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Photoinhibition in outdoor *Spirulina platensis* cultures assessed by polyphasic chlorophyll fluorescence transients

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Abstract

Photoinhibition in outdoor cultures of *Spirulina platensis* was studied by measuring the polyphasic rise of chlorophyll fluorescence transients, which provide information on the primary photochemistry of PSII. The maximum efficiency of PSII photochemustry (F_v/F_m) declined in response to daily increasing irradiance and recovered as daily irradiance decreased. The greatest inhibition (15%) in F_v/F_m was observed at 12:00 hr which responded to the highest irradiance. The absorption flux, the trapping flux, and the electron transport flux per PSII reaction center increased in response to daily increasing irradiance and decreased as irradiance decreased. The daily change in the concentration of PSII reaction centers followed the same pattern as F_v/F_m. However, no significant changes in the probability of electron transport beyond Q_A (Ψ_o) were observed during the day. The results suggest that the decrease in F_v/F_m induced by photoinhibition in outdoor *Spirulina* cultures was a result of the inactivation of PSII reaction centers. The results also suggest that the measurement of polyphasic fluorescence transients is a powerful tool to study the mechanism of photoinhibition in outdoor *Spirulina* cultures and to screen strains for photoinhibition tolerance.

Introduction

The cyanobacterium *Spirulina platensis* as a potential source of proteins and pharmaceuticals is commercially cultivated (Vonshak, 1990). However, the productivity of *Spirulina* cultures grown outdoors is significantly limited by environmental factors. Vonshak & Guy (1992) have demonstrated that a decrease in photosynthetic activity induced by photoinhibition contributes to a decrease in the outdoor production of *Spirulina*. Such a reduction is due to decreased photosystem II (PSII) activity indicated by a decrease in the maximal efficiency of PSII photochemistry (Vonshak et al., 1994, 1996; Torzillo et al., 1996, 1998). The characteristics of PSII photochemistry induced by photoinhibition in outdoor cyanobacterial and algal cultures are still unclear although the mechanisms of photoinhibition have been studied intensively in these organisms and in higher plants (Baker & Bowyer, 1994).

Recently it has been shown that fluorescence induction transients show a polyphasic rise, including phases O, J, I and P (Strasser et al., 1995). This polyphasic rise can be utilized as a tool to evaluate the behavior of PSII under environmental stresses, such as light (Krüger et al., 1997), high temperature (Srivastava et al., 1997; Strasser, 1997), and elevated CO_2 and O_3 (Meinander et al., 1996). Moreover, those modifications in PSII photochemistry can be quantified through the JIP test (Strasser & Strasser, 1995), which was derived from the polyphasic rise of fluorescence transients based on the theory of energy flux in biomembranes (Strasser, 1978, 1981).

We have used polyphasic rise of fluorescence transients to investigate the characterization of PSII photochemistry in salt-shocked and -adapted *Spirulina* cells

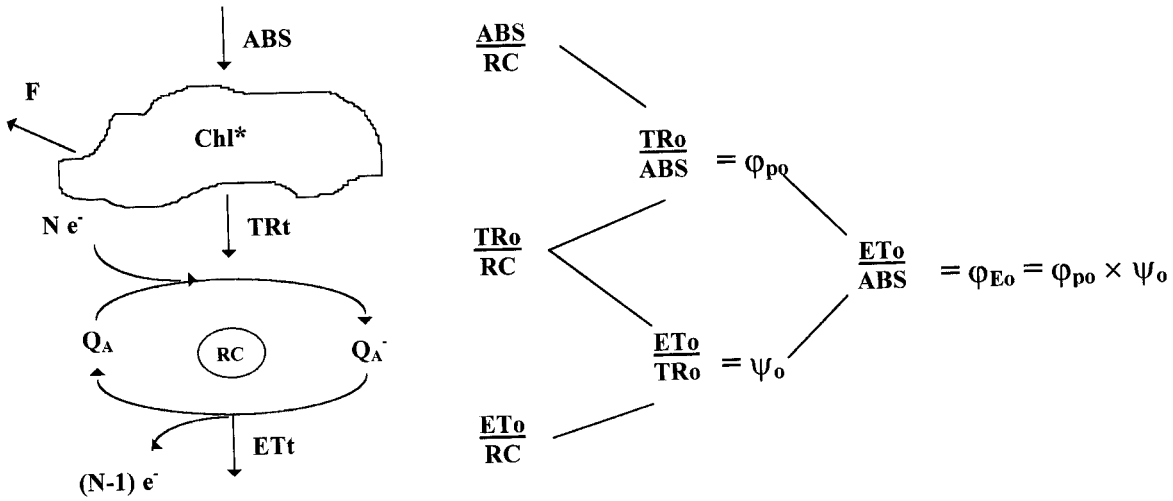

Figure 1. Schematic energy-flux model for PSII. ABS: light absorption flux. TRt, TRo: energy flux trapped by PSII reaction centers at time t and time zero, respectively. ETt, ETo: electron transport flux generated by the reoxidation of Q_A^- to Q_A at time t and time zero, respectively. F: fluorescence emission. TRo/ABS (φ_{po}): maximum efficiency of PSII photochemistry. ETo/TRo (Ψ_o): the probability of electron transport beyond Q_A. ETo/ABS (φ_{Eo}): the maximum yield of electron transport beyond Q_A. Therefore, $\varphi_{Eo} = \varphi_{po} \times \Psi_o$.

(Lu & Vonshak, 1999; Lu et al., 1999). In this study, we have examined the daily changes in several fluorescence parameters determined from the polyphasic fluorescence transients in outdoor *Spirulina platensis* cultures. The aim of this work was to investigate the characteristics of PSII photochemistry induced by photoinhibition and also to evaluate the use of different fluorescence parameters as possible indicators for photoinhibition in outdoor algal cultures.

Materials and methods

Organism and culture conditions

Spirulina platensis strain M_2 was used in this study. Outdoor cultures were grown as previously described (Vonshak & Guy, 1992). Culture depth was 12 cm and mixing of the culture was provided by a paddle wheel at 17 rpm.

Polyphasic rise of chlorophyll a fluorescence transients and the JIP test

The polyphasic rise of chlorophyll a fluorescence transients was measured by using a Plant Efficiency Analyzer (PEA, Hansatech Instruments Ltd, King's Lynn, Norfolk PE32 1JL, UK) as previously described (Lu & Vonshak, 1999). Illumination was provided with an array of six high-intensity light emitting diodes (with a maximum at 650 nm), which were focused on the sample surface to provide homogeneous illumination over an area of 4 mm in diameter. The fluorescence signals were received by a high performance Pin photodiode detector associated with an amplifier circuit. The detector responded maximally to the longer wavelength fluorescence signal while blocking reflected, shorter wavelength light from light-emitting-diodes. The fluorescence signals were recorded within a time scan from 10 μs to 1 s with a data acquisition rate of 10^5 readings s^{-1} for the first 2 ms and of 10^3 readings s^{-1} after 2 ms.

All oxygenic photosynthetic organisms tested so far exhibit a polyphasic rise of fluorescence transients during the first second of illumination. These phases are labeled as O, J, I and P (Strasser et al., 1995). As described previously, *Spirulina platensis* also shows typical polyphasic rise transients including phase O, J, I and P (Lu & Vonshak, 1999).

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

According to the model of energy fluxes in this test (Figure 1), photons absorbed by the antenna pigments are referred to as absorption flux (ABS). Part

of this excitation energy is dissipated as fluorescence, but most of it is transferred as the trapping flux (TR) to the reaction centres (RC). In the RCs, the excitation energy is converted to redox energy by reducing Q_A to Q_A^- which is then reoxidized to Q_A, thus leading to an electron transport flux (ET) which maintains the metabolic reactions of the photosynthetic apparatus.

To carry out the JIP test, several parameters calculated from the measurements of the polyphasic fluorescence transients are needed. They are: (1) the minimal fluorescence yield, F_o, (2) the maximal fluorescence yield, F_m, (3) the initial slope at the beginning of the variable fluorescence transients theoretically at time zero), dV/dto [$=(F_{300\mu s}-F_o)/(F_m-F_o)$]; and (4) the relative variable fluorescence at phase J, V_J [$=(F_J-F_o)/(F_m-F_o)$].

According to the JIP test, the energy fluxes for ABS, TR and ET per PSII reaction center (RC) can be given by equations 1–3, respectively. The probability of electron transport beyond Q_A (Ψ_o) and the concentration of PSII reaction centers (RC/CS) are given by equations 4 and 5, respectively.

$$ABS/RC = [(dV/dto)/V_J]/[1-(F_o/F_m)] \quad (1)$$

$$TRo/RC = (dV/dto)/V_J \quad (2)$$

$$ETo/RC = [(dV/dto)/V_J] \cdot (1-V_J) \quad (3)$$

$$\Psi_o = ETo/TRo = 1-V_J \quad (4)$$

$$RC/CS = [V_J/(dV/dto)] \times [1-(F_o/F_m)] \times F_o \quad (5)$$

For a detail derivation of the formulae for the various energy fluxes and for the flux ratios in the JIP test, see Strasser & Strasser (1995) and Krüger et al. (1997).

All samples were dark-adapted for 10 min prior to the measurement of polyphasic rise fluorescence transients.

Results

The maximum efficiency of PSII photochemistry, i.e. the ratio of variable to maximum fluorescence (F_v/F_m), declined in response to daily increasing irradiance and recovered as irradiance decreased in the late afternoon in the outdoor cultures. This is demonstrated in Figure 2A where changes in F_v/F_m through typical summer days are shown. The greatest inhibition in F_v/F_m (around 15%) was observed at midday (12:00) which responded to the highest irradiance (Figure 2B). It seems that the decrease in F_v/F_m was

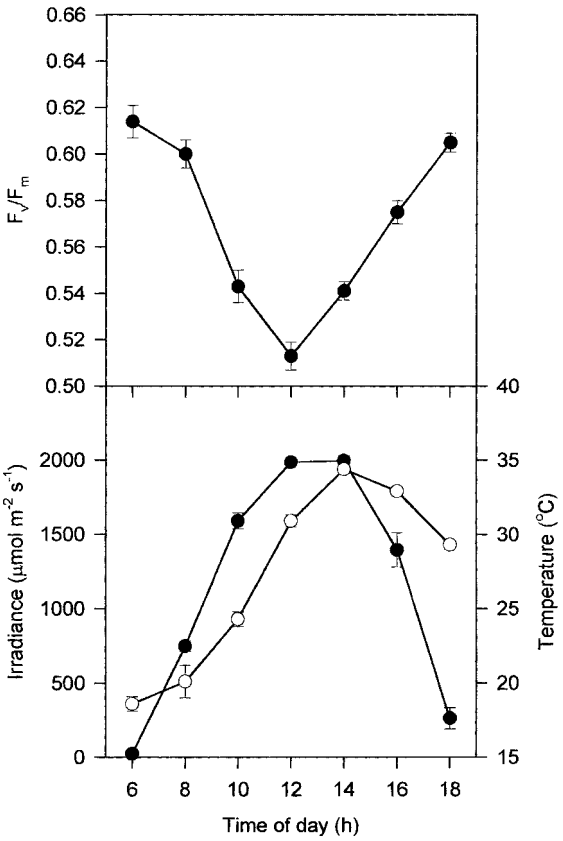

Figure 2. (A) Diurnal patterns of photoinhibition (expressed as F_v/F_m) in outdoor *Spirulina platensis* cultures as a function of daily irradiance. (B) Diurnal changes in daily irradiance (●) and temperatures (○); Data are the means of seven separate days' measurements ± SE.

mainly associated with the changes in daily irradiance but not with the changes in temperature since the temperature at midday was almost optimal for *Spirulina* cells growth (Figure 2B).

In order to examine what caused a decrease in the ratio F_v/F_m in photoinhibited cells, we investigated the characteristics of PSII photochemistry through the analyses of the JIP test. We thus followed the polyphasic rise of fluorescence transients at different daily times. Table 1 shows the values of F_o, F_m, dV/dto, and V_J calculated from the measurements of the polyphasic fluorescence transients.

The results of the JIP test are shown in Figure 3. Figure 3A shows the diurnal changes in the trapping flux, the absorption flux and the electron transport flux per PSII reaction center, i.e. ABS/RC, TRo/RC, and ETo/RC. The changes in ABS/RC, TRo/RC, and ETo/RC followed the same pattern as that of irra-

Table 1. Diurnal changes in several basic parameters in outdoor *Spirulina platensis* cultures determined from measurements of the polyphasic rise of chlorophyll fluorescence transients: (1) the minimal fluorescence yield, F_o; (2) the maximal fluorescence yield, F_m; (3) the initial slope at the beginning of the variable fluorescence transients, dV/dto [=($F_{300\mu s}$-F_o)/(F_m-F_o)]; and (4) the relative variable fluorescence at phase J, V_J [=(F_J-F_o)/(F_m-F_o)]. Data are the means of seven separate days' measurements ± SE

Time of day (h)	F_o	F_m	dV/dt_o	V_J
6:00	69.8 ± 1.6	180.9 ± 1.2	0.180 ± 0.005	0.206 ± 0.008
8:00	69.6 ± 1.5	180.2 ± 1.2	0.186 ± 0.004	0.221 ± 0.007
10:00	71.2 ± 1.6	167.0 ± 2.0	0.212 ± 0.007	0.211 ± 0.008
12:00	73.3 ± 2.2	165.1 ± 1.5	0.241 ± 0.001	0.228 ± 0.008
14:00	76.5 ± 2.2	172.2 ± 3.2	0.231 ± 0.001	0.237 ± 0.014
16:00	76.4 ± 2.2	184.5 ± 2.0	0.226 ± 0.001	0.248 ± 0.012
18:00	77.9 ± 1.8	186.6 ± 2.5	0.200 ± 0.007	0.254 ± 0.011

diance. ABS/RC, TRo/RC, and ETo/RC increased during the morning with the increase in irradiance and reached the maximum levels at 12:00. In the afternoon, they recovered gradually to the early morning levels.

Figure 3B shows the time course of the probability of electron transport beyond Q_A (Ψ_o). No significant changes in Ψ_o were observed during the day.

The diurnal changes in the concentration of PSII reaction centers (RC/CS) is shown in Figure 3C. The changes in RC/CS were inversely proportional to irradiance. RC/CS reached the lowest value at 12:00 and recovered completely in the late afternoon.

Discussion

Photoinhibition, indicated mainly by a decrease in the maximum efficiency of PSII photochemistry (F_v/F_m), has been observed in outdoor *Spirulina* cultures (Vonshak & Guy, 1992; Vonshak et al., 1996; Torzillo et al., 1996, 1998). The results in this study show that *Spirulina platensis* cells in outdoor cultures are susceptible to photoinhibition, and that reduced F_v/F_m towards midday is correlated with the daily pattern of irradiance, further confirming that photoinhibition takes place in outdoor *Spirulina* cultures. However, what caused a decrease in F_v/F_m in outdoor *Spirulina* cultures is not clear yet although the mechanism of photoinhibition has been studied intensively in the laboratory.

A constant value in the probability of electron transport beyond Q_A (Ψ_o) during the day suggests that electron transport at the acceptor of PSII was not affected in photoinhibited *Spirulina* cells (Figure 3B). Our results clearly show that the decrease in F_v/F_m can be explained by the inactivation of PSII reaction centers since the changes in the concentration of PSII reaction centers followed the same pattern as that of F_v/F_m (Figure 3C).

A significant increase in TRo/RC, ABS/RC, and ETo/RC was observed with increasing irradiance (Figure 3A). TRo/RC expresses the rate by which an exciton trapped by an open RC results in reduction of Q_A to Q_A^-. In this sense, TRo/RC here refers only to the *active* (Q_A to Q_A^- reducing) centers. Similarly, ABS/RC and ETo/RC also refer only to the *active* (Q_A to Q_A^- reducing) centers since their derivation is based on the expression for TRo/RC (Krüger et al., 1997). Therefore, an increase in ABS/RC, TRo/RC and ETo/RC during the day was actually a result of an inactivation of PSII reaction centers. It seems that through such a down-regulation of PSII reaction centers, the PSII apparatus in outdoor *Spirulina* cultures thus maintained the high efficiency of excitation energy conversion, indicated by an increase in the trapping flux, the absorption flux and the electron transport flux per PSII reaction center, i.e. an increase in ABS/RC, TRo/RC and ETo/RC (Figure 3A).

The results show that by measuring the polyphasic fluorescence transients we are able not only to estimate the degree of photoinhibition but also to analyze the functional changes of PSII in outdoor *Spirulina* cultures. These results suggest that polyphasic chlorophyll fluorescence transients, within the measuring time of one second, may be used as a tool to investigate

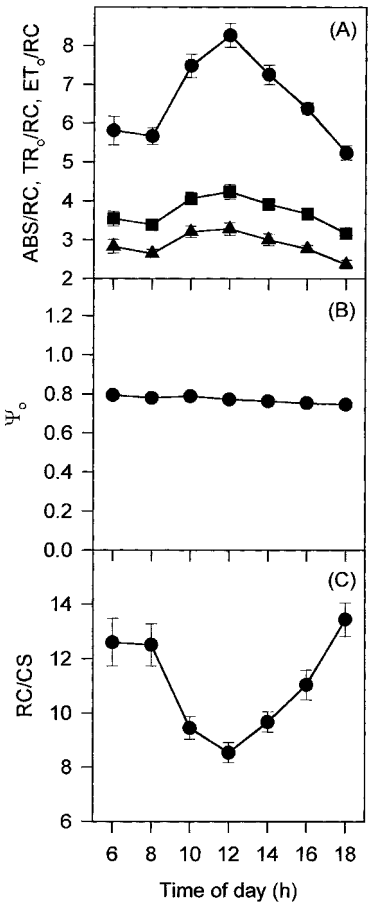

Figure 3. Diurnal changes: (A) in the absorption flux per PSII reaction center (ABS/RC, ●), the trapping flux (TRo/RC, ■), and the electron transport flux per PSII reaction center (ETo/RC, ▲); (B) in the probability of electron transport beyond Q_A (Ψ_o); (C) in the concentration of PSII reaction centers (RC/CS) in outdoor *Spirulina platensis* cultures. Data are the means of seven separate days' measurements ± SE.

mechanisms of light stress on photosynthetic apparatus in outdoor algal cultures and also to screen strains for light stress tolerance.

References

Baker NR, Bowyer JR (eds) (1994) Photoinhibition of Photosynthesis from Molecular Mechanisms to the Field. Bios Scientific Publishers, Oxford.

Krüger GHJ, Tsimilli-Michae M, Strasser RJ (1997) Light stress provokes plastic and elastic modifications in structure and function of photosystem II in camellia leaves. Physiol. Plant. 101: 265–277.

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

Lu C-M, Torzillo G, Vonshak A (1999) Kinetic response of photosystem II photochemistry in cyanobacterium *Spirulina platensis* to high salinity is characterized by two distinct phases. Aust. J. Plant Physiol. 26: 283–292.

Meinander O, Somersalo S, Holopainen T, Strasser RJ (1996) Scots pines after exposure to elevated ozone and carbon dioxide probed by reflectance spectra and chlorophyll a fluorescence transients. J. Plant Physiol. 148: 229–236.

Srivastava A, Guissé B, Greppin H, Strasser RJ (1997) Regulation of antenna structure and electron transport in photosystem II of *Pisum sativum* under elevated temperature probed by the fast polyphasic chlorophyll a fluorescence transient: OKJIP. Biochim. Biophys. Acta 1320: 95–106.

Strasser BJ (1997) Donor side capacity of photosystem II probed by chlorophyll a fluorescence transients. Photosynth. Res. 52: 147–155.

Strasser RJ (1978) The grouping model of plant photosynthesis. In Akoyunoglou G, Argyroudi-Akoyunoglou J (eds), Chloroplast Development. Elsevier/North-Holland Biomedical Press, Amsterdam: 513–524.

Strasser RJ (1981) The grouping model of plant photosynthesis: heterogeneity of photosynthetic units in thylakoids. In Akoyunoglou G (ed.), Photosynthesis III. Structure and Molecular Organization of the Photosynthetic Apparatus. Balaban International Science Services, Philadelphia: 727–737.

Strasser RJ, Srivastava A, Govindjee (1995) Polyphasic chlorophyll a fluorescence transients in plants and cyanobacteria. Photochem. Photobiol. 61: 32–42.

Strasser BJ, Strasser RJ (1995) Measuring fast fluorescence transients to address environmental questions: the JIP test. In Mathis P (ed.), Photosynthesis from Light to Biosphere, Vol. 5. Kluwer, Dordrecht: 977–980.

Torzillo G, Accolla P, Pinzani E, Masojidek J (1996) *In situ* monitoring of chlorophyll fluorescence to assess the synergistic effect of low temperature and high irradiance stresses in *Spirulina* cultures grown outdoors in photobioreactors. J. appl. Phycol. 8: 282–291.

Torzillo G, Bernardini P, Masojidek J (1998) On-line monitoring of chlorophyll fluorescence to assess the extent of photoinhibition of photosynthesis induced by high oxygen concentration and low temperature and its effect on the productivity of outdoor cultures of *Spirulina platensis* (cyanobacteria). J. Phycol. 34: 504–510.

Vonshak A (1990) Recent advances in microalgal biotechnology. Biotechnol. Adv. 8: 709–727.

Vonshak A, Guy R (1992) Photodadaptation, photoinhibition and productivity in the blue-green alga *Spirulina platensis* grown outdoors. Plant Cell Environ. 15: 613–616.

Vonshak A, Torzillo G, Accolla P, Tomaselli L (1996) Light and oxygen stress in *Spirulina platensis* (cyanobacteria) grown outdoors in tubular reactors. Physiol. Plant. 97: 175–179.

Vonshak A, Torzillo G, Tomaselli L (1994) Use of chlorophyll fluorescence to estimate the effect of photoinhibition in outdoor cultures of *Spirulina platensis*. J. appl. Phycol. 6: 31–34.