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Biochimica et Biophysica Acta 1708 (2005) 201–208
BIOCHIMICA ET BIOPHYSICA ACTA
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Cellular energization protects the photosynthetic machinery against salt-induced inactivation in *Synechococcus*

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Received 30 November 2004; accepted 5 January 2005
Available online 28 January 2005

Abstract

The effects of the energization of cells by light and by exogenous glucose on the salt-induced inactivation of the photosynthetic machinery were investigated in the cyanobacterium *Synechococcus* sp. PCC 7942. The incubation of the cyanobacterial cells in a medium supplemented with 0.5 M NaCl induced a rapid decline with a subsequent slow decline, in the oxygen-evolving activity of Photosystem (PS) II and in the electron-transport activity of PSI. Light and exogenous glucose each protected PSII and PSI against the second phase of the NaCl-induced inactivation. The protective effects of light and glucose were eliminated by an uncoupler of phosphorylation and by lincomycin, an inhibitor of protein synthesis. Light and glucose had similar effects on the NaCl-induced inactivation of Na^+/H^+ antiporters. After photosynthetic and Na^+/H^+-antiport activities had been eliminated by the exposure of cells to 0.5 M NaCl in the darkness, both activities were partially restored by light or exogenous glucose. This recovery was prevented by lincomycin. These observations suggest that cellular energization by either photosynthesis or respiration, which is necessary for protein synthesis, is important for the recovery of the photosynthetic machinery and Na^+/H^+ antiporters from inactivation by a high level of NaCl.

Keywords: Salt stress; Cellular energization; Photosynthesis; Respiration; Na^+/H^+-antiporter; *Synechococcus*

1. Introduction

Salt stress is one of the main environmental factors that limit photosynthesis and, thus, the productivity of higher plants and microorganisms [1]. We have been studying the molecular mechanisms responsible for the salt-induced inactivation of the photosynthetic machinery, in particular Photosystem II (PSII), in vitro [2,3] and in vivo [4,7]. When PSII membranes are exposed to high concentrations of NaCl, the extrinsic proteins of the oxygen-evolving machinery of PSII dissociate from the complex, with resultant impairment of the photosynthetic evolution of oxygen [6]. In cyanobacterial cells, high concentrations of NaCl inactivate Na^+/H^+ antiporters in addition to the oxygen-evolving machinery [4,7].

In recent studies, we compared the effects of high-salt stress due to NaCl and hyperosmotic stress due to sorbitol on the photosynthetic machinery of the cyanobacterium *Synechococcus* sp. PCC 7942 (*Anacystis nidulans* R2-SPc; hereafter abbreviated *Synechococcus*) [5,7]. Hyperosmotic conditions decreased the volume of the cytoplasm via the efflux of H_2O through water channels and reversibly inactivated the photosynthetic machinery [5]. By contrast, after salt shock Na^+ ions leaked into the cytoplasm through K^+/Na^+ channels but there was no significant shrinkage of the cytoplasm [7]. This phenomenon had a strong ionic effect and irreversible inactivated the photosynthetic machinery, in particular, the photosynthetic oxygen-evolving machinery. It is likely that the

Abbreviations: BQ, 1,4-benzoquinone; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Chl, chlorophyll; DAD, 2,3,5,6-tetramethyl-1.4-phenylenediamine; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; MV, methyl viologen; PS, photosystem

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doi:10.1016/j.bbabio.2005.01.002

ionic effect also inhibits the synthesis of proteins, in particular the Na^+/H^+ antiporters. Such inhibition might lead to an increase in the intracellular concentration of NaCl, which would further inhibit the synthesis de novo of the Na^+/H^+ antiporters [7].

Most previous studies of the effects of salt stress on the photosynthetic machinery were performed in the darkness, and very little attention has been paid to the possible role of light in the tolerance of the photosynthetic machinery to salt stress. In the present study, we investigated the effects of NaCl on the activities of PSII and PSI, with special emphasis on the role of cellular energization in the tolerance of *Synechococcus* cells to salt stress. We found that cellular energization due to light or to the presence of exogenous glucose protected the photosynthetic machinery and Na^+/H^+ antiporters against salt-induced inactivation.

2. Materials and methods

2.1. Organisms, culture conditions and exposure of cells to NaCl

A strain of *Synechococcus* sp. PCC 7942 (*A. nidulans* R2-SPc) was obtained from Dr. W. E. Borrias (University of Utrecht, the Netherlands). Cells were grown photoautotrophically in glass tubes (80 mL) at 32 °C, with aeration with sterile air that contained 1% CO_2 and under constant illumination at 70 μE m^{-2} s^{-1} from incandescent lamps, in BG-11 medium supplemented with 20 mM Hepes–NaOH (pH 7.5) [8]. This medium contained 20 mM Na^+ ions. After cultivation for 4 days, cells were harvested by centrifugation at 6000×*g* for 5 min and resuspended in fresh BG-11 medium (pH 7.5) at a density of 10 μg Chl mL^{-1}. After a recovery period of 2 h under growth conditions, the cell suspensions were then incubated at 32 °C with gentle stirring at 20-min intervals in BG-11 medium in the presence of 0.5 M NaCl or its absence. In some experiments, at designated times, aliquots were withdrawn and cells were washed twice with fresh BG-11 medium by centrifugation at 6000×*g* for 5 min and resuspension. Finally, cells were suspended in fresh BG-11 medium and incubated in glass tubes (40 mL) in the darkness or under illumination at 70 μE m^{-2} s^{-1}.

2.2. Measurement of electron-transport and respiratory activities

The electron-transport activities of PSII and PSI in *Synechococcus* cells were measured at 32 °C by monitoring the light-induced evolution and uptake of oxygen, respectively, with a Clark-type oxygen electrode (Hansatech Instruments, Kings Lynn, Norfolk, UK) as described previously [4,7]. The oxygen-evolving activity of PSII was measured in the presence of 1.0 mM 1,4-benzoquinone (BQ). The electron transport activity of PSI was determined in the presence of 15 μM 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU), 5 mM sodium ascorbate, 0.5 mM 2,3,5,6-tetramethyl-1.4-phenylenediamine (DAD) and 0.1 mM methyl viologen (MV). Concentrations of Chl were determined as described by Arnon et al. [9].

The respiratory activity was measured at 32 °C by monitoring the uptake of oxygen in the darkness as described above. No electron carriers nor inhibitors were added.

2.3. Measurement of Na^+/H^+-antiport activity

The activity of Na^+/H^+ antiporters in *Synechococcus* cells was measured by monitoring the fluorescence of acridine orange as described previously [10,11] with minor modification [7]. Fluorescence was monitored at room temperature with a spectrofluorometer (RF-500; Shimadzu, Kyoto, Japan) at excitation and emission wavelengths of 495 nm and 540 nm, respectively.

2.4. Protein labelling and separation

During the cultivation under salt stress (0.5 M NaCl) and low salt conditions (recovery period) of *Synechococcus* 5 ml aliquots were taken directly from the cultivation vessels and incubated with 0.56 MBq labelled L-[^{35}S]methionine (specific activity >37 TBq/mmol, Amersham Pharmacia Biotech) for 30 min under the same culture conditions. Labelled cells were harvested by centrifugation. Total proteins were extracted from the cell pellets by sonication (two times for 1 min at 30 W under ice-cooling) in 10 mM HEPES/NaOH buffer (pH 7.3) containing the protease inhibitor phenylmethylsulfo-nylfluoride (PMSF). Aliquots of total protein extracts containing similar amounts of radioactivity were separated in SDS-containing polyacrylamide gradient gels (7.5–15% polyacrylamide, w/v, separating gels of 14×18 cm, 0.8 mm thick) using the discontinuous buffer system according to Laemmli [12]. After electrophoresis, the gels were stained and dried. The labelled protein bands were detected using a phosphoimager (BAS1000, Fuji). The molecular masses of protein bands were determined in comparison to labelled rainbow protein standard (Amersham Pharmacia Biotech).

3. Results

3.1. Effects of light on the NaCl-induced inactivation of Photosystem II and Photosystem I

We examined the effects of light on the NaCl-induced inactivation of PSII and PSI in *Synechococcus* cells by monitoring the evolution and the uptake of oxygen, respectively. Fig. 1A shows changes in the oxygen-evolving activity of PSII during incubation of cells in

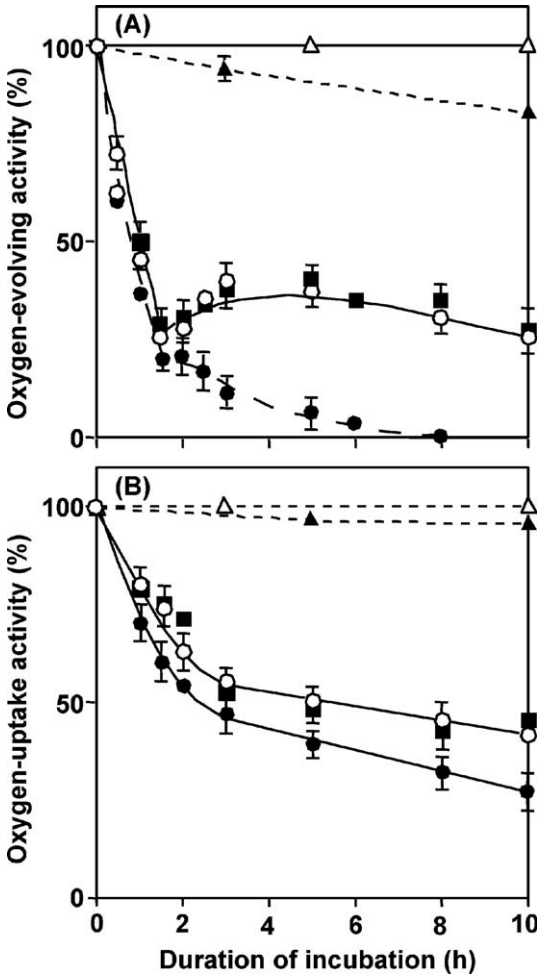

Fig. 1. Effects of light and exogenous glucose on changes in the photosynthetic transport of electrons in PSII and PSI during the incubation of *Synechococcus* cells in the medium supplemented with 0.5 M NaCl. At designated times during the incubation, a portion of the suspension of cells was withdrawn and the activities of PSII and PSI were determined by monitoring the evolution and the uptake of oxygen, respectively. (A) The oxygen-evolving activity of PSII was determined after the addition of 1.0 mM BQ to the suspension of cells. The activity that corresponded to 100% was 508±32 μmol O_2 mg^{-1} Chl h^{-1}. (B) The electron-transport activity of PSI was determined by monitoring the uptake of oxygen after the addition of 15 μM DCMU, 0.5 mM DAD, 5 mM sodium ascorbate and 0.1 mM MV to the suspension of cells. The oxygen-uptake activity of that corresponded to 100% was 298±26 μmol O_2 mg^{-1} Chl h^{-1}. ●, In the presence of 0.5 M NaCl in the darkness; ○, in the presence of 0.5 M NaCl in light at 70 μE m^{-2} s^{-1}; ■, in the presence of 0.5 M NaCl and 5 mM glucose in the darkness; ▲, in the absence of NaCl in the darkness; △, in the absence of NaCl in light. Each point represents the average of results from four independent experiments. Bars in this and other figures show S.E. in all cases where the S.E. was longer than the respective symbol.

the darkness and in light in the absence and in the presence of 0.5 M NaCl. As observed previously [7] the oxygen-evolving activity declined in two phases during incubation with 0.5 M NaCl in the darkness: the activity fell rapidly to 30% of the original level in 1.5 h and then it decreased gradually until it finally disappeared at 8 h. When these experiments were performed under illumination at 70 μE m^{-2} s^{-1}, the activity also declined rapidly as it did in the darkness, but it remained at about 30% of the original level for 8 h (Fig. 1A).

Fig. 1B shows the NaCl-induced inactivation of PSI, as monitored in terms of the light-induced uptake of oxygen in the presence of DAD, sodium ascorbate, MV and DCMU. During incubation with 0.5 M NaCl in the darkness, the oxygen-uptake activity declined to 50% of the original level within 2 h and then continued to decrease gradually. During the incubation in light, the decrease in the activity occurred more slowly than in the darkness. However, the ability of light to restore PSI activity was not as great as its ability to restore PSII activity (Fig. 1B).

These results indicated that, during the incubation of cells in a medium supplemented with 0.5 M NaCl, PSII and PSI were inhibited within 2 h both in the darkness and in light. During the subsequent slow decline in activity, light protected PSII and PSI, albeit to a more limited extent, against NaCl-induced inactivation.

3.2. Effects of glucose on the NaCl-induced inactivation of Photosystem II and Photosystem I

To investigate the possible contribution of respiration to the tolerance to salt stress, we examined the effects of glucose on the NaCl-induced inactivation of PSII and PSI in the darkness (Fig. 1). During the incubation of *Synechococcus* cells in a medium supplemented with 0.5 M NaCl in the presence of 5 mM glucose, the activity of PSII initially declined rapidly, as it did in the absence of glucose, but no further slow decline in the darkness was observed. This result was essentially the same as that observed during the incubation in light (Fig. 1A). When a similar experiment was performed in the presence of 50 mM glucose, we observed essentially the same protective effects as in those observed in the presence of 5 mM glucose (data not shown). The presence of 5 mM glucose had a slight protective effect on PSI against NaCl-induced inactivation, as did light (Fig. 1B).

3.3. Effects of uncouplers and lincomycin on the NaCl-induced inactivation of Photosystem II

To investigate the possible contribution of cellular energization to the tolerance to salt stress, we examined the effects of an uncoupler of phosphorylation, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), on the NaCl-induced inactivation of PSII. When 5 μM CCCP was present in the incubation medium, the ability of light to restore PSII activity was completely eliminated, while the rapid phase of NaCl-induced inactivation was unaffected (Fig. 2). We observed a similar effect when cells were incubated in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCCD, 10 μM), carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP, 10 μM), nigericin plus valinomycin (Nig/Val, 2 μM+2 μM) (data not shown).

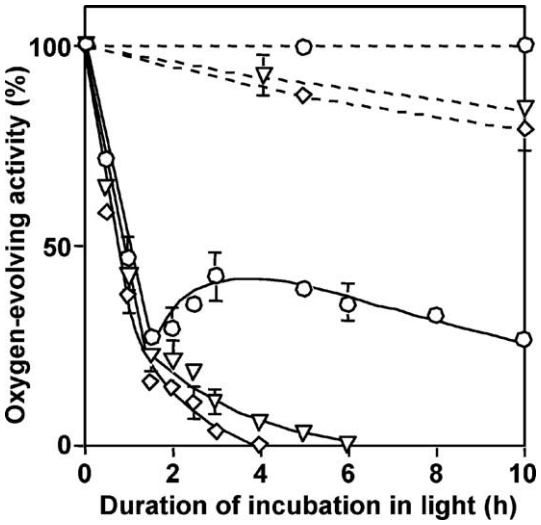

Fig. 2. Effects of CCCP and lincomycin on the NaCl-induced inactivation of PSII activity in *Synechococcus* cells. Cells were incubated in the presence of 0.5 M NaCl (solid line) or in its absence (dashed line) in light at 70 μE m^{-2} s^{-1} in the absence and in the presence of lincomycin or CCCP. At designated times during the incubations, a portion of the suspension of cells was withdrawn and the activity of PSII was determined by monitoring the evolution of oxygen. The oxygen-evolving activity of PSII was determined after the addition of 1.0 mM BQ to the suspension. The activity that corresponded to 100% was 532±41 μmol O_2 mg^{-1} Chl h^{-1}. ○, In the presence of 0.5 M NaCl; ▽, in the presence of 0.5 M NaCl and 200 μg mL^{-1} lincomycin; ◇, in the presence of 0.5 M NaCl and 5 μM CCCP. Each point represents the average of results from four independent experiments.

To clarify the possible involvement of protein synthesis in the tolerance to salt stress, we examined the effects of lincomycin, an inhibitor of protein synthesis, during the incubation of cells in the medium supplemented with 0.5 M NaCl in light and in the darkness (Fig. 2). Although lincomycin had no effect on the NaCl-induced inactivation of PSII in the darkness [4,7], it prevented the restoration of PSII activity by light during the slow phase, when cells were incubated with 0.5 M NaCl in light (Figs. 1A and 2). We observed a similar effect of lincomycin when cells were incubated with 0.5 M NaCl in the darkness in the presence of 5 mM glucose (data not shown).

3.4. Effects of removal of NaCl on the recovery of the activities of Photosystem II and Photosystem I

Fig. 3 shows the recovery of the activities of PSII and PSI after the removal of NaCl from the culture medium. In this experiment, we incubated cells in a medium supplemented with 0.5 M NaCl in light for 12 h, reducing the PSII and PSI activities to 10% and 45% of the original levels, respectively. After cells had been washed with BG-11 medium by centrifugation and resuspension, they were incubated in light at 70 μE m^{-2} s^{-1}. We observed restoration of approximately 50% and 80% of the original activities of PSII and PSI, respectively, within 15 h. In this case also, lincomycin and CCCP completely eliminated the light-induced recovery of the activities of both PSII and PSI. The recovery of these activities in the darkness was much less prominent than in light (Fig. 3).

3.5. Alterations of protein synthesis pattern during NaCl-stress and the recovery under light or dark conditions

The protein synthesis inhibitor lincomycin inhibited the recovery from salt stress, suggesting that the synthesis of

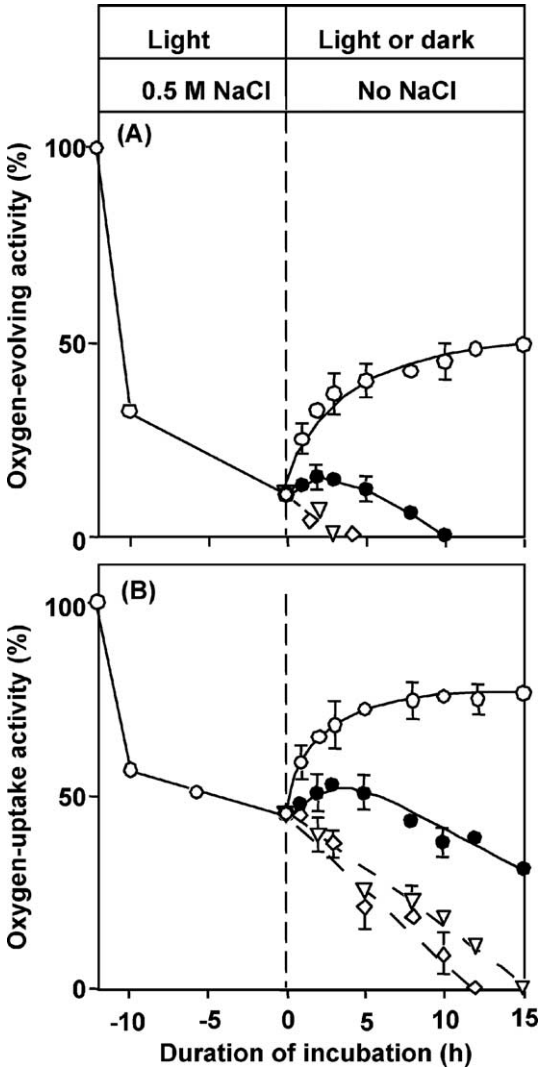

Fig. 3. Effects of light, lincomycin and CCCP on the recovery of the activities of PSII (A) and PSI (B) in *Synechococcus* cells after the removal of NaCl. After the cells had been incubated for 12 h in the medium supplemented with 0.5 M NaCl in light at 70 μE m^{-2} s^{-1}, they were collected by centrifugation and resuspended in fresh BG-11 medium. They were then incubated in the darkness (●), in light at 70 μE m^{-2} s^{-1} (○), in light at 70 μE m^{-2} s^{-1} in the presence of 200 μg mL^{-1} lincomycin (▽) or in light at 70 μE m^{-2} s^{-1} in the presence of 5 μM CCCP (◇). The oxygen-evolving of PSII that corresponded to 100% was 512±35 μmol O_2 mg^{-1}. The oxygen-uptake activity that corresponded to 100% was 311±28 μmol O_2 mg^{-1} Chl h^{-1}. Each point represents the average of results from four independent experiments. For other conditions, see legend to Fig. 1.

proteins was involved in the light-promoted recovery of the activities of PSII and PSI in NaCl-treated cells. In order to investigate directly changes in the protein synthesis pattern, *Synechococcus* cells were labelled by [^{35}S]methionine during salt stress and the following recovery period under light or dark conditions. After the separation of the labelled cellular proteins revealed remarkable differences between control cells, salt-shocked cells and cells recovered in the light or darkness became obvious (Fig. 4). Immediately after salt shock, a fast and nearly complete stop of protein synthesis was observed. In cells shocked for a few hours by 0.5 M NaCl, protein synthesis recovered to some extent but predominantly a typical set of salt shock proteins was synthesized, most of them are absent in the synthesis pattern of control cells [13]. During the recovery phase in light, the protein synthesis pattern quickly returns to that characteristic for control cells before the application of a salt shock. Most of these proteins were also resynthesized when the recovery takes place in the darkness. However, at least 6 proteins which are accumulated during recovery in the light are absent or much less synthesized during dark recovery (Fig. 4). Particularly, an about 23 kDa and an about 39 kDa protein band accumulated during the recovery period in the light and are absolutely non induced under dark conditions. The identification and characterization of these proteins will be the focus of future research.

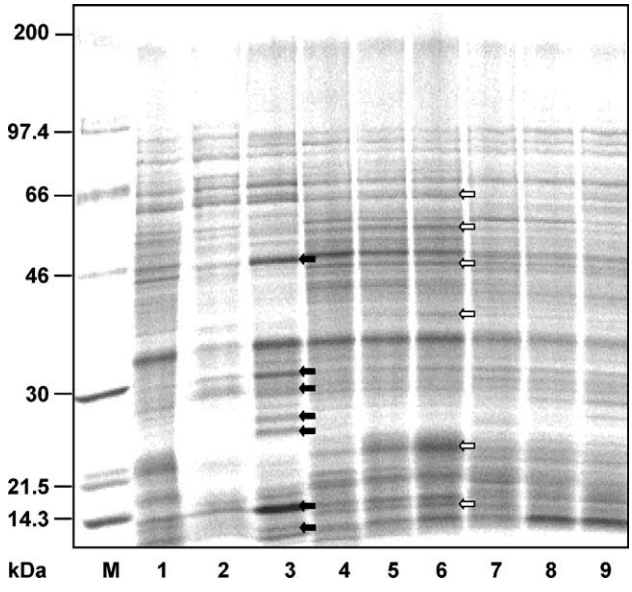

Fig. 4. Protein synthesis pattern of *Synechococcus* under salt stress conditions (0.5 M NaCl in the light) and during its recovery (0 M NaCl) at light or darkness, which was analyzed by the electrophoretic separation of radio-labelled protein extracts obtained after the pulse-labelling of cells by [^{35}S]methionine. Salt stress proteins are marked by black arrows, while proteins induced during the recovery in light but not in dark are highlighted by white arrows. (M—^{14}C-labelled rainbow protein molecular mass marker; 1—control cells; 2—salt shock for 0.5 h; 3—salt shock for 2.5 h; 4—1 h recovery in light; 5—4 h recovery in light; 6—12 h recovery in light; 7—1 h recovery in the darkness; 8—4 h recovery in the darkness; 9—12 h recovery in the darkness).

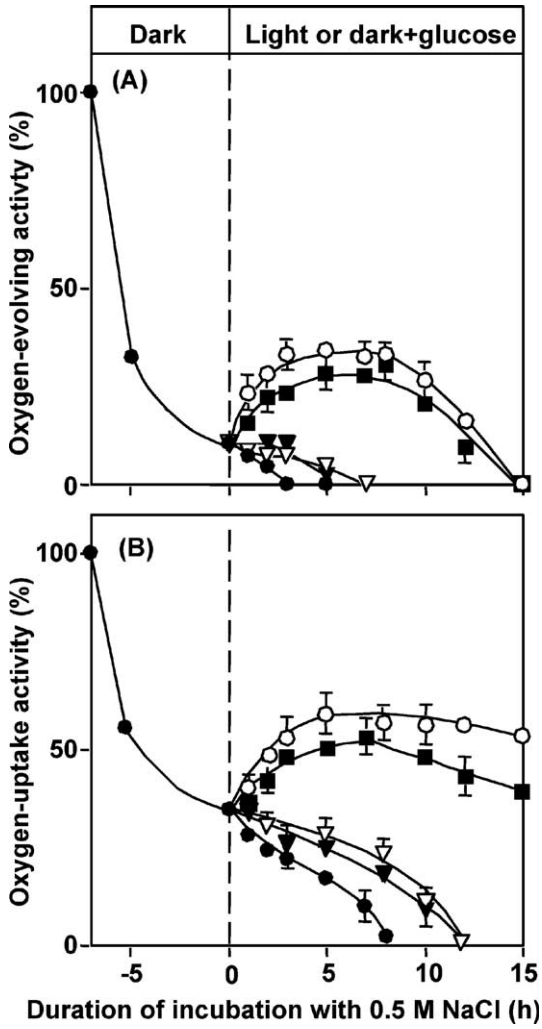

Fig. 5. Effects of light and exogenous glucose on the recovery of the activities of PSII (A) and PSI (B) in *Synechococcus* cells during incubation in medium supplemented with NaCl. After the cells had been incubated for 7 h in the medium supplemented with 0.5 M NaCl in the darkness, they were exposed to light at 70 μE m^{-2} s^{-1} in the absence (○) and presence (▽) of 200 μg mL^{-1} lincomycin, or they were incubated in the darkness with 5 mM glucose in the absence (■) and in the presence (▼) of 200 μg lincomycin mL^{-1}. ●, in the darkness in the absence of glucose. The oxygen-evolving activity that corresponded to 100% was 542±22 μmol O_2 mg^{-1} Chl h^{-1}. The oxygen-uptake activity that corresponded to 100% was 311±28 μmol O_2 mg^{-1} Chl h^{-1}. Each point represents the average of results from five independent experiments. For other details, see legend to Fig. 1.

3.6. Effects of light and glucose on the recovery of the activities of Photosystem II and Photosystem I

To investigate the contribution of photosynthesis and respiration to the recovery of the activities of PSII and PSI, we examined the effects of light and the presence of exogenous glucose on recovery (Fig. 5). Cells were incubated in a medium supplemented with 0.5 M NaCl in

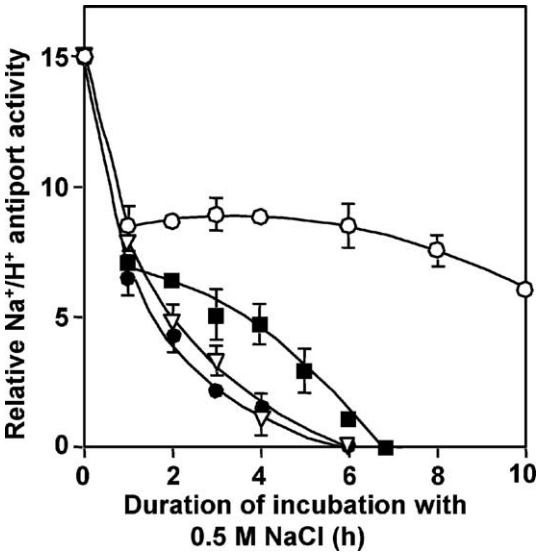

Fig. 6. Effects of light, glucose and lincomycin on changes in the activity of Na^+/H^+ antiporters in *Synechococcus* cells during incubation in the medium supplemented with 0.5 M NaCl. At designated times during the incubation, 20-μL aliquots of the suspension of cells were withdrawn and diluted 100-fold with NaCl-free medium that contained 5 μM acridine orange. Then, Na^+/H^+-antiport activity was measured as described in Materials and methods. The Na^+/H^+-antiport activity was calculated from the initial rate of recovery of fluorescence quenching upon the addition of NaCl, divided by the difference between the fluorescence before the addition of NaCl and the steady-state fluorescence 1 min after the addition of Triton X-100. ●, in the darkness; ○, in light at 70 μE m^{-2} s^{-1}; ▽, in light at 70 μE m^{-2} s^{-1} in the presence of 200 μg mL^{-1} lincomycin; ■, in the darkness in the presence of 5 mM glucose. Each point represents the average±S.E. of results from four independent experiments.

the darkness for 7 h and this treatment reduced the activities of PSII and PSI to 10% and 35%, respectively, of the original levels. Then cells were exposed to light at 70 μE m^{-2} s^{-1}. Under these conditions, the activities of PSII and PSI returned to 35% and 55% of the original levels, respectively, within 5 h. Subsequently, the activity of PSII started to decrease and was completely eliminated at 15 h (Fig. 5A). The activity of PSI was still apparent at 15 h (Fig. 5B). Lincomycin completely eliminated the light-induced recovery (Fig. 5).

Fig. 5 also shows that, in the darkness, the presence of 5 mM glucose restored the activities of PSII and PSI in a manner similar to light. The activity of PSII in the presence of 50 mM glucose was restored, just as it was in the presence of 5 mM glucose, but it remained at 20% of the original level after incubation for 15 h (data not shown). Lincomycin completely eliminated the effects of glucose on the recovery of PSII and PSI activities (Fig. 5).

3.7. Effects of light and glucose on the NaCl-induced inactivation of Na^+/H^+ antiporters

Although Na^+/H^+ antiporters are important for the tolerance of cyanobacterial cells to salt stress (see [10,11,14] and references therein), they are also sensitive to salt stress ([4,11,14] and references therein). Therefore, we examined the effects of light and the presence of exogenous glucose changes in antiporter activity during incubation with NaCl. Fig. 6 shows that the Na^+/H^+ antiporters of *Synechococcus* cells were strongly inactivated during the incubation of cells in the medium supplemented with 0.5 M NaCl. The decline in the activity of Na^+/H^+ antiporters was much more rapid in the darkness than in light. In the darkness, the activity was totally lost within 6 h. In light (70 μE m^{-2} s^{-1}), the activity fell rapidly to 50% of the original level within 1 h and then it remained at about the same level during subsequent incubation for 9 h. The addition of 200 μg mL^{-1} lincomycin to the medium completely eliminated the protective effect of light such that loss of activity in light resembled that in the darkness (Fig. 6). The rate of inactivation of Na^+/H^+ antiporters by incubation of cells in the medium supplemented with 0.5 M NaCl in the darkness was reduced by the presence of 5 mM glucose, but the effect of 5 mM glucose was not as significant as that of light (Fig. 6).

3.8. Effects of light and glucose on the recovery of the activity of Na^+/H^+ antiporters

Fig. 7 shows that the activity of Na^+/H^+ antiporters was restored by light after it had been reduced to 90% of the original level by the incubation of cells in the medium

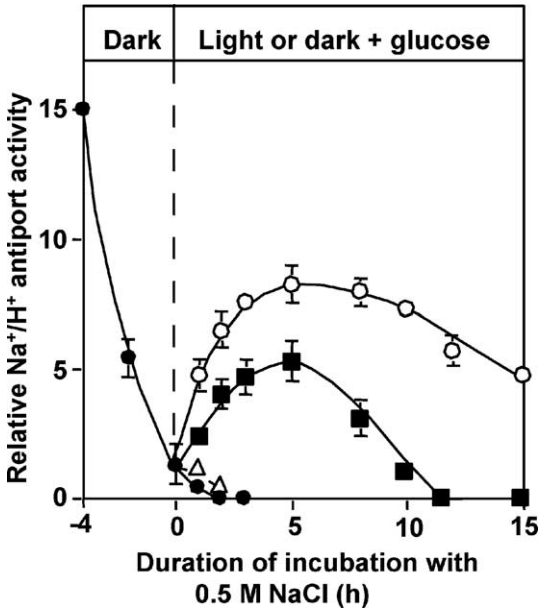

Fig. 7. Effects of light, glucose and lincomycin on the recovery of the activity of Na^+/H^+ antiporters in *Synechococcus* cells during incubation in medium supplemented with 0.5 M NaCl. After the cells had been incubated for 4 h in the darkness, they were exposed to light at 70 μE m^{-2} s^{-1} in the presence (▽) of 200 μg mL^{-1} lincomycin or in its absence (○), or they were incubated in the darkness in the presence (■) of 5 mM glucose or in its absence (●). Each point represents the average±S.E. of results from four independent experiments. For other details, see legend to Fig. 6.

supplemented with 0.5 M NaCl for 4 h in the darkness. Light at 70 μE m^{-2} s^{-1} restored the activity of Na^+/H^+ antiporters to 50% of the original level within 3 h. Lincomycin completely prevented the light-induced recovery of the Na^+/H^+-antiport activity. Fig. 7 also shows that the presence, in the darkness, of 5 mM glucose restored the activity of Na^+/H^+ antiporters, although glucose was not as effective as light.

3.9. Effects of NaCl and glucose on the respiratory activity

Fig. 8 shows the effects of exogenous glucose on the respiratory activity during incubation with NaCl. The addition of 0.5 M NaCl to the medium (at −7 h) increased the respiratory activity by 30%. During the incubation of cells with NaCl, the respiratory activity transiently increased and then gradually decreased to less than 10% of the original activity after the incubation for 15 h. When 5 mM glucose was exogenously added after the incubation with 0.5 M NaCl for 7 h, the respiratory activity immediately increased three-fold, and then gradually decreased. However, levels of the respiratory activity in the presence of exogenous glucose was always much higher than that in its absence. These observations confirmed that the restoration of PSII, PSI and Na^+/H^+-antiport activities upon the addition of glucose (Figs. 5 and 7) was associated with an enhancement of the respiratory activity of *Synechococcus* cells.

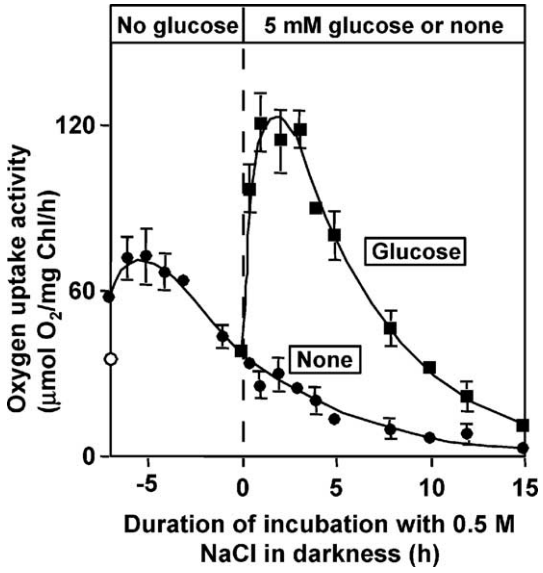

Fig. 8. Effects of exogenous glucose on changes in the respiratory activity in *Synechococcus* cells during incubation in the medium supplemented with 0.5 M NaCl. After the cells had been incubated for 7 h in the darkness, they were further incubated in the presence of 5 mM glucose (■) or its absence (●). The respiratory activities were determined by monitoring the uptake of oxygen in the darkness. The respiratory activity prior to the addition of NaCl is indicated by (○). Each point and bar represent the average±S.E. of results from four independent experiments. Other conditions were the same as in Fig. 5.

4. Discussion

We demonstrated previously that the inhibition of the photosynthetic activity of cyanobacterial cells by the incubation of cells with NaCl is caused by two kinds of effect: osmotic effects and ionic effects [7]. Osmotic effects reduce the amount of water in the cytosol and induce a rapid and reversible decline in the activities of PSII and PSI. Ionic effects, due to the influx of Na^+ ions through K^+/Na^+ channels, induce a slow and irreversible decline in the activities of PSII and PSI. In both the rapid and the slow phases, the site that is sensitive to inactivation by NaCl is the oxygen-evolving machinery of PSII [7].

In the previous studies, salt stress experiments were mainly performed under dark conditions to get clear information about the primary site of action during salt-stress inhibition. However, *Synechococcus* as a cyanobacterium is a photoautotrophic organism, which depends on light to drive energy production and carbon assimilation via photosynthesis, making it impossible to start central acclimation processes in the absence of light. For another cyanobacterial strain, it was shown that the inability to adapt to high salt shocks was mainly based on the insufficient synthesis of the internal compatible solute [15]. In the present study, we demonstrated that light and the presence of exogenous glucose enabled *Synechococcus* to adapt to salt stresses of 0.5 M NaCl even after long exposure, leading to formerly irreversible inhibition of cellular functions by the action of inorganic ions [7].

The incubation at conditions under which energy production was supported restored the activities of PSII and PSI. Furthermore, light and the presence of exogenous glucose resulted in a regeneration of the activity of the Na^+/H^+ antiporters, which showed also a NaCl-induced inactivation in *Synechococcus* cells consisting of rapid and slow phases. The intensity of light, 70 μE m^{-2} s^{-1}, that we used for the restoration and recovery of the activities of PSII, PSI and the Na^+/H^+ antiporters was the same as that used for the growth of *Synechococcus* cells. It is likely that the effects of light were mediated by photosynthesis. It is also likely that the exogenously added glucose activated respiration, since a high but only transient stimulation of dark oxygen consumption was measured after the addition of glucose.

Both photosynthesis and respiration generate energy, for example, such as ATP and proton gradients on cytoplasmic as well as thylakoid membranes in cells. The view that energization of cells by light or glucose might have allowed the recovery of the activities of PSII and PSI and the Na^+/H^+ antiporters is supported by the action of the inhibitors CCCP, DCCD, FCCP, Nig/Val, each of which induces the de-energization of cells. The addition of these substances prevented the restoration and the recovery of the activities of PSII and PSI in light and in the presence of exogenous glucose. It has been shown for salt-stressed *Synechococcus* cells that the energization of ion export mediated mainly by

Na^+/H^+-antiporters is based on the respiratory electron transport chain and cytochrome oxidase activity localized on the cytoplasmic membrane [16].

However, the restoration of PSII, PSI and Na^+/H^+-antiport activity is not only the result of the energy supply by photosynthesis and respiration in the light and the presence of exogenous glucose. The rapid phase of inactivation induced by long term salt stress on *Synechococcus* cells resulted obviously in the irreversible damage of proteins necessary for photosynthesis and ion export activities, since their recovery was completely eliminated by lincomycin, an inhibitor of protein synthesis. NaCl-shock conditions result in a clear inhibition of overall protein synthesis in *Synechococcus* cells (about 80% at 0.5 M NaCl), preventing the continuous synthesis of most cellular proteins, with the exceptions of a set of salt shock proteins [13]. The involvement of altered protein synthesis pattern during recovery leading to the resynthesis of proteins characteristic for control cells was confirmed by protein labelling experiments. Furthermore, it could be shown that light has not only a stimulating effect on overall protein synthesis rate (about three times higher under light than dark conditions) but induced several specific protein bands, which were absent under dark conditions in which no recovery occurred. Thus, our results clearly show that, when cyanobacterial cells are exposed to salt stress, cellular energization and protein synthesis are important for the restoration and recovery of the photosynthetic machinery and the Na^+/H^+ antiporters.

Acknowledgments

The financial support from Russian Foundation for Basic Research (to S.I.A.) is gratefully acknowledged.

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