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Respiration accumulates Calvin cycle intermediates for the rapid start of photosynthesis in *Synechocystis* sp. PCC 6803

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We tested the hypothesis that inducing photosynthesis in cyanobacteria requires respiration. A mutant deficient in glycogen phosphorylase (ΔGlgP) was prepared in *Synechocystis* sp. PCC 6803 to suppress respiration. The accumulated glycogen in ΔGlgP was 250–450% of that accumulated in wild type (WT). The rate of dark respiration in ΔGlgP was 25% of that in WT. In the dark, P700⁺ reduction was suppressed in ΔGlgP, and the rate corresponded to that in (2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone)-treated WT, supporting a lower respiration rate in ΔGlgP. Photosynthetic O₂-evolution rate reached a steady-state value much slower in ΔGlgP than in WT. This retardation was solved by addition of d-glucose. Furthermore, we found that the contents of Calvin cycle intermediates in ΔGlgP were lower than those in WT under dark conditions. These observations indicated that respiration provided the carbon source for regeneration of ribulose 1,5-bisphosphate in order to drive the rapid start of photosynthesis.

Key words: cyanobacteria; respiration; photosynthesis; glycogen phosphorylase

For the last two decades, much attention has been paid to the interplay between photosynthesis and respiration in photosynthetic organisms. Previous studies have shown that leaf mitochondria in higher plants may have several functions during photosynthesis.¹) Mitochondria possess many unique components such as the glycol decarboxylase complex, which is involved in the photorespiratory pathway. The respiratory electron transport chain in mitochondria includes non-phosphorylating pathways comprising type II NAD(P)H dehydrogenases and cyanide-resistant alternative oxidase.²,³) In the tobacco (*Nicotiana sylvestris*) mutant CMSII, in which the major mitochondrial NADH dehydrogenase complex I is non-functional, a decreased photosynthesis to respiration ratio was reported compared to that in wild type (WT).⁴) Moreover, the mutant showed prolonged induction of photosynthesis that was exacerbated in conditions favoring photorespiration and was accompanied by increased extractable NADP-malate dehydrogenase activity.⁵) These data indicate that respiration is required for photosynthesis. However, the detailed mechanisms remain unknown.

Because cyanobacteria lack organelles for sub-localization of different metabolic pathways, respiration should have a greater impact on photosynthesis than that in higher plants. The cyanobacterial thylakoid membrane contains both the photosynthetic electron transport chain including photosystems I (PSI), II and the respiratory electron transport chain including type I NAD(P)H dehydrogenase (NDH-1), succinate dehydrogenase (SDH), and cytochrome aa₃-type terminal oxidase (CtaI). These electron transport chains have common electron carriers such as the cytochrome b₆f complex, the plastoquinone (PQ) pool, and soluble redox-active proteins.⁶–⁸) *NdhB*—the gene encoding one of the NDH-1 proteins, defective mutant of the cyanobacterium *Synechocystis* sp. PCC 6803 (S. 6803) has shown suppressed electron donation from either the respiratory electron donors or the photoreductants generated in PSI to P700⁺ through the PQ pool, compared to WT.⁹) In addition, *ndhF1*, which encodes one of the NDH-1 proteins in a defective mutant of *S.* 6803, has shown oxidation of the PQ pool, leading to state transition to state 1, where the maximum yield of chlorophyll fluorescence is high.¹⁰) Furthermore, Cooley and Vermaas constructed a range of *S.* 6803 mutants that were impaired in several combinations of respiratory and photosynthetic electron transport complexes such as NDH-1, SDH, CtaI, and PSI and demonstrated that

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Abbreviations: GlgP, glycogen phosphorylase; WT, wild type; Chl, chlorophyll; N, nitrogen.

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removing SDH, NDH-1, or NDH-2 led to a more oxidized PQ pool in the dark using a quinone electrode. These recent data suggested that defects in respiratory electron transport components have greater impacts on photosynthetic electron transport activity in cyanobacteria, compared to higher plants.

The primary glycolysis reaction is regulated by degradation of glycogen. As the genomic sequence has been elucidated for many cyanobacterial strains, it is possible to speculate on the essential enzymatic repertoire responsible for synthesis and degradation of glycogen. The presence of genes homologous to bacterial glycogen synthase (glgA: sll0945, slr1393), a branching enzyme (glgB: sll0158), ADP-glucose pyrophosphorylase (glgC: sll1176), the debranching enzyme (glgX: sllr0237, s1r1857), and glycogen phosphorylase (glgP: slr1356, s1r1367) suggests that the glycogen metabolism pathway functions in vivo in S. 6803.

GlgP transfers orthophosphate to the non-reducing end of the glucose residue in glycogen, resulting in the release of glucose-1-phosphate. Genes encoding GlgPs have been cloned from many organisms, and most of these phosphorylases exhibit the activity. Two glgP homologs in S. 6803 are found in the genome: slr1356, which is essential for growth at high temperatures and plays a more important role in glycogen degradation than s1r1367 under autotrophic growth; s1r1367, the key factor in glycogen degradation under phototrophic or phototrophic growth.

Elucidation of glycogen metabolism in cyanobacteria contributes to not only the understanding of the above physiological function of respiration, but also biotechnological aspects. Glycogen is the energy compound, which is synthesized in photosynthesis, and could be utilized for carbon source in producing primary industrial compounds, for example ethanol, isobutanol, and butanol. The usage of photosynthesizing, glycogen, is the strategy for renewable and carbon neutral factory, which alternate petroleum fuels which have long been used and would be almost consumed in near future. Different from plants and trees, refinery efficiency of carbon from algae is higher. Enhanced storage of glycogen in cyanobacteria would contribute to the production of carbon source for bioenergy.

In the present study, we constructed and analyzed the glgP-defective mutant (ΔGlgP) to test the hypothesis that suppressing respiration retards the induction of photosynthesis in S. 6803, similarly to the CMSII mutant. WT and ΔGlgP were grown under various growth conditions. Growth, chlorophyll (Chl), nitrogen (N), and glycogen contents and the respiratory and photosynthetic activities in both strains were investigated. Furthermore, we investigated metabolites in WT and ΔGlgP under dark conditions. The present results indicated that respiration in this S. 6803 cyanobacterium contributes to the enhancement of photosynthesis by supplying Calvin cycle intermediate metabolites.

Materials and methods

S. 6803 culture and determination of Chl concentration. S. 6803 cells were cultured in BG-11 medium on a rotary shaker (100 rpm) with 2% CO2 under two different photoperiods: light–dark cycle (25 °C, 16 h, 100 μmol photons m−2 s−1, fluorescent lamp)/(23 °C, 8h, dark) or continuous light (25 °C, 100 μmol photons m−2 s−1, fluorescent lamp). Unless otherwise stated, the cells used in the measurements are those grown under the light–dark cycle.

Only in Fig. 10, the cells were cultured under another light–dark cycle (25 °C, 12 h, 100 μmol photons m−2 s−1, fluorescent lamp)/(23 °C, 12 h, dark) with atmospheric CO2 concentration.

For the determination of Chl concentration, the cells were harvested and resuspended by vortexing in 1 mL of 100% methanol. After 5 min room temperature incubation, the suspension was centrifuged at 10,000 rpm for 5 min. Chl a was determined in the cyanobacteria by the method reported by de Marsac and Houmard.

Generation of mutants. Total DNA was extracted from the S. 6803 cells. The genomic regions coding the glycogen phosphorylase genes (glgP: slr1356, s1r1367) were amplified by PCR using oligonucleotide primers (sll1356: P1, P2; slr1367: P3, P4; Supplemental Table. S1, see Biosci. Biotechnol. Biochem. Web site) and were respectively cloned into the pTA2 vector (TOYOBO, Tokyo, Japan). The recombinant plasmids were respectively linearized and amplified by inverse PCR using oligonucleotide primers (sll1356: P5, P6; slr1367: P7, P8; Supplemental Table S1) and ligated with the kanamycin and chloramphenicol resistance genes (Kan′, Cm′), respectively, which were obtained from the pUC4-KIXX and pACYC184 vectors by SmaI digestion. The resulting plasmids were designated pAsl1356::Kan′ (Fig. 1(a)) and pAslr1367::Cm′ (Fig. 1(b)).

Transformation of S. 6803 was conducted using the pAsl1356::Kan′ and pAslr1367::Cm′ plasmids and a standard procedure. The transformants were selected on 0.5% BG-11 medium agar plates containing 20 μg mL−1 kanamycin or 25 μg mL−1 chloramphenicol. The ΔAsl1356/slr1367 double mutant (ΔGlgP) was generated by second transformation of the ΔAsl1356 mutant with the mutated pAslr1367::Cm′. The transformant was selected on 0.5% BG-11 medium agar plates containing both 20 μg mL−1 kanamycin and 25 μg mL−1 chloramphenicol.

Immunoassay. The cells were harvested and resuspended in 500 μL extraction buffer (50 mM HEPES-KOH, pH 7.5, 1 mM MgCl2, 2 mM EDTA, 1 mM dithiothreitol, 2% [w/v] polyvinylpolypryridone, 1 mM phenylmethylsulfonyl fluoride, and 10 μL leupeptin). The suspension was homogenized with glass beads and centrifuged at 15,000 rpm for 15 min at 4 °C. The supernatant was treated as the extracted soluble fraction. Concentration of the protein in the fraction was determined with the Pierce 660 nm Protein Assay (Thermo Scientific, Rockford, IL, USA) using bovine serum albumin as the standard. The soluble fraction containing 5 μg proteins was analyzed by SDS-PAGE. After electrophoresis, the proteins were electrotransferred to a polyvinylidene fluoride membrane and detected by protein-specific antibodies. The Sll1356 polyclonal antibody was raised against amino acids.
552–565 of Sll1356 protein of S. 6803. The Slr1367 polyclonal antibody was raised against amino acids 41–54 of Slr1367 protein of S. 6803. These antibodies were a commercial product from Hokkaido System Science (Japan).

Glycogen phosphorylase assay. The cells in dark period were harvested and resuspended in the reaction buffer (18 mM KH₂PO₄, 27 mM Na₂HPO₄, 15 mM MgCl₂, 100 μM EDTA, pH 6.8). The cells were homogenized with glass beads and centrifuged at 15,000 rpm for 15 min at 4 °C. The supernatant was treated as the extracted protein, and protein in the supernatant was quantified as described above (see Immunoassay). The GlgP activities of extracted protein in WT and ΔGlgP were measured in 1 mL of the reaction buffer containing 40 μg proteins, 0.34 mM NADP⁺, 4 μM glucose 1,6-bisphosphate, 4 units glucose 6-phosphate dehydrogenase, 0.8 units phosphoglucomutase, and 2 mg glycogen. The same mixture without the glycogen was used as a control. The GlgP activities were determined by measuring the increase in the absorbance at 340 nm reflecting the production of NADPH.¹⁴

Nitrogen and glycogen measurements. The amount of nitrogen in the S. 6803 cells was determined with Nessler’s reagent after Kjeldahl digestion with sulfuric acid and addition of sodium potassium tartrate.²⁵ The amount of glycogen in the cyanobacterial cells was determined by the method reported by Suzuki et al.²⁶ The cells were harvested by centrifugation at 5000 rpm for 10 min, resuspended in 1 mL of 100% methanol, and kept at −20 °C for 24 h. After centrifugation, the pellet was dried overnight. The dried pellet was resuspended in 1 mL of distilled water and incubated at 100 °C for 1 h. A 200 μL aliquot of the suspension was mixed with 100 μL of 2.5 mM sodium acetate (pH 5) containing 0.5 mg mL⁻¹ glucoamylase (from Rhizopus niveus; Wako, Osaka, Japan) and incubated at 40 °C for 1 h. After centrifugation at 5000 rpm for 5 min, a portion (20–50 μL) of the supernatant was mixed with
reaction buffer (50 mM HEPES-NaOH [pH 7.5], 10 mM MgSO4, 1.5 mM ATP, 0.5 mM NADP+, 3 U of hexokinase [from yeast; Roche, Basel, Switzerland], 2 U of glucose 6-phosphate dehydrogenase [from yeast; Roche, Basel, Schweiz]) up to 1 mL. The amount of glucose moieties derived from glycogen was determined as the increase in the absorbance at 340 nm reflecting the production of NADPH.

Oxygen exchange measurements. Uptake and evolution of O2 were measured with an oxygen electrode (Hansatech, King’s Lynn, U.K.). The reaction mixture (2 mL) containing 50 mM HEPES-KOH (pH 7.5), 10 mM NaHCO3, and 10 μg Chl mL−1 of cyanobacterial cells was illuminated with red actinic light (>620 nm) at the indicated light intensity and 25 °C.27

P700+ absorbance measurements. P700+ absorbance was measured with a KLAS-100 kinetic LED-array spectrophotometer (Walz, Effeltrich, Germany).28 The amplitude of full P700 oxidation was measured in the dark before beginning illumination. P700 is in a reduced state in darkness, and full oxidation of P700 [P700] total was achieved by illumination with far-red (FR) light, which only excites PSI. Oxidation of P700 [P700+] was monitored by the absorbance change at 830–875 nm. The same amount of oxidizable P700 should be available during illumination, unless the PSI electron acceptors are already reduced and cannot accept additional electrons. The fraction of reduced P700, [P700], or the PSI acceptor (A−) during illumination, was determined by short saturating light pulses, which yielded fully oxidized P700, followed by a “dark pulse,” which yielded fully reduced P700. The difference between the P700 amplitude obtained by the short saturating light pulse in the light and the FR-induced amplitude determined in the dark-adapted state was attributed to A−.29 P700 turnover rate was determined by the method of Laisk et al.30

Metabolic profile analysis sampling procedure. Cell sampling was performed according to a method reported previously with minor modifications.20 Cyanobacterial cells, equivalent to 5 or 10 mg dry weight, were removed from cultivation vessels and filtered with 1-μm pore size Omnipore filter disks (Millipore, Billerica, MA, USA). After washing with 20 mM ammionium bicarbonate pre-chilled to 4 °C, the cells retained on the filters were immediately placed in 2 mL of pre-cooled (−30 °C) methanol containing 190 mM (+)-10-camphorsulfonic acid, 31 μM L-methionine sulfone, and 31 μM piperazine-1,4-bis(2-ethanesulfonic acid) as internal standards for mass analysis. Intracellular metabolites were extracted using a cold 10:3:1 (v/v/v) methanol:chloroform:water solution, as described previously.20 The cells were suspended by vortexing, and 1 mL of the cell extract obtained as the supernatant was transferred to a clean tube. After adding 440 μL of water, the aqueous and organic layers were separated by centrifugation at 14 000 × g for 5 min at 4 °C. After filtration with a Millipore 5 kDa cutoff filter to remove solubilized proteins, the aqueous-layer extracts were evaporated under vacuum using a FreeZone 2.5 Plus freeze-dry system (Labconco, Kansas City, MO, USA). The dried extracts were stored at −80 °C until used for CE/MS analysis.

CE/MS metabolite analysis. Dried metabolites were dissolved in 20 μL of Milli-Q water before the CE/MS analysis. The CE/MS experiments were performed using an Agilent G7100 CE system (Palo Alto, CA, USA), an Agilent G6224AA LC/MSD time-of-flight (TOF) system, and an Agilent 1200 series isocratic high-performance liquid chromatography pump equipped with a 1:100 splitter for delivery of the sheath liquid. Agilent ChemStation software for CE and MassHunter software for the Agilent TOF-MS was used for system control and data acquisition, respectively. The analytical conditions were as described previously.20

Results

Isolation of the GlgP-defective mutant

To examine the effects of respiration on photosynthesis in S. 6803, we constructed the ΔGlgP mutant (Fig. 1(a) and (b)) deficient in two GlgP protein homologs (SII1356 and SII1367). Polymerase chain reaction (PCR) analyses showed that WT copies of both sll1356 and slr1367 were inactivated in ΔGlgP (Fig. 1(c)), and an immunobass showed that the sll1356 and slr1367 gene products were absent in ΔGlgP (Fig. 1(d)). Furthermore, the GlgP activity of the soluble fractions was detected in WT but not in ΔGlgP (Fig. 1(e)).

ΔGlgP exhibits decreased nitrogen content and increased glycogen content

Growth of ΔGlgP was slower than that of WT during the early logarithmic growth phase under light–dark cycle but not under continuous light (Fig. 2(a)). Chlorophyll (Chl) content in the BG-11 medium containing each of the cell types increased with growth (Fig. 2(b)). The Chl content was significantly lower in the ΔGlgP mutant than in WT under light–dark cycle. On the other hand, under continuous light, Chl contents in WT and ΔGlgP were almost the same each other (Fig. 2(b)).

We measured the optical density at 750 nm (OD750) and the contents of Chl, nitrogen, and glycogen in WT and ΔGlgP during the logarithmic growth phases (OD750 = 2–5) in two growth conditions, light–dark cycle and continuous light (Table 1). In contrast to continuous light, the ratio of OD750 to Chl content in WT is about 60% of that in ΔGlgP under light–dark cycle. The nitrogen contents based on Chl in ΔGlgP were the same as those in WT in both growth conditions, indicating that the nitrogen content in ΔGlgP was significantly lower than that in WT under light–dark cycle, similarly to the Chl content.
Glycogen much accumulated in ΔGlgP grown under light–dark cycle, indicating that the two GlgP homologs did not function in glycogen degradation in ΔGlgP. The glycogen content of ΔGlgP was about 400% of that of WT at the end of the dark phase. On the other hand, glycogen that accumulated in WT and ΔGlgP grown under continuous light were almost the same each other.

ΔGlgP exhibits decreased respiratory activity

The dark respiration rate of ΔGlgP per Chl was 25% of that of WT (Fig. 3). Dark-adapted cells (5 h in dark) grown under light–dark cycle were used for this experiment. We added D-glucose to ΔGlgP and re-evaluated the respiratory O₂-uptake. The 5-min incubation with D-glucose dramatically enhanced the O₂-uptake rate of ΔGlgP (Fig. 3), which indicates that suppression of respiration in ΔGlgP is derived from the shortage of glucose or glucose phosphate caused by the defect of GlgP.

Suppressed respiration was reflected in the slower donation rate of electrons to PQ in the thylakoid membranes. ΔGlgP showed suppressed reduction of oxidized P700 (P700⁺) in PSI. Before illumination of multiple-turnover (MT) flash (300 ms) at time zero, FR light was illuminated to get the steady-state P700⁺. Next, the FR light and MT flash were turned off simultaneously. In the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), the amount of P700⁺ produced by the MT flash reflected the total amount of P700.29) The steady-state amount of P700⁺ in the absence of DCMU under FR light was the same as that in the presence of DCMU in WT (Fig. 4(a)). These results suggest that PQ is reduced by NDH using NAD(P)H produced during respiration and that respiration

![Fig. 2](image1.png)

**Notes:** (a) Growth was monitored as an optical density at 750 nm (OD750). (b) Chl contents in BG-11 medium of WT and ΔGlgP. Open circles, WT grown under light–dark cycle; open triangles, ΔGlgP grown under light–dark cycle; closed circles, WT grown under continuous light; closed triangles, ΔGlgP grown under continuous light. Data are means ± SD of three independent experiments.

![Fig. 3](image2.png)

**Notes:** The cells were in the logarithmic growth phase of the growth under light–dark cycle (OD750 = 2–5). The reaction mixture contained the cells (10 μg Chl mL⁻¹). Light gray bars, WT; dark gray bars, ΔGlgP; black bars, ΔGlgP with D-glucose. Data are means ± SD of three independent experiments. Differences between WT and ΔGlgP were analyzed by Student’s t-test. Asterisks indicate statistically significant differences compared WT with ΔGlgP at *p < 0.05.

|                  | OD₇₅₀/Chl (L mg⁻¹) | N/Chl (mg mg⁻¹) | Glycogen/Chl (mg mg⁻¹) |
|------------------|--------------------|-----------------|------------------------|
|                  | The end of light phase | The end of dark phase | The end of light phase | The end of dark phase | The end of light phase | The end of dark phase |
| WT (light/dark)  | 0.39 ± 0.04      | 0.37 ± 0.01    | 10.4 ± 0.6             | 10.7 ± 0.4             | 10.7 ± 0.4             | 5.4 ± 1.0               |
| ΔGlgP (light/dark) | 0.59 ± 0.09  | 0.69 ± 0.08    | 10.7 ± 0.1             | 10.9 ± 0.4             | 20.1 ± 0.9             | 22.6 ± 3.0               |
| WT (cont–light)  | 0.53 ± 0.11      | 11.8 ± 2.5     | 9.7 ± 0.1              | 18.4 ± 1.4             |                      |                      |
| ΔGlgP (cont–light) | 0.57 ± 0.001 | 17.2 ± 7.8    |                       |                      |                      |                      |

**Notes:** We used the cells in logarithmic growth phases (OD₇₅₀ = 2–5). WT and ΔGlgP were grown in two growth conditions, the light–dark cycle (light/dark) and the continuous light (cont–light, see Materials and methods). Data are means ± SD of three independent experiments.
The rate of P700+ reduction does not depend on DCMU. In the absence of DCMU, the amount of P700+ decreased during the MT flash, suggesting that the P700 oxidation rate by the MT flash was less than the reduction rate of P700+ by electrons from photoexcited PSII. A decrease in P700+ was not observed during MT flash in the presence of DCMU. The decay rate of P700+ (t_{1/2}, 1–2 ms) was larger in the absence of DCMU than in the presence of DCMU. In the presence of DCMU, the t_{1/2} was about 100–200 ms, which reflected electron flow from the reduced PQ pool by NDH. NDH uses NAD(P)H as an electron donor, which is produced during respiration. Furthermore, adding 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB), which inhibits the electron flow from PQ to Cyt b_{6f}, significantly decreased the decay rate. In contrast, the steady-state amount of P700+ after illuminating ΔGlgP cells with FR light was higher in both the presence and absence of DCMU compared with that in WT cells (Fig. 4(b)). These results correspond to a preceding study, indicating that suppression of respiratory electron transport induces oxidation of the PQ pool under FR light. The decay rate of P700+ in the presence of DBMIB in ΔGlgP cells was the same as that in the presence of DCMU. These results indicate that suppressed respiration through NDH was reflected as slower donation of electrons to PQ in thylakoid membranes. Similarly to the O_{2}-uptake rate, the slower donation rate of electrons through NDH to PQ in the thylakoid membranes in ΔGlgP was complemented by the 5-min incubation with d-glucose (Fig. 4(c)). In the presence of DCMU, the decay rate of P700+ in ΔGlgP with d-glucose (t_{1/2}, 57 ± 5 ms, n = 3) was larger than that in WT (t_{1/2}, 152 ± 23 ms, n = 3), which corresponds to the O_{2}-uptake rates in these strains (Fig. 3).

ΔGlgP showed the same photosynthetic activity as WT at the steady states

The steady-state O_{2}-evolution rates of WT and ΔGlgP were the same in a wide range of light intensities (Fig. 5), indicating that suppression of respiration derived from disruption of glycogen degradation does not affect the steady-state photosynthetic activity. For this measurement, we used dark-adapted cells (5 h in dark) grown in light–dark cycle.

ΔGlgP showed prolonged induction of photosynthesis

Suppression of respiration affected the induction of photosynthesis in the dark-adapted cells (5 h in dark) grown under light–dark cycle (Fig. 6). Upon illumination (red light, 180 μmol photons m^{-2} s^{-1}), the O_{2}-evolution rate in WT increased rapidly and reached a steady-state value of approximately 150 μmol O_{2} mg Chl^{-1} h^{-1}. The half-time to reach the steady state was about 30 s. In contrast, the O_{2}-evolution rate of ΔGlgP increased slowly and reached a steady state about the same rate as that of WT. Half-time was approximately 120 s, which was about fourfold longer than that of WT. These data show that the induction of photosynthesis in ΔGlgP was delayed compared with photosynthesis in WT. Next, we measured the
Fig. 6. O₂-evolution rates in WT, ΔGlgP, and ΔGlgP with D-glucose during the induction phase of photosynthesis.

Notes: The cells were in the logarithmic growth phase of growth under continuous light (OD750 = 2 ± 5). The reaction mixture contained the cells (10 μg Chl mL⁻¹) and 10 mM NaHCO₃. Open circles, WT cells; open triangles, ΔGlgP cells; open diamond, ΔGlgP with D-glucose. Data are means ± SD of six independent experiments. Light intensity used is 180 μmol photons m⁻² s⁻¹ (red light).

O₂-evolution of ΔGlgP after 5-min incubation with D-glucose under dark (Fig. 6). Interestingly, the induction of photosynthesis of the ΔGlgP was similar to that of WT.

To confirm the relationship between respiration and induction of photosynthesis, we measured the O₂-evolution rates in WT and ΔGlgP grown under continuous light. The cells were rapidly harvested just before the measurements not to be exposed to dark for a long time. ΔGlgP grown under continuous light showed the rapid induction of photosynthesis, similarly to WT (Fig. 7(a)). We transferred the cells under continuous light to dark conditions and measured the O₂-evolution rates after 8-h dark incubation. Dark-adapted ΔGlgP cells showed the prolonged induction of photosynthesis (Fig. 7(b)).

Metabolic profile of glycolysis and Calvin cycle intermediates

Cyanobacteria have no organelles for sub-localization of each metabolic pathway; thus, several organic acids are shared between the glycolysis and the Calvin cycle. We extracted and identified the intracellular metabolites in WT, ΔGlgP, and ΔGlgP after 10-min incubation with D-glucose during the dark period to elucidate why ΔGlgP showed a slow induction of photosynthesis. Dark-adapted cells (5 h in dark) were used for this experiment. The glycolysis and the Calvin cycle intermediates were identified by capillary electrophoresis–mass spectrometry (CE/MS). Metabolite levels not only of the glycolysis but also of the Calvin cycle decreased in ΔGlgP compared with those in WT (180 μmol photons m⁻² s⁻¹, fluorescent lamp)/(23 °C, 12 h, dark, see “Materials and methods”). The amount of glycogen in ΔGlgP was two times higher than that in WT, but no difference was observed in growth (Fig. 10).

Glycogen accumulates in ΔGlgP under atmospheric CO₂ condition

We found that ΔGlgP showed no activity of GlgP and glycogen accumulation, particularly at the end of dark phase (Table 1). In this study, we evaluated the accumulation of glycogen in ΔGlgP growing under ambient air condition (25 °C, 12 h, 100 μmol photons m⁻² s⁻¹, fluorescent lamp)/(23 °C, 12 h, dark, see “Materials and methods”). The amount of glycogen in ΔGlgP was two times higher than that in WT, but no difference was observed in growth (Fig. 10).

Discussion

We researched the effect of respiration on photosynthesis in the cyanobacterium S. 6803. We constructed a mutant that was deficient in two glgP homologs, sll1356 and slr1367, to inhibit glycogen degradation. The dark respiration rate in ΔGlgP was significantly lower, and the glycogen in ΔGlgP much accumulated, compared to those in WT (Fig. 3 and Table 1). ΔGlgP showed suppressed reduction of P700⁺ (Fig. 4). Furthermore, ΔGlgP showed a slower induction of photosynthesis compared with that in WT (Figs. 6 and 7). WT and ΔGlgP metabolic profiles suggested that the slower induction of photosynthesis in ΔGlgP was due
to the limited supply of the intermediates of the Calvin cycle in the dark (Fig. 8). These results indicate that dark respiration supplies the Calvin cycle intermediates, which makes it possible that photosynthesis rapidly starts to fix CO₂.

The present results are the first evidence that degradation of glycogen by GlgP was coupled with respiratory O₂-uptake. The dark respiration rate of ΔGlgP, which was 25% of that of WT, suggested that S. 6803 consumed mainly glycogen as a respiratory substrate; glycolysis was regulated by GlgP activity. Higher plants such as Arabidopsis thaliana use mainly starch as the respiratory substrate. In Arabidopsis, plastidial α-glucan phosphorylase, the apparent ortholog of GlgP, is not required for starch degradation. The activity of GlgP would not be inherited from cyanobacteria to higher plants due to the shift of storage polysaccharide from glycogen to starch. S. 6803 has two glycogen degradation pathways such as GlgP and glycogen isomerase (GlgX). The residual respiratory activity of ΔGlgP might be due to GlgX-dependent degradation of glycogen and other respiratory pathways that depend on sources other than glycogen.

Reduction of P700⁺ in the presence of DCMU reflected the respiration rate of S. 6803. In S. 6803 WT, DCMU delayed the reduction of P700⁺ (Fig. 4(a)). Half-time of the decay of P700⁺ was about 150 ms, which gave a P700 turnover number of 4.6 ± 0.8 s⁻¹ (n = 3). In contrast, the turnover number was 340 ± 20 s⁻¹ (n = 3) in the absence of DCMU. Assuming that light intensity of MT was saturated against photosynthesis, the maximum photosynthetic O₂-evolution rate would be more than 350 μmol O₂ mg Chl⁻¹ h⁻¹ (Fig. 5). The subsequent respiration rate would be estimated to be about 5 (350 × 4.6/340) μmol O₂ mg Chl⁻¹ h⁻¹, which was closer to the respiration rate (Fig. 3). However, we could not determine the P700 turnover number for ΔGlgP because the P700⁺ decay rate in the presence of DCMU was almost the same as that in the presence of DBMIB, which was due to oxidation of the PQ pool in ΔGlgP.
These results show that the donation rate of electrons to PQ by NDH in the presence of DCMU reflects the respiration rate. Furthermore, the P700 oxidation also reflected the PQ reduction rate by NDH in respiration.

Rapid induction of photosynthesis requires chemical energy compounds in photosynthetic electron transport (NADPH and ATP) and Calvin cycle intermediates. Calvin cycle intermediates were shared with those in glycolysis in cyanobacteria. ΔGlGP showed prolonged induction of photosynthesis (Figs. 6 and 7), and the amount of intermediates was significantly lower in ΔGlGP compared with that in WT (Fig. 8). But, NADP⁺ content was almost the same in WT and ΔGlGP (Fig. 9). Furthermore, adenylate levels of ATP and ADP were almost the same between them (Fig. 9). The AMP level was higher in ΔGlGP than that in WT. The lower contents of Calvin cycle intermediates were the reason for the delayed start of photosynthesis in ΔGlGP. The accumulation of the intermediates in ΔGlGP would take time before photosynthesis would start because the primary photosynthetic product of the Rubisco-catalyzed reaction, 3-phosphoglycerate (3-PGA), would be used to regenerate RuBP. These results suggest that glycolysis intermediates produced during respiration in the dark will be used for RuBP regeneration in the Calvin cycle during the induction phase of photosynthesis. In fact, addition of D-glucose enhanced the increase in dark respiration rate and accelerated the induction of photosynthesis. RuBP was undetectable in both WT and ΔGlGP maintained in the dark, indicating that the amount of Calvin cycle intermediates was kept low during the dark reactions. Until O₂-evolution rates reach a steady-state value, these intermediates are supplied by photosynthetic CO₂ assimilation to drive the induction of photosynthesis. The tobacco mutant, CMSII, also showed suppressed respiratory activity and prolonged induction of photosynthesis.⁴,⁵ In higher plants, the glycolysis and the Calvin cycle separately function in the cytosol and chloroplast, respectively. The mechanism of slow induction of photosynthesis in the CMSII mutant might be different from that in ΔGlGP.

N assimilation functioned with glycogen-dependent respiration in S. 6803. N content in ΔGlGP was lower

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**Fig. 9.** The amounts of ATP, ADP, AMP, and NADP⁺ in WT, ΔGlGP, and ΔGlGP with D-glucose in the dark.

Notes: The cells were in the logarithmic growth phase of growth under light-dark cycle (OD₅₅₀ = 2–5). Light gray bars, WT; dark gray bars, ΔGlGP; black bars, ΔGlGP with D-glucose. Data are means ± SD of four independent experiments. Differences between WT and ΔGlGP were analyzed by Student’s t-test. Asterisks indicate statistically significant differences compared WT with ΔGlGP at *p < 0.05.

**Fig. 10.** Growth and the amount of glycogen in WT and ΔGlGP grown under light-dark cycle with atmospheric CO₂ concentration. Open circles, WT cells; open triangle, ΔGlGP cells.

Note: Data are means ± SD of three independent experiments.
than that in WT under light-dark cycle (Table 1). N assimilation is coupled with the glutamine synthetase-glutamate synthase pathway, which acquires a carbon skeleton in the form of 2-oxoglutarate (OGT). OGT is produced from 3-PGA in a cooperative manner between the cytosol and mitochondria in higher plants. 3-PGA is supplied by glycolysis and photosynthesis. Cyanobacteria appear to have a unique tricarboxylic acid (TCA) cycle, where 2-oxoglutarate decarboxylase, which is found in the mitochondria of higher plants, is deficient, and two alternative enzymes such as 2-oxoglutarate decarboxylase and succinic semialdehyde dehydrogenase function in the TCA cycle.

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