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# Inhibition of quantum yield of PS II electron transport in *Spirulina platensis* by osmotic stress may be explained mainly by an increase in the proportion of the $Q_B$-non-reducing PS II reaction centres

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**Abstract.** Modulated chlorophyll fluorescence and fluorescence induction kinetics were used to evaluate the PS II photochemistry in *Spirulina platensis* exposed to osmotic stress (0–0.8 M mannitol). Osmotic stress decreased the efficiency of excitation energy capture by open PS II reaction centres ($F_v'/F_m'$) and more significantly, decreased photochemical quenching ($q_p$). Osmotic stress also decreased the maximal efficiency of PS II photochemistry ($F_v/F_m$). There was no significant change in non-photochemical quenching ($q_N$), indicating that the decreased $F_v'/F_m'$ was not due to an increase in $q_N$. Analyses of the fast fluorescence induction kinetics indicated that osmotic stress caused a significant increase in the proportion of the QB-non-reducing PS II reaction centres. Based on the results in this study, we suggest that a substantial increase in the proportion of the QB-non-reducing PS II reaction centres may be responsible for the decrease in $q_p$ and $F_v'/F_m'$, of which both resulted in the decrease in the quantum yield of PS II electron transport ($\Phi_{PS\ II}$).

*Keywords:* chlorophyll fluorescence, cyanobacterium, osmotic stress, PS II, $Q_B$-non-reducing PS II reaction centres, *Spirulina platensis*.

## Introduction

Photosynthesis by algae is inhibited by osmotic stress (Vonshak and Richmond 1981; Gilmour *et al.* 1984; Kirst 1989; Endo *et al.* 1995). Several studies have shown that such a decrease in photosynthesis may be associated with the inhibition of PS II* activity (Gilmour *et al.* 1984, 1985; Endo *et al.* 1995). In *Dunaliella tertiolecta*, osmotic stress inhibits non-cyclic electron transport and stimulates cyclic electron transport and fluorescence emission arising from PS I at 77 K, suggesting that the inhibition of PS II activity may be involved in the ΔpH-dependent down-regulation and state 2 transition (Gilmour *et al.* 1984, 1985). Endo *et al.* (1995) have recently shown that, in *Chlamydomonas reihardtii*, the inhibition of quantum yield of PS II photochemistry by osmotic stress is due to an increase in non-photochemical quenching which is attributable to a state 2 transition. In the red alga *Porphyra perforata*, Satoh *et al.* (1983) demonstrated that the decrease in excitation energy reaching PS II reaction centres and the inhibition of the oxidizing side of PS II by salt stress resulted in a decrease in PS II activity. Additionally, by drying several marine algae, Wiltens *et al.* (1978) found that the decrease in PS II activity by desiccation was possibly due to the loss of the water-splitting system of PS II.

*Spirulina platensis*, a filamentous cyanobacterium, has been isolated from a wide range of habitats largely varying in their salinity (Ciferri 1983). Recently, considerable interest has 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 climates, daily evaporation amounts to 1–2 cm of water level and leads to a progressive increase in the salt concentration in the culture, and the cells are thus subjected to a salinity stress (Vonshak 1987). We have previously demonstrated that *S. platensis* is capable of adapting to high NaCl. An increase in the respiratory activity of the cells and a partial or nearly full recovery of the photosynthetic activity, depending on the strain and the level of salinity, were observed after they became fully adapted to the salinity stress (Vonshak *et al.* 1988, 1995). We also observed that the inhibition of PS II photochemistry induced by high salinity (NaCl) was due mainly to the osmotic toxicity of salt (C. Lu

*Abbreviations used: $F_i$, intermediate level in the fast fluorescence induction kinetics curve; $F_o$, minimal fluorescence in dark-adapted state; $F_o'$, minimal fluorescence in light-adapted state; $F_m$, maximal fluorescence in dark-adapted state; $F_m'$, maximal fluorescence in light-adapted state; $F_v$, maximum variable fluorescence in dark-adapted state; $F_v'$, maximum variable fluorescence in light-adapted state; $F_s$, steady-state fluorescence yield at $q_p > 0$; $F_v/F_m$, maximum quantum efficiency of PS II photochemistry; $F_{PS\ II}$, actual quantum yield of PS II electron transport; $F_v'/F_m'$, efficiency of excitation energy capture by open PS II reaction centres; PFD, photon flux density; $q_p$, photochemical quenching coefficient; $q_N$, non-photochemical quenching coefficient.

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and A. Vonshak, unpublished data). Obviously, a better understanding of osmotic stress on photosynthesis should help optimize the productivity of the algal cultures grown outdoors.

In this study, we investigated the causes for the inhibition of the quantum yield of PS II electron transport after *Spirulina platensis* cells were exposed to hyperosmotic stress (up to 0.8 M mannitol) using chlorophyll fluorescence. Our results demonstrated that the decreased quantum yield of PS II electron transport induced by osmotic stress may be mainly involved in an increase in the proportion of the $Q_B$-non-reducing PS II reaction centres.

## Material and methods

*Cell culture*

*Spirulina platensis* $M_2$, of the culture collection of the Centro di Studio dei Microrganismi Autotrofi of Florence, was grown at 32°C in Zarouk's medium, containing 200 mM sodium bicarbonate (Vonshak *et al.* 1982) at a photon flux density (PFD) of 50 µmol $m^{-2}$ $s^{-1}$ provided by fluorescent day-light lamps.

*Osmotic treatment*

Exponentially grown cells were harvested and resuspended in a 100 mL fresh medium containing different mannitol concentrations of 0.2, 0.4, 0.6, 0.8 M in 250 mL flasks. Cell density of the cultures were 5 µg chlorophyll $mL^{-1}$ and incubated at 50 µmol $m^{-2}$ $s^{-1}$ PFD and 32°C for 48 h.

*Measurements of chlorophyll fluorescence*

Chlorophyll fluorescence quenching analysis was carried out at room temperature (25°C) with a portable fluorometer (PAM-2000, Walz, Germany). The fluorescence probe was imposed on the surface of a 1 mL sample in a 2 mL tube, which was taken directly from the different mannitol treatments. The fluorometer was connected to a computer with data acquisition software (DA-2000, Heinz, Walz).

The minimal fluorescence level in the dark-adapted state ($F_o$) was measured using the modulation 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. 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 $m^{-2}$ $s^{-1}$ PFD) to close all reaction centres and drive photochemical quenching to zero. The true maximum fluorescence was measured by adding 10 µM DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) to the cell suspensions to allow non-photochemical quenching to collapse. The steady-state value of fluorescence ($F_s$) was also recorded after 5 min illumination at 50 µmol $m^{-2}$ $s^{-1}$ PFD using the actinic light.

Using both light and dark fluorescence parameters, we calculated: (1) the maximum efficiency of PS II photochemistry in the dark-adapted state ($F_v/F_m$), (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 quantum yield of PS II electron transport, $F_{PS\ II} = (F_m' - F_s)/F_m'$, (5) the efficiency of excitation energy capture by open PS II reaction centres, $F_v'/F_m' = (F_m' - F_o')/F_m'$ (Genty *et al.* 1989). Here, fluorescence nomenclature was according to van Kooten and Snel (1990).

The fast fluorescence induction kinetics were measured using a PAM-2000 in the dark-adapted samples suddenly illuminated with moderate red light (30 µmol $m^{-2}$ $s^{-1}$ PFD, 655 nm) at a sampling rate of 1 ms/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 prior to the measurements of the fluorescence induction kinetics.

The slow fluorescence induction kinetics were also measured using a PAM-2000 with the dark-adapted samples illuminated by white light (280 µmol $m^{-2}$ $s^{-1}$ PFD) at a sampling rate of 10 ms/point.

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

## Results and discussion

Chlorophyll fluorescence quenching analysis has been proven a non-invasive, powerful and reliable method to assess the changes of the functions of PS II in the steady state of photosynthesis, e.g. the energy absorption, utilization and dissipation of excess excitation energy by PS II, in response to different environmental stresses (Krause and Weis 1991; Schreiber *et al.* 1994). We, thus, examined the effects of osmotic stress on the fluorescence characteristics under the steady state of photosynthesis. Fig. 1 shows the changes in the efficiency of excitation energy capture by open PS II reaction centres ($F_v'/F_m'$), photochemical quenching ($q_P$), non-photochemical quenching ($q_N$), and the quantum yield of PS II electron transport ($\Phi_{PS\ II}$) after *Spirulina platensis* cells were exposed to

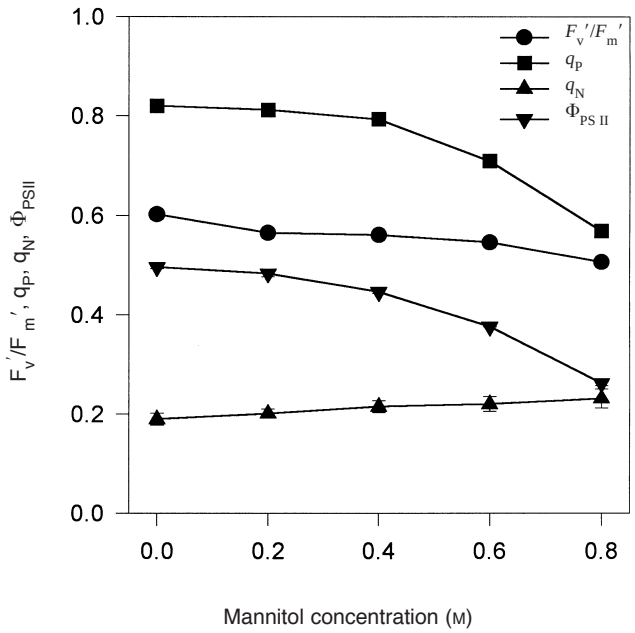


**Fig. 1.** Changes in the efficiency of excitation capture by open PS II reaction centres ($F_v'/F_m'$), photochemical quenching ($q_P$), non-photochemical quenching ($q_N$), and the quantum yield of PS II electron transport ($\Phi_{PS\ II}$) in *Spirulina platensis* exposed to different mannitol concentrations for 48 h. Values are means ± SE for five replicates.

different concentrations of mannitol (0–0.8 M) for 48 h. The results showed that osmotic stress caused a decrease in $F_v'/F_m'$, $q_P$ and $\Phi_{PS\ II}$ but the relative degrees of their decreases were different. $\Phi_{PS\ II}$ was reduced the most followed by $q_P$ and then $\Phi_v'/F_m'$. Because $\Phi_{PS\ II}$ is determined by both $F_v'/F_m'$ and $q_P$ (i.e. $\Phi_{PS\ II} = F_v'/F_m' \times q_P$) (Genty *et al.* 1989), the decreased $\Phi_{PS\ II}$ was the result of the decrease in both $F_v'/F_m'$ and $q_P$. Obviously, the decrease in $q_P$ made a greater contribution to the decreased $\Phi_{PS\ II}$ as the extent to which $q_P$ decreased was greater than that observed for $F_v'/F_m'$. On the other hand, it appeared that the osmotic stress had no significant effect on $q_N$, which increased very slightly with the increase of mannitol concentration (Fig. 1).

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 center. Thus, there are two possible factors which may be responsible for the decrease in $F_v'/F_m'$. One is the decrease in the maximal efficiency of PS II photochemistry ($F_v/F_m$) which may be caused by damage in the PS II reaction center. The other is an increase in the non-photochemical quenching deactivation of PS II, which has often been shown to result in a decrease in $F_v'/F_m'$ (Genty *et al.* 1990). The fact that osmotic stress did not induce an increase in $q_N$ may indicate that the decrease in $F_v'/F_m'$ induced by osmotic stress indeed was not related to any non-photochemical quenching process. Since the antenna system (i.e. the phycobilisome) in cyanobacteria lacks the capacity for non-photochemical quenching, Campbell and Öquist (1995) have shown that non-photochemical quenching in cyanobacterium is involved mainly in a state transition. This state transition causes a down-regulation of the transfer of excitation energy from phycobilisomes/PS II complex to PS II reaction centres. Thus, the excitation energy distribution between PS I and PS II shifts favourably to PS I at the expense of PS II. The above result also suggests that the state transition might not be involved in down-regulation of the quantum yield of PS II electron transport.

In order to evaluate whether the decreased $F_v'/F_m'$ was due to a decrease in the maximal efficiency of PS II photochemistry ($F_v/F_m$), the effect of osmotic stress on $F_v/F_m$ was investigated. As shown in Fig. 2, $F_v/F_m$ decreased with increasing mannitol concentration and a 15% decrease in $F_v/F_m$ was observed when the cells were exposed to 0.8 M mannitol. Fig. 2 also showed that the osmotic stress had little effect on the minimal fluorescence yield, $F_o$. The decreased $F_v/F_m$ was therefore due mainly to the decrease of the maximal fluorescence yield, $F_m$. This result suggests that the decreased $F_v'/F_m'$ was a result of the decrease in $F_v/F_m$.

In an attempt to characterize the nature of the changes in PS II photochemistry induced by osmotic stress and to identify the causes for the decrease in $F_v/F_m$, we further investigated the effects of osmotic stress on the

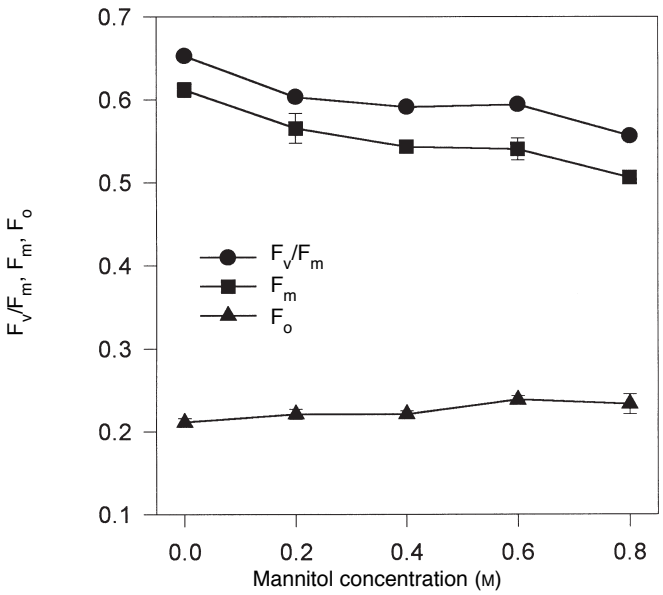


**Fig. 2.** Changes in the maximum photochemical efficiency of PS II ($F_v/F_m$) and the minimal fluorescence ($F_o$) and the maximal fluorescence ($F_m$) in *Spirulina platensis* exposed to different mannitol concentrations for 48 h. Values are means ± SE for five replicates.

characteristics of the slow fluorescence induction kinetics, which may be considered as an indicator of photosynthetic functions beginning from the PS II photochemistry and ending with the final process of $CO_2$ fixation reactions. Fig. 3 shows the changes in the slow fluorescence kinetics induced by osmotic stress. Curve 1 is a typical time course of fluorescence induction kinetics in control cells, which displayed phases O, P, S, M and T following the terminology of Govindjee and Papageorgiou (1971). The peak P is referred to as the maximal extent of the $Q_A$ reduction at a given actinic light intensity (Duysens and Sweers 1963; Munday and Govindjee 1969). The second peak M is suggested to be linked to $CO_2$ fixation (Sivak and Walker 1985). The decline from the peak P to the phase S is due to the light-activation of ferredoxin-NADP-oxidoreductase which results in acceleration of $Q_A$ reoxidation by PS I (Satoh 1981).

It can be seen from Fig. 3 that osmotic stress induced a modification in the patterns of the fluorescence induction curves. The most significant change was that the P peak decreased progressively with the increase of the mannitol concentration. Again, there was no significant change in $F_o$ level. Osmotic stress thus resulted in a decrease in the variable fluorescence, $F_v$ ($F_m - F_o$), suggesting that osmotic stress induced an inhibition of PS II photochemistry linked to $Q_A$ reduction. A similar decrease in $F_v$ was also observed in higher plants (Bukhov *et al.* 1989), red algae (Wiltens *et al*. 1978) and green algae (Chen and Hsu 1995).

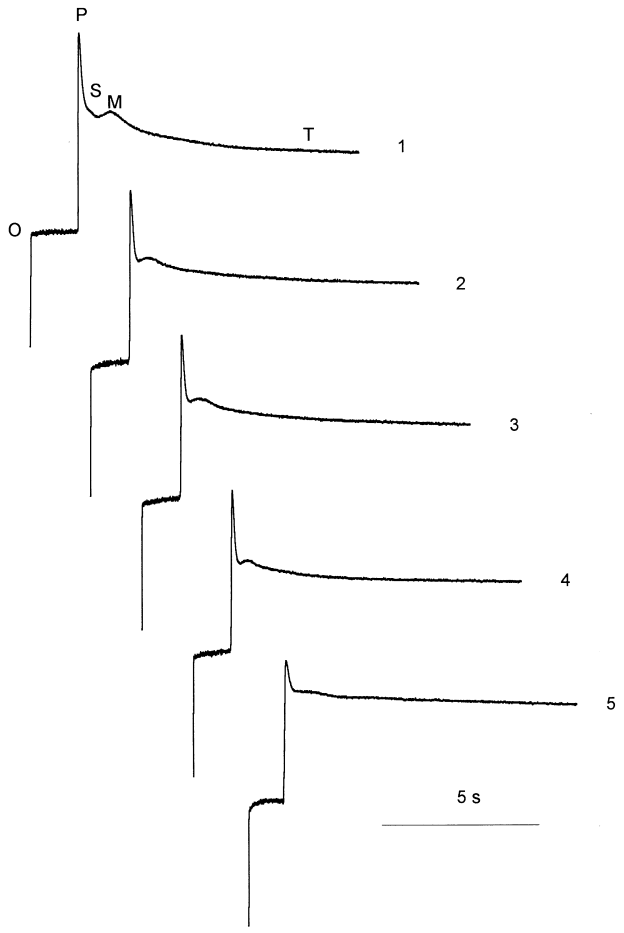


**Fig. 3.** Slow fluorescence induction curves in *Spirulina platensis* exposed to different mannitol concentrations for 48 h. Curves 1–5 were determined from the cells treated with 0, 0.2, 0.4, 0.6, 0.8 M mannitol, respectively.

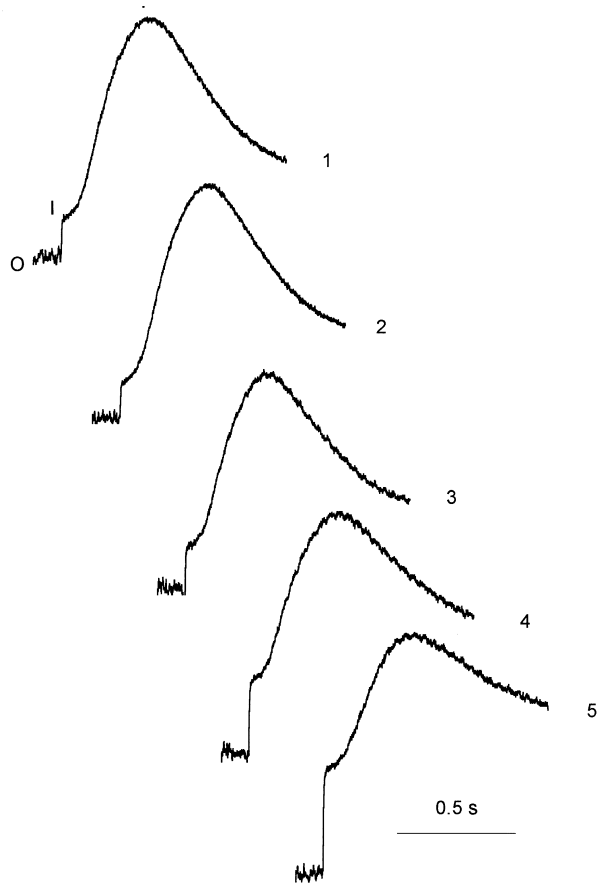


**Fig. 4.** Fast fluorescence induction curves in *Spirulina platensis* exposed to different mannitol concentrations for 48 h. Curves 1–5 were determined from the cells treated by 0, 0.2, 0.4, 0.6, 0.8 M mannitol, respectively.

Fig. 3 also shows that the peak M almost disappeared when the cells were exposed to 0.8 M mannitol, suggesting that the $CO_2$ fixation was sensitive to osmotic stress. In addition, the decline of the phase P to S was slowed down with the increase in mannitol concentration, indicating that electron transport through PS I might be retarded. The inhibition of the electron transport between PS II and PS I has also been observed during dehydration (Wiltens *et al.* 1978; Matorin *et al.* 1982).

$F_v/F_m$ is most frequently used as a measure of the maximal photochemical efficiency of PS II (Krause and Weis 1991); however, it gives no direct information on the heterogeneity of PS II reaction centres. In order to study whether osmotic stress induced modification in the PS II reaction centres, we investigated the changes in the fast fluorescence induction kinetics after the cells were exposed to different concentrations of mannitol.

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 maximum level (P) (refer to the control in Fig. 4). The results showed that osmotic stress had little effects on $F_o$ and $F_p$ but induced a significant increase in $F_i$ (Fig. 4, also in Fig. 5*A*). Consequently, a significant increase in the ratio $(F_i–F_o)/(F_p–F_o)$ occurred (Fig. 5*B*). It has previously been shown that the O–I phase has been attributed to the PS II reaction centres, which lack the ability to reduce $Q_B$ ($Q_B$-non-reducing PS II). 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). An increase in the ratio $(F_i–F_o)/(F_p–F_o)$ in the osmotically stressed cells may indicate an increase in the proportion of the $Q_B$-non-reducing PS II reaction centres.

The above results have shown that the decrease in $F_v/F_m$ induced by osmotic stress was accompanied by a substantial increase in the proportion of the $Q_B$-non-reducing PS II

reaction centres. This suggests that the decreased $F_v/F_m$ was a result of the increased proportion of the $Q_B$-non-reducing PS II reaction centres. Cao and Govindjee (1990) also observed similar results in heat-treated thylakoids. It is possible that the increase in the proportion of the $Q_B$-non-reducing PS II reaction centres and the decrease in the maximal efficiency of PS II photochemistry may be two different consequences of the same process induced by osmotic stress. Whether the increase in the proportion of the $Q_B$-non-reducing PS II reaction centres and the decrease in $F_v/F_m$ may have a common origin deserves further investigation.

Our results showed that osmotic stress resulted in a significant decrease in $q_P$, 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 $Q_A$ in osmotic stressed cells (Dietz *et al.* 1985; Genty *et al.* 1989). Such an increase in the proportion of the reduced state of $Q_A$ will certainly result in a decrease in the proportion of available excitation energy used for photochemistry (Havaux *et al.* 1991). How could osmotic stress induce an increase in the proportion of the reduced state of $Q_A$? As shown in this study, osmotic stress induced a substantial increase in the proportion of the $Q_B$-non-reducing PS II reaction centres. It has been shown that the key characteristic of the $Q_B$-non-reducing PS II reaction centres is an inability to transfer electrons from $Q_A$ to $Q_B$ (Chylla and Whitmarsh 1989; Cao and Govindjee 1990). It is evident that 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 $q_P$, induced by osmotic stress, can be explained by an increase in the proportion of $Q_B$-non-reducing PS II reaction centres.

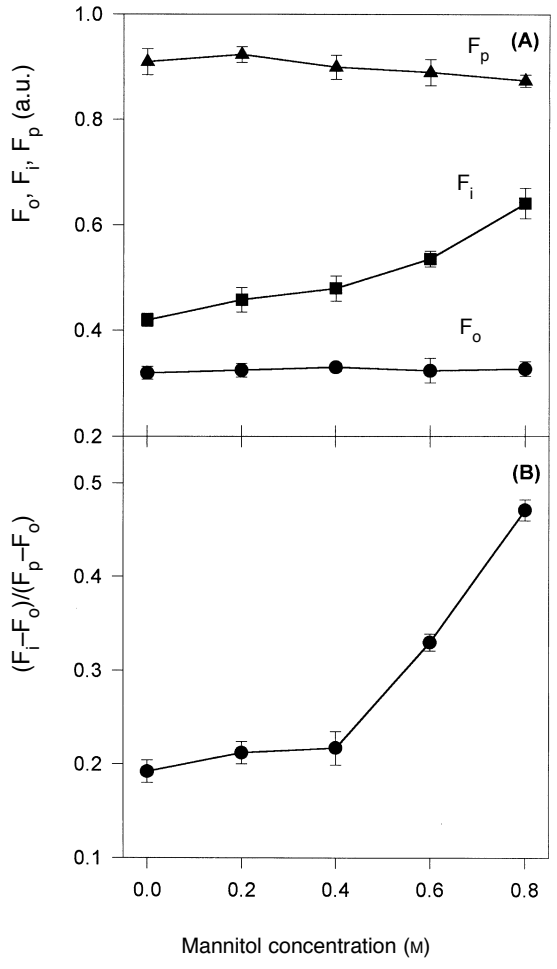


**Fig. 5.** Changes in the fluorescence parameters $F_o$, $F_i$, $F_p$ (*A*) and the ratio $(F_i-F_o)/(F_p-F_o)$ (*B*) in *Spirulina platensis* exposed to different mannitol concentrations for 48 h, which were determined from the fast fluorescence induction kinetics. Values are means ± SE for five replicates.

In conclusion, we have shown that osmotic stress reduced the efficiency of excitation energy capture by open PS II reaction centres ($F_v'/F_m'$) and photochemical quenching ($q_P$). We have also shown that osmotic stress brought about no significant change in non-photochemical quenching ($q_N$), indicating that the decreased $F_v'/F_m'$ was not due to an increase in $q_N$. Instead, the decrease in $F_v'/F_m'$ was associated with a decrease in the maximal efficiency of PS II photochemistry ($F_v/F_m$). Our results suggest that the substantial increase in the proportion of the $Q_B$-non-reducing PS II reaction centres may be responsible for the decrease in $q_P$ and $F_v'/F_m'$, of which both resulted in the decrease in the quantum yield of PS II electron transport ($\Phi_{PS\,II}$).

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