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Effects of salinity stress on photosystem II function in cyanobacterial *Spirulina platensis* cells

Congming Lu[a,b] and Avigad Vonshak[b,*]

[a]*Photosynthesis Research Center, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China*
[b]*Microalgal Biotechnology Laboratory, the Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Sede Boker Campus, 84990, Israel*
**Corresponding author, e-mail: avigad@bgumail.bgu.ac.il*

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The changes in PSII photochemistry in *Spirulina platensis* cells exposed to salinity stress (0–0.8 *M* NaCl) for 12 h were studied. Salinity stress induced a decrease in oxygen evolution activity, which correlated with the decrease in the quantum yield of PSII electron transport (Φ_{PSII}). Phycocyanin content decreased significantly while chlorophyll content remained unchanged in salt-stressed cells. Salinity stress induced an increase in non-photochemical quenching (q_N) and a decrease in photochemical quenching (q_P). Analyses of the polyphasic fluorescence transients (OJIP) showed that with the increase in salt concentration, the fluorescence yield at the phases J, I and P declined sharply and the transient almost levelled off at salt concentration of 0.8 *M* NaCl. The effects of DCMU on the polyphasic rise of fluorescence transients decreased significantly. Salinity stress resulted in a decrease in the efficiency of electron transfer from Q_A^- to Q_B. The slope at the origin of the relative variable fluorescence curves (dV/dt_o) and the relative variable fluorescence at phase J (V_J) increased in the absence of DCMU, but decreased in the presence of DCMU. The shape of the relative variable fluorescence transients in salt-stressed cells was comparable to that of the control cells incubated with DCMU. The results in this study suggest that salt stress inhibited the electron transport at both donor and acceptor sides of PSII, resulted in damage to phycobilisome and shifted the distribution of excitation energy in favour of PSI.

Introduction

Exposing algae to a salinity stress results in a significant inhibition in their photosynthetic activity (Kirst 1989). Such an inhibition seems to be located in the PSII complex. In the green alga *Dunaliella tertiolecta*, the decreased PSII activity was associated with state-2 transition (Gilmour et al. 1984, 1985). This was confirmed by Endo et al. (1995), who also suggested that the inhibition of quantum yield of PSII electron transport in *Chlamydomonas reinhardtii* induced by salinity stress is related to state-2 transition. In the red alga *Porphyra perforata*, Satoh et al. (1983) demonstrated that a decrease in PSII activity induced by salinity stress was due to the decrease in excitation energy reaching PSII reaction centres and damage to the oxidizing side of PSII. In cyanobacteria, the effect of salt stress on PSII has still been controversial. Some reports have shown that salinity stress decreased PSII activity (Schubert and Hagemann 1990, Schubert et al. 1993), while others demonstrated that salinity stress had no significant effect on PSII activity (Jeanjean et al. 1993).

Spirulina platensis, a filamentous cyanobacterium, has been isolated from a wide range of habitats largely varying in their salinity (Vonshak and Tomaselli 2000). Considerable interest has been invested in outdoor cultivation of *S. platensis* for commercial biomass production because of its potential source of protein and valuable chemicals (Vonshak 1997). In cultures grown outdoors in open ponds under arid and semiarid conditions, daily evaporation amounts to 1–2 cm leading to a progressive increase in the salt concentration in the culture (Vonshak 1987). Thus, the *Spirulina* cells cultivated under outdoor conditions are exposed to continuous salinity stress. A

Abbreviations – F_v'/F_m', efficiency of excitation energy trapping by open PSII reaction centres in the light-adapted state; Q_A, primary quinone electron acceptor of PSII; Q_B, secondary quinone electron acceptor of PSII; q_P, photochemical quenching coefficient; q_N, non-photochemical quenching coefficient; Φ_{PSII}, quantum yield of PSII electron transport in the light-adapted state.

better understanding of the effect of salt stress on photosynthesis may help in optimizing the productivity of outdoor *Spirulina* cultures.

Previously, we have demonstrated that *S. platensis* is capable of adapting to high NaCl concentrations. Such adaptation is associated with an increase in respiratory activity and a partial recovery of photosynthetic activity in the *Spirulina* cells after the initial decline due to exposure to high salinity (Vonshak et al. 1988). Recently, we have further investigated whether this adaptation of the photosynthetic activity is associated with adaptation of the PSII apparatus by characterization of the PSII photochemistry of *S. platensis* after being adapted to high salinity (Lu and Vonshak 1999). We showed that the salinity-adapted cells can maintain a high conversion efficiency of excitation energy through the down-regulation of PSII reaction centres.

Most of the studies on the effect of salt stress on PSII in cyanobacterial cells were carried out using cells in the steady-state adapted stage (Schubert and Hagemann 1990, Jeanjean et al. 1993, Schubert et al. 1993). Very little is known about changes in PSII photochemistry during the initial stages of the responses to salinity stress. These early responses may be crucial since they may determine whether the organism will be able to survive the fast transition and then undergo a somewhat longer metabolic stage of acclimation to elevated salinity. Our previous results showed that the adaptation of PSII photochemistry to salinity stress is a complex process (Vonshak et al. 1996, Lu et al. 1999). We proposed that the responses of PSII photochemistry in *S. platensis* cells in the first 12 h of their exposure to high salinity are composed of two phases. The first phase, which was independent of light, was characterized by a rapid decrease (about 50%) in PSII efficiency in the first 15 min, followed by a recovery up to about 80% of the initial level within the next 2 h. The second phase took place after 4 h, in which a further decline in PSII efficiency occurred only in the light, reaching about 45% of the initial level after 12 h. Yet the site of damage induced by the salinity stress in PSII apparatus was not identified.

The objective of the present study was to investigate the short-term (12 h) effect of salinity stress on PSII function. We have analysed the photosynthetic O_2 evolution activity, chlorophyll and phycocyanin contents, the fluorescence quenching under steady-state photosynthesis, and the polyphasic rise in fluorescence transients (OJIP) in an attempt to understand how salinity stress affects PSII photochemistry of *S. platensis*.

Materials and methods

Alga and growth conditions

Spirulina platensis M_2 was grown in Zarouk's medium containing 0.2 *M* sodium bicarbonate (Vonshak et al. 1982), incubated in a gyratory shaker at a constant temperature (35°C), and illuminated by fluorescence lamps (GRO-Lux) at photon flux density of 50 μmol m^{-2} s^{-1}.

Salinity stress treatment

Exponentially grown cells were harvested and resuspended in 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 the same conditions as described above for 12 h.

Photosynthetic O_2 evolution activity

Oxygen evolution activity was measured as previously described (Vonshak et al. 1988), using a Clark-type oxygen electrode. The temperature was kept constant at 35°C and illumination was provided by a projector lamp at PFD of 120 μmol m^{-2} s^{-1}.

Measurement of pigments

Chlorophyll *a* was determined according to Bennet and Bogorad (1973). The absorbance of c-phycocyanin was measured spectrophotometerically at 620 nm and its concentration was then calculated from the specific absorption coefficient E1% = 73 (Boussiba and Richmond 1979).

Chlorophyll fluorescence under steady-state photosynthesis

Chlorophyll fluorescence quenching analysis was carried out at room temperature with a portable fluorimeter (PAM-2000, Walz, Effeltrich, Germany). The fluorimeter was connected to a computer with data acquisition software (DA-2000, Walz).

The procedure followed in general that of Campbell et al. (1998) which was applied to the analyses of fluorescence quenching in cyanobacteria. The minimal fluorescence level in the dark-adapted state (F_o) was measured by the measuring modulated light (ML) which was sufficiently low (< 0.1 μmol m^{-2} s^{-1}) not to induce any significant variable fluorescence. A 0.8-s flash of saturating white light (SP) (8000 μmol m^{-2} s^{-1}) was then given to determine the maximal fluorescence in the dark-adapted state, $F_{m(dark)}$. The actinic light (AL) (50 μmol m^{-2} s^{-1}) was thereafter turned on. The steady-state fluorescence, F_s, was reached within 2.5 min and thereafter a saturating light flash was given again to determine the maximal fluorescence in the light-adapted state, F_m'. After F_s was established again, the minimal fluorescence level in the light-adapted state, F_o', was measured by briefly interrupting the actinic light and illuminating the cells with far-red light (FR) for 3 s (7 μmol m^{-2} s^{-1}). Thereafter, the actinic light was turned on again. After the steady-state fluorescence was achieved, the maximum fluorescence, F_m, was determined by adding 3–3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) (10 μ*M* final) to the cuvette. We found that $F_{m(dark)}$ was 8% lower than F_m in this study.

Using fluorescence parameters of both light-and dark-

adapted cells, we calculated: (1) the photochemical quenching coefficient

$q_P = (F_m' - F_s)/(F_m' - F_o')$

(2) the non-photochemical quenching coefficient

$q_N = 1 - (F_m' - F_o')/(F_m - F_o)$

(3) the quantum yield of PSII electron transport

$\Phi_{PSII} = (F_m' - F_s)/F_m'$

(Genty et al. 1989). Fluorescence nomenclature was according to van Kooten and Snel (1990).

Polyphasic rise of Chl *a* fluorescence transients (OJIP)

Polyphasic rise of Chl *a* fluorescence transients was measured by using a Plant Efficiency Analyser (PEA, Hansatech Instruments Ltd, King's Lynn, UK) according to Strasser et al. (1995). Illumination was provided with an array of six high-intensity light emitting diodes (with a maximum at 650 nm), which were focused on the sample surface to provide homogeneous illumination over an area of 4 mm in diameter. The fluorescence signals were received by a high performance PIN photodiode detector associated with an amplifier circuit. The detector responded maximally to the longer wavelength fluorescence signal while blocking reflected, shorter wavelength light from light-emitting-diodes. The fluorescence signals were recorded within a time scan from 10 μs to 1 s with a data acquisition rate of 10^5 readings per second for the first 2 ms and of 10^3 readings per second after 2 ms.

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

Results

Photosynthetic oxygen evolution activity

The effect of exposing *S. platensis* cells to elevated concentrations of NaCl on the photosynthetic activity was evaluated by measurement of oxygen evolution rate of the cells. Figure 1 shows that the oxygen evolution rate decreased significantly with increasing salt concentrations reaching 29% of the control value in cells exposed to 0.8 *M* NaCl for 12 h.

Chlorophyll and phycocyanin

Changes in the contents of chlorophyll and phycocyanin in salt-stressed cells are shown in Fig. 2. Phycocyanin content decreased significantly with the increase in salt concentration, while chlorophyll content was largely unchanged.

Fluorescence quenching analyses

Chlorophyll fluorescence has been shown to be a non-invasive, powerful and reliable method to assess the changes in the function of PSII under different environmental conditions (Krause and Weis 1991, Schreiber et al. 1994). In higher plants and green algae, the PAM fluorometry has been usually used to assess (1) the maximal efficiency of PSII photochemistry (F_v/F_m); (2) the efficiency of excitation energy capture by open PSII centres (F_v'/F_m'); (3) photochemical quenching (q_P), a measure of the proportion of open PSII centres; (4) non-photochemical quenching (q_N), a measure of the dissipation of energy from PSII not resulting in photochem-

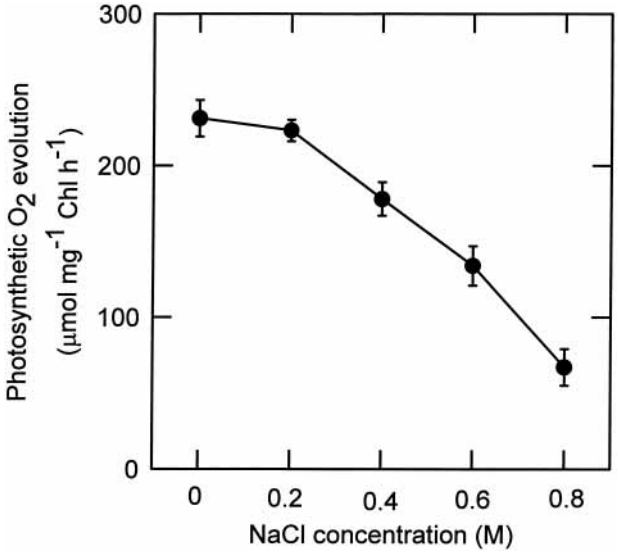

Fig. 1. Changes in the photosynthetic oxygen evolution activity in *S. platensis* cells exposed to various concentrations of NaCl for 12 h. Values are means ± SE of four measurements.

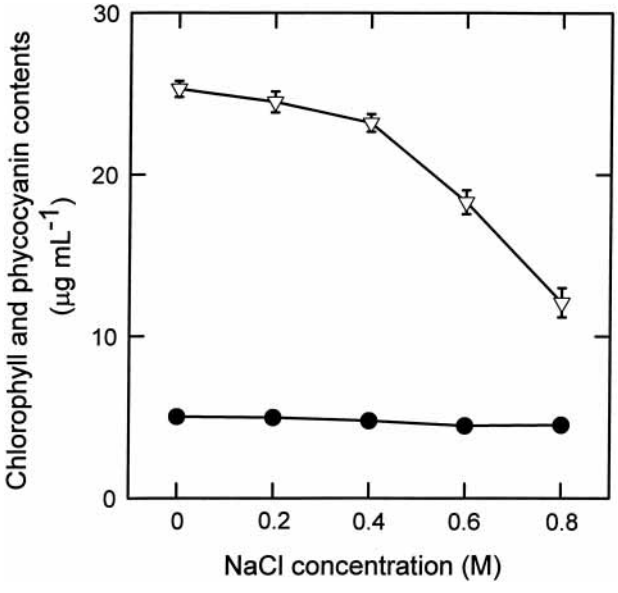

Fig. 2. Changes in chlorophyll (●) and phycocyanin (∇) contents in *S. platensis* cells exposed to various concentrations of NaCl for 12 h. Values are means ± SE of three measurements.

ical reaction; (5) the quantum yield of PSII electron transport (Φ_{PSII}). Because of distinct physiological differences between higher plants and cyanobacteria, special care is needed when the same interpretation of fluorescence as in green algae and higher plants is applied in cyanobacteria (Büchel and Wilhelm 1993).

A fundamental property in cyanobacteria is phycobilisome, which significantly influences the F_o fluorescence and consequently affects the F_v/F_m and F_v'/F_m' parameters. It has been shown that the F_o fluorescence increases with an increase in the phycocyanin/chlorophyll ratio in *Synechococcus* sp. PCC 7942, particularly once the phycocyanin content is increased above a threshold level (Campbell et al. 1996). F_o fluorescence is also affected by changes in the redox state of plastoquinone in the dark by respiration (Büchel and Wilhelm 1993). In addition, cyanobacteria in the dark are generally in State 2, with high excitation transfer to PSI, high non-photochemical quenching and low PSII fluorescence, thus the F_m value is difficult to be measured. However, the fluorescence quenching technique has also proved useful with cyanobacteria when the method is modified and special care is taken. Figure 3 presents the fluorescence traces in control *Spirulina* cells and the cells treated with 0.8 *M* NaCl basically according to the protocol of Campbell et al. (1998) which has been extensively validated with many different cyanobacteria under a range of different growth conditions.

The relatively high F_v/F_m ratio (0.65–0.70) obtained in control *Spirulina* culture suggests that the influence of the phycobilisome on F_o is small. In fact, Fig. 3 shows that the fluorescence traces of *Spirulina* cells were different from those normally obtained from *Synechoccus* cells with very low $F_{m(dark)}$ and low F_v/F_m ratio (0.4–0.5) and are similar to these observed in high plants. Furthermore, it has been shown that the distortion of F_o fluorescence is pronounced only at high phycocyanin content (Campbell et al. 1996), however, we observed that phycocyanin content decreased with increasing salt concentration (Fig. 2), suggesting that the influence of the phycobilisome on F_o in salt-stressed cells should be small. Thus, the effects of the phycobilisome on F_o in *Spirulina* cells in this study should be relatively small.

The F_m value was measured in the presence of DCMU which prevents induction of O_2-dependent electron transport beyond PSII (Fig. 3), thus the F_m can be obtained accurately though salt stress may increase the respiratory activity which increases the redox state of electron transport chain and excitation transfer to PSI.

As defined

$$q_P = (F_m' - F_s)/(F_m' - F_o') = (F_m' - F_s)/F_v'$$

and

$$q_N = 1 - (F_m' - F_o')/(F_m - F_o) = 1 - (F_v'/F_v).$$

q_P and q_N require only minimal mechanistic assumptions (van Kooten and Snel 1990). It can be seen that the calculation of q_P and q_N is scaled to variable fluorescence $F_v = F_m - F_o$ and $F_v' = F_m' - F_o'$. They measure changes in variable fluorescence. In cyanobacteria, variable fluorescence arises essentially from PSII while F_o and F_o' fluorescence levels arises only partly from PSII. As discussed above, F_o and F_o' fluorescence levels contain a major contribution from phycocyanin fluorescence and possibly a contribution from the redox state of the plastoquinone but they do not contribute to variable fluorescence. Furthermore, the underlying background fluorescence from phycocyanin and the redox state of the plastoquinone is subtracted out as an equivalent of both F_o and F_o' (Campbell et al. 1998). Thus, the absolute level of F_o or F_o' does not distort the calculation of q_P and q_N (Campbell et al. 1998).

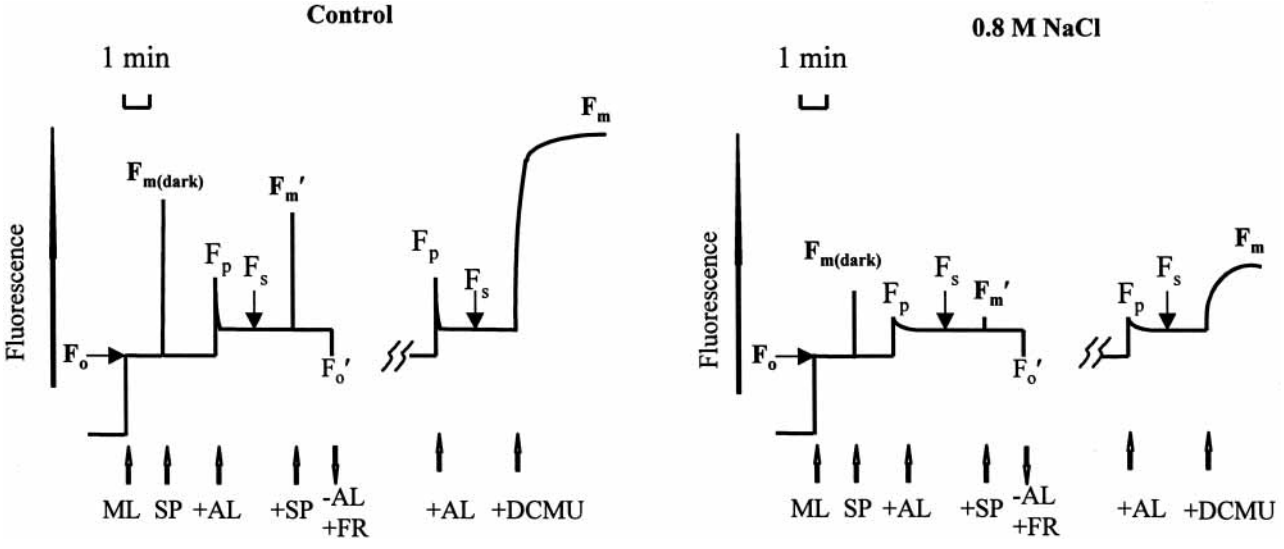

Fig. 3. Representative fluorescence traces for quenching analyses in control cells and cells exposed to 0.8 *M* NaCl for 12 h (for details, see Materials and methods).

It seems that the parameter Φ_{PSII} can be used in cyanobacteria because its calculation is not involved in the F_o and F_o' fluorescence.

Therefore, we believe that the parameters q_P, q_N and Φ_{PSII} of chlorophyll fluorescence in cyanobacteris can be used to evaluate PSII photochemistry in *Spirulina* cells in this study though there are distinct physiological differences between higher plants and cyanobacteria.

The fluorescence characteristics under steady-state photosynthesis, i.e. in the light-adapted state, were examined using modulated fluorescence. Cells exposed for 12 h to salinity stress showed a decrease in Φ_{PSII} and q_P but an increase in q_N (Fig. 4A). Figure 4B shows that salt stress resulted in a decrease in the maximal fluorescence in the dark-adapted state (F_m) and in the light-adapted state (F_m'), while the minimal fluorescence in the dark-adapted state (F_o) and in the light-adapted state (F_o') as well as the steady-state fluorescence (F_s) remained unchanged.

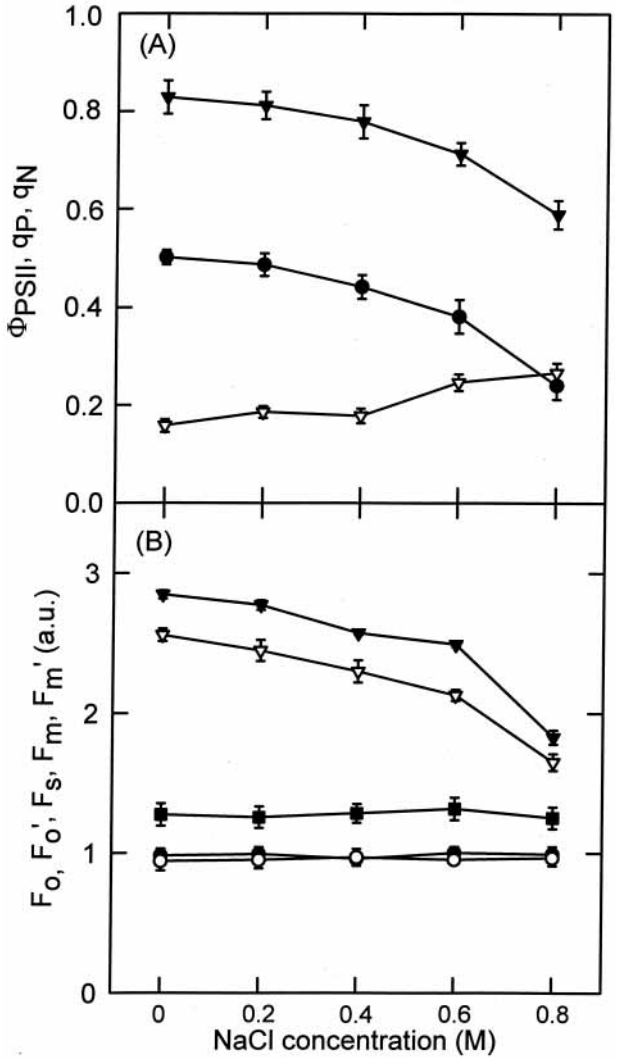

Fig. 4. Changes in (A) the photochemical quenching coefficient (q_P, ▽), the quantum yield of PSII electron transport (Φ_{PSII}, ●), the non-photochemical quenching coefficient (q_N, ∇); (B) the minimal fluorescence in dark-(F_o, ●) and light-adapted states (F_o', ○), the maximal fluorescence in dark-(F_m, ▽) and light-adapted states (F_m', ∇), the steady-state fluorescence (F_s, ■) in *S. platensis* cells exposed to various concentrations of NaCl for 12 h. Values in the figure are means ± SE of three measurements.

The polyphasic rise of fluorescence transients (OJIP)

In order to evaluate the effect of salt stress on the electron transport of PSII, we followed the polyphasic rise of Chl *a* fluorescence transients in *S. platensis* cells exposure for 12 h to elevated concentrations of NaCl. *Spirulina* cells exhibited a typical polyphasic rise of fluorescence induction, called the OJIP fluorescence transient, similar to that described previously for plants, green algae and cyanobacteria (Neubauer and Schreiber 1987, Srivastava et al. 1995, 1997 1998, Strasser et al. 1999) (see Fig. 5A). The J, I and P steps occurred at about 2 ms, 30 ms, and 400 ms, respectively. The OJIP transient represents the successive reduction of the electron acceptor pools of PSII (Govindjee 1995). The J step reflects an accumulation of $Q_A^-Q_B$, i.e. the reduction of Q_A to Q_A^- as demonstrated by experimental results and theoretical simulations (Lazar 1999). It has been suggested that the I step reflects an accumulation of $Q_A^-Q_B^-$, whereas the P step an accumulation of $Q_A^-Q_B^{2-}$ (Strasser et al. 1995). Addition of DCMU leads to a quick rise of fluorescence from steps O to J, and the transient levels off at the step J (see Fig. 5A). This reflects the fact that addition of DCMU results in a complete closure of PSII reaction centres during the first 2 ms of the fluorescence induction curve and an accumulation of Q_A^- at step J. In the absence of DCMU, a complete closure of PSII reaction centres is observed only after 400 ms.

Figure 5 shows that with the increase in salt concentration, the minimal fluorescence level (O) did not change significantly, but the fluorescence yield at phases J, I and P declined sharply, and the transient almost levelled off at salt concentration of 0.8 *M* NaCl. Figure 5 also shows that the effects of DCMU on the polyphasic rise of Chl *a* fluorescence transients decreased significantly with the increase in salt concentration. In control cells, DCMU resulted in a quick rise of fluorescence from steps O to J, and the transient levels off at the step J (Fig. 5A). However, when exposed to 0.8 *M* NaCl, DCMU almost had no effects on the polyphasic rise of Chl *a* fluorescence transients (Fig. 5E).

To localize the action of salt stress in the electron transport chain from the donor side to the acceptor side, we compared the kinetics of the relative variable fluorescence (V) in control and salt-stressed cells in the absence and presence of DCMU (Fig. 6). The relative variable fluorescence is purely geometrical and does not correspond to any theory about the origin of the fluorescence emission. It is also independent of F_o (Strasser 1996). The relative variable fluorescence V at any given time t is defined as:

$$V_t = (F_t - F_o)/(F_m - F_o)$$

This expression can be taken as a measure of the fraction

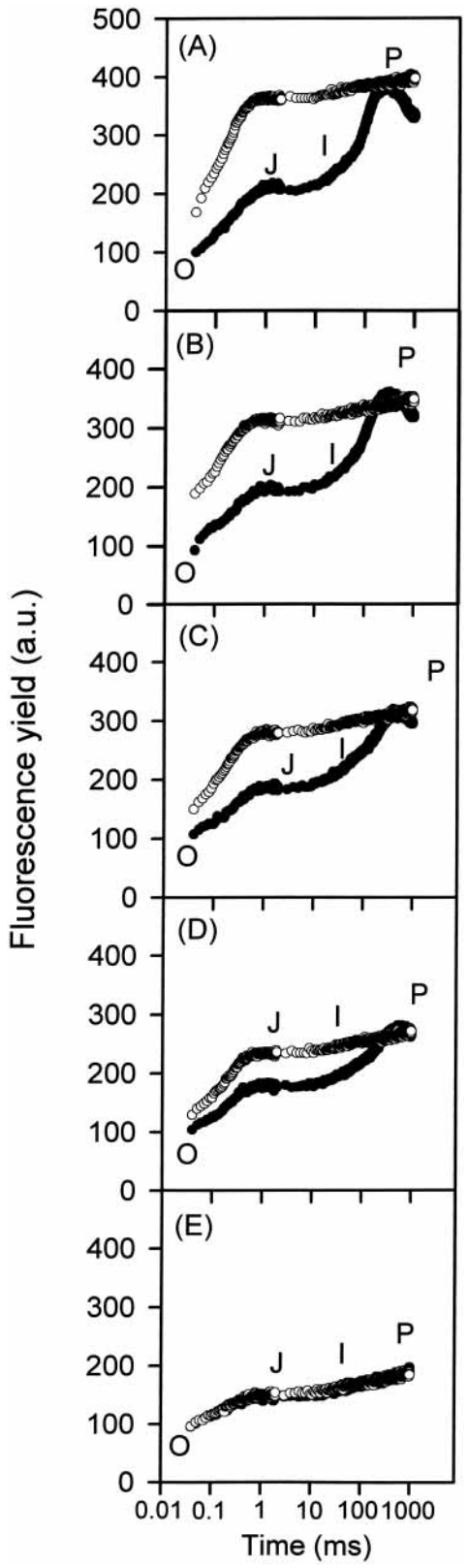

Fig. 5. Polyphasic chlorophyll fluorescence transients (OJIP) without (●) or with (○) 10 μ*M* DCMU in *S. platensis* cells exposed to 0 (A), 0.2 (B), 0.4 (C), 0.6 (D), 0.8 *M* NaCl (E) for 12 h.

of the primary quinone electron acceptor of PSII being in its reduced state $[Q_A^-/Q_{A(total)}]$. The slope at the origin of the relative variable fluorescence curves, dV/dt_o (measured between 50 and 300 μs), is a measure of the rate of primary photochemistry $[(dQ_A^-/Q_{A(total)})/dt_o]$ (Strasser et al. 1995). dV/dt_o is the difference between the Q_A reduction rate and the Q_A^- oxidation rate after the onset of illumination. At the phase J (about 2 ms) the dV_J/dt is practically zero, which means that at the phase J, the reduction rate of Q_A and the oxidation rate of Q_A^- are equal (Strasser et al. 1995). In the presence of DCMU, the reoxidation of Q_A^- is mainly blocked (Trebst 1980), thus, dV/dt_o, after illumination, represents only the electron flux from the donor side to Q_A.

Figure 6A shows the effects of salt stress on the kinetics of the relative variable fluorescence in the absence of

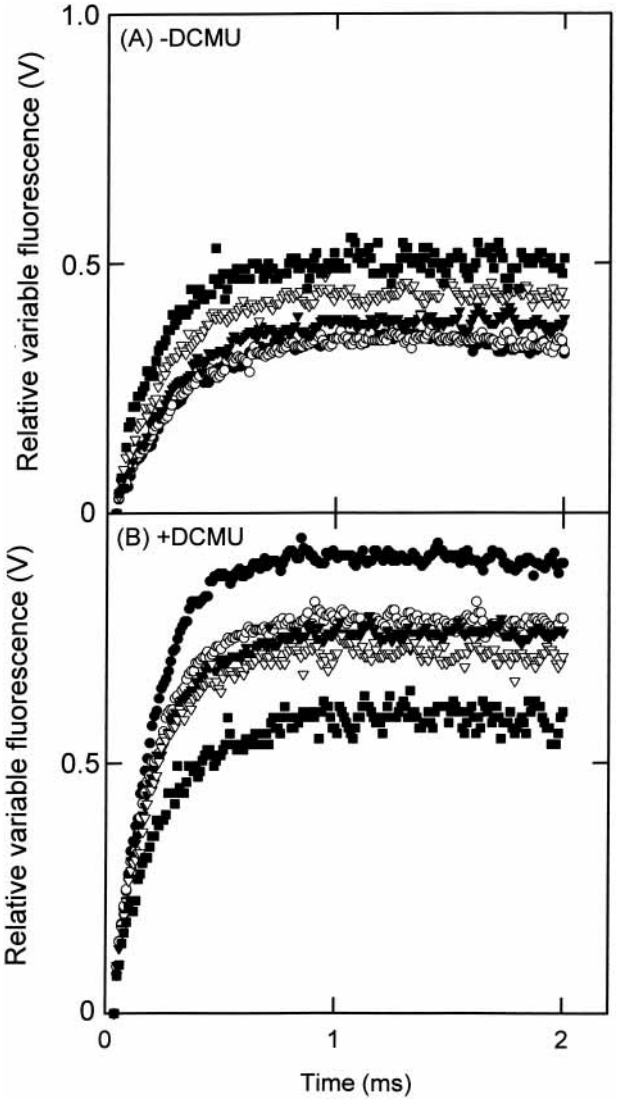

Fig. 6. The fast rise of the relative variable fluorescence yield in the first 2 ms without (A) and with (B) 10 μ*M* DCMU in control cells (●) and cells exposed to 0.2 (○), 0.4 (▽), 0.6 (∇), 0.8 (■) *M* NaCl for 12 h.

DCMU. The relative variable fluorescence in the control cells increased almost linearly during the first 200 μs, and then levelled off. However, the relative variable fluorescence in the stressed cells showed a faster increase than in the control cells. Furthermore, the level of fluorescence was higher at the salt stressed cells as compared to the control. Figure 6B shows the effects of salt stress on the kinetics of the relative variable fluorescence in the presence of DCMU. The relative variable fluorescence rise during the first 200 μs decreased with increasing salt concentration.

Figure 7 further demonstrates the quantitative changes in dV/dt_o and V_J in salt-stressed cells. With increasing salt concentration, dV/dt_o and V_J increased in the absence of DCMU (Fig. 7A) but decreased in the presence of DCMU (Fig. 7B).

An increase in the relative variable fluorescence yield may be indicative of a loss of Q_A^- re-oxidation capacity (Krause and Weis 1991, Govindjee 1995, Strasser et al. 1995). Figure 8 shows the effects of salt stress on the efficiency of electron transfer from Q_A^- to Q_B (Ψ_o) (Srivastava et al. 1998). Indeed the data presented in Fig. 8 demonstrates that incubating the algal cells in elevated salt concentrations resulted in a significant decrease in Ψ_o, suggesting that salt stress resulted in a decrease in the relative Q_A^- re-oxidation capacity.

Discussion

The results in the present study suggest that salt stress induced an inhibition of the electron transport in the acceptor side of PSII. There are several lines of evidence supporting this suggestion. Firstly, a gradual increase of the relative variable fluorescence yield (Fig. 6A) indicates that the accumulation of Q_A^- is strongly increased, thus suggesting that salt stress resulted in a gradual decrease in the electron transport rate after Q_A. Furthermore, we observed that salt stress resulted in a decrease in the efficiency of electron transfer from Q_A^- to Q_B (Ψ_o) (Fig. 8), indicating an inhibition of electron transport after Q_A^-. In addition, the shape of the fluorescence transients induced by salt stress compared to that of DCMU-treated cells, where the maximal fluorescence yield is achieved at the J step, suggests that salt stress inhibits the electron transfer the same way as DCMU does, i.e. from Q_A^- to Q_B (Fig. 6). It seems that the effects of salt stress on the polyphasic

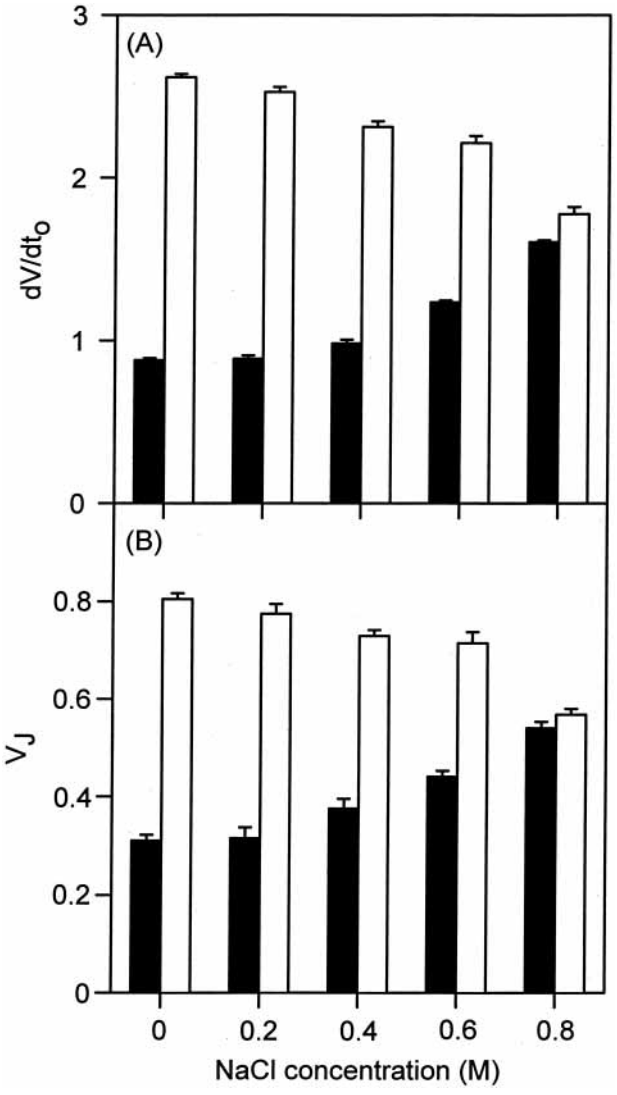

Fig. 7. Changes in (A) the initial slope at the beginning of the relative variable fluorescence transients, dV/dto $[=(F_{300\mu s}-F_o)/(F_m-F_o)]$ and (B) the relative variable fluorescence at step J, V_J $[=(F_J-F_o)/(F_m-F_o)]$ in *S. platensis* cells incubated without (solid column) or with (open column) 10 μ*M* DCMU exposed to various concentrations of NaCl for 12 h. Values in the figures are means ± SE of five measurements.

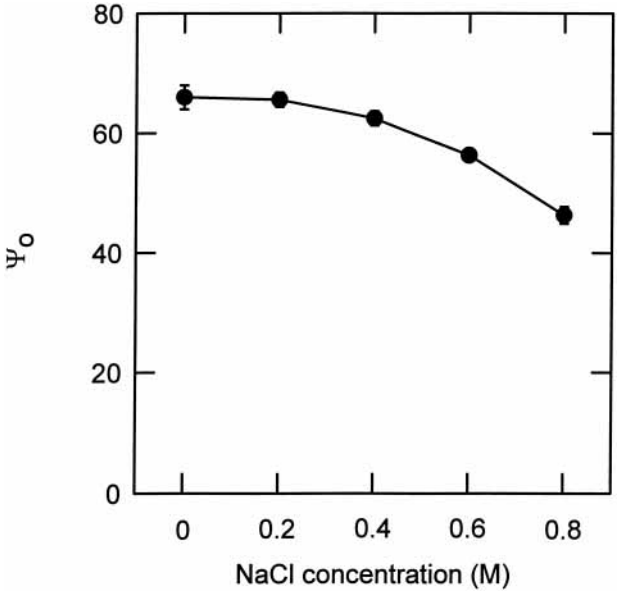

Fig. 8. Changes in the efficiency of electron transfer from Q_A^- to Q_B (Ψ_o) in *S. platensis* cells exposed to various concentrations of NaCl for 12 h. Values in the figures are means ± SE of five measurements. The calculation of Ψ_o was according to Srivastava et al. (1997), $\Psi_o = 1 - V_J$.

fluorescence transients indicate that the electron transfer from Q_A^- to Q_B is only partially inhibited (Fig. 5). This phenomenon most likely suggests that salt stress induces a modification of the Q_B site, which decreases the binding affinity of both plastoquinone and DCMU. However, the direct evidence how salt stress inhibits the electron transport at the acceptor side remains to be further studied by examining the reoxidation kinetics of Q_A^-.

Our results show that salt stress resulted in a decrease in F_m (Fig. 4). Salt stress also resulted in decreases in the fluorescence levels at phases J, I and P. The transient almost levelled off at salt concentration of 0.8 *M* NaCl (Fig. 5). These effects were observed both in the presence and absence of DCMU and can not be explained by the inhibition of electron transport at the acceptor side of PSII. The decreases in F_m and fluorescence levels at phases J, I, and P are normally explained by the inhibition of electron transport at the donor side of PSII, which results in the accumulation of $P680^+$, a strong fluorescence quencher (Neubauer and Schreiber 1987, Schreiber and Neubauer 1987, Govindjee 1995). These results observed in the salt-stressed cells may thus indicate that salt stress had inhibitory effects on the donor side of PSII.

To further evaluate the effects of salt stress on the donor side of PSII, the kinetics of the relative variable fluorescence in control and salt-stressed cells was compared (Fig. 6). In the presence of DCMU, the reoxidation of Q_A^- is blocked, thus the kinetics of the relative variable fluorescence reflects the electron flux from the donor side to Q_A. Our results show that salt stress significantly inhibited the rise kinetics of the relative variable fluorescence, which was exhibited by a decrease in the slope at the origin of the relative variable fluorescence curves (dV/dt_o). dV/dt_o is a measure of the rate of Q_A reduction (Strasser et al. 1995). It depends on the rate of excitation capture and photochemical efficiency of the first charge separation. Thus, the decrease in dV/dt_o in the present of DCMU in salt-stressed cells (Figs 6B, 7A) may be due to the inhibition of electron transfer at the donor side of PSII and the decrease of the antenna efficiency caused by the decreased phycocyanin content (Fig. 2). However, the results that the effects of salt stress on the kinetics of the relative variable fluorescence in the presence of DCMU were significantly different from these in the absence of DCMU further suggest that salt stress resulted in the decrease in the electron transfer from the donor side.

In cyanobacteria, photochemical quenching (q_P) is a general index of the balance between excitation of PSII and electron transport (Campbell et al. 1998). The decreased q_P in salt-stressed cells indicates that electron transport could not efficiently remove electrons from PSII which could be associated with inhibition of electron transport at the acceptor side as discussed above.

Non-photochemical quenching (q_N) reflects the processes that compete with PSII photochemistry for absorbed excitation energy. In cyanobacteria, there is no evidence for energy-dependent quenching mechanisms, which are the predominant components of q_N in higher plants (Campbell and Öquist 1996). It has been shown by many studies that q_N in cyanobacteria largely reflects changes in the PSII fluorescence yield as a result of the state transition mechanism, which regulates the equilibration of excitation energy between PSII and PSI (Öquist et al. 1995, Campbell and Öquist 1996, Campbell et al. 1998). Thus, an increased q_N in salt-stressed cells may suggest the state transition mechanism with an increased transfer of excitation energy to PSI at the expense of PSII.

Our previous study has shown that a significant increase in respiratory activity is induced after *Spirulina* cells exposed to salinity stress for 12 h (Vonshak et al. 1995). Because respiratory and photosynthetic flow share several electron transport intermediates, an increased respiratory activity would lead to an increased redox status of the electron transport chain. The inhibition of electron transport at the acceptor side of PSII as suggested in the present study may also lead to an increased redox status of the electron transport chain. Since state transitions are regulated by the redox status of electron transport chain (Mullineaux and Allen 1990, Vernotte et al. 1990), an increase in respiratory activity and inhibition of electron transport at the acceptor side would result in a distribution of excitation energy in favour of PSI as reflected by an increased q_N.

In conclusion, the results in this study suggest that a salinity shock (12 h) had multiple effects on PSII function. It inhibited the electron transport at both donor and acceptor sides of PSII, resulted in a damage to phycobilisome reflected by a significant decrease in phycocyanin contents and shifted the distribution of excitation energy in favour of PSI as suggested by an increase in q_N. In a previous study, we have studied the characterization of PSII photochemistry of *Spirulina* cells adapted to salinity stress for more than 2 weeks. In such cells a down-regulation of PSII reaction centres was observed without any inhibition of electron transfer on the donor and acceptor sides of PSII (Lu and Vonshak 1999), suggesting that the PSII of *Spirulina* cells undergoes different responses in the course of adaptation to salinity stress. The effects of salt stress on PSII function in *Spirulina* cells may be due to a direct interaction of high salt with PSII or a more complex interaction through unknown cell components, which remain to be further studied by evaluating the effects of high salt on isolated thylakoids, the PSII particles and PSII reaction centres.

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