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The Effects of Excess Irradiance on Photosynthesis in the Marine Diatom *Phaeodactylum tricornutum*[1]

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The response of *Phaeodactylum tricornutum* to excess light was remarkably similar to that observed in higher plants and green algae and was characterized by complex changes in minimal fluorescence yields of fully dark-adapted samples and declines in maximum variable fluorescence levels and oxygen evolution rates. In our study the parallel decreases in the effective rate constant for photosystem II (PSII) photochemistry, the variable fluorescence yield of a dark-adapted sample, and light-limited O_2 evolution rates after short (0–10 min) exposures to photoinhibitory conditions could not be attributed to damage or down-regulation of PSII reaction centers. Instead, these changes were consistent with the presence of nonphotochemical quenching of PSII excitation energy in the antennae. This quenching was analogous to that component of nonphotochemical quenching studied in higher plants that is associated with photoinhibition of photosynthesis and/or processes protecting against photoinhibition in that it did not relax readily in the dark and persisted in the absence of a bulk transthylakoid proton gradient. The quenching was most likely associated with photoprotective processes in the PSII antenna that reduced the extent of photoinhibitory damage, particularly after longer exposures. Our results suggest that a large population of damaged, slowly recovering PSII centers did not form in *Phaeodactylum* even after 60 min of exposure to excess actinic light.

Photoinhibition is a complex multistep process in which the inactivation of PSII reactions is manifested as a decrease in the quantum yield of photochemistry at both limiting and saturating light intensity. Exposure of plants to excess light (where the rate of light absorption exceeds the capacity of the dark reactions to utilize the products of light-driven electron transport) frequently results in PSII photoinactivation due to reactions on both the PSII acceptor and donor sides and in the degradation of the reaction center D1 protein (Barber and Andersson, 1992; Prasil et al., 1992). Although many studies have examined the effects of excess light on photosynthesis in higher plants and green algae, relatively few have examined these effects in other algal classes. In view of the fact that the PSII reaction center has been highly conserved throughout evolution (Thornber, 1986), it is probable that the molecular mechanisms of photoinhibitory damage are similar among higher plants and the different algal classes. It is also likely that all organisms have a variety of photoprotective and repair processes to limit the amount of PSII damage (Barber and Andersson, 1992; Prasil et al., 1992). However, the extent to which any single process contributes is dependent on many factors including the previous light history and photosynthetic physiology of the organism.

Our purpose is to study the effects of exposure to excess actinic light on photosynthesis in the marine diatom *Phaeodactylum tricornutum*. Two practical reasons form the basis for undertaking this project. First, conditions conducive to the development of photoinhibition occur frequently in the aquatic environment, where diatoms are a dominant class of primary producers. Second, in recent years models of primary production rates in the ocean have relied increasingly on in vivo Chl fluorescence measurements as indicators of algal biomass, productivity, photosynthetic capacity, and physiology (Samuelsson and Oquist, 1977; Heath, 1988). However, there exists no solid experimental basis justifying the use of fluorescence parameters for these purposes, since the processes affecting fluorescence yields in different algal classes have not been elucidated (Owens, 1991). Photoprotective processes and photoinhibitory damage are dominant factors affecting photochemical and Chl fluorescence yields in vivo (Krause and Weis, 1991). In higher plants the contribution of these factors to total q_N of PSII excitation energy increases with increasing excess light absorption under either high-light conditions or other environmental stresses that depress the rate of photosynthesis (Horton and Hague, 1988; Krause and Weis, 1991; Owens, 1994a).

One of the major components involved in q_N is q_E (Briantais et al., 1979; Gilmore and Yamamoto, 1993). There is strong evidence that q_E is heterogeneous in origin, arising from several different processes. Although some studies have in-

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Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; D1, 32-kD PSII reaction center polypeptide; F_m, maximal fluorescence yield of a fully dark-adapted sample; F_o, minimal fluorescence yield of a fully dark-adapted sample; F_v, variable fluorescence yield of a dark-adapted sample ($F_v = F_m - F_o$); q_E, a component of nonphotochemical quenching associated with the establishment of a transthylakoid proton gradient; q_I, a component of nonphotochemical quenching associated with photoinhibition of photosynthesis and/or processes protecting against photoinhibition; q_N, nonphotochemical quenching of PSII excitation energy; q_P, photochemical quenching of PSII excitation energy.

dicated that q_E occurs primarily in the antennae and is associated with an increase in thermal deactivation (Krause et al., 1983; Genty et al., 1989; Horton et al., 1991; Noctor et al., 1993), others have proposed that the main site of q_E is in the reaction center (Weis and Berry, 1987; Krieger et al., 1992). Although there is evidence that antenna-dependent q_E may be related to thylakoid zeaxanthin and antheraxanthin content under some conditions (Demmig-Adams, 1990; Gilmore and Yamamoto, 1993), the relationship between q_E and carotenoid content is very complex (Gilmore and Yamamoto, 1991a, 1991b, 1992, 1993; Noctor et al., 1991).

Another component of q_N is q_I. Distinctions between q_I and q_E are difficult to establish under some conditions, since q_I is also heterogeneous in origin. Like q_E, q_I includes photoprotective component(s) involving increased thermal dissipation of absorbed light energy (Demmig and Winter, 1988; Demmig-Adams, 1990; Krause and Weis, 1991). However, in contrast to q_E, q_I is not readily reversed with the addition of uncouplers and exhibits slower relaxation kinetics (Horton and Hague, 1988; Hodges et al., 1989). In addition, q_I is associated with the formation of damaged (Cleland et al., 1986; Krause et al., 1990) and/or down-regulated PSII reaction centers (Krause and Weis, 1991; Krieger et al., 1992).

The regulation of q_N by underlying physiological processes is likely to differ among the algal classes because one of the main sites of q_N action, the PSII antennae, exhibits great diversity among algae with regard to both pigment and protein composition. In contrast to green algae, diatoms possess both a major Chl *a*/*c*-fucoxanthin complex and a minor Chl *a*/*c* complex (Owens and Wold, 1986). Under physiological light conditions, diatoms exhibit values of q_N that are similar to those reported in higher plants and green algae. However, at saturating photon flux densities, the amount of steady-state q_N was reported to be significantly less than in higher plants (Ting and Owens, 1993). In addition, q_E has been observed in the dark-adapted state as well as in the light in the presence of DCMU (Ting and Owens, 1993).

Preliminary work has suggested that after exposure of diatoms to excess light, decreases in carbon fixation rates result from photooxidative damage to components of both the light and dark reactions (Belay and Fogg, 1978). However, the direct effects of excess actinic light on PSII photochemistry or on photoprotective processes were not examined. In the present study we conclude that excess light-dependent changes in oxygen evolution rates, Chl fluorescence parameters, and the kinetics of PSII photochemistry in *P. tricornutum* are consistent with the presence of q_N in the antennae of PSII. This q_N may serve to limit damage to the PSII reaction center under photoinhibitory conditions.

MATERIALS AND METHODS

Phaeodactylum tricornutum Bohlin (UTEX 646) was grown in unialgal batch cultures (2 L) in natural seawater containing "f/2" nutrients at 15°C and 90 μmol photons m^{-2} s^{-1} in a 14/10 h light/dark cycle as previously described (Ting and Owens, 1992). Cells were harvested in the early log phase of growth by centrifugation (5000*g*, 3 min) at 15°C and resuspended in growth medium to a concentration of 6 μg Chl *a* mL^{-1}. Chl was extracted in 90% (v/v) acetone, and Chl *a* concentrations were determined spectrophotometrically (Jeffrey and Humphrey, 1975).

Photoinhibition treatments were conducted at 15°C and 2000 μmol photons m^{-2} s^{-1}. Resuspended cells were placed in an open-top, water-jacketed, glass reaction vessel and supplemented with freshly prepared $NaHCO_3$ (9 mM final concentration) prior to initiation of the photoinhibitory treatment. Cells were stirred gently to ensure uniform exposure. At specific time points after the onset of the high-light treatment, cells were transferred to a Hansatech DW2 oxygen electrode chamber for fluorescence and oxygen evolution measurements or were allowed to recover. During the recovery period cells were either returned to their original growth conditions (15°C, 90 μmol photons m^{-2} s^{-1}) or placed at 15°C in the dark.

Measurements of F_o, F_m, and F_v were conducted at 15°C using a pulse amplitude-modulated Chl fluorometer (PAM Fluorometer, Heinz Walz, Effeltrich, Germany) interfaced to a Hewlett-Packard Series 200 computer. Samples in the Hansatech DW2 electrode chamber were dark adapted for 5 min in the presence of 5 μM CCCP to remove quenching associated with chlororespiration (Ting and Owens, 1993). The effects of other uncouplers (nigericin) and ionophores (valinomycin-K^+) on dark-adapted F_o and F_m levels in *P. tricornutum* have been examined previously (Ting and Owens, 1993). In addition, samples were pre-illuminated for 20 s with 704-nm light (33 μmol photons m^{-2} s^{-1}) immediately before fluorescence measurements were conducted to ensure that the primary quinone acceptor of PSII and the plastoquinone pool were fully oxidized (Owens, 1986b). The saturating pulses (1.5 s, 6800 μmol photons m^{-2} s^{-1}, Corion [Holliston, MA] 650-nm short-pass filter) for measurement of F_m were produced using a 1000-W tungsten-halogen lamp and an electronic shutter (Uniblitz [Rochester, NY], effective opening time = 5 ms).

The effective rate constant for PSII photochemistry was estimated from the kinetics of the variable fluorescence rise measured in the presence of DCMU using a nonmodulated induction fluorometer as described by Owens (1986a). Samples suspended in original growth medium to a concentration of 6 μg Chl *a* mL^{-1} were treated with 20 μM DCMU and 5 μM CCCP (final concentrations) for about 1.5 min in the dark prior to measurement. Blue-green actinic illumination was provided by a 150-W tungsten-halogen lamp (blocked by a CS 4–96 filter [Corning, Corning, NY]), the intensity of which was attenuated using neutral-density filters. A Hamamatsu R636 photomultiplier tube (operated at 700 V) protected by three CS 2–64 sharp-cut red filters was used to detect the fluorescence. Signal output from the photomultiplier was converted to voltage, amplified, and then transferred to the Hewlett-Packard Series 200 computer. The kinetics of PSII photochemistry were analyzed as described by Owens (1986a) except that, after calculation of the normalized area growth over the fluorescence induction curve, standard nonlinear least-squares analysis rather than graphic analysis was used to determine the effective photochemical rate constants.

O_2 evolution rates were measured using a polarographic O_2 electrode (Hansatech, Norfolk, England) at 15°C. Illumination was provided by a 150-W tungsten-halogen lamp, the

intensity of which (1500 μmol photons m^{-2} s^{-1}, maximum) was adjusted using neutral-density filters. Photon flux densities were measured 1 mm away from the end of the fiber-optics cable using a calibrated IL1700 Research Radiometer/Photometer (International Light, Newburyport, MA).

RESULTS

Changes in O_2 Evolution Rates and Chl Fluorescence

The response of *P. tricornutum* to photoinhibitory conditions was investigated by exposing cells to photon flux densities several times greater in intensity than required for saturation of photosynthetic rates. For cells grown under the conditions of our experiments, O_2 evolution rates saturated at approximately 500 μmol photons m^{-2} s^{-1} (Fig. 1, controls). Exposure of cells to 2000 μmol photons m^{-2} s^{-1} resulted in reductions in both the light-limited (180 μmol photons m^{-2} s^{-1}) and light-saturated (580 μmol photons m^{-2} s^{-1}) rates of O_2 evolution (Fig. 1; Table I). After a 10-min exposure to photoinhibitory conditions, the decline in light-limited O_2 evolution rates was greater than the corresponding decrease in light-saturated rates (Table I), whereas the declines in F_v (73%) and F_v/F_m (59%) were similar to those of light-limited O_2 evolution rates (60%) (Fig. 2; Table I). The decrease in F_v/F_m was due to both a decrease in F_v and an increase in F_o. F_o levels increased during the first 20 min of photoinhibitory treatment to a maximum that was 55% greater than controls before beginning to decline with longer exposure (Fig. 2).

Increasing the photoinhibitory treatment from 10 to 60 min led to additional declines in O_2 evolution rates, with the total decline in the light-limited and light-saturated rates being about equal (Table I). However, whereas the 10-min treatment affected mainly light-limited rates, increasing the treatment to 60 min had a larger effect on light-saturated rates (Table I). During the same period, a continuous decline in F_v was observed but at a rate that was much slower than during the first 10 min of treatment (Fig. 2). The decline in F_v (approximately 90%) after 60 min of treatment was greater than that for both limiting and saturating O_2 evolution rates

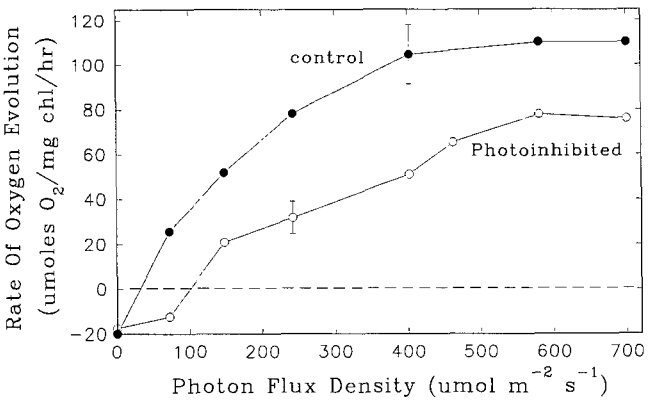

Figure 1. Rate of O_2 evolution at different photon flux densities of control cells (●) and of cells photoinhibited for 40 min (○) at 2000 μmol photons m^{-2} s^{-1}. SD values of representative data points are shown for control and photoinhibited cells.

Table I. *Changes in O_2 evolution rates and Chl fluorescence parameters after exposure to excess light*

Percent change in light-limited and light-saturated O_2 evolution rates and in Chl fluorescence levels compared to control values after exposure of *P. tricornutum* to 2000 μmol photons m^{-2} s^{-1} for 10 or 60 min. Fluorescence quenching parameters q_N and q_P were measured in the light at the end of the 10- or 60-min illumination periods just before dark adaptation.

Parameter	Percent Change	
	10-min Treatment	60-min Treatment
Light-limited O_2 evolution	−60	−67
Light-saturated O_2 evolution	−33	−63
F_o	+44	+14
F_v	−73	−90
F_v/F_m	−59	−78
q_N	0.82	0.93
q_P	0.10	0.08

(Table I). F_o levels decreased after 20 min of exposure to photoinhibitory conditions and reached a level that was about 14% greater than controls after 60 min of treatment (Fig. 2).

Steady-state levels of q_N and q_P were also measured at the end of the 10- and 60-min treatments. After 10 min of treatment, 11% of the total quenching present in the light relaxed in the dark. After 60 min of treatment, this value decreased to only 3% (Table I). These values were measured in the absence of CCCP to estimate the full extent of q_N in the sample but were scaled to F_o and F_m values measured in the presence of CCCP on parallel samples. In *P. tricornutum*, F_o and F_m must be measured in the presence of uncouplers such as CCCP to relax the significant pH gradient that exists in the dark (Ting and Owens, 1993), presumably the result of chlororespiration.

Effects of Exposure to Excess Light on the Kinetics of PSII Photochemistry

To determine whether short exposures to photoinhibitory conditions led primarily to damage (Krause et al., 1990; Barber and Andersson, 1992) or down-regulation (Weis and Berry, 1987; Krieger et al., 1992) of PSII reaction centers or to photoprotective processes in the antennae (Demmig-Adams, 1990; Horton et al., 1991), we next examined the effects of photoinhibition on the kinetics of PSII photochemistry determined from the rate of area growth over the fluorescence induction curve measured in the presence of DCMU under limiting actinic illumination (Melis and Homann, 1976).

In control cells, significant differences were observed in the kinetics of PSII photochemistry compared to higher plants and green algae. At an actinic intensity of 160 μmol photons m^{-2} s^{-1}, the kinetics were monophasic (Fig. 3), with an effective first-order rate constant of 290 s^{-1}. Addition of a second component did not alter the merit function or the distribution of residuals. To confirm that there was no additional fast component that was unresolvable at this intensity, experiments were also conducted at a lower actinic intensity. At 50 μmol photons m^{-2} s^{-1}, the kinetics remained single

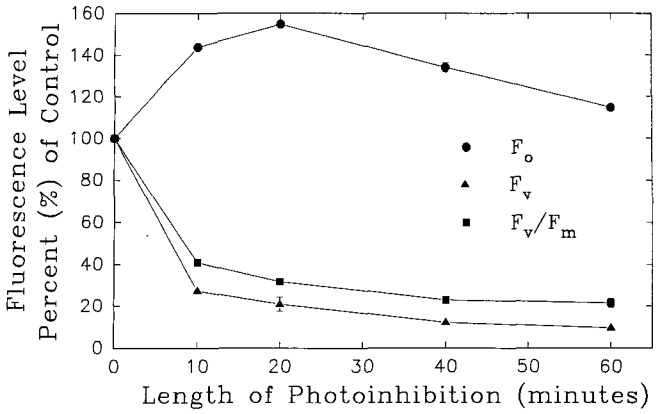

Figure 2. Changes in F_o, F_v, and F_v/F_m (expressed as percent of controls) as a function of the length of the photoinhibitory treatment at 2000 μmol photons m^{-2} s^{-1}. Samples were dark adapted for 5 min prior to measurements. All fluorescence measurements were conducted in the presence of CCCP. The SD values for representative data points are presented.

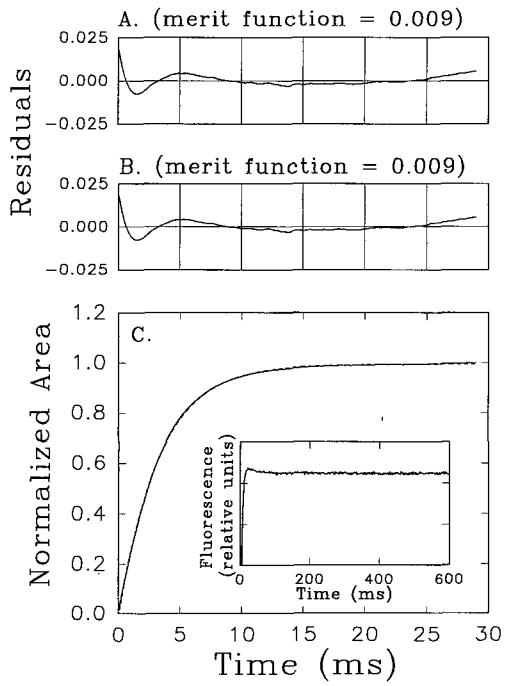

Figure 3. A, Distribution of residuals for a one-exponent fit. B, Distribution of residuals for a two-exponent fit. C, The normalized area growth over the fluorescence induction curve for control cells at 160 μmol photons m^{-2} s^{-1} (solid line) and a standard nonlinear least-squares analysis fit of the data to one exponent (dotted line). Inset, Fluorescence induction rise for control cells showing the rapid quenching of fluorescence from the F_m level. Fluorescence induction measurements were conducted in the presence of DCMU and CCCP.

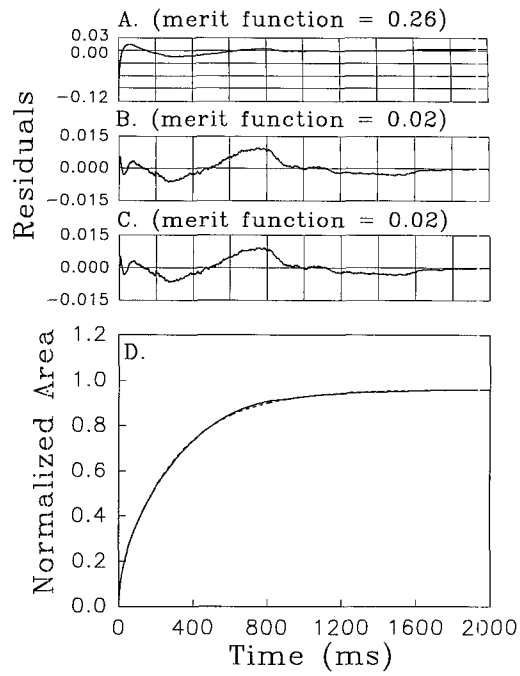

Figure 4. The normalized area growth over the fluorescence induction curve for photoinhibited cells. A, Distribution of residuals for a one-exponent fit. B, Distribution of residuals for a two-exponent fit. C, Distribution of residuals for a three-exponent fit. Note the scale change of the y axis in plot A. D, The normalized area growth over the fluorescence induction curve (+DCMU, +CCCP) at 160 μmol photons m^{-2} s^{-1} for cells photoinhibited (10 min) at 2000 μmol m^{-2} s^{-1} (solid line) and a standard nonlinear least-squares analysis fit of the data to two exponents (dotted line).

exponential, with an effective first-order rate constant that decreased in proportion to the actinic intensity (not shown). The fluorescence levels of control cells were quenched soon after reaching the F_m level (Fig. 3C, inset). This was observed at a range of actinic intensities from 50 to 330 μmol photons m^{-2} s^{-1}. No similar response was observed in intact cells of *Chlamydomonas* (not shown). This quenching was not attributable to the formation of a bulk pH gradient across the thylakoid membrane, since all of our fluorescence measurements were conducted in the presence of CCCP to dissipate any proton gradient that formed during dark adaptation (Ting and Owens, 1993).

After a photoinhibitory treatment of 10 min, two significant differences were observed in the kinetics of PSII photochemistry compared to control cells. First, the kinetics were biphasic, consisting of the sum of two exponential components (Fig. 4). In addition, the effective rate constants of the two components were much lower than the control value. At an actinic intensity of 160 μmol photons m^{-2} s^{-1}, the dominant slow component (amplitude = 85%) had an effective rate constant of 4 s^{-1} and the minor fast component (amplitude = 15%) had a rate constant of 68 s^{-1}. The weighted mean rate constant (13.6 s^{-1}) declined by 95% relative to control cells, a value that is similar to the decline in F_v but larger than the decline in O_2 evolution rates (Table I).

To ensure that CCCP was not having any secondary effects that may have resulted in the large reduction in the rate constants of PSII photochemistry in either control or photoinhibited cells, we examined the fluorescence induction kinetics in the presence and absence of CCCP. In controls incubated with DCMU, the kinetics of PSII photochemistry were identical and monophasic in both the presence and absence of CCCP (Fig. 5, inset). In photoinhibited cells measured in the presence of DCMU and CCCP, the PSII kinetics were biexponential (Fig. 4) and the variable fluorescence rise was smooth (Fig. 5). In the absence of CCCP, the half-time for the variable fluorescence rise was the same as for CCCP-treated cells but the kinetics of the rise were complex, exhibiting a clear inflection at about 0.4 s (Fig. 5). Thus, the effect of CCCP was not to alter the overall rate at which PSII traps close but to inhibit a process of unknown origin in photoinhibited cells that caused an inflection in the fluorescence rise. This inflection, measured in the presence of DCMU, has not been previously reported in control or photoinhibited cells of green algae or higher plants.

The Recovery Process

The changes observed in O_2 evolution rates and Chl fluorescence after exposure of *P. tricornutum* to 2000 μmol photons m^{-2} s^{-1} were largely reversible when cells were returned to control conditions (90 μmol photons m^{-2} s^{-1}). For cells photoinhibited for 10 min, light-limited and light-saturated O_2 evolution rates increased by about 50% during the 60-min recovery period (Fig. 6A). The total increase in F_v (49%) after 60 min of recovery was also similar to the overall increases in light-limited and light-saturated O_2 rates. During the same recovery period, F_o levels declined steadily (Fig. 6B). Both O_2 evolution and F_v returned to within 5% of their control values within 4 h at 90 μmol photons m^{-2} s^{-1} (not shown).

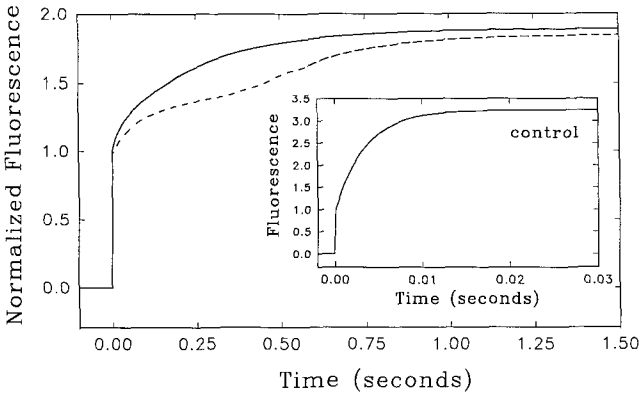

Figure 5. The effects of CCCP (5 μM) on fluorescence induction kinetics measured in photoinhibited (10 min at 2000 μmol photons m^{-2} s^{-1}) cells in the presence of 20 μM DCMU. Solid line, +5 μM CCCP; dashed line, −CCCP. Inset, Kinetics of fluorescence induction measured in control cells treated with 20 μM DCMU ± 5 μM CCCP. Kinetics are indistinguishable in the presence or absence of CCCP. Note the scale change in the *x* axis (time) between control and photoinhibited cells.

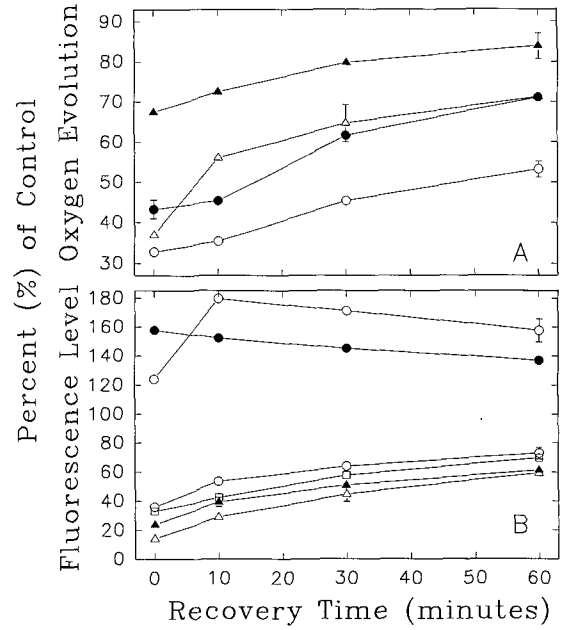

Figure 6. Recovery of O_2 evolution rates and fluorescence parameters after photoinhibition treatments. All values are expressed as percent of controls. Treatments were for 10 min (open symbols) or 60 min (filled symbols) at 2000 μmol photons m^{-2} s^{-1}. SD values of representative data points are shown for control and photoinhibited cells. A, O_2 evolution rates measured at limiting (180 μmol photons m^{-2} s^{-1}, circles) and saturating (580 μmol photons m^{-2} s^{-1}, triangles) light intensity. B, Fluorescence parameters F_o (circles), F_v (triangles), and F_v/F_m (squares). All fluorescence measurements were done in the presence of 20 μM DCMU and 5 μM CCCP.

For cells exposed to photoinhibitory conditions for 60 min, a continuous recovery of O_2 evolution rates was also observed. In this case, light-limited and light-saturated rates were 53 and 71% of control levels, respectively, after 1 h of recovery (Fig. 6A). Recovery after 60 min of exposure to photoinhibitory conditions was also characterized by a steady increase in F_v levels (Fig. 6B). However, F_o levels increased during the first 10 min of recovery and then decreased during the next 50 min; final F_o levels were still 37% larger than control values after 1 h of recovery (Fig. 6B). O_2 evolution rates and F_v both required 5 h to return to within 5% of control values, whereas F_o required at least 8 h to return to control levels (not shown).

A comparison of the recovery of O_2 evolution rates between the 10- and 60-min photoinhibitory treatments indicates that there was greater similarity in the extent of recovery of light-saturated rates than of light-limited rates. During the 1-h recovery period, light-saturated rates increased by 50 and 54% for the 10- and 60-min photoinhibition treatments, respectively (Fig. 6A). The recovery of F_v was also very similar for both the 10- and 60-min photoinhibition treatments. The overall increase in F_v after 1 h of recovery was 49% for the 10-min treatment and 52% for the 60-min treatment; final F_v levels were about 60% of controls for both treatments after 1 h of recovery (Fig. 6B). In addition, the total decrease in F_o during the latter part of the recovery period (from 10 to 60

min) was very similar (approximately 29%) for the two different photoinhibitory treatments. Overall, the extent of recovery of F_v/F_m was similar for the 10- and 60-min photoinhibitory treatments, and increases in F_v and increases/decreases in F_o both contributed to the steady increase in F_v/F_m (Fig. 6B).

DISCUSSION

The overall response of *P. tricornutum* to photoinhibitory conditions is remarkably similar to that observed in green algae and shade leaves of higher plants. This response was characterized by complex changes in F_o and a continuous decline in maximum variable fluorescence levels and O_2 evolution rates during the photoinhibitory treatment. The observation that F_v was suppressed after dark adaptation and in the presence of uncouplers suggests that the primary effect of the excess light treatment was an increase in the q_I component of q_N (Krause and Weis, 1991). q_N associated with q_I is likely to be heterogeneous in origin (Horton and Hague, 1988; Krause and Weis, 1991) and, depending on stress conditions and the previous growth history of the plant, may contain contributions from damaged PSII reaction centers (Cleland et al., 1986; Krause et al., 1990), down-regulated PSII reaction centers (Weis and Berry, 1987; Krieger et al., 1992), and energy-dependent quenching in the antenna involving carotenoids of the xanthophyll cycle (Demmig and Winter, 1988; Demmig-Adams, 1990).

A problem that arises in studies involving q_I is determining whether the q_N of F_v is the result of damage to the PSII reaction center or to slowly relaxing photoprotective components functioning in either the reaction center (down-regulation) or antenna complexes. Such a distinction is important because it determines if the responses to excess light occur before or after damage to PSII. Damage to the PSII reaction center results in a loss of variable fluorescence in that complex by an unknown (nonphotochemical) quenching process that competes with normal photochemistry and linear electron transport. Repair of damaged PSII requires de novo chloroplast protein synthesis and a complex cycle to excise and replace the damaged protein (Guenther and Melis, 1990). The formation of inactive or down-regulated PSII reaction centers analogous to those associated with q_E (Weis and Berry, 1987; Krieger et al., 1992) may serve a photoprotective role by quenching excited states nonphotochemically in competition with reactions that damage PSII. Loss of variable fluorescence in down-regulated PSII has been suggested to occur as the result of normal photochemistry followed by a rapid dissipative process such as charge recombination or cyclic electron transport (Krieger et al., 1992). Finally, a loss of variable fluorescence due to excess light treatment may also result from thermal energy dissipation in the antenna (Demmig and Winter, 1988; Demmig-Adams, 1990). In higher plants and green algae this component of q_N serves a photoprotective role by quenching excited states in competition with damaging reactions by a mechanism that may involve light-harvesting complex II aggregation (Horton et al., 1991) or direct quenching by zeaxanthin and/or antheraxanthin (Yamamoto and Gilmore, 1993; Owens, 1994b).

Butler (1978) originally proposed that quenching in the PSII antenna or reaction center could be distinguished based on its effects on F_o and F_v levels. Whereas antenna quenching results in a decrease in both F_v and F_o, reaction center quenching affects only F_v. However, interpreting the origin(s) of q_N induced by excess light treatment is not possible using Butler's models because a rise in F_o was not predicted (Kirilovsky et al., 1990; Setlik et al., 1990; Franklin et al., 1992; Prasil et al., 1992). Increases in F_o resulting from other, independent processes may mask the decrease in F_o associated with antenna quenching mechanisms.

Another method that can be used to distinguish the quenching of PSII excitation energy in the reaction center from that in the antennae involves measurement of the effective rate constant for PSII photochemistry by analysis of the kinetics of area growth over the fluorescence induction curve in samples treated with DCMU (Melis and Homann, 1976). Because damaged or down-regulated PSII reaction centers remain effective (nonphotochemical) quenchers of excitation energy (the fluorescence yield is approximately that of open PSII reaction centers, approximately F_o), the presence of these reaction centers alone will not alter the kinetics of the variable fluorescence rise; it will only decrease the amplitude of F_v. In several studies, excess light treatment induced a decrease in F_v and O_2 evolution without affecting the effective rate constant for PSII photochemistry (Cleland et al., 1986; Krause et al., 1990). In contrast, quenching in the antennae decreases the effective optical cross-section for photon absorption and results in both a slower fluorescence rise and a decrease in F_v. This decrease in F_v is expected because excitation energy will be dissipated effectively by pathways other than photochemistry. Furthermore, quenching in the antennae should result in a greater inhibition of light-limited PSII electron transport rates compared to light-saturated rates.

In our study we observed parallel decreases in the effective rate constant for PSII photochemistry, F_v, and light-limited O_2 evolution rates after short (0–10 min) exposures to excess light (Table I), which suggest that reaction center damage or down-regulation was not the primary cause of the changes observed. Instead, these changes are consistent with the presence of q_N in the antennae. The larger decrease observed in light-limited O_2 evolution rates compared to light-saturated rates after short exposures to excess light is also consistent with the presence of antennal quenching. This quenching is analogous to the q_I component studied in higher plants and green algae in that it did not relax readily in the dark and persisted in the presence of uncouplers. The insensitivity of the quenching to uncouplers is also consistent with assignment of the quenching site to the antennae rather than to down-regulated reaction centers.

Although photoinhibitory damage may develop during longer exposures to excess light, the extent of PSII reaction center damage during the first 60 min of treatment was small compared to the total amount of q_N. This is supported by the similarity in the kinetics and the extent of recovery observed after 10 and 60 min of photoinhibition. These similarities suggest that there was not a large increase in the proportion of damaged, slowly recovering PSII reaction centers between the 10- and 60-min treatments. This lack of damage to the PSII reaction center is clearly the result of the extensive q_N in

the antennae (dissipation > 90% of absorbed light in PSII after 60 min) that developed during the photoinhibitory treatment.

Fluorescence quenching attributable to q_E was present during the illumination phase of the photoinhibitory treatment, although it contributed at most about 10% of the total q_N measured in the light (Table I). For all other fluorescence measurements reported (Table I; Figs. 2–6), cells were dark-adapted for 5 min prior to measurements, during which time q_E probably would have relaxed (half-time < 1 min) (Demmig and Winter, 1988; Horton and Hague, 1988; Hodges et al., 1989). More importantly, measurements of F_o and F_m levels and fluorescence induction kinetics were conducted in the presence of CCCP to dissipate any pH gradient that may have formed during the dark-adaptation period as a result of chlororespiration (Ting and Owens, 1993). The total q_N measured at 2000 μmol photons m^{-1} s^{-1} in this experiment is very similar to that previously reported for this organism (0.85; Ting and Owens, 1993). In those previous experiments it was observed that at intensities above light saturation (approximately 500 μmol photons m^{-2} s^{-1}) the amount of q_N measured at steady-state illumination in the absence of CCCP increased linearly with light intensity; below light saturation the amount of q_N was only a weak function of intensity (Ting and Owens, 1993). Similarly, we observed that at approximately 800 μmol photons m^{-2} s^{-1} CCCP addition did not result in the relaxation of a large proportion of the total amount of steady-state q_N present. Together these data indicate that at intensities above light saturation, although some q_E (pH dependent) is present in the light, the total quenching is dominated by a component of q_N that is independent of changes in pH.

Using Butler's (1978) models, our observation that q_N in *P. tricornutum* under excess light conditions is dominated by antenna quenching processes suggests that a significant decrease in F_o should be observed. In contrast, a large initial increase in F_o was observed; only after 20 min of treatment did F_o begin to decline (Fig. 2). We conclude that this increase in F_o was most likely masking the expected decrease. An initial photoinhibition-induced increase in F_o has been reported in many higher plants and green algae and was assigned to the first phase of photoinhibition (Kirilovsky et al., 1990; Setlik et al., 1990; Franklin et al., 1992; Prasil et al., 1992). The decrease observed in F_o after longer (20–60 min) exposures to photoinhibitory conditions is consistent with the continued development of antenna quenching and the occurrence of a second phase of photoinhibition similar to that reported in many higher plants and green algae (Franklin et al., 1992; Prasil et al., 1992). The rate of q_N increase in this second phase was slower than in the first phase because the corresponding decline in F_v was much less.

Significant differences have been observed in the kinetics of PSII photochemistry in *P. tricornutum* compared to measurements in green algae and higher plants. In the present study the kinetics in control cells were single exponential, whereas higher plant and green algal systems are biphasic, with the faster component being sigmoidal (Melis and Homann, 1976). In addition, our present results differ from previous studies in this laboratory (Owens, 1986a) in which control cells of *P. tricornutum* exhibited biexponential kinetics. A comparison of results measured at the same actinic intensity indicates that the effective rate constant (290 s^{-1}) measured in the present study is approximately equal to the weighted mean of those determined previously (220 s^{-1}; Owens, 1986a). These differences may be due in part to variation in the growth conditions of the cells. It has been reported for algae that the proportions of the components of PSII photochemistry depend strongly on factors such as CO_2 availability (Falk et al., 1992).

After exposure to photoinhibitory conditions, our results show a switch to biexponential kinetics and a large decrease in the effective rate constants for PSII photochemistry. The effective rate constant of the slow component was 17 times smaller than that of the fast component, which in turn was about 4 times smaller than that of controls. These results suggest that it is very unlikely that the primary effect of photoinhibition was a decrease in the physical size of the PSII antennae and/or a decrease in the rate constants for energy transfer among adjacent antenna or reaction center complexes. In either case, the extent of the decrease necessary to account for the slow fluorescence rise would have resulted in much larger increases in the F_o and F_m fluorescence yields. As was previously discussed, these changes are consistent with the presence of q_N in the antennae. The heterogeneity observed in PSII after exposure to photoinhibitory conditions may have resulted in part from changes in the yield of photochemistry; this in turn could also be associated with the presence of heterogeneous q_N in PSII.

The overall effect of photoinhibition on photosynthesis reflects the involvement of multiple reactions, which may occur in parallel or in sequence, with different or overlapping time constants. These reactions may in turn be affected by selection of the sample (whole plant or algal cell, intact chloroplast, thylakoid membranes), the photosynthetic physiology of the organism, and the conditions of the photoinhibitory treatment (Cleland et al., 1986; Demmig and Winter, 1988; Krause et al., 1990; Krause and Weis, 1991). In *P. tricornutum*, short (10 min) exposures to excess irradiance levels did not result primarily in photoinhibitory damage of PSII reaction centers. The parallel decreases observed in the effective rate constant for PSII photochemistry, F_v, and light-limited O_2 evolution rates were consistent with extensive q_N in the antennae. This q_N did not relax readily in the dark and persisted in the presence of uncouplers. We conclude that this q_N was most likely associated with photoprotective processes, because longer (60 min) exposures to excess irradiance did not result in a detectable increase in the amount of damaged, slowly recovering PSII reaction centers. In the natural environment, the role of protective processes in reducing the amount of reaction center damage under photoinhibitory conditions may be particularly important to diatoms and other algae, which encounter frequent fluctuations in light intensity because of vertical mixing within the water column and which may become trapped in surface layers due to short-term thermal stratification.

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LITERATURE CITED

Barber J, Andersson B (1992) Too much of a good thing: light can be bad for photosynthesis. Trends Biochem Sci **17:** 61–66

Belay A, Fogg GE (1978) Photoinhibition of photosynthesis in *Asterionella formosa* (Bacillariophyceae). J Phycol **14:** 341–347

Briantais JM, Vernotte C, Picaud M, Krause GH (1979) A quantitative study of the slow decline of chlorophyll *a* fluorescence in isolated chloroplasts. Biochim Biophys Acta **548:** 128–138

Butler WL (1978) Energy distribution in the photochemical apparatus of photosynthesis. Annu Rev Plant Physiol **29:** 345–378

Cleland RE, Melis A, Neale PJ (1986) Mechanism of photoinhibition: photochemical reaction center inactivation in system II of chloroplasts. Photosynth Res **9:** 79–88

Demmig B, Winter K (1988) Characterization of three components of nonphotochemical quenching and their response to photoinhibition. Aust J Plant Physiol **15:** 163–177

Demmig-Adams B (1990) Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. Biochim Biophys Acta **1020:** 1–24

Falk S, Leverenz JW, Samuelsson G, Oquist G (1992) Changes in photosystem II-fluorescence in *Chlamydomonas reinhardtii* exposed to increasing levels of irradiance in relationship to the photosynthetic response to light. Photosynth Res **31:** 31–40

Franklin LA, Levavasseur G, Osmond CB, Henley WJ, Ramus J (1992) Two components of onset and recovery during photoinhibition of *Ulva rotunda*. Planta **186:** 399–408

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta **990:** 87–92

Gilmore AM, Yamamoto HY (1991a) Zeaxanthin formation and energy-dependent fluorescence quenching in pea chloroplasts under artificially mediated linear and cyclic electron transport. Plant Physiol **96:** 635–643

Gilmore AM, Yamamoto HY (1991b) Zeaxanthin-dependent and independent nonphotochemical quenching are quantitatively related to Δ-pH (abstract No. 229). Plant Physiol **96:** S-41

Gilmore AM, Yamamoto HY (1992) Dark induction of zeaxanthin-dependent nonphotochemical quenching mediated by ATP. Proc Natl Acad Sci USA **89:** 1899–1903

Gilmore AM, Yamamoto HY (1993) Linear models relating xanthophylls and lumen acidity to non-photochemical fluorescence quenching. Evidence that antheraxanthin explains zeaxanthin-independent quenching. Photosynth Res **35:** 67–78

Guenther JE, Melis A (1990) The physiological significance of photosystem II heterogeneity in chloroplasts. Photosynth Res **23:** 105–109

Heath MR (1988) Interpretation of in vivo fluorescence and cell division rates of natural phytoplankton using a cell cycle model. J Plankton Res **10:** 1251–1272

Hodges M, Cornic G, Briantais JM (1989) Chlorophyll fluorescence from spinach leaves: resolution of non-photochemical quenching. Biochim Biophys Acta **974:** 289–293

Horton P, Hague A (1988) Studies on the induction of chlorophyll fluorescence in isolated barley protoplasts. IV. Resolution of non-photochemical quenching. Biochim Biophys Acta **932:** 107–115

Horton P, Ruban AV, Rees D, Pascal AA, Noctor G, Young AJ (1991) Control of the light-harvesting function of chloroplast membranes by aggregation of the LHCII chlorophyll-protein complex. FEBS Lett **292:** 1–4

Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls *a, b,* c_1 and c_2 in algae, phytoplankton and higher plants. Biochem Physiol Pflanzen **167:** 191–194

Kirilovsky DL, Vernotte C, Etienne AL (1990) Protection from photoinhibition by low temperature in *Synechocystis* 6714 and in *Chlamydomonas reinhardtii*: detection of an intermediary state. Biochemistry **29:** 8100–8106

Krause GH, Briantais JM, Vernotte C (1983) Characterization of chlorophyll fluorescence quenching in chloroplasts by fluorescence spectroscopy at 77K. I. δpH-dependent quenching. Biochim Biophys Acta **723:** 169–175

Krause GH, Somersalo S, Zumbusch E, Weyers B, Laasch H (1990) On the mechanism of photoinhibition in chloroplasts. Relationship between changes in fluorescence and activity of photosystem II. J Plant Physiol **136:** 472–479

Krause GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Plant Mol Biol **42:** 313–349

Krieger A, Moya I, Weis E (1992) Energy-dependent quenching of chlorophyll *a* fluorescence: effect of pH on stationary fluorescence and picosecond relaxation kinetics in thylakoid membranes and photosystem II preparations. Biochim Biophys Acta **1102:** 167–176

Melis A, Homann PH (1976) Heterogeneity of the photochemical centers in system II chloroplasts. Photochem Photobiol **23:** 343–350

Noctor G, Rees D, Young A, Horton P (1991) The relationship between zeaxanthin, energy-dependent quenching of chlorophyll fluorescence, and transthylakoid pH gradient in isolated chloroplasts. Biochim Biophys Acta **1057:** 320–330

Noctor G, Ruban AV, Horton P (1993) Modulation of delta-pH dependent nonphotochemical quenching of chlorophyll fluorescence in spinach chloroplasts. Biochim Biophys Acta **1183:** 339–344

Owens TG (1986a) Photosystem II heterogeneity in the marine diatom *Phaeodactylum tricornutum*. Photochem Photobiol **43:** 535–544

Owens TG (1986b) Light-harvesting function in the diatom *Phaeodactylum tricornutum*. II. Distribution of excitation energy between photosystems. Plant Physiol **80:** 739–746

Owens TG (1991) Energy transformation and fluorescence in photosynthesis. *In* S Demers, ed, Particle Analysis in Oceanography. Springer-Verlag, Berlin, pp 101–140

Owens TG (1994a) In vivo chlorophyll fluorescence as a probe of photosynthetic physiology. *In* R Alscher, A Wellburn, eds, Gaseous Pollutants and Plant Metabolism. Elsevier Science Publishers, Amsterdam (in press)

Owens TG (1994b) Excitation energy transfer between chlorophylls and carotenoids: a proposed molecular mechanism for nonphotochemical quenching. *In* NR Baker, JR Bowyer, eds, Photoinhibition of Photosynthesis: From Molecular Mechanisms to the Field. Bios Scientific Publishers, Oxford, UK, pp 95–109

Owens TG, Wold ER (1986) Light-harvesting function in the diatom *Phaeodactylum tricornutum*. I. Isolation and characterization of pigment-protein complexes. Plant Physiol **80:** 732–738

Prasil O, Adir N, Ohad I (1992) Dynamics of photosystem II: mechanism of photoinhibition and recovery processes. *In* J Barber, ed, The Photosystems: Structure, Function, and Molecular Biology. Topics in Photosynthesis, Vol 11. Elsevier Science Publishers, Amsterdam, pp 295–348

Samuelsson G, Oquist G (1977) A method for studying photosynthetic capacities of unicellular algae based on in vivo chlorophyll fluorescence. Physiol Plant **40:** 315–319

Setlik I, Allakhverdiev SI, Nedbal L, Setlikova E, Klimov VV (1990) Three types of photosystem II photoinactivation. I. Damaging processes on the acceptor side. Photosynth Res **23:** 39–48

Thornber JP (1986) Biochemical characterization and structure of pigment-proteins of photosynthetic organisms. *In* AL Staehelin, CJ Arntzen, eds, Encyclopedia of Plant Physiology, Vol 19. Springer-Verlag, Berlin, pp 98–142

Ting CS, Owens TG (1992) Limitations of the pulse-modulated technique for measuring the fluorescence characteristics of algae. Plant Physiol **100:** 367–373

Ting CS, Owens TG (1993) Photochemical and nonphotochemical fluorescence quenching processes in the diatom *Phaeodactylum tricornutum*. Plant Physiol **101:** 1323–1330

Weis E, Berry JA (1987) Quantum efficiency of photosystem II in relation to "energy" dependent quenching of chlorophyll fluorescence. Biochim Biophys Acta **894:** 198–208