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*

**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; Kirsch, 1990). Such a decrease can be associated with the state-2 transition and a decrease in PSI 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).
On the other hand, it has been shown that salt stress has no effect on PSI 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 PSI activity in *Spirulina platensis*. This is due to an inactivation of PSI reaction centers (Lu and Vonshak, 1999; Lu et al., 1999; Lu and Vonshak, 2002). In addition, our studies have shown that the inhibition of PSI activity by salt stress is affected significantly by light intensity (Lu et al., 1999).

Salt stress results in a greater decrease in PSI 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 PSI 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 PSI 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, 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* M2 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 O₂ evolution with 0.9 mM pBQ as an electron acceptor. PSI activity was measured as O₂ uptake in the presence of 0.1 mM 2,6-dichlorophenol indophenol (DCPIP), 0.1 mM MV, 5 mM NaN₃ 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 (QA) to QA⁻, which is then reoxidized to QA⁺, 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 O₂ mg⁻¹ Chl h⁻¹ from 760 μmol O₂ mg⁻¹ Chl h⁻¹ in control cells.
Fig. 1. Effects of salt stress on photosynthetic oxygen evolution activity in *S. platensis* cells exposed to different NaCl concentrations for 12 h. The data represent the mean ± SD of 4 independent measurements. Error bars are not shown if smaller than symbols.

Low-temperature (77 K) Chl fluorescence emission spectra

To investigate the effects of salt stress on excitation energy transfer between PBS and PSII and PSI, we examined the changes in the 77 K Chl fluorescence emission spectra in salt-stressed *S. platensis* cells. Fig. 2A shows Chl fluorescence emission spectra obtained with excitation at 436 nm, which was absorbed mainly by Chl a. The control cells exhibited the characteristic emission peaks at 695 and 725 nm. Two shoulders at around 685 and 751 nm were also visible. As can be seen in Fig. 2A, salt stress resulted in a decrease in the peak heights at 695 and 725 nm. Fig. 2B shows the effects of salt stress on Chl fluorescence emission spectra obtained with excitation at 580 nm, which is absorbed mainly by PBS. The control cells showed the characteristic emission peaks at 643, 664, 693, and 725 nm. In addition, there were two shoulders at around 685 and 751 nm. We observed that the peak heights at 643, 664, 693, and 725 nm decreased significantly with increasing salt concentration.

To reveal the parts of the photosynthetic apparatus that were responsible for the changes in the related Chl fluorescence emission peaks, we fitted the emission spectra by Gaussian components according to the assignments of emission bands to different components of the photosynthetic apparatus (Murata and Satoh, 1986; Krause and Weis, 1991; Govindjee, 1995; Gilmore et al., 2000). As shown in our previous study (Wen et al., 2005), a typical Gaussian deconvolution for Chl fluorescence spectra of *S. platensis* with excitation at 580 nm can be fitted well by 10 Gaussian sub-bands. The 643 and 664 nm emissions originate from C-phycocyanin (CPC) and allophycocyanin (APC), respectively. The 685 and 695 nm emissions originate from the core antenna complexes of PSII, CP43 and CP47, respectively. APC also contributed a small fraction to the Chl fluorescence at 685 nm. The 725 nm band is most effectively produced by PSI. The long-wavelength band at 751 nm is suggested to be emitted by a specific Chl form associated with PSI, which is characteristic of many cyanobacteria (Mohanty et al., 1997). The 600 and 620 nm bands originate from excitation light itself.

Fig. 3 shows the effects of salt stress on Chl fluorescence yields of F685, F695, F725, and F751 with excitation at 436 nm after *S. platensis* cells were exposed to different salt concentrations for 12 h. We observed that F685, F695 F725, and F751 decreased significantly with increasing salt concentration.

Fig. 4 shows the effects of salt stress on Chl fluorescence yields, i.e. F643, F664, F685, F695, F725, and F751 with excitation at 580 nm after *S. platensis* cells were exposed to different salt concentrations for 12 h. Our results show that there was no significant change in F664 with increasing salt concentration. F643, F685, and F751 decreased slightly with increasing salt concentration. However, F695 and F725 decreased considerably with increasing salt concentration.

To investigate the effects of salt stress on excitation energy transfer from PBS to PSI and PSII, we examined the changes in the ratio of Chl fluorescence yield of F725 in the spectra excited at 580 nm to that in the spectra excited at 436 nm, i.e. F725(580)/F725(436), as well as the ratio of Chl fluorescence yields of PBS to PSII, i.e. (F643 + F664)/(F685 + F695). Our results show that salt stress resulted in an increase in the ratio of F725(580)/F725(436) and (F643 + F664)/(F685 + F695) (Fig. 5). Since F725(580)/F725(436) has been considered an indicator of the efficiency of energy transfer from PBS to PSI (Misra and Mahajan, 2000), a significant increase in the ratio F725(580)/F725(436) indicates that salt stress led to an increase in the efficiency of energy transfer from PBS to PSI. An increase in the ratio of (F643 + F664)/(F685 + F695) suggests that salt stress resulted in a decrease in excitation energy transfer from PBS to PSII.
Fig. 3. Effects of salt stress on the Chl fluorescence yields at 77 K of F685 and F695 (A); F725 and F751 (B) with excitation at 436 nm in *S. platensis* cells exposed to different NaCl concentrations for 12 h. The yields were calculated as areas below the emission spectra normalized on Chl concentrations. Values represent mean ± SD of 5 independent measurements. Error bars are not shown if smaller than symbols.

**Polyphasic rise of Chl a fluorescence transient (OJIP) and the JIP test**

In order to evaluate the effect of salt stress on the electron transport of PSII and utilization efficiency per PSII reaction center, we determined the polyphasic rise of Chl a fluorescence transients in *S. platensis* cells after exposed to different NaCl concentrations (Fig. 6). In the absence of DCMU, the control cells exhibited a typical polyphasic rise of fluorescence induction, i.e. the OJIP fluorescence transient, similar to that described previously for plants, green algae and cyanobacteria (Srivastava et al., 1995, 1997, 1998; Strasser et al., 1999). Phases J, I and P occurred at about 2 ms, 30 ms, and 300 ms, respectively. The OJIP transient represents the successive reduction of the electron acceptor pools of PSII (Govindjee, 1995). Phase J reflects an accumulation of QA−QB (Lazar, 1999). Phase I reflects an accumulation of QA−QB−2 (Strasser et al., 1995). Fig. 6A shows that, with salt concentration increasing, the minimal fluorescence level (O) decreased slightly and the fluorescence yield at phases J, I and P decreased considerably. The transient almost leveled off in *S. platensis* cells when exposed to 0.8 M NaCl.

- In the presence of DCMU, there was a quick rise of fluorescence from phase O to J, the transient leveled off at phase J, and phases I and P were replaced by phase J in the control cells (Fig. 6B). This is because addition of DCMU results in a complete closure of PSII reaction centers during the first 2 ms of the fluorescence induction curve and an accumulation of QA− (Strasser et al., 1995). Fig. 6B shows that, with an increase in salt concentration, the minimal fluorescence level (O) decreased slightly and the fluorescence yield at phase J decreased significantly.

- By following the changes in the polyphasic Chl a fluorescence transients during salt stress, we investigated the changes in the maximal efficiency of PSII photochemistry (ϕpo) and electron transport in the acceptor side of PSII, which can be assessed by the probability of electron transfer beyond QA (ϕe) and the yield of electron transport beyond QA (ϕeo) (Fig. 7A–C). Fig. 7A shows that ϕpo decreased with an increase in salt concentration, either in the presence of DCMU or in the absence of DCMU. Fig. 7B and C shows that, in the absence of DCMU, ϕe and ϕeo decreased with an increase in salt concentration. In the presence of DCMU, the values of ϕe and ϕeo were very small; this is because electron transport at the acceptor side of PSII was inhibited by DCMU.

- To further evaluate the effects of salt stress on the primary photochemistry of PSII, we examined the absorption flux (ABS), trapping flux (TR) and electron transport flux (ET) per PSII reaction center (RC), i.e. ABS/RC, TR/RC, ET/RC (Fig. 7D–F). Our
Fig. 5. Effects of salt stress on (A) the ratio of Chl fluorescence yield of F725 in the spectra excited at 580 to that in the spectra excited at 436 nm, i.e. F725(580)/F725(436) and (B) the ratio of Chl fluorescence yields of PBS to PSII, i.e. (F643 + F664)/(F685 + F695), in \textit{S. platensis} cells exposed to different NaCl concentrations for 12 h. Values represent mean ± SD of 5 independent measurements.

Results show that ABS/RC did not change significantly at 0.2–0.6 M NaCl and increased significantly at 0.8 M NaCl either in the presence of DCMU or in the absence of DCMU. There was no significant change in TRo/RC with an increase in salt concentration, either in the presence or in the absence of DCMU. On the other hand, ETo/RC decreased with an increase in salt concentration in the absence of DCMU. In the presence of DCMU, the value of ETo/RC was small, which was due to the inhibition of electron transport at the acceptor side of PSII by DCMU.

• Using the JIP test, we also investigated the effects of salt stress on the concentration of the active PSI reaction centers (RC/CS). Our results show that RC/CS decreased with an increase in salt concentration either in the presence or in the absence of DCMU (Fig. 8).

**PSI and PSII electron transport**

We further investigated the effects of salt stress on PSI and PSII electron transport activities. PSI activity decreased, while PSII activity increased with an increase in the salt concentration (Fig. 9).

**Discussion**

Our previous study has shown that salt stress leads to an inhibition of electron transport at both donor and acceptor sides of PSII in \textit{S. platensis} (Lu and Avigad, 2002). Our recent study has further shown that the inhibition of electron transport at the acceptor is due to a modification of the Q\textsubscript{b} niche and damage to the donor side is associated with a dissociation of PsbO protein (Gong et al., 2008).

In the present study, we investigated how salt stress affects excitation energy transfer from PBS to PSI and PSII in the cyanobacterium \textit{S. platensis}.

Unlike other cyanobacteria, the cyanobacterium \textit{S. platensis} contains only two phycobiliproteins, allophycocyanin (APC) and C-phycocyanin (CPC) (Boussiba and Richmond, 1979). CPC is the major light-harvesting pigment protein present in the antenna rods of \textit{S. platensis}, while APC is a minor component present only at the core. Therefore, energy transfer in the PSII of \textit{S. platensis} proceeds in the direction from the tip to the core through CPC → APC → PSII core complexes (CP47 + CP43) → the PSII reaction center complexes.

In order to investigate how salt stress affects excitation energy transfer from PBS to PSII, we determined Chl fluorescence emission spectra with excitation at 580 nm that is absorbed mainly by PBS. Our results demonstrate that salt stress induced an increase in the ratio of (F643 + F664)/(F685 + F695), which indicates that salt stress resulted in a decrease in excitation energy transfer from PBS to PSII (Fig. 4). By determining room temperature fluorescence emission spectra of intact \textit{S. platensis} cells, Sudhir et al. (2005) observed that salt stress resulted in a decrease in the emission intensity at 654 and 680 nm, suggesting that salt stress resulted in a decrease in the excitation energy transfer from PBS to PSII. However, we observed that...
Fig. 7. Effects of salt stress on the maximal efficiency of PSII photochemistry ($\phi_{po}$, A), the probability of electron transport beyond $Q_a$ ($\psi_o$, B), the maximum yield of electron transport beyond $Q_a$ ($\phi_{Eo}$, C), the absorption flux per reaction center (ABS/RC, D), the trapping flux per reaction center (TR$_t$/RC, E), and the electron transport flux per reaction center (ET$_t$/RC, F) in *S. platensis* cells exposed to different NaCl concentrations for 12 h. Values represent mean ± SD of 5 independent measurements. Error bars are not shown if smaller than symbols.

There was no significant change in F664 in salt-stressed *S. platensis* cells (Fig. 4A). If salt stress resulted in a decrease in excitation energy transfer from APC to PSII core complexes, an increase in F664 should be expected. Thus, no significant change in F664 during salt stress suggests that salt stress has no effect on energy transfer from APC to PSII core complexes (CP47 + CP43).

How does salt stress affect excitation energy transfer between PSII core complexes (CP47 + CP43) and PSII reaction center complexes? To answer this question, we measured the 77 K Chl fluorescence emission spectra with excitation at 436 nm which was absorbed mainly by Chl a and thus rule out the contribution of fluorescence from APC to the Chl fluorescence at 685 nm (Gantt, 1981).

Our results show that F685 and F695 decreased significantly with increasing salt concentration as reflected in the changes in the 77 K Chl fluorescence spectra at excited at 436 nm (Fig. 3). F685 and F685 emit from CP43 and CP47 of the PSII core complex, respectively. Our previous study has shown that salt stress has no significant effect on the contents of CP47 and CP43 (Gong et al., 2008). If there were an interruption in energy transfer from CP43 and CP47 to the PSII reaction centers, an increase in F685 and F695 would be expected. The decrease in F685 and F695 observed in this study may suggest that salt stress has no effect on excitation energy transfer from CP43 and CP47 to the PSII reaction centers, i.e. that the decreased Chl fluorescence yield from CP43 and CP47 is not due to the decreased energy transfer from CP43 and CP47 to PSII reaction centers. Thus, our results suggest that a decrease in excitation energy transfer from PBS to PSII in salt stressed cells was not due to an inhibition of excitation energy transfer either from APC to PSII core complexes (CP47 + CP43) or from PSII core complexes (CP47 + CP43) to PSII reaction center complexes. Since we observed that there were a slight decrease in F643 and no significant change in F664, there should be no inhibition of excitation energy transfer from CPC to APC. Thus, the possibility for the decreased Chl fluorescence yields from CP43 and CP47 could be that salt stress results in a shift of excitation energy transfer in favor of PSI.

Our results show that salt stress resulted in a decrease in the maximal efficiency of PSII photochemistry ($\phi_{po}$) (Fig. 7). Our previous study has shown that the decrease in $\phi_{po}$ in salt-stressed cells was greater if light intensity was greater (Lu and Zhang, 2000), suggesting that the decrease in $\phi_{po}$ observed in this study was resulting from salt stress induced photoinhibition. Our previous study has also shown that there was a decrease in photochemical quenching ($q_p$) in salt-stressed cells, suggesting that PSII in salt-stressed cells is under high pressure of excitation energy (Lu and Vonshak, 2002). If excess excitation energy on PSII cannot be dissipated or redistributed, PSII in salt-stressed cells would be inevitably subjected to more photodamaged.

Here, we investigated whether salt stress induces a shift of excitation energy transfer from PSB to PSI. Salt stress resulted in a significant decrease in F725 and a slight decrease in F751 (Fig. 4).
However, we observed that there was an increase in the ratio of F725/(S80)/F725/(436), which has been considered an indicator of the efficiency of energy transfer from PBS to PSI (Fig. 5). Our results suggest that salt stress induced an increase in the efficiency of excitation energy transfer from PBS to PSI.

We observed that PSI activity in salt-stressed cells increased significantly (Fig. 9). Sudhir et al. (2005) also observed that salt stress induced an increase in PSI activity. The increased PSI activity may be due to an increase in the content of P700 reaction centers (Sudhir et al., 2005). Understandably, the higher excitation pressure on PSI could be overcome by increased PSI activity. The increased PSI activity would result in a decrease in energy transfer between PBS and PSI and shift the distribution of excitation energy more in favor of PSI. In addition, our results show that salt stress resulted in an inhibition of electron transport at the acceptor of PSI (Fig. 7B and C). The inhibition of electron transport at the acceptor side of PSI may lead to an increased redox status of the electron transport chain. Since excitation energy transfer is regulated by the redox status of the electron transport chain (Mullineaux and Allen, 1990; Vernotte et al., 1990), inhibition of electron transport at the acceptor side of PSI would result in a distribution of excitation energy in favor of PSI.

An increase in PSI activity should increase cyclic electron transport. Several reports have shown that cyclic electron flow increases under salinity stress (Jeanjean et al., 1993; Hbibino et al., 1996). Thus, it seems that an increase in PSI activity in salt-adapted cells may protect PSI from excessive excitation energy under salt stress. On the other hand, the increases in PSI activity in salt-stressed cells, together with an increase in the respiratory rate, may provide more energy for the synthesis of organic osmolytes and for the exclusion of Na+ in cells to maintain osmotic balance (Vonshak et al., 1995; Allakhverdiev and Murata, 2008).

In this study, we observed that there was a down-regulation of PSI reaction centers either in the presence or in the absence of DCMU (Fig. 8). However, our results show that there was no significant change in the trapping flux per PSI reaction center (TRO/RC) in salt-stressed cells (Fig. 7E). TRO/RC represents the maximum rate of reduction of Qa (Strasser and Strasser, 1995). No change in TRO/RC in salt-stressed cells suggests that the high conversion efficiency of excitation energy was maintained. According to the results in this study, it is suggested that through a down-regulation of PSI reaction centers and a shift of excitation energy transfer to PSI, the PSI apparatus was thus protected from further excess excitation energy.

It should be noted that we have previously investigated the effects of heat stress on excitation energy transfer from PBS to PSI and PSI in S. platensis cell. We found that heat stress had no effect on excitation energy transfer from PBS to PSI (Wen et al., 2005). However, the results of the present study demonstrated that salt stress induced an increase in excitation energy transfer from PSI (Fig. 5). Thus, it is suggested that there may be different mechanisms for excitation energy transfer from PBS to PSI in S. platensis under different environmental conditions.

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References


