Ionic and Osmotic Effects of NaCl-Induced Inactivation of Photosystems I and II in *Synechococcus* sp.[1]

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We report here that osmotic effects and ionic effects are both involved in the NaCl-induced inactivation of the photosynthetic machinery in the cyanobacterium *Synechococcus* sp. PCC 7942. 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. An Na^+-channel blocker protected both PSII and PSI against the slow, but not the rapid, inactivation. The rapid decline resembled the effect of 1.0 M sorbitol. The presence of both an Na^+-channel blocker and a water-channel blocker protected PSI and PSII against the short- and long-term effects of NaCl. Salt stress also decreased cytoplasmic volume and this effect was enhanced by the Na^+-channel blocker. Our observations suggested that NaCl had both osmotic and ionic effects. The osmotic effect decreased the amount of water in the cytosol, rapidly increasing the intracellular concentration of salts. The ionic effect was caused by an influx of Na^+ ions through potassium/Na^+ channels that also increased concentrations of salts in the cytosol and irreversibly inactivated PSI and PSII.

High-salt stress is a major environmental factor that limits plant growth and productivity (Boyer, 1982). The detrimental effects of high concentrations of salt on plants can be observed at the whole-plant level as the death of plants and/or decreases in productivity. Reductions in plant growth due to salt stress are often associated with decreases in photosynthetic activities, such as the electron transport (Greenway and Munns, 1980). Effects of salt stress have been examined in various salt-sensitive and -tolerant plants, including some crops (Cheeseman, 1988) and a facultative halophyte (Adams et al., 1992), as well as in cultured cells (Sumaryati et al., 1992), but mechanisms of inhibition of photosynthesis by salt stress remain poorly defined.

Cyanobacteria provide a suitable model for studies of effects of salt stress on photosynthesis since these prokaryotes perform oxygenic photosynthesis using photosynthetic apparatus similar to that in chloroplasts of algae and higher plants (Pfenning, 1978; Öquist et al., 1995). Moreover, cyanobacterial cells can be exposed directly to environmental stress conditions (Blumwald et al., 1983, 1984; Reed and Stewart, 1988; Joset et al., 1996; Hagemann and Erdmann, 1997; Papageorgiou et al., 1998; Allakhverdiev et al., 1999, 2000) and they are able to acclimate to a wide range of environmental stresses (Tandeau de Marsac and Houmard, 1993; Nishida and Murata, 1996; Hagemann and Erdmann, 1997). Thus, using such cells, we can study the direct effects of salt stress and osmotic stress on the photosynthetic machinery.

We demonstrated recently that Na^+/H^+ antiporters play an important role in the tolerance of the photosynthetic machinery to salt stress in *Synechocystis* sp. PCC 6803 (Allakhverdiev et al., 1999). The synthesis of Na^+/H^+ antiporters de novo is regulated by the unsaturation of fatty acids in membrane lipids, and the apparent activity of the antiporters is controlled by the photosynthetic and/or respiratory activity of the cell (Allakhverdiev et al., 1999).

Salt stress involves both osmotic stress and ionic stress (Hagemann and Erdmann, 1997; Hayashi and Murata, 1998). We, therefore, attempted to study these two kinds of stress separately. We demonstrated previously that osmotic stress reversibly inactivates photosynthetic electron transport via shrinkage of the intracellular space, which is due to the efflux of water through water channels in the plasma membrane (Allakhverdiev et al., 2000). By contrast, under salt stress due to NaCl, Na^+ ions leak into the cytosol (Papageorgiou et al., 1998) and inactivate both photosynthetic and respiratory electron transport (Allakhverdiev et al., 1999).

In the present study, we examined effects of NaCl on intact cells of *Synechococcus* sp. PCC 7942. We monitored activities of photosystem (PS)II and PSI in relation to the activities of K^+/Na^+ channels, water channels, and Na^+/H^+ antiporters.

RESULTS

NaCl-Induced Inactivation of the Oxygen-Evolving Machinery in PSII

We examined the effects of salt stress on PSII by monitoring the evolution of oxygen in intact cells.

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Cells that had been grown in BG-11 medium were transferred to fresh BG-11 medium supplemented with 0.5 M NaCl or 0.5 M LiCl. Figure 1 shows changes in the oxygen-evolving activity of cells during incubation in darkness in the presence of NaCl or sorbitol. The oxygen-evolving activity of cells in the presence of 1,4-benzoquinone (BQ) declined to about 30% of the original level in 1 h. It then continued to decrease gradually until it disappeared at 8 h. Essentially the same results were obtained when BQ was replaced by 2,6-dichloro-1,4-benzoquinone as the artificial acceptor of electrons (data not shown). Cells incubated in the presence of 0.5 M LiCl gave similar results, although PSII was inactivated more rapidly (data not shown).

To examine whether the effects of NaCl and LiCl might be related to osmotic effects, we examined the effects of 1.0 M sorbitol, which has approximately the same osmotic effect as NaCl or LiCl at 0.5 M. During the first 2 h of incubation with 1.0 M sorbitol, the evolution of oxygen declined to about 45% of the control level. It remained almost unchanged for 8 h (Fig. 1). These results suggested that the rapid decline in oxygen-evolving activity that occurred within 1.5 h in the presence of NaCl, LiCl, and sorbitol was caused by osmotic pressure, whereas the subsequent slow decline was due to ionic effects.

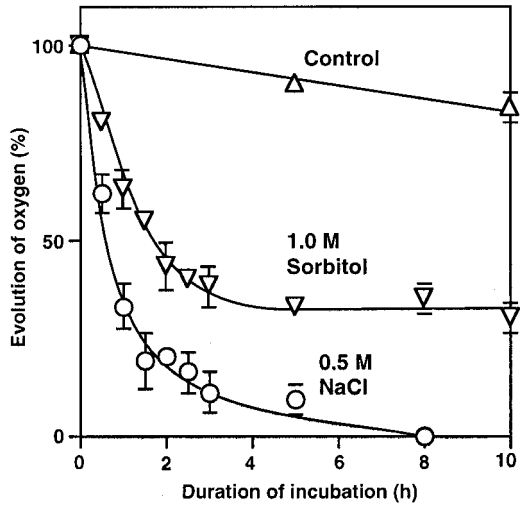

Figure 1. Changes in the photosynthetic oxygen-evolving activity of PSII in intact cells during incubation with NaCl or sorbitol. Cells were incubated in the presence of 0.5 NaCl or 1.0 M sorbitol at 32°C. At designated times, aliquots were withdrawn and oxygen evolution was measured at 32°C after addition of 1.0 mM BQ. The activity that corresponded to 100% was 594 ± 38 μmol O_2 mg^{-1} Chl h^{-1}. △, Control (no addition); ○, 0.5 M NaCl; ▽, 1.0 M sorbitol. Each point and bar represent the average ± SE of results from four independent experiments.

Reversibility of the NaCl-Induced Inactivation of the Oxygen-Evolving Machinery

To examine the reversibility of the NaCl-induced inactivation of the oxygen-evolving machinery in PSII, we released cells that had been incubated with 0.5 M NaCl from salt stress by washing them with fresh BG-11 medium. The oxygen-evolving activity recovered fully in fresh medium after the 0.5-h incubation with NaCl, when only the rapid decline had been observed (Fig. 2). When incubation with NaCl was extended to 1 h, oxygen-evolving activity recovered only partially in the absence of NaCl. When initial incubation with NaCl was extended to 5 h, no recovery was observed. These findings were consistent with the hypothesis that the rapid decline in oxygen-evolving activity resembled that caused by sorbitol (i.e. an osmotic effect) and was reversible (Allakhverdiev et al., 2000), whereas slow inactivation was an irreversible process due to ionic effects.

Effects of K^+/Na^+-Channel Blockers and Water-Channel Blockers on the NaCl-Induced Inactivation of the Oxygen-Evolving Machinery

The K^+ and Na^+ channels in *Synechococcus* sp. remain to be fully characterized. However, the genome of *Synechocystis* sp. (Kaneko et al., 1996) includes at least three putative genes for K^+ channels. The K^+ channels in prokaryotes (Murata et al., 1996; Nakamura et al., 1998) and in higher plants (Schachtman et al., 1991; Murata et al., 1994; Tyerman et al., 1997) are permeable to Na^+ ions. Thus, such channels in *Synechocystis* sp. are referred to as $K^+(Na^+)$ channels.

To clarify the roles of $K^+(Na^+)$ channels and water channels in the NaCl-induced inactivation of PSII, we examined the effects of specific channel blockers (Fig. 3). During incubation with 0.5 M NaCl, inactivation of the oxygen-evolving activity of PSII was significantly suppressed by 100 μM phenytoin, a blocker of Na^+ channels (Muramatsu et al., 1990; Ju et al., 1992). Two other blockers of Na^+ channels, lidocaine and quinidine (Muramatsu et al., 1990; 100 μM), also protected the oxygen-evolving machinery against NaCl-induced inactivation (data not shown). The extent of the NaCl-induced inactivation of PSII was also significantly reduced by 100 μM *p*-chloromercuriphenylsulfonic acid, a blocker of water channels (Pfeuffer et al., 1998; Tyerman et al., 1999, and refs. therein). When blockers of the two kinds of channels wereapplied together, the slow phase of NaCl-induced inactivation of PSII almost disappeared (Fig. 3).

The K^+-channel blocker, tetraethylammonium chloride (Schroeder, 1988; Tyerman et al., 1997; Gaymard et al., 1998; Zhang and Tyerman, 1999), at 500 μM, also markedly suppressed the NaCl-induced in-

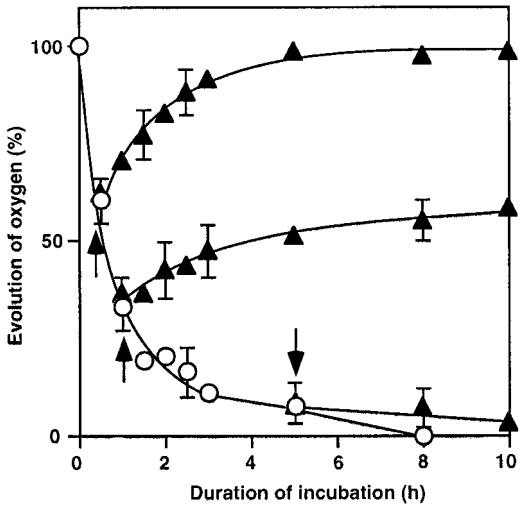

Figure 2. Reversibility of the effects of NaCl on the photosynthetic oxygen-evolving activity of PSII in intact cells. Cells were incubated for 0.5, 1, and 5 h with 0.5 M NaCl at 32°C. Aliquots were withdrawn at times indicated by arrows and cells were washed twice with fresh BG-11 medium. The washed cells were incubated at 32°C in BG-11 medium. A small aliquot of each suspension was withdrawn and oxygen evolution was measured at 32°C after addition of 1.0 mM BQ. The activity that corresponded to 100% was 612 ± 48 μmol O_2 mg^{-1} Chl h^{-1}. ○, Incubation with 0.5 M NaCl; ▲, incubation without NaCl after washing. Each point and bar represent the average ± SE of results from four independent experiments.

activation of PSII (data not shown). The results indicated that $K^+(Na^+)$ channels and water channels played important roles in the NaCl-induced inactivation of the oxygen-evolving machinery.

NaCl-Induced Changes in Chlorophyll (Chl) Fluorescence

To relate the NaCl-induced inactivation of the oxygen-evolving machinery to partial reactions within PSII, we examined changes in the maximum fluorescence of Chl (F_{max}) during incubation of cells with 0.5 M NaCl (data not shown). F_{max} declined in two phases, i.e. rapid and slow, as the oxygen-evolving activity. When dithionite was added to reduce the primary electron acceptor of the PSII complex (Q_A; see "Discussion"), F_{max} did not decline (data not shown), suggesting that the site of NaCl-induced inactivation was not the photochemical reaction center, but the electron-donating side of PSII. This possibility was confirmed with 3-(3′,4′-dichlorophenol)-1,1-dimethylurea (DCMU), whose effects were similar to those of dithionite.

NaCl-Induced Inactivation of the Oxygen-Evolving Machinery in Vitro

Figure 4 shows the effects of NaCl on the oxygen-evolving activity of isolated thylakoid membranes. During incubation of thylakoid membranes with 0.5 M NaCl, transport of electrons from water to 2,6-dichloroindpphenol (DCIP) was inhibited much more rapidly than in intact cells (Fig. 4). The time required for 50% inactivation was 50 min. The transport of electrons from diphenylcarbazide (DPC) to DCIP, which bypasses the oxygen-evolving site (Yamashita and Butler, 1969), was inhibited considerably less during the incubation with 0.5 M NaCl. Thus, incubation with NaCl resulted primarily in damage to the oxygen-evolving site in PSII.

Inactivation of PSI during Salt Stress in Vivo

We examined the effects of NaCl, LiCl, and sorbitol on the activity of PSI in intact cells. When cells were incubated with 0.5 M NaCl, nearly 50% of PSI activity was lost within 2 h (Fig. 5). The decline in PSI activity was less rapid than that in PSII (Fig. 1). The activity of PSI also was markedly affected by 0.5 M LiCl (data not shown), declining within 2 h to 30% of the original level. Thus, the effect of LiCl was greater than that of NaCl.

We next investigated the effects of 1.0 M sorbitol on PSI activity, which decreased by only 25% in 2 h and then remained at about the same level for 8 h (Fig. 5).

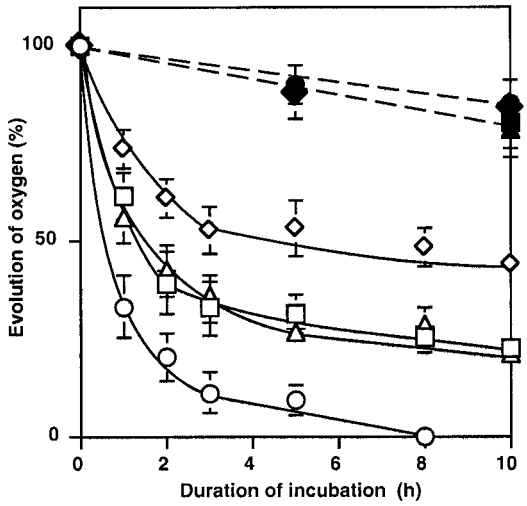

Figure 3. Effects of a Na^+-channel blocker (phenytoin) and a water-channel blocker (*p*-chloromercuriphenyl-sulfonic acid) on the NaCl-induced inactivation of the oxygen-evolving machinery in intact cells. Cells were incubated with 0.5 M NaCl at 32°C in the presence of 100 μM phenytoin, or of 100 μM phenytoin plus 100 μM *p*-chloromercuriphenyl-sulfonic acid, or in their absence. At designated times, aliquots were withdrawn and oxygen evolution was measured at 32°C after addition of 1.0 mM BQ. The activity that corresponded to 100% was 572 ± 37 μmol O_2 mg^{-1} Chl h^{-1}. ○, Cells in the presence of 0.5 M NaCl; □, cells in the presence of 0.5 M NaCl and 100 μM phenytoin; △, cells in the presence of 0.5 M NaCl and 100 μM *p*-chloromercuriphenyl-sulfonic acid; ◇, cells in the presence of 0.5 M NaCl, 100 μM phenytoin, and 100 μM *p*-chloromercuriphenyl-sulfonic acid. Black symbols correspond to white symbols of the same shapes but in the absence of added NaCl. Each point and bar represent the average ± SE of results from five independent experiments.

Thus, the PSI-mediated transport of electrons in intact cells was sensitive to salt stress, albeit to a lesser extent than the oxygen-evolving activity of PSII.

Reversibility of the NaCl-Induced Inactivation of the PSI-Mediated Transport of Electrons

The possible reversibility of the effects of NaCl on PSI in intact cells was examined. When cells were washed after a 1.5-h incubation with 0.5 M NaCl, full recovery of PSI-mediated electron transport activity occurred in fresh medium (data not shown). When cells were incubated for longer periods with 0.5 M NaCl, the inhibition of PSI electron transport could not be reversed, and increased with time of incubation with NaCl (data not shown). These observations suggested that the rapid decline in PSI activity was reversible and, thus, similar to that caused by osmotic stress (Allakhverdiev et al., 2000), whereas the slow irreversible decline was due to ionic effects of NaCl.

Effects of Channel Blockers on the NaCl-Induced Inactivation of PSI Activity

The effects of various channel blockers on the NaCl-induced inactivation of PSI were examined. The effects on oxygen uptake of 0.5 M NaCl were significantly suppressed by 100 μM phenytoin (Fig. 6). Furthermore, the extent of the NaCl-induced inactivation of PSI was significantly reduced in the presence of both *p*-chloromercuriphenyl-sulfonic acid and phenytoin (Fig. 6).

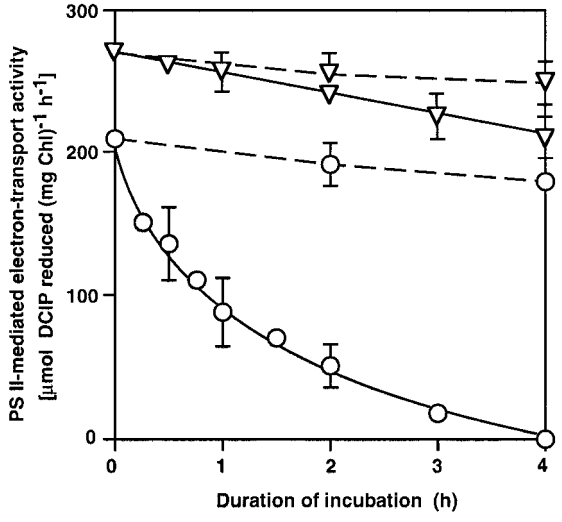

Figure 4. Changes in PSII-mediated electron transport activity in isolated thylakoid membranes during incubation with NaCl. Thylakoid membranes (10 μg Chl mL^{-1}) were incubated at 32°C in the presence of 0.5 M NaCl and in its absence. At designated times, aliquots were withdrawn and PSII-mediated electron transport activity from water to DCIP (○) and from DPC to DCIP (▽) was measured at 25°C by monitoring the light-induced reduction of DCIP after addition of 0.1 mM DCIP or 0.1 mM DCIP plus 0.5 mM DPC. Solid line, In the presence of 0.5 M NaCl; dashed line, in the absence of NaCl. Each point and bar represent the average ± SE of results from five independent experiments.

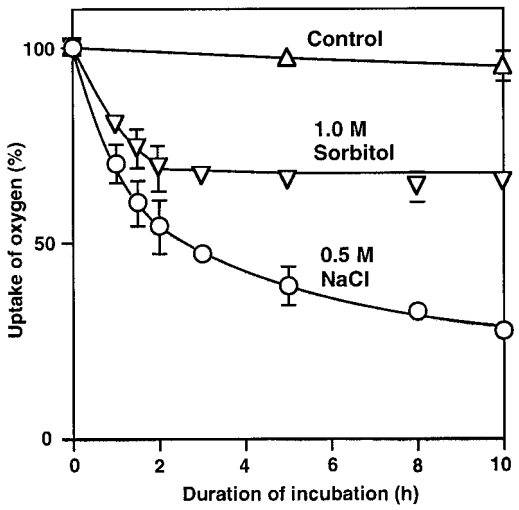

Figure 5. Changes in the photosynthetic electron transport activity of PSI in intact cells during incubation with NaCl or sorbitol. Cells were incubated in the presence of 0.5 NaCl or 1.0 M sorbitol or in their absence at 32°C. At designated times, aliquots were withdrawn and activity of the PSI complex was monitored at 32°C by measuring the uptake of oxygen after addition of 15 μM DCMU, 0.1 mM DCIP, 5 mM sodium ascorbate, and 0.1 mM MV. The oxygen-uptake activity that corresponded to 100% was 312 ± 46 μmol O_2 mg^{-1} Chl h^{-1}. △, Control (no addition); ○, 0.5 M NaCl; ▽, 1.0 M sorbitol. Each point and bar represent the average ± SE of results from four independent experiments.

NaCl-Induced Inactivation of PSI-Mediated Transport of Electrons in Vitro

To examine differences between PSII and PSI in terms of tolerance to salt stress in vitro, we also monitored the PSI-mediated transport of electrons in isolated thylakoid membranes. Figure 7 illustrates the effects of salt stress on the PSI-driven transport of electrons from reduced DCIP to methyl viologen (MV). During incubation of isolated thylakoid membranes for 4 h in the presence of 0.5 M NaCl in darkness, PSI activity decreased by 45%. In the absence of NaCl, nearly 90% of the activity of PSI remained after a similar incubation (Fig. 7). Thus, the PSI-mediated transport of electrons in isolated thylakoid membranes was inactivated by salt stress.

Effects of Salt Stress on Cytoplasmic Volume

Changes in cytoplasmic volume during incubation with 0.5 M NaCl were examined by monitoring electron paramagnetic resonance signals. After incubation for 2 h in medium that contained 0.5 M NaCl, cytoplasmic volume fell by 25% to 30% and then it gradually decreased to 55% of the initial volume in 10 h (Fig. 8). In the presence of 100 μM phenytoin,

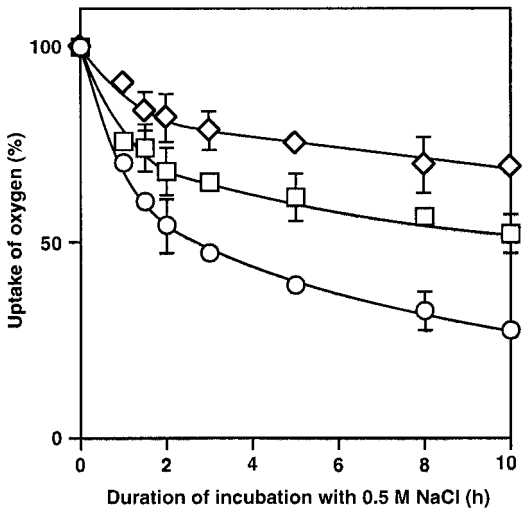

Figure 6. Effects of a Na^+-channel blocker (phenytoin) and a water-channel blocker (*p*-chloromercuriphenyl-sulfonic acid) on the NaCl-induced inactivation of the PSI complex in intact cells. Cells were incubated in the presence of 0.5 M NaCl at 32°C with 100 μM phenytoin or with 100 μM phenytoin plus 100 μM *p*-chloromercuriphenyl-sulfonic acid, or in their absence. At designated times, aliquots were withdrawn and the activity of PSI was monitored by measuring the uptake of oxygen at 32°C after addition of 15 μM DCMU, 0.1 mM DCIP, 5 mM sodium ascorbate, and 0.1 mM MV. The oxygen uptake activity that corresponded to 100% was 337 ± 45 μmol O_2 mg^{-1} Chl h^{-1}. ○, Cells in the presence of 0.5 M NaCl; □, cells in the presence of 0.5 M NaCl and 100 μM phenytoin; ◇, cells in the presence of 0.5 M NaCl, 100 μM phenytoin, and 100 μM *p*-chloromercuriphenyl-sulfonic acid. Each point and bar represent the average ± SE of results from five independent experiments.

cytoplasmic volume fell by 45% to 50% and 60% during incubation with 0.5 M NaCl for 2 and 10 h, respectively, suggesting that this Na^+-channel blocker enhanced the NaCl-induced decrease in cytoplasmic volume. By contrast, the decrease in cytoplasmic volume in response to salt stress was significantly minimized when both 100 μM *p*-chloromercuriphenyl-sulfonic acid (water-channel blocker) and 100 μM phenytoin (Na^+-channel blocker) were included in incubation medium (Fig. 8).

NaCl-Induced Decreases in Na^+/H^+ Antiport Activities

Na^+/H^+ exchange in intact cells was monitored by a fluorometric method using acridine orange. When cells were incubated with 0.5 M NaCl, the Na^+/H^+ antiport activity decreased, whereas it remained close to the maximum level for 10 h in the absence of NaCl or in the presence of 1.0 M sorbitol (Fig. 9). These findings suggested that NaCl, but not sorbitol, inactivated the Na^+/H^+ antiport activity.

DISCUSSION

The present study has demonstrated that salt stress due to 0.5 M NaCl inactivated both the PSII- and PSI-mediated electron transport (Figs. 1–3, 5, and 6). The NaCl-induced inactivation involved rapid and slow phases, with one-half-decay times of about 1 and 5 h, respectively. Since NaCl has both osmotic and ionic effects (Joset et al., 1996; Hagemann and Erdmann, 1997; Hayashi and Murata, 1998), it was necessary to analyze these effects separately. To mimic the osmotic effects of NaCl we used sorbitol at 1.0 M, which has approximately the same osmotic effect as NaCl at 0.5 M.

The rapid phase of the NaCl-induced inactivation of PSII and PSI (Figs. 1–3, 5, and 6) appeared to correspond to the time course of osmotic stress-induced inactivation (Allakhverdiev et al., 2000), suggesting that the rapid decline in the activities of PSII and PSI might have been caused by osmotic pressure. The slow phase, which occurred in the presence of NaCl but not of sorbitol, appeared to be specific to ionic effects, as verified with specific channel blockers. The blockers of ion channels protected PSII and PSI against the NaCl-induced slow inactivation, but not against the rapid inactivation. In their presence, the NaCl-induced inactivation resembled the sorbitol-induced inactivation (Figs. 1, 3, 5, and 6). Since the osmotic effect was reversible but the ionic effect was irreversible (Fig. 2), it is likely that Na^+ ions damaged the machinery that is necessary for the recovery of PSII from NaCl-induced damage.

We examined the oxygen-evolving activity of intact cells in the presence of BQ as an artificial acceptor of electrons (Figs. 1–3). In this system, electrons are transported from water to BQ through the Mn clus-

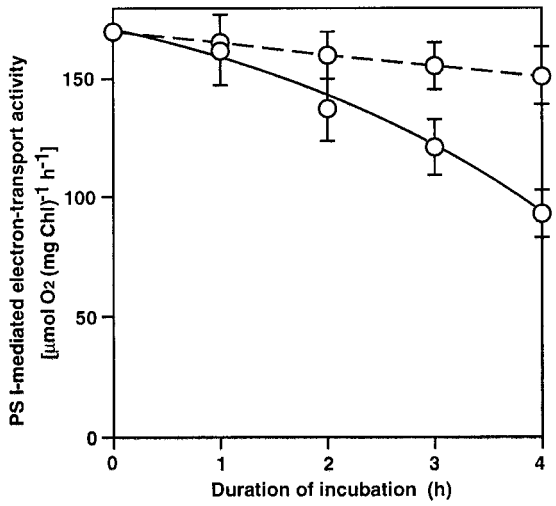

Figure 7. Changes in PSI-mediated electron transport activity in isolated thylakoid membranes during incubation with NaCl. Thylakoid membranes (10 μg Chl mL^{-1}) were incubated at 32°C in the presence of 0.5 M NaCl and in its absence. At designated times, aliquots were withdrawn and transport of electrons by PSI from reduced DCIP to MV was measured at 25°C by monitoring the light-induced uptake of oxygen after addition of 15 μM DCMU, 0.1 mM DCIP, 5 mM sodium ascorbate, and 0.1 mM MV. Solid line, In the presence of 0.5 M NaCl; dashed line, in the absence of NaCl. Each point and bar represent the average ± SE of results from four independent experiments.

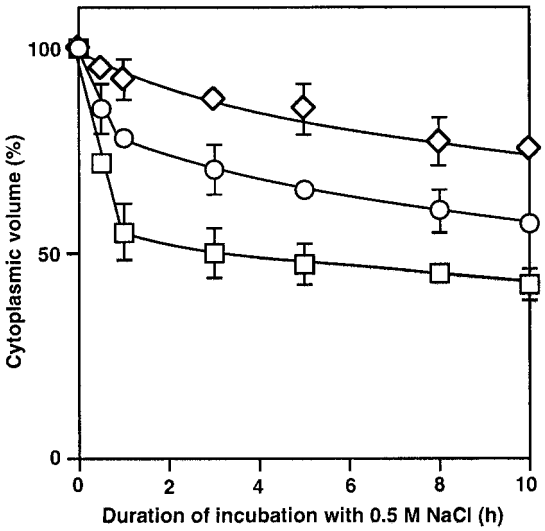

Figure 8. Effects of a Na^+-channel blocker (phenytoin) and a water-channel blocker (*p*-chloromercuriphenyl-sulfonic acid) on cytoplasmic volume during incubation with NaCl. Cells were incubated with 0.5 M NaCl at 32°C in the presence of phenytoin or of *p*-chloromercuriphenyl-sulfonic acid or in their absence. At designated times, aliquots were withdrawn and cytoplasmic volume was determined. The cytoplasmic volume that corresponded to 100% was 0.75 ± 0.05 fL. ○, In the presence of 0.5 M NaCl; □, in the presence of 0.5 M NaCl and 100 μM phenytoin; ◇, in the presence of 0.5 M NaCl, 100 μM phenytoin, and 100 μM *p*-chloromercuriphenyl-sulfonic acid. Each point and bar represent the average ± SE of results from five independent experiments.

ter, P680 (a form of Chl at the photochemical reaction center), pheophytin *a*, Q_A (the primary electron acceptor of plastoquinone), and Q_B (the secondary electron acceptor of plastoquinone). Our analysis of Chl fluorescence suggested that the photochemical reaction center complex that includes Q_A, pheophytin, and P680 was undamaged in NaCl-treated cells. Therefore, it is likely that the transport of electrons from water to P680 was blocked in such cells.

NaCl interfered with the PSII-mediated transport of electrons from water to DCIP, but not from DPC to DCIP (Figs. 4 and 7). It is likely that the oxygen-evolving machinery in PSII was damaged by the ionic effects. These findings are consistent with results obtained with *Synechocystis* sp., where exposure of intact cells and isolated thylakoid membranes to salt stress inactivated the oxygen-evolving machinery of PSII (Allakhverdiev et al., 1999).

The ion channels and water channels in *Synechococcus* sp. have not been fully characterized. However, Kaneko et al. (1996) analyzed the *apqZ* gene for the water channel in *Synechocystis* sp. A gene homologous of the *apqZ* gene has been found in the genome of *Synechococcus* sp. (M. Sugita, personal communication). There are at least three putative genes for $K^+(Na^+)$ channels in *Synechocystis* sp. (Kaneko et al., 1996) and we can assume that $K^+(Na^+)$ channels are also present in *Synechococcus* sp.

Salt stress due to 0.5 M NaCl decreased the cytoplasmic volume by about 25%, and such shrinkage was enhanced by a Na^+-channel blocker (Fig. 8). The time course of shrinkage in the presence of the Na^+-channel blocker was similar to that due to the effects of osmotic stress caused by the presence of 1.0 M sorbitol (Allakhverdiev et al., 2000). The water-channel blocker *p*-chloromercuriphenyl-sulfonic acid (Pfeuffer et al., 1998; Tyerman et al., 1999, and refs. therein), when applied together with the Na^+-channel blocker phenytoin (Muramatsu et al., 1990; Ju et al., 1992), markedly suppressed cell shrinkage (Fig. 8). The water-channel blocker, the Na^+-channel blocker, and a blocker of K^+-channels, tetraethylammonium chloride (Schroeder, 1988; Murata et al., 1994; Tyerman et al., 1997; Gaymard et al., 1998; Zhang and Tyerman, 1999), also markedly suppressed the NaCl-induced inactivation of photosynthetic activities (Figs. 3 and 6). These observations suggest that the initial event after the onset of salt stress due to 0.5 M NaCl might be the influx of Na^+ ions through K^+ channels and the efflux of water through water channels, both located in the plasma membrane. These events might increase the intracellular concentrations of Na^+, K^+ and, possibly, Cl^- ions, leading to inactivation of PSI and PSII. We demonstrated previously that increases in the concentration of NaCl inactivate the oxygen-evolving

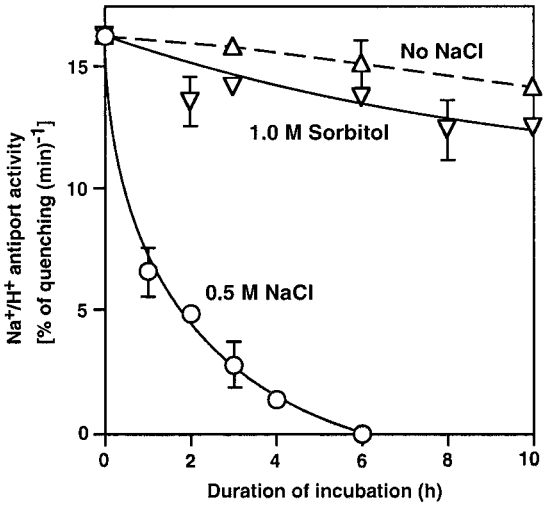

Figure 9. Changes in Na^+/H^+ antiport activity during incubation with NaCl or sorbitol. Cells (200 μg Chl mL^{-1}) were incubated in the presence of 0.5 M NaCl or of 1.0 M sorbitol or in their absence. At designated times, 20-μL aliquots were withdrawn and diluted 100-fold with Na^+-free medium that contained 5 μM acridine orange. Then the Na^+/H^+ antiport activity was measured as described previously (Allakhverdiev et al., 1999). The Na^+/H^+ antiport activity was calculated from the initial rate of recovery of fluorescence quenching upon addition of NaCl, divided by the difference between the level of fluorescence before the addition of NaCl and the steady-state level of fluorescence 1 min after addition of Triton X-100. △, Control (no addition); ▽, in the presence of 1.0 M sorbitol; ○, in the presence of 0.5 M NaCl. Each point and bar represent the average ± SE of results from four independent experiments.

PSII complex in vitro (Kuwabara and Murata, 1983; Miyao and Murata, 1983; Murata and Miyao 1985).

In a previous study (Allakhverdiev et al., 1999), we demonstrated that the tolerance of *Synechocystis* sp. to salt stress from NaCl is related to the activity of Na^+/H^+ antiporters. The present study demonstrated that the decrease in the activity of Na^+/H^+ antiporters in *Synechococcus* sp. in 0.5 M NaCl was caused by the ionic, and not the osmotic effects, of NaCl (Fig. 9). The inactivation of Na^+/H^+ antiporters might be involved in the NaCl-induced inactivation of PSI and PSII.

A hypothetical model that might explain the NaCl-induced inactivation of the photosynthetic machinery is shown in Figure 10. $K^+(Na^+)$ channels and water channels are located in the plasma membrane. The oxygen-evolving machinery of PSII is located on the lumenal side of thylakoid membranes. In cyanobacteria this machinery is stabilized by three extrinsic proteins: a 33-kD protein, cytochrome c_{550}, and PsbU (Enami et al., 1998; Shen et al., 1998; Nishiyama et al., 1999). Cytochrome c_{550} and PsbU are loosely bound on the donor side of the core complex of PSII (Nishiyama et al., 1999). These proteins are easily dissociated from the cyanobacterial PSII complex in the presence of high concentrations of salts (Stewart et al., 1985; Shen et al., 1992). When the extracellular concentration of Na^+ ions increases, about 25% of the water in intracellular spaces leaks out of the cell through water channels and Na^+ ions flow into the cytoplasm, with a resultant increase in cytosolic concentrations of Na^+ and K^+ ions. The Na^+/H^+ antiport system, which is assumed to pump Na^+ ions out of the cell to maintain an appropriately low concentration of Na^+ ions in the cytosol, is rapidly inactivated during incubation with NaCl. As a consequence, the Na^+/H^+ antiport system becomes inoperative, with a resultant increase in the cytosolic concentration of Na^+ ions. Na^+ ions then leak through the thylakoid membranes to increase the concentration of Na^+ ions in the intrathylakoid space (lumen). As a result, extrinsic proteins dissociate from PSII and the oxygen-evolving machinery is partially inactivated. A similar mechanism can be postulated for the NaCl-induced inactivation of PSI. An increase in the intrathylakoid concentration of Na^+ ions might lead to the dissociation of plastocyanin or cytochrome c_{553} from the PSI complex, causing a decrease in the rate of PSI-mediated electron transport.

MATERIALS AND METHODS

Growth Conditions and Exposure of Cells to Salt Stress

A strain of *Synechococcus* sp. PCC 7942 was obtained from W.E. Borrias (University of Utrecht, The Netherlands). Cells were grown photoautotrophically in glass tubes (80-mL) at 32°C under constant illumination at 70 μmol m^{-2} s^{-1} from incandescent lamps in BG-11 medium (Stanier et al., 1971), which contained 20 mM Na^+ ions and was supplemented with 20 mM HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid]-NaOH (pH 7.5). Cultures were aerated with sterile air that contained 1% (v/v) CO_2 (Ono and Murata, 1981). After 4 d, cells were harvested by centrifugation at 9,000*g* for 10 min and resuspended in fresh BG-11 medium (pH 7.5) at a density of 10 μg Chl mL^{-1}. Cells were incubated in glass tubes (40-mL) with gentle stirring every 20 min at 32°C in darkness in the presence of 0.5 M NaCl, 0.5 M LiCl, or 1.0 M sorbitol or in their absence. At designated times, aliquots were withdrawn for analysis of reversibility from salt stress, and cells were washed twice with fresh BG-11 medium by centrifugation at 9,000*g* for 10

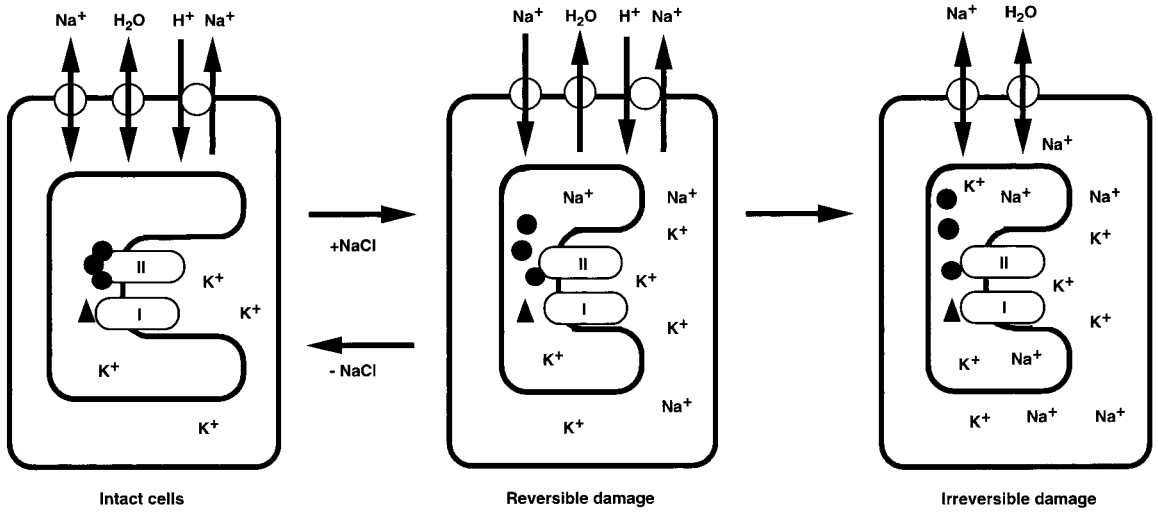

Figure 10. A hypothetical model of the NaCl-induced inactivation of PSI and PSII in cyanobacterial cells. ●, Extrinsic proteins of the oxygen-evolving machinery of PSII, namely, the 33-kD protein, cytochrome c_{550}, and PsbU; ▲, a protein associated with the PSI complex, namely, plastocyanin or cytochrome c_{553}. I, PSI complex; II, PSII complex.

min and resuspended. Finally, cells were suspended in fresh BG-11 medium.

Determination 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 (2 mmol 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 BQ or 1.0 mM 2,6-dichloro-1,4-benzoquinone as artificial acceptor of electrons. The electron transport activity of PSI was determined in the presence of 15 μM DCMU, 5 mM sodium ascorbate, 0.1 mM DCIP, and 0.1 mM MV (Allakhverdiev et al., 1999, 2000).

Thylakoid membranes were isolated from intact cells, as described previously (Allakhverdiev et al., 2000). The isolated thylakoid membranes were incubated at 32°C in darkness in 50 mM HEPES-NaOH (pH 7.5) that contained 400 mM Suc and 5 mM $CaCl_2$, and the light-induced reduction of DCIP was monitored at 25°C by following changes in A_{580}, with a reference beam at 500 nm, in a dual-wavelength spectrophotometer (UV-300, Shimadzu, Kyoto) as described previously (Murata et al., 1992; Allakhverdiev et al., 1999, 2000). The transport of electrons from water to DCIP was monitored in the presence of 0.1 mM DCIP and that from DPC to DCIP was monitored in the presence of 0.1 mM DCIP and 0.5 mM DPC.

Electron transport from reduced DCIP to MV (i.e. the activity of PSI) by thylakoid membranes was measured at 25°C by monitoring the uptake of oxygen with the Clark-type oxygen electrode in the same reaction mixture as described above after the addition of 15 μM DCMU, 5 mM sodium ascorbate, 0.1 mM DCIP, and 0.1 mM MV (Allakhverdiev et al., 2000). Concentrations of Chl were determined as described by Arnon et al. (1974).

Measurement of Chl Fluorescence

The yield of Chl fluorescence from intact cells was measured with a pulse amplitude modulation fluorometer (PAM-101, Walz, Effeltrich, Germany) according to Schreiber et al. (1993) in the presence and absence of dithionite at 1 mg mL^{-1} (Allakhverdiev et al., 2000).

Measurement of Cytoplasmic Volume and Na^+/H^+ Antiport Activity

Cytoplasmic volume was determined by electron paramagnetic resonance spectroscopy as described previously (Blumwald et al., 1983), with minor modifications (Allakhverdiev et al., 2000). The Na^+/H^+ antiport activity of intact cells was determined by monitoring the fluorescence of acridine orange as described previously (Blumwald et al., 1984; Allakhverdiev et al., 1999).

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