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ORIGINAL ARTICLE

The PsbJ protein is required for photosystem II activity in centers lacking the PsbO and PsbV lumenal subunits

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Abstract

Photosystem II (PS II) of oxygenic photosynthesis is found in the thylakoid membranes of plastids and cyanobacteria. The mature PS II complex comprises a central core of four membrane proteins that bind the majority of the redox-active cofactors. In cyanobacteria the central core is surrounded by 13 low-molecular-weight (LMW) subunits which each consist of one or two transmembrane helices. Three additional hydrophilic subunits known as PsbO, PsbU and PsbV are found associated with hydrophilic loops belonging to the core proteins protruding into the thylakoid lumen. During biogenesis the majority of the LMW subunits are known to initially associate with individual pre-assembly complexes consisting of one or more of the core proteins; however, the point at which the PsbJ LMW subunit binds to PS II is not known. The majority of models for PS II biogenesis propose that the three extrinsic proteins and PsbJ bind in the final stages of PS II assembly. We have investigated the impact of creating the double mutants ΔPsbJ:ΔPsbO, ΔPsbJ:ΔPsbU and ΔPsbJ:ΔPsbV to investigate potential cooperation between these subunits in the final stages of biogenesis. Our results indicate that PsbJ can bind to PS II in the absence of any one of the extrinsic proteins. However, unlike their respective single mutants, the ΔPsbJ:ΔPsbO and ΔPsbJ:ΔPsbV strains were not photoautotrophic and were unable to support oxygen evolution suggesting a functional oxygen-evolving complex could not assemble in these strains. In contrast, the PS II centers formed in the ΔPsbJ:ΔPsbU strain were capable of photoautotrophic growth and could support oxygen evolution when whole-chain electron transport was supported by the addition of bicarbonate.

Keywords Cyanobacteria · Extrinsic proteins · Photosystem II · PsbJ · PsbO · PsbU · PsbV

Introduction

Photosystem II (PS II) is a multisubunit complex that catalyses the light-driven splitting of water in the thylakoid membrane of oxygenic phototrophs (Shen 2015; Vinyard and Brudvig 2018). High resolution X-ray-derived PS II structures (~ 1.9–2.1 Å) from thermophilic cyanobacteria have shown PS II to be dimeric, with each monomer containing the chlorophyll *a*-binding core antenna proteins CP43 and CP47 together with the D1/D2 reaction center subunits and at least 16 additional polypeptides and 70 cofactors (Umena et al. 2011; Suga et al. 2015, 2017; Kern et al. 2018). Detailed structures have shown conservation of these intrinsic components of PS II between cyanobacteria, algae and higher plants (Ago et al. 2016; Wei et al. 2016).

Thirteen low-molecular-weight (LMW) subunits have been identified on the periphery of each PS II monomer (Ferreira et al. 2004; Umena et al. 2011). Mutagenesis studies have shown the absence of these subunits frequently results in strains with impaired photoautotrophic growth or PS II assembly (Müh et al. 2008; Shi et al. 2012; Luo et al. 2014; Fagerlud et al. 2020). The assembly of PS II involves the formation of different pre-complexes that contain distinct groups of PS II proteins along with multiple assembly factors that are not present in mature PS II centers (Komenda et al. 2012; Mabbitt et al. 2014). Most of the LMW subunits

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have been found to be associated with different pre-complexes (Nickelsen and Rengstl 2013; Eaton-Rye and Sobotka 2017).

The PsbJ subunit has not been identified in any of the assembly pre-complexes; however, in mature PS II centers, PsbJ is a single transmembrane helix that is located near the PsbE and PsbF proteins that comprise the cytochrome b_{559} complex (Heinz et al. 2016; Müh and Zouni 2016). In *Thermosynechococcus elongatus* deletion of PsbJ led to the accumulation of an intermediate monomeric PS II complex which contained the Psb27 and Psb28 assembly factors (Nowaczyk et al. 2012).

In addition to the membrane-spanning subunits of PS II, three hydrophilic extrinsic proteins, PsbO, PsbU and PsbV, are present on the lumenal face of the photosystem (Bricker et al. 2012). The PsbJ protein was absent from a PS II structure obtained by cryo-electron microscopy that accumulated in a *Thermosynechococcus vulcanus* strain in which the *psbV* gene, encoding PsbV, had been deleted (Huang et al. 2021). The ΔPsbV strain accumulated PS II complexes that contained the assembly factor Psb27 and these Psb27-PS II complexes formed dimers which exhibited structural changes compared to mature PS II centers. This included conformational shifts associated with PsbE and PsbF that may have resulted in the loss of PsbJ (Huang et al. 2021). These observations are consistent with late addition of PsbJ to PS II complexes.

In *Synechocystis* sp. PCC 6803 PsbJ is not required for photoautotrophic growth; however, the deletion of PsbJ affected the efficiency of PS II electron flow between the primary and secondary plastoquinone electron acceptors Q_A and Q_B and back electron transfer to the oxidized Mn_4CaO_5 cluster of the oxygen-evolving complex (OEC) (Regel et al. 2001). An additional Q_C-binding site has also been identified in *Thermosynchococcus elongatus* (Guskov et al. 2009). The PsbJ protein, along with the cytochrome b_{559} complex, are located close to the Q_C site and have been suggested to contribute to a second channel allowing exchange of plastoquinone or plastoquinol with the pool of plastoquinone in the thylakoid membrane (Müh and Zouni 2016).

As in the case of PsbJ, the extrinisic proteins are predicted to be added late to the PS II complex (Bricker et al. 2012; Nickelsen and Rengstl 2013). The extrinsic proteins form a stabilising structure around the catalytic Mn_4CaO_5 of the OEC protecting it from bulk reductants and contributing to putative channels associated with the access of substrate H_2O and the egress of O_2 and H^+ (Ho and Styring 2008; Roose et al. 2016).

In *Synechocystis* sp. PCC 6803, strains lacking any one of the PsbO, PsbU or PsbV proteins are able to grow photoautotrophically (Burnap and Sherman 1991; Shen et al. 1995a, 1997) but strains lacking two of these proteins either grow photoautotrophically (ΔPsbU:ΔPsbV mutant; Eaton-Rye et al. 2003), are unable to grow phototrophically (ΔPsbO:ΔPsbV mutant; Shen et al. 1995b) or exhibit conditional growth at elevated pH (ΔPsbO:ΔPsbU mutant; Eaton-Rye et al. 2003; Summerfield et al. 2007). This double mutant approach identified that each of these extrinsic subunits is able to bind in the absence of one other extrinsic protein and that the presence of at least two of the proteins are required for stable assembly of PS II centers (except at elevated pH) (Morris et al. 2016, 2019). Due to the predicted late addition of PsbJ and the extrinsics proteins to PS II, we have employed a similar approach to investigate whether the absence of the extrinsic proteins altered the impact of removing PsbJ in *Synechocystis* sp. PCC 6803.

Materials and methods

Cyanobacterial strains and culture conditions

The glucose-tolerant GT-O1 substrain of *Synechocystis* sp. PCC 6803 was used and referred to throughout as wild type (Williams 1988; Morris et al. 2014). The mutant strains were made by transforming the GT-O1 substrain. The ΔPsbJ strain was constructed with the *psbJ* gene replaced with a spectinomycin-resistance cassette introduced 57 bp after the start codon and 63 bp from the stop codon. The *psbO* gene was inactivated by inserting a kanamycin-resistance cassette 503 bp after the start codon and 267 bp before the stop codon. Both *psbU* and *psbV* were also inactivated by the insertion of a kanamycin-resistance cassette. In the case of *psbU*, the antibiotic-resistance cassette was inserted 36 bp after the start codon and 151 bp before the stop codon. In the case of *psbV,* the antibiotic-resistancc cassette was located 68 bp after the start condon and 120 bp from the stop codon. The double mutants ΔPsbJ:ΔPsbO, ΔPsbJ:ΔPsbU and ΔPsbJ:ΔPsbV were made by transforming the ΔPsbJ strain using the corresponding plasmids in which *psbO*, *psbU* or *psbV* had been inactivated.

All strains were grown on BG-11 media agar plates containing 5 mM glucose, 20 µM atrazine, 10 mM TES-NaOH (pH 8.2) and 0.3% sodium thiosulfate. Liquid cultures were grown mixotrophically in unbuffered BG-11 media containing 5 mM glucose. In both solid and liquid media antibiotics were added at 25 µg mL^{-1} and cultures were maintained at 30 °C under constant illumination at 30 µmol photons m^{-2} s^{-1} (Eaton-Rye 2011). Cells in liquid culture were harvested when they reached mid-logarithmic growth by centrifugation at 5000×*g* for 10 min at room temperature. Photoautotrophic growth curves were carried out as described in Forsman and Eaton-Rye (2021).

Oxygen evolution assays in cells

Cells grown mixotrophically were washed and then resuspended in BG-11 containing 25 mM HEPES–NaOH (pH 7.5) at a chlorophyll concentration of 10 µg mL^{-1} and 30 °C. Oxygen evolution was measured with a Clark-type electrode (Hansatech, King's Lynn, U.K.) at 30 °C using 15 mM $NaHCO_3^-$ or a combination of 0.2 mM 2,5-dimethyl-1,4-benzoquinone (DMBQ) or 2,5-dichloro-1,4-benzoquinone (DCBQ) and 1 mM $K_3Fe(CN)_6$. Saturating actinic light (2 mmol photons m^{-2} s^{-1}) was provided by an FLS1 light source (Hansatech, King's Lynn, U.K.) passed through a Melis Griot OG 590 sharp cutoff red glass filter.

Variable chlorophyll *a* fluorescence induction

Cells were washed and resuspended in BG-11 (pH 7.5) at a chlorophyll concentration of 5 µg mL^{-1} and dark adapted at room temperature for 5 min. Variable chlorophyll *a* fluorescence was measured with a FL-3000 double modulation kinetic fluorometer (PSI Instruments, Brno, Czech Republic) using a blue 455 nm measuring light. The actinic voltage was set to 50% for all samples and the measuring light voltage was set to 80%. When present, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was at a final concentration 40 µM, and added 1 min prior to measurement. The decay of variable fluorescence following a single actinic flash was measured according to Forsman and Eaton-Rye (2021).

77 K chlorophyll *a* fluorescence emission spectroscopy

Low-temperature (77 K) fluorescence emission spectroscopy was performed as previously described (Jackson et al. 2014). Spectra were normalized to the Photosystem I peak at 725 nm.

Protein analyses

For protein analyses, cultures of the wild type and mutant strains were grown photomixotrophically to mid-exponential phase at 30 °C and 40 µmol of photons m^{-2} s^{-1}. Isolation of thylakoid membranes, blue-native polyacrylamide gel electrophoresis and western blotting were performed as previously described (Jackson et al. 2014).

Pigment extraction

Chlorophyll concentrations were determined according to MacKinney (1941) and carotenoids were extracted according to Chamovitz et al. (1993).

Results

Removal of PsbJ from strains lacking PsbO, PsbU or PsbV abolishes or reduces photoautotrophic growth

Strains lacking either PsbJ, PsbO, PsbU, PsbV grew photoautotrophically (Fig. 1). The photoautotrophic doubling time for the wild-type and ΔPsbU strains was 12 h and this increased to 17 h in ΔPsbV cells, 18 h in ΔPsbJ cells and was extended to 20 h in the ΔPsbO strain. In contrast, the doubling time in the ΔPsbJ:ΔPsbU mutant was slowed to 60 h (Fig. 1b) while removal of PsbO or PsbV in the ΔPsbJ background prevented photoautotrophic growth (Fig. 1a, b).

Oxygen evolution is impaired in strains lacking PsbJ and one of the extrinsic proteins PsbO, PsbU or PsbV

The rates of oxygen evolution observed for the ΔPsbJ strain and wild type were similar in the presence of bicarbonate but were reduced to ~ 59% of the wild-type rate in the ΔPsbJ strain in the presence of either DCBQ or DMBQ which are PS II-specific electron acceptors (Table 1). In contrast, the ΔPsbJ:ΔPsbO strain did not evolve oxygen under any any conditions; however, the ΔPsbJ:ΔPsbU strain exhibited an initial rate in the presence of bicarbonate that was similar to the observed rate for wild-type cells, but had less than 20% the wild-type rate with either PS II-specific electron acceptor (Table 1). In ΔPsbJ:ΔPsbV cells, oxygen evolution was reduced to ~ 34% of the wild-type rate when supported by bicarbonate and essentially abolished when supported by either DCBQ or DMBQ (Table 1). This impaired oxygen evolution indicates a specific effect on PS II activity following the removal of PsbJ when an extrinsic protein is also absent.

Chlorophyll *a* fluorescence is altered in the ΔPsbJ strain in the presence and absence of extrinsic proteins

Variable chlorophyll *a* fluorescence induction assays were performed, in the presence and absence of the PS II-specific herbicide DCMU, to assess the impact of removing PsbJ. The ΔPsbJ strain exhibited chlorophyll *a* fluorescence induction kinetics with typical O, J, I and P features: O indicates the F_o fluorescence origin, the rise from O to the J peak

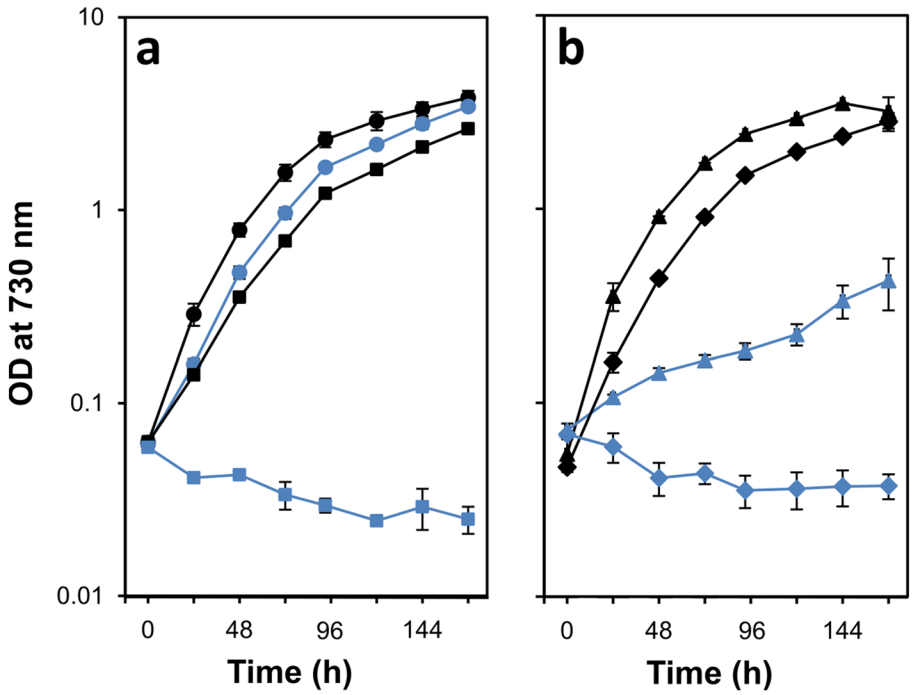

Fig. 1 Photoautotrophic growth of *Synechocystis* sp. PCC 6803 strains in BG-11 medium was measured by optical density at 730 nm. **a** Wild type (black circles), ΔPsbJ (blue circles), ΔPsbO (black squares) and ΔPsbJ:ΔPsbO (blue squares). **b** ΔPsbU (black triangles), ΔPsbJ:ΔPsbU (blue triangles), ΔPsbV (black diamonds) and ΔPsbJ:ΔPsbV (blue diamonds). Error bars represent standard error of the mean for three separate experiments

Table 1 Maximum oxygen evolution rates in wild-type *Synechocystis* sp. PCC 6803 and strains lacking PsbJ and the extrinsic proteins PsbO, PsbU and PsbV

Strains	Oxygen evolution (μmol O_2 per mg of Chl *a* per h)[a]		
	DCBQ	DMBQ	Bicarbonate
Wild type	361 ± 22	287 ± 22	220 ± 29
ΔPsbJ	210 ± 27	175 ± 31	256 ± 60
ΔPsbO	190 ± 13	139 ± 23	236 ± 24
ΔPsbU	284 ± 30	213 ± 26	234 ± 24
ΔPsbV	217 ± 18	195 ± 15	266 ± 25
ΔPsbJ:ΔPsbO	0 ± n/a	0 ± n/a	0 ± n/a
ΔPsbJ:ΔPsbU	67 ± 1	29 ± 6	246[b] ± 18
ΔPsbJ:ΔPsbV	19 ± 3	0 ± n/a	75 ± 3

[a]Data are presented as the standard error from three independent experiments

[b]Initial rate only, photoinactivation of strain occurred within 30 s

reflects the photoreduction of Q_A to Q_A^-, and the rise from J to the intermediary inflection I and the maximum fluorescence (F_m or peak (P)) reflects the reduction of the plastoquinone pool (Govindjee 1995). The O to J and I to P transitions were reduced in the ΔPsbJ strain compared to wild type (Fig. 2a). As previously reported, removal of the extrinsic proteins altered chlorophyll fluorescence induction kinetics (Summerfield et al. 2013). The removal of PsbU flattened the O to J rise, but a substantial I to P rise remained; in contrast, removal of either PsbO or PsbV resulted in a reduced J level and almost no I to P rise. Combined removal of PsbJ and one of the extrinsic proteins further suppressed fluorescence induction including the absence of an I to P rise for the ΔPsbJ:ΔPsbO and ΔPsbJ:ΔPsbV mutants and a greatly reduced I to P rise in the ΔPsbJ:ΔPsbU strain (Fig. 2a, b).

In the presence of DCMU, all strains showed a rapid fluorescence rise to F_m due to the inhibition of forward electron transfer from Q_A^-. The variable fluorescence yield in the presence of DCMU is typically indicative of the number of active PS II centers present. The fluorescence yield from the ΔPsbJ, ΔPsbO, ΔPsbU and ΔPsbV mutants was reduced compared to wild type by different amounts, ranging between 96% of the wild-type F_m for ΔPsbU cells (least affected), and 58% of the F_m for the ΔPsbV strain (most affected) (Fig. 2c, d). The The F_m for the ΔPsbO and ΔPsbJ strains was similar at ~68% of the wild-type level (Fig. 2c); however, the ΔPsbJ:ΔPsbU double mutant retained only 35% of the wild-type level and the ΔPsbJ:ΔPsbO and ΔPsbJ:ΔPsbV strains showed a further reduction to ~ 24%. The results in Fig. 2 indicate that the absence of an O–J–I–P transient and, in the presence of DCMU, an Fm value 35% of the wild-type level does not preclude photoautotrophic growth, as the ΔPsbJ:ΔPsbU strain grew photoautotrophically.

To further investigate PS II assembly, low-temperature fluorescence emission spectra were measured following direct excitation of chlorophyll *a* at 440 nm (Fig. 3). Following 440 nm excitation, the PS II-specific emission peaks at 685 nm and 695 nm were lower in the ΔPsbJ mutant than in wild type when normalized to the PS I peak at 725 nm. The PS II emission at 685 nm from the

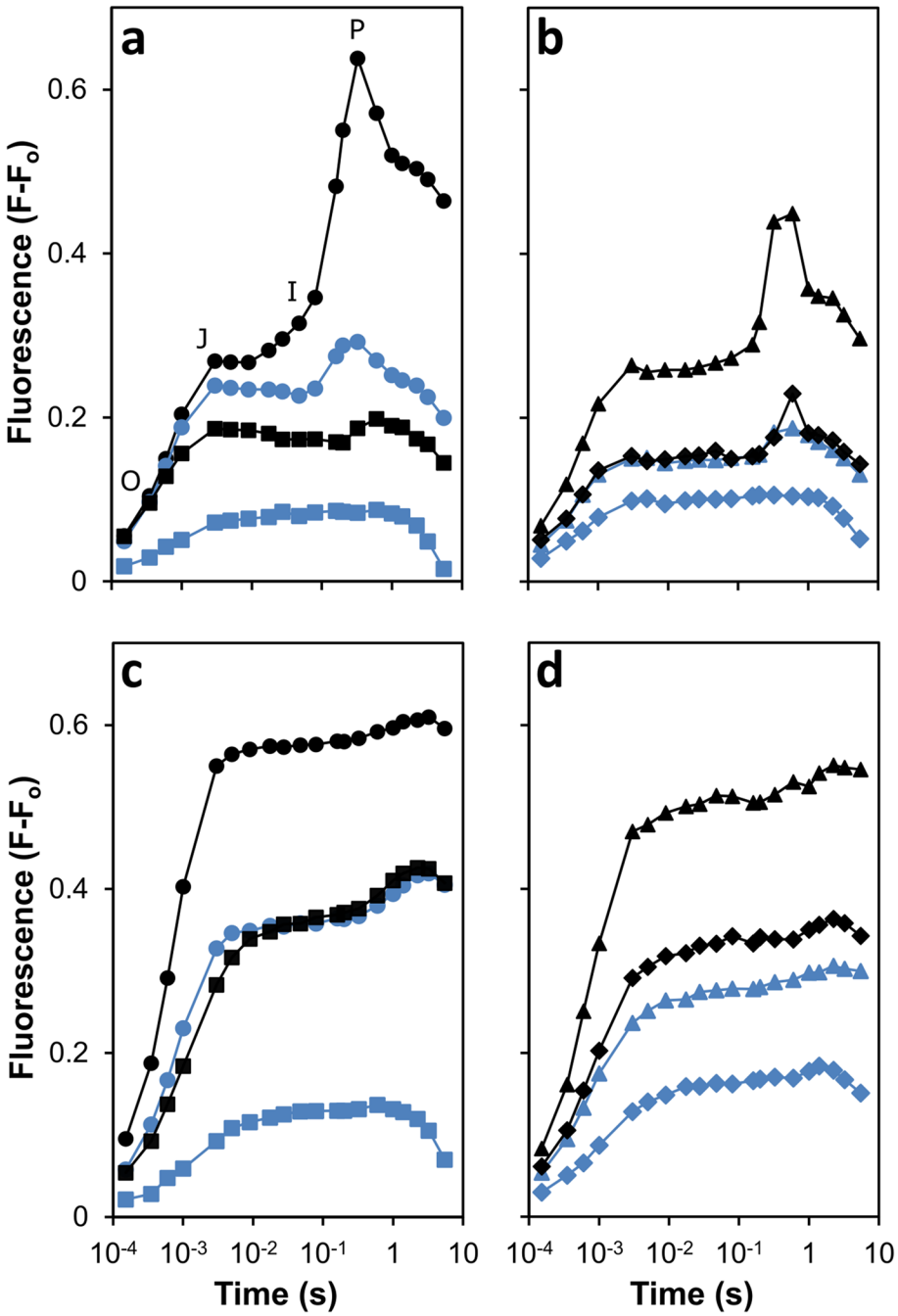

Fig. 2 Chlorophyll *a* fluorescence induction transients are altered in strains lacking PsbO, PsbU and PsbV following removal of PsbJ. **a, c** Wild-type cells (black circles), ΔPsbJ cells (blue circles), ΔPsbO cells (black squares), ΔPsbJ:ΔPsbO cells (blue squares). **b, d** ΔPsbU cells (black triangles), ΔPsbJ:ΔPsbU cells (blue triangles), ΔPsbV cells (black diamonds), ΔPsbJ:ΔPsbV cells (blue diamonds). Cells have no addition (**a**, **b**), or 40 µM DCMU (**c**, **d**). Data are the average of at least three independent experiments

ΔPsbO and ΔPsbJ:ΔPsbO strains was increased, compared to wild type, with a shoulder at 695 nm for the ΔPsbO strain but not for the ΔPsbJ:ΔPsbO strain. The ΔPsbU strain had a similar spectrum to wild type but a slightly reduced 695 nm peak, this peak was further reduced in the ΔPsbJ:ΔPsbU strain. The ΔPsbV mutant had a higher 685 nm peak, similar to the ΔPsbO strain but a 695 nm shoulder that was less pronounced than the ΔPsbO strain, and the ΔPsbJ:ΔPsbV strain had a slightly elevated 685 nm peak and no distinct 695 nm shoulder. The reduced 695 nm emission in the ΔPsbJ:ΔPsbU strain and further reduction in the ΔPsbJ:ΔPsbO and ΔPsbJ:ΔPsbV strains is consistent with the reduction of variable fluorescence in the presence of DCMU and indicates reduced accumulation of PS II centers in the ΔPsbJ:ΔPsbU strain and greater reduction in the ΔPsbJ:ΔPsbO and ΔPsbJ:ΔPsbV strains. The reduced levels of assembled PS II in the double mutants was also supported by low-temperature fluorescence emission spectra where the phycobilisomes were excited directly with 580 nm light (Fig. S1).

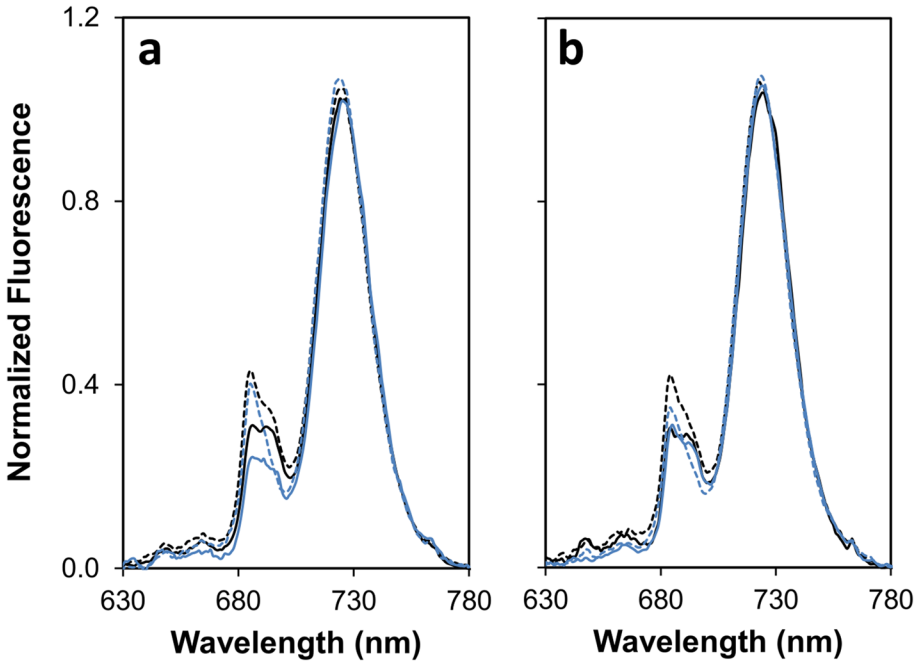

Fig. 3 Low temperature (77 K) fluorescence emission spectra following excitation at 440 nm. **a** wild type (black solid line), ΔPsbJ (blue solid line), ΔPsbO (black dashed line) and ΔPsbJ:ΔPsbO (blue dashed line), **b** ΔPsbU (black solid line), ΔPsbV (black dashed line), ΔPsbJ:ΔPsbU (blue solid line), ΔPsbJ:ΔPsbV (blue dashed line). Results are the average of at least three individual biological replicates. All spectra were normalized to the fluorescence emission from PS I at 725 nm

Dimerisation of PS II is reduced in the combined mutants

The abolished or impaired photoautotrophic growth and reduced level of PS II centers in the strains lacking both PsbJ and one of the extrinsic proteins indicated PsbJ may be important for assembly or stability of PS II. Therefore we investigated whether changes to PS II in these mutants included altered dimer abundance. To assess PS II assembly, blue native-polyacrylamide gel electrophoresis (BN-PAGE) followed by immunodetection using D1-specific, D2-specific and CP43-specific antibodies was used to determine the relative abundance of PS II dimers, monomers and other assembly intermediates. Detection of the PS II native complexes in the ΔPsbJ, ΔPsbU and ΔPsbV strains showed a slight reduction in dimers compared to wild type (Fig. 4). The

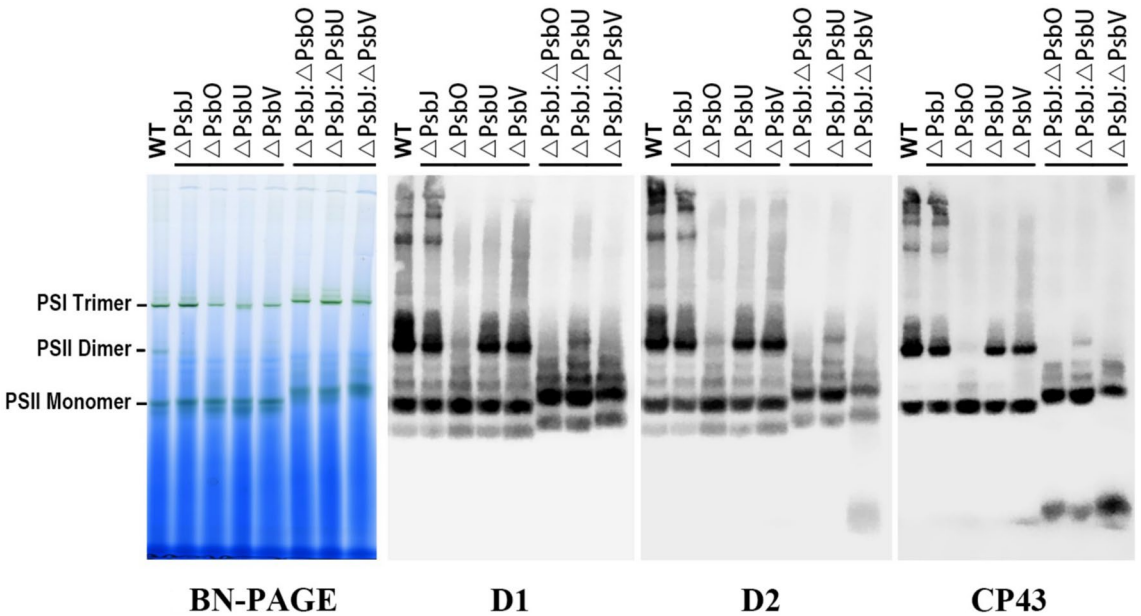

Fig. 4 Analysis of PSII assembly by BN-PAGE and western blotting. The different assembly complexes were separated in **a** on a 3–12% gradient gel, followed by identification using antibodies raised against the core reaction center proteins: **b** D1, **c** D2, and **d** CP43

ΔPsbO strain showed greatly decreased dimer abundance, as did all three double mutants. The destabilization of PS II dimer formation in the absence of PsbO is consistent with earlier reports (Bentley and Eaton-Rye 2008; Komenda et al. 2010). Only the ΔPsbJ:ΔPsbU strain had any detectable PS II dimers, this strain also had increased levels of PS II monomers compared to the ΔPsbJ:ΔPsbO and ΔPsbJ:ΔPsbV strains. The three double mutants also showed a shift in the size of the PS II monomer; however, the gel shift was also observed for the PS I trimer band in the BN-PAGE gel consistent with stress-induced accumulation of carotenoids (Paerl 1984; Toth et al. 2015; Vajravel et al. 2017). Elevated levels of carotenoids in the double mutants were confirmed by absorption spectra of *N*,*N*-dimethylformamide-extracted pigments (Fig. S2). In addition, each of the double mutants accumulated a putative assembly intermediate complex containing CP43 that was observed at the bottom of the western blot probed with the CP43-specific antibody (Fig. 4). A similar signal was also obtained with an antibody to CP43 in a double mutant lacking the CyanoP and Ycf48 PS II proteins which are both lumenal assembly factors (Jackson and Eaton-Rye 2015).

Discussion

The *psbJ* gene is in the operon encoding cytochrome b_{559} and PsbL: in cyanobacteria this operon is found in a gene cluster with *ycf48* and *rubA* that both encode assembly factors in PS II biogenesis (Yu et al. 2018; Garcia-Cerdán et al 2019). It has been suggested that cooperation between the LMW and extrinsic subunits of PS II may have arisen in the ancestral photosystem that gave rise to PS II (Cardona 2016). Since the other proteins encoded in the *psbEFLJ* operon play essential roles in PS II biogenesis (reviewed in Nickelsen and Rengstl (2013)), we probed the role of PsbJ by constructing double mutants inactivating *psbJ* and the genes encoding the extrinsic proteins of PS II. Consistent with the previous report by Regel et al. (2001), we found the absence of PsbJ impaired PS II-specific oxygen evolution and reduced the number of PS II centers. We also observed a slightly elevated rate of bicarbonate-supported electron transport in our ΔPsbJ and ΔPsbV mutants when compared to wild-type cells; however, the mechanism for this effect is not yet understood but it is possible that alternative pathways for PQH_2 oxidation are upregulated in these strains (Lea-Smith et al. 2016).

As expected, removal of any one of the three extrinsic proteins did not prevent photoautotrophic growth but did impact PS II activity and the number of assembled active centers as previously reported (Burnap and Sherman 1991; Shen et al. 1995a, 1997; Eaton-Rye et al. 2003). The combination of removing PsbJ with any one of the three extrinsic proteins, however, prevented or impaired photoautotrophic growth and decreased oxygen evolution, the number of assembled PS II centers, and the detectable level of PS II dimers. The difference in phenotype between the single and double mutants indicates that PsbJ can bind PS II in the absence of any one of the extrinsic proteins. Support for this conclusion is also evident in the chlorophyll *a* fluorescence decay kinetics obtained after a single actinic flash (Figs. S3 and S4; and Tables S1 and S2).

The observation that PsbJ can bind in the absence of one of the extrinic proteins is in contrast with the reported cryo-EM-derived structure of PS II from a *Thermosynechococcus vulcanus* strain lacking PsbV where PsbJ was absent (Huang et al. 2021). In the case of the *Thermosynechococcus vulcanus* strain lacking PsbV, it was suggested that conformational shifts of PsbE and PsbF may result in the loss of PsbJ from the structure (Huang et al. 2021). In addition, in our ΔPsbJ mutant the intensity and mobility of the PS II dimer and monomer bands obtained by BN-PAGE were similar to those observed for wild type. This suggests that there is little or no accumulation of an intermediate PS II complex that retains the Psb27 and Psb28 assembly factors as reported for the ΔPsbJ strain of *Thermosynechococcus elongatus* (Nowaczyk et al. 2012; Zabret et al. 2021). Our results indicate PsbJ is functionally present in the ΔPsbV strain in vivo in *Synechocystis* sp. PCC 6803. Similarly, the ΔPsbJ strain had a phenotype distinct from that of the double mutants consistent with the extrinsic proteins binding in the absence of PsbJ. Our results also contrast with the situation in higher plants where deletion of *psbJ* altered the binding of the PsbP and PsbQ extrinsic proteins and prevented photoautotrophic growth in Δ*psbJ* plants (Hager et al. 2002; Swiatek et al. 2003; Suorsa et al. 2004).

Conclusion

The PsbJ protein can bind to PS II in cells lacking any one of the PsbO, PsbU and PsbV extrinsic proteins and the extrinsic proteins can bind to PS II in the absence of PsbJ in *Synechocystis* sp. PCC 6803 cells. Photoautotrophic growth, however, was prevented in the ΔPsbJ:ΔPsbO and ΔPsbJ:ΔPsbV strains and substantially slowed in the ΔPsbJ:ΔPsbU strain. In addition, 77 K fluorescence emission spectra, variable fluoresence induction and oxygen evolution indicate low numbers of functional PS II centers in the double mutants. Taken together, our data suggest that an active OEC could not assemble in the absence of PsbJ when either PsbO or PsbV was missing.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11120-021-00862-y.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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