Journal of Plant Physiology 167 (2010) 951–958

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Salt stress induces a decrease in excitation energy transfer from phycobilisomes to photosystem II but an increase to photosystem I in the cyanobacterium *Spirulina platensis*

Tao Zhang, Hongmei Gong, Xiaogang Wen, Congming Lu *

Photosynthesis Research Center, Key Laboratory of Photobiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, PR China

ARTICLE INFO

Article history:
Received 5 November 2009
Received in revised form 2 December 2009
Accepted 2 December 2009

Keywords:
Chlorophyll fluorescence
Excitation energy transfer
Photosystem II photochemistry
Salt stress
Spirulina platensis

ABSTRACT

The effects of salt stress (0–0.8 M NaCl) on excitation energy transfer from phycobilisomes to photosystem I (PSI) and photosystem II (PSII) in the cyanobacterium *Spirulina platensis* were investigated. Salt stress resulted in a significant decrease in photosynthetic oxygen evolution activity and PSII electron transport activity, but a significant increase in PSI electron transport activity. Analyses of the polyphasic fluorescence transients (OJIP) showed that, with an increase in salt concentration, the fluorescence yield at the phases J, I and P declined considerably and the transient almost leveled off at 0.8 M NaCl. Analyses of the JIP test demonstrated that salt stress led to a decrease in the maximal efficiency of PSII photochemistry, the probability of electron transfer beyond Q_A, and the yield of electron transport beyond Q_A. In addition, salt stress resulted in a decrease in the electron transport per PSII reaction center, but an increase in the absorption per PSII reaction center. However, there was no significant change in the trapping per PSII reaction center. Furthermore, there was a decrease in the concentration of the active PSII reaction centers. Analyses of 77 K chlorophyll fluorescence emission spectra excited either at 436 or 580 nm showed that salt stress inhibited excitation energy transfer from phycobilisomes to PSII but induced an increase in the efficiency of energy transfer from phycobilisomes to PSI. Based on these results, it is suggested that, through a down-regulation of PSII reaction centers and a shift of excitation energy transfer in favor of PSI, the PSII apparatus was protected from excess excitation energy.

Introduction

Salt stress is a major environmental factor that limits plant growth and productivity (Boyer, 1982; Pitman and Läuchli, 2002). Reductions in plant growth under salt stress conditions are often associated with a decrease in photosynthetic activity and often occurs in many plants (Munns et al., 2006; Chaves et al., 2009). The decrease in photosynthetic activity commonly observed under salt stress may be due to limitations in photosynthetic electron transport and partial stomatal closure (Flexas et al., 2004; Zurita et al., 2005; Stepien and Johnson, 2009). Although the effects of salt stress on photosynthesis have been studied intensively, the mechanisms of inhibition of photosynthesis by salt stress remain unclear (Allakhverdiev and Murata, 2008; Munns and Tester, 2008).

Cyanobacteria provide suitable model systems for investigating the effects of environmental stresses on photosynthesis (Allakhverdiev and Murata, 2008). This is because cyanobacteria perform oxygenic photosynthesis using a photosynthetic apparatus similar to that found in chloroplasts of higher plants and algae (Öquist et al., 1995). In addition, cyanobacterial cells can easily be exposed directly to defined stress conditions in culture (Joset et al., 1996; Hagemann and Erdmann, 1997). Moreover, cyanobacteria are able to acclimate to a wide range of environmental stresses (Nishida and Murata, 1996; Hagemann and Erdmann, 1997).

In cyanobacteria, it has been shown that photosynthetic oxygen evolution is inhibited by salt stress (Vonshak et al., 1988; Kirst, 1990). Such a decrease can be associated with the state-2 transition and a decrease in PSII activity (Schubert and Hagemann, 1990; Schubert et al., 1993; Allakhverdiev and Murata, 2004; Allakhverdiev et al., 2005; Sudhir et al., 2005). It has also been reported that salt stress inhibits photosystem I (PSI) activity (Allakhverdiev et al., 2000, 2005; Allakhverdiev and Murata, 2008).

Abbreviations: ABS, absorption; APC, allophycocyanin; Chl, chlorophyll; CPC, C-phycocyanin; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; ET, energy flux for electron transport; PBS, phycobilisome; PSI, photosystem I; PSII, photosystem II; Q_A, primary quinine electron acceptor of PSII; Q_B, secondary quinone electron acceptor of PSII; TR, energy flux for trapping; ψ_o, probability of electron transport beyond Q_A; φ_{Eo}, maximum yield of electron transport beyond Q_A; φ_{po}, maximal efficiency of PSII photochemistry.

* Corresponding author at: Photosynthesis Research Center, Institute of Botany, Chinese Academy of Sciences, 20# Nanxincun, Xiangshan, Haidian District, Beijing 100093, PR China. Tel.: +86 10 6283 6554; fax: +86 10 6259 5516.
E-mail address: lucm@ibcas.ac.cn (C. Lu).

0176-1617/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2009.12.020

On the other hand, it has been shown that salt stress has no effect on PSII activity when *Synechocystis* cells are exposed to high salinity (0.55 M NaCl) (Jeanjean et al., 1993). Our previous studies have shown that salt stress significantly inhibits PSII activity in *Spirulina platensis*. This is due to an inactivation of PSII reaction centers (Lu and Vonshak, 1999; Lu et al., 1999; Lu and Vonshak, 2002). In addition, our studies have shown that the inhibition of PSII activity by salt stress is affected significantly by light intensity (Lu et al., 1999). Salt stress results in a greater decrease in PSII activity in *S. platensis* cells when exposed to higher light intensity (Lu and Zhang, 1999, 2000). In *Synechocystis*, it has also been reported that the combination of light and salt stress has a strong synergistic and damaging effect on PSII, which is due to the fact that salt stress inhibits the recovery of PSII from light-induced inactivation (Allakhverdiev et al., 2002). Although the possible mechanisms for inhibited PSII activity in salt-stressed cyanobacterial cells have been investigated in several studies (Allakhverdiev et al., 2002; Marin et al., 2004; Ohnishi and Murata, 2006), how salt stress affects excitation energy transfer from phycobilisomes (PBS) to PSII and PSI remains unclear.

S. platensis, a filamentous cyanobacterium, has been used in outdoor cultivation for commercial biomass production owing to its high protein content and other nutritional elements (Vonshak, 1990; Borowitzka, 1994). *S. platensis* has been isolated from a wide range of habitats varying greatly in salinity (Ciferri, 1983). In cultures grown outdoors in open ponds under arid and semiarid climates, daily evaporation amounts to 1–2 cm of the water level, leading to a progressive increase in the salt concentration in the culture, and the cells are thus subjected to salinity stress (Vonshak, 1987). Obviously, a better understanding of salt stress on photosynthesis should help optimize the productivity of the algal cultures grown outdoors.

The objective of this study was to investigate how salt stress affects excitation energy transfer from PBS to PSII and PSI in the cyanobacterium *S. platensis*. To this end, we investigated the changes in 77 K chlorophyll (Chl) fluorescence spectroscopy, polyphasic Chl fluorescence rise transients, and PSI and PSII electron transport activities in this alga during salt stress.

Materials and methods

Alga and growth conditions

The cyanobacterium *Spirulina platensis* M_2 was grown at 30 °C in Zarouk's medium supplemented with 0.2 M sodium bicarbonate (Vonshak et al., 1982). Illumination of 100 μmol photons m^{-2} s^{-1} was provided by cool daylight tubes (TLD 30W/865, Philips).

Salt stress treatment

Exponentially grown cells were harvested and resuspended in a fresh medium containing different concentrations of NaCl (0.2, 0.4, 0.6, 0.8 M exclusive of 0.017 M NaCl already present in the medium) and incubated at 30 °C for 12 h at growing light intensity (100 μmol m^{-2} s^{-1}).

Measurements of photosynthetic oxygen evolution

Photosynthetic oxygen evolution activity was measured at 30 °C using a Clarke-type electrode. Cells were harvested and resuspended in fresh medium containing the same NaCl concentration as that to which cells were adapted. Measuring light intensity was 1000 μmol m^{-2} s^{-1} using a 100 W Halogen lamp that was saturated for photosynthesis. The concentration of Chl for each sample was 5 μg ml^{-1}.

Assay of electron transport activities

PSI and PSII electron transport activities were assayed as described in our previous study (Lu and Vonshak, 1999). PSII activity was determined by O_2 evolution with 0.9 mM pBQ as an electron acceptor. PSI activity was measured as O_2 uptake in the presence of 0.1 mM 2,6-dichlorophenol indophenol (DCPIP), 0.1 mM MV, 5 mM NaN_3 as an inhibitor of respiration, 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) as an inhibitor of PSII, 5 mM ascorbate and 1 mM potassium cyanide as an inhibitor of superoxide dismutase.

Low-temperature Chl fluorescence emission spectra measurements

Low-temperature (77 K) Chl fluorescence emission spectra were recorded with a fluorescence spectrophotometer (Hitachi F-4500). The excitation wavelength was at 580 and 436 nm (slit 5 nm) and the emission was between 600 and 780 nm (slit 2.5 nm). For the measurements, the cell samples at a concentration of 5 μg Chl ml^{-1} were put into a cuvette and were then quickly dipped into liquid nitrogen. At the cell concentrations used, re-absorption of emitted Chl fluorescence was negligible. The Chl fluorescence data were analyzed with a Gaussian deconvolution program and the Chl fluorescence areas for all sub-bands were calculated.

Measurements of the polyphasic Chl a fluorescence transients and the analysis of the JIP test

The polyphasic Chl a fluorescence transients were measured as described in our previous studies (Lu et al., 1999). The polyphasic rise in fluorescence transients due to Chl a was measured by a Plant Efficiency Analyzer (PEA, Hansatech Instruments Ltd., King's Lynn, Norfolk PE32 1JL, England) with an actinic light of about 3000 μmol quanta m^{-2} s^{-1}. Using the theory of energy fluxes in biomembranes in a photosynthetic apparatus in combination with the data from measurements of the polyphasic rise of fluorescence transient, Strasser and Strasser (1995) developed the JIP test, in which the formulae for the calculation of the energy fluxes and for the flux ratios have been derived. According to the model of energy fluxes in this test, the photons absorbed by the antennae pigments are referred to as absorption flux (ABS). Part of this excitation energy is dissipated as fluorescence, but most of it is transferred as trapping flux (TR) to the reaction centers (RCs). In the RCs, the excitation energy is converted to redox energy by reducing primary quinine electron acceptor of PSII (Q_A) to Q_A^-, which is then reoxidized to Q_A, leading to an electron transport flux (ET), which maintains the metabolic reactions of photosynthetic apparatus. The detailed derivation for the formulae for the various energy fluxes and for the flux ratios in the JIP test is derived from Strasser and Strasser (1995) and Krüger et al. (1997).

All samples were dark-adapted for 10 min prior to measurement of fluorescence transients.

Results

Photosynthetic oxygen evolution

Fig. 1 shows the effects of salt stress on photosynthetic oxygen evolution in *S. platensis* cells. Photosynthetic oxygen evolution activity decreased significantly with increasing salt concentration. After *S. platensis* cells were exposed to 0.8 M NaCl for 12 h, photosynthetic oxygen evolution decreased to 200 μmol O_2 mg^{-1} Chl h^{-1} from 760 μmol O_2 mg^{-1} Chl h^{-1} in control cells.