



Net light-induced oxygen evolution in photosystem I deletion mutants of the cyanobacterium *Synechocystis* sp. PCC 6803[☆]

Qing Jun Wang^{a,*}, Abhay Singh^{b,1}, Hong Li^{b,2}, Ladislav Nedbal^{c,d}, Louis A. Sherman^b, Govindjee^{a,e,f,**}, John Whitmarsh^{a,e,g}

^a Center for Biophysics & Computational Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

^b Department of Biological Sciences, Purdue University, Lilly Hall, West Lafayette, IN 47907, USA

^c Institute of Systems Biology and Ecology AVCR, Zámek 136, CZ-37333 Nové Hradky, Czech Republic

^d Institute of Physical Biology JU, Zámek 136, CZ-37333 Nové Hradky, Czech Republic

^e Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

^f Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

^g USDA/ARS, USA

ARTICLE INFO

Article history:

Received 15 September 2011

Received in revised form 29 December 2011

Accepted 3 January 2012

Available online 12 January 2012

Keywords:

Photosynthesis
Electron transport
CO₂ assimilation
Photosystem I
Oxygen evolution
Synechocystis

ABSTRACT

Oxygenic photosynthesis in cyanobacteria, algae, and plants requires photosystem II (PSII) to extract electrons from H₂O and depends on photosystem I (PSI) to reduce NADP⁺. Here we demonstrate that mixotrophically-grown mutants of the cyanobacterium *Synechocystis* sp. PCC 6803 that lack PSI (Δ PSI) are capable of net light-induced O₂ evolution *in vivo*. The net light-induced O₂ evolution requires glucose and can be sustained for more than 30 min. Utilizing electron transport inhibitors and chlorophyll *a* fluorescence measurements, we show that in these mutants PSII is the source of the light-induced O₂ evolution, and that the plastoquinone pool is reduced by PSII and subsequently oxidized by an unidentified electron acceptor that does not involve the plastoquinol oxidase site of the cytochrome *b₆f* complex. Moreover, both O₂ evolution and chlorophyll *a* fluorescence kinetics of the Δ PSI mutants are highly sensitive to KCN, indicating the involvement of a KCN-sensitive enzyme(s). Experiments using ¹⁴C-labeled bicarbonate show that the Δ PSI mutants assimilate more CO₂ in the light compared to the dark. However, the rate of the light-minus-dark CO₂ assimilation accounts for just over half of the net light-induced O₂ evolution rate, indicating the involvement of unidentified terminal electron acceptors. Based on these results we suggest that O₂ evolution in Δ PSI cells can be sustained by an alternative electron transport pathway that results in CO₂ assimilation and that includes PSII, the plastoquinone pool, and a KCN-sensitive enzyme.

© 2012 Elsevier B.V. All rights reserved.

Abbreviations: Chl, chlorophyll; PSII, photosystem II; Cyt *b₆f*, cytochrome *b₆f*; Cyt *c₆*, cytochrome *c₆*; PSI, photosystem I; Fd, ferredoxin; FNR, ferredoxin-NADP⁺-oxidoreductase; Pheo, pheophytin; FQR, ferredoxin-plastoquinone-oxidoreductase; PQ, plastoquinone; PQH₂, plastoquinol; P680, special pair of Chl *a* molecules for primary photochemistry in PSII; P700, special pair of Chl *a* molecules for primary photochemistry in PSI; Q_A, first plastoquinone electron acceptor of PSII; Q_B, second plastoquinone electron acceptor of PSII; Q_o, the plastoquinol oxidase site of the Cyt *b₆f* complex; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; KCN, potassium cyanide; FeCN, potassium ferricyanide; DCBQ, 2,6-dichloro-*p*-benzoquinone; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCPIP, 2,6-dichlorophenolindophenol; MV, methyl viologen; WT, wild type; Δ PSI, PSI deletion; PEP, phosphoenol pyruvate; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase

[☆] This work was supported by the USDA/Agricultural Research Service (J.W.), grant-FG02-99ER20342 from DOE (L.A.S.), grants MSM6007665808 from MSM and AV0260870520 from USBE AVCR (L.N.), and 2P20 RR020171 from NIH NCR (Q.J.W.).

* Correspondence to: Q.J. Wang, Department of Molecular and Cellular Biochemistry, University of Kentucky, College of Medicine, B163 BBSRB, 741 South Limestone, Lexington, KY 40536-0509, USA. Tel.: +1 859 323 5335.

** Correspondence to: Govindjee, Department of Plant Biology, University of Illinois at Urbana-Champaign, 265 Morrill Hall, 505 South Goodwin Avenue, Urbana, IL 61801, USA. Tel.: +1 217 337 0627; fax: +1 217 244 7246.

E-mail addresses: qingjun.wang@uky.edu (Q.J. Wang), gov@illinois.edu (Govindjee).

¹ Current address: MOgene Green Chemicals, 4633 World Parkway Circle, Saint Louis, MO 63134, USA.

² Current address: VaxInnate Corporation, 3 Cedar Brook Dr., Cranbury, NJ 08512, USA.

1. Introduction

The light-driven reactions of photosynthesis in cyanobacteria, algae, and plants are described by a Z-scheme, the linear electron transport pathway from H₂O to ferredoxin (Fd) and NADP⁺ that generates O₂ as a byproduct and drives the assimilation of CO₂ [1–3]. This linear electron transport pathway depends on photosystem II (PSII) [4], photosystem I (PSI) [5] and the cytochrome *b₆f* (Cyt *b₆f*) complex [6]. Although the Z-scheme serves as the dominant pathway in oxygenic photosynthesis, PSII-driven electron transport from H₂O without the involvement of PSI has been suggested [7–10]. Govindjee et al. [7] proposed an alternative electron transport pathway in algal cells in which PSII could reduce NADP⁺ independent of PSI – a pathway similar to that in photosynthetic bacteria. Arnon [9] and the references therein) proposed a more complicated pathway for the reduction of Fd by PSII – a pathway that required plastocyanin, but not PSI. Several PSI deletion mutants of *Chlamydomonas reinhardtii* have been shown to evolve O₂ in the light [11–13]. However, the rate of light-induced O₂ evolution in these mutants was lower than

that of respiration, resulting in net O₂ uptake. In addition, neither CO₂ assimilation nor H₂ production was observed in these mutants.

The observation of net light-induced O₂ evolution in the absence of PSI was first reported in two PSI deletion mutants (Δ PSI) of the cyanobacterium *Synechocystis* sp. PCC 6803 ([8] and L. B. Smart, personal communication). Smart et al. [8] speculated that the plastoquinone (PQ) pool, NADP⁺, H⁺ and/or O₂ may serve as the terminal electron acceptor(s) for the PSI-independent O₂ evolution. Additional Δ PSI mutants of *Synechocystis* 6803 have been generated in the laboratories of Himadri Pakrasi (Washington University, St. Louis, MO) and Wim F. J. Vermaas (Arizona State University, Tempe, AZ) [14–17]. Vermaas and co-workers (see e.g. [18–20]) proposed PSII-driven electron transfer from H₂O to O₂ via a pathway that involved PQ and potassium cyanide (KCN)-sensitive respiratory terminal oxidases. However, Vermaas and co-workers did not observe net light-induced O₂ evolution in the absence of PSI.

In the present work, we report that in several mixotrophically-grown Δ PSI mutants of *Synechocystis* 6803, the rate of PSII water oxidation in the light in the presence of glucose and NaHCO₃ is significantly faster than the respiration rate, resulting in net O₂ evolution. Inhibitor studies and chlorophyll (Chl) *a* fluorescence measurements demonstrate the presence of electron transport from H₂O to the PQ pool and the involvement of KCN-sensitive enzyme(s) in the Δ PSI mutants. We show that although the Δ PSI mutants are capable of light-minus-dark CO₂ assimilation, the rate is about half of that required to account for the rate of net O₂ evolution.

2. Materials and methods

2.1. Mutants and growth conditions

The glucose tolerant wild type (WT) strain of *Synechocystis* sp. PCC 6803 and six PSI deletion mutant strains were gifts from Himadri Pakrasi (E. Zak and H. Pakrasi, personal communication) and W. F. J. Vermaas [14–17]. Details of these mutants are summarized in Table 1. These cyanobacterial strains were maintained according to the methods provided by the laboratories donating the strains. The antibiotics and glucose concentrations used for the BG-11 plates were as follows: 50 μ g/ml kanamycin and 10 mM glucose for Δ AB; 25 μ g/ml spectinomycin and 10 mM glucose for Δ VIII–XI; 25 μ g/ml chloramphenicol and 15 mM glucose for Δ PSI/WV; 10 μ g/ml erythromycin, 25 μ g/ml chloramphenicol and 15 mM glucose for Δ PSI Δ ApcE; 15 μ g/ml zeocin, 25 μ g/ml erythromycin, 35 μ g/ml chloramphenicol, 25 μ g/ml spectinomycin and 15 mM glucose for Δ PSI Δ NdbABC; 25 μ g/ml erythromycin, 35 μ g/ml chloramphenicol, 25 μ g/ml spectinomycin and 15 mM glucose for Δ PSI Δ CtaDIIIEII Δ CydAB.

The WT cells were cultured in the BG-11 medium in the presence or the absence of 5 mM glucose at 30 °C under 65 μ mol photons·m^{−2}·s^{−1} fluorescent light. All of the Δ PSI mutant strains

were grown in the presence of 5 mM glucose at 30 °C without antibiotics. All of the Δ PSI mutant strains except for the Δ PSI Δ ApcE strain were grown under ca. 1.5 μ mol photons·m^{−2}·s^{−1} fluorescent light. The Δ PSI Δ ApcE strain was grown under 65 μ mol photons·m^{−2}·s^{−1} fluorescent light. The cell cultures were bubbled with water-saturated air through a sterilized filter (Gelman #4210, 0.3 μ m pore size). Cells were grown in the BG-11 medium either with or without the supplement of 1 μ M Cu²⁺ (in the form of CuSO₄), which has been previously reported to be sufficient for these cells to express either plastocyanin or Cyt c₆, respectively [21]. The absence of plastocyanin in the cells grown without the Cu²⁺ supplement³ was indirectly examined by the presence of the light-minus-dark absorption peak of Cyt c₆ at 553 nm. The cell density was monitored by measuring the optical density of the cell cultures at 730 nm [22].

2.2. Measuring the net light-induced O₂ evolution and dark respiration rates and the PSII activity

Cells were harvested during exponential growth phase by centrifugation at 5000 g for 5 min at room temperature and kept in fresh BG-11 medium in the presence of 5 mM glucose (unless noted otherwise) prior to the measurements. This glucose concentration was sufficient for maintaining the activities during the measurements. Based on the growth curves, it was also sufficient to sustain cell growth until stationary phase. The rates of the net light-induced O₂ evolution and the dark respiration of whole cells were monitored at 30 °C by a Clark-type electrode (YSI 5331 oxygen probe, Yellow Springs, OH) with a polarizing voltage of −0.7 V [23,24]. The actinic light was provided by a pair of Tungsten-halogen lamps (EG&G, 250 W) illuminating from both sides of the reaction chamber after being filtered through pairs of heat-reflecting filters (Melles Griot 03MHG007), heat absorbing blocking filters (Corning CS I-75, Rochester, NY), red blocking filters (Corning CS 2-63, Rochester, NY) and appropriate neutral density filters. The light intensity for reaching maximal net O₂ evolution rate was measured to be 500 μ mol photons·m^{−2}·s^{−1} (same on each side) by a Licor Quantum Photometer LI-185B (Lincoln, NE). Sodium dithionite was used to calibrate the electrode. NaHCO₃ was added at 10 mM concentration to the cell suspensions in the reaction chamber right before the measurements.

For testing 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) adaptation, the WT cells were also grown in the presence of micromolar DBMIB and 5 mM glucose under ca. 1.5 μ mol photons·m^{−2}·s^{−1} light intensity for a total of two inoculation cycles: the concentration of DBMIB on the day of each inoculation was 10 μ M; an extra dose of DBMIB (equivalent to 2 μ M final concentration) was supplied everyday afterwards to compensate for the light-sensitive loss of DBMIB. For titrating the net O₂ evolution rates of these DBMIB-adapted cells with DBMIB, the cells were washed with fresh BG-11 three times to remove DBMIB that was present during the cultivation.

The PSII activity was measured as the saturated light-induced O₂ evolution rate in the presence of artificial PSII electron acceptors (i.e., 1.2 mM potassium ferricyanide (FeCN) and 600 μ M 2,6-dichloro-*p*-benzoquinone (DCBQ)) and with the saturating actinic light intensity as high as 3600 μ mol photons·m^{−2}·s^{−1}.

2.3. Membrane preparation

Membranes from the *Synechocystis* 6803 cells were prepared as previously described [25] with minor modifications. All steps were carried out at 4 °C. The cell pellet from 50 ml cell culture was resuspended in 1 ml breakage buffer containing 50 mM HEPES/NaOH (pH 7), 0.5 M sucrose, 15 mM NaCl, 5 mM MgCl₂, 0.1% (v/v) Protease

Table 1
The Δ PSI mutants used in this study.

Name	Mutation	Reference
Δ AB	A significant portion of the <i>psaAB</i> operon was deleted	E. Zak and H. Pakrasi, personal communication
Δ VIII–XI	A part of the <i>psaB</i> gene that encoded the VIIIth to XIth helices of the PsaB protein was deleted	E. Zak and H. Pakrasi, personal communication
Δ PSI/WV	PSI was deleted	[14]
Δ PSI Δ ApcE	Both PSI and the phycobilisome linker protein ApcE were deleted	[15]
Δ PSI Δ NdbABC	Both PSI and the type 2 NADH dehydrogenase Ndb were deleted	[16]
Δ PSI Δ CtaDIIIEII Δ CydAB	Both PSI and the alternative terminal oxidases CtaII and Cyd were deleted	[17]

³ No special procedure was employed to remove trace amounts of Cu²⁺.

Inhibitor Cocktail (Sigma), and 4 $\mu\text{g}/\text{mL}$ DNase I. These cells were broken by glass beads (size 150–212 μm , acid-washed, Sigma) with 5 cycles of 2 min of vortexing followed by 2 min of cooling on ice. After the final cycle, the glass beads were allowed to settle and the homogenate was collected. The glass beads were washed several times and the homogenates were pooled. Debris spin was carried out at 8160 g (Eppendorf, model Centrifuge 5415 C) for 5 min. The supernatant was collected and centrifuged again at 16,000 g for 30 min. The membrane pellet was collected and re-suspended in the breakage buffer.

2.4. Chl concentration, Chl-to-PSII ratio and PSII concentration determination

Concentration of Chl in *Synechocystis* 6803 cells was spectrophotometrically determined [26]. The Chl-to-PSII ratios of the membrane preparations were determined by measuring their O_2 evolution activity driven by single-turnover flashes [23]. The actinic light was provided by 600 flashes (frequency 10 Hz, width 6 μs) of a Xe-flash lamp (FX-200; EG&G, Salem, MA). The actinic flashes were filtered by a yellow filter (Corning CS 3-71, Rochester, NY) and 1 cm of water. The Xe-flash lamp was driven by 700 V to yield saturating light intensity. The O_2 evolution rate of the membranes was measured at 30 °C using a Clark-type oxygen electrode, as described in Section 2.2. The reaction buffer contained 50 mM MES-NaOH (pH 6.0), 10 mM MgCl_2 , 5 mM CaCl_2 , 5 mM NaCl and 0.5 M sucrose. The optimal O_2 evolution rate was reached when the reaction mixture contained 250 μM FeCN and 100 μM DCBQ. Varying the saturating single-turnover flash number between 400 and 800 or the flash frequency between 5 and 20 Hz had no effect on the O_2 evolution rate. When calculating the Chl-to-PSII ratios, we assumed that one O_2 molecule was evolved per PSII per four flashes. The PSII concentration of each sample was determined by converting the Chl concentration using corresponding Chl-to-PSII ratio. In our work, the net light-induced O_2 evolution rate and light-minus-dark CO_2 assimilation were calculated on a PSII basis, because the PSI-less mutants of *Synechocystis* 6803 had a much lower Chl-to-PSII ratio than WT. In WT, 80–85% of all Chl is associated with the PSI core complex, which is lost in the PSI-less mutants [15]. Because of the low Chl-to-PSII ratio, most PSI-less mutants could be grown only under low light, except for the $\Delta\text{PSI}\Delta\text{ApeC}$ strain in which the light harvesting complex was impaired in addition to PSI.

2.5. The kinetics of PSI reaction center, P700

Flash-induced P700 oxidation and re-reduction kinetics of the membrane preparations were measured at room temperature using a laboratory-built single-beam spectrophotometer as described earlier [27]. The saturating single-turnover actinic flashes (width, 6 μs) were provided by a pair of Xe-flash lamps (FX-200; EG&G, Salem, MA) from both sides of the sample cuvette to induce a rapid oxidation of the PSI primary donor P700. The actinic flashes were filtered by two broad-band interference filters (DT-Blau, Balzers) and two blocking filters (Corning CS 4-96, Rochester, NY). The flash-induced P700 oxidation and re-reduction kinetics were recorded at discrete wavelengths ranging from 665 nm to 830 nm. Typically, at each wavelength, data from 8 runs were averaged. The reaction buffer for P700 measurements contained 50 mM Tris-HCl (pH 8.3), 33 μM 2,6-dichlorophenolindophenol (DCPIP), and 1.7 mM sodium ascorbate. Chl concentration was adjusted to be between 20 and 30 μM for best signal-to-noise ratio and to get desirable PSII concentration. The samples were stirred after measuring absorbance at each wavelength. The experiments were carried out at 24 ± 1 °C.

2.6. Chl *a* fluorescence kinetics

Chl *a* fluorescence kinetics was monitored using a dual-modulation kinetic fluorometer (PSI, Brno, Czech Republic). The single-turnover, saturating flashes were provided by diodes (LEDs) emitting at 660 nm. The measuring flashes were provided by LEDs emitting at 620 nm [28]. Cells at exponential growth phase were resuspended in fresh BG-11 medium in the presence of 5 mM glucose. The cell density was adjusted so that Chl concentration was ca. 1 μM for best signal-to-noise ratio. Cells were dark adapted for 10 min before each experiment. For measuring the Chl *a* fluorescence induction kinetics, three data points (1 ms apart) were collected in darkness for measuring the F_0 level. Afterwards, a train of 5000 saturating, single-turnover flashes were fired at 1 kHz for 5 s. For measuring the Chl *a* fluorescence dark relaxation kinetics, the actinic light was turned off at 370 ms when the Chl *a* fluorescence reached the maximum, and subsequently the redox states of Q_A^- and the PQ pool were probed for ca. 20 s by logarithmically spaced measuring flashes. The duration and intensity of the measuring flashes were adjusted to minimize their actinic effect. The experiments were carried out at 24 ± 1 °C.

2.7. Measurements of the CO_2 assimilation rates

The CO_2 assimilation rates were measured using ^{14}C -labeled NaHCO_3 [29]. Cells at the exponential growth phase were harvested. The cell pellet was re-suspended in freshly prepared HCO_3^- -less BG-11 medium. $\text{NaH}^{14}\text{CO}_3$ was diluted with cold NaHCO_3 . The final NaHCO_3 concentration in the reaction mixtures was either 5 mM or 10 mM for several independent experiments. An aliquot of 0.85 ml cell suspension combined with 0.85 ml diluted $\text{NaH}^{14}\text{CO}_3$ was used for O_2 evolution measurement and another aliquot was kept in the dark as the control. After the O_2 evolution measurement, both the sample and the control were quickly treated with a strong acidic solution containing 0.7 ml of 4 M formic acid and 1 M HCl to break the cells in order to release un-incorporated inorganic carbon. Both the sample and the control were dried overnight in the hood at 65 °C with air blowing over them. Then 0.5 ml of 0.1 M HCl was added to each vial to dissolve the organic forms of carbon. After addition of the scintillation cocktail to the sample, radioactivity of the sample was counted in a liquid scintillation counter (liquid scintillation analyzer, Packard, model tri-carb 1600tr). The specific activity was calculated from three replicas of the diluted $\text{NaH}^{14}\text{CO}_3$ aliquots. The calculated specific activities for these experiments ranged from 200 to 1000 cpm/nmol $\text{NaH}^{14}\text{CO}_3$.

3. Results

3.1. Spectroscopic measurements confirm the absence of PSI activity in *Synechocystis* 6803 mutant strains lacking PSI

We characterized six ΔPSI mutants, which were generated in the laboratories of Himadri Pakrasi and Wim F. J. Vermaas (Table 1). In each of these mutants, a significant portion of either the *psaB* gene (encoding for the PSI core protein PsaB) or the *psaAB* operon (encoding for the PSI core proteins PsaA and PsaB) was replaced by an antibiotic resistance marker. These ΔPSI mutant strains were completely segregated and the absence of PSI was confirmed in their original laboratories by Southern and Western blots (E. Zak and H. Pakrasi, personal communication and [14–17]) (see discussion in Supplementary Material S1). We further examined the absence of PSI in these mutant strains by measuring their P700 oxidation and re-reduction kinetics. When a saturating single-turnover flash was applied to the WT membranes, a large absorbance change reflecting a rapid light-induced oxidation of P700 (peak 703 nm), followed by its re-reduction in the dark, was observed. In contrast, such P700 oxidation transient was not detected in the membranes prepared from the ΔPSI (ΔAB)

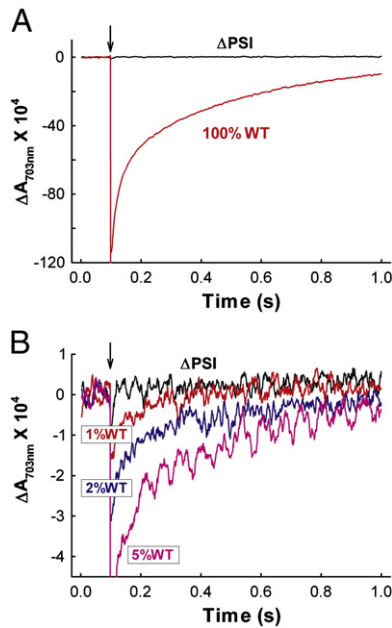


Fig. 1. The absence of PSI in the PSI deleted Δ AB mutant strain of *Synechocystis* 6803 is confirmed by the lack of P700 oxidation-induced absorbance change at 703 nm in the Δ AB membrane preparation. (A) Absorption transient at 703 nm reflecting the P700 oxidation and re-reduction kinetics of the WT *Synechocystis* 6803 membranes and lack of it in the Δ AB membranes. (B) Absorption transient at 703 nm (on an expanded scale) demonstrating the lack of the P700 response in the Δ AB membranes as compared to that of the Δ AB membranes with 1%, 2% and 5% WT admixtures. In both (A) and (B), the arrows indicate the times when the actinic flashes were fired. The reaction buffer contained 50 mM Tris-HCl (pH 8.3), 33 μ M DCPIP, 1.7 mM sodium ascorbate and 50 nM PSII. The experiments were carried out at 24 ± 1 °C.

mutant (Fig. 1A). To determine the detection limit of our instrument, we ‘titrated’ the Δ AB membranes with small amounts of the WT membranes. As shown in Fig. 1B, the light-induced absorbance change resulting from as little as 1% WT P700 oxidation (on a PSII basis) can be detected with our instrument. Therefore, within a maximal experimental error of 1%, there was no detectable PSI activity in the Δ AB mutant. Similar conclusions were reached with the other Δ PSI strains, where there was no detectable PSI activity within maximal experimental errors of 1–5% (data not shown).

3.2. Δ PSI mutants show net light-induced O_2 evolution in the presence of glucose and $NaHCO_3$

Despite the lack of PSI, all the Δ PSI cells showed a net light-induced O_2 evolution *in vivo* in the presence of 5 mM glucose and 10 mM $NaHCO_3$.⁴ For example, Fig. 2 shows a representative chart recorder trace of net light-induced O_2 evolution in the Δ AB cells that was sustained for 30 min. The trace, which is similar to that for the other mutants, shows a net O_2 uptake in the dark due to respiration, followed by a net oxygen evolution in the light, indicating that the rate of light-induced O_2 evolution exceeds the rate of respiration in the light.

Here we define the net light-induced O_2 evolution rate (i.e., the observed O_2 evolution rate, not adjusted for respiration) as P_{obs} , the dark respiration rate (i.e., O_2 consumption rate, of positive values) as R , and the O_2 evolution rate that is adjusted for dark respiration as P_{adj} (Fig. 2). Thus $P_{adj} = R + P_{obs}$, assuming the respiration rate in the light was the same as that in the darkness.⁵ Classically, O_2 evolution rate is calculated as P_{adj} with the above assumption. We note that

⁴ In the present work, both O_2 and CO_2 were monitored in the presence of 5 mM glucose and 10 mM $NaHCO_3$ unless stated otherwise.

⁵ In the formula $P_{adj} = R + P_{obs}$, respiration (R) is positive.

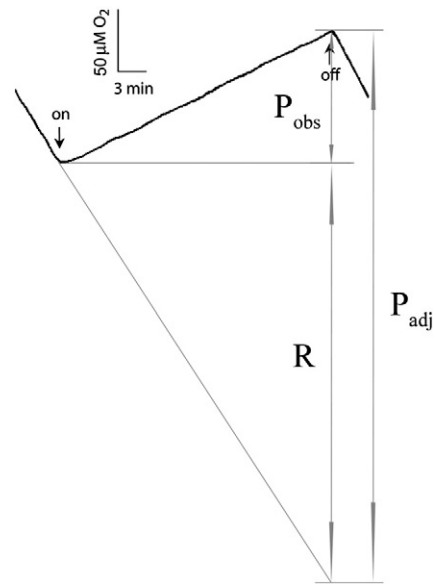


Fig. 2. A typical chart recorder trace of the light-induced O_2 evolution of the Δ PSI (Δ AB) cells in the presence of 5 mM glucose and 10 mM $NaHCO_3$. The rates of the dark respiration (R) and of the light-induced O_2 evolution either with (P_{adj}) or without (P_{obs}) adjustment for dark respiration are labeled. The down- and up-arrows indicate the times when the actinic light was turned on and off, respectively. This figure shows that the Δ AB cells were able to maintain substantial and constant rates of net light-induced O_2 evolution P_{obs} for 30 min. The Chl concentration of this sample was 1.3 μ M. The experiment was carried out at 30 °C.

O_2 uptake in the light could also be inhibited (e.g., ‘Kok effect’) or stimulated (e.g., as reported in another cyanobacterium *Synechococcus elongatus* at medium and high light intensities [30]) in the Δ PSI cells. Therefore, in this work, we primarily used P_{obs} as the readout for O_2 evolution. As summarized in Table 2, on a PSII basis, the net light-induced O_2 evolution rate P_{obs} was 4 ± 1 mol O_2 · mol PSII^{−1} · s^{−1} in the PSI deleted Δ AB cells, amounting to about 13% of that in the WT

Table 2

Comparison of the O_2 evolution and CO_2 assimilation rates of WT *Synechocystis* 6803 (grown either in the absence or in the presence of 5 mM glucose, Glc) and the PSI deleted Δ AB (grown in the presence of 5 mM glucose) cells. Both the O_2 evolution and the CO_2 assimilation rates were measured in fresh BG-11 growth medium supplemented with 10 mM $NaHCO_3$ and the corresponding glucose content. As shown in Fig. 2, P_{obs} is the net light-induced O_2 evolution rate obtained from the above-zero portion of the O_2 evolution traces and P_{adj} is the O_2 evolution rate adjusted for respiratory O_2 consumption, assuming that the respiration rate in the light is the same as in the dark (positive values for O_2 consumption). Since the Δ AB cells differed vastly from the WT cells in their Chl-to-PSII ratios, both the O_2 evolution and the CO_2 assimilation rates are normalized to the PSII content, utilizing the measured Chl-to-PSII ratios. The rates of corresponding electron fluxes can be obtained by multiplying the O_2 evolution rates by 4. The numbers of independent experiments performed are indicated in parentheses. Detailed experimental conditions are described in Materials and methods. Despite the high variation in the dark CO_2 assimilation rates, the light-minus-dark CO_2 assimilation rate of the Δ PSI cells is highly statistically significant, as examined by the paired *t*-test (one-tailed, $p = 0.0003$) for 7 independent measurements.

Parameters measured	WT + Glc	WT-Glc	Δ PSI + Glc
Net O_2 evolution rate P_{obj} (mmol O_2 · mol Chl ^{−1} · s ^{−1})	68 ± 10 (6)	82 ± 15 (7)	56 ± 14 (7)
Chl-to-PSII ratio	490 ± 25 (2)	518 (1)	78 ± 4 (2)
Net O_2 evolution rate P_{obj} (mol O_2 · mol PSII ^{−1} · s ^{−1})	34 ± 5 (6)	42 ± 8 (7)	4 ± 1 (7)
Dark respiration rate R (mol O_2 · mol PSII ^{−1} · s ^{−1})	5 ± 1 (6)	4 ± 2 (7)	6 ± 2 (7)
Adjusted O_2 evolution rate P_{adj} (mol O_2 · mol PSII ^{−1} · s ^{−1})	39 ± 5 (6)	46 ± 8 (7)	10 ± 3 (7)
PSII activity (mol O_2 · mol PSII ^{−1} · s ^{−1})	66 (1)	—	110 (1)
Dark CO_2 assimilation rate (mol CO_2 · mol PSII ^{−1} · s ^{−1})	1.4 ± 0.7 (3)	1.4 ± 0.8 (5)	5 ± 2 (7)
Light-minus-dark CO_2 assimilation rate (mol CO_2 · mol PSII ^{−1} · s ^{−1})	16 ± 7 (3)	34 ± 6 (5)	2.2 ± 0.9 (7)

cells ($34 \pm 5 \text{ mol O}_2 \cdot \text{mol PSII}^{-1} \cdot \text{s}^{-1}$); the dark respiration rate R was $6 \pm 2 \text{ mol O}_2 \cdot \text{mol PSII}^{-1} \cdot \text{s}^{-1}$ in the ΔAB cells, similar to that in the WT cells ($5 \pm 1 \text{ mol O}_2 \cdot \text{mol PSII}^{-1} \cdot \text{s}^{-1}$); and P_{adj} was $10 \pm 3 \text{ mol O}_2 \cdot \text{mol PSII}^{-1} \cdot \text{s}^{-1}$ in the ΔAB cells, amounting to about 26% of that in the WT cells ($39 \pm 5 \text{ mol O}_2 \cdot \text{mol PSII}^{-1} \cdot \text{s}^{-1}$). Similarly, significant net light-induced O_2 evolution was also observed in all other ΔPSI strains tested (e.g., the net light-induced O_2 evolution rates were $4 \pm 1 \text{ mol O}_2 \cdot \text{mol PSII}^{-1} \cdot \text{s}^{-1}$ in $\Delta PSI \Delta NdbABC$ and $2.2 \pm 0.4 \text{ mol O}_2 \cdot \text{mol PSII}^{-1} \cdot \text{s}^{-1}$ in $\Delta PSI \Delta CtaDIIIEII \Delta CydAB$). Our data are consistent with the previous observation of net light-induced O_2 evolution in the absence of supplemental electron acceptors for either the *psaA*- or the *psaB*-deletion mutant of *Synechocystis* 6803 – the ADK9 [8] or BDK8 (L. B. Smart 2006, personal communication) strains.

The significant amount of net light-induced O_2 evolution of the ΔPSI mutants suggests the existence of alternative electron transport pathway(s) downstream of oxygenic PSII water oxidation in the absence of PSI. This net light-induced O_2 evolution in the ΔPSI mutants also indicated that the electron sinks of the alternative electron transport pathways in the absence of PSI are not limited to O_2 , which has previously been suggested to be a sink for the PSII-generated electrons via a respiratory terminal oxidase in the $\Delta PSI/WV$ mutant [19,20].

3.3. O_2 evolution in the absence of PSI requires glucose

Synechocystis sp. PCC 6803 is known to be a facultative photoheterotroph in the presence of glucose [31,32]. Consistent with this observation, we found that the PSI deleted ΔAB mutant did not grow either photoautotrophically or mixotrophically with non-glucose carbon sources such as sucrose, fructose, glycerol or mannose. We next examined whether glucose was required to sustain O_2 evolution in the ΔAB cells. We found that upon depleting glucose from the medium, P_{obs} (Fig. 3A), R (Fig. 3B) and P_{adj} (Fig. 3C) dropped gradually to basal levels with similar time constants (ca. 8 h). After 42 h of glucose depletion, we added 5 mM glucose back to the ΔAB cell culture. While respiration recovered almost instantly, the net light-induced O_2

evolution P_{obs} was restored slowly and partially (Fig. 3D). In contrast, adding back non-metabolic glucose analogs, including 3-O-methyl glucose and 2-deoxy glucose, was not effective in restoring either the net light-induced O_2 evolution or the dark respiration of the glucose-depleted ΔAB cells. These results suggest the requirement of glucose and/or its metabolite(s) for sustaining the net light-induced O_2 evolution in the absence of PSI. In addition, after incubating the ΔAB cells with added glucose for ca. 30 min, we re-deprived these cells of glucose. Again, P_{obs} , R and P_{adj} dropped gradually to basal levels. However, the decay of these rates during the second glucose deprivation was much faster (time constant ca. 10 min) than that during the first glucose deprivation (time constant ca. 8 h), suggesting depletion of the unidentified electron sinks as a result of the prior prolonged glucose deprivation. To further characterize the net light-induced O_2 evolution of the mixotrophically-grown ΔPSI mutants of *Synechocystis* 6803, we examined whether several known linear electron transport pathway components were involved and whether the ΔPSI mutants were capable of CO_2 assimilation, as will be described in Sections 3.4 to 3.8.

3.4. PSII is the source of O_2 evolution in the absence of PSI

To examine the involvement of PSII in O_2 evolution in the absence of PSI, we titrated the ΔPSI cells with the PSII inhibitor, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). We found that oxygen evolution was completely inhibited in the ΔPSI cells by 10 μM DCMU, while respiration was insensitive. This observation is consistent with the reported DCMU sensitivity of O_2 evolution in the ΔPSI mutant strain ADK9 [8]. The observed DCMU sensitivity of O_2 evolution in the ΔPSI cells was similar to that in the WT cells ($I_{50} \sim 100 \text{ nM}$ for both), suggesting that O_2 evolution of the ΔPSI cells originated from oxygenic PSII water oxidation.

To directly assess the PSII activity in the absence of PSI, we measured the light-induced O_2 evolution rate of the ΔAB cells in the presence of the artificial PSII electron acceptors DCBQ and FeCN. The PSII

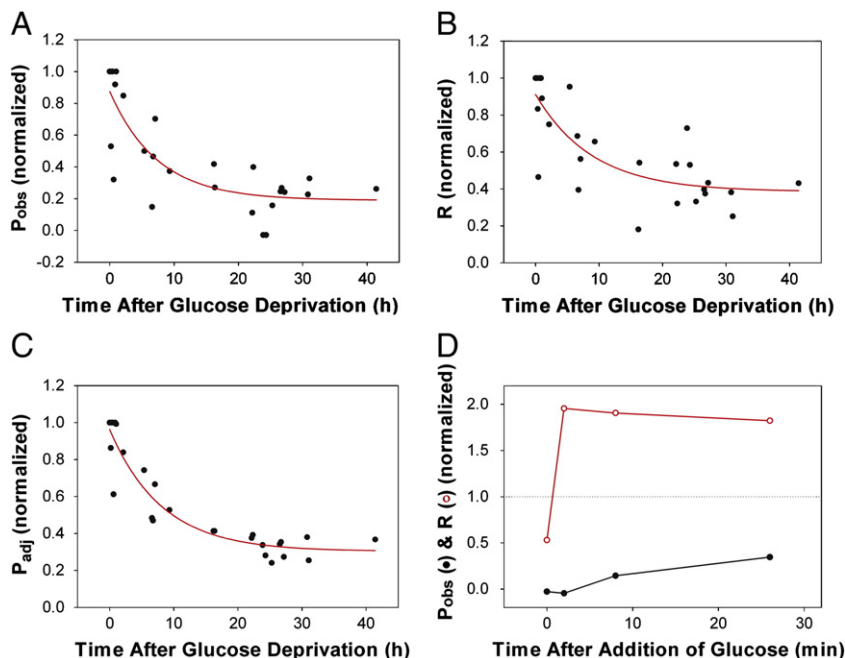


Fig. 3. Requirement of glucose for both O_2 evolution and dark respiration in the ΔPSI (ΔAB) mutant. The net light-induced O_2 evolution rate P_{obs} (A), dark respiration rate R (B) and the adjusted O_2 evolution rate P_{adj} (C) of the ΔAB cells dropped gradually after glucose deprivation (time constant ca. 8 h). The exponentially grown cells were harvested, washed and re-suspended in fresh BG-11 medium without glucose and kept at 30°C and under $1 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ light prior to experiments. A final concentration of 10 mM NaHCO_3 was added before each measurement. For each panel, data from 3 independent experiments were normalized to the maximal rate (right before glucose deprivation) and combined. For each rate, 100% activity is the activity before the sample was deprived of glucose. (D) Recovery of the net light-induced O_2 evolution rate (P_{obs}) (closed circles) and the dark respiration rate (R) (open circles) after glucose (5 mM) was supplied to the 42 h glucose-depleted ΔAB cells. Since 5 mM glucose was enough to maintain cell growth until stationary phase, the remaining glucose concentration during/after the measurements was assumed to be sufficient.

activity of the ΔAB cells ($110 \text{ mol O}_2 \cdot \text{mol PSII}^{-1} \cdot \text{s}^{-1}$) was comparable to that of the WT cells ($66 \text{ mol O}_2 \cdot \text{mol PSII}^{-1} \cdot \text{s}^{-1}$) (Table 2). This result is consistent with the reported similarity of the light-induced O_2 evolution rates (on a per cell basis) in the presence of 1 mM DCBQ between the WT and the ΔPSI mutant (ADK9) strains [8], suggesting un-impaired PSII in the ΔPSI cells.

To characterize PSII activity in the ΔPSI cells, we monitored Chl *a* fluorescence induction kinetics (the Kautsky effect) [33,34]. Chl *a* fluorescence emission yield reflects the redox state of Q_A , the first PQ electron acceptor in PSII: It increases with the reduction of Q_A and decreases with the oxidation of Q_A^- [35,36]. In the absence of DCMU, hundreds of milliseconds of illumination was required to reduce Q_A and the PQ pool to reach the fluorescence maxima F_P ($\approx F_M$) (Fig. 4A, closed symbols; ca. 170 ms in WT, ca. 320 ms in the ΔAB cells, and ca. 560 ms in the $\Delta PSI\Delta ApcE$ cells). In contrast, in the presence of DCMU, Q_A was fully reduced to give maximal Chl *a* fluorescence emission yield (F_M) within 2 ms of illumination by actinic flashes (Fig. 4A, open symbols). These results show that DCMU has the same effect on Chl *a* fluorescence induction kinetics in the WT cells (Fig. 4A, circles) and ΔPSI cells (Fig. 4A, squares and triangles), demonstrating the PSII-origin of O_2 evolution in the ΔPSI cells. Furthermore, these results indicate that the lower net light-induced O_2 evolution observed the ΔPSI cells compared to that in the WT cells, is the result of a limitation in electron transport downstream of PSII, rather than an impaired PSII capacity.

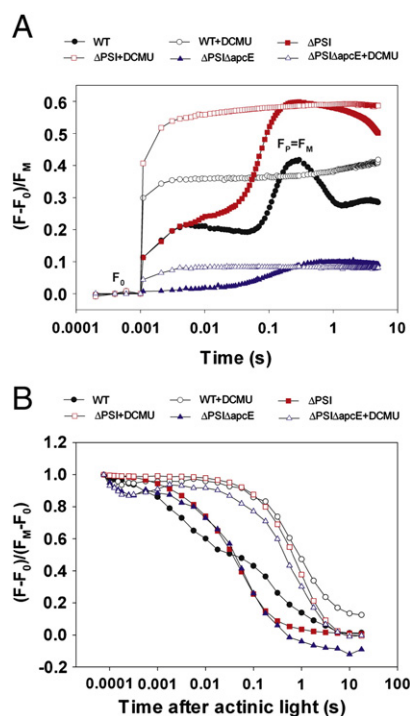


Fig. 4. DCMU effects on Chl *a* fluorescence kinetics of the WT and the ΔPSI cells. (A) Chl *a* fluorescence induction curves of the WT (circles), ΔAB (squares, labeled as ΔPSI) and $\Delta PSI\Delta ApcE$ (triangles) cells show the same effect of DCMU on the WT and ΔPSI cells: The illumination time needed to fully reduce Q_A decreased from hundreds of milliseconds (WT: 170 ms; ΔAB : 320 ms; $\Delta PSI\Delta ApcE$: 560 ms) in the absence of DCMU to 2 milliseconds or less when DCMU is present. (B) Chl *a* fluorescence dark relaxation curves of the WT (circles), ΔAB (squares, labeled as ΔPSI) and $\Delta PSI\Delta ApcE$ (triangles) cells show the same slow kinetics in both the WT and ΔPSI cells in the presence of DCMU (open symbols, $T^{DCMU} \approx 1 \text{ s}$) and yet there is drastically different kinetics between the WT (black closed circles, bi-phasic, $T_{slow}^{WT} = 0.1\text{--}1 \text{ s}$ and $T_{fast}^{WT} \approx 1\text{--}10 \text{ ms}$) and ΔPSI (red closed squares for ΔAB and blue closed triangles for $\Delta PSI\Delta ApcE$, monophasic, $T^{\Delta PSI} \approx 10\text{--}100 \text{ ms}$) cells in the absence of DCMU. A total of 370 saturating actinic flashes were given at the frequency of 1 kHz to maximally reduce Q_A and the PQ pool prior to recording the dark relaxation kinetics. The time when the actinic flashes were turned off was set as zero. DCMU was applied at a final concentration of $10 \mu\text{M}$.

3.5. The PQ pool is involved in O_2 evolution of the ΔPSI cells

To investigate the involvement of the PQ pool in O_2 evolution, we monitored the oxidation kinetics of Q_A^- and the plastoquinol (PQH_2) pool in the WT and ΔPSI (ΔAB and $\Delta PSI\Delta ApcE$) cells by recording their Chl *a* fluorescence dark relaxation kinetics after their Q_A and the PQ pool were reduced (Fig. 4B). In the presence of $10 \mu\text{M}$ DCMU, Chl *a* fluorescence of the WT and the ΔPSI cells relaxed slowly in the darkness with the same time constant $T^{DCMU} \approx 1 \text{ s}$ (Fig. 4B, open symbols). This slow Chl *a* fluorescence dark relaxation kinetics in the presence of DCMU was also reported previously in the ΔPSI /WT cells and is attributed to the Q_A^- oxidation through the back reaction of PSII [20].

In contrast, in the absence of DCMU, the Chl *a* fluorescence dark relaxation curves of the WT and ΔPSI mutants were significantly different (Fig. 4B, closed symbols): The relaxation kinetics were bi-phasic in the WT cells (Fig. 4B, closed circles) with time constants of $T_{slow}^{WT} = 0.1\text{--}1 \text{ s}$ and $T_{fast}^{WT} \approx 1\text{--}10 \text{ ms}$, respectively, whereas the relaxation kinetics of both the ΔAB (Fig. 4B, closed squares) and the $\Delta PSI\Delta ApcE$ (Fig. 4B, closed triangles) cells were monophasic with an intermediate time constant of $T^{\Delta PSI} \approx 10\text{--}100 \text{ ms}$. The slow phase of the Chl *a* fluorescence dark relaxation kinetics in the WT cells in the absence of DCMU is attributed to the Q_A^- oxidation through the back reaction of PSII [20]. Alternatively, it may reflect the cyclic electron transport around PSI which reduces the PQ pool [37,38]. The fast phase of Chl *a* fluorescence dark relaxation kinetics in the WT cells in the absence of DCMU indicates a more oxidized PQ pool as a result of the PSI activity before the actinic light is switched off. In contrast, the intermediately-fast time constant of the monophasic Chl *a* fluorescence dark relaxation kinetics in the ΔPSI cells in the absence of DCMU reflects a more reduced PQ pool, possibly as a result of less effective PQH_2 oxidation pathway(s) than PSI.

These observations show that the PQ pool is involved in O_2 evolution in the ΔPSI cells, and that PQH_2 -oxidizing pathway(s) other than PSI must exist to sustain the prolonged O_2 evolution in the absence of PSI.

3.6. PQH_2 oxidation in the ΔPSI cells does not involve the Q_o site of the Cyt *bf* complex

At low concentrations ($\leq 1 \mu\text{M}$), DBMIB inhibits PQH_2 oxidation at the plastoquinol oxidase (Q_o) site of the Cyt *bf* complex [39], whereas at higher concentrations ($\geq 10 \mu\text{M}$), DBMIB can inhibit Q_A^- oxidation in a DCMU-like manner (reviewed in [40]). To examine whether the Cyt *bf* complex could effectively oxidize PQH_2 in the absence of PSI, we titrated O_2 evolution of the ΔPSI cells with DBMIB. Our data show that O_2 evolution of the ΔPSI cells was much less sensitive to DBMIB than that of the WT cells. The DBMIB concentration leading to 50% inhibition (I_{50}) of the net O_2 evolution rate P_{obs} was $0.5 \mu\text{M}$ for the WT cells (Fig. 5A, closed circles) and $4.2 \mu\text{M}$ for the ΔPSI cells (Fig. 5A, open circles), respectively. Significantly different I_{50} values for the DBMIB inhibition of the adjusted O_2 evolution rate P_{adj} were also observed for the WT ($0.6 \mu\text{M}$; Fig. 5C, closed circles) and ΔPSI cells ($23.7 \mu\text{M}$; Fig. 5C, open circles). In contrast, the dark respiration rate *R* was not inhibited by DBMIB ($50 \mu\text{M}$) in either WT (Fig. 5B, closed circles) or ΔPSI (Fig. 5B, open circles) cells. These results indicate that the Q_o site of the Cyt *bf* complex is unlikely to be involved in the PQH_2 -oxidizing pathway(s) that contributes to the net light-induced O_2 evolution in the ΔPSI cells.

3.7. Oxygen evolution in the ΔPSI cells is highly sensitive to KCN

Cyanide is a potent inhibitor of the respiratory cytochrome oxidase and other metalloproteins including plastocyanin [41] and Rubisco [42,43]. To examine the possible involvement of KCN-sensitive redox components in the PQH_2 -oxidizing pathway(s) in

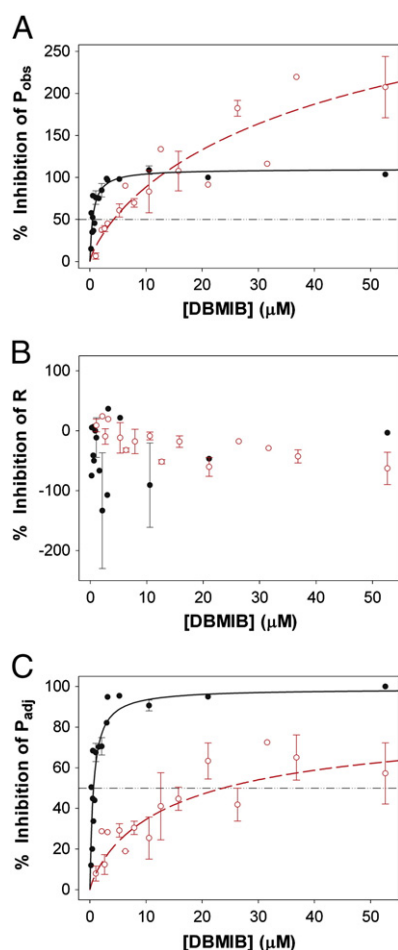


Fig. 5. DBMIB titration of (A) P_{obs} , (B) R and (C) P_{adj} of the WT and the Δ PSI cells. P_{obs} and P_{adj} of the Δ PSI cells were significantly less sensitive to DBMIB than those of the WT cells, whereas the rates of R in both the WT and the Δ PSI cells were not sensitive to DBMIB. The I_{50} values for DBMIB inhibition were 0.5 μ M for P_{obs} of the WT cells (Panel A, black closed circles), 4.2 μ M for P_{obs} of the Δ PSI cells (Panel A, red open circles), 0.6 μ M for P_{adj} of the WT cells (Panel C, black closed circles), and 23.7 μ M for P_{adj} of the Δ PSI cells (Panel C, red open circles). P_{obs} , R and P_{adj} of both the WT and Δ PSI cells were measured in BG-11 medium in the presence of 5 mM glucose and 10 mM NaHCO_3 . The titration curves of the WT cells (black closed circles) are plotted from 3 data sets. The titration curves of the Δ PSI cells (red open circles, for Δ AB, Δ VIII–XI, and Δ PSI/WV strains) are plotted from 4 data sets. Standard errors are indicated. In addition, we grew the Δ PSI cells either in the presence or in the absence of 1 μ M Cu^{2+} supplement so that they expressed either plastocyanin or Cyt c_6 , respectively [21]. Both plastocyanin- and Cyt c_6 -expressing Δ PSI cells exhibited indistinguishable DBMIB titration curves: The data shown include those from cells grown both with and without Cu^{2+} supplement. For each rate, 100% inhibition by DBMIB means the rate is reduced from the control value (with no DBMIB) to zero. An inhibition that is greater than 100% in the case of P_{obs} means that in the presence of DBMIB, the cells show net O_2 consumption instead of displaying net O_2 evolution.

the Δ PSI cells, we titrated O_2 evolution and dark respiration with KCN. Our data showed that P_{obs} of the Δ PSI cells was highly KCN-sensitive ($I_{50} \sim 1.7 \mu\text{M}$, Fig. 6A, open circles), as compared to that of the WT cells ($I_{50} \sim 49 \mu\text{M}$, Fig. 6A, closed circles). Similarly, P_{adj} of the Δ PSI cells was also highly KCN-sensitive ($I_{50} \sim 6 \mu\text{M}$, Fig. 6C, open circles), as compared to that of the WT cells ($I_{50} \sim 46 \mu\text{M}$, Fig. 6C, closed circles). In contrast, dark respiration of the Δ PSI cells was much less sensitive to KCN, with the I_{50} values of 30 μM in both the WT (Fig. 6B, closed circles) and Δ PSI (Fig. 6B, open circles) cells.

To further investigate the KCN-sensitive PQH₂-oxidizing pathway(s) in the Δ PSI cells, we examined the possible involvement of PSII, plastocyanin and respiratory terminal oxidases. By monitoring O_2 evolution in the presence of the artificial PSII electron acceptors FeCN and DCBQ, we found that the PSII activities of both the WT cells and the PSI deleted Δ AB cells were not inhibited by 500 μM

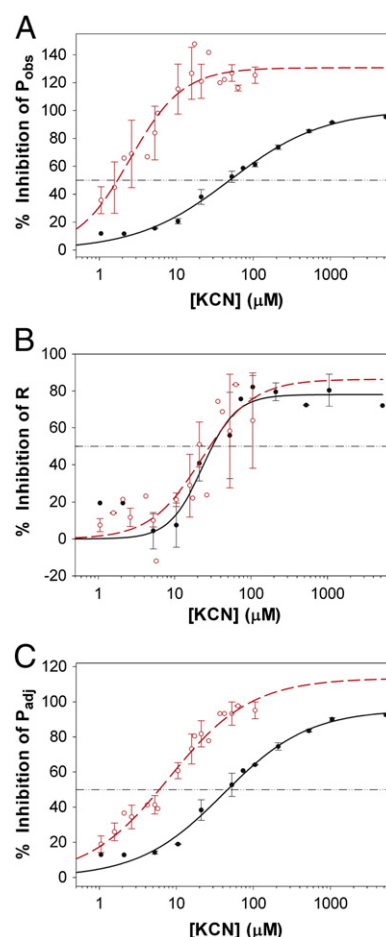


Fig. 6. KCN titration of (A) P_{obs} , (B) R and (C) P_{adj} of the WT and the Δ PSI cells. P_{obs} and P_{adj} of the Δ PSI cells were much more sensitive to KCN than those of the WT cells, whereas R of the Δ PSI cells had the same sensitivity to KCN as that of the WT cells. The I_{50} values for KCN inhibition were 49 μ M for P_{obs} of the WT cells (Panel A, black closed circles), 1.7 μ M for P_{obs} of the Δ PSI cells (Panel A, red open circles), 46 μ M for P_{adj} of the WT cells (Panel C, black closed circles), 6 μ M for P_{adj} of the Δ PSI cells (Panel C, red open circles) and 30 μ M for R of both the WT (Panel B, black closed circles) and Δ PSI (Panel B, red open circles) cells. P_{obs} , R and P_{adj} of both the WT and the Δ PSI cells were measured in BG-11 medium in the presence of 5 mM glucose and 10 mM NaHCO_3 . The titration curves of the WT cells (black closed circles) are plotted from 2 data sets. The titration curves of the Δ PSI cells (red open circles, for Δ AB, Δ VIII–XI, and Δ PSI/WV strains) are plotted from 5 data sets. Standard errors are indicated. In addition, the KCN sensitivities of both the WT and Δ PSI cells were not affected by the supplemental Cu^{2+} in the growth medium, as the data shown are for cells grown both with and without Cu^{2+} supplement. For each rate, 100% inhibition by KCN means the rate is reduced from the control value (with no KCN) to zero. An inhibition that is greater than 100% in the case of P_{obs} means that in the presence of KCN, the cells showed net O_2 consumption instead of displaying net O_2 evolution.

KCN. Therefore, the KCN inhibition of both P_{obs} and P_{adj} of the WT and Δ PSI cells does not result from the KCN inhibition of PSII.

Plastocyanin has been previously shown to react readily with high concentrations of KCN (> 10 mM) [41]. We grew the Δ PSI cells either in the presence or in the absence of 1 μ M Cu^{2+} supplement so that they expressed either plastocyanin or Cyt c_6 , respectively [21]. Both plastocyanin- and Cyt c_6 -expressing cells exhibited indistinguishable KCN titration curves (Fig. 6), further supporting that the PQH₂-oxidizing pathway(s) in the absence of PSI does not involve plastocyanin.

Lastly, we monitored the KCN effect on Chl *a* fluorescence dark relaxation kinetics of the Δ PSI cells (Fig. 7A). Chl *a* fluorescence dark relaxation in the Δ PSI mutants was severely inhibited by 1.5 mM KCN (Fig. 7A, cf. closed squares with closed circles). We note that, Chl *a* fluorescence dark relaxation kinetics of the Δ PSI mutants in the presence of KCN (Fig. 7A, closed squares) was similar to that in the

presence of DCMU (Fig. 7A, open circles), suggesting effective inhibition of the oxidation of Q_A^- and the PQH_2 pool by KCN in the Δ PSI cells. In contrast, in the WT cells, KCN did not cause significant inhibition of the PQH_2 pool oxidation (Fig. 7B, closed squares); rather, it induced a small light-to-dark transient increase of the Chl *a* fluorescence yield which was previously attributed to the delayed reduction of the PQ pool by the KCN-independent cyclic electron transport around PSI [37,38]. In spite of the fact that KCN inhibits the oxidation of Q_A^- and the PQH_2 pool, which was previously attributed to the KCN-sensitive PQH_2 -oxidizing activity of respiratory cytochrome oxidases in the Δ PSI/WT cells [20], we suggest that the KCN-sensitivity of the net light-induced O_2 evolution in the Δ PSI cells is independent of direct involvement of respiratory terminal oxidases (see the Discussion and the Supplementary Material S2). In addition, the observed KCN-sensitivity of the net light-induced O_2 evolution in the WT cells has an I_{50} value close to the reported I_{50} value for the cyanide inhibition of Rubisco [42,43]; thus, it may be attributed to the KCN inhibition of the Calvin–Benson cycle.

3.8. The Δ PSI cells are capable of light-minus-dark CO_2 assimilation in the presence of glucose

We examined CO_2 assimilation in the Δ PSI cells by performing *in vivo* radioisotope-labeling studies using $NaH^{14}CO_3$. Our data show that the Δ PSI cells take up CO_2 both in the darkness and upon illumination, resulting in significant amount of light-minus-dark CO_2 uptake (Table 2 and Fig. 8A). Since acid-stable intermediates instead of acid-labile carbamates of proteins or bicarbonate of carbon

concentrating mechanism (CCM) [44–47] were monitored in these experiments, the observed CO_2 uptake in the Δ PSI cells was in fact CO_2 assimilation. The light-minus-dark CO_2 assimilation rate was measured to be 2.2 ± 0.9 mol $CO_2 \cdot mol$ PSII $^{-1} \cdot s^{-1}$ in the PSI deleted Δ AB cells, when the CO_2 assimilation rate in the darkness was surprisingly as high as 5 ± 2 mol $CO_2 \cdot mol$ PSII $^{-1} \cdot s^{-1}$. The light-minus-dark CO_2 assimilation rate in the Δ AB cells was likely under-estimated because the dilution of $^{14}CO_2$ as a result of the release of non-labeled CO_2 by respiration was ignored in the analysis. This notion was supported by the increase of the measured light-minus-dark CO_2 assimilation rate of the WT cells from 16 ± 7 mol $CO_2 \cdot mol$ PSII $^{-1} \cdot s^{-1}$ to 34 ± 6 mol $CO_2 \cdot mol$ PSII $^{-1} \cdot s^{-1}$ when the reaction media were changed from those containing 5 mM glucose to those without glucose. Therefore, we conclude that the PSI deleted Δ AB cells assimilate CO_2 at a significant rate as compared to the O_2 evolution rates: The light-

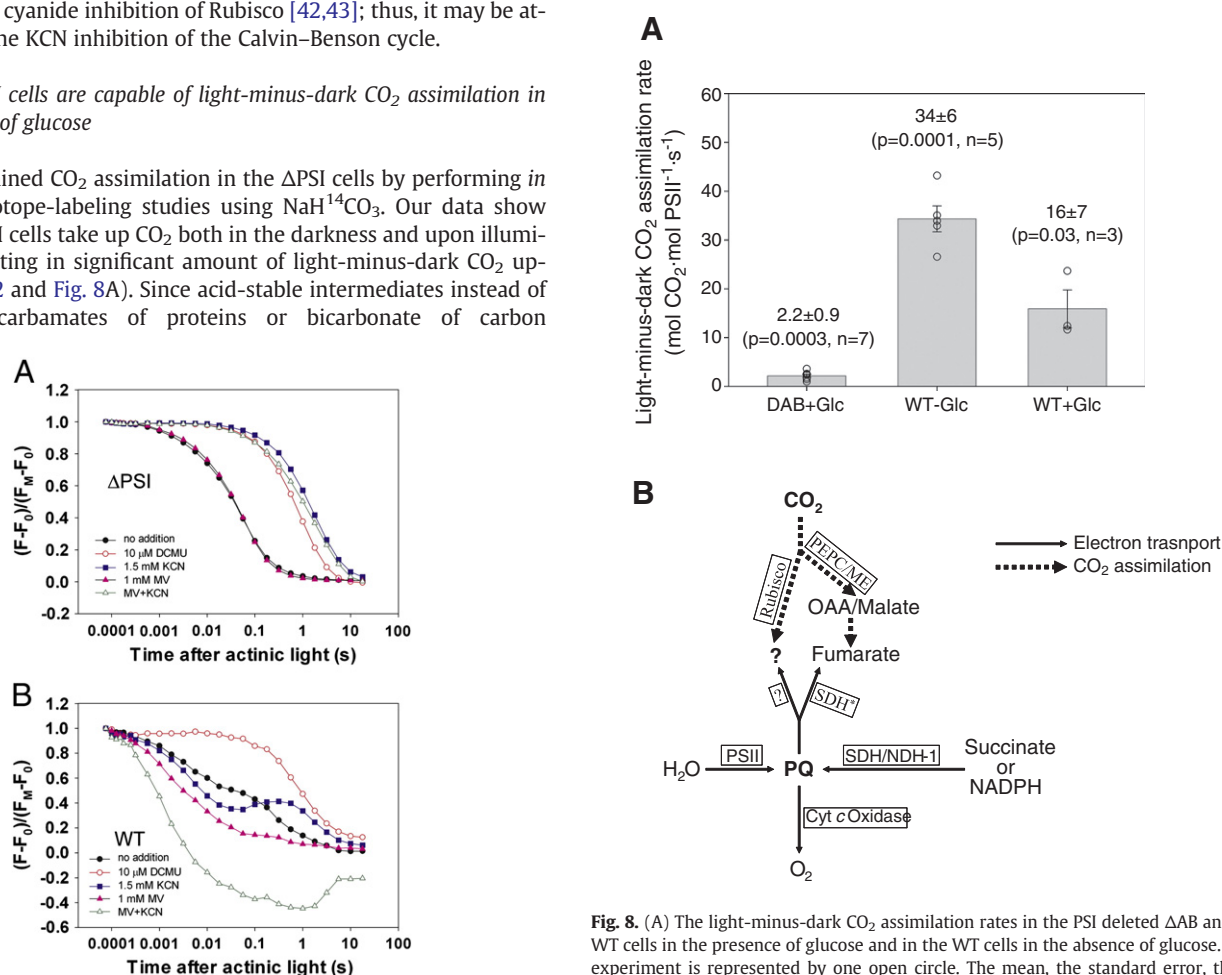


Fig. 7. KCN effects on Chl *a* fluorescence dark relaxation kinetics of the (A) Δ PSI (Δ AB) and (B) WT cells. Like DCMU (Panel A, red open circles), KCN severely inhibited Chl *a* fluorescence dark relaxation in the Δ PSI cells (Panel A, blue closed squares), as compared to without KCN (Panel A, black closed circles), suggesting that KCN may effectively slow down the oxidation of Q_A^- and the PQH_2 pool in the Δ PSI cells. In contrast, in the WT cells, KCN did not cause inhibition of the PQH_2 pool oxidation; rather, it induced a small light-to-dark transient increase of the Chl *a* fluorescence yield (Panel B, blue closed squares) which was absent in the Δ PSI cells. This result is consistent with the lack of PSI and the cyclic electron transport around PSI in the Δ PSI cells. This result was further supported by the capability of methylviologen (MV), an electron acceptor for PSI [56], to reverse the KCN inhibition of the PQH_2 pool oxidation in the WT cells (Panel B, green open triangles), but not in the Δ PSI cells (Panel A, green open triangles). A total of 370 saturating actinic flashes were given at the frequency of 1 kHz to maximally reduce Q_A and the PQ pool prior to recording the dark relaxation kinetics. The time when the actinic flashes were turned off was set as zero. The concentrations of the inhibitors and electron acceptor used were as follows: 10 μ M DCMU, 1.5 mM KCN, and 1 mM MV.

Fig. 8. (A) The light-minus-dark CO_2 assimilation rates in the PSI deleted Δ AB and the WT cells in the presence of glucose and in the WT cells in the absence of glucose. Each experiment is represented by one open circle. The mean, the standard error, the *p*-value, and the number of independent experiments are indicated. The light-minus-dark CO_2 assimilation rates were calculated as the difference between the light and the dark CO_2 assimilation rates. Despite the high variation in the dark CO_2 assimilation rates, the light-minus-dark CO_2 assimilation rates are statistically highly significant, as evaluated by the paired *t*-test. (B) A working model for the O_2 evolving electron transport (thin solid-line arrows) and light-minus-dark CO_2 assimilation (thick dotted-line arrows) in the Δ PSI cells. The PQ pool serves as the “hub” of the electron flow with the incoming electrons from H_2O (via PSII) as well as succinate (via succinate dehydrogenase) and/or NADPH (via type 1 NAD(P)H dehydrogenase) and with the outgoing electrons to O_2 (via cytochrome *c* oxidase) and CO_2 (via Rubisco-dependent mechanisms and/or a glycolysis-dependent mechanism). The glycolysis-dependent CO_2 assimilation may involve PEP carboxylase and/or malic enzyme, which generate malate and/or oxaloacetate via carboxylation reactions, followed by a succinate dehydrogenase working in reverse as a fumarate reductase to reduce fumarate to succinate. Abbreviations used in this model are: PSII — photosystem II, PQ — the plastoquinol pool, SDH — succinate dehydrogenase, SDH* — succinate dehydrogenase working in reverse as fumarate reductase, NDH-1 — type 1 NAD(P)H dehydrogenase, Cyt *c* oxidase — cytochrome *c* oxidase, OAA — oxaloacetate, PEP — PEP carboxylase, ME — malic enzyme.

minus-dark CO₂ assimilation rate ($2.2 \pm 0.9 \text{ mol CO}_2 \cdot \text{mol PSII}^{-1} \cdot \text{s}^{-1}$) is at least 55% of the light-minus-dark O₂ evolution rate ($4 \pm 1 \text{ mol O}_2 \cdot \text{mol PSII}^{-1} \cdot \text{s}^{-1}$).

We tested the effects of the electron transport inhibitors and glucose deprivation on CO₂ assimilation of the Δ PSI cells. Our data show that the light-minus-dark CO₂ assimilation rate was inhibited by 10 μM DCMU (90% inhibition) or 1 mM KCN (100% inhibition). In addition, the light-minus-dark CO₂ assimilation rate dropped upon glucose deprivation and recovered upon adding back glucose (e.g., in one experiment, the light-minus-dark CO₂ assimilation rate dropped to 29% after 24 h glucose deprivation and recovered to 66% after adding back glucose for 3.5 h). The similarity between the light-minus-dark CO₂ assimilation and O₂ evolution in their responses to the electron transport inhibitors and glucose deprivation indicates that the observed light-minus-dark CO₂ assimilation of the Δ PSI cells is a key PQH₂-oxidizing pathway enabling the net light-induced O₂ evolution in the Δ PSI cells.

4. Discussion

In this paper, we have demonstrated the following: (1) a net light-induced O₂ evolution is present in *Synechocystis* 6803 cells lacking PSI; (2) this net light-induced O₂ evolution of the Δ PSI cells requires glucose and can be sustained for over 30 min; (3) the rate of net light-induced O₂ evolution (P_{obs}) of the Δ PSI cells is ca. 13% of the WT rate on a PSII basis; (4) this net light-induced O₂ evolution of the Δ PSI cells is driven by PSII and the pathway of electrons includes the PQ pool but not the Q_B site of the Cyt bf complex; and (5) this net light-induced O₂ evolution of the Δ PSI cells is highly sensitive to KCN addition.

We also looked for the terminal electron acceptor pools of this PSI-independent, net light-induced O₂ evolution. As will be discussed below, we suggest that CO₂, but not the PQ pool or O₂, is a key terminal electron acceptor. A working model depicting O₂ evolving electron transport and CO₂ assimilation in the Δ PSI cells is shown in Fig. 8B.

First, with the limited size of the PQ pool (ca. 10 molecules PSII⁻¹) [34], all PQ molecules can be reduced within 2 s. Therefore, our observation that the Δ PSI cells could sustain the net light-induced O₂ evolution for 30 min at a rate as high as $4 \pm 1 \text{ O}_2 \cdot \text{PSII}^{-1} \cdot \text{s}^{-1}$ strongly argues against the hypothesis of Smart et al. [8] that the PQ pool serves as the main terminal electron acceptor for the net light-induced O₂ evolution in the Δ PSI cells.

Second, O₂ is a logical candidate to be an electron sink for the PQH₂-oxidizing pathway(s) in the PSI deletion mutants of *Synechocystis* 6803, as respiration and photosynthesis in cyanobacteria share common components such as the Cyt bf complex, the PQ pool and NADPH [48]. Vermaas et al. [20] and Vermaas [19] suggested that a respiratory terminal oxidase may direct the electrons generated by PSII to O₂ in the Δ PSI/WV cells. Notably different from our results, Vermaas [19] showed that the Δ PSI/WV cells did not display net O₂ evolution in the light. However, we observed net light-induced O₂ evolution in the Δ PSI (including Δ PSI/WV) cells; if all the PSII-generated electrons were directed to O₂, there would have been no net O₂ evolution [49–52]. Thus, to explain our data, other electron acceptors must also exist (see the Supplementary Material S2 for preliminary data and a detailed discussion). We speculate that this discrepancy may be due to the differences in growth conditions, including light intensities and glucose concentrations.⁶ Of note, in this work, we primarily use the net light-induced O₂ evolution rate P_{obs} as the readout. However, because the O₂ evolution rate is classically calculated with adjustment for dark respiration (i.e., P_{adj}), we also report in this work P_{adj} , assuming that respiration does not change

in light. As respiration and photosynthesis are intertwined in cyanobacteria, O₂ uptake possibly varies during the light phase in the Δ PSI cells. For instance, as a result of enhanced oxidase activity, which is likely in the absence of PSI, O₂ uptake in the light could be stimulated. Further, mass spectroscopic data exist on another cyanobacterium *S. elongatus* (previously known as *Anacystis nidulans*) that at medium and high light intensities, O₂ uptake is enhanced in the light [30]. If O₂ uptake in the light is indeed enhanced in the Δ PSI cells, true O₂ evolution in these cells would be much higher than reported by P_{obs} .

Third, we have further demonstrated that CO₂ may serve as a key terminal electron acceptor of the PQH₂-oxidizing pathway(s) in the Δ PSI cells: Upon illumination, the Δ PSI cells can assimilate CO₂ at a rate that accounts for ca. 55% of the net light-induced O₂ evolution rate, and this light-minus-dark CO₂ assimilation is DCMU- and KCN-sensitive. These observations were made in the presence of 5 mM glucose and 10 mM NaHCO₃. We also found that the O₂ evolution rate in the Δ PSI cells decreased when CO₂ was depleted by bubbling NaHCO₃-less BG-11 medium with air filtered through Soda Lime (containing >75% Ca(OH)₂ and <3.5% NaOH). This finding is consistent with CO₂ being a key terminal electron acceptor in the Δ PSI O₂ evolution pathway. In *Synechocystis*, it is known that CO₂ assimilation is intertwined with a network of carbon metabolism. In this network, glycolysis and the tricarboxylic acid (TCA) cycle are connected not only to the Calvin–Benson cycle, but also to the oxidative pentose phosphate pathway, the glyoxylate pathway and nitrogen storage. It is also known that TCA cycle is incomplete in *Synechocystis*, lacking 2-ketoglutarate dehydrogenase or 2-ketoglutarate ferredoxin oxidoreductase [53]. Based on our pilot studies (see Supplementary Material S3) that attempted to address the mechanism responsible for the net light-induced O₂ evolution and the light-minus-dark CO₂ assimilation in the Δ PSI cells and the reported metabolic flux analysis showing the operation of a C₄-like pathway involving PEP carboxylase and malic enzyme in the mixotrophically-grown *Synechocystis* 6803 for the delivery of substantial carbon flow from the TCA cycle to the glycolysis pathway [54], we speculate that (1) the CO₂-assimilating PQH₂-oxidizing pathway in the Δ PSI cells may involve a glycolysis-dependent pathway with a glucose metabolite serving as the immediate electron acceptor after the PQ pool, but not the Calvin–Benson cycle or the cyanophycin synthesis pathway that assimilates both carbon and nitrogen at the same time; and (2) the alternative glycolysis-dependent CO₂-assimilating pathway in the Δ PSI cells may involve fumarate generation via PEP carboxylase/malic enzyme and subsequent fumarate reduction via succinate dehydrogenase working in reverse (Fig. 8B) (see Supplementary Material S3 for preliminary data and an elaborate discussion).

Finally, our efforts to identify additional terminal electron acceptor pools (e.g., H⁺) (see Supplementary Material S4) were unsuccessful. Due to the complex nature of this newly discovered alternative PQH₂-oxidizing pathways in the Δ PSI cells, we suggest that techniques such as mass spectrometry-based metabolic flux analysis utilizing isotopically labeled O₂/CO₂ and GC/MS [54,55] as well as genomic and/or proteomic studies will be particularly valuable to dissect the intercalated network of the metabolite pools and enzymes for the final solution of the mechanism of the net light-induced O₂ evolution in PSI deletion mutants of the cyanobacterium *Synechocystis* sp. PCC 6803.

Acknowledgment

We thank Himadri Pakrasi for providing us with the Δ VIII–XI and Δ AB mutant strains, and Wim F. J. Vermaas for the Δ PSI Δ ApcE, Δ PSI Δ NdbABC, and Δ PSI Δ CtaDIIIEIIICydAB mutant strains. Q.J.W. thanks Elena Zak for her help. We thank Archie Portis, Donald Ort, David Krogmann, William Cramer, Aaron Kaplan, Jean-David Rochaix and Kevin Redding for helpful discussions. We also thank Lawrence B. Smart for sharing his unpublished results. Finally, we give special thanks to Wim F. J. Vermaas for discussions that led to significant improvement of this paper.

⁶ Light intensities: 5 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ for Δ PSI/WV and 1.5 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in the present work. Glucose concentrations: 15 mM for Δ PSI/WV and 5 mM in the present work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at [doi:10.1016/j.bbabo.2012.01.004](https://doi.org/10.1016/j.bbabo.2012.01.004).

References

- [1] L.N. Duysens, J. Ames, B.M. Kamp, Two photochemical systems in photosynthesis, *Nature* 190 (1961) 510–511.
- [2] R. Hill, F. Bendall, Function of the two cytochrome components in chloroplasts – a working hypothesis, *Nature* 186 (1960) 136–137.
- [3] R. Blankenship, *Molecular Mechanisms of Photosynthesis*, Blackwell Science, Oxford, 2002.
- [4] T.J. Wydrzynski, K. Satoh (Eds.), *Photosystem II: The Light-Driven Water: Plastoquinone Oxidoreductase*, in: *Advances in Photosynthesis and Respiration Series*, Springer, Dordrecht, The Netherlands, 2006.
- [5] J.H. Golbeck (Ed.), *Photosystem I: The Light-Driven Plastocyanin: Ferredoxin Oxidoreductase*, in: *Advances in Photosynthesis and Respiration Series*, Springer, Dordrecht, The Netherlands, 2006.
- [6] W.A. Cramer, H. Zhang, Consequences of the structure of the cytochrome b6f complex for its charge transfer pathways, *Biochim. Biophys. Acta* 1757 (2006) 339–345.
- [7] Govindjee, J.C. Munday Jr., G.C. Papageorgiou, fluorescence studies with algae: changes with time and preillumination, *Brookhaven Symp. Biol.* 19 (1966) 434–445.
- [8] L.B. Smart, S.L. Anderson, P. McIntosh, Targeted genetic inactivation of the photosystem I reaction center in the cyanobacterium *Synechocystis* sp. PCC 6803, *EMBO J.* 10 (1991) 3289–3296.
- [9] D.I. Arnon, Divergent pathways of photosynthetic electron transfer: the autotrophic oxygenic and anoxygenic photosystems, *Photosynth. Res.* 46 (1995) 47–71.
- [10] G. Peltier, L. Courmac, Chlororespiration, *Annu. Rev. Plant Biol.* 53 (2002) 523–550.
- [11] L. Courmac, K. Redding, P. Bennoun, G. Peltier, Limited photosynthetic electron flow but no CO₂ fixation in *Chlamydomonas* mutants lacking photosystem I, *FEBS Lett.* 416 (1997) 65–68.
- [12] L. Courmac, K. Redding, J. Ravenel, D. Rumeau, E.M. Josse, M. Kuntz, G. Peltier, Electron flow between photosystem II and oxygen in chloroplasts of photosystem I-deficient algae is mediated by a quinol oxidase involved in chlororespiration, *J. Biol. Chem.* 275 (2000) 17256–17262.
- [13] K. Redding, L. Courmac, I.R. Vassiliev, J.H. Golbeck, G. Peltier, J.-D. Rochaix, Photosystem I is indispensable for photoautotrophic growth, CO₂ fixation, and H₂ photoproduction in *Chlamydomonas reinhardtii*, *J. Biol. Chem.* 274 (1999) 10466–10473.
- [14] S. Boussiba, W.F.J. Vermaas, Creation of a mutant with an enriched photosystem II/pigment ratio in the cyanobacterium *Synechocystis* sp. PCC 6803, in: N. Murata (Ed.), *Research in Photosynthesis*, 1992, pp. 429–432.
- [15] G. Shen, S. Boussiba, W.F.J. Vermaas, *Synechocystis* sp. PCC 6803 strains lacking photosystem I and phycobilisome function, *Plant Cell* 5 (1993) 1853–1863.
- [16] C.A. Howitt, P.K. Udall, W.F.J. Vermaas, Type 2 NADH dehydrogenases in the cyanobacterium *Synechocystis* sp. strain PCC 6803 are involved in regulation rather than respiration, *J. Bacteriol.* 181 (1999) 3994–4003.
- [17] C.A. Howitt, W.F.J. Vermaas, Quinol and cytochrome oxidases in the cyanobacterium *Synechocystis* sp. PCC 6803, *Biochemistry* 37 (1998) 17944–17951.
- [18] S. Berry, D. Schneider, W.F.J. Vermaas, M. Rogner, Electron transport routes in whole cells of *Synechocystis* sp. strain PCC 6803: the role of the cytochrome *bd*-type oxidase, *Biochemistry* 41 (2002) 3422–3429.
- [19] W.F.J. Vermaas, Molecular-genetic approaches to study photosynthetic and respiratory electron transport in thylakoids from cyanobacteria, *Biochim. Biophys. Acta* 1187 (1994) 181–186.
- [20] W.F.J. Vermaas, G. Shen, S. Styring, Electrons generated by photosystem II are utilized by an oxidase in the absence of photosystem I in the cyanobacterium *Synechocystis* sp. PCC 6803, *FEBS Lett.* 337 (1994) 103–108.
- [21] L. Zhang, B. McSpadden, H.B. Pakrasi, J. Whitmarsh, Copper-mediated regulation of cytochrome *c₅₅₃* and plastocyanin in the cyanobacterium *Synechocystis* 6803, *J. Biol. Chem.* 267 (1992) 19054–19059.
- [22] J.G.K. Williams, Construction of specific mutations in photosystem II photosynthetic reaction center by genetic engineering methods in *Synechocystis* 6803, *Methods Enzymol.* 167 (1988) 766–778.
- [23] L. Nedbal, C. Gibas, J. Whitmarsh, Light saturation curves show competence of the water splitting complex in inactive photosystem II reaction centers, *Photosynth. Res.* 30 (1991) 85–94.
- [24] R.M. Mannan, J. Whitmarsh, P. Nyman, H.B. Pakrasi, Directed mutagenesis of an iron-sulfur protein of the photosystem I complex in the filamentous cyanobacterium *Anabaena variabilis* ATCC 29413, *Proc. Natl. Acad. Sci. U. S. A.* 88 (1991) 10168–10172.
- [25] K.J. Nyhus, M. Ikeuchi, Y. Inoue, J. Whitmarsh, H.B. Pakrasi, Purification and characterization of the photosystem I complex from the filamentous cyanobacterium *Anabaena variabilis* ATCC 29413, *J. Biol. Chem.* 267 (1992) 12489–12495.
- [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] R.M. Mannan, W.Z. He, S.U. Metzger, J. Whitmarsh, R. Malkin, H.B. Pakrasi, Active photosynthesis in cyanobacterial mutants with directed modifications in the ligands for two iron-sulfur clusters on the PsaC protein of photosystem I, *EMBO J.* 15 (1996) 1826–1833.
- [28] M. Trtílek, D.M. Kramer, M. Kobližek, L. Nedbal, Dual-modulation LED kinetic fluorometer, *J. Luminescence* 72–74 (1997) 597–599.
- [29] N.A. Eckardt, G.W. Snyder, A.R. Portis Jr., W.L. Orger, Growth and photosynthesis under high and low irradiance of *Arabidopsis thaliana* antisense mutants with reduced ribulose-1,5-bisphosphate carboxylase/oxygenase activase content, *Plant Physiol.* 113 (1997) 575–586.
- [30] G. Hoch, O.V. Owens, B. Kok, Photosynthesis and respiration, *Arch. Biochem. Biophys.* 101 (1963) 171–180.
- [31] R. Rippka, J. Deruelles, J.B. Waterbury, M. Herdman, R.Y. Stanier, Generic assignments, strain histories and properties of pure cultures of cyanobacteria, *J. Gen. Microbiol.* 111 (1979) 1–61.
- [32] S. Kahlon, K. Beeri, H. Ohkawa, Y. Hihara, O. Murik, I. Suzuki, T. Ogawa, A. Kaplan, A putative sensor kinase, Hik31, is involved in the response of *Synechocystis* sp. strain PCC 6803 to the presence of glucose, *Microbiology* 152 (2006) 647–655.
- [33] Govindjee, Sixty-three years since Kautsky, *Aust. J. Plant. Biol.* 22 (1995) 131–160 (now *Funct. Plant Biol.*).
- [34] G.C. Papageorgiou, Govindjee (Eds.), *Chlorophyll a Fluorescence: A Signature of Photosynthesis*, in: *Advances in Photosynthesis and Respiration Series*, Springer, Dordrecht, The Netherlands, 2005.
- [35] L.N.M. Duysens, H.E. Sweers, Mechanism of the two photochemical reactions in algae as studied by means of fluorescence, *Studies on Microalgae and Photosynthetic Bacteria*, Japanese Soc. Plant Physiol., University of Tokyo Press, Tokyo, 1963, pp. 353–372.
- [36] M. Kobližek, D. Kaftan, L. Nedbal, On the relationship between the non-photochemical quenching of the chlorophyll fluorescence and the photosystem II light harvesting efficiency. A repetitive flash fluorescence induction study, *Photosynth. Res.* 68 (2001) 141–152.
- [37] H. Mi, T. Endo, T. Ogawa, K. Asada, Thylakoid membrane-bound, NADPH-specific pyridine nucleotide dehydrogenase complex mediates cyclic electron transport in the cyanobacterium *Synechocystis* sp. PCC 6803, *Plant Cell Physiol.* 36 (1995) 661–668.
- [38] H. Mi, T. Endo, U. Schreiber, T. Ogawa, K. Asada, NAD(P)H dehydrogenase-dependent cyclic electron flow around photosystem I in the cyanobacterium *Synechocystis* PCC 6803: a study of dark-starved cells and spheroplasts, *Plant Cell Physiol.* 35 (1994) 163–173.
- [39] R.W. Jones, J. Whitmarsh, Inhibition of electron transfer and the electrogenic reaction in the cytochrome b/f complex by 2-n-nonyl-4-hydroxyquinoline N-oxide (NQNO) and 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMB), *Biochim. Biophys. Acta* 933 (1988) 258–268.
- [40] A. Trebst, Inhibitors in electron flow: tools for the functional and structural localization of carriers and energy conservation sites, *Methods Enzymol.* 69 (1980) 675–715.
- [41] R. Ouitrakul, S. Izawa, Electron transport and photophosphorylation in chloroplasts as a function of the electron acceptor. II. Acceptor-specific inhibition by KCN, *Biochim. Biophys. Acta* 305 (1973) 105–118.
- [42] M. Wishnick, M.D. Lane, Inhibition of ribulose diphosphate carboxylase by cyanide. Inactive ternary complex of enzyme, ribulose diphosphate, and cyanide, *J. Biol. Chem.* 244 (1969) 55–59.
- [43] A.M. Charles, B. White, Ribulose biophosphate carboxylase from *Thiobacillus A2*. Its purification and properties, *Arch. Microbiol.* 108 (1976) 195–202.
- [44] M.R. Badger, G.D. Price, CO₂ concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution, *J. Exp. Bot.* 54 (2003) 609–622.
- [45] A. Kaplan, L. Reinhold, CO₂ concentrating mechanisms in photosynthetic microorganisms, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50 (1999) 539–570.
- [46] J.V. Moroney, A. Somanchi, How do algae concentrate CO₂ to increase the efficiency of photosynthetic carbon fixation? *Plant Physiol.* 119 (1999) 9–16.
- [47] G.D. Price, S.I. Maeda, T. Omata, M.R. Badger, Modes of active inorganic carbon uptake in the cyanobacterium, *Synechocystis* sp. PCC7942, *Funct. Plant Biol.* 29 (2002) 131–149.
- [48] G. Schmetterer, Cyanobacterial respiration, in: D.A. Bryant (Ed.), *The Molecular Biology of Cyanobacteria*, Kluwer Academic Publishers, The Netherlands, 1994, pp. 409–435.
- [49] H. Yamamoto, C. Miyake, K. Dietz, K. Tomizawa, N. Murata, A. Yokota, Thioredoxin peroxidase in the cyanobacterium *Synechocystis* sp. PCC 6803, *FEBS Lett.* 447 (1999) 269–273.
- [50] K. Asada, The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50 (1999) 601–639.
- [51] C. Miyake, F. Michihata, K. Asada, Scavenging of hydrogen peroxide in prokaryotic and eukaryotic algae: Acquisition of ascorbate peroxidase during the evolution of cyanobacteria, *Plant Cell Physiol.* 32 (1991) 33–43.
- [52] K. Asada, Production and scavenging of reactive oxygen species in chloroplasts and their functions, *Plant Physiol.* 141 (2006) 391–396.
- [53] M.A. Huynen, T. Dandekar, P. Bork, Variation and evolution of the citric-acid cycle: a genomic perspective, *Trends Microbiol.* 7 (1999) 281–291.
- [54] C. Yang, Q. Hua, K. Shimizu, Metabolic flux analysis in *Synechocystis* using isotope distribution from ¹³C-labeled glucose, *Metab. Eng.* 4 (2002) 202–216.
- [55] Y. Helman, E. Barkan, D. Eisenstadt, B. Luz, A. Kaplan, Fractionation of the three stable oxygen isotopes by oxygen-producing and oxygen-consuming reactions in photosynthetic organisms, *Plant Physiol.* 138 (2005) 2292–2298.
- [56] J.C. Munday Jr., Govindjee, Light-induced changes in the fluorescence yield of chlorophyll A *in vivo*. III. The dip and the peak in the fluorescence transient of *Chlorella pyrenoidosa*, *Biophys. J.* 9 (1969) 1–21.