Biochimica et Biophysica Acta 1777 (2008) 488–495

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Characterization of photosystem II in salt-stressed cyanobacterial *Spirulina platensis* cells

Hongmei Gong, Yunlai Tang, Jia Wang, Xiaogang Wen, Lixin Zhang, Congming Lu *

Photosynthesis Research Center, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, China
Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, China

ARTICLE INFO

Article history:
Received 1 December 2007
Received in revised form 4 March 2008
Accepted 20 March 2008
Available online 29 March 2008

Keywords:
Chlorophyll fluorescence
Photosystem II
PsbO protein
Salt stress
Spirulina platensis
Thermoluminescence

ABSTRACT

PSII activity was inhibited after *Spirulina platensis* cells were incubated with different salt concentrations (0–0.8 M NaCl) for 12 h. Flash-induced fluorescence kinetics showed that in the absence of DCMU, the half time of the fast and slow components decreased while that of the middle component increased considerably with increasing salt concentration. In the presence of DCMU, fluorescence relaxation was dominated by a 0.6s component in control cells. After salt stress, this was partially replaced by a faster new component with half time of 20–50 ms. Thermoluminescence measurements revealed that $S_2Q_A^-$ and $S_2Q_B^-$ recombinations were shifted to higher temperatures in parallel and the intensities of the thermoluminescence emissions were significantly reduced in salt-stressed cells. The period-four oscillation of the thermoluminescence B band was highly damped. There were no significant changes in contents of CP47, CP43, cytochrome *c550*, and D1 proteins. However, content of the PsbO protein in thylakoid fraction decreased but increased significantly in soluble fraction. The results suggest that salt stress leads to a modification of the Q_B niche at the acceptor side and an increase in the stability of the S_2 state at the donor side, which is associated with a dissociation of the PsbO protein.

1. Introduction

Salt stress is an important environmental factor that restricts plant growth and productivity [1]. The decline in growth observed in many plants subjected to salt stress is often associated with a decrease in their photosynthetic capacity [2,3]. This decreased photosynthetic capacity is associated with the partial stomatal closure and/or the non-stomatal limitation which is involved in the dark enzymatic processes of CO_2 assimilation, such as the decrease in Rubisco activity and content, RuBP or Pi regeneration capacity [4–7]. Since it has been considered that one of the primary sites of damage to the photosynthetic apparatus by environmental stress is located in photosystem II (PSII) [8], the effects of salt stress on PSII in plants have been investigated by many studies. Some studies have shown that salt stress inhibits PSII activity [9–11] whereas others have demonstrated that salt stress has no effect on PSII [6,7,12–15]. These studies suggest that the sensitivity of PSII to salt stress may be different in different plant species [16].

Photosynthesis of algae is often inhibited by salt stress [17] (for a review). Such an inhibition may be explained by a decrease in PSII activity. In the green algae, salt stress inhibits PSII activity in *Dunaliella tertiolecta* and *Chlamydomonas reinhardtii* that is associated with a state-2 transition [18,19]. In the red alga *Porphyra perforata*, salt stress also results in a decrease in PSII activity which is due to the decrease in excitation energy reaching PSII reaction centers [20]. In the cyanobacteria, it has also been reported that salt stress inhibits PSII activity [21–24], although there is a report showing that salt stress has no effect on PSII activity when *Synechocystis* cells exposed to 0.55M NaCl [25]. However, in general, it is still unclear for the mechanism of salt-stress-induced inhibition of PSII activity in algae.

In the cyanobacteria, it has been suggested that the decreased PSII activity in salt-stressed *Synechocystis* cells is associated with the state-2 transition [21,22]. Our previous studies have shown that salt stress significantly inhibit the maximal efficiency of PSII photochemistry (F_v/F_m) in *Spirulina platensis* cells and this inhibition is increased with increasing light intensity [24,26,27]. The decreased F_v/F_m may be due to an inactivation of PSII reaction centers, an inhibition of electron transport at both donor and acceptor sides of PSII, and a distribution of excitation energy transfer in favor of PSI [23,24,28]. In *Synechocystis*, it has been reported that the decreased PSII activity by salt stress can be explained by the fact that salt stress inhibits the repair of photodamaged PSII by suppressing the transcription and translation of *psbA* genes [29]. However, it has not been shown how salt stress affects primary photochemistry of PSII or induces the modifications of the donor and acceptor sides of PSII either in green algae or in the cyanobacteria.

S. platensis, a filamentous cyanobacterium, has been isolated from a wide range of habitats [30]. It has long been cultured for production of health food because of its high content of protein and other nutritional

---

* Corresponding author. Tel./fax: +86 10 62595516.
E-mail addresses: lucm@ibcas.ac.cn, congminglu@yahoo.com (C. Lu).

0005-2728/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2008.03.018

elements [31]. In addition, much attention has also been paid to its potential employment for production of high value chemicals, such as phycocyanin, carotenoids and γ-linolenic acid [32]. Therefore, considerable interest has been invested in outdoor cultivation of *S. platensis* for commercial biomass production [33]. However, 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 the culture, which results in a decrease in algal growth and photosynthesis [34,35]. Our previous studies have shown that PSII activity is significantly inhibited in salt-stressed *S. platensis* [23,24,28]. However, the exact mechanism for the decreased PSII activity remains unclear. A better understanding of salt stress on PSII may help optimize the productivity of the algal cultures grown outdoors.

In the present study, we have used thermoluminescence (TL) and flash-induced chlorophyll (Chl) fluorescence measurements and Western-blotting analyses to evaluate more precisely the electron transfer and charge recombination events between the acceptor and donor sides of PSII in salt-stressed *S. platensis* cells. Our results demonstrate that salt stress leads to a modification of the Q_B niche at the acceptor side. Our results also show that, salt stress results in an increase in the stability of the S_2 state at the donor side, which is associated with a dissociation of the PsbO protein from the thylakoid membranes.

2. Materials and methods

2.1. Cell culture and growth conditions

The cyanobacterium, *S. platensis* M_2, was grown at 30 °C in Zarouk's medium supplemented with 0.2M sodium bicarbonate [36]. Illumination of 80 μmol photons m^{-2} s^{-1} was provided by cool daylight tubes (TLD 30W/865, Philips).

2.2. Salt stress treatments

Exponentially growing cells were harvested and resuspended at a concentration of 10 μg Chl mL^{-1} in a fresh medium containing different concentrations of NaCl (0, 0.2, 0.4, 0.6, 0.8 M exclusive of 0.017M NaCl already present in the medium) and incubated at the same conditions as described above for 12 h.

2.3. Maximal efficiency of PSII photochemistry

The maximal efficiency of PSII photochemistry was determined as the ratio of variable to maximal chlorophyll fluorescence (F_v/F_m) [37] with a portable fluorometer (PAM-2100, Walz, Germany), where $F_v=(F_m-F_o)$ and F_m and F_o are the maximal and minimal fluorescence yield, respectively, of a dark-adapted suspension. F_o was measured by using modulated measuring light that was of sufficiently low intensity (0.1 mol m^{-2} s^{-1}) not to induce any significant variable fluorescence and F_m was determined by applying 0.8s saturating pulse at 8000 mol m^{-2} s^{-1} in dark-adapted cells.

2.4. Oxygen evolution measurements

Steady-steady rates of oxygen evolution of PSII were determined using a Hansatech O_2 electrode at a light intensity of 900 μmol m^{-2} s^{-1} in the presence of 0.6mM 2,6-dichloro-*p*-benzoquinone and 5mM $K_3Fe(CN)_6$ as an electron acceptor system.

2.5. TL measurements

TL measurements of whole cells were performed with the thermoluminescence extension of the Double-Modulated Fluorometer FL2000-S/F, consisting of Thermoregulator TR2000 (Photon Systems Instruments, Brno, Czech Republic). After 30min dark adaptation at 30 °C, the cells were cooled to 0 °C and illuminated with one or multiple number of single-turnover flashes. Then the cells were warmed up to 60 °C at a heating rate of 1 °C·s^{-1} and the TL light emission was measured during the heating. The volume of the samples for each measurement was 0.4 ml and the Chl concentration of the sample was 10 μg Chl·$^{-1}$. To detect period-four oscillation of the B band, cells were illuminated with a series of single-turnover flashes. For $S_2Q_A^-$ recombination studies, cells were measured in the presence of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU, 10μM) before the flash illumination. The nomenclature of Vass and Govindjee [38] was used for characterization of the flash-induced TL glow peaks.

2.6. Chl fluorescence relaxation kinetics

The decay of Chl *a* fluorescence yield after a single-turnover flash was measured with a double-modulation fluorescence fluorometer (model FL-200, Photon Systems Instruments, Brno, Czech Republic) [39] according to the method used in the cyanobacterium *Synechocystis* sp. PCC 6803 cells as described by Vass et al. [40]. The instrument contained red LEDs for both actinic (20 μs) and measuring (2.5 μs) flashes, and was used in the time range of 100 μs to 100 s. With this type of measurement, it is important to avoid distortion of the relaxation kinetics due to the actinic effect of measuring flashes. This was carefully checked, and the intensity of the measuring flashes was set at a value that was low enough to avoid reduction of Q_A in the presence of DCMU.

2.7. SDS-PAGE and immunological analyses

Samples were solubilized in the presence of 6M urea and separated by SDS-PAGE [41] using 15% (w/v) acrylamide gels with 6M urea. For immunoblotting, polypeptides were electrophoretically transferred to PVDF membranes (Millipore, Saint-Quentin, France) and proteins were detected with antibodies raised against CP43, CP47, D1, and *psb*O proteins.

2.8. Detection of cytochromes

Cytochromes were detected by 3,3′,5,5′-tetramethylbenzidine (TMBZ) staining which was performed in 10–18% gradient gels of LDS-PAGE as described previously [42].

2.9. Determinations of protein and chlorophyll

Protein content was determined by the dye-binding assay according to Bradford [43]. Chl *a* was determined according to Bennet and Bogorad [44].

3. Results

3.1. Effects of salt stress on PSII activity

We examined the changes in the PSII activity of *S. platensis* cells after incubated at various NaCl concentrations for 12 h. The maximal efficiency of PSII photochemistry (F_v/F_m) [37] did not show a considerable decrease even when NaCl concentration was increased to 0.6 M NaCl and decreased significantly by 25% only at 0.8 M NaCl (Fig. 1). However, the activity of oxygen evolution of PSII either by whole cells or thylakoid membranes decreased almost linearly with increasing salt concentration. For example, at 0.8 M NaCl, the activity of oxygen evolution in whole cells and thylakoid membranes decreased by about 75 and 80%, respectively, as compared with that of control cells (Fig. 2).

3.2. Effects of salt stress on flash-induced Chl fluorescence kinetics

The relaxation of the flashed-induced increase in variable Chl fluorescence yield monitors the oxidation of Q_A^-, which reflects the reoxidation of Q_A via forward electron transport to Q_B (and Q_B^-) and back

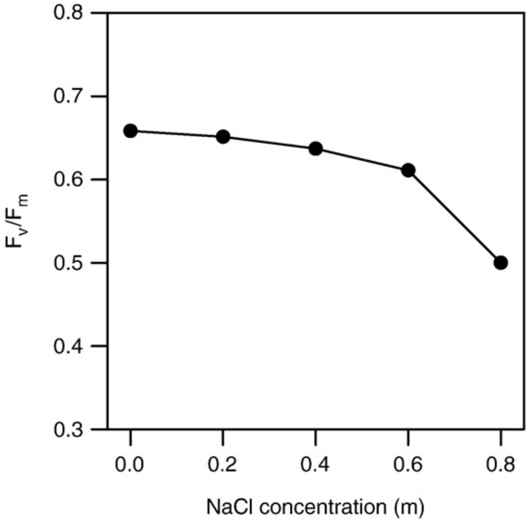

Fig. 1. Effects of salt stress on the maximal efficiency of PSII photochemistry (F_v/F_m) in *S. platensis* cells. The values are mean ± SE of five independent experiments.

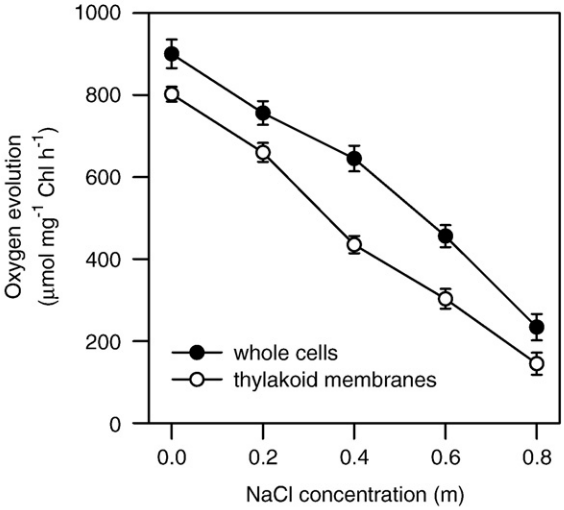

Fig. 2. Effects of salt stress on oxygen evolution of PSII by whole cells or thylakoid membranes isolated from control and salt-stressed *S. platensis* cells. The values are mean ± SE of five independent experiments. Bars indicate standard errors.

reaction with donor-side components [45,46]. Due to technical development, it is possible to measure the relaxation of the variable fluorescence in the wide time range of 100 μs to 100 s after single-flash

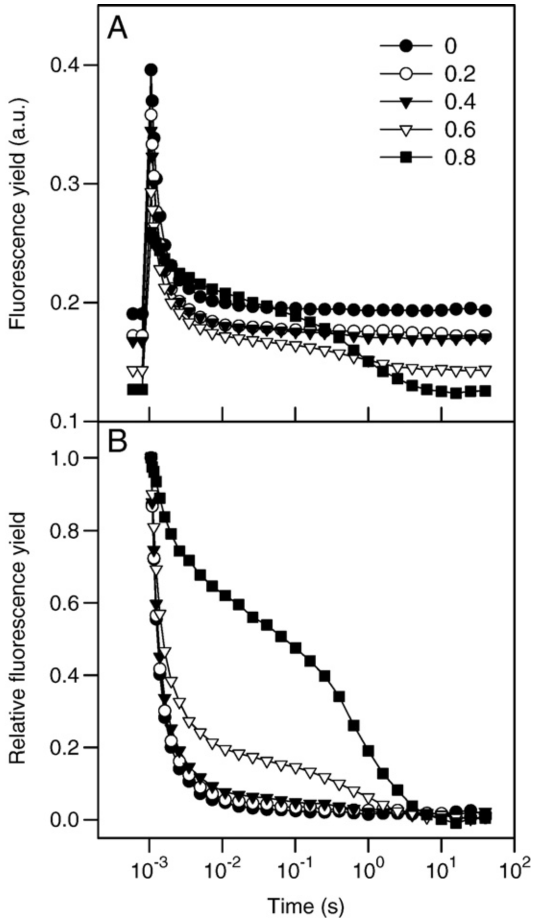

Fig. 3. Effects of salt stress on the decay of Chl fluorescence yield in *S. platensis* cells. Fluorescence decays induced by single-turnover flash were measured in the absence of 10 μM DCUM. (A) The curves are the actual data of the fluorescence signals; (B) The curves were normalized relative to the total variable fluorescence.

excitation [39] and thus obtain simultaneous information for the donor- and acceptor-side modifications [40,47]. The relaxation of the flashed-induced increase in variable Chl fluorescence yield in control cells exhibited complex kinetics that could be resolved into three different exponential decay components (Fig. 3, Table 1), which was similar to those reported by others [48,49]. The fluorescence yield relaxation in control cells is dominated by the fast phase ($t_{1/2}$ = 220 μs), whose relative amplitude is about 72%. The contribution of the middle phase ($t_{1/2}$ = 2ms) was about 25%, and that of the slow phase ($t_{1/2}$ = 10s) was about 3%. Salt stress resulted in a gradual decrease in the total fluorescence amplitude with increasing salt concentration (Fig. 3, Table 1), suggesting that salt stress resulted in a loss of Q_A reduction. In addition, salt stress resulted in a significant increase in the decay half time of the fast phase but a significant decrease in the amplitude of the fast phase. The $t_{1/2}$ of the fast phase increased from 217 μs in control cells to 876 μs in the cells treated with 0.8 M NaCl. For the middle phase, salt stress resulted in a significant increase in the decay half time and showed no significant effect on the amplitude. The $t_{1/2}$ of the middle phase increased from 2.1 μs in control cells to 25.8 μs in the cells treated with 0.8 M NaCl. However, salt stress led to a significant decrease in the decay half time and a significant increase in the amplitude of the slow phase. The $t_{1/2}$ of the slow phase decreased from 10.2s in control cells to 1.0s in the cells treated with 0.8 M NaCl (Fig. 3, Table 1). These results suggest that salt stress slowed down the middle phase but accelerated the slow phase. These results also suggest that salt stress induced a modification of the PQ binding at the Q_B site and/or a decrease in the apparent equilibrium constant for sharing the electron transport between Q_A and Q_B.

In order to investigate if the modification of the Q_B site induced by salt stress also affects the binding of PSII electron transport inhibitor, inhibitor titration experiments were carried out either in control cells or in salt-stressed cells. Table 2 shows the half-inhibitory concentration (pI_{50}) of DCMU that was calculated from the inhibitor titration curves of the slowly decaying component (0.6 s) which reflects the amount of centers in which $S_2Q_A^-$ recombination occurs. Salt stress resulted in a about 6-fold increase in the half-inhibitory concentration

Table 1
Effects of salt stress on the parameters of decay kinetics of flash-induced variable fluorescence in *Spirulina platensis* cells

NaCl concentration	Total amplitude	Fast phase	Middle phase	Slow phase
(M)	(%)	$t_{1/2}$ (μs) [A (%)]	$t_{1/2}$ (ms) [A (%)]	$t_{1/2}$ (s) [A (%)]
Without DCMU				
0	100[a]	217 ± 12 (72 ± 1.4)[b]	2.1 ± 0.3 (25 ± 1.2)	11.2 ± 0.8 (3 ± 0.1)
0.2	91 ± 2	218 ± 10 (70 ± 1.7)	2.4 ± 0.2 (26 ± 1.7)	8.7 ± 0.3 (4 ± 0.1)
0.4	86 ± 3	230 ± 16 (67 ± 2.2)	2.7 ± 0.3 (27 ± 2.1)	5.5 ± 0.3 (6 ± 0.1)
0.6	73 ± 2	279 ± 19 (56 ± 2.1)	3.2 ± 0.4 (26 ± 2.1)	3.2 ± 0.1 (18 ± 0.5)
0.8	65 ± 2	876 ± 90 (29 ± 1.4)	25.8 ± 4.8 (25 ± 1.5)	1.8 ± 0.1 (46 ± 1.0)
With DCMU				
0	100	−(0)	−(0)	0.61 ± 0.02 (100)
0.2	89 ± 2	−(0)	22.2 ± 1.2 (4.3 ± 1.5)	0.57 ± 0.04 (95.7 ± 2.4)
0.4	82 ± 1	−(0)	32.4 ± 1.4 (10.2 ± 1.0)	0.60 ± 0.03 (89.8 ± 2.6)
0.6	66 ± 2	−(0)	45.5 ± 2.0 (16.4 ± 1.1)	0.56 ± 0.02 (83.6 ± 1.8)
0.8	47 ± 1	−(0)	52.3 ± 1.8 (21.4 ± 1.7)	0.62 ± 0.04 (78.6 ± 1.7)

S. platensis cells were exposed to different salt concentrations for 12 h and the relaxation of the flashed-induced fluorescence yield was measured without or with 10 DCMU. Exponential analysis yielded either three or two phasic kinetics with different half times ($t_{1/2}$) and amplitudes (A). Mean ± SE values were calculated from four to six independent experiments.

[a] Values represent the amplitude of total variable fluorescence as a percentage of that in non-stressed control cells.

[b] Values in parentheses are relative amplitude as a percentage of total variable fluorescence obtained from non-stressed control cells and different salt-stressed cells.

Table 2
Effects of salt stress on the half-inhibitory concentrations of DCMU in *Spirulina platensis* cells

	MCMU (μM)	
	Control (0 M NaCl)	0.8 M NaCl
pI_{50} for the amplitude of the slow phase	0.31 ± 0.012	1.82 ± 0.015
pI_{50} for the activity of oxygen evolution	0.30 ± 0.013	1.55 ± 0.013

S. platensis cells were exposed to 0.8 M NaCl for 12 h. The activity of oxygen evolution and the relaxation of the flashed-induced fluorescence yield were measured after addition of various concentrations of DCMU. The fluorescence curves were analyzed by using three exponential components. The activities of oxygen evolution and the amplitude of the slow phase that reflects the recombination of Q_A^- with the S_2 state were plotted as a function of DCMU concentration and thus the half-inhibitory concentrations were determined. Mean ± SE values were calculated from four to six independent experiments.

of DCMU. To further confirm this result, the inhibitor titration curves of oxygen evolution were also performed. Table 2 shows that compared to control cells, there was a 5-fold increase in the pI_{50} value of DCMU in the cells treated with 0.8 M NaCl.

When Chl fluorescence induction kinetics is determined in the presence of 10μM DCMU, the fluorescence relaxation reflects the reoxidation of Q_A^- via recombination with donor-side components. Salt stress resulted in a decrease in the initial amplitude and accelerated the decay kinetics (Fig. 4). The analyses of these fluorescence relaxation curves showed that in control cells, a slow component with about 0.6s time constant dominated the decay (Table 1). With increasing salt concentration, there was no significant change in the time constant for this slow component but its relative amplitude decreased to 78.6% at 0.8 M NaCl. On the other hand, salt stress induced an appearance of a fast phase with 20–50 ms time constant besides the slow component. Its relative amplitude increased with increasing salt concentration and reached 21.4% at 0.8 M NaCl. By performing inhibitor titration of oxygen evolution in the control and salt-stressed cells, we have checked that electron transport through PSII was completely inhibited at 10μM DCMU. Thus, electron transfer from Q_A to Q_B was fully blocked in the oxygen-evolving centers. Therefore, the fast phase induced by salt stress observed in this study might arise from forward leak of electrons through the DCMU blocking in centers, which do not evolve oxygen. To investigate this possibility, we examined the effects of hydroxylamine on the fluorescence relaxation kinetics. Hydroxylamine releases the Mn cluster from PSII and it reduces Tyr-Z$^+$ after its light-induced oxidation [50]. Thus, the electrons that are stabilized on Q_A^- by DCMU have no recombination partner. Therefore, the flashed-induced increase in Chl fluorescence yield in control cells stayed almost constant and both the fast and slow phases shown in the presence of DCMU were eliminated by hydroxylamine (Fig. 5). Similar results were also observed in salt-stressed cells. These results suggest that the fast phase induced by salt stress should arise from the recombination reactions of Q_A^- with PSII donor-side component(s).

3.3. Effects of salt stress on TL characteristics

TL was further used to investigate the effects of salt stress the redox properties of the acceptor and donor sides of PSII, since it is a useful tool for studying charge stabilization and subsequent recombination in PSII in higher plants and cyanobacteria. Recombination of positive charges stored in the S_2 and S_3 oxidation states of the water-oxidizing complex with electrons stabilized on the reduced Q_A and Q_B acceptors of PSII results in characteristic TL emissions [38,51]. The TL intensity reflects the amount of recombining charges, whereas the peak temperature is indicative of the energetic stabilization of the separated charge pair; the higher the peak temperature, the greater the stabilization [52]. Illumination of single-turnover flash with the plant or cyanobacterial sample after a short dark adaptation induces a major TL band, called the B band which appears around at 30 °C and arises from $S_2/S_3Q_B^-$ recombination [51,53,54]. According to Govindjee et al. [55], TL emission following single-flash excitation of dark-adapted cyanobacterial cells results largely from the recombination of

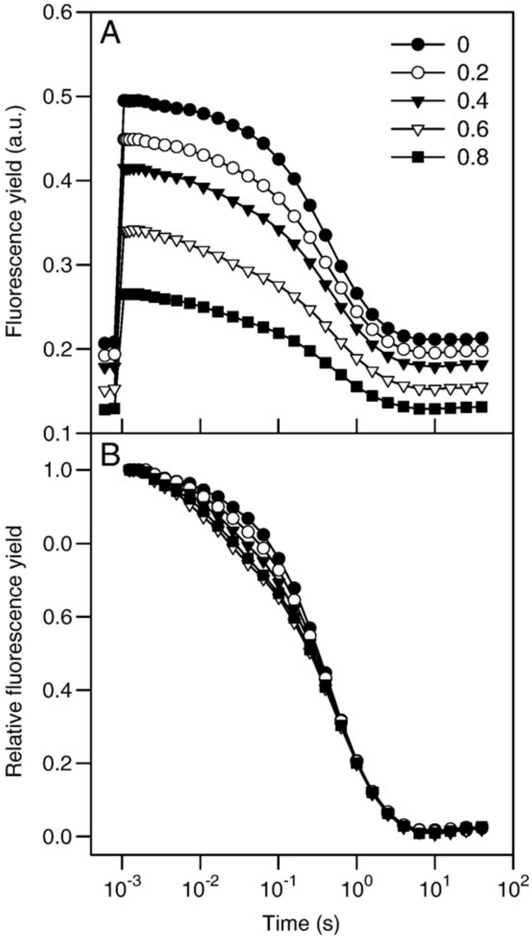

Fig. 4. Effects of salt stress on the decay of Chl *a* fluorescence yield in *S. platensis* cells. Fluorescence decays induced by single-turnover flash were measured in the presence of 10 μM DCUM. (A) The curves are the actual data of the fluorescence signals; (B) The curves were normalized relative to the total variable fluorescence.

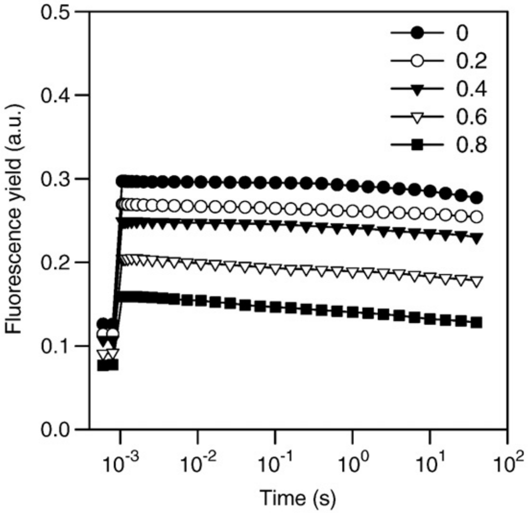

Fig. 5. Effects of salt stress on the decay of Chl *a* fluorescence yield in *S. platensis* cells. Fluorescence decays induced by single-turnover flash were measured in the presence of 10 μM DCUM and 5 mM hydroxylamine.

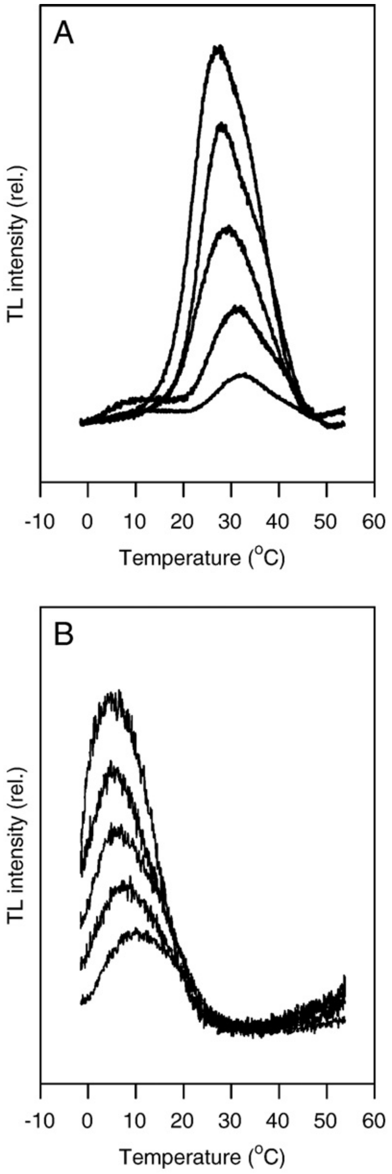

Fig. 6. Effects of salt stress on thermoluminescence glow curves in *S. platensis* cells. The cells were excited with a single flash in the absence (A) and presence (B) of 10 μM DCMU. The curves from the top to bottom were recorded from control cells and the cells treated with 0.2, 0.4, 0.6, and 0.8 M NaCl, respectively.

the $S_2Q_B^-$ charge pair. If electron transfer between Q_A and Q_B is blocked by DCMU, the B band is replaced by the so-called Q-band arising from $S_2Q_A^-$ recombination at around 10^{o}C [51,53].

Table 3
Peak emission temperatures of the TL curves in control and salt-stressed *Spirulina platensis* cells

NaCl concentration (M)	Peak temperature (°C)	
	Without DCMU	With DCMU
0.0	26.5 ± 0.1	5.1 ± 0.1
0.2	27.0 ± 0.1	5.5 ± 0.2
0.4	28.8 ± 0.2	6.5 ± 0.1
0.6	30.8 ± 0.1	8.7 ± 0.1
0.8	32.5 ± 0.1	10.3 ± 0.1

The measurements were performed in the absence and presence of 10 μM DCMU. Mean ± SE values were calculated from four to six independent experiments.

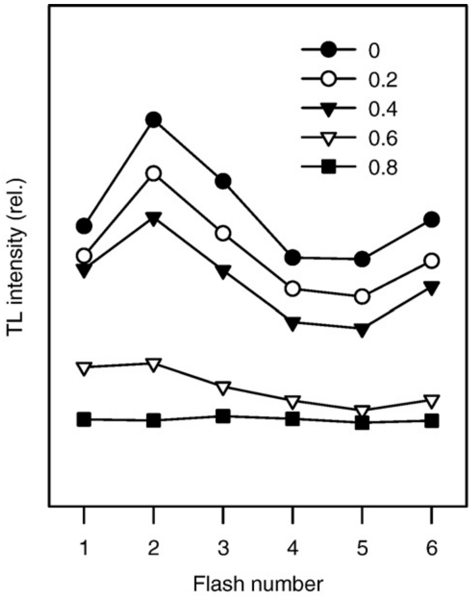

Fig. 7. Effects of salt stress on the flash-induced oscillation of the B thermoluminescence band in *S. platensis* cells. Values represent mean of 8 independent measurements.

Fig. 6 shows the TL glow curves in control and salt-stressed cells. The control cells exhibited TL emission maxima for the $S_2Q_B^-$ and $S_2Q_A^-$ charge recombination at approximately 26 and 5 °C, respectively. Salt stress resulted in an upshift in the peak temperatures for the $S_2Q_B^-$ and $S_2Q_A^-$ charge recombinations, relative to control cells. At 0.8 M NaCl, the peak temperatures for the $S_2Q_B^-$ and $S_2Q_A^-$ charge recombination were at approximately 32 and 105 °C, respectively (Fig. 6, Table 3). Since the temperature at which maxima luminescence occurs is a function of the free energy of stabilization of the charge-separated state, the upshifts in the peak temperatures of the $S_2Q_B^-$ and $S_2Q_A^-$ recombination observed in salt-stressed cells indicate that salt stress resulted in an increase in the stabilities of both charge-separated states. Moreover, since the increased stabilizations of the $S_2Q_B^-$ and $S_2Q_A^-$ recombination occurred approximately in parallel, it seems most likely that these changes were due largely to a modification of the oxygen-evolving complex specifically affecting the charge transfer characteristics of the S_2 state.

Fig. 7 shows the oscillation of the B band in control and salt-stressed cells as a function of the number of flashes given prior to recording the TL curves. In control cells, the intensity of the B band oscillated with a periodicity of four with the maximum emission occurring on the second flash. This is the same type of oscillation as observed in the cyanobacteria [55,56] and for whole leaf tissue [57]. With increasing salt concentration, the oscillatory pattern was gradually damped compared to control cells. At 0.6 and 0.8 M NaCl, the period-four oscillation almost could not be observed.

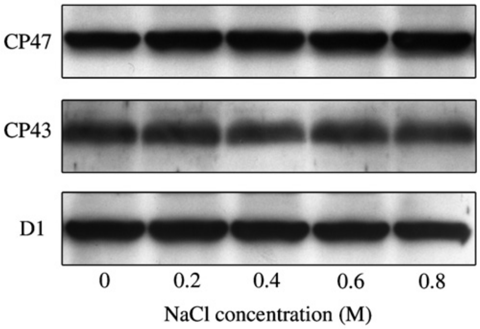

Fig. 8. Effects of salt stress on the contents of CP47, CP43 and D1 proteins in *S. platensis* cells.

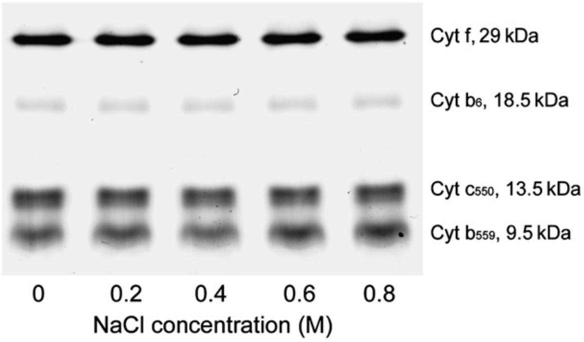

Fig. 9. Effects of salt stress on the TMBZ-staining polypeptides of thylakoids isolated control and salt-stressed *S. platensis* cells.

3.4. Effects of salt stress on CP47, CP43, D1, cytochrome c550, and psbO proteins

In order to investigate if the decreased PSII activity in salt-stressed cells was associated with the changes in the contents of PSII proteins, we further examined the effects of salt stress on several major proteins of PSII. Salt stress had no significant effects on the content of CP47 and CP43. The content of D1 protein did not show a significant change until salt concentration reaching to 0.6 M NaCl and showed a slight decrease only a 0.8 M NaCl (Fig. 8).

We also examined the effects of salt stress on the contents of TMBZ-staining cytochromes. TMBZ staining exploits the inherent peroxidase activity of cytochromes after gel electrophoresis and is in particular useful for detection of c-type cytochromes [42]. Four cytochromes were observed in the present study (Fig. 9). According to their molecular masses and patterns of electrophoresis, these four cytochromes were identified as cytochrome *f*, cytochrome *b*, cytochrome *c550*, and cytochrome *b559* [42,58,59]. Our results show that salt stress had no effects on the contents of cytochrome *f*, cytochrome *b*, cytochrome *c550*, and cytochrome *b559*.

Since it has been reported that a cyanobacetrium *Synechocystis* sp. PCC6803 genetically lacking the manganese-stabilizing protein, i.e. the PsbO protein, of PSII shows an increased stability of the S_2 state [56], we explored if the change in the S_2 state induced by salt stress was associated with a decrease in the content of the PsbO protein in the thylakoid membranes. We examined the changes in the content of the PsbO protein in the thylakoids fraction and the soluble fraction in control and salt-stressed cells. Fig. 10 shows that with increasing salt concentration, the content of the PsbO protein in the thylakoid fraction decreased significantly while the content of the PsbO protein in the soluble fraction increased significantly, suggesting that salt stress induces a dissociation of the PsbO protein from the thylakoid membranes.

4. Discussion

Our previous studies have shown that salt stress inhibits the PSII activity in *S. platensis* cells [23,24,28]. In the present study, we extended our investigation to the possible mechanisms of this decreased PSII activity. Thus, we have determined the changes in the characteristics of TL curves and flash-induced Chl fluorescence kinetics in salt-stressed cells to examine how salt stress affects the electron transfer at the donor and acceptor sides of PSII.

Flash-induced Chl fluorescence kinetics is a useful to study the electron transfer at the donor and donor sides of PSII. The double-modulation technique [39] makes it possible to measure fluorescence yield changes in a very broad time range from 100 μs to 100 s and thus to study the reoxidation processes of Q_A^- by both forward and back reactions. Analyses of fluorescence decay kinetics show that a significant increase in the decay half time of the fast phase and a significant decrease in its amplitude (Fig. 3, Table 1), suggesting that salt stress results in an inhibition of electron transfer from Q_A to Q_B. Such an inhibition may be due to a decrease in the apparent equilibrium constant (K_{app}) for sharing the electron between Q_A and Q_B. To check this possibility, here we discuss how salt stress affects the K_{app}.

In the absence of DCMU, the $t_{1/2}$ of the slow phase in control cells was about 11s. However, in the presence of DCMU, the $t_{1/2}$ of the slow phase decreased to about 0.6s in control cells (Table 1). The slow phase reflects the reoxidation of Q_A^- via charge recombination with the S_2 state. In the presence of DCMU, the slow phase occurs from the $S_2Q_A^-$ recombination in PSII centers. Thus, the differences in the $t_{1/2}$ indicate that in the absence of DCMU, the recombination should occur from the $Q_AQ_B^-$ state, which is in charge equilibrium with the $Q_A^-Q_B$ state. Therefore, the relationship of recombination half time occurring through charge equilibrium can be described as $t_{1/2}(S_2Q_AQ_B^-)=t_{1/2}(S_2Q_A^-)$ (1 + K_{app}) [60]. Using a $t_{1/2}(S_2Q_AQ_B^-)$ of 11s in the absence of DCMU and a $t_{1/2}$ ($S_2Q_A^-$) of 0.6 s in the presence of DCMU in control cells, we calculated that K_{app} was about 17. This calculated value of K_{app} agrees well with previous reported values of K_{app} being 15–20 [61,62], which supports the analyses of fluorescence relaxation kinetics used in this study. Our results show that salt stress resulted in a decrease in the $t_{1/2}$ of the slow phase in the absence of DCMU ($S_2Q_AQ_B^-$ recombination) but had no effects on the $t_{1/2}$ of the slow phase in the presence of DCMU ($S_2Q_A^-$ recombination) (Table 1). Using these data, we calculated that the values of K_{app} were about 13, 8, 4, 2 in the cells treated with 0.2, 0.4, 0.6 and 0.8 M, respectively, indicating that K_{app} decreased with increasing salt concentration.

The decreased K_{app} in salt-stressed cells might be associated with a decreased affinity of Q_B binding pocket. Indeed, we observed that salt stress induced an increase in the $t_{1/2}$ of the middle phase in the absence of DCMU (Table 1). This decay component is assigned to reoxidation of Q_A^- in the centers in which a vacant Q_B site has to be reoccupied by a PQ molecule before the Q_A^- to Q_B electron transfer can take place. Thus, an increase in the $t_{1/2}$ of the middle phase in salt-stressed cells suggests that salt stress may result in modifications of the Q_B niche by which PQ binding is slowed down. To confirm if salt stress induces a modification of the Q_B niche, we performed inhibitor titration experiments either in control cells or in salt-stressed cells to calculate the half-inhibitory concentrations (pI_{50}) of DCMU. We observed that there was a 6-fold increase in the Q_B/herbicide binding niche in the pI_{50} value in the cells treated with 0.8 M NaCl (Table 2). Thus, the inhibitor titration experiments further confirm that salt stress induces a modification of Q_B niche.

In this study, we were surprised to observe that the kinetics of fluorescence yield relaxation exhibited an obvious acceleration when measured in salt-stressed cells in the present of DCMU and that salt stress induced an appearance of a fast phase with 20–50 ms time constant besides the slow component (Fig. 4, Table 1). This fast phase could be eliminated by hydroxylamine (Fig. 5), suggesting that this fast phase in salt-stressed cells was associated with new recombination reactions of Q_A^- with the donor-side components. Similar results have been reported in the cyanobacterium *Synechocystis* sp. PCC 6803

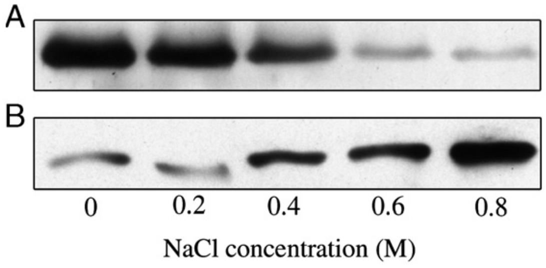

Fig. 10. Effects of salt stress on the contents of the PsbO protein in the thylakoid fractions (A) and the soluble fractions (B) isolated from the control and salt-stressed *S. platensis* cells. Thylakoid fractions and soluble fractions were isolated from the broken cells.

mutants lacking a functional Mn cluster. The half times of the fast component in the mutants range from 3.5 to 50 ms by fitting with an exponential component and this fast phase is assigned to the recombination of Q_A^- and Tyr-Z^+ [63]. This fast phase assigned as the Tyr-$Z^+Q_A^-$ recombination was also observed in dark-grown *Chlamydomonas* cells lacking the function Mn cluster with the half time of 90 ms and in isolated BBY particles in which the Mn cluster was inactivated with the half time of 36ms [64,65]. Based on the half time of the fast component observed in this study and previous similar reports, we propose that the appearance of the fast decay of fluorescence relaxation in the presence of DCMU in salt-stressed cells may also reflect charge recombination between Tyr-Z^+ and Q_A^-. Stabilization of Tyr-Z^+ in salt-stressed cells is most likely the consequence of inhibited electron transfer from the Mn cluster which is inactivated. Thus, our results suggest that salt stress induces an inactivation of the Mn cluster.

To investigate the effects of salt stress on the function of the Mn cluster, we measured the changes in the TL profiles in salt-stressed cells. We observed a parallel increase in the stability of the $S_2Q_A^-$ and $S_2Q_B^-$ charge recombination in salt-stressed cells as compared with control cells (Fig. 6). TL measurements also show that with increasing salt concentration, the period-four oscillation was much more highly damped compared to control cells (Fig. 7). These results indicate that salt stress induces an increase in the redox stability of the S_2 state. How could salt stress increase the stability of the S_2 state? It ahs been reported that the removing of the PsbO protein from PSII preparations in vitro by measuring TL profiles and by monitoring deactivation kinetics of the S states have shown that the loss of the PsbO protein is accompanied by an increase in the stability of the S_2 state [66,67]. In addition, it has been reported that the mutant lacking the PsbO protein results in upshifts in the peak temperatures of $S_2Q_A^-$ and $S_2Q_B^-$ recombination by determining TL profiles and thus leads to an increase in the stability of the S_2 state in a cyanobacterium *Synechocystis* sp. PCC 6803 [56]. In addition, earlier studies have demonstrated the release of the PsbO protein in response to salt stress by using isolated PSII particles [68–70]. These studies and the results from the TL profiles in salt-stressed cells prompt us to propose that the increased stability of the S_2 state in the salt-stressed may be due to the decreased PsbO protein in the thylakoid membranes. To investigate this possibility, we examined the effects of salt stress on the content of the PsbO protein. We observed that salt stress indeed resulted in a significant decrease in the content of psbO protein in the thylakoid fraction. On the other hand, we observed that the content of PsbO protein in the soluble fraction increased significantly with increasing salt concentration (Fig. 10). The changes in the content of the PsbO protein in the thylakoid fraction and in the soluble fraction suggest that salt stress induces a dissociation of the PsbO protein from the thylakoid membranes. Therefore, the increased stability of the S_2 state in salt-stressed cells is associated with the dissociation of the PsbO protein from the thylakoid membranes.

The results in this study clearly demonstrate that salt stress induced significant effects on electron transfer of both the donor and acceptor sides of PSII. It seems that the relationship between the donor- and acceptor-side damage is complex. It has been reported that the modifications of the PSII donor side may result in the changes in the redox properties of the acceptor side components [71]. However, the inhibitor binding experiments in this study show that the Q_B niche can be still changed in salt-stressed cells, which retain a functional oxygen-evolving complex (Table 2). Thus, our data suggest that salt-stress-induced changes in the donor and acceptor sides of PSII in *S. platensis* are the separated events and that the Q_B binding niche is already modified by salt stress prior to inactivation of the oxygen-evolving complex.

Our results demonstrated that the levels of CP47, CP43, and D1 proteins were unchanged in *S. platensis* cells after exposure to different salt concentrations up to 0.8 M NaCl (Fig. 8). However, the oxygen-evolving activity of PSII decreased significantly with increasing salt concentration (Fig. 2) and the maximal efficiency of PSII photochemistry showed a significant decrease at 0.8 M NaCl (Fig. 1). These results suggest that there was a decrease in the functional activities of PSII though the levels of CP47, CP43, and D1 remained unchanged. The similar discrepancy on the D1 level and the oxygen-evolving activity of PSII was also observed in previous studies [72,73], which was explained by the fact that immunoblotting analysis revealed the impaired form of D1 in addition to the active form [74,75]. Accordingly, the discrepancy on the levels of CP47, CP43, and D1 and PSII activity observed in this study could be similarly explained that immunoblotting analyses in this study might reveal the impaired forms of CP47, CP43, and D1 in addition to the active forms [74,75]. In addition, our results show that salt stress resulted in a release of the PsbO protein (Fig. 10). Thus, the significant decrease in the oxygen-evolving activity of PSII could be also explained by the release of the PsbO protein even though there was no significant change in the levels of CP47, CP43, and D1 proteins.

In conclusion, the results in this study have demonstrated that salt stress induces the modifications of the donor- and acceptor side of PSII in *S. platensis* cells by measuring TL profiles and flash-induced fluorescence kinetics and analyzing major proteins of PSII. Our results show that salt stress leads to a modification of the Q_B niche at the acceptor side. Our results also show that salt stress results in an increase in the stability of the S_2 state at the donor side, which is associated with a dissociation of the PsbO protein from the thylakoid membranes.

Acknowledgement

This work was supported by the Frontier Project of the Knowledge Innovation Engineering of Chinese Academy of Sciences (KSCX2-YW-N-042).

References

[1] J.S. Boyer, Plant productivity and environment, Science 218 (1982) 443–448.

[2] S.P. Long, N.R. Baker, Saline terrestrial environments, in: B.R. Baker, S.P. Long (Eds.), Photosynthesis in Contrasting Environments, Elsevier Science Publishers, Amsterdam, 1986, pp. 63–102.

[3] R. Munns, A. Termaat, Whole plant responses to salinity, Aust. J. Plant Physiol. 13 (1986) 143–160.

[4] W.J. Downton, W.J.R. Grant, S.P. Robinson, Photosynthetic and stomatal response of spinach leaves to salt stress, Plant Physiol. 77 (1985) 85–88.

[5] L.H. Ziska, J.R. Seemann, T.M. DeJong, Salinity induced limitations on photosynthesis in *Prunus salicina*, a deciduous tree species, Plant Physiol. 93 (1990) 864–870.

[6] E. Brugnoli, O. Björkman, Growth of cotton under continuous salinity stress: influence on allocation pattern, stomatal and non-stomatal components of photosynthesis and dissipation of excess light energy, Planta 187 (1992) 335–345.

[7] S. Delfine, A. Alvino, M.C. Villani, F. Loreto, Restrictions to CO_2 conductance and photosynthesis in spinach leaves recovering from salt stress, Plant Physiol. 119 (1999) 1101–1106.

[8] N.R. Baker, A possible role for photosystem II in environmental perturbations of photosynthesis, Physiol. Plant. 81 (1991) 563–570.

[9] G. Bongi, F. Loreto, Gas-exchange properties of salt-stressed olive (*Olea europea* L.) leaves, Plant Physiol. 90 (1989) 1408–1416.

[10] R. Belkhodja, F. Morales, A. Abadia, J. Gomez-Aparisi, J. Abadia, Chlorophyll fluorescence as a possible tool for salinity tolerance screening in barley (*Hordeum vulgare* L.), Plant Physiol. 104 (1994) 667–673.

[11] J.D. Everard, R. Gucci, S.C. Kann, J.A. Flore, W.H. Loescher, Gas exchange and carbon partitioning in the leaves of celery (*Apium graveolens* L.) at various levels of root zone salinity, Plant Physiol. 106 (1994) 281–292.

[12] C. Lu, J. Zhang, Thermostability of photosystem II is increased in salt-stressed sorghum, Aust. J. Plant Physiol. 25 (1998) 317–324.

[13] C. Lu, N. Qiu, Q. Lu, B. Wang, T. Kuang, Does salt stress lead to increased susceptibility of photosystem II to photoinhibition and changes in photosynthetic pigment composition in halophyte *Suaeda salsa* grown outdoors? Plant Sci. 163 (2002) 1063–1068.

[14] C. Lu, G. Jiang, B. Wang, T. Kuang, Photosystem II photochemistry and photosynthetic pigment composition in salt-adapted halophyte *Artimisia anethifolia* grown under outdoor conditions, J. Plant Physiol. 160 (2003) 403–408.

[15] C. Lu, N. Qiu, B. Wang, J. Zhang, Salinity treatment shows no effects on photosystem II photochemistry but increases the resistance of photosystem II to heat stress in halophyte *Suaeda salsa*, J. Exp. Bot. 54 (2003) 851–860.

[16] F. Loreto, M. Centritto, K. Chartzoulakis, Photosynthetic limitations in olive cultivars with different sensitivity to salt stress, Plant Cell Environ. 26 (2003) 595–601.

[17] G.O. Kirst, Salinity tolerance of eukaryotic marine algae, Annu. Rev. Plant Physiol. Plant Mol. Biol. 41 (1990) 21–53.

[18] D.J. Gilmour, M.F. Hipkins, A.N. Webber, N.R. Baker, A.D. Boney, The effect of ionic stress on photosynthesis in *Dunaliella tertiolecta*, Planta 163 (1985) 250–256.

[19] T. Endo, U. Schreiber, K. Asada, Suppression of quantum yield of photosyntem II by hyperosmotic stress in *Chlamydomonas reinhardtii*, Plant Cell Physiol. 36 (1995) 1253–1258.

[20] K. Satoh, C.M. Smith, D.C. Fork, Effects of salinity on primary processes of photosynthesis in the red alga *Porphyra perforate*, Plant Physiol. 73 (1983) 643–647.

[21] H. Schubert, S. Fluda, M. Hagemann, Effects of adaptation to different salt concentrations on photosynthesis and pigmentation of the cyanobacterium *Synechocystis sp*. PCC 6803, J. Plant Physiol. 142 (1993) 291–295.

[22] H. Schubert, M. Hagemann, Salt effects on 77K fluorescence and photosynthesis in the cyanobacterium *Synechocystis sp*. PCC 6803, FEMS Microbiol. Lett. 71 (1990) 169–172.

[23] C. Lu, A. Vonshak, Characterization of PSII photochemistry in salt-adapted cells of cyanobacterium *Spirulina platensis*, New Phytol. 141 (1999) 231–239.

[24] C. Lu, T. Giuseppe, A. Vonshak, Kinetic response of photosystem II photochemistry in cyanobacterium *Spirulina platensis* to high salinity is characterized by two distinct phases, Aust. J. Plant Physiol. 26 (1999) 283–292.

[25] R. Jeanjean, H.C.P. Matthijs, B. Onana, M. Havaux, F. Joset, Exposure of the cyanobacterium *Synechocystis* PCC 6803 to salt stress induction concerted changes in respiration and photosynthesis, Plant Cell Physiol. 34 (1993) 1073–1079.

[26] C. Lu, J. Zhang, Effects of salt stress on PSII function and photoinhibition in cyanobacterium *Spirulina platensis*, J. Plant Physiol. 155 (1999) 740–745.

[27] C. Lu, J. Zhang, Role of light in the response of PSII photochemistry in the cyanobacterial *Spirulina platensis* to salt stress, J. Exp. Bot. 51 (2000) 911–917.

[28] C. Lu, A. Vonshak, Effects of salinity stress on photosystem II function in cyanobacterial *Spirulina platensis* cells, Physiol. Plant. 114 (2002) 405–413.

[29] S.I. Allakhverdiev, Y. Nishiyama, S. Miyairi, H. Yamamoto, N. Inagaki, Y. Kanesaki, N. Murata, Salt stress inhibits the repair of photodamaged photosystem II by suppressing the transcription and translation of *psbA* genes in *Synechocystis*, Plant Physiol. 130 (2002) 1443–1453.

[30] O. Ciferri, *Spirulina*, the edible microorganism, Microbiol. Rev. 38 (1983) 36–40.

[31] A. Richmond, *Spirulina*, in: M.A. Borowitzka, L.J. Borowitzka (Eds.), Micro-algal Biotechnology, Cambridge University Press, Cambridge, 1988, pp. 85–121.

[32] M.A. Borowitzka, Products from algae, in: S.M. Phang, Y.K. Lee, M.A. Borowitzka, B.A. Whitton (Eds.), Algal Biotechnology in Asia-Pacific Region, University of Malaya, Kuala Lumpur, 1994, pp. 5–15.

[33] A. Vonshak, Recent advances in microalgal biotechnology, Biotechnol. adv. 8 (1990) 709–727.

[34] A. Vonshak, Biological limitation in developing the biotechnology for algal mass cultivation, Science De L'eau 6 (1987) 99–103.

[35] A. Vonshak, R. Guy, M. Guy, The response of the filamentous cyanobacterium *Spirulina platensis* to salt stress, Arch. Microbiol. 150 (1988) 417–420.

[36] A. Vonshak, A. Abeliovich, S. Boussiba, A. Richmond, Production of *Spirulina* biomass: effect of environmental factors and population density, Biomass 2 (1982) 175–185.

[37] G.H. Krause, E. Weis, Chlorophyll fluorescence and photosynthesis: the basics, Annu. Rev. Plant Physiol. Plant Mol. Biol. 42 (1991) 313–349.

[38] I. Vass, Govindjee, Thermoluminescence from the photosynthetic apparatus, Photosynth. Res. 48 (1996) 117–126.

[39] M. Trtilek, D.M. Kramer, M. Koblizek, Dual-modulation LED kinetic fluorometer, J. Lumin. 72–74 (1997) 597–599.

[40] I. Vass, D. Kirilovsky, A-L. Etienne, UV-B radiation-induced donor- and acceptor-side modifications of photosystem II in the cyanobacterium *Synechocystis* sp. PCC 6803, Biochem. 38 (1999) 12786–12794.

[41] U.K. Laemmli, Cleavage of structural proteins during the assembly of head of bacteriophage T4, Nature 227 (1970) 680–685.

[42] J.A. Guikema, L.A. Sherman, Electrophoretic profiles of cyanobacterial membrane polypeptides showing heme-dependent peroxidase activity, Biochim. Biophys. Acta 637 (1980) 189–201.

[43] M.M. Bradford, A rapid and sensitive method for the quantification of microgram quantities of protein using the principal of protein–dye binding, Anal. Biochem. 72 (1976) 248–254.

[44] J. Bennet, L. Bogorad, Complementary chromatic adaptation in a filamentous blue-green alga, J. Cell Biol. 58 (1973) 419–435.

[45] A.R. Crofts, C.A. Wraight, The electrochemical domain of photosynthesis, Biochim. Biophys. Acta 726 (1983) 49–185.

[46] H. Dau, Molecular mechanisms and quantitative models of variable photosystem II fluorescence, Photochem. Photobiol. 60 (1994) 1–23.

[47] I. Vass, E. Turcsanyi, E. Touloupakis, D. Ghanotakis, V. Petrouleas, The mechanism of UV-A radiation-induced inhibition of photosystem II electron transport studied by EPR and chlorophyll fluorescence, Biochem. 41 (2002) 10200–10208.

[48] J. Cao, Govindjee, Chlorophyll a fluorescence transients as an indicator of active and inactive photosystem II in thylakoid membranes, Biochim. Biophys. Acta 1015 (1990) 180–188.

[49] Govindjee, P. Eggenberg, K. Pfister, R.J. Strasser, Chlorophyll a fluorescence decay in herbicide-resistant D1 mutants of *Chlamydomonas reinhardtii* and the formate effects, Biochim. Biophys. Acta 1101 (1992) 352–358.

[50] G.M. Cheniae, I.F. Martin, Effects of hydroxylamine on photosystem II. I. Factors affecting the decay of O_2 evolution, Plant Physiol. 47 (1971) 568–575.

[51] Y. Inoue, Photosynthetic thermoluminescence as a simple probe of photosystem II electron transport, in: J. Amesz, J. Hoff (Eds.), Biophysical Techniques in Photosynthesis, Advances in Photosynthesis, vol. 3, Kluwer Academic Publishers, Dordrecht, 1996, pp. 93–107.

[52] I. Vass, G. Horvath, T. Herczeg, S. Demeter, Photosynthetic energy conservation investigated by thermoluminescene. Activation energies and half-lives of thermoluminescence bands of chloroplasts determined by mathematical resolution of glow curves, Biochim. Biophys. Acta 634 (1981) 140–152.

[53] A.W. Rutherford, A.R. Crofts, Y. Inoue, Thermoluminescence as a probe of photosystem II photochemistry: the origin of the flash-induced glow peaks, Biochim. Biophys. Acta 682 (1982) 457–465.

[54] S. Demeter, I. Vass, Charge accumulation and recombination in photosystem II studied by thermoluminescence. I: participation of the primary acceptor Q and secondary acceptor B in the generation of thermoluminescence of chloroplasts, Biochim. Biophys. Acta 764 (1984) 24–32.

[55] Govindjee, H. Koike, Y. Inoue, Thermoluminescence and oxygen evolution from a thermophilic blue-green alga obtained after a single-turnover flashes, Photochem. Photobiol. 42 (1985) 579–585.

[56] R.L. Burnap, J.R. Shen, P.A. Jurinic, Y. Inoue, L.A. Sherman, Oxygen yield and thermoluminescence characteristics of a cyanobacterium lacking the manganese-stabilizing protein of photosystem II, Biochem. 31 (1992) 7404–7410.

[57] A.W. Rutherford, Govindjee, Y. Inoue, Charge accumulation and photochemistry in leaves studied by thermoluminescence and delayed light emission, Proc. Natl. Acad. Sci. U. S. A. 81 (1984) 1107–1111.

[58] T.M. Bricker, L.A. Sherman, Triton X-114 phase fraction of membrane proteins of the cyanobacterium *Anacystis niduans* R2, Arch. Biochem. Biophys. 15 (1984) 204–211.

[59] T.M. Bricker, J. Morvant, N. Masri, H.M. Sutton, L.K. Frankel, Isolation of a highly active photosystem II preparation from *Synechocystis* 6803 using a histidine-tagged mutant of CP 47, Biochim. Biophys. Acta 1409 (1998) 50–57.

[60] R.J. Shopes, C.A. Wraight, The acceptor quinone complex of *Rhodopseudomonas viridis* reaction centers, Biochim. Biophys. Acta 806 (1985) 348–356.

[61] H.H. Robinson, A.R. Crofts, Kinetics of the oxidation-reduction reactions of the photosystem II quinone acceptor complex, and the pathway for deactivation, FEBS Lett. 153 (1983) 221–226.

[62] S. Demeter, I. Vass, É. Hideg, A. Sallai, Comparative thermoluminescence study of triazine-resistant and -susceptible biotypes of *Erigeron canadensis* L, Biochim. Biophys. Acta 806 (1985) 16–24.

[63] H.-A. Chu, A.P. Nguyen, R.J. Debus, Site-directed photosystem II mutants with perturbed oxygen-evolving properties. 1. Instability or inefficient assembly of the manganese cluster in vivo, Biochem. 33 (1994) 6137–6149.

[64] C.A. Buser, L.K. Thompson, B.A. Diner, G.W. Brudvig, Electron-transfer reactions in manganese-depleted photosystem II, Biochem. 29 (1990) 8977–8985.

[65] M. Rova, F. Mamedov, A. Magnuson, P.-O. Fredriksson, S. Styring, Coupled activation of the donor and the acceptor side of photosystem II during photoactivation of the oxygen evolving cluster, Biochem. 37 (1998) 11039–11045.

[66] I. Vass, T. Ono, Y. Inoue, Stability and oscillation properties of thermoluminescent charge pairs in the O_2-evolving system depleted of Cl^- or the 33kDa extrinsic protein, Biochim. Biophys. Acta 892 (1987) 224–235.

[67] M. Miyao, N. Murata, J. Lavorel, B. Maison-Peteri, A. Boussac, A.-L. Etienne, Effect of the 33-kDa protein on the S-state transitions in photosynthetic oxygen evolution, Biochim. Biophys. Acta 890 (1987) 151–159.

[68] T. Kuwabara, N. Murata, Quantitative analysis of the inactivation of photosynthetic oxygen evolution and the release of polypeptides and manganese in the photosystem II particles of spinach chloroplasts, Plant Cell Physiol. 24 (1983) 741–747.

[69] M. Miyao, N. Murata, Role of the 33-kDa polypeptide in preserving Mn in the photosynthetic oxygen-evolution system and its replacement by chloride ions, FEBS Lett. 170 (1984) 350–354.

[70] M. Miyao, N. Murata, The mode of binding of three extrinsic proteins of 33kDa, 23kDa and 18kDa in the photosystem II complex of spinach, Biochim. Biophys. Acta 977 (1989) 315–321.

[71] A. Krieger, E. Weis, S. Demeter, Low-pH-induced Ca^{2+} ion release in the water-splitting system is accompanied by a shift in the midpoint redox potential of the primary quinone acceptor Q_A, Biochim. Biophys. Acta 1144 (1993) 411–418.

[72] S.I. Allakhverdiev, Y. Nishiyama, S. Miyairi, H. Yamamoto, N. Inagaki, Y. Kanesaki, N. Murata, Salt stress inhibits the repair of photodamaged photosystem II by suppressing the transcription and translation of *psb*A genes in *Synechocystis*, Plant Physiol. 130 (2002) 1443–1453.

[73] K. Al-Taweel, T. Iwaki, Y. Yabuta, S. Shigeoka, N. Murata, A. Wadano, A bacterial transgene for catalase protects translation of D1 protein during exposure of salt-stressed tobacco leaves to strong light, Plant Physiol. 145 (2007) 258–265.

[74] J. Barber, B. Andersson, Too much of a good thing: light can be bad for photosynthesis, Trends Biochem. Sci. 17: 61-66.

[75] E.-M. Aro, I. Virgin, B. Andersson, Photoinhibition of photosystem II: inactivation, protein damage and turnover, Biochim. Biophys. Acta 1143 (1993) 113–134.