

# Oxygen evolution in the thylakoid-lacking cyanobacterium *Gloeobacter violaceus* PCC 7421

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## Abstract

The oxygen-evolving reactions of the thylakoid-lacking cyanobacterium *Gloeobacter violaceus* PCC 7421 were compared with those of *Synechocystis* sp. PCC 6803. Four aspects were considered: sequence conservation in three extrinsic proteins for oxygen evolution, steady-state oxygen-evolving activity, charge recombination reactions, i.e., thermoluminescence and oscillation patterns of delayed luminescence on a second time scale and delayed fluorescence on the nanosecond time scale at  $-196\text{ }^{\circ}\text{C}$ . Even though there were significant differences between the amino acid sequences of extrinsic proteins in *G. violaceus* and *Synechocystis* sp. PCC 6803, the oxygen-evolving activities were similar. The delayed luminescence oscillation patterns and glow curves of thermoluminescence were essentially identical between the two species, and the nanosecond delayed fluorescence spectral profiles and lifetimes were also very similar. These results indicate clearly that even though the oxygen-evolving reactions are carried out in the periplasm by components with altered amino acid sequences, the essential reaction processes for water oxidation are highly conserved. In contrast, we observed significant changes on the reduction side of photosystem II. Based on these data, we discuss the oxygen-evolving activity of *G. violaceus*.

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## 1. Introduction

Photosystem (PS) II is a unique protein complex that oxidizes two water molecules and evolves molecular oxygen using light energy. Although several crystal structures for this complex have been reported [1–4], its reaction processes and mechanisms remain unresolved [5–7]. Sequence analysis of 16 S rRNA has indicated that the cyanobacterium *Gloeobacter violaceus* PCC 7421 is an early-branching species within the cyanobacterial clade [8]. Due to its phylogenetic position, it is

thought to retain ancestral properties. It lacks intracytoplasmic membranes [9], and the photosynthetic and respiratory systems co-localize on the cell membrane. It is assumed that both systems share a common electron transfer component, i.e. plastoquinone (PQ), by analogy with overlap of the two electron transfer chains on the thylakoid membranes in other cyanobacteria [10]. Only low levels of chlorophyll (Chl) are found in cell membranes in other cyanobacteria [11].

We have studied several biochemical properties of PS I in this bacterium, specifically in relation to gene sequences [12]. In contrast to the 11 subunits found in other cyanobacteria, PS I of *G. violaceus* PCC 7421 comprises 9 subunits, including the novel subunit Psaz [13]. Psab is one of the major subunits in PS I and contains a unique C-terminal motif that exhibits considerable similarity to a peptidoglycan-binding motif in eubacteria [14]. In addition, the secondary electron acceptor of PS I is menaquinone-

**Abbreviations:** Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PC, plastocyanin; PQ, plastoquinone; PS, photosystem

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4, rather than phylloquinone, which is found more commonly in oxygenic photosynthetic organisms [15]. Furthermore, phytoene desaturase, which is the key enzyme in carotenoid biosynthesis, is of a bacterial type, rather than a cyanobacterial/plant type [16]. These properties demonstrate that *G. violaceus* retains some primitive and ancestral characteristics and thus confirm its position as a primordial species.

Genome information for this cyanobacterium indicates other unusual properties within PS II [12]. The genes encoding four subunits of PS II (*psbQ*, *psbY*, *psbZ* and *psb27*) are absent, and three genes for extrinsic proteins in oxygen evolution (*psbO*, *psbU* and *psbV*) are poorly conserved. Extrinsic proteins perform several functions in oxygen evolution. PsbO is primarily responsible for stabilization of the Mn-cluster [17–19], and in cooperation with PsbU and PsbV, protects the cluster from exposure to reducing substances [20]. In the absence of PsbU and/or PsbV, requirements for  $\text{Ca}^{2+}$  and  $\text{Cl}^-$  are higher in *Synechocystis* sp. PCC 6803 (hereafter referred to as *Synechocystis*), hence these proteins probably play a role in the accumulation of  $\text{Ca}^{2+}$  and  $\text{Cl}^-$  [21]. In addition, a cyanobacterial homolog of PsbQ, the extrinsic protein found in higher plants, green algae, and red algae, is absent in *G. violaceus* [22]. Similarly, there is significantly lower conservation of sequence in two polypeptides localized in the lumen in other cyanobacteria, PetE (plastocyanin, PC) and PsbF (PC docking protein), which are expected to localize in the periplasm in *G. violaceus*. Since the oxygen-evolving system is localized in the periplasm where ionic conditions are different from those on the luminal side of the thylakoid membranes, the functional structures of the extrinsic proteins in the periplasm might be modified through modifications in the amino acid sequences, which may affect PS II activity. Indeed, it is known that *G. violaceus* can grow only under very low light conditions [23,24], a characteristic that might be related to its PS II activity.

In this study, we compared the amino acid sequences of three extrinsic proteins involved in cyanobacterial oxygen-evolving processes, and investigated interactions between the PS II subunits in *G. violaceus* and *Synechocystis* as a control. In order to characterize PS II (including the Mn-cluster), we examined oxygen-evolving activity, thermoluminescence, delayed luminescence at 1.6 s after the flash and delayed fluorescence on the nanosecond time scale at  $-196^\circ\text{C}$ . Based on these findings, we discuss the effects of PS II composition on oxygen-evolving activity in *G. violaceus*.

## 2. Materials and methods

### 2.1. Algal culture

*Gloeobacter violaceus* PCC 7421 and *Synechocystis* sp. PCC 6803 were grown photosynthetically in BG-11 medium [25] under continuous illumination using light intensities less than 5 and approximately  $15\ \mu\text{E}/(\text{m}^2 \times \text{s})$ , respectively. The temperature was maintained at  $25^\circ\text{C}$  and air was supplied continuously via an air filter (Millex-FG, Millipore).

### 2.2. Measurements of oxygen-evolving activities

Oxygen-evolving activity was measured using a Teflon-coated oxygen electrode (Rank Brothers, England). Light from a  $\text{W}_2$  lamp was guided via a glass fiber (Ikemori, Japan) to the front of the electrode vessel and the light

intensity was then adjusted using neutral density filters (Toshiba, Japan). The average oxygen yield per flash was measured on excitation with a Xenon lamp (MS-230 S, Sugawara Lab, Japan) with a  $4\ \mu\text{s}$  half-duration. An appropriate UV-cut filter (Toshiba, Japan) was inserted between the Xenon lamp and the electrode vessel. Chl contents of algal suspensions were maintained at approximately  $2\ \mu\text{g}/\text{ml}$  in order to attenuate actinic light at the rear of the electrode vessel. Following methanol extraction, Chl contents of individual samples were determined using a Hitachi U-2010 spectrophotometer and the extinction coefficient reported by Porra et al. [26]. 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was purchased from Sigma.

### 2.3. Measurements of thermoluminescence and delayed luminescence on a second time scale

Thermoluminescence was measured according to procedures reported previously [27]. Cell suspensions containing  $7\ \mu\text{g}$  Chl *a* were layered onto filter paper ( $1.5 \times 1.5\ \text{cm}$ ), illuminated by white light ( $1\ \text{mW}/\text{m}^2$ ) at  $25^\circ\text{C}$  for 30 s and then maintained in the dark for 5 min. Samples were illuminated by xenon flash once at  $5^\circ\text{C}$ , and then nitrogen gas was used to cool the cells down to  $-180^\circ\text{C}$ . Samples were warmed in the dark at  $5^\circ\text{C}/\text{min}$  and the thermoluminescence curves measured.

For measurement of delayed luminescence, cell concentrations were adjusted to  $20\ \mu\text{g}$  Chl *a*/ml. Cells were illuminated by white light ( $1\ \text{mW}/\text{m}^2$ ) at  $20^\circ\text{C}$  for 1 min, then incubated in the dark until no signal from  $\text{S}_2\text{Q}_\text{B}$  or  $\text{S}_3\text{Q}_\text{B}$  was detected. A series of xenon flashes was then applied at 2 s intervals, and delayed luminescence was determined 1.6 s after the flashes.

### 2.4. Measurements of the time-resolved fluorescence spectrum

Time-resolved fluorescence spectrum was measured at  $-196^\circ\text{C}$  using a time-correlated single photon counting method and a custom-made Dewar bottle [24,28]. The second harmonic of the Ti:Sapphire laser, whose fundamental oscillation was 800 nm, provided preferential excitation of Chl *a* (400 nm). A 2.9 MHz pulse repetition rate was used. The time resolution of the time-to-amplitude converter was 51.7 ps/channel. The zero time was taken as the time when an excitation pulse showed maximum counts on a time-to-amplitude converter. Since the excitation pulse (pulse width 150 fs) was observed with a width of approximately 30 ps on a time-to-amplitude converter, a negative time was assigned to signals occurring prior to the maximum of the excitation pulse. Fluorescence lifetimes were estimated using a convolution calculation [28].

### 2.5. Protein sequences

Amino acid sequences were deduced from DNA sequence information deposited for *G. violaceus* [12], *Synechocystis* [29] and *Thermosynechococcus elongatus* [30] at the Kazusa DNA Research Center (accession numbers NC\_005125, NC\_000911 and NC\_004113, respectively). Extracted gene numbers for *G. violaceus* were *glr2324* (CP43), *glr2999* (CP47), *glr3691* (PsbO), *gll2873* (PsbU) and *gll2338* (PsbV). The corresponding numbers for *Synechocystis* were *slr0851* (CP43), *slr0906* (CP47), *slr0427* (PsbO), *slr1194* (PsbU) and *slr0258* (PsbV), while those for *T. elongatus* were *tlr1631* (CP43), *tlr1530* (CP47), *tlr0444* (PsbO), *tlr2409* (PsbU) and *tlr1285* (PsbV). The presence of N-terminal signal peptides in PsbO, PsbU and PsbV of *G. violaceus* was predicted using the Signal P program (<http://www.cbs.dtu.dk/services/SignalP/>) [31] because N-terminal sequences for extrinsic proteins of *G. violaceus* have not been experimentally determined. N-terminal sequences for extrinsic proteins of *Synechocystis* [32] and *T. elongatus* [4,33] were taken from the literature. Multiple alignments were built using the T-coffee program (<http://tcoffee.vital-it.ch/cgi-bin/Tcoffee/tcoffee.cgi/index.cgi>) [34].

## 3. Results

The two cyanobacterial species used in this study are very different in color and this difference is evident in the absorption spectra of whole cells (Fig. 1). *G. violaceus* is rich in phycoerythrin, whereas *Synechocystis* is rich in phycocyanin. In

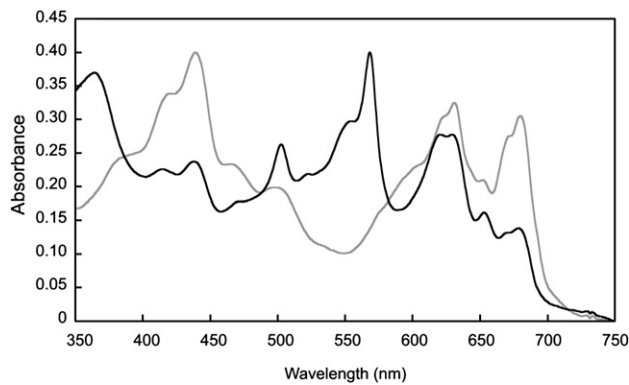


Fig. 1. Absorption spectra of two species of cyanobacteria at  $-196^{\circ}\text{C}$  *G. violaceus* (thick line) and *Synechocystis* (thin line).

addition, the ratios of Chl *a* to phycobiliproteins are very different between the two species, with *G. violaceus* containing less Chl *a*. It is possible that such differences could affect the PS II activity in these species.

### 3.1. Comparison of amino acid sequences and predicted structures

The amino acid sequences of the three extrinsic proteins PsbO, PsbU and PsbV are well conserved between *Synechocystis*, *T. elongatus* and *Anabaena* sp. PCC 7120 (PsbO, 52–62%; PsbU, 45–47%; and PsbV, 42–66%). However, inclusion of *G. violaceus* in this comparison reduced the sequence identity significantly (PsbO, 23–25%; PsbU, 18–20%; and PsbV, 18–31%) [12]. Since such reduced sequence identity indicates an altered structure in *G. violaceus*, we aligned the sequences and extracted features required for molecular interactions with other PS II proteins, using the 3.0 Å crystal structure from *T. elongatus* (Fig. 2) [4].

### 3.2. PsbO (33 kDa protein)

Fig. 2 (upper panel) shows an alignment of PsbO proteins from *G. violaceus*, *Synechocystis* and *T. elongatus*. Shaded residues represent PsbO-binding sites to D1, D2, CP43 and CP47, as derived from the 3.0 Å crystal structure of the *T. elongatus* protein. There were remarkable differences between the PsbO sequences of *G. violaceus* and the other two species, including two deletions (residues 7–11 and 50–60) and a short insertion (residues 174–176) (Fig. 2; regions differing are boxed). In *G. violaceus*, the N-terminus (residues 1–14) was poorly conserved, although this region is known to be functionally significant for binding and oxygen-evolving activity in spinach, and is conserved in the other cyanobacteria [35].

With respect to other modifications in PsbO, residues 56–70 form a  $\beta 1$ – $\beta 2$  loop in other cyanobacteria (Fig. 2) [36], but residues 56–60 were absent in *G. violaceus*. This deletion would affect the formation or structure of the  $\beta 1$ – $\beta 2$  loop. In addition, most residues in the DPKGR region are involved in binding to D1, D2 and CP47 [36]. Nevertheless, there was an insertion (residues 174–176) detected within this region in *G. violaceus*, and in addition, approximately half of

the residues (13 out of 29) differed from the consensus. Since a one-residue insertion between Leu<sub>159</sub> and Asp<sub>160</sub> had deleterious effects on binding to PS II [37], the short insertion (Gln<sub>174</sub>–Thr<sub>176</sub>), unique to *G. violaceus*, should lead to significant modifications of the PsbO structure, and possibly reduced binding ability to PS II. It has been reported that substitution of Lys<sub>162</sub> with Arg reduces binding affinity and oxygen evolution [37] and *G. violaceus* PsbO has Arg<sub>162</sub> in fact. This residue also would affect the ability of binding to PS II.

We surveyed the amino acid sequences of D1, D2, CP43 and CP47 to determine whether corresponding alterations may have occurred in the binding partners. A long insertion was observed in CP43 (residues 373–387) and four insertions were found in CP47 (residues 83–89, 96–100, 184–191 and 194–202; Fig. 2, lower panel). We predicted the structure of *G. violaceus* PsbO using its amino acid sequence and the crystal structure of *T. elongatus*, and then examined regions involved in interactions with CP43 or CP47 (data not shown). The insertion in *G. violaceus* CP43 (residues 373–387) was predicted to form a loop that might function in binding with PsbO. In this model, the residue Met<sub>376</sub> in the loop is located in only 3.1 Å distant from Glu<sub>12</sub> of PsbO. A similar situation was observed for the PsbO-binding site of CP47, whereby an insertion in CP47 (residues 83–100) was predicted to form a loop structure located in the vicinity of the binding site. These observations strongly suggest modification of the interactions between PsbO and CP43 and between PsbO and CP47. These changes may result in differences in the three-dimensional structure that could affect PS II functions and reaction processes.

Extrinsic proteins are known to be involved in the accumulation of  $\text{Ca}^{2+}$  and  $\text{Cl}^{-}$ , and in many cyanobacterial species,  $\text{Ca}^{2+}$  binds PsbO at the exit of the proton release pathway [38]. In line with a number of differences in the PsbO sequences between *G. violaceus* and the other two cyanobacteria, among the three proposed binding sites for  $\text{Ca}^{2+}$ , only the most highly conserved residue (Glu<sub>116</sub>) was found to be present in *G. violaceus* PsbO.

### 3.3. PsbU (12 kDa protein)

PsbU is known to interact with PsbO and PsbV in cyanobacteria [39]. Fig. 3 shows an alignment of the PsbU amino acid sequences of *G. violaceus*, *T. elongatus* and *Synechocystis*. Three significant differences were found between the PsbU sequences of *G. violaceus* and the other cyanobacteria: a N-terminal elongation (residues 1–20), an insertion (residues 40–62), and a deletion (residues 126–139, Fig. 3; regions differing are boxed). In *T. elongatus*, the crystal structure indicates that the N- and C-terminal regions of PsbU are in close proximity to CP47 and CP43, respectively [4]. However, the *G. violaceus* PsbU sequence shows two insertions and a deletion in the N- and C-terminal regions. We determined the sites of interaction of PsbU with PsbV, CP43 and CP47 using the *T. elongatus* crystal structure. Of five interaction sites predicted from the crystal structure, only Asn<sub>142</sub> shows sequence identity with *T. elongatus* and *Synechocystis*.



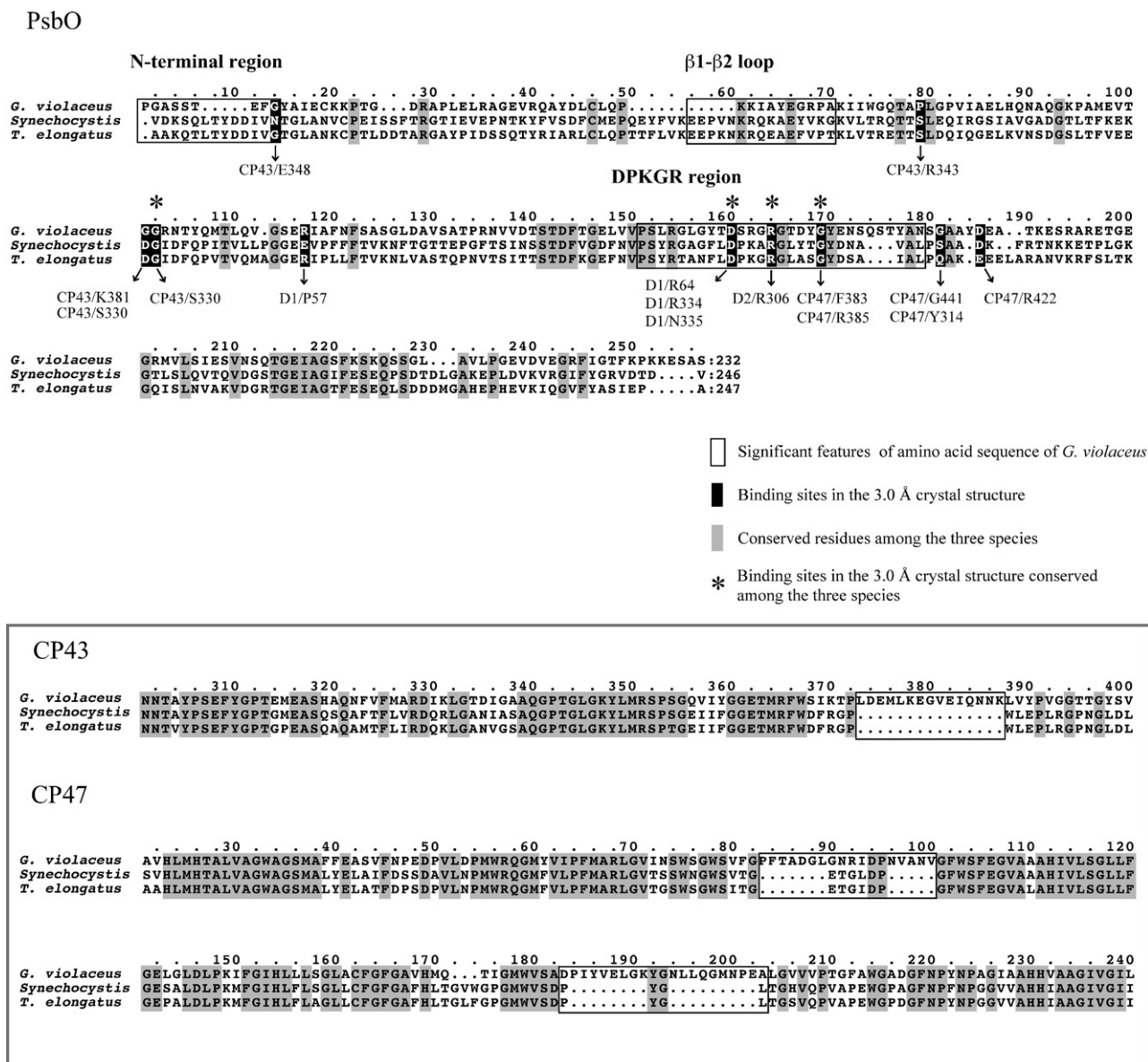


Fig. 2. Multiple alignments of PsbO sequences and interaction sites with CP43 and CP47. Alignment of deduced amino acid sequences for PsbO (upper panel), CP43 and CP47 (lower panel). Residues shaded in gray represent conserved residues among the three species. Residues shaded in black with white lettering represent PsbO interaction sites with D1, CP43 and CP47, as determined from the 3.0 Å crystal structure from *T. elongatus*. For example, CP43/E348 refers to the site that interacts with residue Glu<sub>348</sub> in CP43. These residues are located within 3.0 Å of each other. Asterisks above these residues represent complete conservation among the three species. Black boxes are used to identify features in the sequence of *G. violaceus* PsbO (upper panel). Major insertions in the sequences of *G. violaceus* CP43 and CP47 are boxed (lower panel). Note that the residue numbers used in the text correspond to the numbers shown above the alignment sequences.

### 3.3.1. PsbV (cyt *c*<sub>550</sub>)

Two genes within the *G. violaceus* genome sequence were annotated as PsbV (*gll2338* and *gll2337*). The former was used for sequence comparison, since it contained two conserved His residues suitable for heme binding, whereas one of these was lacking in the latter, suggesting a possible non-functional status (Fig. 4; conserved His residues are boxed). In all species, sequence conservation is lower in PsbV than in PsbO or PsbU, and no particular feature could be identified as responsible for binding with other proteins. In the absence of PsbV and/or PsbU, higher concentrations of Ca<sup>2+</sup> or Cl<sup>−</sup> are required for proper oxygen-evolving activity [39]. Although it is thought that these extrinsic

proteins contain specific binding sites for Ca<sup>2+</sup> and Cl<sup>−</sup>, the exact locations of these sites remain unknown. Following site-directed mutagenesis at heme ligands of *Synechocystis* PsbV, mutants have been shown to exhibit a higher redox potential and reduced growth in Ca<sup>2+</sup>-depleted medium [40]. In addition, binding of three Ca<sup>2+</sup> ions has been detected in the crystal structure of *Synechocystis* cyt *c*<sub>550</sub> [41]. However, none of these binding sites are conserved in *G. violaceus* PsbV. The binding sites for Cl<sup>−</sup> remain unknown.

In summary, we found significant modification of the amino acid sequences in the three extrinsic proteins of *G. violaceus*, which may cause differences in PS II activity including the water oxidizing reaction. Thus, we measured a several characteristic

## PsbU

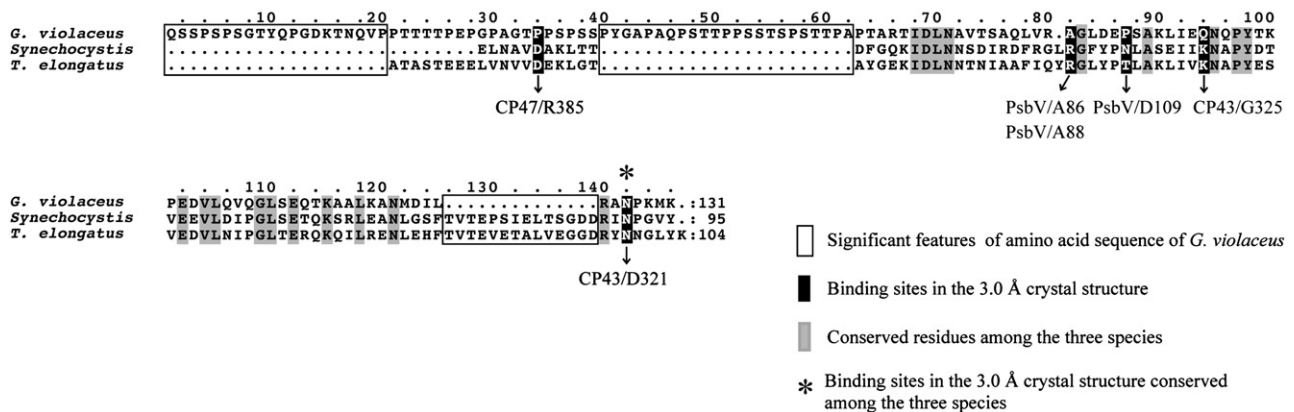


Fig. 3. Alignment of PsbU. Deduced amino acid sequences for PsbU from *G. violaceus*, *T. elongatus* and *Synechocystis*. Residues shaded in gray represent conserved residues among the three species. Residues shaded in black with white lettering represent PsbU interaction sites with PsbV, CP43 and CP47, as determined from the 3.0 Å crystal structure from *T. elongatus*. Asterisks above these residues represent complete conservation among the three species. Note that the residue numbers used in the text correspond to the numbers shown above the alignment sequences.

features reflecting properties of oxygen evolution in intact cells of *G. violaceus*.

### 3.4. Oxygen-evolving activity

We compared the steady-state oxygen-evolving activities of *G. violaceus* and *Synechocystis* (Fig. 5A). *Synechocystis* exhibited light intensity-dependent oxygen evolution, with maximum activity (250  $\mu\text{mol O}_2/(\text{mg Chl} \times \text{h})$ ) observed at light intensities lower than 200  $\mu\text{E}/(\text{m}^2 \times \text{s})$ ; no further increase in activity was observed at higher intensities (higher than 700  $\mu\text{E}/(\text{m}^2 \times \text{s})$ ). DCMU inhibited oxygen-evolving activity completely, and the respiration rate was very low (approximately 5  $\mu\text{mol O}_2/(\text{mg Chl} \times \text{h})$ ; Fig. 5A and B). This low respiration activity may have arisen as a result of photoautotrophic growth conditions (25 °C) [42,43]. *G. violaceus* also exhibited light intensity-dependent oxygen evolution, with its maximum

activity (approximately 125  $\mu\text{mol O}_2/(\text{mg Chl} \times \text{h})$ ) observed at a light intensity of 200  $\mu\text{E}/(\text{m}^2 \times \text{s})$ . However, there was a remarkable difference in respiration activity, which was estimated at approximately 100  $\mu\text{mol O}_2/(\text{mg Chl} \times \text{h})$  for *G. violaceus*. Therefore, the net oxygen-evolving activity in *G. violaceus* was 230  $\mu\text{mol O}_2/(\text{mg Chl} \times \text{h})$ ; which was approximately 90% that of *Synechocystis*; the light compensation point was observed at 35  $\mu\text{E}/(\text{m}^2 \times \text{s})$  (Fig. 5A). DCMU suppressed the oxygen-evolving activity in *G. violaceus* by approximately 70% (160  $\mu\text{mol O}_2/(\text{mg Chl} \times \text{h})$ ) (Fig. 5B), a value which was larger than the apparent oxygen evolution (125  $\mu\text{mol O}_2/(\text{mg Chl} \times \text{h})$ ).

### 3.5. Thermoluminescence and delayed luminescence on a second time scale

To monitor the redox properties and S-state transition of the Mn-cluster in *G. violaceus*, we compared thermoluminescence

## PsbV

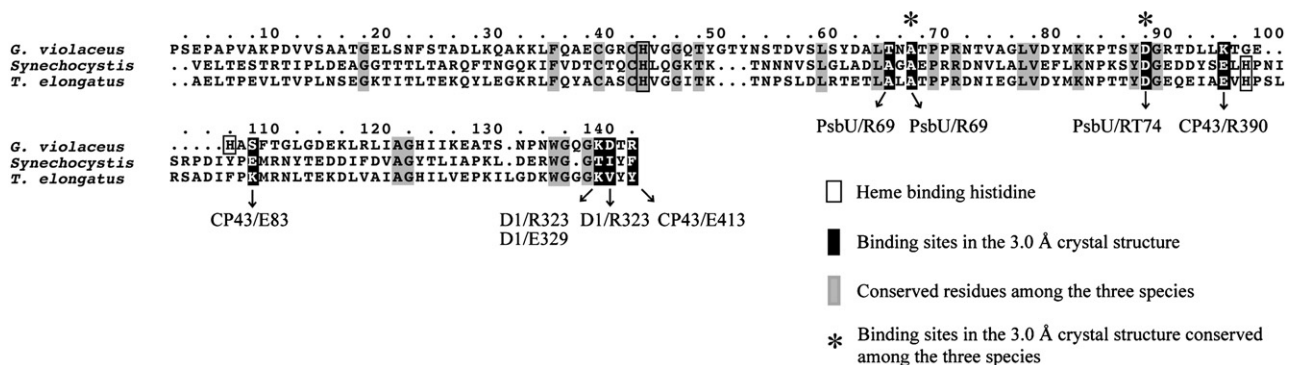


Fig. 4. Alignment of PsbV. Deduced amino acid sequences for PsbV from *G. violaceus*, *T. elongatus* and *Synechocystis*. In *G. violaceus*, two genes corresponding to PsbV are known; the gene demonstrating the greater sequence identity (*gll2338*) was chosen for analysis. Residues shaded in gray represent conserved residues. Residues shaded in black with white lettering represent PsbV interaction sites with PsbU, CP43 and CP47, as determined from the 3.0 Å crystal structure from *T. elongatus*. Asterisks above these residues represent complete conservation among the three species. Note that the residue numbers used in the text correspond to the numbers shown above the alignment sequences.

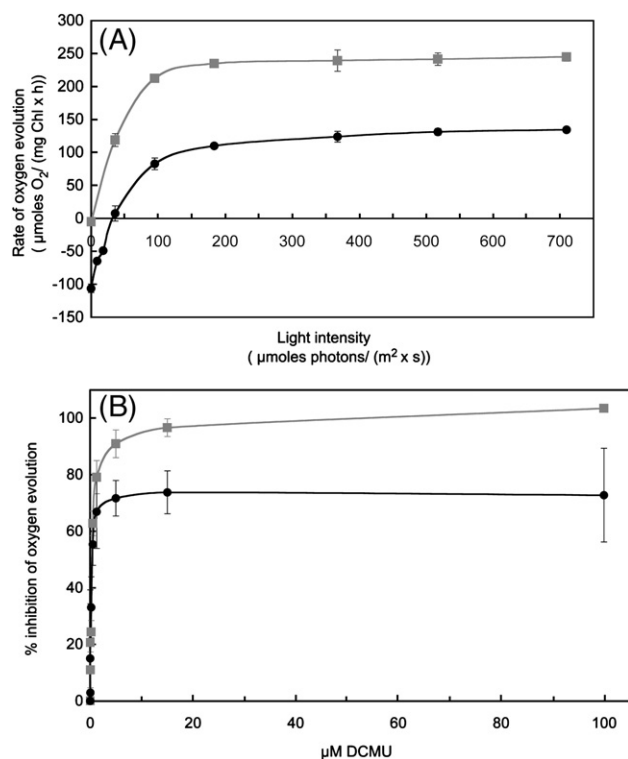


Fig. 5. Steady-state oxygen-evolving activity of two cyanobacteria (A) and the effect of DCMU (B). *G. violaceus* (black line, circles) and *Synechocystis* (gray line, squares). The assay was performed in BG11 and HEPES pH 7.0. DCMU ethanol stocks of varying concentrations were added to the assay medium to a final concentration of 0.2% (v/v). The light intensity used in this measurement was approximately  $530 \mu\text{E}/(\text{m}^2 \times \text{s})$ . Standard deviations were estimated from three independent measurements.

(glow curve) and delayed luminescence oscillation patterns of *G. violaceus* with those of *Synechocystis* intact cells. Fig. 6A shows thermoluminescence glow curves after a single flash. A peak observed at  $34^\circ\text{C}$  in *G. violaceus* was assigned to the B-band, originating from charge recombination between the  $\text{S}_2$  and  $\text{Q}_\text{B}^-$  states. Although the temperature at which the maximum intensity was obtained was slightly lower than for *Synechocystis* ( $39^\circ\text{C}$ ), it was still within the reported variation for this band [44]. Alternatively, differences in the extrinsic proteins for oxygen evolution may be responsible for the lower temperature, since a lower maximum has been observed in a *psbO*-mutant of *Synechocystis* [45]. Although the second band (C-band) was discernible at approximately  $45^\circ\text{C}$ , it disappeared after 5 flashes (data not shown). Fig. 6B shows changes in delayed luminescence intensity as a function of flash number. Delayed luminescence at 1.6 s after a flash derives from charge recombination between the  $\text{S}_2$  and  $\text{Q}_\text{B}^-$  states or  $\text{S}_3$  and  $\text{Q}_\text{B}^-$  states. Peaks were observed at 2 and 8 flashes for *G. violaceus* and at 2 and 7 flashes for *Synechocystis*, indicating almost the same oscillation pattern in oxygen evolution between the two species. *G. violaceus* clearly has a normal oxygen-evolving machinery, as shown by the S-state transition in delayed luminescence and by measurement of thermoluminescence. Taken together with the steady-state oxygen-evolving activity data, it is reasonable to conclude that the oxygen-evolving system of *G. violaceus* functions in essentially the same manner as in other cyanobacteria.

### 3.6. Delayed fluorescence on the nanosecond time scale

Fig. 7 shows time-resolved fluorescence spectra of *G. violaceus* intact cells at  $-196^\circ\text{C}$ . Typical PS I fluorescence was not detected [23,24] and no fluorescence was observed beyond 710 nm, even after direct excitation of Chl *a* at 400 nm. At  $-196^\circ\text{C}$ , delayed fluorescence arises from charge recombination between P680 and the primary electron acceptor in PS II (Pheophytin *a*). We found that all fluorescence bands from phycobiliproteins disappeared within 30 ns of excitation, and delayed fluorescence was detected at 684 nm (Fig. 7). Significant intensity was also detected at 694 nm, however, this was due to overlap of the 684-nm fluorescence band. Lifetime of the delayed fluorescence was estimated by convolution calculations, and was found to be 17.1 ns with an amplitude of 0.3% of the total fluorescence intensity. These values were consistent with those obtained on intact cyanobacterial cells and spinach chloroplasts [46,47]. Thus, the charge separation and recombination processes in PS II were essentially identical to those of *Synechocystis*. In addition, a preliminary survey of pigment content (Pheophytin *a* and Chl *a'*) indicated no difference between these organisms (Koyama et al., unpublished).

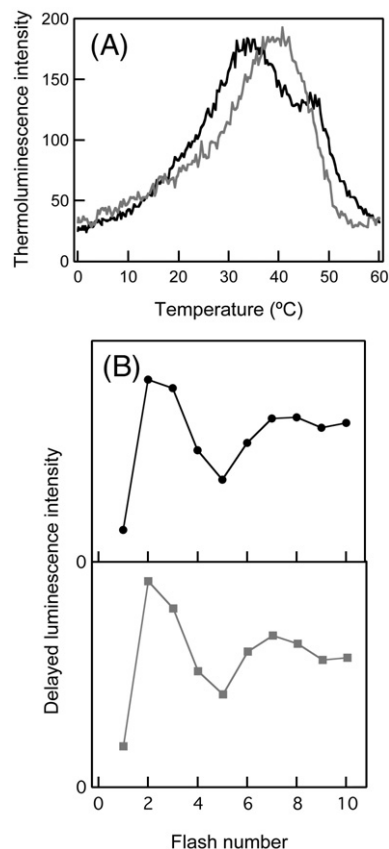


Fig. 6. Thermoluminescence and delayed luminescence in *G. violaceus* and *Synechocystis*. (A) Thermoluminescence glow curves for two species after one flash, sampling the  $\text{S}_2\text{Q}_\text{B}^-$  state. *G. violaceus* (black line) and *Synechocystis* (gray line). (B) Oscillation patterns of delayed luminescence derived from the  $\text{S}_2\text{Q}_\text{B}^-$  and  $\text{S}_3\text{Q}_\text{B}^-$  states. *G. violaceus* (upper panel), *Synechocystis* (lower panel). Luminescence was detected at 1.6 s after the flash. The data show the average of five independent measurements.



### 3.7. Average oxygen yields per flash

We analyzed turnover of the PQ pool by average oxygen yields per flash. Fig. 8 shows yields as a function of repetition rate in the two cyanobacteria, after correction for respiration. *Synechocystis* exhibited a constant yield at a low flash frequency, although it decreased at higher than 10 Hz (dark interval 100 ms). At 20 Hz,

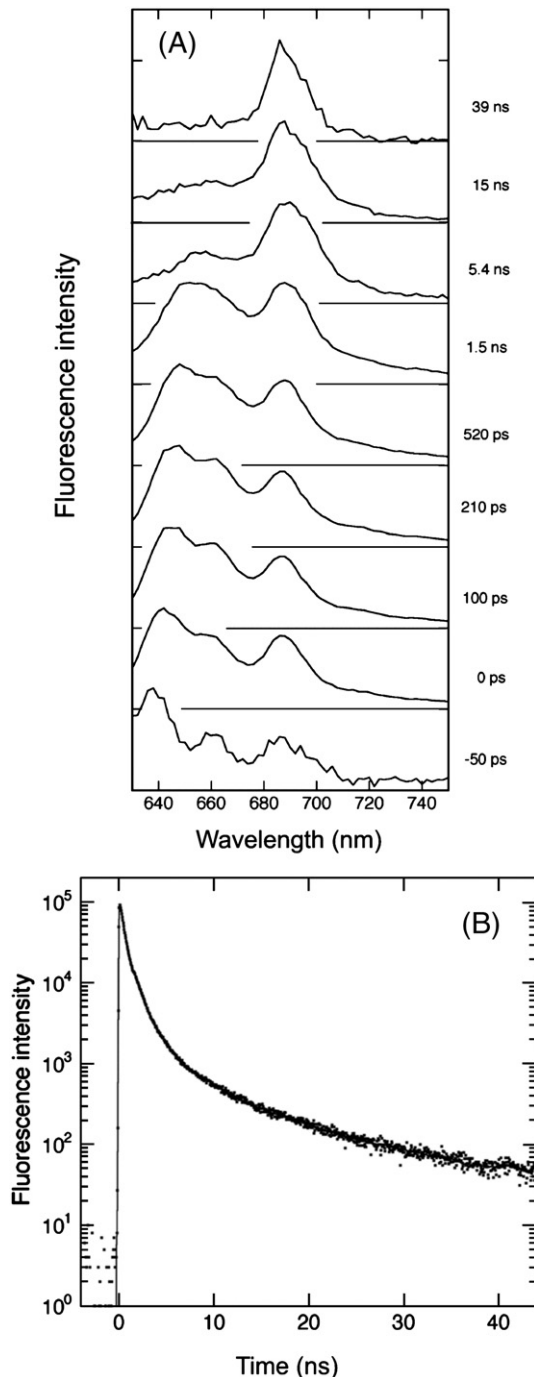


Fig. 7. Time-resolved fluorescence spectra (A) and delayed fluorescence (B) for intact *G. violaceus* cells at  $-196\text{ }^{\circ}\text{C}$ . (A) The spectra were normalized to the maximum intensities of individual spectra. Numbers on the right y-axis show times after the excitation pulse. (B) Lifetime of the delayed fluorescence was estimated from the fluorescence decay curve.

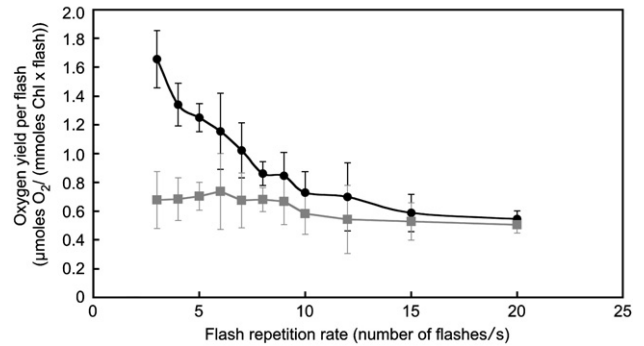


Fig. 8. Average oxygen yields per flash for two cyanobacteria *G. violaceus* (black line, circles) and *Synechocystis* (gray line, squares). Standard deviations were estimated from three independent measurements.

the yield was approximately half the maximum, a finding that reflects limitation of PQ turnover under high repetition rate conditions. This pattern is almost the same as that reported for *Anabaena variabilis* (M-2) [48], and indicated the presence of a single rate-limiting step. In contrast, the yield in *G. violaceus* was approximately twice that of *Synechocystis* at lower frequency. The actual yield was less than zero under these conditions, but after correction for respiratory activity, we were able to detect oxygen-evolving activity. The yield decreased steadily at repetition rates higher than 3 Hz (dark interval 333 ms), reaching the same level as that of *Synechocystis* at a high repetition rate (20 Hz). This pattern in *G. violaceus* is very unusual, in that the yield started to decrease at low repetition rates, but the yield itself was higher than for *Synechocystis*. These data indicate that the PQ pool turnover rate is limited in *G. violaceus*, which is most likely a consequence of electron supply from the respiratory chain on the cell membranes.

Based on these results, we conclude that in *G. violaceus*, there is no significant defect on the oxidizing side of PS II, although some defects may be present on the reducing side. Differences in the locus for water oxidation, i.e. periplasm or lumen of thylakoid membranes, did not appear to exert a significant effect on water oxidation activity.

## 4. Discussion

### 4.1. Stabilization of the Mn-cluster by extrinsic proteins with less conserved sequences

The extrinsic proteins for oxygen evolution in *G. violaceus* showed a number of modifications in amino acid sequence, such as insertions and deletions, which were not observed in more conserved homologs from other cyanobacteria (Figs. 2–4); many of the modified regions were identified as interaction sites with other proteins. In the deleted regions (residues 7–11 and 50–60 of PsbO) of amino acid sequences in *G. violaceus*, there were several conserved Asp and Glu residues. The absence of these conserved hydrophilic residues in PsbO may alter the hydrogen bond networks in this protein, as suggested in a previous report [49]. It is likely that such differences would cause changes in three-dimensional structure of the protein, as

well as in interactions with other system components. Therefore, it might be expected that the oxygen-evolving activity of *G. violaceus* would be affected by these structural differences. However, we were unable to detect any deficits in activity either in terms of steady-state oxygen yield (Fig. 5A) or thermoluminescence and delayed luminescence (Fig. 6). It appears that the oxygen-evolving reaction processes in *G. violaceus* are essentially identical to those of *Synechocystis*. Furthermore, the results indicate that the Mn-cluster is stabilized by these proteins despite their modified structures. Thus, although the actual crystal structure has yet to be resolved, these results suggest the possibility that there might be multiple modes of cluster stabilization. In general, the structures of extrinsic proteins such as PsbO have been conserved among many species of cyanobacteria [36]. However, it is interesting to find that the Mn-cluster in *G. violaceus* can be stabilized by modified proteins, since the extrinsic proteins for oxygen evolution in this species are very different from those of other cyanobacteria.

#### 4.2. Biological significance of modified protein structures localized in the periplasm

It is reasonable to assume that the ionic conditions in the periplasm are different from those in the lumen of thylakoid membranes. Furthermore, it must be expected that protein structures can undergo significant amino acid sequence modification in order to adapt to these different conditions. In *G. violaceus*, the amino acid sequences of the extrinsic proteins involved in oxygen evolution are different from those found in other cyanobacteria, and these changes are a reflection of differences in the cellular loci of oxygen evolution. *G. violaceus* does not possess *psbQ* [12], and the absence of PsbQ has been shown to destabilize binding of PsbV [50]. Thus, it is possible that additional functional roles are associated with the modifications observed in the extrinsic proteins of *G. violaceus* (i.e., alternative interactions between extrinsic proteins).

Sequence modifications were also found in the periplasmic region of core proteins in PS II (CP43 and CP47). A low degree of amino acid sequence identity was observed for PsbO/U/V between *G. violaceus* and *Synechocystis*, at 25, 16 and 21%, respectively. Although the identities for PsbA/B/C/D between *G. violaceus* and *Synechocystis* (74–84, 63, 65 and 82%, respectively) were high, there were obvious differences in the periplasmic regions of PsbB and PsbC between the two species. These can be interpreted as meaning that the level of conservation in these proteins depends upon their localization, as modifications in the amino acid sequence would be required for the proteins to function under different conditions.

Changes in amino acid sequence were also observed in PS I. The periplasmic loops in PsaA contain three insertions and one deletion, whereas those of PsaB contain two deletions and one insertion; PsaB also contains one deletion in the cytoplasmic loop. Furthermore, PsaB contains a long 155-amino acid C-terminal tail that is localized in the periplasm. In principal proteins comprising reaction center in *G. violaceus*, modifications were frequently found in periplasmic loops rather than cytoplasmic loops. The two proteins PetE (PC) and PsaF (PC

docking protein), which localize in the periplasm, were also less conserved. These protein modifications appear to reflect adaptation to the different conditions in the periplasm of *G. violaceus*.

#### 4.3. Linkage between photosynthesis and respiration for electron transfer chains on the cytoplasmic membranes

*G. violaceus* exhibited a very high respiration activity (approximately  $100 \mu\text{mol O}_2/(\text{mg Chl} \times \text{h})$ ), 20 times higher than that observed in *Synechocystis* (Fig. 5A). Since *G. violaceus* is grown under relatively low light intensities, it is probable that respiration is its primary free energy source for growth, although the substrate for respiration is enigmatic; this point is an open question at the moment. However, at this point we cannot exclude the possibility that quinol oxidase is involved in the electron transfer chain, leading to a high oxygen uptake activity. The compensation point for oxygen evolution was approximately  $35 \mu\text{E}/(\text{m}^2 \times \text{s})$ , which is greater than the light conditions required for normal growth ( $5 \mu\text{E}/(\text{m}^2 \times \text{s})$ ). This would lead to an anaerobic state in a liquid medium when air is not supplied, and might cause a slow growth rate.

DCMU-insensitive oxygen evolution indicates the presence of two types of reactions relating to oxygen evolution. This is interpreted as inhibition of oxygen uptake due either to suppression of electron flow to cytochrome oxidase, or light-induced increase in electron flow to PS I. Differences in the effect of DCMU clearly indicate modification in the reducing side of PS II in *G. violaceus*. We made an alignment of PsbA of *Synechocystis*, *T. elongatus* and five PsbAs of *G. violaceus*, and compared the region containing the herbicide-binding pocket (Phe<sub>211</sub>-Leu<sub>275</sub> of PsbA) (data not shown); we found that three PsbAs of *G. violaceus* (Glr0779, Glr2322 and Glr3144) has no significant difference from PsbAs of *Synechocystis* or *T. elongatus*, however Glr1706 and Glr2656 have several substitutions. The substituted loci of the Glr1706 are not assigned to the herbicide-sensitive amino acids, on the contrary, the Glr2656 contains several amino acids that are assigned to the herbicide-sensitive substitution including replacement of Ser<sub>264</sub> to Ala, that is assigned to the most effective residue for the DCMU resistance. Furthermore, the Glr2656 contains two inserted amino acids close to the Ser<sub>264</sub>, suggesting some effect on the herbicide binding [51,52]. However, it is reported that the *glr2656* was expressed at trace level among five *psbA* genes in *G. violaceus* irrespective of the light conditions for growth, i.e. normal light condition, UVB exposure and high light condition [52]. Therefore, it is not necessarily reasonable that the observed insensitivity is attributed to difference in amino acid sequences. There were no significant differences in the *G. violaceus* amino acid sequences of PsbE, PsbF, PsbJ and PsbL, responsible for formation of the Q<sub>B</sub> pocket. Thus, the observed partial DCMU effect on oxygen evolution in *G. violaceus* would be related not to the amino acid sequence, but to other factor(s). The basis for changes on the reducing side of PS II is thus not clear at this stage.

The average oxygen yield per flash in *G. violaceus* was strongly affected by the dark interval between flashes (Fig. 8). This probably resulted from a prolonged PQ pool turnover time due to a substantial fraction of reduced PQ molecules. Since the



respiration rate is very high, it is reasonable to assume that the PQ pool is reduced under normal photosynthetic conditions. The pattern of the average oxygen yield per flash cannot be explained by a single rate-limiting process, in contrast to *Synechocystis*. There may be an additional limiting step, as reflected by a monotonic decrease in the yield at low frequency. The delayed luminescence intensities converged at higher intensity than in *Synechocystis* (Fig. 6B). This probably indicated the accumulation of  $Q_A^-$  during flashes, reflecting equilibration of the PQ pool at more reducing conditions in *G. violaceus* than in *Synechocystis* and leading to incomplete re-oxidation of  $Q_A^-$ . These unique features of *G. violaceus* may arise from a different degree of sharing of the two electron transfer activities than in other cyanobacteria. In addition to these observations, we have found several differences on the reducing side of PS II in *G. violaceus* (Koyama et al. unpublished).

We were able to estimate the size of the photosynthetic unit (Chl basis) based on average oxygen yield per flash, although this is an average value depending on the stoichiometry of the two PSs. It was approximately 1320 for *Synechocystis*, a value consistent with previous reports for other cyanobacteria grown under low light intensity (e.g. 1470 for *Anabaena cylindrica*, 1140 for *Anabaena variabilis* (M-2) and 1480 for *Synechococcus* sp. PCC 6301 (formerly *Anacystis nidulans*) [48]). In contrast, the unit size under the lowest flash interval was estimated to be only 740 in *G. violaceus*. The basis for the small value is not clear. As shown by absorption spectra of intact cells (Fig. 1), the ratio of Chl to phycobiliproteins is small, suggesting that a small unit size may be compensated by a large phycobilin antenna. This unique feature of the pigment system might support functioning of the photosynthetic systems of *G. violaceus*.

Based on the results described above, we concluded that the oxygen-evolving system of *G. violaceus* functions normally, even though this species lacks thylakoid membranes and oxygen evolution is performed in the periplasm. *Acaryochloris marina*, the Chl *d*-dominated cyanobacteria whose primary electron donor of PS II is Chl *d* dimer [53], also has a normal oxygen-evolving system [54]. Our conclusions are that the oxygen-evolving complex is almost invariant in all oxygenic photoautotrophs, as discussed in a previous report [55]. Although the structures of both core and extrinsic proteins in *G. violaceus* were altered by changes in their amino acid sequences, these modifications did not cause a defect in the oxygen-evolving reaction. Although we do not know the structure of the PS II core in *G. violaceus*, the protein modifications may provide new insights into the stabilization of the Mn-cluster in PS II. Our understanding of this system will be improved further by EPR spectroscopy.

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## References

- [1] A. Zouni, H.T. Witt, J. Kern, P. Fromme, N. Krauß, W. Saenger, P. Orth, Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 Å resolution, *Nature* 409 (2001) 739–743.
- [2] N. Kamiya, J.-R. Shen, Crystal structure of oxygen-evolving photosystem II from *Thermosynechococcus vulcanus* at 3.7 Å resolution, *Proc. Natl. Acad. Sci. U. S. A.* 100 (2003) 98–103.
- [3] K.N. Ferreira, T.M. Iverson, K. Maghlaoui, J. Barber, S. Iwata, Architecture of the photosynthetic oxygen-evolving center, *Science* 303 (2004) 1831–1838.
- [4] B. Loll, J. Kern, W. Saenger, A. Zouni, J. Biesiadka, Towards complete cofactor arrangement in the 3.0 Å resolution structure of photosystem II, *Nature* 438 (2005) 1040–1044.
- [5] K. Sauer, V.K. Yachandra, The water-oxidation complex in photosynthesis, *Biochim. Biophys. Acta* 1655 (2004) 140–148.
- [6] J.P. McEvoy, G.W. Brudvig, Water-splitting chemistry of photosystem II, *Chem. Rev.* 106 (2006) 4455–4483.
- [7] J. Kern, G. Renger, Photosystem II: structure and mechanism of the water: plastoquinone oxidoreductase, *Photosynth. Res.* 94 (2007) 183–202.
- [8] B. Nelissen, Y. Van de Peer, A. Wilmotte, R. De Wachter, An early origin of plastids within the cyanobacterial divergence is suggested by evolutionary trees based on complete 16 S rRNA sequences, *Mol. Biol. Evol.* 12 (1995) 1166–1173.
- [9] R. Rippka, J. Waterbury, G. Cohen-Bazire, A cyanobacterium which lacks thylakoids, *Arch. Microbiol.* 100 (1974) 419–436.
- [10] G.A. Peschek, C. Obinger, M. Paumann, The respiratory chain of blue green algae (cyanobacteria), *Physiol. Plant.* 120 (2004) 358–369.
- [11] N. Keren, M. Liberton, H.B. Pakrasi, Photochemical competence of assembled photosystem II core complex in cyanobacterial plasma membrane, *J. Biol. Chem.* 280 (2005) 6548–6553.
- [12] Y. Nakamura, T. Kaneko, S. Sato, M. Mimuro, H. Miyashita, T. Tsuchiya, S. Sasamoto, A. Watanabe, K. Kawashima, Y. Kishida, C. Kiyokawa, M. Kohara, M. Matsumoto, A. Matsuno, N. Nakazaki, S. Shimpo, C. Takeuchi, M. Yamada, S. Tabata, Complete genome structure of *Gloeobacter violaceus* PCC 7421, a cyanobacterium that lacks thylakoids, *DNA Res.* 10 (2003) 137–145.
- [13] H. Inoue, T. Tsuchiya, S. Satoh, H. Miyashita, T. Kaneko, S. Tabata, A. Tanaka, M. Mimuro, Unique constitution of photosystem I with a novel subunit in the cyanobacterium *Gloeobacter violaceus* PCC 7421, *FEBS Lett.* 578 (2004) 275–279.
- [14] S. Grizot, S.K. Buchanan, Structure of the OmpA-like domain of RmpM from *Neisseria meningitidis*, *Mol. Microbiol.* 51 (2004) 1027–1037.
- [15] M. Mimuro, T. Tsuchiya, H. Inoue, Y. Sakuragi, Y. Itoh, T. Gotoh, H. Miyashita, D.A. Bryant, M. Kobayashi, The secondary electron acceptor of photosystem I in *Gloeobacter violaceus* PCC 7421 is menaquinone-4 that is synthesized by a unique but unknown pathway, *FEBS Lett.* 579 (2005) 3493–3496.
- [16] T. Tsuchiya, S. Takaichi, N. Misawa, T. Maoka, H. Miyashita, M. Mimuro, The cyanobacterium *Gloeobacter violaceus* PCC 7421 uses bacterial-type phytoene desaturase in carotenoid biosynthesis, *FEBS Lett.* 579 (2005) 2125–2129.
- [17] T. Ono, Y. Inoue, Reconstitution of photosynthetic oxygen evolving activity by rebinding of 33 kDa protein to  $\text{CaCl}_2$ -extracted PS II particles, *FEBS Lett.* 166 (1984) 381–384.
- [18] R.L. Burnap, J.-R. Shen, P.A. Jursinic, Y. Inoue, L.A. Sherman, Oxygen yield and thermoluminescence characteristics of a cyanobacterium lacking the manganese-stabilizing protein of photosystem II, *Biochemistry* 31 (1992) 7404–7410.
- [19] A. Seidler, The extrinsic polypeptides of photosystem II, *Biochim. Biophys. Acta* 1277 (2004) 35–60.
- [20] S.F. Al-Khaldi, J. Coker, J.-R. Shen, R.L. Burnap, Characterization of site-directed mutants in manganese-stabilizing protein (MSP) of *Synechocystis* sp. PCC 6803 unable to grow photoautotrophically in the absence of cytochrome *c*-550, *Plant Mol. Biol.* 43 (2000) 33–41.
- [21] J.-R. Shen, M. Qian, Y. Inoue, R.L. Burnap, Functional characterization of *Synechocystis* sp. PCC 6803  $\Delta psbU$  and  $\Delta psbV$  mutants reveals important roles of cytochrome *c*-550 in cyanobacterial oxygen evolution, *Biochemistry* 37 (1998) 1551–1558.

- [22] L.E. Thornton, H. Ohkawa, J.L. Rooses, Y. Kashino, N. Keren, H.B. Pakrasi, Homologs of plant PsbP and PsbQ proteins are necessary for regulation of photosystem II activity in the cyanobacterium *Synechocystis* 6803, *Plant Cell* 16 (2004) 2164–2175.
- [23] F. Koenig, M. Schmidt, *Gloeobacter violaceus*-investigation of an unusual photosynthetic apparatus. Absence of the long wavelength emission of photosystem I in 77 K fluorescence spectra, *Physiol. Plant.* 94 (1995) 621–628.
- [24] M. Mimuro, T. Ookubo, D. Takahashi, T. Sakawa, S. Akimoto, I. Yamazaki, H. Miyashita, Unique fluorescence properties of a cyanobacterium *Gloeobacter violaceus* PCC 7421: reasons for absence of the long-wavelength PSI Chl *a* fluorescence at  $-196^{\circ}\text{C}$ , *Plant Cell Physiol.* 43 (2002) 587–594.
- [25] M.M. Allen, Simple conditions for growth of unicellular blue-green algae on plates, *J. Phycol.* 4 (1968) 1–4.
- [26] R.J. Porra, W.A. Thompson, P.E. Kriedemann, Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy, *Biochim. Biophys. Acta* 975 (1989) 384–394.
- [27] T. Noguchi, M. Katoh, Y. Inoue, A new system for detection of thermoluminescence and delayed luminescence from photosynthetic apparatus with precise temperature control, *Spectroscopy* 16 (2002) 89–94.
- [28] S. Akimoto, M. Yokono, M. Ohmae, I. Yamazaki, N. Nagata, R. Tanaka, A. Tanaka, M. Mimuro, Excitation energy transfer in the antenna system with divinyl-chlorophylls in the vinyl reductase expressing *Arabidopsis*, *Chem. Phys. Lett.* 409 (2005) 167–171.
- [29] T. Kaneko, S. Sato, H. Kotani, A. Tanaka, E. Asamizu, Y. Nakamura, N. Miyajima, M. Hirose, M. Sugiura, S. Sasamoto, T. Kimura, T. Hosouchi, A. Matsuno, A. Muraki, N. Nakazaki, K. Naruo, S. Okumura, S. Shimpo, C. Takeuchi, T. Wada, A. Watanabe, M. Yamada, M. Yasuda, S. Tabata, Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions, *DNA Res.* 3 (1996) 109–136.
- [30] Y. Nakamura, T. Kaneko, S. Sato, M. Ikeuchi, H. Katoh, S. Sasamoto, A. Watanabe, M. Iriguchi, K. Kawashima, T. Kimura, Y. Kishida, C. Kiyokawa, M. Kohara, M. Matsumoto, A. Matsuno, N. Nakazaki, S. Shimpo, M. Sugimoto, C. Takeuchi, M. Yamada, S. Tabata, Complete genome structure of the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1, *DNA Res.* 9 (2002) 123–130.
- [31] J.D. Bendtsen, H. Nielsen, G. von Heijne, S. Brunak, Improved prediction of signal peptides—SignalP 3.0, *J. Mol. Chem.* 340 (2004) 783–795.
- [32] Y. Kashino, W.M. Lauber, J.A. Carroll, Q. Wang, J. Whitmarsh, K. Satoh, H.B. Pakrasi, Proteomic analysis of a highly active photosystem II preparation from the cyanobacterium *Synechocystis* sp. PCC 6803 reveals the presence of novel polypeptides, *Biochemistry* 41 (2002) 8004–8012.
- [33] J. Kern, B. Loll, C. Lüneberg, D. DiFiore, J. Biesiadka, K.-D. Irrgang, A. Zouni, Purification, characterization and crystallisation of photosystem II from *Thermosynechococcus elongatus* cultivated in a new type of photobioreactor, *Biochim. Biophys. Acta* 1706 (2005) 147–157.
- [34] C. Notredame, D.G. Higgins, J. Heringa, T-Coffee: a novel method for fast and accurate multiple sequence alignment, *J. Mol. Biol.* 302 (2002) 205–217.
- [35] H. Popelkova, I.M. Michael, C.F. Yocum, N-terminal truncations of manganese stabilizing protein identify two amino acid sequences required for binding of the Eukaryotic protein to photosystem II and reveal the absence of one binding-related sequence in cyanobacteria, *Biochemistry* 41 (2002) 10038–10045.
- [36] J. De Las Rivas, J. Barber, Analysis of the structure of the PsbO protein and its implications, *Photosynth. Res.* 81 (2004) 329–343.
- [37] A. Motoki, M. Usui, T. Shimazu, M. Hirano, S. Katoh, A domain of the manganese stabilizing protein from *Synechococcus elongatus* involved in functional binding to photosystem II, *J. Biol. Chem.* 277 (2002) 14747–14756.
- [38] J.W. Murray, J. Barber, Identification of a Calcium-binding site in the PsbO protein of photosystem II, *Biochemistry* 45 (2006) 4128–4130.
- [39] J.-R. Shen, Y. Inoue, Binding and functional properties of two new extrinsic components, cytochrome *c*-550 and a 12 kDa protein, in cyanobacterial photosystem II, *Biochemistry* 32 (1993) 1825–1832.
- [40] Z. Li, H. Andrews, J.J. Eaton-Rye, R.L. Burnap, In situ effects of mutations of the extrinsic cytochrome *c*<sub>550</sub> of photosystem II in *Synechocystis* sp. PCC6803, *Biochemistry* 43 (2004) 14161–14170.
- [41] C. Frazão, F.J. Enguita, R. Coelho, G.M. Sheldrick, J.A. Navarro, M. Hervas, M.A. De la Rosa, M.A. Carrondo, Crystal structure of low-potential cytochrome *c*<sub>549</sub> from *Synechocystis* sp. PCC 6803 at 1.21 Å resolution, *J. Biol. Inorg. Chem.* 6 (2001) 324–332.
- [42] C.A. Howitt, W.F.J. Vermaas, Quinol and cytochrome oxidases in the cyanobacterium *Synechocystis* sp. PCC 6803, *Biochemistry* 37 (1998) 17944–17951.
- [43] T. Endo, Cytochrome *b/f* complex is not involved in respiration in the cyanobacterium *Synechocystis* PCC 6803 grown photoautotrophically, *Biosci. Biotech. Biochem.* 61 (1997) 1770–1771.
- [44] I. Vass Govindjee, Thermoluminescence from the photosynthetic apparatus, *Photosynth. Res.* 48 (1996) 117–126.
- [45] I. Vass, K.M. Cook, Z. Deak, S.R. Mayes, J. Barber, Thermoluminescence and flash-oxygen characterization of the IC2 deletion mutant of *Synechocystis* sp. PCC 6803 lacking the photosystem II 33 kDa protein, *Biochim. Biophys. Acta* 1102 (1992) 195–201.
- [46] M. Mimuro, S. Akimoto, I. Yamazaki, H. Miyashita, S. Miyachi, Fluorescence properties of chlorophyll *d*-dominating prokaryotic alga, *Acaryochloris marina*: studies using time-resolved fluorescence spectroscopy on intact cells, *Biochim. Biophys. Acta* 1412 (1999) 37–46.
- [47] F. van Mieghem, K. Brettel, B. Hillmann, A. Kamlowski, A.W. Rutherford, E. Schlöder, Charge recombination reactions in photosystem II. 1. Yields, recombination pathways, and kinetics of the primary pair, *Biochemistry* 34 (1995) 4798–4813.
- [48] M. Kawamura, M. Mimuro, Y. Fujita, Quantitative relationship between two reaction centers in the photosynthetic system of blue-green algae, *Plant Cell Physiol.* 20 (1979) 697–705.
- [49] T. Shutova, K.-D. Irrgang, V. Shubin, V.V. Klimov, G. Renger, Analysis of pH-induced structural changes of the isolated extrinsic 33 kilodalton protein of photosystem II, *Biochemistry* 36 (1997) 6350–6358.
- [50] Y. Kashino, N. Inoue-Kashino, J.L. Roose, H.B. Pakrasi, Absence of the PsbQ protein results in destabilization of the PsbV protein and decreased oxygen evolution activity in cyanobacterial photosystem II, *J. Biol. Chem.* 281 (2006) 20834–20841.
- [51] W. Oettmeier, Herbicide resistance and supersensitivity in photosystem II, *Cell. Mol. Life. Sci.* 55 (1999) 1255–1277.
- [52] C.I. Sicora, C.M. Brown, O. Cheregi, I. Vass, D.A. Campbell, The *psbA* gene family responds differentially to light and UVB stress in *Gloeobacter violaceus* PCC 7421, a deeply divergent cyanobacterium, *Biochim. Biophys. Acta* 1777 (2008) 130–139.
- [53] T. Tomo, T. Ohkubo, S. Akimoto, M. Yokono, H. Miyashita, T. Tsuchiya, T. Noguchi, M. Mimuro, Identification of the special pair of photosystem II in a chlorophyll *d*-dominated cyanobacterium, *Proc. Natl. Acad. Sci. U. S. A.*, 104 (2007) 7283–7288.
- [54] D. Shevela, B. Nöring, H.-J. Eckert, J. Messinger, G. Renger, Characterization of the water oxidizing complex of photosystem II of the Chl *d*-containing cyanobacterium *Acaryochloris marina* via its reactivity towards endogenous electron donors and acceptors, *Phys. Chem. Chem. Phys.* 8 (2006) 3460–3466.
- [55] M. Karge, K.-D. Irrgang, G. Renger, Analysis of the reaction coordinate of photosynthetic water oxidation by kinetic measurements of 335 nm absorption changes at different temperatures in photosystem II preparations suspended in either H<sub>2</sub>O or D<sub>2</sub>O, *Biochemistry* 36 (1997) 8904–8913.