s. When the cells were returned to the dark after 140 s of illumination, the sharp increase in relative fluorescence was followed by a gradual decrease as the PBS return to PSI. Examination of the mutants Kn2 and Kn5 produced the same overall pattern, confirming that in the absence of PsbZ cells were still capable of performing state transitions. The decrease in fluorescence observed in wild type at 60 s is absent from both of the mutant spectra, indicating that the plastoquinone pool was not as reduced (i.e. more oxidised) in the mutant cells, relative to wild type. This was typical of a shift in the ratio of delivery of excitation energy between PSII and PSI, consistent with previous observations.

To follow the energy transfer to and between the photosystems, 77 K fluorescence emission spectroscopy was performed on wild type and *psbZ* mutant cells grown under low-light conditions (3 μmol photons m^{-2} s^{-1}) and adjusted to 10 μM chlorophyll. Figure 6 shows the resulting spectra for dark-adapted (state 2) cells following excitation at 435 nm (chlorophyll *a*; Fig. 6A) and 600 nm (allophycocyanin; Fig. 6B). Examination of the 435 nm fluorescence emission spectra revealed a decrease in the peaks at 685 nm (fluorescence of chlorophyll molecules associated with PSII) and 695 nm (fluorescence of chlorophyll molecules specifically associated with CP47). The reduction was greatest for the Kn2 mutant. The same fluorescence measurements were performed for the cells in state 1. Excitation at 435 and 600 nm confirmed the findings from the state 2 measurements (data not shown). Thus, while energy transfer between PSII and PSI is not compromised in the mutants, the absence of PsbZ appears to result in an increase in the antenna compliment relative to wild type.

Discussion

The PsbZ protein has been shown to be an intrinsic component of PSII of higher plants and *C. reinhardtii* (Swiatek

et al. 2001). In a study with tobacco, PsbZ was connected to CP26 (Ruf et al. 2000), a component of plant light-harvesting antenna of PSII. However, the presence of PsbZ was also revealed in cyanobacterial PSII (Kashino et al. 2002), whose light-harvesting complex lacks any component resembling CP26. This makes *Synechocystis* PCC 6803 an interesting model to study the role of PsbZ. Here, we have employed reverse genetics to unravel the function of the PsbZ protein in *Synechocystis* PCC 6803.

Although *psbZ* mutants of *Synechocystis* PCC 6803 were capable of photoautotrophic growth, the growth rates were impaired when compared to wild type. Surprisingly, in contrast to wild type, the *psbZ* mutants were not capable of growth in the presence of glucose. Photomixotrophic growth conditions are known to produce excess photooxidative damage (Hihara and Sonoike 2001). Such damage to the thylakoid membrane can usually be avoided by dispersal of the excess excitation energy via photoprotection mechanisms, which are intimately linked through the xanthophyll cycle in plants. There are a number of photosynthetic organisms, like cyanobacteria, that do not perform the xanthophyll cycle, that do not accumulate zeaxanthin, or lack both processes (Demmig-Adams 1990; Horton 1996). However, these organisms still express PsbZ and are capable of photoprotection. It is, therefore, possible that PsbZ plays a role in protection against photoinhibition. It is interesting that a similar role has been proposed for *psbK* following the isolation of glucose-sensitive *psbK*-distruptants of *Synechocystis* PCC 6803 (Kobayashi et al. 2005).

To reveal the reasons for reduced growth in mutants we analysed the partial reactions of photosynthetic electron transfer. EPR spectroscopic analysis of the *psbZ* mutants showed a normal $Y_D^{\cdot}$ spectrum. Further, the oxygen-evolution capacity of PSII was similar for wild type and mutants, confirming that the absence of PsbZ does not significantly affect the assembly of the PSII reaction centre core and that PsbZ is not required for the oxidation of Y_D via $P680^+$. Examination of PSI function, on the other hand, showed some abnormality. However, reasons for the significantly accelerated post-illumination reduction of $P700^+$ are difficult to evaluate: in addition to the linear PSI electron transfer and the PSI back reaction, the water–water cycle (Makino et al. 2002), the NDH-1 (Zhang et al. 2004) and a PGR5-dependent cyclic electron pathway (Munekage et al. 2002) may be involved. It is possible that these accelerated electron transfer pathways, in the absence of PsbZ, results in strong protection around PSI. Under normal light conditions this change has little impact; however, under low-light conditions when linear electron transfer will be slower and NADPH production reduced, the influence of such an increase in protection is expected to be more conspicuous.

Consistent with the tobacco mutants, the *Synechocystis* PCC 6803 *psbZ* mutants presented a phenotype similar to wild type under standard growth conditions, but showed impaired growth at low irradiances. In one case it was concluded that the tobacco *psbZ* knockout had reduced light-harvesting capacity relative to wild type. These plants were shown to have reduced amounts of the CP26 light-harvesting antenna component (Ruf et al. 2000). In contrast, Baena-Gonzalez et al. (2001) showed that, under low-light conditions, where the mutant phenotype became obvious, CP26 clearly accumulates in plants lacking PsbZ. In cyanobacteria, the light-harvesting antenna of PSII differs from that of plants (Sidler 1994) and lacks any subunits homologous to CP26. Importantly, despite the retarded growth at low irradiance, no differences in the percentage decrease of PSII activities between the *psbZ* mutants and the wild type was observed when the measurements were performed at limiting light intensities. Further, comparison with the corresponding activities under saturating light conditions, argues against impairment of the efficiency of energy transfer from the light-harvesting antenna to the reaction centre in the absence of PsbZ in *Synechocystis* PCC 6803. Similarly, energy transfer to and between the photosystems takes place in the same way in the mutants and the wild type.

Retarded growth at low irradiances of cyanobacterial *psaE* (Zhao et al. 1993) and *ndhF* (Schluchter et al. 1993) mutants has been suggested to result from an inability to produce enough ATP under those conditions. Interestingly, the tobacco) *psbZ* mutant (Baena-Gonzalez et al. 2001) had defects in electron transfer routes alternative to linear photosynthetic electron transfer (Bendall and Manasse 1995), which may result in impairment of growth under sub-optimal conditions. The slight change in the PSII/PSI ratio in favour of PSI in the *Synechocystis* PCC 6803 *psbZ* mutants may reflect an adjustment of the ATP/NADPH ratio necessary for mutant growth under sub-optimal conditions such as low-light intensities. Acclimation of plants to low light conditions is known to involve reduction of the PSII/PSI ratio (Anderson et al. 1995) aiming at adjustment of the ATP/NADPH ratio through cyclic electron flow (Finazzi et al. 1999).

In summary, we present the characterisation of *Synechocystis* PCC 6803 *psbZ* mutants supporting a model in which PsbZ functions in the regulation of electron transfer activity through the two photosystems, and particularly in dissecting the electron flow in PSI.

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