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ORIGINAL ARTICLE

NADPH production in dark stages is critical for cyanobacterial photocurrent generation: a study using mutants deficient in oxidative pentose phosphate pathway

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Abstract

Live cyanobacteria and algae integrated onto an extracellular electrode can generate a light-induced current (i.e., a photocurrent). Although the photocurrent is expected to be correlated with the redox environment of the photosynthetic cells, the relationship between the photocurrent and the cellular redox state is poorly understood. Here, we investigated the effect of the reduced nicotinamide adenine dinucleotide phosphate [NADP(H)] redox level of cyanobacterial cells (before light exposure) on the photocurrent using several mutants (Δ*zwf*, Δ*gnd*, and Δ*glgP*) deficient in the oxidative pentose phosphate (OPP) pathway, which is the metabolic pathway that produces NADPH in darkness. The NAD(P)H redox level and photocurrent in the cyanobacterium *Synechocystis* sp. PCC 6803 were measured noninvasively. Dysfunction of the OPP pathway led to oxidation of the photosynthetic NADPH pool in darkness. In addition, photocurrent induction was retarded and the current density was lower in Δ*zwf*, Δ*gnd*, and Δ*glgP* than in wild-type cells. Exogenously added glucose compensated the phenotype of Δ*glgP* and drove the OPP pathway in the mutant, resulting in an increase in the photocurrent. The results indicated that NADPH accumulated by the OPP pathway before illumination is a key factor for the generation of a photocurrent. In addition, measuring the photocurrent can be a non-invasive approach to estimate the cellular redox level related to NADP(H) pool in cyanobacteria.

Keywords Oxidative pentose phosphate pathway · Photocurrent · Extracellular electron transfer · Biophotovoltaics · Cyanobacteria

Introduction

Light-induced current can be observed in an electrochemical cell containing cyanobacteria or microalgae. This so-called photocurrent is derived from the photosynthetic electron transport system and the respiratory glucose metabolism systems can be detected by electrodes via extracellular electron transfer (EET) of photosynthetic organisms (Cereda et al. 2014; McCormick et al. 2015). Because of its potential benefit in utilizing solar energy, this photocurrent has attracted wide interest in various engineering fields as a component of "biophotovoltaic" technology (Rosenbaum et al. 2010; McCormick et al. 2015; Ng et al. 2017; Tschörtner et al. 2019; Liu and Choi 2021). Various studies have successfully improved the photocurrent yield through the modification of the access of photosynthetic cells to electrodes and the screening of the electrochemical mediators (Sawa et al. 2017; Bombelli et al. 2015; Lemaître et al. 2021; Çevik et al.

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2018; Wenzel et al. 2018). According to the literature, the electrons are possibly transferred from photosynthetic cells to an electrode via reduced nicotinamide adenine dinucleotide phosphate (NADPH) and/or other electron mediator(s) (Zhang et al. 2018; Thirumurthy et al. 2020; Shlosberg et al. 2021). Further, Saper et al. (2018) has analyzed the cells impaired in photosystem II (PSII) to show the important implication that respiratory metabolisms are related to photocurrent in cyanobacteria.

In addition to its biotechnological aspects targeting the foundation for energy transduction in biological systems, the photocurrent is one of attractive physiological responses of photosynthetic cells to the illumination and should reflect the physiological states of the cyanobacterial or algal cells. In photosynthetic organisms, EET is assumed to play roles in nutrition uptake (Kranzler et al. 2011; Tanaka et al. 2021a), extracellular signaling between cells [e.g., in a bacterial mat (Michelusi et al. 2014)], and the dissipation of excess electrons if the electron flux is sufficiently large (Lea-Smith et al. 2016). Because EET is expected to be dependent on the cellular redox level, the current density can reflect the physiological states in vivo. Indeed, changes in photocurrent are associated with the circadian clock controlled by the redox state of the plastoquinone pool (Nishio et al. 2015; Tanaka et al. 2019). However, the electron transfer pathway from the photosynthetic electron transport system in cells to an extracellular electrode remains poorly understood, and the relationship between the photocurrent and the cellular redox level has not yet been elucidated.

Cyanobacteria, the progenitors of oxygenic phototrophs, are among the most commonly used photosynthetic organisms in photocurrent research. Because prokaryotic cells develop no organelles, two central energy metabolisms, i.e., respiration and photosynthesis, proceed in one cell and share many metabolic intermediates and electron transport components (Binder 1982; Peschek et al. 2004; Mullineaux 2014) (also see Fig. 1 and the legend). Although the dark respiration rate is 10 times smaller than the photosynthetic O_2 evolution rate, both rates are mutually dependent on the redox states of shared pools of plastoquinone and NADPH (Pils and Schmetterer 2001; Bolychevtseva et al. 2015) and on the amounts of shared metabolic intermediates (Shimakawa et al. 2014). NADPH, a candidate electron source for EET, is produced by photosynthetic electron transport and is mainly consumed by the reductive pentose phosphate pathway, the so-called Calvin–Benson-Bassham (CBB) cycle, under

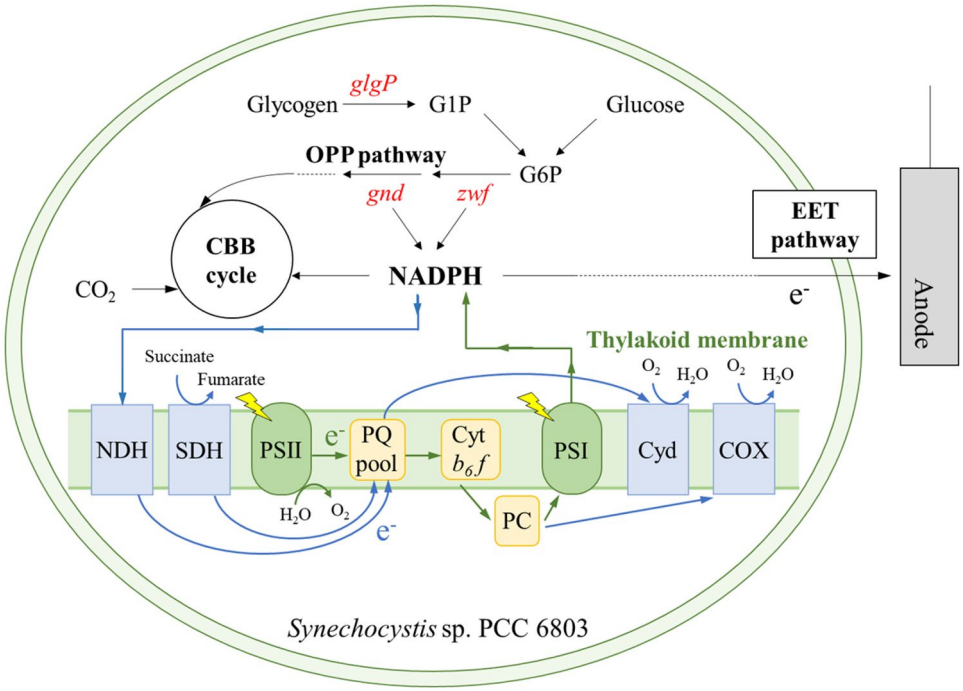

Fig. 1 Respiration, photosynthesis, and extracellular electron transfer (EET) for the cyanobacterium Syn6803. Blue and green arrows indicate respiratory electron transport and photosynthetic electron transport, respectively. To simplify the illustration, the stoichiometry of each reaction is disregarded here. *OPP* oxidative pentose phosphate, *CBB* Calvin-Benson-Bassham, *glgP* glycogen phosphorylase, *zwf* glucose-6-phosphate 1-dehydrogenase, *gnd* 6-phosphogluconate dehydrogenase, *G1P* glucose-1-phosphate, *G6P* glucose-6-phosphate, *NDH* NAD(P)H dehydrogenase, *SDH* succinate dehydrogenase, *PSII and PSI* photosystems II and I, respectively, *PQ* plastoquinone, *Cyt* b_6f cytochrome b_6f complex, *PC* plastocyanin, *Cyd* cytochrome *bd*-type quinol oxidase, *COX* cytochrome *c* oxidase. Details of the electron transport from NADPH to the PQ pool are yet not fully understood (see the "Discussion")

illumination. In the dark, the oxidative pentose phosphate (OPP) pathway dominantly produces NADPH (Welkie et al. 2019), which is consumed by respiratory electron transport. Thus, both photosynthesis and respiration are expected to potentially affect EET, implying that the amplitude of the photocurrent can reflect these physiological activities.

Here, we investigated the effect of changes in the redox state of the NADPH pool on the photocurrent in the cyanobacterium *Synechocystis* sp. PCC 6803 (Syn6803). In the dark, the mutants deficient in the OPP pathway showed a more oxidized NADPH pool than the wild-type cells. The induction of photocurrent in these mutants was retarded, and the current density was small compared with that observed for the wild type, which suggests that the generation of a photocurrent requires the production of NADPH via the OPP pathway before illumination.

Materials and methods

Cultures and mutant strains

The cyanobacterium Syn6803 was cultured under continuous fluorescent lighting (30 °C, 20 µmol photons m^{-2} s^{-1}) on BG-11 agar plates and liquid media bubbled with ambient air.

A mutant of Syn6803 deficient in glycogen phosphorylase (Δ*glgP*) was previously constructed (Shimakawa et al. 2014), and mutants deficient in the genes *zwf* (*slr1843*) and *gnd* (*sll0329*), which encode glucose 6-phosphate 1-dehydrogenase and 6-phosphogluconate dehydrogenase (Δ*zwf* and Δ*gnd*), respectively, were constructed in the present study following the previously reported method (Shimakawa et al. 2021). To disrupt these genes, we replaced each portion of the coding region with streptomycin and erythromycin resistance cassettes (Sm^r and Em^r) respectively derived from pRL453 and pRL425 (Elhai and Wolk 1988; Supplemental Fig. S1A). For the preparation of the construct of *zwf* with Sm^r, the coding region was cloned into a pTA2 vector (TOYOBO, Otsu, Japan) using the primer set (F, 5′-CAG AACCCCTCATTTTGACCATTTT-3′; R, 5′-CAGCGACGG CCATCTTTATTAATTA-3′). The plasmid was linearized by an inverse polymerase chain reaction (PCR) with the primer set (F, 5′-CAATATAAAGCTGGTTGGATGGGAG-3′; R, 5′-ACATGATCAACAAACTGACGGTTC-3′) and ligated with Sm^r. To prepare the construct of *gnd* with Em^r, two separated PCR fragments for the coding regions were obtained with the primer sets (F1, 5′-TATGACTAAGCGAACTTT TGGGGTA-3′; R1, 5′-TTTTTCGTGTGCCTACGATTA GTTGCATATCGCCATACTC-3′; F2, 5′-GTAATACAA ACGGGATGATGAGTGGTTTGGAATTAGGAGT-3′; R2, 5′-CTTATCAGTCCGTTCATAGGTGTGG-3′), which were linked with Em^r by a splicing with overlap extension PCR. Transformants were selected on 0.5% (w/v) agar plates of BG-11 medium containing antibiotics. Segregations of the mutants were confirmed by PCR with the primers for cloning each gene (Supplemental Fig. S1B).

NAD(P)H fluorescence measurements

The in vivo NAD(P)H fluorescence originating from NAD(P)H was measured using the NADPH/9-AA module of a Dual-PAM-100 instrument (Heinz Walz, Effeltrich, Germany) following the method reported in our previous study (Tanaka et al. 2021b). The reaction mixture (2 mL) contained fresh BG-11 medium (pH 7.5) and cyanobacterial cells cultured in the liquid medium (10 µg chlorophyll mL^{-1}). The chlorophyll *a* concentration was determined in 100% (v/v) methanol (Grimme and Boardman 1972). The NADPH/9-AA module consisted of an emitter unit (DUAL-ENADPH) and a detector unit (DUAL-DNADPH). NADPH fluorescence was excited by UV-A (365 nm) irradiation from the DUAL-ENADPH unit and was detected using a blue-sensitive photomultiplier with a filter transmitting light between 420 and 580 nm in the DUAL-DNADPH unit. The intensity of the measuring light was on a scale from 1 to 20, and the intensity was set at 10 in the present study. The frequency of the measuring light in the absence and presence of red actinic light (220 µmol photons m^{-2} s^{-1}) was set at 50 and 500 Hz, respectively.

Electrochemical measurements

The electrochemical setup consisted of a cylindrical glass chamber (⌀20 × 30 mm^2; geometrical surface area, 3.14 cm^2), indium tin oxide (ITO)-coated glass (GEOMATEC, Yokohama, Japan) as a working electrode placed at the bottom of the chamber, a platinum wire as a counter electrode, and an Ag/AgCl electrode (saturated KCl) with saturated KCl as a reference electrode (HOKUTO DENKO, Tokyo, Japan). A schematic of the setup is shown in Supplemental Fig. S2A. In this study, we applied the cyanobacterial cell pellets cultured on agar plates were directly pasted on the electrode (Supplemental Fig. S2B) to the electrode without any chemical modifications. The electrochemical chamber was filled up to 4 mL (pH 7.0) with BG-11 liquid media from the cultures (Supplemental Fig. S2C). The electrode was fully covered by the cell samples, but the local concentrations of the cells on the electrode cannot be strictly controlled in both cases. Current was detected without any artificial mediators on the glass electrode with an ITO film at 0.25 V (vs Ag/AgCl) at 30 °C using a CHI1000A potentiostat (CH Instruments). The sample was illuminated from the bottom of the electrochemical chamber using an LED light source LC-LED450W (TAITEC) at 100 µmol photons m^{-2} s^{-1}.

Results

Effects of OPP pathway on the redox state of NAD(P)H pool

In cyanobacteria, the OPP pathway plays a main role in the production of NADPH in the dark (Welkie et al. 2019). To study the relationship between the redox state of the NADPH pool and the photocurrent, we constructed two mutants of Syn6803 deficient in the OPP pathway (Δ*zwf* and Δ*gnd*) and investigated the redox state of the NADPH pool in the light–dark transition. The in vivo NAD(P)H fluorescence was measured in response to 1 min illumination with a red actinic light at 220 µmol photons m^{-2} s^{-1}. In the wild type, the fluorescence intensity immediately increased when the light was turned on and then remained approximately constant under illumination (Fig. 2). When the light was turned off, the NAD(P)H fluorescence rapidly decayed. These light-responsive rapid changes in fluorescence are attributed to the production and consumption of NADPH by photosynthetic linear electron transport and the CBB cycle, respectively (Tanaka et al. 2021b; Kauny and Sétif 2014). Thereafter, a post-illumination transient increase in fluorescence intensity was observed in the dark in the wild type (Fig. 2). All of these observations are typical responses for in vivo NAD(P)H fluorescence in cyanobacteria (Mi et al. 2000; Feilke et al. 2017; Kauny and Sétif 2014). Notably, the NAD(P)H fluorescence level was higher before illumination than immediately after the light was turned off (Fig. 2), indicating that the NAD(P)H pool remained partially reduced even in the dark before illumination. In Δ*zwf* and Δ*gnd*, the pre-illumination fluorescence level was the same as that observed after the light was turned off. The magnitude of the increase in fluorescence in the light was larger in Δ*zwf* and Δ*gnd* than in the wild type. That is, the NADPH pool was more oxidized in these mutants. In addition, no post-illumination transient fluorescence rise was observed in these mutants, suggesting that the OPP pathway participates in reducing the NADPH pool in the dark. The fluorescence induction in the light–dark transition was similar between the wild type and the mutants (Supplemental Fig. S4), indicating that photosynthetic linear electron transport was not affected by the OPP pathway deficiency in Syn6803.

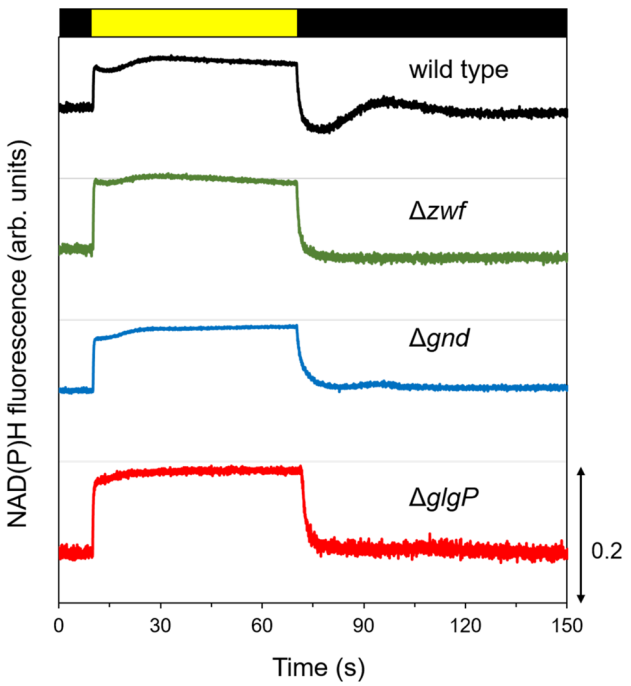

Fig. 2 Changes in the in vivo NAD(P)H fluorescence in the light–dark transition for the Syn6803 wild type (black line) and the mutants Δ*zwf* (green line), Δ*gnd* (blue line), and Δ*glgP* (red line). The black band indicates darkness, and the yellow band indicates illumination. Representative traces of three independent experiments are shown. The other traces are shown in Supplemental Fig. S3

In cyanobacteria, the OPP pathway is initiated by the substrate glucose provided by glycogen degradation (Shinde et al. 2020). Here, we investigated the effect of the glycogen degradation activity on the NAD(P)H redox level in Syn6803 using the mutant deficient in glycogen phosphorylase (Δ*glgP*). Different from the wild type, Δ*glgP* showed the same NAD(P)H fluorescence level before illumination as that shown just after the light was turned off. In addition, the post-illumination increase in fluorescence intensity was not observed in Δ*glgP* (Fig. 2). That is, the OPP pathway was suppressed in Δ*glgP*. Because Syn6803 can uptake extracellular glucose, the addition of glucose at 1 mM compensated the phenotype of Δ*glgP* and kept the NADPH pool more reduced in the dark state, as reflected in the NAD(P)H fluorescence (Supplemental Fig. S5A), indicating that the lack of the OPP pathway in Δ*glgP* was due to a substrate shortage. Exogenously added glucose drives the respiratory metabolisms to accumulate the intermediates at a high concentration (Shimakawa et al. 2014). In Δ*glgP* with glucose, the magnitude of the increase in fluorescence in the light was smaller and the fluorescence level was kept higher after the light was turned off (Supplemental Fig. S5A), probably due to the enhanced generation of NADPH from the OPP pathway.

Effects of OPP pathway on photocurrent

From the results in Fig. 2, in the dark state, the OPP-pathway-deficient mutants kept the NADPH pool oxidized more than the wild type. In the present study, we investigated the effect of the cellular NADP(H) redox level on the current observed using an extracellular electrode in response to illumination. The current detected using an ITO electrode at 0.25 V (vs Ag/AgCl, saturated KCl)

was observed already in the dark state in the presence of the wild type cells but not in their absence (Fig. 3A and Supplemental Fig. S6), which suggests that the cyanobacterium Syn6803 exhibits EET activity in the dark. In response to illumination with white light, the Syn6803 wild type showed a gradual increase in current density, followed by a decrease to an approximately constant level (Fig. 3A). That is, EET from the cyanobacterial cells to the extracellular electrode was enhanced by the illumination. Immediately after the light was turned off, the current density showed a transient peak, although the mechanism remains unclear. The current density slowly decreased thereafter (Fig. 3A). The current density in the light largely decreased in the presence of the inhibitor to PSII, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (Supplemental Fig. S7), which suggests that PSII is involved in the photocurrent observed in the Syn6803 wild type. Like the wild type, the Δ*zwf* and Δ*gnd* mutants showed EET in the dark state; however, the current densities were smaller than that in the wild type (Fig. 3B and C). In the transition from dark to light, these mutants showed a substantially smaller current density and longer time to reach constant values (Fig. 3B and C). A transient decrease in current was observed just after the start of illumination, especially in the mutants impaired in the OPP pathway, although the molecular mechanism was still not understood.

Similar to Δ*zwf* and Δ*gnd*, Δ*glgP* exhibited a lower current density than the wild type in the dark and illuminated states (Fig. 3D). Compared with the current density in the wild type, the current density in Δ*glgP* slowly increased during illumination (Fig. 3D). The addition of glucose, which initiated the OPP pathway, increased the current density under both dark and light conditions (Supplemental Fig. S5B). In addition, the photocurrent was rapidly induced in Δ*glgP* with exogenously added glucose (Supplemental Fig. S5B). These results are consistent with those for Δ*zwf* and Δ*gnd* and indicate that NADPH production via the OPP pathway in the dark is required for photocurrent generation in Syn6803. The current densities in the mutants impaired in the OPP pathway were not affected by DCMU (Supplemental Fig. S7). Possibly, the photocurrent in these mutants is independent of PSII.

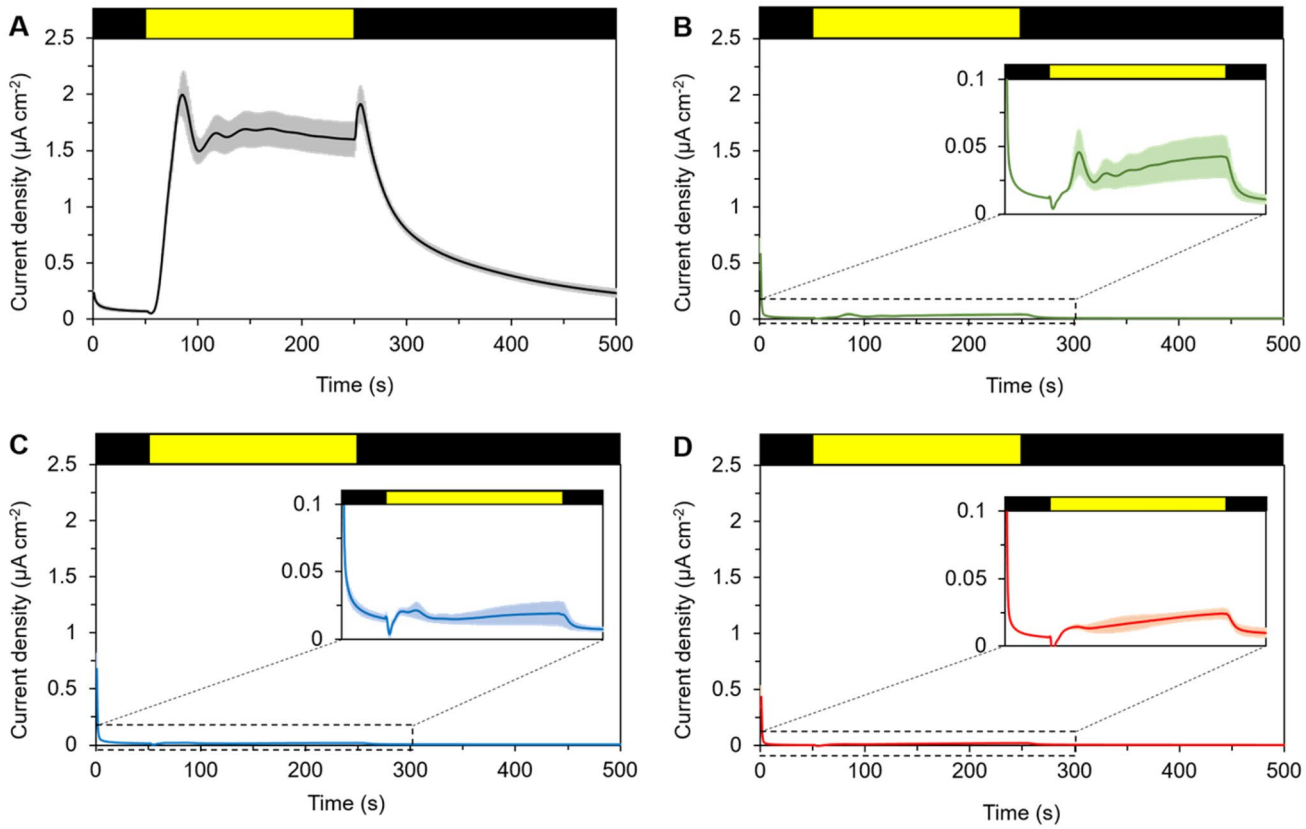

Fig. 3 Changes in the photocurrent in the light–dark transition for the Syn6803 wild type (**A**) and the mutants Δ*zwf* (**B**), Δ*gnd* (**C**), and Δ*glgP* (**D**). The black band at the top of each figure indicates darkness, and the yellow band indicates illumination. The traces are shown as the mean of three measurements, and the shaded areas show the standard deviation. In Fig. 3B, C, and D, the changes in photocurrent are enlarged to be shown in each inset

Discussion

Here, we found that the OPP pathway is critical for photocurrent generation in the cyanobacterium Syn6803. A photocurrent should be triggered by the photosynthetic electron transport system and should be related to the redox states of intracellular and extracellular electron transfer components. Therefore, the redox state of the EET intermediates is expected to affect the photocurrent. Nevertheless, the details of the electron transport pathway from thylakoid membranes to the electrode remain elusive (McCormick et al. 2015); even the relationship between the cellular redox state and the photocurrent has not yet been elucidated. NADPH is one of the most important reductants for both photosynthetic and respiratory metabolisms and is assumed to function for EET in photosynthetic organisms (Shlosberg et al. 2021). Cyanobacteria are known to produce NADPH via the OPP pathway in the dark (Welkie et al. 2019). Here, we hypothesized that NADPH accumulated under dark conditions contributes to the photocurrent in Syn6803. Different from the wild type, the mutants Δ*zwf*, Δ*gnd*, and Δ*glgP* showed approximately the same NAD(P)H fluorescence levels before and immediately after illumination (Fig. 2), indicating that the photosynthetic NADPH pool remained almost fully oxidized in these mutants in the dark state. Compared with the wild type, all three OPP-pathway-impaired mutants showed a lower current density and a slower induction of photocurrent during illumination (Fig. 3). Overall, the reduction of the NADPH pool via the OPP pathway in the darkness is an indicator to the rapid start and large capacity of the photocurrent. Notably, the absolute values of the current density are not completely accurate in this study because the local concentrations of the cyanobacterial cells and electron mediator(s), which were likely secreted by cyanobacteria (Zhang et al. 2018), were difficult to control in the experimental setup. We therefore focused on the kinetics of the current density during illumination and on the large differences in the current density (> 0.5 μA cm^{-2}). Electrochemical measurements for EET are a useful physiological tool in investigating the redox information of cyanobacteria and microalgae. In the previous research, through the measurement of the current with a phospholipid redox polymer, the redox state of the in vivo plastoquinone pool was evaluated and also the circadian oscillation was observed in cyanobacteria (Nishio et al. 2015; Tanaka et al. 2019). In the present work, we propose that the photocurrent can be used as an indicator for a cell redox level associated to the NADP(H) redox level in cyanobacterial cells.

How the NADP(H) redox level in the dark state is associated with the current in the illuminated state remains unclear. It should be noted that the current densities in Δ*zwf*, Δ*gnd*, and Δ*glgP* were significantly lower than that in the wild type during the illumination (Fig. 3) even after the photosynthetic NADPH pool was reduced (Fig. 2). NADPH produced by the OPP pathway in the dark is utilized for respiratory electron transport and also a variety of metabolisms. That is, the redox level should be shared between the NADPH pool and respiratory metabolisms. Indeed, many intermediates are shared by respiratory and photosynthetic metabolisms in cyanobacteria, and reducing sugars are accumulated in the dark by the OPP pathway initiated by glycogen degradation (Shimakawa et al. 2014). Overall, it is possible that the redox state of the NADPH pool measured in Fig. 2 is just an indicator to the other redox pool associated with EET in Syn6803, and we guess that the photosynthetic NADPH pool and the EET-related redox pool share the redox level at the slower time scale than photosynthetic linear electron transport. Meanwhile, the photocurrent should be mainly derived from the leakage and/or proactive release of electrons via photosynthetic electron transport with a regulatory mechanism. Therefore, another possibility is that the reduction of the NADPH pool in the dark condition might switch the physiological states of the cyanobacterial cells to increase their EET capacity through, for example, thiol redox regulation mechanisms (Oktyabrsky and Smirnova 2007). Here, we clearly showed that respiratory metabolisms are the key factor for photocurrent in cyanobacteria, which is in agreement with the recent report from Saper et al. (2018). Meanwhile, PSII was also involved in the photocurrent of the Syn6803 wild type (Supplemental Fig. S7), as reported by previous studies (Bombelli et al. 2011; Cereda et al. 2014; Zhang et al. 2018). The molecular details in the relationship of cyanobacterial respiration with photocurrent should be further assessed in future works.

In the present study, we observed the same fluorescence level for NAD(P)H in the dark state between pre- and post-illumination in the Syn6803 mutants Δ*zwf* and Δ*gnd* (Fig. 2), which suggests that the OPP pathway is the main metabolic pathway to partially keep the NADPH pool reduced in the cyanobacterium in the dark. This is consistent with the latest report showing the exogenous glucose effect on the NAD(P)H fluorescence in the wild type and Δ*gnd* of Syn6803 (Ogawa et al. 2021). In addition, a post-illumination fluorescence rise, which has been assumed to be derived from the NADPH generation dependent on the accumulation of the CBB cycle intermediates after illumination (Mi et al. 2000), was not detected in either Δ*zwf* or Δ*gnd*. It is plausible from the present results that the OPP pathway was driven by the transiently accumulated CBB cycle intermediates to produce NADPH after the light was turned off. The NADPH pool was kept reduced in the dark state at the same level as in the illuminated state in the case of the mutant of Syn6803 deficient in

NAD(P)H dehydrogenase-1 (NDH-1) complex (Δ*ndh*) (Mi et al. 2000; Sétif et al. 2020). In addition, a mutant of Syn6803 that over-expressed tobacco plastid terminal oxidase showed the oxidized pool of NADPH in the dark state, similar to Δ*zwf* and Δ*gnd* (Feilke et al. 2017), which suggests that chlororespiration plays a role in oxidizing the NADPH pool. Overall, the NADPH pool is dominantly reduced by the OPP pathway with glucose and is oxidized by respiratory electron transport with O_2 in Syn6803 in the dark state. We note that the details of the respiratory electron transport from NAD(P)H to plastoquinone is still controversial in cyanobacteria. NDH-1 complex possibly donates electrons to plastoquinone from NADPH via ferredoxin-$NADP^+$ reductase and ferredoxin (Schuller et al. 2019). Meanwhile, NDH-2 possibly has a contribution to respiratory electron transport although it has been reported to be small in cyanobacteria (Howitt et al. 1999). Bradley et al. (2013) found that the Syn 6803 mutant deficient in NDH-1 complex shows a larger dark current than the wild type, which may be also observed in the mutant that lacks NDH-2.

Similar to Δ*zwf* and Δ*gnd*, Δ*glgP* showed oxidation of the NADPH pool before illumination, likely because of a shortage of intermediates driving the OPP pathway (Shimakawa et al. 2014; Shinde et al. 2020). The post-illumination transient rise of the NADPH fluorescence intensity was hardly recognized in Δ*glgP* without glucose added (Fig. 2). Meanwhile, the addition of exogenous glucose accumulates the intermediates of the glycolysis, the CBB cycle, and the OPP pathway in the cells (Shimakawa et al. 2014), which complements the reduction of the NADPH pool in the darkness and the rapid start of photocurrent induction (Supplemental Fig. S5). Once the actinic light was turned off, the NADPH fluorescence decreased but then gradually increased to keep the higher level in Δ*glgP* with glucose (Supplemental Fig. S5A). The addition of glucose accumulates the CBB cycle intermediates at a high concentration in Syn6803 (Shimakawa et al. 2014), resulting in the highly reduced NADPH pool in the dark to mask the post-illumination fluorescence rise (Supplemental Fig. S5A).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11120-022-00903-0.

Author contributions S.N. and G.S. conceived the research plans; J.H. and G.S. performed all experiments; S.K. provided technical assistance to J.H.; K.T., A.K., and C.M. provided technical assistance to G.S.; J.H., S.N., and G.S. designed the experiments, analyzed the data, and wrote the article with support from all other authors.

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Declarations

Conflict of interest The authors have no conflict of interest to declare.

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