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Kinetic response of photosystem II photochemistry in the cyanobacterium *Spirulina platensis* to high salinity is characterized by two distinct phases

Congming Lu[A], Giuseppe Torzillo[B] and Avigad Vonshak[AC]

[A]*Microalgal Biotechnology Laboratory, The Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Sede Boker Campus, 84990, Israel.*
[B]*Centro di Studio dei Microrganismi Autotrofi del CNR, Piazzale delle Cascine, 27, 50144 Florence, Italy.*
[C]*Corresponding author; email: avigad@bgumail.bgu.ac.il*

Abstract. The kinetic response of photosystem II (PS II) photochemistry in *Spirulina platensis* (Norstedt M_2) to high salinity (0.75 M NaCl) was found to consist of two phases. The first phase, which was independent of light, was characterized by a rapid decrease (15–50%) in the maximal efficiency of PS II photochemistry (F_v/F_m), the efficiency of excitation energy capture by open PS II reaction centres (F_v'/F_m'), photochemical quenching (q_P) and the quantum yield of PS II electron transport ($\Phi_{PS\ II}$) in the first 15 min, followed by a recovery up to about 80–92% of their initial levels within the next 2 h. The second phase took place after 4 h, in which further decline in above parameters occurred. Such a decline occurred only when the cells were incubated in the light, reaching levels as low as 45–70% of their initial levels after 12 h. At the same time, non-photochemical quenching (q_N) and Q_B-non-reducing PS II reaction centres increased significantly in the first 15 min and then recovered to the initial level during the first phase but increased again in the light in the second phase. The changes in the probability of electron transfer beyond Q_A (ψ_o) and the yield of electron transport beyond Q_A (φ_{Eo}), the absorption flux (ABS/RC) and the trapping flux (TR_o/RC) per PS II reaction centre also displayed two different phases. The causes responsible for the decreased quantum yield of PS II electron transport during the two phases are discussed.

Introduction

Exposure of algal cells to salt stress results in a decline in their photosynthetic activity. It has been suggested that a reduction in photosystem II (PS II*) activity induced by salt stress may be one of the major factors responsible for this decrease (Kirst 1990). In the green alga *Dunaliella tertiolecta*, the decrease in PS II activity was associated with the formation of a pH gradient across thylakoid membranes and with state-2 transition after the cells had been exposed to high salinity for 5 min in the dark (Gilmour *et al.* 1984, 1985). Endo *et al.* (1995) showed that the inhibition of the quantum yield of PS II in *Chlamydomonas reinhardtii* induced by salt stress can be attributed to the state-2 transition. In the red alga *Porphyra perforata* stressed by high salinity for 5 min in the dark, Satoh *et al.* (1983) demonstrated that the decrease in the excitation energy reaching PS II reaction centres and the inhibition of the oxidizing side of PS II resulted in a decrease in PS II activity. In cyanobacteria, many studies have focused on the changes in the PS II activity at steady state after cells became adapted to the salinity stress (Schubert *et al.* 1993; Jeanjean *et al.* 1993). However, less information is available on the initial responses of PS II photochemistry to salt stress and on the mechanism of such responses.

Chlorophyll fluorescence is a non-invasive, powerful and reliable method to assess the changes in the function of PS II under different environmental conditions (Krause and Weis 1991; Schreiber *et al.* 1994; Govindjee 1995; Strasser *et al.* 1995; Krüger *et al.* 1997). By the analyses of fluorescence quenching, it is possible to obtain the information on the fundamental processes of energy absorption, utilization and dissipation of excess excitation energy by PS II.

Spirulina platensis, a filamentous cyanobacterium, has been isolated from a wide range of habitats largely varying in their salinity (Ciferri 1983). Considerable interest has recently been invested in outdoor cultivation of *S. platensis* for commercial biomass production (Vonshak 1990). In cultures grown outdoors in open ponds under arid and semiarid conditions, daily evaporation amounts to 1–2 cm, thus leading to a progressive increase in the salt concentration in

*Abbreviations used: ABS, absorption; ET, energy flux for electron transport; Chl, chlorophyll; F_i, intermediate level in the fast fluorescence induction kinetics curve; F_o, F_o', minimal fluorescence in dark- and light-adapted state, respectively; F_m, F_m', maximal fluorescence in dark- and light-adapted state; F_v, F_v', maximal variable fluorescence in dark- and light-adapted state; F_s, steady-state fluorescence yield at q_P >0; F_v/F_m, maximal efficiency of PS II photochemistry; $\Phi_{PS\ II}$, quantum yield of PS II electron transport; F_v'/F_m', efficiency of excitation energy capture by open PS II reaction centres; PS I, photosystem I; PS II, photosystem II; q_P, photochemical quenching coefficient; q_N, non-photochemical quenching coefficient; RC, reaction centre; TR, energy flux for trapping; ψ_o, probability of electron transport beyond Q_A; φ_{Eo}, maximum yield of electron transport beyond Q_A.

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the culture (Vonshak 1987). Moreover, these cultures also suffer from photoinhibition in addition to salt stress (Vonshak and Guy 1992). A better understanding of the interaction of light and salt stress on photosynthesis may help optimize the productivity of the algal cultures grown outdoors.

Previously, our studies have demonstrated that photosynthetic oxygen evolution activity in *S. platensis* cells was inhibited significantly by high salinity (0.5–1.0 M NaCl) and that salinity increased the sensitivity to photoinhibition (Vonshak *et al.* 1988, 1995). In the present paper, we describe the kinetic response of PS II photochemistry in *S. platensis* cells incubated not only in the light but also in the dark in the first 12 h of their exposure to salt stress by the analyses of fluorescence quenching, of the rapid fluorescence induction kinetics (Kautsky effect), and of the polyphasic rise of fluorescence transients (O-J-I-P). It is found that the response was characterized by two phases distinguished in their behavior in the light or dark. An attempt is made to explain the mechanism of such responses in relation to the interaction of salt and light stresses in *S. platensis*.

Material and methods

Cell culture

S. platensis (Norstedt) Geitler strain M_2 of the Culture Collection of the Centro di Studio dei Microrganismi Autotrofi of Florence was grown in Zarouk's medium, containing 200 mM sodium bicarbonate at 50 μmol photons m^{-2} s^{-1} provided by fluorescent lamps (GRO-Lux), at 35°C and with CO_2-enriched (1%) air.

Salt stress treatment

Exponentially growing cells were harvested and resuspended at a concentration of 5 μg Chl mL^{-1} in a fresh medium containing 0.75 M NaCl (exclusive of 0.017 M NaCl already present in the medium).

Photosynthetic oxygen evolution activity

Oxygen evolution rate was measured as previously described (Vonshak *et al.* 1988), using a Clark-type oxygen electrode.

Analyses of fluorescence quenching

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

The minimal fluorescence level in the dark-adapted state (F_o) was determined by the measuring modulated light which was sufficiently low (< 0.1 μmol m^{-2} s^{-1}) not to induce any significant variable fluorescence. The minimal fluorescence level in the light-adapted state (F_o') was measured after turning off 50 μmol m^{-2} s^{-1} PFD of the actinic light, which was equivalent of the growth light intensity and illuminating with far-red light for 3 s (11 μmol m^{-2} s^{-1}, emission peak at 735 nm). The maximal fluorescence level in the dark-adapted (F_m) and light-adapted (F_m') states were determined before or after addition of the actinic light by 0.8 s saturating white light (8000 μmol m^{-2} s^{-1}) to close all reaction centres and drive photochemical quenching to zero. In the dark, cyanobacteria are normally in State-2, with high non-photochemical quenching and low PS II fluorescence, since the PQ pool is reduced by respiratory electron transport. When illuminated, they rapidly shift to State-1, with lower non-photochemical quenching and increased PS II fluorescence (Öquest *et al.* 1995). The reversion of the dark-adapted cells to State-1 can be promoted by far-red illumination which is preferentially absorbed by photosystem I (PS I). A single, high-intensity flash produces a F_m value in dark-adapted *Spirulina* cells that is 15–20 % lower than the true F_m. The true maximal fluorescence was measured under red light illumination (150 μmol m^{-2} s^{-1} PFD) in the presence of 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) to allow non-photochemical quenching to collapse. The steady-state value of fluorescence (F_s) under actinic light was also recorded. The actinic light intensity was 50 μmol m^{-2} s^{-1} which was equivalent of the growth light intensity to minimize the effect of dark adaptation on the measurements of the non-photochemical quenching (Campbell and Öquest 1996).

Using fluorescence parameters measured in both light- and dark-adapted states, we calculated: (1) the maximal efficiency of PS II photochemistry (F_v/F_m), (2) the photochemical quenching coefficient, $q_P = (F_m'–F_s)/(F_m'–F_o')$, which measures the proportion of open PS II reaction centres (van Kooten and Snel 1990), (3) the non-photochemical quenching coefficient, $q_N = 1 – (F_m'–F_o')/(F_m–F_o)$, (4) the efficiency of excitation energy capture by open PS II reaction centres, $F_v'/F_m' = (F_m'–F_o')/F_m'$, (5) the quantum yield of PS II electron transport, $\Phi_{PS\ II} = (F_m'–F_s)/F_m'$, which is the product of F_v'/F_m' and q_P, i.e $\Phi_{PS\ II} = F_v'/F_m' \times q_P$ (Genty *et al.* 1989). Here, fluorescence nomenclature was according to van Kooten and Snel (1990).

Rapid fluorescence induction kinetics (Kautsky effect)

The rapid fluorescence induction kinetics was measured by PAM-2000 in the dark-adapted samples suddenly illuminated with moderate red light (30 μmol m^{-2} s^{-1}, 655 nm) at a sampling rate of 1000 μs/point. In order to avoid an incomplete reoxidation of the plastoquinone pool in the dark which could result in an increase in fluorescence level at phase I, the dark-adapted samples were illuminated for 3 s with far-red light (11 μmol m^{-2} s^{-1}) prior to the measurements of the fluorescence induction kinetics.

Polyphasic rise of Chl a *fluorescence transients (O-J-I-P) and the JIP test*

Chl *a* fluorescence transients were measured by a Plant Efficiency Analyzer (PEA, Hansatech Instruments Ltd, King's Lynn, Norfolk PE32 1JL, England) with an actinic light of 3000 μmol m^{-2} s^{-1} by the method described by Strasser *et al.* (1995). Illumination was provided by an array of six high intensity, light-emitting-diodes (LED, a peak at 650 nm), which were focused on the sample surface to provide homogeneous illumination over the exposed area of a sample with a 4 mm diameter. The fluorescence signals were received by a detector, a high performance pin photodiode associated with an amplifier circuit, after passing through a long pass filter. The detector, responding maximally to the longer wavelength fluorescence signal, blocked the reflected shorter wavelength LED light used as the source of illumination. All the fluorescence transients 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 per second after 2 ms.

All oxygenic photosynthetic materials investigated till now exhibit a polyphasic rise of chlorophyll fluorescence transient during the first second illumination. These phases are labeled as O, J, I, P (Strasser *et al.* 1995). The key for recording a complete fluorescence transient showing phase O, J, I, P is the sampling rate so high that intermediate steps J and I between the initial (F_o) and the maximal (F_p) fluorescence can be visualized on the log time scale (Strasser *et al.* 1995).

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 (see Fig. 1), 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

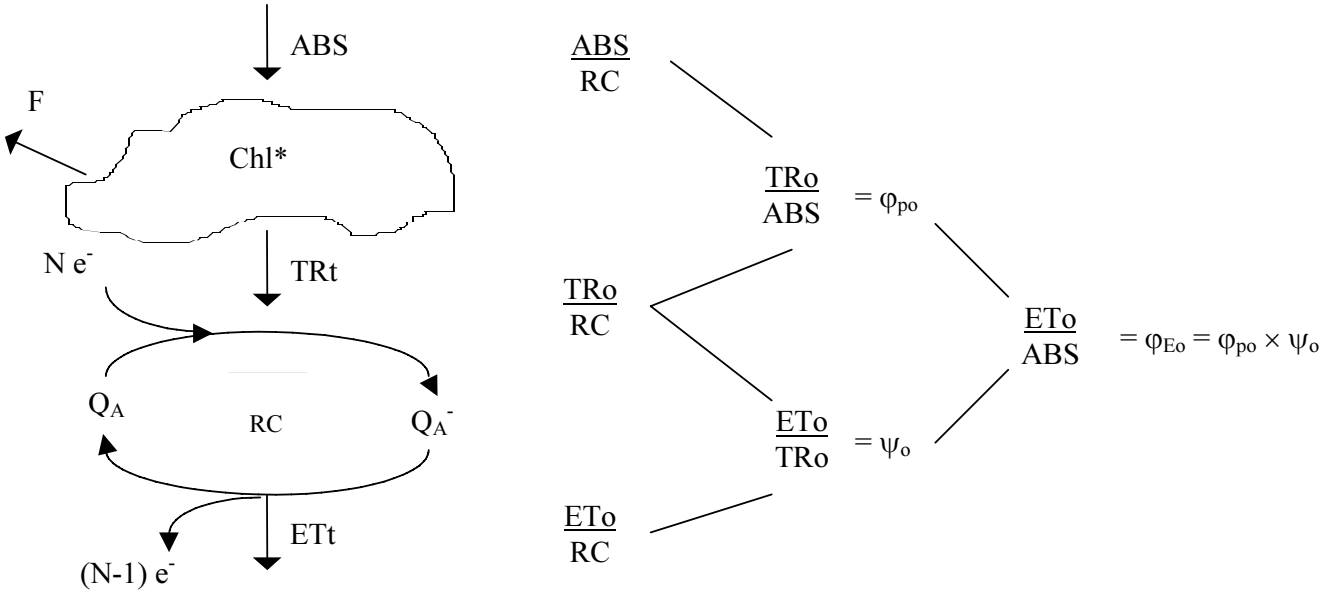

Fig. 1. Schematic energy-flux model for PS II. ABS: light absorption flux. TR_t, TR_o: energy flux trapped by PS II reaction centres at time *t* and time zero, respectively. ET_t, ET_o: electron transport flux generated by the reoxidation of Q_A^- to Q_A at time *t* and time zero, respectively. *F*: fluorescence emission. TR_o/ABS (φ_{po}): maximum efficiency of PS II photochrmistry. ET_o/TR_o (ψ_o): the probability of electron transport beyond Q_A. ET_o/ABS (φ_{Eo}): the maximum yield of electron transport beyond Q_A. Therefore, $\varphi_{Eo} = \varphi_{po} \times \psi_o$.

of it is transferred as trapping flux (TR) to the reaction centres (RC). In the RCs, the excitation energy is converted to redox energy by reducing Q_A to Q_A^- which is then reoxidized to Q_A, thus leading to an electron transport flux (ET), which maintains the metabolic reactions of photosynthetic apparatus. We can therefore evaluate PS II photochemistry during salt stress through the JIP test by measuring of the polyphasic rise of fluorescence transients. The detailed derivation for the formulae for the various energy fluxes and for the flux ratios in the JIP test was from Strasser and Strasser (1995) and Krüger *et al.* (1997).

The JIP test was originally developed for higher plants. It is based on the relative variable fluorescence, which is purely geometrical, and does not correspond to any theory about the origin of the fluorescence emission. It is independent of F_o (Strasser 1996). Our previous study demonstrated that the cyanobacterium *S. platensis* shows a typical polyphasic fluorescence transient like that of higher plants and that the results obtained from the JIP test were in line with these obtained from the biochemical analyses after *Spirulina* cells became adapted to salt stress (Lu and Vonshak 1999). We therefore believe that the JIP test can be used to evaluate PS II photochemistry in the cyanobacterium *S. platensis*.

All samples were dark adapted for 10 min before chlorophyll fluorescence was determined.

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).

Results

Photosynthetic oxygen evolution activity

The effect of salt stress on photosynthetic activity was evaluated by measurement of oxygen evolution (Fig. 2). Addition of 0.75 M NaCl caused an immediate inhibition of photosynthetic activity. After 15 min of exposure of to salt shock, the oxygen evolution decreased to about 30% of the initial values in both light- and dark-incubated cells. Thereafter, the oxygen evolution was partially recovered to 50 and 36% of their initial values in light- and dark-incubated cells, respectively. After 4 h, the oxygen evolution decreased

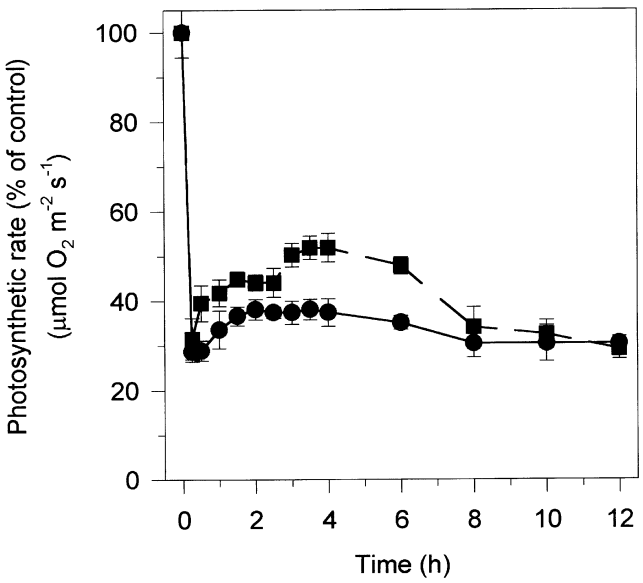

Fig. 2. Time courses of the changes in photosynthetic oxygen evolution rate in *S. platensis* cells exposed to 0.75 M NaCl and incubated in the dark (●) and light (■).The initial value of oxygen evolution rate (as 100%) was 655.1 ± 20.2 (μmol O_2 mg^{-1} Chl h^{-1}). Values are mean ± SE (n = 3).

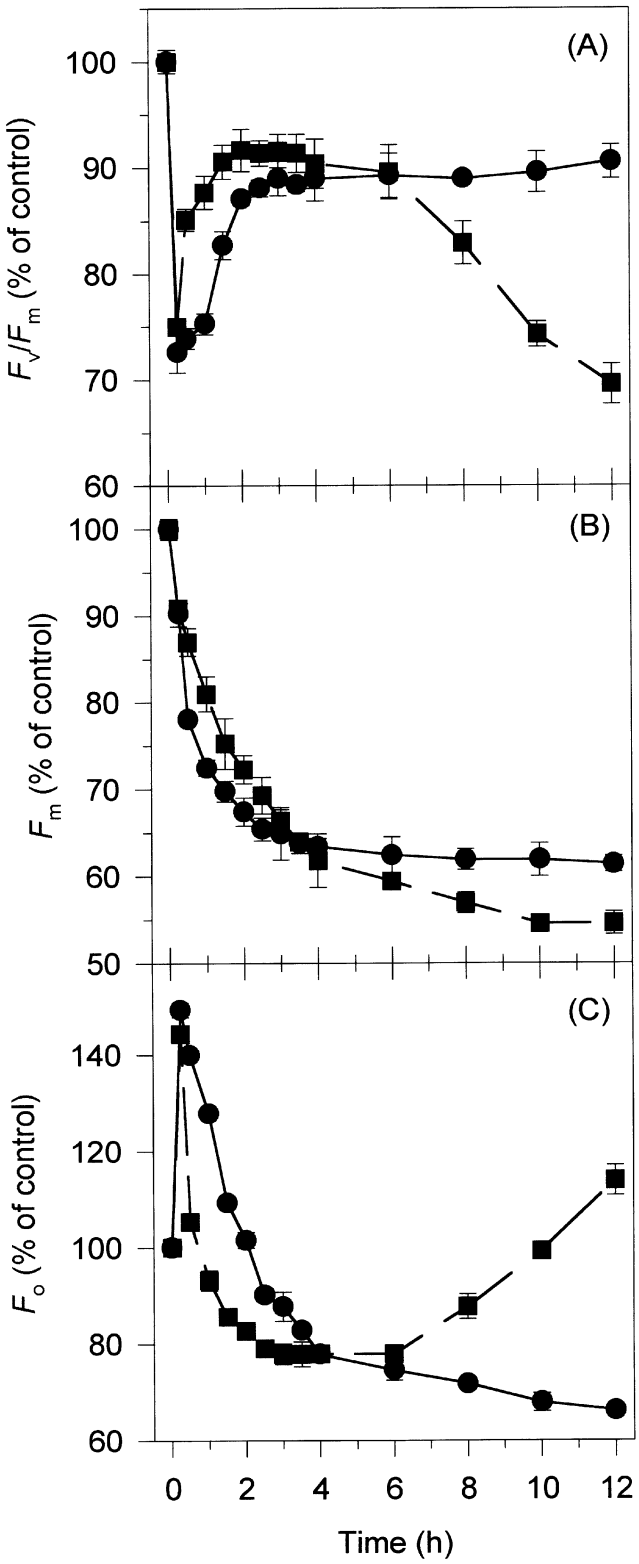

Fig. 3. Time courses of the changes in fluorescence parameters, F_v/F_m (*A*), F_m (*B*), and F_o (*C*) in *S. platensis* cells exposed to 0.75 M NaCl and incubated in the dark (●) and light (■). The initial values of F_v/F_m, F_m and F_o (as 100%) were 0.7 ± 0.012, 201.9 ± 3.5, 60.4 ± 0.62, respectively. Values are mean ± SE (n = 4).

again to 30% of the initial value in light-incubated cell but remained constant in dark-incubated cells.

Maximal quantum efficiency of PS II photochemistry (F_v/F_m)

Addition of 0.75 M NaCl to a culture of *S. platensis* incubated in the light resulted in an immediate decrease in the maximal quantum efficiency of PS II photochemistry (F_v/F_m) of about 26% in the first 15 min, followed by a partial recovery to about 92% of the original level after 2 h of incubation. After 4 h from the beginning of the salt treatment, another decline in F_v/F_m was observed, which reached about 70% of the original level after 12 h (Fig. 3*A*). The maximal fluorescence, F_m, decreased significantly in the first 4 h to a level of 64% of the original (Fig. 3*B*). The pattern of the response of minimal fluorescence, F_o, to 0.75 M NaCl was somewhat opposite to that of F_v/F_m. It increased remarkably in the first 15 min, reaching a value of 142% of control, then declined rapidly to 81% of control in 4 h, after which it increased again, reaching a value of 120% of control after 12 h (Fig. 3*C*).

The response of cells incubated in the dark to 0.75 M NaCl with respect to the above three fluorescence parameters was similar to that in cells incubated in the light in the first 4 h. Nevertheless, the second decline in F_v/F_m and in F_m as well as the increase in F_o seen in the light were not observed after 4 h in the dark (Fig. 3).

The changes in F_v/F_m in response to salinity stress in *S. platensis* cells incubated in the light or dark indicate the occurrence of two distinct phases. The first phase took place in the first 4 h of exposure to salinity. A transient decrease of F_v/F_m and subsequent recovery were observed regardless of light conditions, suggesting that this phase is independent of light. The second phase proceeded after the first 4 h of salt stress, the changes in F_v/F_m in this phase being light-dependent. This result suggests that photoinhibition was induced by the salinity stress. It should be noted that the two-phase response of F_v/F_m to salinity was observed only when *S. platensis* cells were exposed to high salinity, not lower than 0.25 M (data not shown).

PS II photochemistry under steady state photosynthesis

We further investigated the time course of PS II photochemistry under steady state photosynthesis, i.e. in the light-adapted state, in response to 0.75 M NaCl. Fig. 4*A* shows the changes in the efficiency of excitation energy capture by open PS II reaction centres (F_v'/F_m'). The patterns of the responses of F_v'/F_m' to salt stress in the dark and light were similar to these of F_v/F_m. During the first phase, i.e. in the first 4 h, there was a 40% decrease in F_v'/F_m' in the first 15 min, followed by a partial recovery to about 85% of the original level after 2 h of incubation. No significant difference between the effect of light and dark on F_v'/F_m' was observed. During the second phase, i.e. after 4 h from the beginning of

the salt treatment, a second decline in F_v'/F_m' was observed only in the light, which reached about 65% of the original level after 12 h (Fig. 4*A*). Fig. 4*B* shows that the response pattern of the quantum yield of PS II electron transport ($\Phi_{PS\ II}$) to 0.75 M NaCl was also similar to that of F_v/F_m.

The responses of photochemical quenching (q_P) and non-photochemical quenching (q_N) to 0.75 M NaCl also showed two phases which were similar to that of F_v/F_m (Fig. 5). During the first phase, q_P decreased by 15% in the first 15 min, which was followed by a recovery to about 92% of the original values, independent of whether the *S. platensis* cells were kept in the light or dark. During the second phase, q_P was maintained at the level of 92% of the original when the cells were kept in the dark. However, if the cells were exposed to light, q_P started to decrease again and reached to 66% of the original value 12 h after the cell suspension exposed to 0.75 M NaCl (Fig. 5*A*).

In contrast to the change in q_P, during the first phase, q_N increased by 40% in the first 15 min, thereafter, it showed a recovery to the original value in about 2 h. The data also show that the change in q_N in this phase was independent of light (Fig. 5*B*). During the second phase, q_N increased significantly in the light but was maintained in the dark.

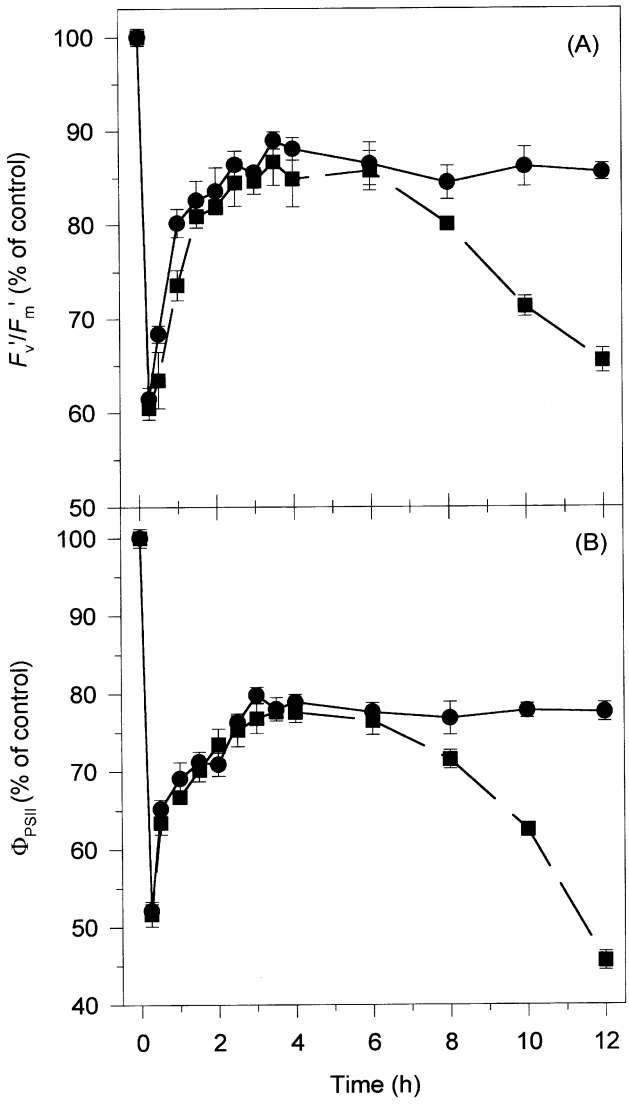

Fig. 4. Time courses of the responses of the efficiency of excitation energy capture by open PS II reaction centres (F_v'/F_m', *A*) and the quantum yield of PS II electron transport ($\Phi_{PS\ II}$, *B*) to 0.75 M NaCl in *S. platensis* cells incubated in the dark (●) and light (■). The initial value of Φ_v'/F_m' and $F_{PS\ II}$ (as 100%) were 0.618 ± 0.011 and 0.581 ± 0.016, respectively. Values are mean ± SE (n = 3).

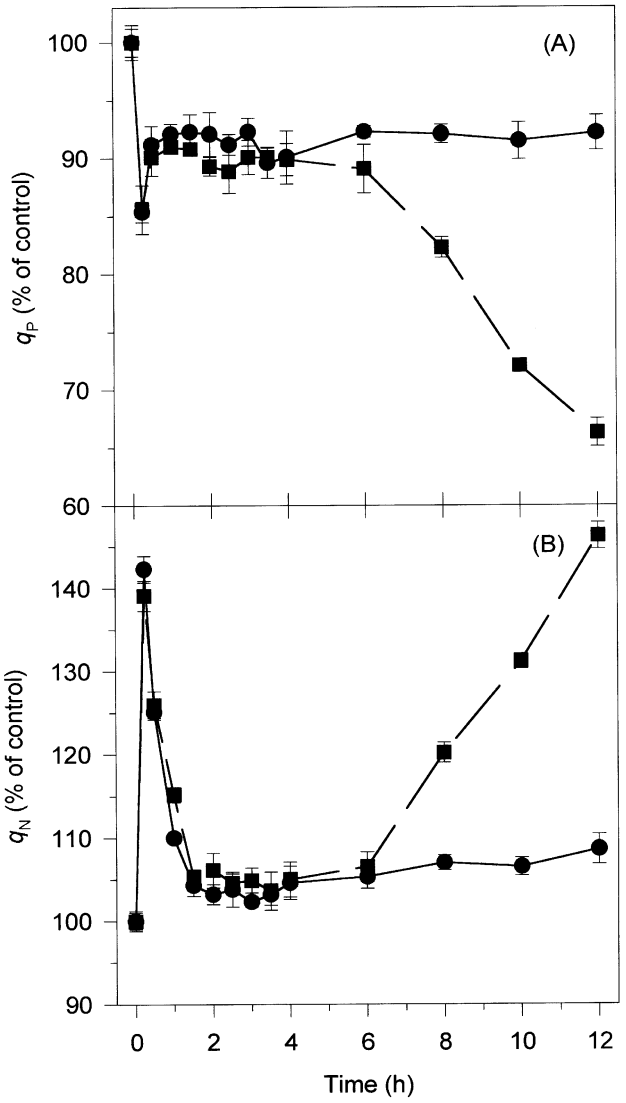

Fig. 5. Time courses of the responses of the photochemical quenching coefficient (q_P, *A*) and the non-photochemical quenching coefficient (q_N, *B*) to 0.75 M NaCl in *S. platensis* cells incubated in the dark (●) and light (■). The initial values of q_P and q_N (as 100%) were 0.939 ± 0.031 and 0.164 ± 0.009, respectively. Values are mean ± SE (n = 3).

Rapid fluorescence induction kinetics (Kautsky effect)

When a dark-adapted sample is illuminated with red light at a moderate intensity, a typical Kautsky curve is observed, which displays a rapid rise of chlorophyll fluorescence from the minimal level (O) to an intermediate level (I) followed by a very fast rise to the maximal level (P). The O–I phase has been attributed to Q_A reduction in the Q_B-non-reducing PS II reaction centres, in which the electron transfer from Q_A^- to Q_B is inhibited. The ratio $(F_i–F_o)/(F_p–F_o)$ can thus be considered as a measure of the proportion of those Q_B-non-reducing PS II reaction centres (Chylla and Whitmarsh 1989; Cao and Govindjee 1990).

The responses of the ratio $(F_i–F_o)/(F_p–F_o)$ to salt stress are demonstrated in Fig. 6. During the first phase, there was an increase in this ratio to 30% from the control value 20% in the first 15 min, followed by a recovery to the control values in both dark and light. During the second phase, the ratio showed no significant change in the dark but increased significantly in the light.

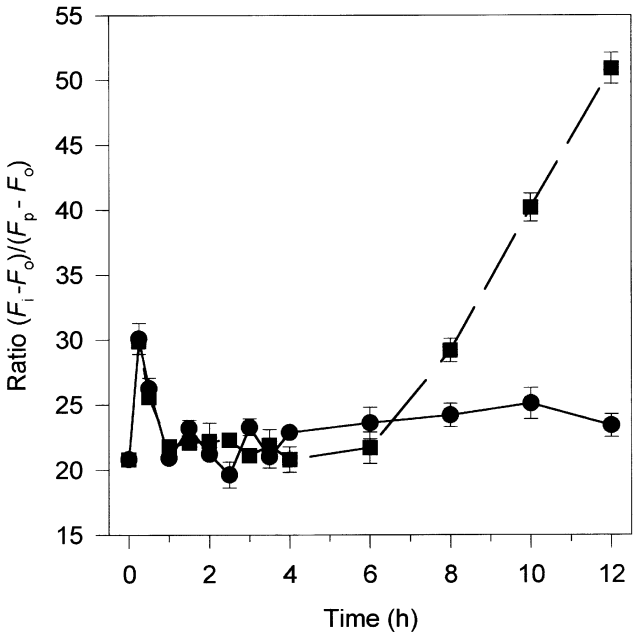

Fig. 6. Time course of the responses of the ratio $(F_i–F_o)/(F_p–F_o)$ expressed as percentage to 0.75 M NaCl in *S. platensis* cells incubated in the dark (●) and light (■). The initial value of $(F_i–F_o)/(F_p–F_o)$ was 20.8 ± 1.6. Values are mean ± SE (n = 3).

Polyphasic rise of Chl a *fluorescence transient (O-J-I-P) and the JIP test*

It has been demonstrated that, in response to a high intensity of actinic light, the fluorescence transient shows a polyphasic rise including phase O, J, I, and P (Strasser *et al.* 1995). This polyphasic rise of fluorescence transient may provide more information on PS II photochemistry, such as the electron transport in both donor and acceptor sides of PS II as well as excitation utilization efficiency per PS II reaction centre (Strasser *et al.* 1995; Krüger *et al.* 1997; Strasser 1997).

By following the changes in the polyphasic Chl *a* fluorescence transients during salt stress, we evaluated the changes in the electron transport in the acceptor side of PS II, which can be assessed by the probability of electron transfer beyond Q_A (ψ_o) and the yield of electron transport beyond Q_A (φ_{Eo}).

When *S. platensis* cells were exposed to 0.75 M NaCl and incubated in the dark or light, a 10% decrease in ψ_o was observed. Subsequently it recovered to about the original level after 2 h in both light and dark. After 4 h, ψ_o stayed constant in the dark but decreased significantly in the light, reaching a value of 75% of the control after 12 h (Fig. 7*A*). The pattern of response of φ_{Eo} to 0.75 M NaCl was similar to that of F_v/F_m, but after 4 h, a greater decrease in φ_{Eo} than in F_v/F_m in the light was observed (Fig. 7*B*).

To further evaluate the changes in the primary photochemistry of PS II during salt stress, we examined the absorption flux (ABS), trapping flux (TR) and electron transport flux (ET) per PS II reaction centre (RC), i.e. ABS/RC, TR_o/RC, ET_o/RC (Fig. 8). The results show that ABS/RC and TR_o/RC increased in the first 15 min and then declined, reaching the original level after 2 h in salt-stressed cells incubated in both light and dark. After 4 h, an increase in ABS/RC and TR_o/RC in the light-incubated cells was observed, while no significant change in the dark-incubated cells was observed (Fig. 8*A*, *B*). No significant changes in ET_o/RC were observed during the first phase regardless of illumination conditions, but a decrease in ET_o/RC was observed in light-incubated cells during the second phase (Fig. 8*C*).

Chlorophyll and phycocyanin

Changes in the contents of chlorophyll and phycocyanin during salt stress were also investigated (Fig. 9). Chlorophyll content decreased slightly by 3% in the first 2 h. After 12 h it decreased by 10 and 20 % in the dark-and light-incubated cells, respectively, (Fig. 9*A*). The content of phycocyanin decreased sharply by 55% in the first 2 h no matter if the S. platensis cells were kept in the light or dark. After 4 h, it remained constant in the light and decreased slightly in the dark (Fig. 9*B*).

Discussion

The results in this study reveal that the responses of PS II photochemistry in *S. platensis* cells to high salinity are composed of two phases. In the first phase, a rapid decrease in F_v/F_m, F_v'/F_m', q_P, $\Phi_{PS\,II}$ and a rapid increase in q_N combined by a subsequent recovery proceeded not only in the light but

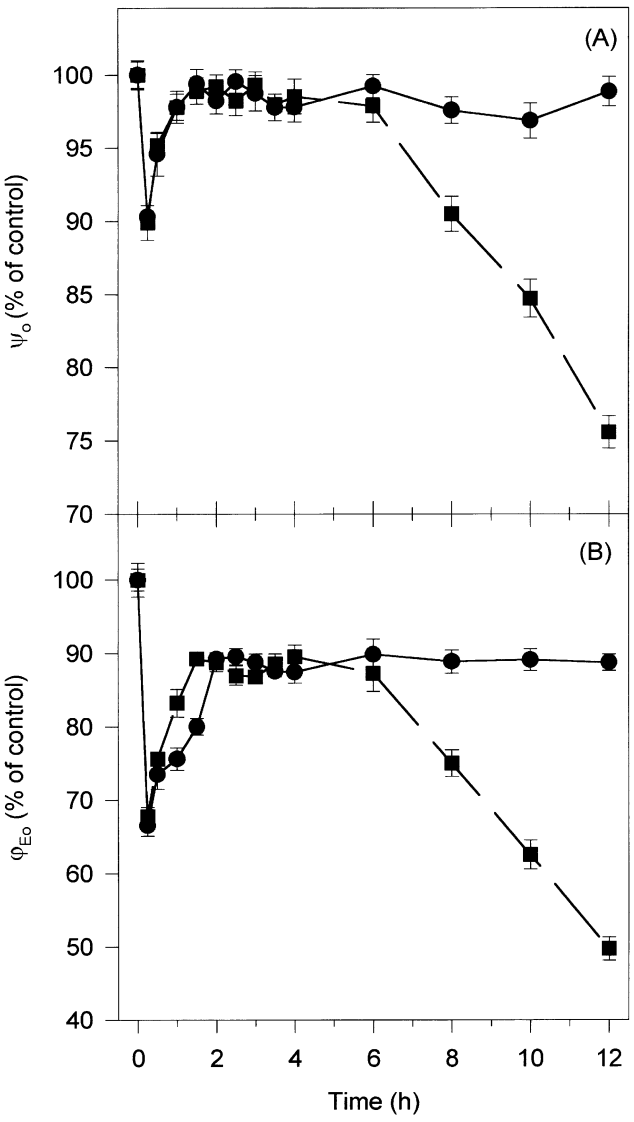

Fig. 7. Time courses of the responses of in the probability of electron transport beyond Q_A (ψ_o, *A*) and the maximum yield of electron transport beyond Q_A (φ_{Eo}, *B*) to 0.75 M NaCl in *S. platensis* cells incubated in the dark (●) and light (■). The initial values of ψ_o and φ_{Eo} (as 100%) were 0.74 ± 0.021 and 0.52 ± 0.012, respectively. Values are mean ± SE (n = 5).

also in the dark, suggesting that the changes in this phase were independent of light. In the second phase, a progressive decrease in F_v/F_m, F_v'/F_m', q_P, $\Phi_{PS\ II}$ and an increase in q_N were observed only in the light, indicating that a photoinhibitory stress was induced in this phase as a result of the salinity stress.

Our results show that salt stress resulted in a significant decrease in q_P during the two phases, which may indicate a significant increase in the proportion of the closed PS II reaction centres or in the proportion of the reduced state of Q_A. As shown salt stress induced a substantial increase in the proportion of the Q_B-non-reducing PS II reaction centres during two phases (Fig. 6). In Q_B-non-reducing PS II reac-

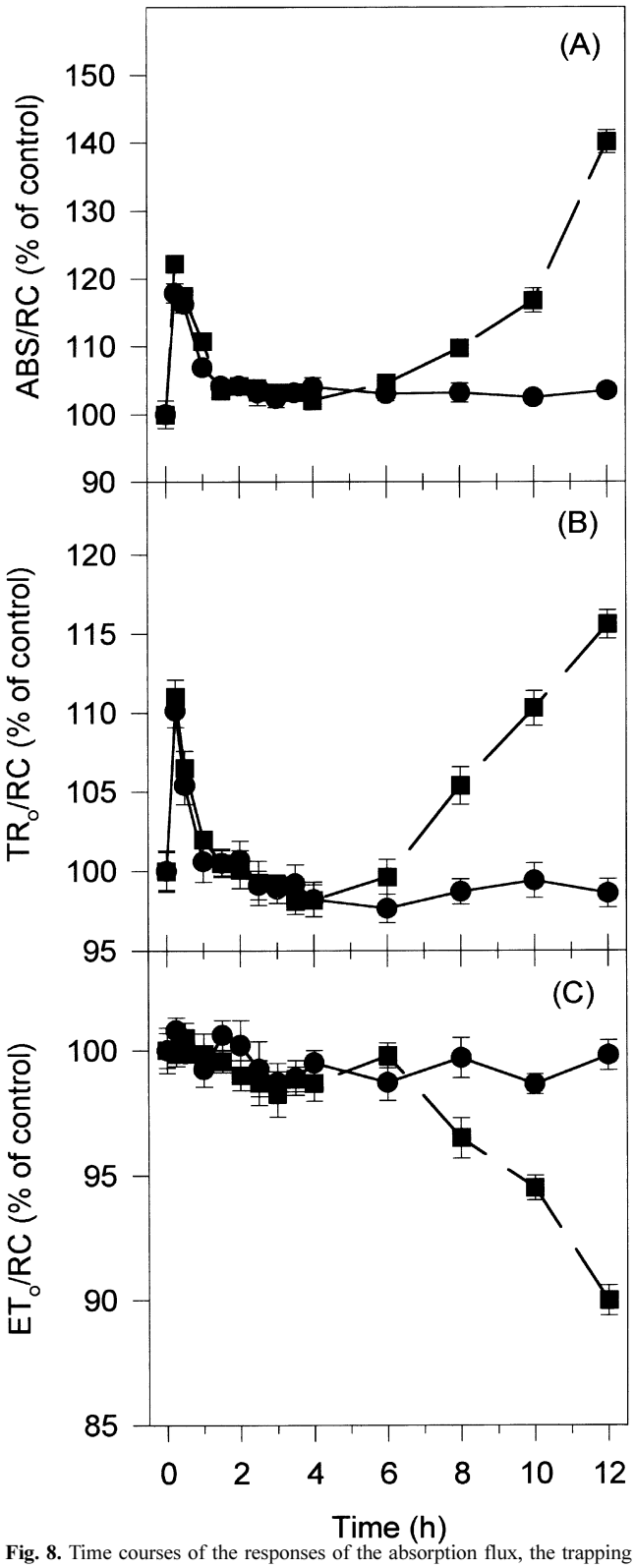

Fig. 8. Time courses of the responses of the absorption flux, the trapping flux and the electron transport flux per reaction centre, i.e. ABS/RC (*A*), TR_o/RC (*B*) and ET_o/RC (*C*) to 0.75 M NaCl in *S. platensis* cells incubated in the dark (●) and light (■).The initial values of ABS/RC, TR_o/RC and ET_o/RC (as 100%) were 0.922 ± 0.022, 0.646 ± 0.022 and 0.478 ± 0.017, respectively. Values are means ± SE (n = 5).

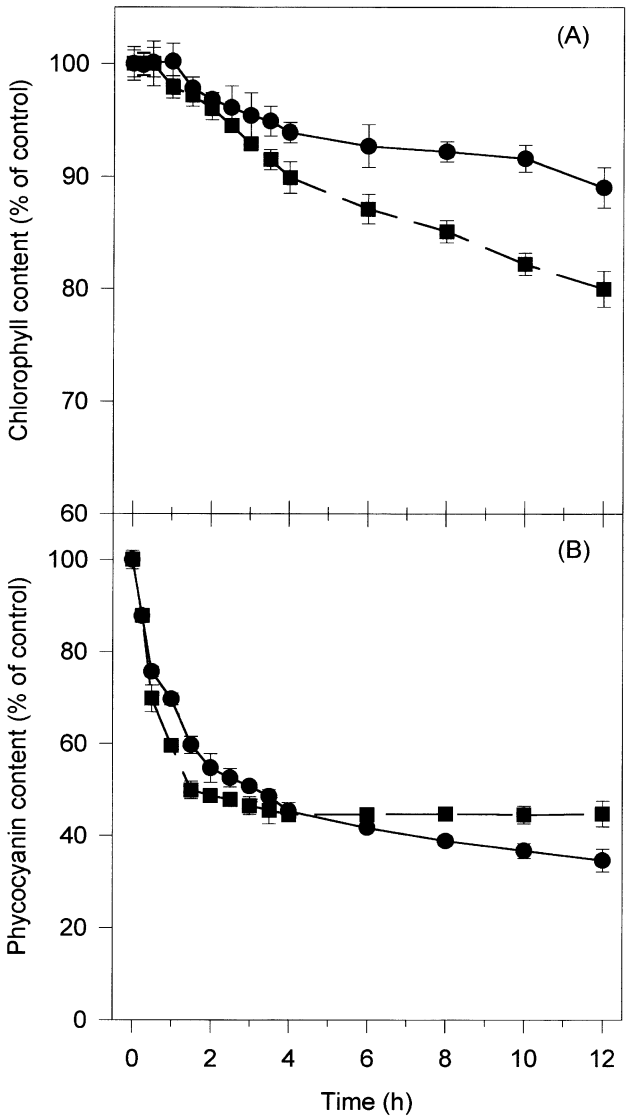

Fig. 9. Time courses of the responses of chlorophyll (*A*) and phycocyanin (*B*) to 0.75 M NaCl in *S. platensis* cells incubated in the dark (●) and light (■). The initial values of chlorophyll content and phycocyanin (as 100%) were 5.1 ± 0.04 mg mL^{-1} and 26.3 ± 0.09 mg mL^{-1}. Values are means ± SE (n = 3).

tion centres the electron transfer electrons from Q_A to Q_B is inhibited (Chylla and Whitmarsh 1989; Cao and Govindjee 1990). Obviously, a blocking of electron transfer from Q_A to Q_B will inevitably result in an accumulation of reduced Q_A. Therefore, an increase in the proportion of reduced Q_A indicated by a decreased q_P could be explained by an increase in the proportion of Q_B-non-reducing PS II reaction centres. The decrease in the probability of electron transfer beyond Q_A (ψ_o) and the yield of electron transport beyond Q_A (φ_{Eo}) also suggest that a decrease in q_P was associated with an inhibition of electron transport at the acceptor side of PS II (Fig. 7).

Our results also show that salt stress induced a significant decrease in F_v'/F_m' during the two phases. According to the theoretical analysis by Havaux *et al.* (1991), F_v'/F_m' is proportional to the probability of excitation energy transfer between the antennae and the PS II reaction centres, which will decrease when non-photochemical quenching deactivation of PS II increases. Genty *et al.* (1990) have also shown that non-photochemical quenching deactivation of PS II resulted in a decrease in F_v'/F_m'. Correlating to the decreased F_v'/F_m' during the two phases, we observed a significant increase in q_N. Obviously, q_N reflects the processes that compete with PS II photochemistry for absorbed excitation energy. We thus suggested that the decreased F_v'/F_m' is due to increased non-photochemical quenching.

It has been suggested that q_N plays an important role in protecting PS II apparatus from damage by dissipating excess excitation energy under environmental stress conditions (Krause and Weis 1991). In higher plants, excess excitation energy can be mainly dissipated by energy-dependent quenching mechanisms (for reviews see Demmig-Adams and Adams 1992). In cyanobacteria, however, there is no evidence for energy-dependent quenching mechanisms, which are the predominant components of q_N in higher plants (Campbell and Öquist 1996). Instead, in cyanobacteria, it has been shown that q_N reflects the excitation flow from the phycobilisome to photosystem I and can be fully explained through a state transition (Öquist *et al.* 1995; Campbell and Öquist 1996). Our results show that increased q_N during the first phase was induced not only in the dark but also in the light. Yet during the second phase q_N was induced only in the light. This suggests that the mechanisms for increased q_N during two phases may be different.

Endo *et al.* (1995) have demonstrated that addition of salt to *Chlamydomonas reinhardtii* cells induced a transient increase in q_N followed by subsequent recovery either in the light or in the dark, which was similar to the pattern of q_N observed during the first phase in this study. They have also shown that such increased q_N was attributed to the state-2 transition. In addition, Schoor *et al.* (1995) also have shown that salt shock induced the state transition in favor of PS I in *Synechocystis* sp. cells. It is therefore possible that the changes in q_N during the first phase may be involved in the state transition mechanism. Since an increase in q_N during the second phase was observed only in the light, we would like to suggest it reflects a photoinhibitory quenching process. The mechanisms for non-photochemical quenching induced by salt stress observed in this study needs to be further studied.

However, whatever the mechanism of non-photochemical quenching in cyanobacteria is, an increase in q_N induced by salt stress in the light will inevitably dissipate excess excitation energy at the level of PS II reaction centres or/and shift excess excitation energy from the phycobilisomes to PS I, thus causing a down-regulation of excitation energy pressure

imposed on the PS II to avoid the over-reduction of Q_A (Genty *et al*. 1990; Havaux *et al.* 1991) and to match the decreased demand for ATP and NADPH in the CO_2 fixation process as indicated by a significant decrease in the photosynthetic evolution activity (Fig. 2). A smaller recovery of the photosynthetic evolution activity after the first 15 min may suggest that a greater inhibition in the photosynthetic dark-reaction process (the Calvin cycle) was induced during salt stress (Fig. 2).

The analyses of the polyphasic rise of fluorescence transients show that the changes in the probability of electron transfer beyond Q_A (ψ_o), the yield of electron transport beyond Q_A (φ_{Eo}), the absorption flux (ABS/RC) and the trapping flux per reaction centre (TR_o/RC) also demonstrate two different phase. How could salt stress induce the changes in ABS/RC and TR_o/RC? According to the JIP test (Strasser and Strasser 1995; Krüger *et al.* 1997), the RC here refers only to the reaction centres that are able to reduce Q_A to Q_A^- which is then reoxidized to Q_A. Therefore, TR_o/RC and ABS/RC actually refer to the absorption flux and the trapping flux per active RC. As shown in this study, salt stress induced an increase in the Q_B-non-reducing PS II reaction centres which lack the ability to reduce Q_A to Q_A^- (Fig. 6), It is thus possible that an inactivation of some PS II reaction centres by salt stress would also result in an increase in TR_o/RC and ABS/RC. A decrease in ET_o/RC during the second phase indicates that salt stress resulted in a decrease in the electron transport per RC, although there is an increase in TR_o/RC. Our results suggest that the decreased ET_o/RC resulted from a significant inhibition of the electron transport at the acceptor side of PS II as reflected by the decreased ψ_o since $ET_o/RC = \psi_o \times TR_o/RC$ (Fig. 1). No significant changes in ET_o/RC during the first phase were due to a smaller decrease in ψ_o.

The data presented point out a significant increase of F_o followed by a subsequent recovery during the first phase (Fig. 3*C*). It seems that the reversible change in F_o can not be due to the changes in the phycocyanin content, since we observed a continuing decline in the content of phycocyanin during the first phase (Fig. 9*B*). We would like to suggest that the reversible change in F_o in the first phase was a result of the reversible blocking of electron transport at the acceptor side of PS II reaction centres indicated by the changes in the probability of electron transfer beyond Q_A (ψ_o) and the yield of electron transport beyond Q_A (φ_{Eo}) (Fig. 7), which may be associated with a reversible change in Q_B-non-reducing PS II reaction centres (Fig. 6).

A second increase in F_o was observed after 4 h of exposure to salt stress only in the light-incubated cells (Fig. 3*C*). The increase in F_o may be related to a irreversible damage to PS II reaction centres (photoinhibitory damage), which has been reported in several studies suggesting that F_o can be taken as an indicator of reaction centre malfunction (Franklin *et al.* 1992; Park *et al.* 1995).

It should be noted that as defined by van Kooten and Snel (1990), $q_P = (F_m'–F_s)/(F_m'–F_o') = (F_m'–F_s)/F_v'$ and $q_N = 1–(F_m'–F_o')/(F_m–F_o) = 1–(F_v'/F_v)$, it is obvious that the values of q_P and q_N depend mainly on F_o, F_m, and thereby F_o', F_m'. In fact, a significant decrease in F_m accompanied by the changes in F_o indicates that the changes in q_P and q_N were not only a result of the changes in F_o (Fig. 3*C*). More importantly, since F_o and $F_o'$are subtracted out and the calculation of q_P and q_N is scaled to variable fluorescence $F_v' = F_m'–F_o'$ and $F_v = F_m–F_o$, the level of F_o and F_o' does not distort the calculation of q_P and q_N (Campbell *et al.* 1998). Therefore, the changes in F_o should not significantly affect the original meanings of q_P and q_N.

In conclusion, our results show that the responses of PS II photochemistry to high salinity was characterized by two phases. The first phase was a reversible process and independent of light. During the second phase, the changes in PS II photochemistry was a result of photodamage induced by salt stress, suggesting that the changes in PS II photochemistry may not be due to salt stress alone but due to the interaction of light and salt stress. Our results also show that the decrease in the quantum yield of PS II electron transport during two phases can be explained by an increase in both the proportion of the Q_B-non-reducing PS II reaction centres and non-photochemical quenching, both of which resulted in a decrease in q_P and F_v'/F_m', respectively.

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