Carbohydrate Metabolism in Mutants of the Cyanobacterium *Synechococcus elongatus* PCC 7942 Defective in Glycogen Synthesis

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ADP-glucose pyrophosphorylase (AGPase) and glycogen synthase (GS) catalyze the first two reactions of glycogen synthesis in cyanobacteria. Mutants defective in each of these enzymes in *Synechococcus elongatus* PCC 7942 were constructed and characterized. Activities of the corresponding enzymes in the selected mutants were virtually undetectable, and their ability to synthesize glycogen was entirely abolished. The maximal activities of photosynthetic O2 evolution and the rates of respiration in the dark were significantly decreased in the mutants compared to those in wild-type cells. Addition of 0.2 M NaCl or 3 mM H2O2 to liquid cultures markedly inhibited the growth of the AGPase and GS mutants, while the same treatment had only marginal effects on the wild type. These results suggest a significant role for storage polysaccharides in tolerance to salt or oxidative stress.

Cyanobacteria are oxygenic photosynthetic prokaryotes and important biomass producers that are widespread in diverse environments, including freshwater, oceanic, and terrestrial habitats (39). Photosynthetic carbon assimilation in cyanobacteria results in the accumulation of polysaccharides, mostly glycogen (22), which is synthesized by the sequential actions of ADP-glucose pyrophosphorylase (AGPase, EC 2.7.7.27), glycogen synthase (GS, EC 2.4.1.21), and branching enzyme (BE, EC 2.4.1.18) (28). Although the accumulation of storage polysaccharides as intracellular inclusions has been extensively described (32, 33), its physiological significance has not been thoroughly investigated.

It is likely that glycogen in cyanobacteria has a physiological function for adaptation to an unfavorable environment (28). To study the role of storage polysaccharides, we constructed mutants of *Synechococcus elongatus* PCC 7942 defective in glycogen production by disrupting the structural genes coding for AGPase and GS. *S. elongatus* PCC 7942 is a unicellular, obligately phototrophic cyanobacterium, and its genomic sequence is available (accession number NC_007604) (10). The number of genes coding for enzymes in the glycogen biosynthesis pathway is variable among cyanobacterial species, but *S. elongatus* PCC 7942 is one of the simplest examples in that it has just one of each gene, as opposed to *Synechocystis* sp. PCC 6803 (accession number NC_000911) or *Nostoc* (Anabaena) sp. PCC 7120 (accession number NC_003272). (Both organisms have one gene for AGPase but two genes for GS.) It was therefore expected that mutants with the definite phenotype could be obtained through a single mutagenesis manipulation, avoiding possible complementary functions of paralogous genes.

The cyanobacterial glycogen biosynthesis mutants could also provide opportunities to study the mechanism of starch synthesis in plants. Based on a number of experimental observations, it is now thought that a significant proportion of the glycogen biosynthesis system in cyanobacteria is responsible for the evolution of starch biosynthesis in plants (3, 22, 26). Notable similarities have been found in the enzymatic system and its regulation between glycogen synthesis in cyanobacteria and starch synthesis in plants (2). The cyanobacterial mutants will therefore serve as hosts for the expression of heterologous AGPase and starch synthase (SS) derived from plants to examine their in vivo specificity in the absence of otherwise co-existing isozymes.

As an initial characterization, the effect of mutation on the activities of the other enzymes of the glycogen synthesis pathway was examined. Photosynthetic activities of the mutants were determined at various light intensities. Growth rates were also compared between the wild-type (WT) and mutant strains under salt and oxidative stresses due to the presence of 0.2 M NaCl and 3 mM H2O2, respectively. The role of storage polysaccharides in cyanobacteria under environmental stresses is discussed.

MATERIALS AND METHODS

**Culture conditions.** Cells of *S. elongatus* PCC 7942 were grown in modified BG-11 medium at 30°C under continuous illumination at 50 μmol m−2 s−1, as described previously (36). Salt stress was applied by adding 0.88 g of solid NaCl to 75 ml of the liquid culture (final concentration of 0.2 M) (17, 30), followed by prompt agitation until thorough dissolution. For oxidative stress, 25 μl of 30% H2O2 was added to 75 ml of the liquid culture (final concentration of 3 mM) (27).

**Gene disruption.** Total DNA was extracted from the cyanobacterial cells according to the method described by Golden et al. (6). The genomic regions flanking the AGPase gene (gclC) were amplified by PCR using two pairs of oligonucleotide primers, 5′-TGAGGCAGAAGCCTAAGCATTAG-3′ (839B) and 5′-CTCGAGCAGGCTAAACGGGCTAG-3′ (840B) for the upstream

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region and 5’-CTCGAGGTTGTCGAAGGCGGCTTAT-3’ (841B) and 5’-A
TCTTATTATGTTAGTGTGTTG-3’ (842B) for the downstream region (the
XhoI site is underlined). Two DNA fragments (889 and 894 bp for the
upstream and downstream regions, respectively) were independently cloned and then joined on the plasmid pGEM-T Easy (Promega). The recombinant plasmid was linearized by digestion with the unique XhoI site and ligated with the blasticidin S resistance gene (bsd) derived from pEM7/bsd (Invitrogen). The resulting plasmid, pAGEC/bsd, contained the genomic region in which nearly the entire coding sequence of the AGPase gene was replaced with bsd.

The GS gene (glnG) was amplified with PCR primers 5’-CATATGCGGATT
CTGTTGCTG-3’ (761B) and 5’-ACCAACGGCCACCGTACTG-3’ (762B).
The DNA fragment obtained (1,480 bp) was cloned into pGEM-T Easy. The recombinant plasmid was then linearized by digestion with EcoRV within the coding region and ligated with the chloramphenicol resistance gene (cat) derived from pHSG396 (Takara Bio Inc.). The resulting plasmid was designated pAGEC/ cat.

The DNA sequences of the plasmids were confirmed using an ABI PRISM 3100 genetic analyzer (Applied Biosystems).

Transformation of S. elongatus PCC 7942 was carried out using plasmids pAGEC/bsd and pAGEC/cat by the standard procedure (6). The transformants were selected on 1% agar plates of BG-11 medium containing 10 µg/ml blasticidin S (Invitrogen) or 10 µg/ml chloramphenicol (Sigma).

Insertion of the antibiotic resistance cassette at the targeted site was confirmed by PCR using total DNA extracted from the transformants as the template. Replacement of glnC with bsd was verified with PCR primers 5’-TCTCAGCG
CGCTTGACTG-3’ (849B) and 5’-AATAACCGCCATTTGACA-3’ (850B). Insertion of cat into glnG was confirmed with PCR primers 761B (see above) and 5’-ATATCGACCTGACAGGAG-3’ (794B).

Enzyme assay. Enzyme activities were determined with crude extracts that were prepared by disrupting the cells with a French pressure cell as described (23). The reaction mixture consisted of 50 mM HEPES-NaOH (pH 7.5), 2 mM ADP-glucose, 2.4 mM Na pyrophosphate, 1 mM 3-phosphoglycerate, 5 mM MgCl2, 4 mM dithiothreitol (DTT), and enzyme extract (containing 50 µg protein) in a total volume of 400 µl. The reaction, initiated by the addition of the extract, was carried out at 30°C for 20 min and then stopped by heating at 100°C in a water bath for 2 min. After centrifugation, 300 µl of the supernatant was removed and mixed with an equal volume of 0.33 mM NADP+.

Enzymatic activity was measured as an increase in A340 after the addition of 0.2 µl of phosphoglucocinase (Roche) and 1 U of glucose-6-phosphate dehydrogenase (Roche). The extinction coefficient of NADPH at 340 nm (6.22 mol
-1 cm-1) was used for the calculation.

GS activity was determined by the modified method of Nishi et al. (24). The assay was carried out at 30°C in a reaction medium that consisted of 50 mM Tris
HCl (pH 8.0), 20 mM DTT, 2 mM ADP-glucose, 2 mg/ml oyster glycogen (type II; Sigma), and the crude enzyme extract in a reaction volume of 300 µl. The reaction was started by the addition of the extract and the mixture was incubated for 20 min. The reaction was stopped by heating at 100°C in a water bath for 2 min. After centrifugation, 300 µl of the supernatant was removed and mixed with an equal volume of 0.33 mM NADP+.

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-1 cm-1) was used for the calculation.

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Enzymatic activity was measured as an increase in A340 after the addition of 0.2 U of phosphoglucinase (Roche) and 1 U of glucose-6-phosphate dehydrogenase (Roche). The extinction coefficient of NADPH at 340 nm (6.22 mol
-1 cm-1) was used for the calculation.

Growth rates and activities of photosynthesis and respiration in the dark. Growth rates of S. elongatus PCC 7942 cells in liquid cultures were determined by measuring the A330 of the cell suspension. Activities of photosynthetic oxygen evolution in the cyanobacteria were determined by a Clark-type oxygen electrode (Rank Bros. Ltd., Bottonham, UK). The cells were suspended in 50 mM Tricine-KOH (pH 7.5) at a concentration of 5 µg Chl ml
-1 in the presence of 10 mM NaHCO3 and kept at 30°C. Light was provided by a halogen lamp (Iwasaki Electric Co., Ltd., Tokyo, Japan) at various intensities. A measurement at a constant light intensity was carried out for at least 5 min to ensure the linearity of the slope. Respiration in the dark was measured under the same conditions, except that the cell suspension was placed in complete darkness.

Hydrogen evolution. The activities of light-dependent hydrogen evolution were measured by the method of Gutthann et al. (7) using a Clark-type oxygen electrode which was inversely connected so that the platinum electrode was polarized at +600 mV relative to the Ag/AgCl electrode. A cell suspension containing 100 µg Chl ml
-1 was incubated with 40 U of glucose oxidase–50 U of catalase–1 mM glucose for 15 min at 30°C in darkness. After the completion of fermentative hydrogen production, the suspension was illuminated at 800 µmol
m
-2 s
-1 to induce photohydrogen production. For comparison, Synchocystis sp. PCC 6803 was grown and subjected to the assay under the same conditions.

Carbohydrate extraction and analysis. S. elongatus PCC 7942 cells were grown in liquid culture to an A330 of approximately 2.0, and aliquots (1.5 ml) were removed at intervals for the determination of glycogen and sucrose accumulation in the cells. Cells were collected by centrifugation at 10,000 × g for 5 min, resuspended with 1.5 ml of absolute methanol, and kept at −20°C for 24 h. After complete methanolization, the pellet and the supernatant were dissolved in 667 µl of 1 M sulphuric acid, and the dried supernatant was dissolved in 667 µl of 1 M sulphuric acid. The dried pellet was resuspended in 1 ml of distilled water and incubated at 100°C for 40 min. A portion (200 µl) of the suspension was mixed with 100 µl of 2.5 mM Na-acetate (pH 5) containing 0.5 mg/ml glucamylase (from Rhizopus niveus; Seikagaku Kogyo) and incubated at 40°C for 1 h. After centrifugation at 10,000 × g for 5 min, 150 µl of the supernatant was mixed with 375 µl of distilled water and 105 µl of a reaction medium (S1 solution) containing 400 mM HEPES-NaOH (pH 7.5), 10 mM MgSO4, 3 mM NADP+, 10 mM ATP, 3 U of hexokinase (from yeast; Roche), and 2 U of glucose-6-phosphate dehydrogenase (from yeast; Roche). The amount of glucose moieties derived from glycogen was determined as the increase in A340.

The dried pellet was resuspended in 1 ml of distilled water and incubated at 100°C for 40 min. A portion (200 µl) of the suspension was mixed with 100 µl of 2.5 mM Na-acetate (pH 5) containing 0.5 mg/ml glucamylase (from Rhizopus niveus; Seikagaku Kogyo) and incubated at 40°C for 1 h. After centrifugation at 10,000 × g for 5 min, 150 µl of the supernatant was mixed with 375 µl of distilled water and 105 µl of a reaction medium (S1 solution) containing 400 mM HEPES-NaOH (pH 7.5), 10 mM MgSO4, 3 mM NADP+, 10 mM ATP, 3 U of hexokinase (from yeast; Roche), and 2 U of glucose-6-phosphate dehydrogenase (from yeast; Roche). The amount of glucose moieties derived from glycogen was determined as the increase in A340.

RESULTS AND DISCUSSION

Construction of mutants. Genes coding for AGPase (glnC, Synpcc7942_0603) and GS (glnJ, Synpcc7942_2518) in S. elong-
the PCR primers described in Materials and Methods were extracted and examined by PCR for gene replacement. When functional terminator or stream genes, we used antibiotic markers without a transcribing polycistronic units. To preserve the activity of the downstream open reading frame of unknown function, apparently constitutive AGPase and GS genes are immediately followed by another and exogenous genes from occurring.

On the chromosome of *S. elongatus* PCC 7942, both the AGPase and GS genes are in the same operon (25), these genes were distantly located on the chromosome of *S. elongatus* PCC 7942 and were therefore inactivated independently. We used different antibiotic resistance markers (bsd and cat) for disruption of the two genes, leaving the opportunity to construct double mutants in future studies.

We also adopted different procedures for the disruption of the two genes; nearly the entire coding region of AGPase gene was removed and replaced with *bsd*, while *cat* was simply inserted at an EcoRV restriction site in the GS gene. We chose a much more tedious procedure for mutation of the AGPase gene, intending to make a host for heterologous expression of AGPase from plants (in future work). As the conservation of the sequence of the AGPase gene between different organisms is much more substantial than that of the GS/SS gene, deletion of the coding sequence would prevent any possible recombination event (through sequence similarity) between intrinsic and exogenous genes from occurring.

On the chromosome of *S. elongatus* PCC 7942, both the AGPase and GS genes are immediately followed by another open reading frame of unknown function, apparently constituting polycistronic units. To preserve the activity of the downstream genes, we used antibiotic markers without a transcriptional terminator or Ω element.

Antibiotic-resistant colonies were grown, and total DNA was extracted and examined by PCR for gene replacement. When the PCR primers described in Materials and Methods were used, a DNA fragment of 1.3 kb was amplified from the AGPase gene of the WT strain. In contrast, the DNA fragment found in the WT was replaced with a 0.6-kb DNA fragment from a blasticidin S-resistant transformant (AGP5). A portion of the GS gene was amplified as a 0.8-kb DNA fragment from the WT strain, while it was replaced with a 1.8-kb fragment in two independent chloramphenicol-resistant transformants (GS1 and GS2). We concluded that the WT alleles of these genes were completely removed from the transformants. After the establishment of the gene disruption, the mutant strains were maintained without the selective antibiotics.

**Enzymatic activities for glycogen synthesis.** The activities of enzymes involved in glycogen biosynthesis were measured in the crude extracts of WT and mutant *S. elongatus* strain PCC 7942. Figure 1A shows that the AGPase activity in the crude extract of the WT was detected on the gel as a single band within the background level in GS mutant. In the AGPase mutant, the GS activity was decreased to 1/5 of that in the WT. The BE activity of *S. elongatus* was 32 (nmol min⁻¹ mg protein⁻¹), while it was undetectable in the AGPase mutant. In contrast, the DNA fragment found in the WT was replaced with a 0.6-kb DNA fragment from a blasticidin S-resistant transformant (AGP5). A portion of the GS gene was amplified as a 0.8-kb DNA fragment from the WT strain, while it was replaced with a 1.8-kb fragment in two independent chloramphenicol-resistant transformants (GS1 and GS2). We concluded that the WT alleles of these genes were completely removed from the transformants. After the establishment of the gene disruption, the mutant strains were maintained without the selective antibiotics.

**Figure 1.** Enzymatic activities in crude extracts of WT and mutant *S. elongatus* strain PCC 7942. (A) AGPase activity. (B) GS activity. Extracts containing 50 μg of protein were used for the assay. The data shown for each strain are averages of three independent measurements, and standard deviation bars are shown.

**Figure 2.** Enzymatic activity of BE and protein composition in crude extracts of WT and mutant *S. elongatus* strain PCC 7942. (A) The extracts of the cyanobacterial strains (containing 6.7 μg of protein) were run on a nondenaturing polyacrylamide gel along with an extract of immature rice grains (containing 2.2 μg of protein) as a reference. The gel was then subjected to BE activity staining as described in the text. An arrowhead indicates the mobility of the band corresponding to the BE activity of *S. elongatus* PCC 7942. The migration of BE isoforms in rice endosperm (BEI, BEIIa, and BEIIb) is also indicated. The activity of the BEI isoform is visible as a characteristic smeary band (40). (B) Composition of soluble proteins (upper panel) and immunological detection of the photosystem II D1 protein (lower panel) in crude extracts of *S. elongatus* PCC 7942. Crude extract containing 40 μg of protein was loaded onto each lane. The bands indicated by the characters α and β are α- and β-phycocyanin, respectively. The D1 protein was detected using an antiserum raised against the D1 protein of spinach as described in the text.

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bicarbonate-dependent $O_2$ evolution with an oxygen electrode, activities in the WT and mutant strains were measured as

immunoblot detection analysis of the D1 protein, a core sub-
dominantly to photosystem II. To determine whether the level
fore concluded that the activity of BE was specifically de-
as a consequence of increased phycocyanin content. We there-
change in phycocyanin content could disturb the
in this organism, were therefore compared between the WT
of phycocyanin and $\text{Chl}_a$ contents were increased, compared to those in the
and mutant strains (Table 1). In both of the mutants, the
in the WT.

**Composition of pigments and proteins.** Both the AGPase
and GS mutants exhibited a pale blue-green color, suggesting
an altered composition of their pigments. The cellular contents
of phycocyanin and $\text{Chl}_a$, the major photosynthetic pigments
in this organism, were therefore compared between the WT
and mutant strains (Table 1). In both of the mutants, the
phycocyanin contents were increased, compared to those in the
WT, while the $\text{Chl}_a$ contents were comparable. Consequently,
the phycocyanin/$\text{Chl}_a$ ratio was consistently higher in the
mutants. An increased level of phycocyanin was visualized
when the soluble protein was resolved by SDS-PAGE (Fig.
2B). The change in phycocyanin content could disturb the
equality of the amount of the other proteins (e.g., BE shown in
Fig. 2A) loaded on each lane. However, the substantial reduction
in the band intensity of BE could not be explained solely
as a consequence of increased phycocyanin content. We there-
fore concluded that the activity of BE was specifically de-
creased as an enzyme involved in glycogen metabolism.

Light energy absorbed by phycobilisomes is transferred pre-
dominantly to photosystem II. To determine whether the level
of photosystem II is altered in the mutants, we carried out
immunoblot detection analysis of the D1 protein, a core sub-
unit of photosystem II. As shown in Fig. 2B, the amount of D1
protein was not significantly different between the WT and
mutant strains.

**Activities of photosynthesis and respiration.** Photosynthetic
activities in the WT and mutant strains were measured as bicarbonate-dependent $O_2$ evolution with an oxygen electrode at various light intensities. The activity in the WT showed a hyperbolic curve, increasing gradually at high light intensities of up to 500 $\mu$mol m$^{-2}$ s$^{-1}$, and it exceeded 200 $\mu$mol mg Chl$^{-1}$ h$^{-1}$ at a saturating light intensity (Fig. 3). In contrast, the activities in the mutants were saturated at 100 $\mu$mol m$^{-2}$ s$^{-1}$ and the maximum activities in AGPase and GS mutants were approximately 1/3 and 1/4, respectively, of that observed in the WT.

Figure 3 also shows that the activities of respiration in the
mutants placed in darkness were reduced to 1/2 of that in the
WT. This result suggests that glycogen serves as a major form
of respiratory substrate in darkness.

The decreased activity of photosynthetic $O_2$ evolution would be related to a limited capacity to consume reducing equivalents due to the defect in glycogen synthesis. We have shown that the level of photosystem II protein was not altered (Fig.
2B) while that of antenna pigments (phycocyanin) for photo-
system II was increased (Table 1; Fig. 2B) in the mutants. A
possible explanation for these observations is that the efficiency
of energy transfer from the antenna pigments to the reaction
center is impaired in the mutants.

In the absence of adequate consumption of reducing equiv-
ulents through glycogen synthesis, one of the candidates for
alternative electron acceptors is $H^+$, leading to $H_2$ production.
The capacity for light-dependent, anaerobic $H_2$ evolution was therefore measured according to the method described by Gut-
theory et al. (7) with the WT and mutant forms of $S. elongatus$
PCC 7942. The activity in Synechocystis PCC 6803 was also
determined for comparison (7). While a limited but definite
amount of $H_2$ evolution was observed with WT Synechocystis
PCC 6803, no appreciable activity was detectable with $S. elonga-
gus$ strain PCC 7942 under the same conditions (data not
shown). The relative activity of hydrogenase in $S. elongatus$
PCC 7942 may be much lower, and/or other compound(s) may
be primarily responsible for the consumption of reducing

equivalents.

**Carbohydrate contents and responses to salt and oxidative
stresses.** The carbohydrate contents of the WT and mutant
strains were compared during their growth in liquid culture
under continuous light. Growth rates were determined by
measuring the $A_{730}$ of cell suspensions. A cell suspension
with an $A_{730}$ of 1.0 contained $1.1 \times 10^8 \pm 0.1 \times 10^8$ cells
ml$^{-1}$ (average $\pm$ standard deviation; $n = 10$), and no ap-
preciable difference was observed between the values of WT
and mutant cells. In WT cells, the amount of glycogen in-
creased steadily as the cell density increased (Fig. 4A and B),
indicating that a constant amount of polysaccharide was accu-

![FIG. 3. Oxygen evolution by the cells of WT and mutant $S. elongatus$ strain PCC 7942 under illumination at various light intensities. Negative values observed in darkness indicate oxygen consumption. The data shown for each strain are averages of three independent measurements, and standard deviation bars are shown.](http://aem.asm.org/)
mulated during growth under continuous illumination. Figure 4C and E show that the growth rates of AGPase and GS mutants were much lower than that of the WT. In these mutants, glycogen was hardly detectable throughout the experiment (Fig. 4D and F).

The effect of salt stress (addition of 0.2 M NaCl) on the WT and mutant strains was examined next. Although *S. elongatus* is not tolerant to high salt concentrations, it has been reported that sucrose synthesis is induced in moderate salinity (17, 30). It was therefore possible that a significant modulation of carbohydrate metabolism takes place under salt stress. In the presence of 0.2 M NaCl, the A730 of the culture of WT cells increased at a rate comparable to that in the culture without NaCl (Fig. 4A). Addition of 0.2 M NaCl to the culture did not cause a significant change in the glycogen content of WT cells (Fig. 4B). When cells were grown in standard medium without NaCl, sucrose was undetectable. After the addition of NaCl, sucrose rapidly accumulated in the cells in 8 h. Intracellular sucrose attained a steady level (18 μg ml⁻¹ A730⁻¹) and remained constant for at least 2 days. The amount of sucrose was expressed conventionally on a culture volume basis. Assuming that the cell volume to Chl α ratio of this organism is 60 μl mg⁻¹ Chl (29), the intracellular sucrose concentration is estimated to be approximately 0.28 M. If accumulation of sucrose is confined to the cytoplasmic space (excluding the thylakoid lumen), the value would be much higher but still within a physiologically plausible range. Since the fluctuation of the glycogen content upon the addition of NaCl was rather small, a major fraction of the carbon used to synthesize sucrose should be derived from *de novo* CO₂ fixation through the Calvin-Benson cycle. Compared to the WT, the most notable effect of NaCl treatment on the mutants was the substantial reduction of the growth rates (Fig. 4C and E). Addition of NaCl led to the synthesis of sucrose in these cells, as in the WT (Fig. 4D and F). As the ability to synthesize glycogen was lost in these mutants, sucrose should be synthesized solely through *de novo* assimilation of CO₂ instead of conversion from polysaccharide.

The marked inhibition of the growth of the mutants by NaCl raised the possibility that these mutants are susceptible to environmental stress. To see if the sensitivity (growth inhibition) of the mutants is a general effect under environmental stresses, growth rates were measured in the presence of 3 mM H₂O₂. Figure 5A shows that the addition of hydrogen peroxide to a liquid culture hardly affected the growth of the WT. The glycogen content did not show a significant change after the addition of H₂O₂ (Fig. 5B). In contrast to the WT, a severe inhibition of the growth of AGPase and GS mutants during oxidative stress was observed (Fig. 5C and D).

Under salt and osmotic stresses, sucrose is responsible for the protective functions as a compatible solute (9). In addition to the synthesis of the compatible solute(s), other responsive
and adaptive processes are also induced under the salt and osmotic stresses. These include exclusion of Na\(^+\) ion by Na\(^+\)/H\(^+\) antiporter (4, 13, 31, 38) and synthesis of stress-responsive proteins, including enzymes for the production of compatible solutes, heat shock proteins, and enzymes acting on reactive oxygen species (5, 15, 19). Salt and oxidative stresses are therefore causally related in the cells of cyanobacteria, as supported by much experimental evidence (16). The stress response processes described above induce a high demand for ATP synthesis. A considerable proportion of the glycolate that was transiently degraded in the WT upon the addition of NaCl or H\(_2\)O\(_2\) would be responsible for the production of ATP. As supporting evidence, it has been reported that the activities and expression of photosystem I and cytochrome c oxidase were enhanced during salt stress in *Synechocystis* (14). It is plausible that glycolate serves as a substrate for respiration through cytochrome c oxidase. Because of the deficiency of glycolate synthesis and accumulation, AGPase and GS mutants would be unable to synthesize a sufficient amount of ATP to fulfill their cellular needs. These mutants were thus incapable of adapting to salt and oxidative stresses and consequently showed growth inhibition.

**Concluding remarks.** Mutants defective in glycerogen/starch biosynthesis due to the lesion in AGPase or GS (SS) have been characterized in a number of photosynthetic organisms. An AGPase mutant of the cyanobacterium *Synechocystis* PCC 6803 has been isolated (20, 21). In contrast to AGPases in plants and eukaryotic algae, the enzyme in cyanobacteria is a homotetramer (11) and is encoded by a single gene. In *Synechocystis* PCC 6803, however, ADP-glucose serves as the precursor for both glycolate and the primary compatible solute glucoylglycerol (8). The ability to synthesize both of these compounds was simultaneously abolished by the lesion in the AGPase gene in *Synechocystis* PCC 6803 (20). In the AGPase mutant of *Synechocystis* exposed to salt stress, sucrose was accumulated in the place of glucoylglycerol. The carbohydrate metabolism of *S. elongatus* PCC 7942 is thus much simpler than that of *Synechocystis* PCC 6803 and would be suitable for studying the role of sucrose during salt stress. The homologues of GS in plants and algae are SSs, which also show diversification into multiple isoforms. To date, the complete elimination of starch (polysaccharide) synthesis due to the lesion in SS activities has not been reported for any plant species. In conclusion, in this study, we have demonstrated the previously unexplored significance of storage polysaccharides in cyanobacteria. Glycerogen metabolism is thus physiologically important for these organisms to cope with the ever-changing environment.

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