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Aquaporin-deficient mutant of *Synechocystis* is sensitive to salt and high-light stress

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ABSTRACT

Cyanobacterial aquaporins play an important role in the regulation of various physiological functions: cell volume control, osmotic stress responses, gas exchange. We employed the AqpZ-deficient mutant of *Synechocystis* to study the role of aquaporins in responses to salt (NaCl) and high light stress. Electron microscopy and paramagnetic resonance revealed that AqpZ-deficient cells are unable to efficiently regulate the cytoplasmic volume under salt stress. Both photosystems (PSII and, especially, PSI) of these cells are more sensitive to NaCl and to high light. Thus, AqpZ of *Synechocystis* participates in regulation of the photosynthetic activity of PSI and PSII under salt and high-light stress. Our results demonstrate that AqpZ might be necessary for the repair of PSII and PSI after photodamage.

1. Introduction

The unicellular freshwater *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) belongs to the group of moderately halotolerant cyanobacteria [1] that resists up to 0.6 M NaCl, which is equivalent to seawater conditions [2]. Sodium is an essential element for the cell metabolism in cyanobacteria, however, the excess amounts of sodium cause salinity stress. Incubation of the cyanobacterial cells in 0.5 M NaCl induced a rapid and reversible decline and subsequent slow and irreversible loss of the oxygen-evolving activity of photosystem (PS) II and the electron transport activity of PSI [3]. NaCl had both osmotic and ionic effects on cyanobacterial cells.

The osmotic effect caused a rapid outflow of water from cells through water channels [4] with the corresponding rapid increase in the intracellular concentration of salts. The ionic effect was caused by an influx of Na^+ ions through K^+/Na^+ channels that also increased concentrations of salts in the cytosol and irreversibly inactivated PSI and PSII [3,4].

Fast Na^+ export is based on the activation of pre-existing Na^+/H^+ antiporters. In *Synechocystis*, six genes encode Na^+/H^+ antiporters (*sll0273*, *sll0556*, *sll0689*, *slr0415*, *slr1595*, and *slr1727*). Water flux occurs mainly through water-permeable channels, including aquaporins, simultaneously with the transport of ions across the membrane and the change in concentration of the compatible solutes synthesized *de novo* in the cells. *Synechocystis* has only one gene for the water channel, AqpZ (*slr2057*), which regulates responses of cells to hyperosmotic stress [5–7]. AqpZ is located in the cytoplasmic membrane and is involved in regulation of the glucose metabolism during photomixotrophic growth [8]. It might be possible that AqpZ is also permeable to CO_2. The physiological importance of aquaporin mediated CO_2 membrane diffusion was demonstrated for plants, cyanobacteria, and partly for mammals [9]. In land plants, aquaporin-mediated CO_2 permeation has been suggested to play physiological roles in photosynthesis [10]. A study with a water channel blocker suggested that AqpZ may participate in CO_2 uptake in cyanobacterial cells [11]. At least, AqpZ of *Synechococcus* sp. PCC 7942, expressed in *Xenopus laevis* oocytes and proteoliposome systems, in addition to water permeability, was able to transport CO_2. AqpZ-deficient cells of *Synechococcus* grew slower during the exponential phase if compared to wild-type cells [12].

Previously we demonstrated that AqpZ blocker, *p*-chloromercuriphenyl-sulfonic acid, prevented the recovery of PSII after the

Abbreviations: BQ, 1,4-benzoquinone; Chl, chlorophyll; DCBQ, 2,6-dichloro-1,4-benzoquinone; DCIP, 2,6-dichloroindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; EPR, electron paramagnetic resonance; MV, methyl viologen; PSI, photosystem I; PSII, photosystem II.

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illumination with strong light in the presence of NaCl [4]. Here we employed the AqpZ-deficient mutant of *Synechocystis* to study the separate effects of NaCl and high light on the activity of PSI and PSII. Our results show cyanobacterial AqpZ participates in regulation of photosynthetic activity of PSI and PSII under salt and high-light stress.

2. Materials and methods

2.1. Strains and growth conditions

The wild-type glucose tolerant (GT) strain of *Synechocystis* sp. PCC 6803 was used in the experiments. The AqpZ-deficient mutant of *Synechocystis* was produced as described before by the insertion of a spectinomycin-resistant gene into two *Eco*47 III sites of the *aqpZ* (*slr2057*) gene of *Synechocystis* [5]. ORF names correspond to definitions of genes in Cyanobase, http://genome.microbedb.jp/cyanobase/Synechocystis). Transformation of *Synechocystis* cells was done as described elsewhere [13]. Cells were grown at 32 °C in BG11 medium [14] under continuous illumination with light from incandescent lamps at 50 μmol photons m^{-2} s^{-1}, and aeration was done with 1% CO_2. The growth rate of cells was estimated as changes in optical density of the cell suspension at 750 nm (OD_{750}).

2.2. Stress treatments

Salt stress was provided by the addition of 5 M NaCl solution in BG11 medium to the final concentration of 0.5 M. High-light stress was provided by exposure of cells to light of 1500 μE m^{-2} s^{-1} for designated periods of time.

2.3. Electron microscopy

For electron microscopy analysis, cells were withdrawn just before the addition of 0.5 M NaCl (control, time 0) and after 15 and 60 min of salt stress. Cells were collected, fixed and embedded in Epon resin as described earlier [15], and analyzed with the transmission electron microscope (Libra-120, Carl Zeiss, Jena, Germany).

2.4. Measurement of the cytoplasmic volume

The cell volume (cytoplasmic volume) was determined by electron paramagnetic resonance (EPR) spectrometry with 2,2,6,6-tetra methyl-4-oxopiperidinooxy free radical (TEMPO; a spin probe) probe as described earlier [4,5].

2.5. Measurement of electron transport activities

Electron-transport activities of PSII and PSI in intact cells were determined at 32 °C by monitoring the light-induced evolution and uptake of oxygen, respectively, with a Clark-type oxygen electrode (Hansatech Instruments, Kings Lynn, UK). Actinic light (1500 or 2500 μE m^{-2} s^{-1} at the surface of the cuvette) was obtained by passage of light from an incandescent lamp through a red optical filter (R-60, Toshiba, Tokyo) and an infrared-absorbing filter (HA-50, Hoya Glass, Tokyo). The oxygen-evolving activity of PSII was measured in the presence of 1.0 mM 1,4-benzoquinone (BQ) and 1.0 mM 2,6-dichloro-1,4-benzoquinone (DCBQ) as artificial acceptor of electrons. The electron transport activity of PSI (light-induced uptake of oxygen) was determined in the presence of 15 μM DCMU, 5 mM sodium ascorbate, 0.1 mM 2,6-dichloroindophenol (DCIP), and 0.1 mM methyl viologen (MV) [3,4].

2.6. Measurement of chlorophyll fluorescence

The yield of Chl fluorescence from intact cells was measured with a pulse amplitude modulation fluorometer (PAM-101, Walz, Effeltrich, Germany) in the presence and absence of dithionite at 1 mg ml^{-1} [3]. The initial fluorescence of Chl (F_0) was determined after excitation with dim light at 650 nm and 10 μE m^{-2} s^{-1}, which was modulated at 600 Hz. The maximum yield of fluorescence ($F_{max} = F_0 + F_v$) was determined after the addition of continuous actinic light at 2000 μE m^{-2} s^{-1} [16]. Concentration of Chl *a* was determined as described elsewhere [17].

2.7. Quantitative gene transcription analysis

Total RNA was isolated with the hot-phenol method [18]. RNA was further purified from DNA with the DNase I (Fermentas, Vilnius, Lithuania) according to the manufacturer's protocols. Reverse transcription was performed with Superscript III (Invitrogen, Carlsbad, CA, USA), 1 μg of the isolated total RNA and the gene-specific synthetic oligonucleotides used as primers. PCR was performed with the gene-specific primers (2.5 pmol each) using the CFX-96 real-time PCR detection system and iTaq™ Universal SYBR® Green Supermix (Bio-Rad, Hercules, CA, USA). The following genes have been used as reference genes: *rnpB* gene (*slr1469*) for RNase P subunit B, *petB* (*slr0342*) for cytochrome b_6, and *secA* (*sll0616*) for protein translocase [19].

3. Results

3.1. Changes in ultrastructure of Synechocystis wild-type and AqpZ-deficient cells under NaCl stress

Under normal growth conditions wild-type and AqpZ-deficient cells of *Synechocystis* had rather similar ultrastructure, with the exception that mutant cells had thylakoids with wider interthylakoid space compared to wild type (Fig. 1a and d).

After 15 min of NaCl treatment ultrastructure of wild-type cells changed dramatically: lumen space of all thylakoids enlarged significantly, especially in most inner thylakoids, while the central cytoplasmic area with a nucleoid and carboxysomes was condensed to a small island in the center of cells. AqpZ-deficient cells remained mainly unchanged (Fig. 1b and e). They had only few inner thylakoids with increased lumen space.

Wild-type cells mostly restored their ultrastructure within 1 h of NaCl treatment, and they looked just like cells before salt stress (all their thylakoids had normal interthylakoid space), with only difference that they accumulated glycogen granules between thylakoids. After 1 h of salt stress, AqpZ-deficient cells still had several inner thylakoids with enlarged lumen space as they had after 15 min treatment with NaCl. Similarly to normal growth conditions, all thylakoids of the mutant strain had wider interthylakoid space compare to wild type. Mutant cells accumulated glycogen granules to higher extent than wild-type cells.

The multiple cells images of control and salt-treated wild-type and mutant cells are also provided in Supplementary Fig. S1. The statistical analysis of changes in the thylakoid width revealed that, NaCl caused transient increase of this parameters (keritomized, or bloating thylakoids, were not counted). Wild type cells restored the thylakoid width faster than mutant cells (Supplementary Fig. S2).

3.2. NaCl-dependent changes in cytoplasmic volume

The impacts of salt stress caused by the addition of 0.5 M NaCl to the culture media were examined on wild-type and AqpZ-mutant cells. The extent of cellular shrinkage under salt stress due to NaCl was estimated by EPR spectrometry. EPR allows

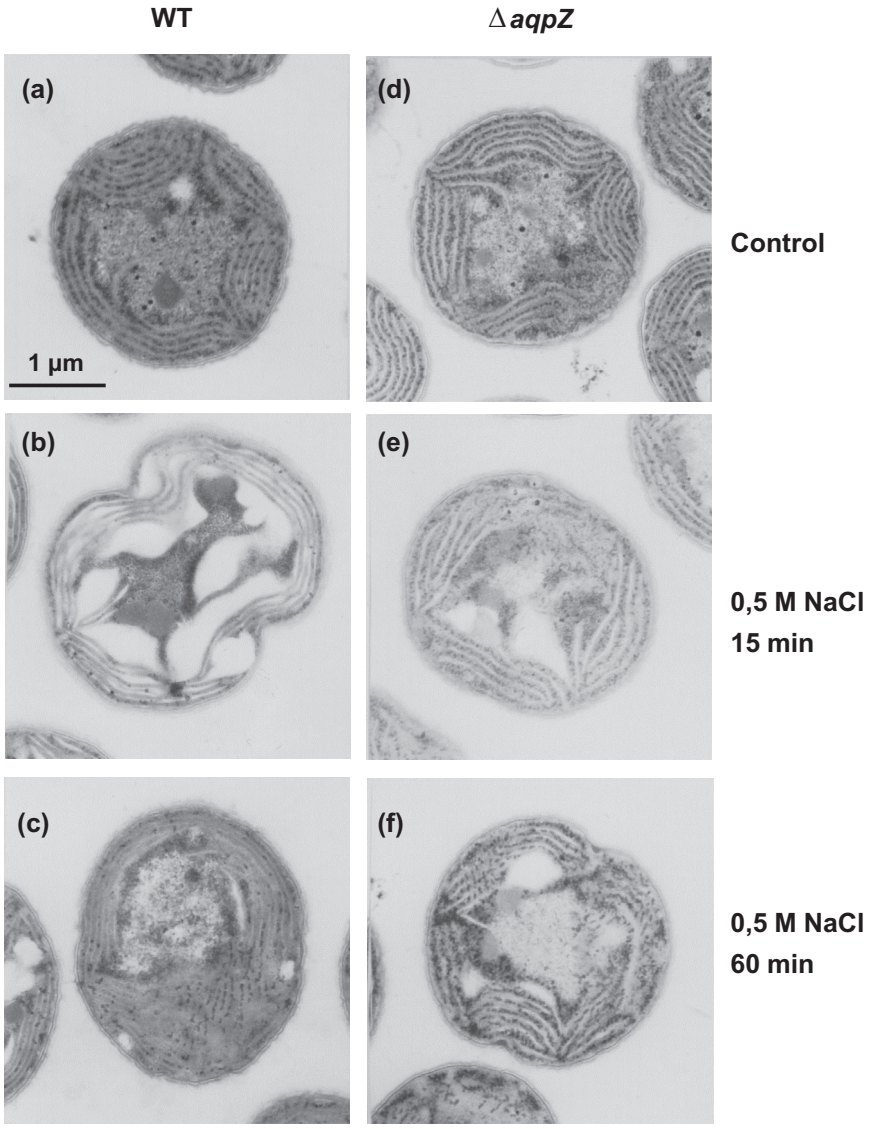


Fig. 1. Electron microphotographs of wild-type (a–c) and AqpZ-deficient (d–f) *Synechocystis* cells incubated in the absence (a and d) or in the presence of 0.5 M NaCl for 15 (b and e) or 60 (c and f) min. A bar corresponds to 1 μm.

to register relative changes in the cytoplasmic volume of cells, which avoids possible artefacts caused by such treatments as fixation for electron microscopy. The results in Fig. 2 indicate the cytoplasmic volume of wild-type cells was rapidly (5–15 min) reduced to 70% of the original level under NaCl stress due to the efflux of water, whereas no shrinkage was detected in AqpZ-deficient cells. These observations confirm the electron microscopic data (Fig. 1) and indicate that aquaporins mediate the rapid outflow of water from the cells under salt stress. This effect of NaCl to cell volume is similar, though as much pronounced, to the previously observed action of sorbitol to the cells of *Synechocystis* and its AqpZ-deficient mutant [5]. The contribution of the lipid bilayer to water conductivity appears to be insignificant at the early stage of NaCl stress similarly to osmotic stress.

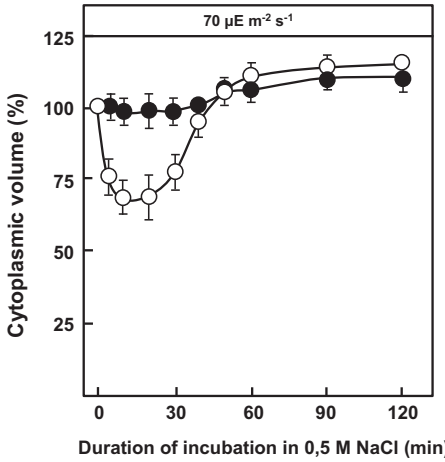


Fig. 2. Changes in cytoplasmic volume of wild-type (open symbols) and the *aqpZ*-deficient mutant (dark symbols) during incubation with 0.5 M NaCl at 70 μE m^{-2} s^{-1}. At designated times, a portion of each cell suspension was withdrawn and the cytoplasmic volume was determined. Measurements were performed with EPR [4,5] and repeated 3 times.

3.3. NaCl-dependent changes in the activities of PSII and PSI

The effect of salt stress on photosystems was monitored by the oxygen evolution (PSII) or uptake (PSI) in intact wild-type and AqpZ-deficient mutant cells of *Synechocystis*. NaCl was added to

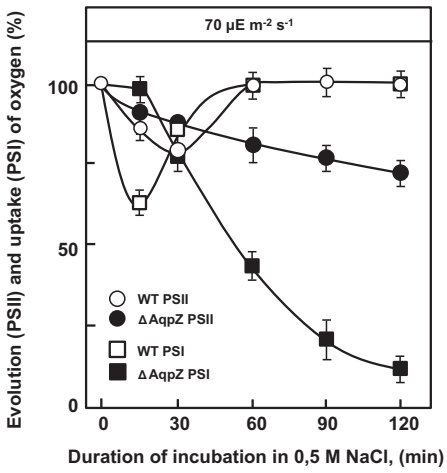


Fig. 3. Effect of NaCl on the photosynthetic electron-transport activities of PSII and PSI in intact wild-type (open symbols) and AqpZ-deficient (dark symbols) cells. Cells were incubated at 32 °C in the presence of 0.5 M NaCl at 70 μE m^{-2} s^{-1}. At designated times, portions of each cell suspension were withdrawn and the activities of PSII (○●) and PSI (□■) were determined. The oxygen-evolving activity of PSII was examined after the addition of 1.0 mM BQ and 1.0 mM DCBQ to the suspension. The activities of wild-type and AqpZ mutant cells that corresponded to 100% were ~423 μmol O_2 mg^{-1} Chl h^{-1} and 411 μmol O_2 mg^{-1} Chl h^{-1}, respectively. The electron-transport activity of PSI was determined by monitoring the uptake of oxygen at 32 °C in the presence of DCMU (15 μM), DCIP (0.1 mM), Na-ascorbate (5 mM), and MV (0.1 mM) in the suspension. The oxygen-uptake activities of wild-type and AqpZ mutant cells that corresponded to 100% was ~342 μmol O_2 mg^{-1} Chl h^{-1} and ~318 μmol O_2 mg^{-1} Chl h^{-1}, respectively. Each point represents the average with SE of results from 3 independent experiments.

cells that had been grown in BG-11 medium to the final concentration of 0.5 M.

Fig. 3 shows changes in the oxygen-evolving activity of PSII of cells during incubation in light in the presence of NaCl. The oxygen-evolving activity of cells in the presence of BQ and DCBQ declined to about 75% of the original level in 30 min and then reached the original level in another 30 min in wild-type cells. In AqpZ-deficient mutant cells, however, the PSII activity gradually decreased in the presence of NaCl with no signs of recovery.

The activity of PSI, was much more sensitive to NaCl in the AqpZ-mutant cells (Fig. 3). Light-induced uptake of oxygen declined to about 55% of the original level in 30 min, and it was completely restored in further 30 min in wild-type cells. By contrast, mutant cells retained the PSI activity at control level within first 15 min, but then lost it almost to zero within 2 h exposure to 0.5 NaCl.

3.4. Analysis of gene expression in wild-type and AqpZ-mutant cells under salt stress

Salt stress strongly induces transcription of dozens of genes in *Synechocystis* [20]. Here we compared the responses of several salt-induced genes in wild-type and AqpZ-deficient mutant. In general, salt-induced genes could be divided into three groups according to the effect of the mutation on their salt-induced transcription: (1) genes that were repressed by the mutation; (2) genes that were induced by the mutation; and (3) unaffected genes.

An operon consisting of two genes, *sll1862* and *sll1863*, of unknown function was strongly induced by NaCl in wild-type cells. However, its inducibility was diminished in the mutant cells (Fig. 4). Similar phenomenon was observed for several genes: small 17 kDa chaperone HspA, histidine kinase Hik34, response regulator Rre37, and glucosylglycerol transport protein GgtB.

The opposite effect of mutation was observed for genes for a high-light-inducible protein, HliB; 40-kDa chaperone, DnaJ; glucosylglycerol-phosphate synthase, GgpS; and glycerol-3-phosphate dehydrogenase, GlpD. These genes were induced to higher extent in the AqpZ-deficient mutant than in the wild-type cells.

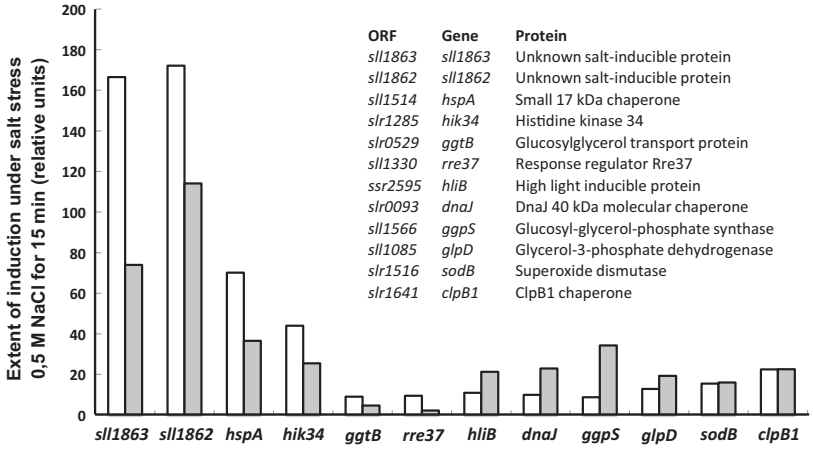
Finally, there was the third group of genes, whose transcription was not affected by the mutation, *e.g.* genes for superoxide dismutase SodB, or chaperone ClpB1.

3.5. High-light-dependent changes in the activities of PSII and PSI

Cells of wild-type and AqpZ-mutant were grown at 70 μE m^{-2} s^{-1} to the OD_{750} = 0.3 and then exposed to 1500 μE m^{-2} s^{-1} for 85 min. During this time the activities of PSII and PSI were inhibited to similar levels: 20–25% of the original level for PSII, and 40–45% for PSI (Fig. 5). After that light intensity was returned to 70 μE m^{-2} s^{-1} and cells were allowed to recover. Within 2 h, wild-type cells were able to completely restore their PSII, and up to 80% – PSI. In mutant cells, the activities were restores up to 60% for PSII and 35% – for PSI. It should be noted that the activity of PSI started to recover slowly and reached 50% of the original level in 1 h. However, after that this activity started to decline.

We also examined the recovery of PSII after exposure of cells to more strong (2500 μE m^{-2} s^{-1}) light by measuring oxygen evolution and chlorophyll fluorescence (Fig. 6). Both methods show that recovery PSII after photoinhibition is very much retarded in AqpZ-mutant cells compared to wild-type cells.

All these results show that the activities of both PSII and PSI were equally inhibited by strong light in wild-type and in AqpZ



ORF	Gene	Protein
sll1863	*sll1863*	Unknown salt-inducible protein
sll1862	*sll1862*	Unknown salt-inducible protein
sll1514	*hspA*	Small 17 kDa chaperone
slr1285	*hik34*	Histidine kinase 34
slr0529	*ggtB*	Glucosylglycerol transport protein
sll1330	*rre37*	Response regulator Rre37
ssr2595	*hliB*	High light inducible protein
slr0093	*dnaJ*	DnaJ 40 kDa molecular chaperone
sll1566	*ggpS*	Glucosyl-glycerol-phosphate synthase
sll1085	*glpD*	Glycerol-3-phosphate dehydrogenase
slr1516	*sodB*	Superoxide dismutase
slr1641	*clpB1*	ClpB1 chaperone

Fig. 4. Relative changes in gene transcription in *Synechocystis* wild-type and AqpZ-deficient cells exposed to 0.5 M NaCl for 15 min.

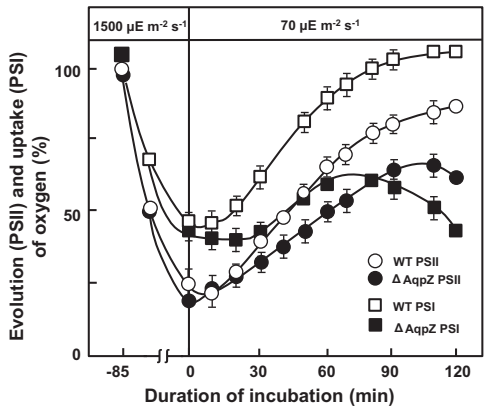


Fig. 5. Effect of high light intensity on the photosynthetic electron-transport activities of PSII and PSI in intact wild-type and AqpZ-deficient cells of *Synechocystis*. Cells were incubated in the light at 1500 μE m^{-2} s^{-1} for 85 min at 32 °C. Then cells were returned to light at 70 μE m^{-2} s^{-1} at 32 °C for recovery. At designated times, a portion of each cell suspension was withdrawn and kept in darkness for 10 min, then the activities of PSII (○●) and PSI (□■) were determined. The oxygen-evolving activity of PSII was examined after the addition of 1.0 mM BQ and 1.0 mM DCBQ to the suspension. The activities of wild-type and AqpZ mutant cells that corresponded to 100% were ~454 μmol O_2 mg^{-1} Chl h^{-1} and 436 μmol O_2 mg^{-1} Chl h^{-1}, respectively. The electron-transport activity of PSI was determined by monitoring the uptake of oxygen at 32 °C in the presence of DCMU (15 μM), DCIP (0.1 mM), Na-ascorbate (5 mM), and MV (0.1 mM) in the suspension. The oxygen-uptake activities of wild-type and AqpZ mutant cells that corresponded to 100% was ~324 μmol O_2 mg^{-1} Chl h^{-1} and ~337 μmol O_2 mg^{-1} Chl h^{-1}, respectively. Each point represents the average with SE of results from 3 independent experiments.

mutant cells, and that, in contrast to wild-type cells, AqpZ-mutant cells could not fully recover the activities of PSII and PSI after photoinhibition.

4. Discussion

Aquaporins play important role in regulation of water status in cells. In cyanobacteria, the role of aquaporins in osmotic acclimation have been studied with inhibitors [3,4] or employing the aquaporin-deficient mutants [5–8]. While AqpZ-like aquaporin of *Synechococcus* [12] revealed sensitivity to mercury inhibitors [3,4], the homolog of *Synechocystis* was insensitive to mercury inhibitor [7].

Involvement of AqpZ in cyanobacterial osmoregulation and photosynthetic activity has been previously demonstrated [3–5,20]. The genome-wide study of gene transcription under hyperosmotic stress in *Synechocystis* suggested that a rapid shrinkage of cells due to a decrease in cytoplasmic volume may trigger the adaptive gene expression [5].

Akai et al. [7] confirmed that the addition of sorbitol and NaCl leads to a slower decrease in cell volume of the AqpZ-deficient cells compared to wild-type. They also demonstrated that NaCl stress had negligible effect on net photosynthesis measured as oxygen evolution [7].

In this work, we visualized salt-stress-induced changes in cell morphology in wild-type and AqpZ-deficient cells. The results show dramatic salt-induced changes in cellular architecture in wild type cells, while AqpZ-mutant cells remained basically unchanged (Fig. 1). This is consistent with the results obtained with EPR method (Fig. 2; [7]).

We separately measured the changes of the activities of PSII and PSI by two methods, oxygen evolution or consumption, and by Chl fluorescence. The results indicated that PSII and PSI of AqpZ-deficient cells were more sensitive to NaCl than PSII and PSI of wild-type cells. Furthermore, PSI of the mutant appeared to be more sensitive to NaCl than its PSII (Fig. 3).

Earlier works revealed the correlation between CO_2 uptake and water channels in *Synechococcus* sp. PCC 7942. CO_2 uptake was inhibited in the presence of water channel blocker, *p*-chloromercuriphenyl sulphonic acid [11]. A direct evidence for the role of PSI as a major energy source for CO_2 uptake has been provided by Vermaas et al. [21]. It is also known that cyclic electron flow around PSI is enhanced under salt stress conditions contributing significantly to the formation of proton gradient which will subsequently lead to ATP synthesis [22]. Taken together, earlier reports and our present results strongly suggest a link between PSI activity under salt stress and CO_2 uptake via water channels. In this view, the sensitivity of PSI to salt stress in AqpZ-deficient mutant may be due to inhibition of CO_2 uptake. Enhanced cyclic electron flow around PSI, probably, helps to cope with such conditions by creating proton gradient which plays a role in CO_2 uptake.

Comparison of salt-induced gene transcription in wild-type and AqpZ-deficient cells revealed that, among, approximately, 200 of salt-induced genes, some of them were affected by the mutation (Fig. 4). Salt-induced transcription of genes for chaperone HspA [23], sensory histidine kinase Hik34 [24], response regulator Rre37, which is involved in regulation of glycogen synthesis [25], glucosylglycerol transporter GgtB [1], was diminished in the mutant cells. At the same time, transcription of other genes was

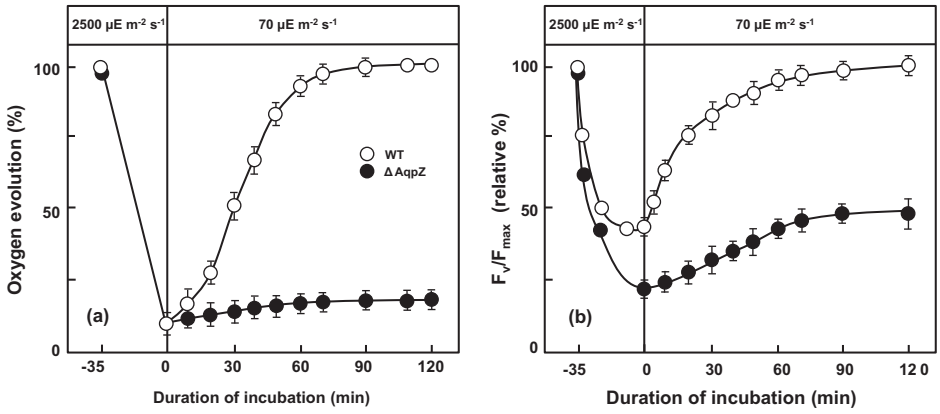


Fig. 6. Recovery of PSII in intact wild-type (○) and AqpZ-deficient (●) cells estimated by measurements of oxygen evolution (a) and Chl fluorescence (b). Cells were incubated in light at 2500 μE m^{-2} s^{-1} for 85 min at 32 °C. Then cells were returned to light at 70 μE m^{-2} s^{-1} at 32 °C for recovery. At designated times, a portion of each cell suspension was withdrawn and kept in darkness for 10 min, then the activities of PSII were determined. The oxygen-evolving activity of PSII was examined after the addition of 1.0 mM BQ and 1.0 mM DCBQ to the suspension. The activities of wild-type and AqpZ mutant cells that corresponded to 100% were ~400 μmol O_2 mg^{-1} Chl h^{-1} and 420 μmol O_2 mg^{-1} Chl h^{-1}, respectively. Each point represents the average with SE of results from 3 independent experiments. F_{max}, F_v, and F_0 were determined as described in Section 2. Each point represents the average with SE of results from 4 independent experiments.

enhanced. The letter set of genes encode chaperone DnaJ, glucosylglycerol-phosphate synthase GgpS, glycerol-3-phosphate dehydrogenase GlpD (GgpS and GlpD are involved in the salt-and osmo-induced synthesis of an osmoprotector, glucosylglycerol [1]). Similar effect of the mutation in the *aqpZ* gene had been observed in *Synechocystis* under hyperosmotic stress [5]. These results suggest that a rapid shrinkage of cells due to a decrease in cytoplasmic volume under salt and hyperosmotic stress may trigger gene expression cascade, which is necessary for acclimation to new environments.

Strong light inhibits PSII in all photosynthetic organisms, which overcome inactivation to PSII by the rapid and efficient repair of the damage by the *de novo* synthesis of required proteins. The activity of PSII depends on the balance between the rates of photodamage and repair, and the photoinhibition of PSII becomes apparent when the rate of photodamage exceeds to the rate of repair. The environmental stresses, such as temperature, salinity, osmotic pressure act primarily by inhibiting the repair of PSII [26]. Here we show that the AqpZ-deficient mutant of *Synechocystis* is more sensitive to photoinhibition by strong light than wild-type cells (Figs. 5 and 6). Since photoinactivation by strong light appears at the same rate in both types of cells, it seems evident that functional AqpZ is necessary for the repair of PSII and PSI after photodamage.

Stress generates reactive oxygen species, among which neutral H_2O_2 may be transported to relatively long distances and appear as a signaling of regulatory molecule through oxidation of SH groups of proteins [26,27]. It was previously demonstrated that some plant and animal aquaporins expressed in yeast may transport H_2O_2 molecules [28]. Thus, the regulatory role of the aquaporins might be more complex in cyanobacterial cells and include not only stress-induced changes of cell volume, water and solute transport, but also the transfer of the reactive oxygen species.

5. Conclusion

In *Synechocystis*, aquaporin AqpZ is involved in regulation of the activity of PSII and PSI under NaCl and high light stress. AqpZ might be necessary for the repair of PSII and PSI after photodamage.

Acknowledgement

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jphotobiol.2015.07.012.

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