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Characterization of PSII photochemistry in salt-adapted cells of cyanobacterium *Spirulina platensis*

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SUMMARY

The changes in pigment composition, photosynthesis and PSII photochemistry were investigated in cells of *Spirulina platensis* adapted to salt stress (<0.75 M NaCl). A decrease in the phycocyanine/chlorophyll and no significant change in the carotenoid/chlorophyll ratio were observed in salt-adapted cells. Salt stress inhibited the apparent quantum efficiency of photosynthesis and PSII activity while stimulating PSI activity and dark respiration significantly. Salt stress also resulted in a decrease in overall activity of the electron transport chain, which could not be restored by diphenylcarbazide, an artificial electron donor to the reaction centres of PSII. Measurements of the polyphasic fluorescence rise in fluorescence transients including phases O, J, I and P showed that salt stress had no effect on the fluorescence yield at phase O but decreased the fluorescence yield at phases J, I and P. Analyses of the JIP test developed from the polyphasic rise of fluorescence transients showed that salt stress led to a decrease in both the maximum quantum efficiency of PSII photochemistry and the maximum quantum efficiency of electron transport beyond the primary quinone electron acceptor. However, salt stress induced no significant changes in the probability of transporting an electron beyond Q_A, the trapping flux per PSII reaction centre, or the electron transport flux per PSII reaction centre. A theoretical analysis of fluorescence parameters indicated a decrease in the rate constant of excitation energy trapping by PSII reaction centres. In addition, salt stress induced an increase in the complementary area above the fluorescence induction curve in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea, suggesting an increase in the proportion of closed PSII reaction centres in salt-adapted cells. Based on these results, it is suggested that modifications in PSII photochemistry in salt-adapted *Spirulina* cells maintained a high conversion efficiency of excitation energy, such that no significant change was observed in either the trapping flux or the electron transport flux per PSII reaction centre.

Key words: cyanobacterium (*Spirulina platensis*), photosystem II (PSII) photochemistry, photosynthesis, polyphasic chlorophyll fluorescence, salt adaptation.

Abbreviations: ETo, electron transport flux beyond Q_A; F_o, F_m, F_v, minimum, maximum, and variable fluorescence in the dark-adapted state; PPFD, photosynthetic photon flux density; TRo, energy trapping flux by PSII reaction centre.

INTRODUCTION

The decline in productivity observed in many plants subjected to excessive salinity is often associated with a decrease in their photosynthetic capacity. It was reported earlier that one of the primary sites of damage to the photosynthetic apparatus by environmental stress is located in PSII (Baker, 1991). Data concerning the effect of salinity on the photochemical efficiency of PSII are limited and conflicting. Some studies have shown that, in higher plants, salt stress inhibits PSII activity (Bongi & Loreto, 1989; Mishra *et al.*, 1991; Masojidek & Hall, 1992; Belkhodja *et al.*, 1994; Everard *et al.*, 1994), whereas others have indicated that salt stress has no effect on PSII (Robinson *et al.*, 1983; Brugnoli & Björkman, 1992; Morales *et al.*, 1992) or even increases PSII activity (Smillie & Nott, 1982). In cyanobacteria, the effect of salt stress of PSII has not been studied as intensively as in higher plants. Jeanjean *et al.* (1993) reported that no significant changes were noticed in the activity of PSII electron

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transport in *Synechocystis* sp. PCC 6803 adapted to 0.55 M NaCl. However, in cells adapted to a higher level of salinity (0.684 M), PSII activity decreased (Schubert & Hagemann, 1990). Furthermore, it has not been shown how salt stress affects the primary photochemistry of PSII, or which components of PSII are modified by salt stress either in higher plants or in algae.

Spirulina platensis, a filamentous cyanobacterium, has been isolated from a wide range of habitats, varying from low to high ionic strength, in salty and alkaline waters (Ciferri, 1983). In a previous work, Vonshak *et al*., (1988) demonstrated that *S. platensis* is capable of adapting to high concentrations of NaCl and that this adaptation is associated with an increase in respiratory activity. Such an increase in respiration associated with salt tolerance was also observed in a marine *Spirulina* strain (Gabbay-Azaria *et al*., 1992). The effect of salinity stress on salt-adapted cells at steady state was studied with respect to photosynthesis (Schubert & Hagemann, 1990), enzyme activity (Hagemann *et al*., 1989), and protein synthesis (Hagemann *et al*., 1990). Less information is available on changes in the primary processes of photosynthesis and possible sites of damage to PSII in cyanobacteria after the cells become adapted to high salinity.

Recently, it has been shown that transients in chlorophyll (Chl) a fluorescence display a more complex polyphasic rise when fluorescence induction is measured with a strong actinic illumination (Neubauer & Schreiber, 1987; Schreiber & Neubauer, 1987; Strasser *et al*., 1995). The polyphasic rise of these transients reflects the filling up of the electron acceptor side of PSII, the primary (Q_A) and secondary (Q_B) quinone electron acceptors, and the plastoquinone (PQ) pool with electrons from the donor side of PSII (Srivastava *et al*., 1995; Strasser *et al*., 1995). Measurements such as these have provided an opportunity to determine whether the primary photochemistry of PSII is modified by salt stress and to identify the possible sites of damage to PSII.

In this study we have examined mainly the changes that adaptation to salinity caused in the primary photochemistry of PSII and in photosynthetic electron transport in cells of *S. platensis*.

MATERIALS AND METHODS

Conditions of growth

Spirulina platensis M_2 was grown at 35°C in air enriched with 1% (v/v) CO_2 in Zarouk's medium supplemented with 0.2 M sodium bicarbonate (Vonshak *et al*., 1982). Illumination of 50 μmol quanta m^{-2} s^{-1} was provided by fluorescence lamps (GRO-Lux, Sylvania, Germany).

Salt adaptation

To obtain a salt-adapted culture, exponentially growing cells were diluted and grown for at least 14 d in Zarouk's medium containing different concentrations of NaCl (0.1, 0.35, and 0.75 M).

Measurement of pigments

Chlorophyll (Chl) a was determined according to Bennet & Bogorad (1973). The absorbance of c-phycocyanin was measured spectrophotometrically at 620 nm and its concentration calculated from the specific absorption coefficient E1% = 73 (Boussiba & Richmond, 1979). For the determination of carotenoids, samples were harvested by centrifugation, and the pellet was saponified by suspension in 30% (v/v) methanol containing 5% (w/v) KOH. The remaining pellet was neutralized by addition of 70% (v/v) acetic acid, after which carotenoids were extracted by addition of pure dimethylsulphoxide and maintained at 70°C for 5 min. The absorbance of the supernatant was measured at 490 nm and the concentration of carotenoids was calculated using the specific absorption coefficient E1% = 2200 (Davies, 1976).

Measurement of photosynthetic quantum efficiency and dark respiration

Evolution of O_2 was measured at 35°C using a Clarke-type electrode. Cells were harvested and resuspended in fresh medium containing the NaCl concentration to which cells were adapted. Light-response curves of photosynthesis (P-I curve) were obtained for cells by measuring the rate of O_2 evolution at different photosynthetic photon flux densities (PPFDs). Illumination was provided by a slide projector and a Halogen lamp (100 W), adjusting the lamp to cuvette distance or by inserting different neutral density filters into the optical path. The slope of the light-response curve of photosynthesis is a reflection of the apparent quantum efficiency of photosynthesis (α) and was calculated by regression, using the rates at 8–10 PPFDs in the range of 10–120 μmol quanta m^{-2} s^{-1}. The strictly linear region of the curves was determined on the basis of the maximum r^2 (>0.95). Dark respiration (R_d) was estimated from O_2 uptake by cells incubated in the dark.

Assay of electron transport activities

After the cells were permeabilized by treatment in the dark with 0.9 mM of p-benzoquinone (pBQ), as previously described (Satoh *et al*., 1992), PSII activity was determined by O_2 evolution with 0.9 mM (pBQ) as an electron acceptor. Overall electron transport chain activity and PSI activity were

assayed in the same cell preparation. Overall electron transport activity, from water to methyl viologen (MV), was determined from the rate of O_2 uptake following addition of 0.1 mM MV, which is reduced by PSI. Electron transport activity in the absence of the water-splitting complex was measured by incorporating 0.50 mM diphenylcarbazide (DPC) as an electron donor in the assay mixture. PSI activity was measured as O_2 uptake in the presence of 0.1 mM 2,6-dichlorophenol indophenol (DCPIP), 0.1 mM MV, 5 mM NaN_3 as an inhibitor of respiration, 10 μM 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea (DCMU) as an inhibitor of PSII, 5 mM ascorbate and 1 mM potassium cyanide as an inhibitor of superoxide dismutase.

According to Robinson *et al*., (1982), intact trichomes of *S. platensis* are permeable to the acceptors and donors used in our measurements.

Measurement of the polyphasic chlorophyll a fluorescence transients

The polyphasic rise in fluorescence transients due to Chl a were measured by a Plant Efficiency Analyser (PEA, Hansatech Instruments Ltd., King's Lynn, Norfolk, UK) with an actinic light of *c*. 3000 μmol quanta m^{-2} s^{-1} (Strasser *et al*., 1995). Illumination was by an array of six high-intensity light-emitting diodes (with a maximum of 650 nm), which were focused on the sample surface to provide homogeneous illumination over an area 4 mm in diameter. The fluorescence signals were received by a high-performance Pin photodiode detector associated with an amplifier circuit. The detector responded maximally to the longer-wavelength fluorescence signal while blocking reflected, shorter-wavelength light from light-emitting-diodes. All 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 readings per second after 2 ms.

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

The JIP test

All of the oxygenic photosynthetic materials so far investigated exhibit a polyphasic rise in fluorescence transients during the first second of illumination. These phases are labelled as O, J, I, P (Strasser *et al*., 1995). To record a complete fluorescence transient showing all these phases the sampling rate should be sufficiently high to enable the vizualization of intermediate steps J and I between the initial (F_o) and the maximal (F_m) fluorescence on a logarithmic time scale (Srivasta *et al*., 1995; Strasser *et al*., 1995). In this study, our results demonstrate that the *S. platensis* cells show a typical polyphasic rise (O-J-I-P) of fluorescence transients (see curve a in Fig. 2a) similar to that of higher plants (Strasser *et al*., 1995).

Strasser and Strasser (1995) developed the JIP test, from which formulae for the calculation of the energy fluxes and flux ratios have been derived, and which is based on the theory of energy fluxes in biomembranes in a photosynthetic apparatus (Strasser, 1978; Strasser, 1981) in combination with data from measurements of the polyphasic rise of fluorescence transients.

According to the model of energy fluxes in this test (Fig. 1), photons absorbed by the antennae pigments are referred to as absorption flux (ABS). Part of this excitation energy is dissipated as fluorescence, but most is transferred as the trapping flux (TR) to the 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) that maintains the metabolic reactions of the photosynthetic apparatus. We can therefore use the JIP test to evaluate the modifications in PSII photochemistry in salt-adapted cells, by measuring the polyphasic rise of fluorescence transients. For a detailed derivation of the formulae for the various energy fluxes and for the flux ratios in the JIP test, see 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). Although the phycobilisome and PSI are responsible for a large F_o in cyanobacteria, they do not contribute to the variable fluorescence (Fock & Mohanty, 1986). The relatively high variable fluorescence F_v/F_m ratio (0.7) obtained in our control culture suggests that the influence of the phycobilisome and PSI on F_o and the effect of dark respiration on F_m are relatively small. Moreover, the mathematical simulation of the OJIP transient, using the model of PSII from which the JIP test is derived and published rate constants, gives a satisfactory fit of the OJIP transient (Stirbet *et al*., 1995). In addition, the cyanobacterium *S. platensis*, like higher plants, shows a typical variable fluorescence transient and the main effect of salinity was on F_m rather than on F_o. Furthermore, the results obtained from the analyses of fluorescence-quenching under steady-state photosynthesis were comparable with these obtained from the analysis of the JIP test after *Spirulina* cells were subjected to salinity shock (Lu & Vonshak, unpublished). In fact, the polyphasic rise of fluorescence has been used successfully in studies of ozone stress (Meinander *et al*., 1996), high-temperature stress (Srivastava & Strasser, 1996), light stress (Krüger *et al*., 1997) and in the characterization of herbicide-resistant D_1 mutants (Srivastava *et al*., 1995). We therefore believe that the JIP test can be used to evaluate PSII photochemistry in the cyanobacterium *S. platensis*.

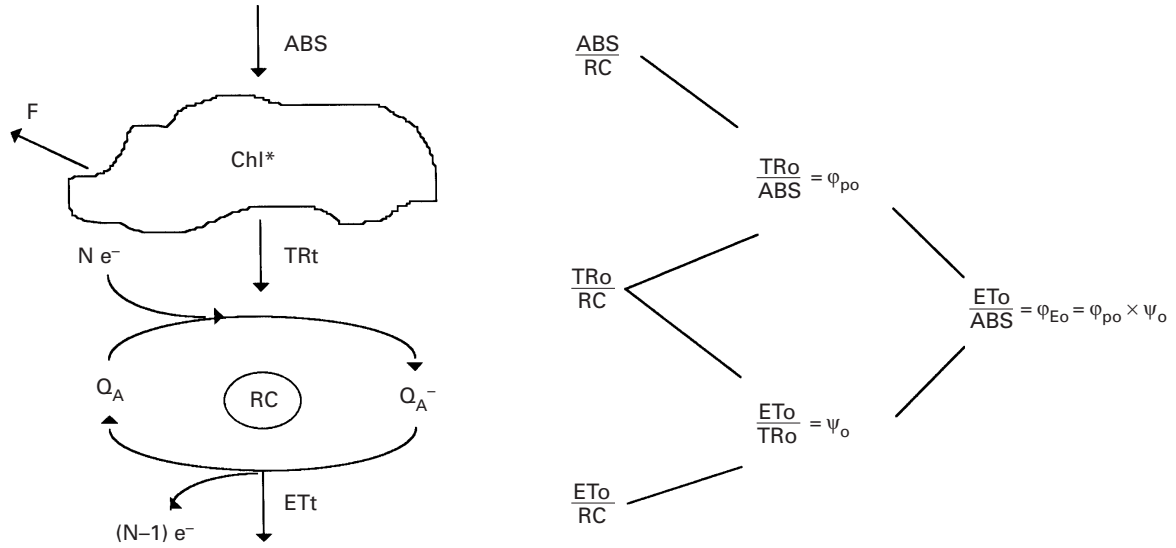

Figure 1. Schematic energy-flux model for PSII. ABS: light absorption flux. TRt, TRo: energy flux trapped by PSII reaction centres at time t and time zero, respectively. ETt, ETo: electron transport flux generated by the reoxidation of Q_A^- to Q_A at time t and time zero, respectively. F: fluorescence emission. TRo/ABS (= $_{po}$): maximum quantum efficiency of PSII photochemistry. ETo/TRo (= Ψ_o): probability that a trapped exciton is moving an electron beyond Q_A. ETo/ABS (= $_{Eo}$): maximum quantum efficiency for movement of electrons further than Q_A^-. Therefore, $_{Eo}$ = $_{po} \times \Psi_o$.

RESULTS

Pigment composition, photosynthetic quantum efficiency, and dark respiration

Changes in the relative pigment composition in salt-adapted cells are shown in Table 1. The ratio of phycocyanin/Chl decreased significantly with increase in salt concentration, whereas the ratio of carotenoid/Chl remained relatively constant in salt-adapted cells.

The apparent quantum efficiency of photosynthesis (α) decreased progressively with increasing salt concentration (Table 1). For example, a 22%

Table 1. *Ratios of carotenoid/Chl a and c-phycocyanin/Chl a, the apparent quantum efficiency of photosynthesis* (α, μ*mol* O_2 [*mg chl h* μ*mol quantum* m^{-2} s^{-1}]$^{-1}$) *and the rate of respiration in the dark* (R_d, μ*mol* O_2 mg^{-1} *chl* h^{-1}) *in cells of* Spirulina platensis *adapted to different concentrations of NaCl*

NaCl concentration (M)	Carotenoid/Chl a	c-Phycocyanin/Chl a	α	R_d
0	0.214±0.004 a (100)	5.14±0.025 a (100)	3.09±0.70 a (100)	25.3±3.7 a (100)
0.1	0.222±0.013 a (104)	5.05±0.035 a (98)	2.94±0.77 ab (95)	27.2±4.2 b (108)
0.35	0.222±0.012 a (104)	4.05±0.035 b (79)	2.84±0.52 b (92)	31.4±5.0 c (124)
0.75	0.204±0.008 a (95)	3.13±0.049 c (61)	2.41±0.66 c (78)	38.5±4.7 d (152)

Numbers in parentheses are percentages of the control value. Values are means±SE from 3–5 different experiments; different lower-case letters indicate values significantly different at $P = 0.05$.

Table 2. *Electron transport activities* (μ*mol* O_2 h^{-1} mg^{-1} *chl*) *of photosynthesis in cells of* Spirulina platensis *adapted to different concentrations of NaCl*

Electron transport activity	NaCl concentration (M)			
	0	0.1	0.35	0.75
PSII (H_2O→pBQ)	574.0±40.1 a (100)	523.0±35.1 b (91)	493.6±46.6 bc (86)	459.8±32.2 cd (80)
PSI (DCPIP→MV)	719.8±46.6 a (100)	1329.5±71.5 b (185)	1597.3±68.1 c (222)	1323.5±80.6 bd (184)
Whole-chain (H_2O→MV)	280.0±23.3 a (100)	248.9±25.9 b (89)	235.2±30.3 bc (84)	219.0±15.9 d (78)
Whole-chain (DPC→MV)	278.2±27.7 a (100)	247.6±18.8 b (89)	234.2±20.2 bc (84)	219.8±23.3 cd (79)

Values are means±SE of 3–4 independent experiments. Numbers in parentheses are percentages of the control value.

decrease in α was observed at 0.75 M NaCl. On the other hand, salt stress resulted in a significant increase in the R_d rate, which increased by 52% at 0.75 M NaCl.

Photosynthetic electron transport

In salt-adapted cells of *S. platensis*, a decrease in PSII activity ($H_2O \rightarrow pBQ$) was observed (Table 2). PSI activity (DCPIP→MV) was, however, markedly stimulated, increasing by 85, 122 and 84% at 0.1, 0.35 and 0.75 M NaCl, respectively. In an attempt to locate the site of induction of these changes in PSII, the activities of the electron transport were measured both in the presence and absence of DPC, which is known to donate electrons to PSII reaction centres, thereby bypassing the oxidizing side of PSII (Izawa, 1980). The response of activity was identical, both in the absence ($H_2O \rightarrow MV$) and in the presence of DPC (DPC→MV) (Table 2). This suggests that the changes in PSII induced by salt stress were most likely to be located in the reaction centres themselves rather than in the oxidizing side of PSII.

Relative rate of reduction of the primary quinone electron acceptor (Q_A) and relative variable fluorescence at phases J and I in polyphasic fluorescence transients

Cells of *S. platensis* exhibit a typical polyphasic rise of fluorescence transients including phases O, J, I, and P (Fig. 2a, curve a). The initial Chl fluorescence at level O (F_o) reflects the minimal fluorescence yield when all molecules of Q_A are in the oxidized state. Level P corresponds to the situation in which all molecules of Q_A are in the reduced state. Steps J and I occur between the more commonly observed steps O and P. The transition from phase O to phase J is controlled by photochemical charge separation (photochemical phase), leading to the reduction of Q_A to Q_A^- while appearance of phases I and P is limited by dark reactions (Neubauer & Schreiber, 1987; Schreiber & Neubauer, 1987; Strasser *et al.*, 1995). Polyphasic Chl a fluorescence transients thus provide information on the primary photochemistry of PSII.

The polyphasic Chl a fluorescence transients were followed in salt-adapted cells. In order to clarify the difference between control cells and salt-adapted cells, Figure 2a shows only the polyphasic rise of Chl a fluorescence transients determined from control cells and from cells adapted to 0.75 M NaCl. It seems that the minimal fluorescence level (O) did not change significantly with increase in salt concentration, but the fluorescence yield at phases J, I and P declined markedly (Fig. 2a).

In order to characterize further the polyphasic rise of fluorescence in salt-adapted cells, the transients

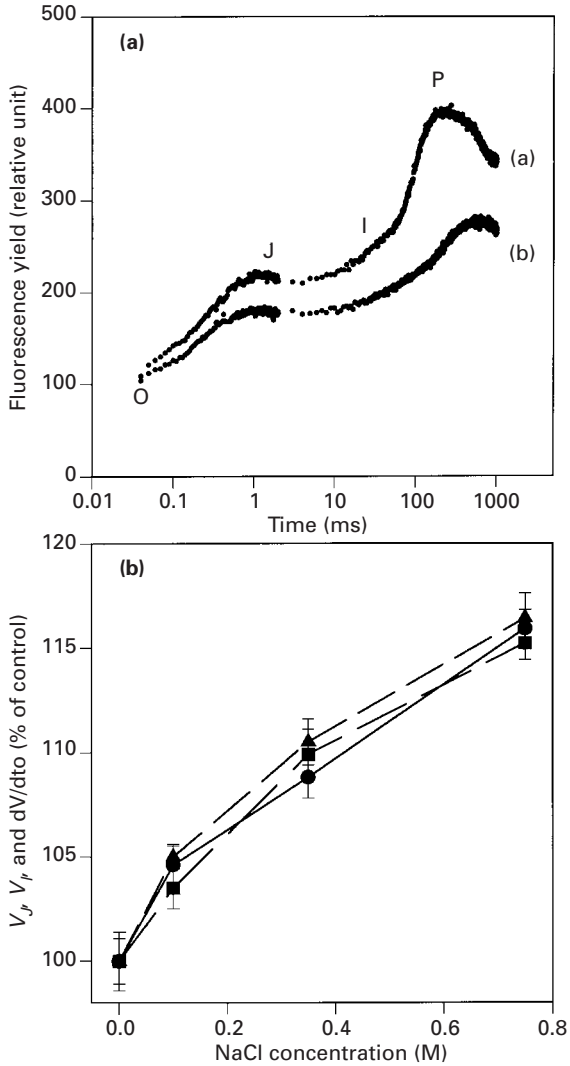

Figure 2. (a) Polyphasic rise of Chl a fluorescence transients in salt-adapted cells of *Spirulina platensis*. Curves a and b represent control cells and cells adapted to 0.75 M NaCl, respectively. (b) Changes in the relative variable fluorescence of Chl a at the intermediate step J (V_J) (●) and step I (V_I) (■) at 2 ms and 30 ms, respectively, and the slope at the origin of the relative variable fluorescence of Chl a (dV/dto) (▲) in *S. platensis* adapted to different concentrations of NaCl. Values are means ± SE ($n = 5$).

were normalized on the basis of variable fluorescence, so that the polyphasic variable fluorescence transients were visualized. The relative variable fluorescence at any given time t is defined as $V_t = (F_t - F_o)/(F_m - F_o)$. According to the concept of Duysens and Sweers (1963), where Q_A in open reaction centres acts as a quencher, and the energy flux theory (Strasser, 1978), the empirical expression of the relative variable fluorescence in a chlorophyll variable fluorescence transient (V) can be considered as a function of the fraction of the closed reaction centres of PSII or as the fraction of reduced Q_A. Thus, the variation of V at a given time (V_t) at the beginning of the variable fluorescence transient (theoretically at time zero), expressed as dV/dto, is

Table 3. *Probability of the transport of an electron beyond Q_A (Ψ_o), the maximum quantum efficiency of PSII electron transport ($_{Eo}$), the trapping flux per PSII reaction centre (TRo/RC), the electron transport flux per PSII reaction centre (ETo/RC), and $(1/F_o)-(1/F_m)$, which can be used as a measure of the photochemical rate constant (k_p), in cells of* Spirulina platensis *adapted to different concentrations of NaCl*

NaCl concentration (M)	Ψ_o	$_{po}$	$_{Eo}$	TRo/RC	ETo/RC	$(1/F_o)-(1/F_m)$
0	0.719±0.004 a (100)	0.702±0.006 a (100)	0.505±0.002 a (100)	0.639±0.003 a (100)	0.461±0.002 a (100)	8.57±0.02 a (100)
0.10	0.705±0.003 a (98)	0.675±0.003 ab (96)	0.477±0.005 ab (94)	0.646±0.004 a (101)	0.451±0.002 a (98)	8.33±0.09 ab (97)
0.35	0.692±0.003 a (96)	0.651±0.007 bc (93)	0.451±0.003 bc (89)	0.642±0.007 a (100)	0.444±0.004 a (96)	7.84±0.17 b (91)
0.75	0.670±0.006 a (93)	0.612±0.005 c (87)	0.413±0.004 c (82)	0.644±0.002 a (101)	0.433±0.003 a (94)	6.76±0.08 c (79)

Numbers in parentheses are percentages of the control value. Values are means±SE (n = 5).

the relative rate of reduction of Q_A, and also represents the relative rate of primary photochemistry (Strasser *et al.*, 1995). The fraction of the closed reaction centres of PSII at phase J and I are represented by V_J and V_I, respectively. The changes in V_J, V_I, and dV/dto in salt-adapted cells are given in Fig. 2b. An increase in V_J, V_I, and dV/dto was observed in salt-adapted cells, suggesting that salt stress induced modifications in PSII photochemistry.

Fluorescence parameters determined from the JIP test

In order to investigate the effect of salt stress on electron transport at the acceptor side of PSII, we examined changes in the probability of electron transfer beyond Q_A (Ψ_o). The results show that no significant change in Ψ_o was observed in salt-adapted cells (Table 3), indicating that salt stress had no effect on the acceptor side of PSII.

We also examined changes in the maximum yield of excitation energy trapping by PSII, $_{Po}$, expressed as TRo/ABS = F_v/F_m, as derived by Havaux *et al.* (1991), and the quantum yield of electron transport beyond Q_A ($_{Eo}$). As the salt concentration increased, $_{Eo}$ and $_{Po}$ decreased progressively (Table 3).

The conversion efficiency of excitation energy in salt-adapted cells was further evaluated by studying the trapping flux (TR) and the electron transport flux (ET) per PSII reaction centre (RC), i.e. TRo/RC and ETo/RC. No significant changes were observed in either TRo/RC or ETo/RC occurred in salt-adapted cells (Table 3).

These results clearly indicate that salt stress resulted in modifications in PSII photochemistry. Whether the photochemical rate constant (k_p) was also modified by salt stress is unclear. According to Havaux *et al.* (1991) and Krüger *et al.* (1997),

$$(1/F_o)-(1/F_m) = k_{2b}/(J_2 \times k_{2F}),$$

where J_2 is the light absorption flux in PSII, k_{2b} is the rate constant of energy trapping by PSII reaction centres and k_{2F} is the rate constant of fluorescence emission from PSII. The value of k_{2F} is considered very small and constant over a wide range of physiological conditions (Butler & Kitajima, 1975), while that of J_2 can be changed by environmental stress. Salt stress resulted in a decrease in the ratio of phycocyanin/chl (Table 1). This would possibly decrease the light absorption flux (J_2) in PSII and increase the value of the fluorescence parameter, $(1/F_o)-(1/F_m)$. However, a decrease in $(1/F_o)-(1/F_m)$ was seen in salt-adapted cells (Table 3), suggesting that k_{2b}decreases. Moreover, because of the decrease in J_2 in salt-adapted cells, it is most likely that the decrease of k_{2b} is even more pronounced than that in the values of $(1/F_o)-(1/F_m)$ shown in Table 3.

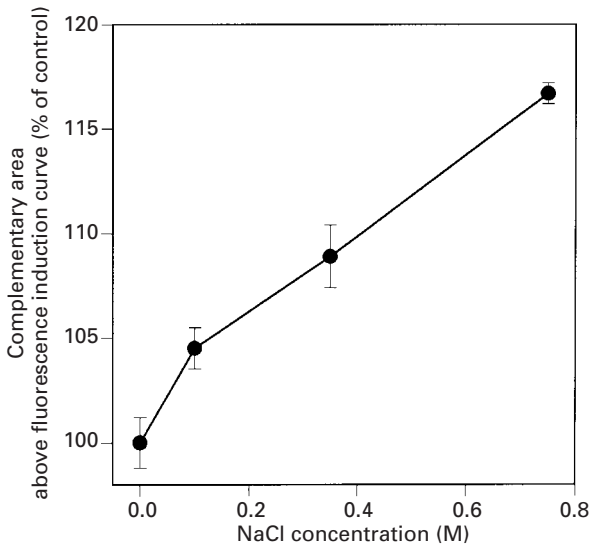

Figure 3. Complementary area above the fluorescence induction curves in the presence of DCMU, normalized with respect to the maximum variable fluorescence in cells of *Spirulina platensis* grown under different concentrations of NaCl. Values represent mean ± SE of 6–7 independent measurements (control value: 9857 relative units).

Complementary area above the fluorescence induction curve in the presence of DCMU

The complementary area above the fluorescence induction curve between F_o and F_m is equal to the fraction of the closed PSII reaction centres when no reoxidation of Q_A^- occurs (Krause & Weis, 1991). Changes in the fraction of the closed PSII reaction centres in salt-adapted cells was therefore evaluated by measurements of the complementary area above the fluorescence induction curve between F_o and F_m in the presence of DCMU. The complementary area above the fluorescence induction curve increased significantly with increasing salt concentration (Fig. 3), suggesting that the fraction of closed PSII reaction centres increased in salt-adapted cells.

DISCUSSION

Our results show no significant change in the trapping flux per PSII reaction centre (TRo/RC) in salt-adapted cells of *S. platensis* (Table 3). The TRo/RC also represents the maximum rate of reduction of Q_A, assuming that no reoxidation of Q_A^- occurs (Strasser & Strasser, 1995). In consequence, TRo/RC is a measure of electron flux only from the donor side of PSII to Q_A. There was also no difference in dV/dto between control and salt-adapted cells when measured in the presence of DCMU, which blocks electron transport beyond Q_A (data not shown). This indicates that no modification was induced by salt stress at the donor side of PSII. In addition, the finding that overall electron transport activity in salt-adapted cells was almost the same in the presence and absence of DPC (Table 2) further confirms that salt stress had no effect on the donor side of PSII, and also suggests that the decrease in PSII activity, seen during adaptation to salt stress, might be due to damage at the acceptor side of PSII and/or in the PSII reaction centres.

As shown in Table 3, there was also no significant decrease in the probability of electron transport beyond Q_A (Ψ_o), indicating that electron transport at the acceptor side was not the main site of damage induced by salt stress. It therefore appears most likely that salt stress causes damage to the PSII reaction centre itself. The decrease in the rate constant of energy trappings by PSII reaction centres in salt-adapted cells, derived from the fluorescence parameter $(1/F_o)-(1/F_m)$ (Table 3), also suggests that the main effect induced by salt stress lies in the PSII reaction centres. An increase in the complementary area above the fluorescence induction curve in salt-adapted cells exposed to DCMU clearly demonstrated that salt stress resulted in an increase in the proportion of closed PSII reaction centres. This disturbance in PSII reaction centres resulted in a decrease in $_{Po}$ that led to a decrease in the yield of electron transport beyond Q_A ($_{Eo}$). This could be inferred from the fact that $_{Eo}$ is the product of $_{Po}$ and Ψ_o (Strasser & Strasser, 1995) and no significant changes in Ψ_o was observed in salt-adapted cells.

An increase in the relative rate of Q_A reduction (dV/dto) was observed in salt-adapted cells. Since dV/dto is the difference between the maximum rate of reduction of Q_A (TRo/RC) and that of Q_A^- reoxidation, and no significant change was observed in TRo/RC, the increased value of dV/dto was obviously due to a decrease in the rate of Q_A^- reoxidation. This would have resulted from the increase in the proportion of closed PSII reaction centres. It has been shown that relative variable fluorescence is proportional to the fraction of closed PSII reaction centres or to the fraction of reduced Q_A when energy transfer between PSII units is ignored. The increase in V_I and V_J in salt-adapted cells (Fig. 2) might therefore indicate an increase in the proportion of closed PSII reaction centres and, in consequence, in the proportion of reduced Q_A at phases *I* and *J*, respectively.

The pronounced increase observed in the respiratory rate in salt-adapted cells could increase electron flux in the electron transport chain and thereby affect PSII, since the respiratory electron transport chain is often coupled with the photosynthetic electron transport chain in cyanobacteria. However, the higher excitation pressure that this would place on PSII could be overcome by a decrease in the absorption cross section of PSII (as reflected by a decrease in the ratio of phycocyanin/Chl), a decrease in the rate constant of excitation-energy trapping by PSII reaction centres and by increased PSI activity. This would result in

a decrease in energy transfer between phycobilisomes and PSII and shift the distribution of excitation energy in favour of PSI. Enhancement in PSI activity should increase cyclic electron transport. Several reports have shown that cyclic electron flow increases under salinity stress (Jeanjean *et al*., 1993; Hibino *et al*., 1996). Thus, it seems that an increase in PSI activity in salt-adapted cells could protect PSII from excessive excitation energy under salt stress. On the other hand, the increases in PSI activity and in the respiratory rate of salt-adapted cells might provide more energy for the synthesis of organic osmolytes and for the extrusion of Na^+ in cells to maintain osmotic balance.

Based on the findings presented in this paper, the adaptation of PSII apparatus to salt stress in *Spirulina* cells appears to involve a decrease in the absorption cross section (decreased ratio of phycocyanin/Chl) and in modifications to PSII photochemistry. An increase in PSI activity parallels the decrease in the maximum quantum efficiency of PSII photochemistry and might regulate excitation-energy equilibration to maintain balanced electron transport in salt-adapted *Spirulina* cells. Through an increase in the proportion of closed PSII reaction centres, the PSII apparatus was thus protected from further excess excitation energy. On the other hand, the high conversion efficiency of excitation energy was maintained, as reflected by no change in TRo/RC or ETo/RC under salt stress.

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