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pH-dependent photoautotrophic growth of specific photosystem II mutants lacking lumenal extrinsic polypeptides in *Synechocystis* PCC 6803

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Abstract The removal of either the PsbU or PsbV protein has been investigated in a cyanobacterial ΔPsbO strain and in mutants carrying deletions or substitutions in lumen-exposed domains of CP47. These experiments have demonstrated a functional interaction between the PsbU protein and photosystem II (PSII) in the absence of the PsbO subunit. The control:ΔPsbO:ΔPsbU strain assembled PSII centers at pH 7.5 but did not evolve oxygen; however, photoautotrophic growth was restored at pH 10.0. In addition, several CP47 mutants, lacking extrinsic proteins, were obligate photoheterotrophs at pH 7.5 but photoautotrophic at pH 10.0, whereas other strains remained photoheterotrophs at alkaline pH.

Key words: CP47; Cytochrome *c*-550; Photosystem II; PsbU; *Synechocystis*

1. Introduction

In oxygenic photosynthesis extrinsic hydrophilic proteins are associated with the oxygen-evolving complex on the lumenal face of photosystem II (PSII) [1,2]. In cyanobacteria three proteins, PsbO, PsbU and PsbV, encoded by the *psbO*, *psbU* and *psbV* genes, respectively, have been identified and based on their respective gene sequences these correspond to ~27, 10 and 15 kDa polypeptides in *Synechocystis* PCC 6803 [3–6]. Here the effect of removing PsbU in a strain lacking PsbO, and the consequences of removing either PsbU or PsbV in mutants carrying specific mutations in the chlorophyll *a*-binding protein CP47, have been investigated.

The CP47 protein has six membrane-spanning helices and serves as a core antenna directing excitation energy to the PSII reaction center [7]. In addition, CP47 has three hydrophilic domains protruding into the thylakoid lumen: loop A (~56 amino acids) spans between helices 1 and 2, loop C (~37 amino acids) joins helices 3 and 4 and loop E (~190 amino acids) joins helices 5 and 6 [7,8]. The C-terminal half of loop E between Glu-364 and Asp-440 has been shown to cross-link to the N-terminal 76 amino acids of PsbO and there are extensive additional biochemical data supporting a close interaction between PsbO and CP47 [2,8,9].

Structural data for PSII are available from *Thermosynechococcus* (formally *Synechococcus*) *elongatus* and *T. vulcanus*, at a resolution of 3.8 Å and 3.7 Å, respectively, and these data support a model where loop E of CP47 is adjacent to PsbO, and PsbV is close to the corresponding loop of the core antenna CP43 protein [5,6]. In this arrangement PsbO and PsbV are linked by the PsbU protein [6]. It is known that the removal of either PsbO or PsbV results in fewer assembled PSII centers in the thylakoid membrane, a susceptibility of oxygen evolution to photoinactivation, and a strict dependence on Ca^{2+} and Cl^- for photoautotrophic growth [10–16]. In addition, the absence of PsbO in *Synechocystis* PCC 6803 slows the kinetics of oxygen release and results in an increase in the relative quantum yield for photoactivation of the oxygen-evolving complex [17–19]. The PsbV protein contains a *c*-type heme, although the physiological role of this heme has yet to be identified [20]. However, the mutant lacking both PsbO and PsbV has been found to be an obligate photoheterotroph [21]. In contrast, the physiological requirement of PsbU is less stringent, although its absence in vivo resulted in slowed growth in the absence of Ca^{2+} and Cl^- and oxygen evolution was reduced by up to 20% when compared to the wild type [22–24].

In *Synechocystis* PCC 6803 the double deletion mutant lacking both *psbU* and *psbV* had a similar phenotype to the *psbV* deletion mutant [22]. This result was in agreement with earlier reconstitution studies performed with isolated PSII complexes from *T. vulcanus* [4]. In addition, a preliminary experiment, in which *psbO* and *psbU* had been inactivated, produced a *Synechocystis* PCC 6803 strain that exhibited severely impaired photoautotrophic growth [25]. In this report a more detailed assessment of this strain has been completed. In addition, a novel pH dependence on photoautotrophic growth in cells lacking both PsbO and PsbU, and several strains carrying mutations in loop E of CP47 and lacking the PsbV protein, is also described.

2. Materials and methods

2.1. Growth of cyanobacterial strains

Synechocystis PCC 6803 cultures were maintained on BG-11 plates in the presence of 5 mM glucose and 20 μM atrazine with the appropriate antibiotics. When included in either solid or liquid media, chloramphenicol was present at a concentration of 15 μg/ml and erythromycin, kanamycin and spectinomycin were present at 25 μg/ml. The control strain contained a kanamycin-resistance cassette located downstream of the *psbB* gene although it was identical to the wild type except for the antibiotic-resistant phenotype [26]. The BG-

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11 solid media were supplemented with 10 mM TES–NaOH (pH 8.2) and 0.3% sodium thiosulfate [27]. For oxygen evolution and herbicide-binding measurements, liquid cultures were grown in BG-11 media containing either 25 mM HEPES (pH 7.5) or 25 mM CAPS (pH 10.0). However, for growth curve experiments starter cultures were grown in un-buffered BG-11 and then transferred into buffered media at the start of the growth measurement. Growth curve measurements were performed in at least three independent experiments for all strains studied, and the photoautotrophic doubling times were reproducible to within 15% of the average. Where appropriate, 5 mM glucose was added and all cultures were maintained at 30°C under constant illumination of 25 μE m^{-2} s^{-1} [16].

2.2. Construction of strains lacking extrinsic proteins

To remove the PsbO protein strains were transformed with a plasmid containing a *psbO* gene that had been interrupted by a spectinomycin-resistance cassette [16]. To construct strains lacking PsbU a plasmid containing the *psbU* gene, interrupted by a chloramphenicol-resistance cassette derived from pBR325, was used to transform the cells [25,28,29]. For strains lacking PsbV, the cells were transformed with a plasmid in which an intragenic 0.4 kb *psbV* fragment had been replaced by an erythromycin-resistance cassette [14,16]. The control:ΔPsbO:ΔPsbU strain was constructed from control:ΔPsbO cells, although identical results were obtained when a similar strain was constructed from the control:ΔPsbU mutant (data not shown).

2.3. Measurement of the relative level of assembled PSII centers

The relative level of assembled PSII centers was estimated on a chlorophyll *a* (hereafter referred to as chlorophyll) basis by herbicide-binding assays employing [^{14}C]atrazine as described in [16]. The specific activity of the [^{14}C]atrazine was 17.1 mCi/mmol, and the chlorophyll concentration was 50 μg/ml, except when the relative amount of assembled PSII centers was below 50% of the control level where the chlorophyll concentration was 100 μg/ml.

2.4. Oxygen evolution assays

A Clark-type electrode was used to measure oxygen evolution at 30°C. Measurements were made in buffered BG-11 at 10 μg/ml chlorophyll. Actinic light was provided by a Schott 1500 light source providing 6.5 mE m^{-2} s^{-1} light passed through a Melles Griot OG 515 sharp cut-off yellow glass filter. The electron acceptors were 1.0 mM $K_3Fe(CN)_6$ and 0.2 mM 2,5-dimethyl-*p*-benzoquinone (DMBQ) for PSII-specific electron transport and 15 mM $NaHCO_3$ for whole-chain measurements. The chlorophyll determinations were performed according to [30].

3. Results

3.1. Verification of mutant strains

To conduct this study six mutants were constructed from either our original control:ΔPsbO and control:ΔPsbV strains or our pre-existing strains, Δ(R384-V392), Δ(R384-V392):ΔPsbO and Δ(G429-T436), that each carry deletions in loop E of CP47 [16]. The successful inactivation of *psbU* in each of these strains and the creation of the control:ΔPsbO:ΔPsbV mutant was confirmed by Southern analysis and the complete segregation of these mutants is shown in Fig. 1. All other strains have been previously described [16,23,31].

3.2. The control:ΔPsbO:ΔPsbU mutant exhibits pH-dependent photoautotrophic growth

Removal of PsbU, in a control:ΔPsbO strain, produced a mutant with impaired photoautotrophic growth when grown in un-buffered BG-11 media. However, PSII centers assembled in this strain and oxygen evolution was retained [25]. Based on the observations that *Synechocystis* PCC 6803 cultures grew better on BG-11 plates buffered to pH 8.2 than on un-buffered plates (typically $<$pH 7.5) and that photoautotrophic growth frequently resulted in liquid media experiencing a 1 or 2 pH unit shift towards alkaline pH (typ-

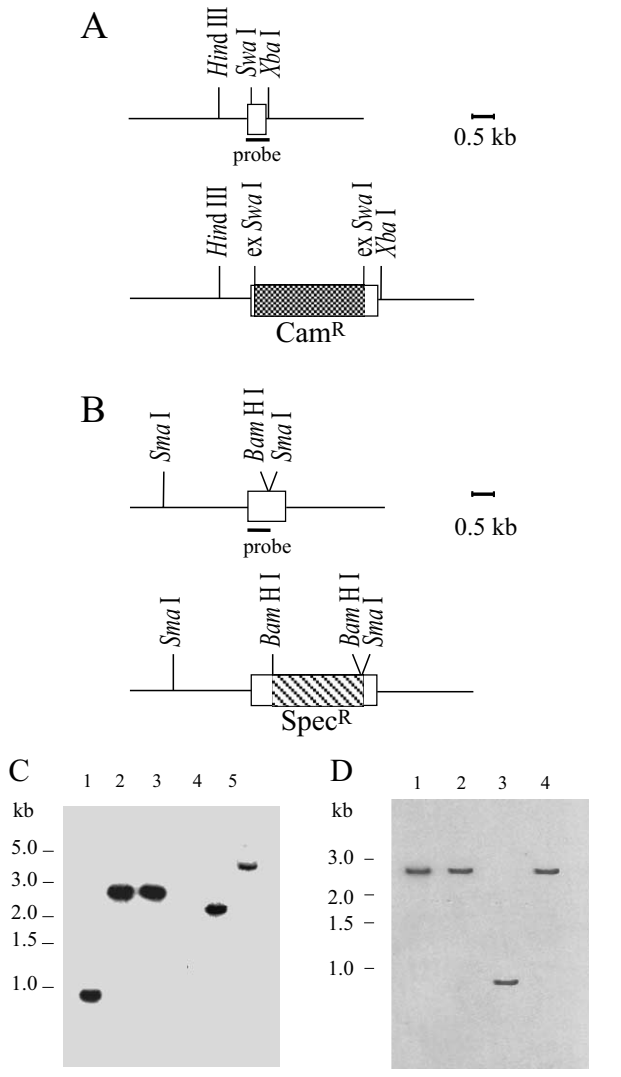

Fig. 1. Construction of mutants lacking PSII extrinsic proteins. A: Restriction map of the *psbU* region in the *Synechocystis* PCC 6803 genome with or without the insertion of a 2.0 kb chloramphenicol-resistance marker (Cam^R) inserted at a *Swa*I site 160 bp from the initial base of the start codon. The position of the intragenic *psbU* probe used for the Southern blots in panels C and D is shown. B: Restriction map of the *psbO* region in the *Synechocystis* PCC 6803 genome with or without the insertion of a 2.0 kb spectinomycin-resistance marker (Spec^R) inserted at a *Bam*HI site 558 bp from the initial base of the start codon. The position of the *psbO* probe used for lanes 4 and 5 of the Southern blot in panel C is indicated. C: Lanes 1–3, Southern blot of the control:ΔPsbO (lane 1), control:ΔPsbO:ΔPsbU (lane 2) and control:ΔPsbU:ΔPsbV (lane 3) mutants. The *Synechocystis* PCC 6803 genomic DNA was cut with *Hin*dIII and *Xba*I and probed with a 496 bp *Bam*HI/*Xba*I intragenic fragment where the *Bam*HI site had been introduced when *psbU* was cloned into pUC 19 [25]. Lanes 4 and 5, Southern blot of the control:ΔPsbV (lane 4) and control:ΔPsbO:ΔPsbV (lane 5) strains. The genomic DNA was cut with *Sma*I and probed with a polymerase chain reaction-generated 541 bp *Eco*RI/*Bam*HI intragenic *psbO* fragment [16]. D: Lanes 1–4, Southern blot of the Δ(R384-V392):ΔPsbU (lane 1), Δ(G429-T436):ΔPsbU (lane 2), Δ(R384-V392):ΔPsbO (lane 3) and Δ(R384-V392):ΔPsbO:ΔPsbU (lane 4) strains. The genomic DNA was cut with *Hin*dIII and *Xba*I and probed with the 496 bp *Bam*HI/*Xba*I *psbU* intragenic fragment.

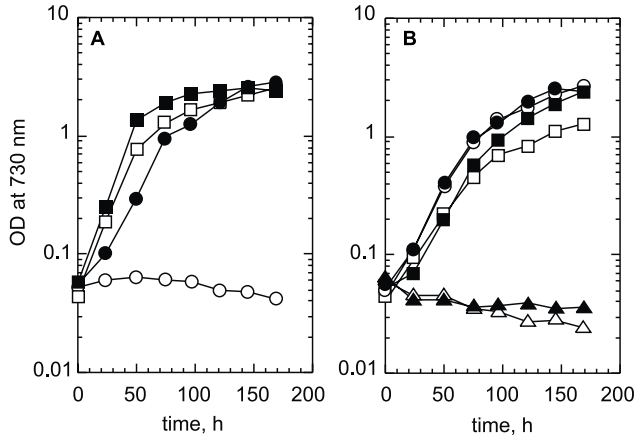

Fig. 2. Alkaline pH restores photoautotrophic growth in the control:ΔPsbO:ΔPsbU mutant. Photoautotrophic growth of *Synechocystis* PCC 6803 strains as measured by the optical density at 730 nm. Open symbols, pH 7.5; closed symbols, pH 10.0. A: Control (squares), control:ΔPsbO:ΔPsbU (circles). B: Control:ΔPsbO (squares), control:ΔPsbU:ΔPsbV (circles) and control:ΔPsbO:ΔPsbV (triangles).

ically ~pH 8.0 to >pH 9.0), we investigated the effect of pH 7.5 and pH 10.0 on the control:ΔPsbO:ΔPsbU mutant.

In Fig. 2A the photoautotrophic growth of the control remained at ~11–12 h at either pH 7.5 or 10.0. However, growth was eliminated at pH 7.5 in the control:ΔPsbO:ΔPsbU mutant; but photoautotrophic growth was restored at pH 10.0, following an initial lag, to a doubling time of ~15 h. In contrast, no major pH effect was observed on photoautotrophic growth in the control:ΔPsbO strain in Fig. 2B, which exhibited a doubling time of ~18 h, nor in the control:ΔPsbU strain, which had a doubling time of ~14 h (data not shown). Furthermore, in Fig. 2B, control:ΔPsbU:ΔPsbV cells grew with a similar doubling time of ~16 h at each pH while the control:ΔPsbO:ΔPsbV strain remained unable to grow photoautotrophically at either pH 7.5 or 10.0.

In Table 1, control:ΔPsbO:ΔPsbU cells, grown photoheterotrophically at pH 7.5, did not evolve oxygen. In addition, control:ΔPsbO:ΔPsbU cells grown in un-buffered BG-11 lost the ability to evolve oxygen when transferred to pH 7.5 media for a further 24 h (data not shown). Furthermore, although at pH 7.5 control:ΔPsbO cells evolved oxygen at 13% of the control rate, when supported by $K_3Fe(CN)_6$, these cells remained photoautotrophic. This rate is ~25% of the rate of oxygen evolution observed for these cells in un-buffered medium [10–12,16]. However, the control:ΔPsbO:ΔPsbU cells, when grown at pH 10.0, exhibited oxygen evolution that was 30% of the control rate supported by $K_3Fe(CN)_6$, and 74% of the control rate with $NaHCO_3$ as the added electron acceptor. Additionally, oxygen evolution, using CO_2 as the terminal electron acceptor, was less susceptible to photoinactivation than the PSII-specific reaction: essentially remaining stable for over 2 min, while the PSII reaction was inactivated during <100 s of continuous actinic illumination.

The control strain was found to have a chlorophyll/PSII ratio of ~460 at pH 7.5 and ~450 at pH 10.0, indicating that the number of assembled PSII centers, when grown at either pH, remained essentially unchanged. In addition, the number of centers measured in the control:ΔPsbU:ΔPsbV mutant was similar at each pH, although the observed rate of oxygen evolution in the presence of $K_3Fe(CN)_6$ was increased >two-fold at pH 10.0. In the control:ΔPsbO:ΔPsbV mutant a chlorophyll/PSII ratio of 1340 was measured at pH 7.5, corresponding to a PSII level that was 34% of the control, whereas at pH 10.0 the number of assembled centers fell to below the level detectable by the assay (typically <20% of the control level in un-buffered BG-11 [16]). In contrast, the control:ΔPsbO:ΔsbU mutant exhibited a chlorophyll/PSII ratio of 830 at pH 7.5 that was reduced to 500 at pH 10.0, thereby suggesting that PSII was more stable at alkaline pH, and a similar result was obtained with the control:ΔPsbO strain.

3.3. *Specific mutations in loop E of CP47 combined with the removal of PsbU exhibit pH-dependent photoautotrophic growth*

In Fig. 3A,B the effect of removing PsbU in two mutants carrying deletions between either Arg-384 and Val-392 or between Gly-429 and Thr-436 in loop E of CP47 is shown. The Δ(R384-V392):ΔPsbU strain remained photoautotrophic with a doubling time of ~15 h at both pH 7.5 and 10.0. Similarly,

Table 1
The effect of pH on oxygen evolution and PSII assembly in *Synechocystis* PCC 6803 strains lacking different combinations of extrinsic proteins

Strain	pH 7.5			pH 10.0		
	Rate of oxygen evolution[a]		Relative level of PSII[b]	Rate of oxygen evolution[a]		Relative level of PSII[b]
	HCO_3^- [c]	$K_3Fe(CN)_6$[d]		HCO_3^- [c]	$K_3Fe(CN)_6$[d]	
Control	1.00	1.00	1.00	1.00	1.00	1.00
Control:ΔPsbO	0.41[e]	0.13[f]	0.57	0.75	0.29[f]	0.91
Control:ΔPsbO:ΔPsbU	0.00	0.00	0.55	0.74	0.30[f]	0.90
Control:ΔPsbU:ΔPsbV	0.72[e]	0.27[f]	0.54	0.78	0.57[f]	0.54
Control:ΔPsbO:ΔPsbV	0.00	0.00	0.34	0.17[f]	0.00	n.d.[g]

These data represent the average of three to five independent measurements and were reproducible to within 15% of the average.
[a]The rate of oxygen evolution was stable for at least 2 min of actinic illumination unless otherwise stated.
[b]These values for PSII abundance are normalized to a chlorophyll/PSII ratio of 460 at pH 7.5 and 450 at pH 10.0 and have been determined by [^{14}C]atrazine binding. The K_d for atrazine binding was between 250 and 333 nM and no dependence on pH was observed.
[c]Oxygen evolution was supported by CO_2 and normalized to the control rates of 266 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 7.5 and 276 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 10.0.
[d]Oxygen evolution was supported by $K_3Fe(CN)_6$ and DMBQ and normalized to the control rates of 557 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 7.5 and 490 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 10.0.
[e]The initial rate of oxygen evolution was not sustained and decreased by ~50% during 3 min of actinic illumination.
[f]The rate of oxygen evolution was completely photoinactivated in <100 s of actinic illumination.
[g]No measurable [^{14}C]atrazine binding could be detected.

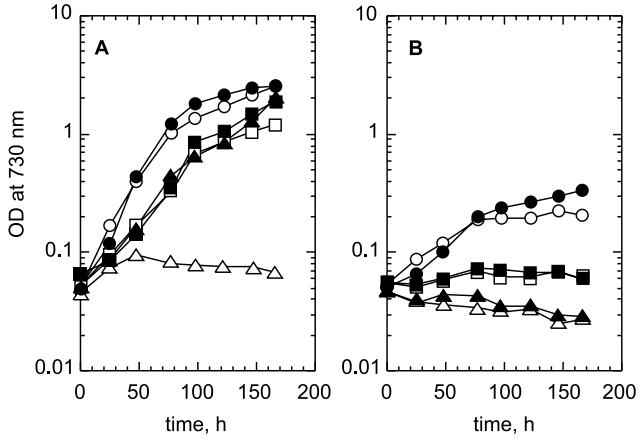

Fig. 3. Alkaline pH restores photoautotrophic growth in specific mutants carrying deletions in loop E of CP47 in the absence of the PsbU protein. Photoautotrophic growth of *Synechocystis* PCC 6803 strains as measured by the optical density at 730 nm. Open symbols, pH 7.5; closed symbols, pH 10.0. A: Δ(R384-V392):ΔPsbO (squares), Δ(R384-V392):ΔPsbU (circles) and Δ(R384-V392):ΔPsbO:ΔPsbU (triangles). B: Δ(G429-T436):ΔPsbO (squares), Δ(G429-T436):ΔPsbU (circles) and Δ(G429-T436):ΔPsbV (triangles).

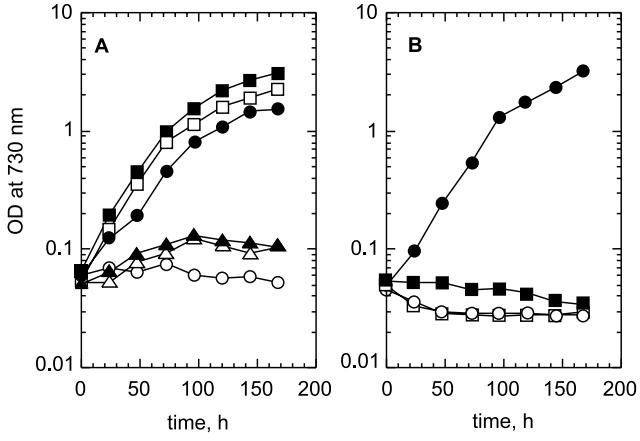

Fig. 4. Alkaline pH restores photoautotrophic growth in specific mutants carrying mutations in lumen-exposed domains of CP47 in the absence of the PsbV protein. Photoautotrophic growth of *Synechocystis* PCC 6803 strains as measured by the optical density at 730 nm. Open symbols, pH 7.5; closed symbols, pH 10.0. A: Control:ΔPsbV (squares), Δ(R384-V392):ΔPsbV (circles), Δ(E184-A188):ΔPsbV (triangles). B: F363R:ΔPsbV (squares) and E364Q:ΔPsbV (circles).

no pH dependence was observed in the Δ(R384-V392):ΔPsbO strain which had a doubling time of ~25 h and oxygen evolution in the mutant was readily photoinactivated (see Table 2). However, although the Δ(G429-T436):ΔPsbU cells were photoautotrophic at both pH 7.5 and 10.0, the growth was not sustained and entered a stationary phase with an $OD_{730\,nm}$ of 0.2–0.3 after 75 h. In this strain oxygen evolution was observed at pH 10.0 when supported by $K_3Fe(CN)_6$ but this was also rapidly photoinactivated. Higher rates of oxygen evolution were obtained in this mutant when HCO_3^- was added, and at pH 10.0 the observed rate was 84% of the control even though only 57% of the control level of PSII centers was detected by herbicide binding. This suggests that the number of centers present was sufficient to support high rates of electron transport to CO_2 when saturating actinic light was provided.

Fewer centers and lower rates of oxygen evolution were observed for Δ(G429-T436):ΔPsbU at pH 7.5, whereas the photoautotrophic Δ(R384-V392):ΔPsbU mutant assembled active centers at both pH 7.5 and 10.0. In agreement with results obtained in un-buffered BG-11, the Δ(R384-V392):ΔPsbO strain assembled impaired centers and the comparison between this mutant and the Δ(G429-T436):ΔPsbU cells indicates that there is not always a direct correlation between oxygen evolution activity, under saturating actinic light, and photoautotrophic growth. Additionally, in the Δ(G429-T436):ΔPsbO and Δ(G429-T436):ΔPsbV strains, pH 10.0 medium was unable to restore photoautotrophic growth, and this correlated with an absence of assembled PSII centers in these mutants (data not shown).

In Fig. 3A and Table 2 the effect of pH on the Δ(R384-V392):ΔPsbO:ΔPsbU strain was also examined. The removal of the PsbO and PsbU proteins produced an obligate photoheterotrophic strain at pH 7.5 and photoautotrophic growth

Table 2
The effect of pH on oxygen evolution and PSII assembly in *Synechocystis* PCC 6803 strains lacking different combinations of the PsbO and PsbU extrinsic proteins and carrying mutations in CP47[a]

Strain	pH 7.5			pH 10.0		
	Rate of oxygen evolution[b]		Relative level of PSII[c]	Rate of oxygen evolution[b]		Relative level of PSII[c]
	HCO_3^- [d]	$K_3Fe(CN)_6$[e]		HCO_3^- [d]	$K_3Fe(CN)_6$[e]	
Control	1.00	1.00	1.00	1.00	1.00	1.00
Δ(R384-V392):ΔPsbO	0.43[f]	0.25[f]	0.59	0.26[f]	0.00	0.59
Δ(R384-V392):ΔPsbU	0.71	0.41[f]	0.73	0.89	0.56[f]	0.57
Δ(G429-T436):ΔPsbU	0.40[f]	0.00	0.29	0.84[f]	0.47[f]	0.57
Δ(R384-V392):ΔPsbO:ΔPsbU	0.18[f]	0.09[f]	0.38	0.32[f]	0.00	0.38

These data represent the average of three to five independent measurements and were reproducible to within 15% of the average.
[a]The data have been normalized to the control values in Table 1.
[b]The rate of oxygen evolution was stable for at least 2 min of actinic illumination unless otherwise stated.
[c]These values for PSII abundance are normalized to a chlorophyll/PSII ratio of 460 at pH 7.5 and 450 at pH 10.0 and have been determined by [^{14}C]atrazine binding. The K_d for atrazine binding was between 250 and 333 nM and no dependence on pH was observed.
[d]Oxygen evolution was supported by CO_2 and normalized to the control rates of 266 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 7.5 and 276 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 10.0.
[e]Oxygen evolution was supported by $K_3Fe(CN)_6$ and DMBQ and normalized to the control rates of 557 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 7.5 and 490 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 10.0.
[f]The rate of oxygen evolution was completely photoinactivated in <100 s of actinic illumination.

was restored at pH 10.0, in this case with a doubling time of ~25 h; but, unlike the control:ΔPsbO:ΔPsbU mutant, alkaline pH did not alter the apparent number of assembled PSII centers. However, in this strain a small amount of oxygen evolution was observed at pH 7.5 in the presence of HCO_3^-, whereas no rate was detected in the control:ΔPsbO:ΔPsbU cells under these conditions. In contrast, no oxygen evolution was detected at pH 10.0 when supported by $K_3Fe(CN)_6$, and oxygen evolution activity in the presence of HCO_3^- also remained unstable in the Δ(R384-V392):ΔPsbO:ΔPsbU strain.

3.4. Alkaline pH restores photoautotrophic growth to strains carrying mutations in loop E of CP47 and lacking the PsbV protein

In Fig. 4A photoautotrophic growth was restored in the Δ(R384-V392):ΔPsbV mutant at pH 10.0 with a doubling time of ~26 h, whereas the mutant Δ(E184-A188):ΔPsbV, which carries a deletion in loop C of CP47, exhibited severely limited photoautotrophic growth at either pH 7.5 or 10.0. These compare with a photoautotrophic doubling time of ~18 h at pH 7.5, and ~16 h at pH 10, in the control:ΔPsbV strain. In Table 3 oxygen evolution was inactivated at both pH 7.5 and 10.0 in the Δ(R384-V392):ΔPsbV mutant even though PSII centers were assembled at 37% of the control level. Strikingly, in the Δ(E184-A188):ΔPsbV strain, oxygen evolution was again supported at pH 10.0 even though photoautotrophic growth was not.

We also investigated the effect of pH on the two strains F363R:ΔPsbV and E364Q:ΔPsbV that possess adjacent single amino acid substitutions. This pair was selected since neither was photoautotrophic in un-buffered BG-11 media [16,23]. The pH 10.0 growth medium only supported photoautotrophic growth in E364Q:ΔPsbV cells, with a doubling time of 21 h, and in fact no assembly of PSII centers was detected in the F363R:ΔPsbV strain. However, oxygen evolution rates in E364Q:ΔPsbV were similar to those obtained in the control:ΔPsbV strain but, as seen for the control:ΔPsbO:ΔPsbU cells, more centers were assembled at pH 10 than at pH 7.5. It was also evident that, along with the control:ΔPsbO:ΔPsbU strain, oxygen evolution supported by HCO_3^- was more stable than PSII activity supported by $K_3Fe(CN)_6$ in both control:ΔPsbV and E364Q:ΔPsbV cells. This was also observed for several other mutants in Tables 1 and 2.

4. Discussion

Studies on PSII mutants in *Synechocystis* PCC 6803 routinely use BG-11 medium for cell culturing. Normally BG-11 medium is ~pH 7.5–8.0; however, we have discovered that several photoheterotrophic mutants that lack one or more hydrophilic extrinsic proteins are in fact photoautotrophic when grown at pH 10.0. The observation of pH-conditional photoautotrophic growth suggests that a metabolic limitation, imposed by the pH of the external medium, may result in inactivation of PSII at pH < 8.0 in these strains. The pH-dependent phenotype was observed when the PsbU protein was absent in either the control:ΔPsbO or the Δ(R384-V392):ΔPsbO genetic backgrounds. Since the PSII centers in the control:ΔPsbU:ΔPsbO strain also lost their ability to evolve oxygen when grown at pH 7.5, these results demonstrate a functional interaction between PsbU and PSII in the absence of PsbO. Previous reconstitution studies, with salt-washed PSII particles obtained from *T. vulcanus*, had indicated that PsbU was unable to rebind to the PSII complex in the absence of PsbO [4]. It is therefore possible that in vivo a weakened binding of PsbU persists in the absence of PsbO that is able to stabilize oxygen evolution and maintain the capacity for photoautotrophic growth at pH 10.0.

Short segment deletions have been introduced into hydrophilic domains of CP47 to identify sub-domains that are important in PSII assembly and function [16,26,31–33]. As part of these studies the removal of PsbO and PsbV was examined in the Δ(R384-V392) and Δ(G429-T436) strains. The Δ(R384-V392):ΔPsbO mutant was photoautotrophic; however, the Δ(G429-T436):ΔPsbO, Δ(R384-V392):ΔPsbV and Δ(G429-T436):ΔPsbV strains were obligate photoheterotrophs [16]. The removal of PsbU in Δ(G429-T436) cells produced a strain that exhibited impaired photoautotrophic growth at both pH

Table 3
The effect of pH on oxygen evolution and PSII assembly in *Synechocystis* PCC 6803 strains lacking the PsbV extrinsic protein and carrying mutations in CP47[a]

Strain	pH 7.5			pH 10.0		
	Rate of oxygen evolution[b]		Relative level of PSII[c]	Rate of oxygen evolution[b]		Relative level of PSII[c]
	HCO_3^- [d]	$K_3Fe(CN)_6$[e]		HCO_3^- [d]	$K_3Fe(CN)_6$[e]	
Control	1.00	1.00	1.00	1.00	1.00	1.00
Δ(R384-V392):ΔPsbV	0.00	0.00	0.37	0.14[f]	0.00	0.37
Δ(E184-A188):ΔPsbV	0.07[f]	0.00	0.30	0.95[f]	0.24[f]	0.31
F363R:ΔPsbV	0.00	0.00	n.d.[g]	0.00	0.00	n.d.[g]
E364Q:ΔPsbV	0.85[h]	0.37[f]	0.35	0.89	0.43[f]	0.58
Control:ΔPsbV	0.95[h]	0.24[f]	0.50	0.95	0.52[f]	0.51

These data represent the average of three to five independent measurements and were reproducible to within 15% of the average.
[a]The data have been normalized to the control values in Table 1.
[b]The rate of oxygen evolution was stable for at least 2 min of actinic illumination unless otherwise stated.
[c]These values for PSII abundance are normalized to a chlorophyll/PSII ratio of 460 at pH 7.5 and 450 at pH 10.0 and have been determined by [^{14}C]atrazine binding. The K_d for atrazine binding was between 250 and 333 nM and no dependence on pH was observed.
[d]Oxygen evolution was supported by CO_2 and normalized to the control rates of 266 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 7.5 and 276 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 10.0.
[e]Oxygen evolution was supported by $K_3Fe(CN)_6$ and DMBQ and normalized to the control rates of 557 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 7.5 and 490 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 10.0.
[f]The rate of oxygen evolution was completely photoinactivated in < 100 s of actinic illumination.
[g]No measurable [^{14}C]atrazine binding could be detected.
[h]The initial rate of oxygen evolution was not sustained and decreased by ~50% during 3 min of actinic illumination.

7.5 and 10.0. However, at pH 10.0 substantial rates of oxygen evolution were detected. Therefore photoheterotrophic growth at pH 10.0 stimulated the capacity for oxygen evolution even though photoautotrophic growth was prevented at this pH. Conversely, in the Δ(R384-V392):ΔPsbO:ΔPsbU and Δ(R384-V392):ΔPsbV mutants alkaline pH restored photoautotrophic growth but did not stabilize oxygen evolution under saturating light. In the 3.7 Å model of cyanobacterial PSII, loop E of CP47 is not in direct contact with PsbV [6]. Therefore indirect protein interactions between these subunits are implied by these data.

We also investigated the specificity of this novel pH dependence in several other strains that contained mutations in lumen-exposed domains of CP47 and which lacked the PsbV protein. In loop E the F363R:ΔPsbV and the E364Q:ΔPsbV pair were investigated along with the Δ(E184-A188):ΔPsbV mutant. In the presence of the extrinsic proteins the F363R and Δ(E184-A188) strains only assemble ~40–50% of the control level of PSII centers while the E364Q strain is similar to the control until the PsbV protein is removed [16,23,31,34]. At pH 10.0 only the E364Q:ΔPsbV mutant exhibited photoautotrophic growth.

The observed pH dependence reported here points to a mechanism for the acclimation of PSII activity in response to the external pH, and the observed specificity suggests that the elucidation of this response will provide novel information on the operation of PSII during environmental conditions that result in pH variation.

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