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Cellular energization protects the photosynthetic machinery against salt-induced inactivation in *Synechococcus*

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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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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