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Synergistic action of light and salt stress to impair photosystem II by inhibition of the expression of *psbA* genes

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Introduction

Salt stress causes a decrease in the photosynthetic activity in higher-plant and algal cells. We demonstrated recently that exposure of *Synechococcus* sp. PCC 7942 (hereafter *Synechococcus*) to salt stress (0.5 M NaCl) or to hyperosmotic stress (1.0 M sorbitol) inhibits the activity of photosystem II (PSII) by inhibiting, in particular, the oxygen-evolving activity of PSII (Allakhverdiev et al., 2000a,b). The hyperosmotic effect of 1.0 M sorbitol decreases the volume of the cytoplasm via the efflux of water through water channels with reversible inactivation of the oxygen-evolving machinery (Allakhverdiev et al., 2000a). By contrast, 0.5 M NaCl does not cause a significant decrease in the volume of the cytoplasm but the ionic effects of Na^+ ions irreversibly damage the oxygen-evolving machinery when these ions leak into the cytoplasm through $K^+(Na^+)$ channels (Allakhverdiev et al., 2000b). It is likely that the ionic effects are also responsible for inhibition of the synthesis of proteins, in particular, the components of the Na^+/H^+ antiport system. This inhibition might lead to an increase in the intracellular concentration of NaCl, which would further inhibit the synthesis *de novo* of the components of the Na^+/H^+ antiport system (Allakhverdiev et al., 2000a,b).

Synechocystis sp. PCC 6803 (hereafter *Synechocystis*) is much more resistant to NaCl than is *Synechococcus*. The oxygen-evolving machinery of *Synechocystis* is almost completely inactivated during incubation of cells with 1.0 M NaCl in darkness for 15 h, but not at all with 0.5 M NaCl (Allakhverdiev et al.,1999), whereas that of *Synechococcus* is completely inactivated after incubation with only 0.5 M NaCl for a mere 3 h (Allakhverdiev et al., 2000b). The strong tolerance of *Synechocystis* to salt stress might be due to the active extrusion of Na^+/H^+ ions from cells, namely, to the activities of the Na^+/H^+ antiport system, as well as to the accumulation of compatible solutes such as glucosylglycerol (Hagemann and Erdmann).

The studies of salt stress whose results are summarized above were performed in darkness and not much is known about the effects of salt stress on the photosynthetic machinery under strong light, which, by itself, damages PSII (Barber and Andersson 1992; Aro et al., 1993). It has been reported that salt stress enhances the inhibition by strong light of photosynthesis in *Chlamydomonas reinhardtii* (Neale and Melis, 1989) and in leaves of barley (*Hordeum vulgare* L.) and sorghum (*Sorghum bicolor* L.) (Sharma and Hall, 1991). However, the mechanism responsible for the effects of salt stress on the photoinhibition of PSII remains to be clarified.

In the present study, we investigated the interaction between the effects of light stress and salt stress on PSII in *Synechocystis*. We found that the combination of light and salt stress had a strong synergistic and damaging effect on PSII and, moreover, that salt stress inhibited the recovery of PSII from light-induced inactivation. Western and Northern blotting analyses suggested that the salt stress inhibited the expression of the *psbA* genes for pre-D1.

Materials and methods

Cells were grown photoautotrophically at 34°C under constant illumination from incandescent lamps at 70 μE m^{-2} s^{-1} in BG-11 medium (Stanier et al., 1971) supplemented with 20 mM Hepes-NaOH (pH 7.5). This medium contained 20 mM Na^+ ions, and is described below as low-salt medium. In contrast, a medium that contained added NaCl is described as high-salt medium. Cultures were aerated with sterile air that contained 1% CO_2 (Ono and Murata, 1981).

Cells from four-day-old cultures were harvested by centrifugation at 8,000 x *g* for 10 min at room temperature and resuspended in fresh BG-11 medium at a chlorophyll (Chl) concentration of 5 μg ml^{-1}. Then suspensions of cells were incubated at 34°C for 1 h in 100-ml glass tubes in growth chambers under conditions identical to the original culture conditions. Salt stress was applied by addition of NaCl at 0.5 or 1.0 M and light stress involved exposure to light at 500 or 2,000 μE m^{-2} s^{-1} In some experiments, protein synthesis was blocked by inclusion in the medium of 250 μg ml^{-1} lincomycin (Sigma Chemical Co., St. Louis, MO), which was added to the culture medium 10 min before the start of incubations.

The activity of PSII was measured in intact cells by monitoring the oxygen-evolving activity at 34°C with a Clark-type oxygen electrode (Hansatech Instruments, Kings Lynn, U.K.) in the presence of 1.0 mM 1,4-benzoquinone (BQ) which accepts electrons from PSII and inhibits respiration (Ono and Murata, 1981; Tasaka et al., 1996), as described previously (Allakhverdiev et al., 1999; 2000a,b). The sample, in a 3-ml cuvette, was illuminated by light that had been passed through a red optical filter (R-60; Toshiba, Tokyo, Japan) and an infrared-absorbing filter (HA-50; Hoya Glass, Tokyo). The intensity of light at the surface of the cuvette was 2,000 μE m^{-2} s^{-1}

Light-induced quenching of the fluorescence of Chl *a* due to reduction of pheophytin (Klimov et al., 1986; Allakhverdiev et al., 1988) in intact cells was monitored with a fluorometer (PAM-101; Heinz Walz, Effeltrich, Germany) in the pulse-amplitude modulation mode. The light-induced quenching of Chl fluorescence was measured at 25°C in the presence of 1 mg ml^{-1} sodium dithionite after the continuous exposure of the sample to actinic light (λ > 520 nm) from an incandescent lamp (KL-1500 Electronic; Schott Glasswerke, Wiesbaden, Germany) at 2,700 μE m^{-2} s^{-1}. The concentration of Chl was determined as described by Arnon et al. (1974).

Results and discussion

Exposure to light at 500 μE m^{-2} s^{-1} under low-salt conditions (20 mM NaCl) resulted in minimal inactivation of PSII: after incubation for 120 min, only about 10% of the original activity had disappeared. In the presence of 0.5 M NaCl, by contrast, inactivation occurred more rapidly and 50% of the original activity had disappeared after incubation for 120 min. In the presence of 1.0 M NaCl, the activity of PSII declined even more rapidly and no activity was detectable after 120 min (data not shown). In darkness, exposure of cells to 1.0 M NaCl did not result in any inactivation over the entire duration of the experiment. These results demonstrated that, while exposure of cells to either light stress or salt stress resulted in

minimal inactivation of PSII, the combination of the two kinds of stress induced marked inactivation of PSII, with apparent synergism between the effects of strong light and high salt.

To examine the contribution of protein synthesis *de novo* to the stress-induced inactivation of PSII, we incubated cells in darkness for 10 min in the presence of 250 μg ml^{-1} lincomycin, an inhibitor of protein synthesis, prior to exposure of cells to light at 500 μE m^{-2} s^{-1} in the presence of 20 mM, 0.5 M or 1.0 M NaCl. The inhibition of protein synthesis by lincomycin markedly accelerated the inactivation of PSII. The inactivation observed in the presence of lincomycin was unaffected by NaCl. Lincomycin had no effect on the inactivation of PSII when 1.0 M NaCl was also present in the medium. However, the extent of inactivation in the presence of lincomycin was only minimal when cells were incubated in the presence of 1.0 M NaCl in darkness. These observations suggested that protein synthesis *de novo* might be involved in the synergistic effects of light stress and salt stress during the inactivation of PSII.

We performed the same set of experiments with light at 250 and 2,000 μE m^{-2} s^{-1}. The rate of inactivation depended on the intensity of light but essentially the same results were obtained with respect to the synergistic effects of light stress and the salt stress.

To monitor the effects of NaCl on the recovery of PSII activity after cells had been exposed to light at 2,000 μE m^{-2} s^{-1} for 100 min, a treatment that reduced the activity of PSII to approximately 10% of the original level, we then incubated the cells in light at 70 μE m^{-2} s^{-1} for 4 h in the presence at various concentrations of NaCl. In low-salt medium (20 mM NaCl) the activity of PSII returned to 90% of the initial value within 2 h and recovery was complete within 3 h. When cells were incubated with 0.5 M NaCl, recovery was slow and only 60% of the original activity was restored after 4 h. However, in the presence of either 1.0 M NaCl or 250 μg ml^{-1} lincomycin, recovery was completely blocked. These results demonstrated that NaCl at the high concentrations inhibited the repair of PSII. This phenomenon might explain the apparent ability of NaCl to accelerate the light-induced damage to PSII.

To identify the site of damage to PSII, we monitored the light-induced quenching of Chl fluorescence in the presence of dithionite. Such quenching corresponds to the reduction of pheophytin *a* in the photochemical reaction center complex in intact cells (Klimov et al., 1986; Allakhverdiev et al., 1988; Ke, 2001). When *Synechocystis* cells were exposed for 150 min to light at 500 μE m^{-2} s^{-1} in the presence of 1.0 M NaCl, the extent of the light-induced quenching decreased to 20% of the original level; in low-salt medium (20 mM NaCl) there was no detectable decrease in light-induced quenching.

We also examined the effects of NaCl on the recovery of the light-induced quenching of Chl fluorescence after cells had been exposed to light at 2,000 μE m^{-2} s^{-1} for 100 min. In low-salt medium, the light-induced quenching returned to normal within 2 h. However, in the presence of 1.0 M NaCl, such recovery was completely suppressed. These results suggested that the site of damage to PSII under light and salt stress might be the photochemical reaction center.

References

Allakhverdiev SI, Klimov VV, Ladygin VG (1988) *Biofizika* **33,** 442-447.

Allakhverdiev SI, Nishiyama Y, Suzuki I, Tasaka Y, Murata N (1999) *Proceeding of the National Academy of Sciences* **96,** 5862-5867.

Allakhverdiev SI, Sakamoto A, Nishiyama Y, Murata N (2000a) *Plant Physiology* **122,** 1201-1208.

Allakhverdiev SI, Sakamoto A, Nishiyama Y, Inaba M, Murata N (2000b) *Plant Physiology* **123,** 1047-1056.

Arnon DI, McSwain BD, Tsujimoto HY, Wada K (1974) *Biochimica et Biophysica Acta* 357, 231-245.

Aro EM, Virgin I, Andersson B (1993) *Biochimica et Biophysica Acta* **1143,** 113-134.

Barber J, Andersson B (1992) *Trends in Biochemical Sciences* **17,** 61-66.
Hagemann M, Erdmann N (1997) In Cyanobacterial Nitrogen Metabolism and Environmental Biotechnology, Rai AK, ed (Springer-Verlag, Heidelberg; Narosa Publishing House, New Delhi), pp. 156-221.
Ke B (2001) In Photosynthesis. Photobiochemistry and Photobiophysics, Ke B, ed, Vol. 10, (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 305-322.
Klimov VV, Allakhverdiev SI, Ladygin VG (1986) *Photosynthesis Research* **10,** 355-361.
Neale PJ, Melis A (1989) *Journal of Plant Physiology* **134,** 619-622.
Ono T, Murata N (1981) *Plant Physiology* **67,** 176-181.
Sharma PK, Hall DO (1991) *Journal of Plant Physiology* **138,** 614-619.
Stanier RY, Kunisawa R, Mandel M, Cohen-Bazire G (1971) Bacteriological Reviews **35,** 171-205.
Tasaka Y, Gombos Z, Nishiyama Y, Mohanty P, Ohba T, Ohki K, Murata N (1996) *EMBO Journal* **15,** 6416-6425.