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Photosynthetic Light Utilization Efficiency, Photosystem II Heterogeneity, and Fluorescence Quenching in *Chlamydomonas reinhardtii* during the Induction of the CO_2-Concentrating Mechanism[1]

Stefan Falk* and Kristin Palmqvist

Department of Plant Physiology, University of Umeå, S-901 87 Umeå, Sweden

ABSTRACT

The photosynthetic light-response curve, the relative amounts of the different photosystem II (PSII) units, and fluorescence quenching were altered in an adaptive manner when CO_2-enriched wild-type *Chlamydomonas reinhardtii* cells were transferred to low levels of CO_2. This treatment is known to result in the induction of an energy-dependent CO_2-concentrating mechanism (CCM) that increases the internal inorganic carbon concentration and thus the photosynthetic CO_2 utilization efficiency. After 3 to 6 h of low inorganic carbon treatment, several changes in the photosynthetic energy-transducing reactions appeared and proceeded for about 12 h. After this time, the fluorescence parameter variable/maximal fluorescence yield and the amounts of both PSIIα and PSIIβ (secondary quinone electron acceptor of PSII-reducing) centers had decreased, whereas the amount of PSIIβ (secondary quinone electron acceptor of PSII-nonreducing) centers had increased. The yield of noncyclic electron transport also decreased during the induction of the CCM, whereas both photochemical and nonphotochemical quenching of PSII fluorescence increased. Concurrent with these changes, the photosynthetic light-utilization efficiency also decreased significantly, largely attributed to a decline in the curvature parameter θ, the convexity of the photosynthetic light-response curve. Thus, it is concluded that the increased CO_2 utilization efficiency in algal cells possessing the CCM is maintained at the cost of a reduced light utilization efficiency, most probably due to the reduced energy flow through PSII.

To understand the control of photosynthetic CO_2 fixation in any photosynthesizing organism, it is important to define the limiting step(s) of photosynthesis under various environmental conditions. In a model proposed by Farquhar et al. (8), photosynthesis in leaves of C_3 plants is limited by the activity of Rubisco when the CO_2 concentration is low, and it is limited by the rate of production of ATP and NADPH on the thylakoid membranes, or ultimately by the rate of ribulose-1,5-bisphosphate regeneration from phosphoglyceric acid, when the light intensity is limiting. It was also predicted that the highest efficiency is obtained when colimitation between the sequential reactions of photosynthesis does not occur or is minimized (8). There is always some degree of colimitation present, however, which can be regarded as an inefficiency of the photosynthetic system. This inefficiency is generally most pronounced in the transition zone of photosynthetic light-, CO_2-, or temperature-response curves, where there is a change from one limiting step to another (5, 8). It has recently been shown that changes in one of these steps, namely the characteristics of PSII, such as antenna size and the ability to photoreduce the Q_B[2] site of the D_1 protein, were tightly coupled with changes in the shape of the photosynthetic light response curve in photoinhibited cells of *Chlamydomonas reinhardtii* (6, 7, 16). This relationship was described by a model consisting of a quadratic equation having three independent variables: Φ, P_{max}, θ (5, 16).

Green algae and cyanobacteria have a CCM that regulates the internal concentration of Ci. The algae can thus maintain an internal concentration of CO_2 that is several times higher than that of the external medium and, as one of the results, the oxygenase activity of the carboxylating enzyme, Rubisco, will be suppressed (2–4, 13). Algal cells grown under high Ci concentrations seem to have a suppressed CCM, whereas the mechanism is induced within a few hours after transfer to low Ci concentrations (2, 4, 22, 23, 27, 31). Several studies have dealt with changes in photosynthetic CO_2-response characteristics and changes in carbonic anhydrase levels that occur when algae are transferred from high to low Ci concentrations (2–4, 13, 17, 22, 23). However, there are few studies dealing with the regulation of the photosynthetic energy transducing reactions (24, 26, 29) or the regulation of the carbon reduction cycle (18, 31) during low-Ci acclimation.

[1] This research was supported by the Swedish Natural Science Resource Council.

[2] Abbreviations: Q_B and Q_A, secondary and primary quinone electron acceptors of PSII; Φ, initial slope of the light response curve, used as an estimate of initial quantum yield; P_{max}, maximum rate of photosynthesis; θ, convexity (rate of bending) of the light-response curve of photosynthesis; CCM, CO_2-concentrating mechanism; Ci, inorganic carbon (CO_2 + HCO_3^-); F_M, F_V, and F_0, dark-adapted maximal, total variable, and nonvariable fluorescence yield; F_{PI}, dark-adapted intermediate fluorescence yield, plateau level; F_M', F_S, and F_0', maximal, steady-state, and nonvariable fluorescence yield at any light-adapted state; q_P, photochemical fluorescence quenching; PQ, plastoquinone; q_N, nonphotochemical fluorescence quenching; q_E, energy-dependent part of nonphotochemical fluorescence quenching; q_T, nonphotochemical fluorescence quenching related to state 1–state 2 transition; q_I, nonphotochemical fluorescence quenching related to photoinhibition.

The CCM is light and energy requiring (27), and it has been suggested that photosynthetically transduced energy may be used to meet the additional demand for ATP (24, 26, 29). In a recent study (24) it was shown that the induction of the CCM in the unicellular green alga *C. reinhardtii* was paralleled by an 80% increase in the rate of spillover of excitation energy from PSII to PSI, together with a 30% increase in the absorption cross-section of PSI. Thus, it was concluded that pronounced alterations in energy distribution between the two photosystems occurred as a consequence of CCM induction.

In this paper, we have analyzed the effects of these changes on the photosynthetic light utilization efficiency, PSII heterogeneity, and fluorescence quenching in *C. reinhardtii* cells during the induction of the CCM. This was done by modeling the photosynthetic light-response curve according to Leverenz et al. (16) and Collatz et al. (5) and by measuring Chl *a* fluorescence induction and quenching (cf. Krause and Weis [14, 15]). To avoid a situation with an initial low-Ci stress, the algae were allowed to gradually decrease the availability of Ci by photosynthetic consumption of the high-Ci pool in their growth medium, which was slowly bubbled with air during the low-Ci treatment. Any changes of the photosynthetic apparatus of the cells may thus be regarded more as adaptive changes related to a gradual induction of the CCM rather than to stress-induced changes.

MATERIALS AND METHODS

Algal Material and Culturing Conditions

The green alga *Chlamydomonas reinhardtii* Dangeard (WT strain 137 c mt+; kindly given by Dr. D.D. Kaska, Department of Biological Sciences, University of California, Santa Barbara) was grown photoautotrophically at 27°C in axenic constant-density cultures (ACC 400, Techtum Instruments AB, Umeå, Sweden) in continuous white light with an incident PPFD of 125 μmol m^{-2} s^{-1}, provided by fluorescent tubes (Philips TL 40W/55S), in a phosphate-buffered medium (pH 7.5). The major components of the medium were: 2.6 mM KH_2PO_4, 8.3 mM K_2HPO_4, 6.25 mM NH_4NO_3, 0.081 mM $MgSO_4$, 0.068 mM $CaCl_2$, 0.037 mM $FeCl_3$, 0.6 mM Na_2-citrate, and the trace elements as in Falk et al. (6). The Chl concentration in the culture was 2 to 3 μg mL^{-1}.

Ci Supply and Induction of the CCM

The algae were slowly bubbled (1200 mL h^{-1}) with 50 mL L^{-1} CO_2 to obtain cells with a suppressed CCM. The CCM was induced by reducing the CO_2 content of the gas supply to ambient levels of CO_2 in the culture room (approximately 450 μL L^{-1}) at an equally slow rate of bubbling. Thus, a gradual decline of the CO_2 content in the culture medium was obtained rather than exposing the cells to the sudden stress of CO_2 depletion.

Photosynthesis Measurements and Modeling of the Light-Response Curve

Rates of photosynthesis were measured polarographically as in Falk and Samuelsson (7) and presented as gross evolution of O_2 in μmol (mg Chl)$^{-1}$ h^{-1}. Photosynthetic light response was measured at 18 PPFDs ranging from 0 to 1140 μmol m^{-2} s^{-1} for five replicates. In all experiments, including the measurements of O_2 evolution, data are given for high-Ci cells (0 h of low-Ci treatment) and for cells treated as described in the previous paragraph for 3, 6, 12, and 23.5 h, respectively. Cells were assayed in their culture medium and supplied with 100 μg mL^{-1} chloramphenicol to arrest protein synthesis in the chloroplast during the measurements and with 4 mM $NaHCO_3$ to supply photosynthesis with substrate. The data from the O_2 evolution measurements were used to model the three independent variables Φ, θ, and P_{max}, that together describe the light-response curve (5, 16). Chl was extracted in 80% acetone and determined as in Falk et al. (6) using the equations of Arnon (1).

Measurements of Chl *a* Fluorescence Kinetics

All measurements of room temperature Chl *a* fluorescence kinetics were done as in Falk et al. (6) using a Plant Stress Meter (PSM, BioMonitor, SCI AB, Umeå, Sweden) to generate and detect the fluorescence signal that was recorded and stored on a computer hard disk using an oscilloscope card (PC-Scope T12840, Intelligent Messtechnik GmbH, Backnang, FRG). The patterns of fluorescence induction in both high- and low-Ci-grown *C. reinhardtii* are described in detail in Palmqvist et al. (24) and the fluorescence parameters F_M, F_V, F_0, F_{pl}, etc. were attained as described in Falk et al. (6) and Guenther and Melis (12). q_N and q_P were determined from pulse-amplitude modulated fluorescence (PAM 100, H. Walz, Effeltrich, FRG) using the liquid-cuvette system supplied by the same manufacturer. The quenching parameters were determined at 12 actinic light intensities, measured at the common fiber end, ranging from 0 to 1750 μmol m^{-2} s^{-1}. Actinic light was provided by a slide projector fitted with a 150-W halogen bulb, and the light intensity was regulated by different combinations of Schott neutral density glass filters. The measuring beam had a light intensity of approximately 0.1 μmol m^{-2} s^{-1} for determination of F_0 and F_0', but was approximately 3 μmol m^{-2} s^{-1} during the measurements of F_M, F_M', and F_S. Saturating flashes of 3700 μmol m^{-2} s^{-1} were provided by the PAM flash light source FL 103, set at 2-s duration. Each measurement started with 5 min of complete darkness, whereupon the measuring beam was turned on for determination of F_0. The saturating flash was thereafter applied and the sample was illuminated with the lowest actinic irradiance (11.5 μmol m^{-2} s^{-1}) until F_S was reached (2–3 min). After the following saturating flash, the actinic light was briefly turned off to measure F_0', whereupon the actinic light was changed to the next light intensity and turned on again. The F_0' achieved in darkness was not significantly different from that measured in the presence of weak far-red illumination (data not shown). The procedure of the measurements of F_S, F_M', and F_0' was repeated for each of the 12 actinic light intensities.

All cells were supplied with 4 mM HCO_3^- during the fluorescence measurements to obtain the same experimental conditions as in the O_2 evolution measurements. It has previously been shown that the addition of HCO_3^- to low-Ci cells of *C. reinhardtii* does not induce Ci-related fluorescence

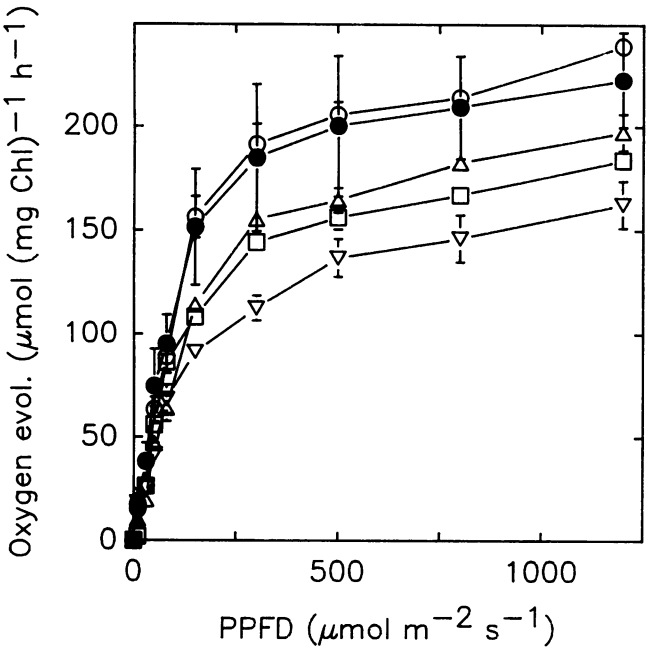

Figure 1. Photosynthetic light-response curves of gross O_2 evolution for *C. reinhardtii* cells transferred from high- to low-Ci-growth conditions, expressed as μmol(mg Chl) $^{-1}$ h^{-1}, after 0 (●), 3 (○), 6 (△), 12 (□) and 24 h (▽), respectively, of low-Ci treatment. The low-Ci treatment was started at time 0 by exchanging the gas-bubbling supply from 5 mL L^{-1} CO_2(g) to ambient air (450 μL L^{-1}). Error bars (±SE) are indicated for $n = 5$ when they exceed the symbol size.

quenching (24), as has been shown to occur in the cyanobacterium *Synechococcus* (21).

Calculation of Fluorescence Quenching Coefficients

Utilizing the nomenclature in Snel and van Kooten (25), q_N was determined by the ratio $\{1-[(F_M'- F_0')/(F_M-F_0)]\}$, whereas q_P was calculated from $[(F_M'-F_S)/(F_M'-F_0')]$. The yield of noncyclic electron transport was calculated from $[q_P \times F_V/F_M]$, according to Genty et al. (10).

Calculations of PSII Heterogeneity

PSII antenna size heterogeneity (PSIIα and -β centers) was estimated from whole cells poisoned with 10 mM DCMU for 5 min in darkness according to Melis and Homann (19, 20) with the alterations described in Falk et al. (6). PSII Q_B-reduction heterogeneity (PSII Q_B-reducing and -nonreducing centers) was estimated from changes in $(F_M-F_{pl})/F_V$ and $(F_{pl}-F_0)/F_V$ of room temperature fluorescence kinetics as in Falk et al. (6). The change in 77K F_V/F_M was used to correct the calculations for changes in the total amount of PSII centers, as in Falk et al. (6).

RESULTS

Low-Ci-Induced Changes in the Shape of the Light-Response Curve

More than 3 h of low-Ci treatment were required before the photosynthetic light-response curve (Fig. 1) and the fluorescence parameter F_V/F_M (Fig. 2) were affected. This may be explained by the fact that the cells were allowed to gradually decrease the CO_2 concentration of their growth medium from 1.5 mM to 10 to 15 μM by their own photosynthesis, implying that the cells were not exposed to CCM-inducing conditions until approximately 3 h had elapsed. The time required to reach CCM-inducing conditions was estimated from the known volume and density of the algal culture, from previously published data of photosynthetic rates (22, 23, 31), and from the assumption that the CCM is triggered when the CO_2 concentration is below 50 to 100 μM (cf. ref. 2). After 6 h and longer of low-Ci treatment, however, there was a significant effect on the light-response curve of gross oxygen evolution (Fig. 1), with a successive decrease in the oxygen evolution at all measured light intensities. The decrease in gross oxygen evolution was in part counteracted by an increased respiration (data not shown).

When the data from the light-response curves were fitted into a model consisting mainly of a quadratic equation (5, 16) (Fig. 2), it was found that P_{max}, which is defined to occur at an infinitely high light intensity, and Φ, the quantum yield at infinitely low light intensity, only fluctuated within the errors of the method during the low-Ci treatment and concomitant CCM induction. These results are not surprising though, provided that the low-Ci treatment did not impose a severe photoinhibitory damage, because the modeled P_{max} has been hypothesized to remain largely unaffected until only 2 to 5% of the PSII centers, relative to the control, are of the PSIIα-type (6, 16) and because Φ reflects the efficiency of the most efficient functional reaction center. The changes in appearance of the light-response curve during the induction of the CCM (Fig. 1) could instead largely be explained by a decrease in θ, which decreased from 0.9 to 0.4 during the low-Ci treatment (Fig. 2).

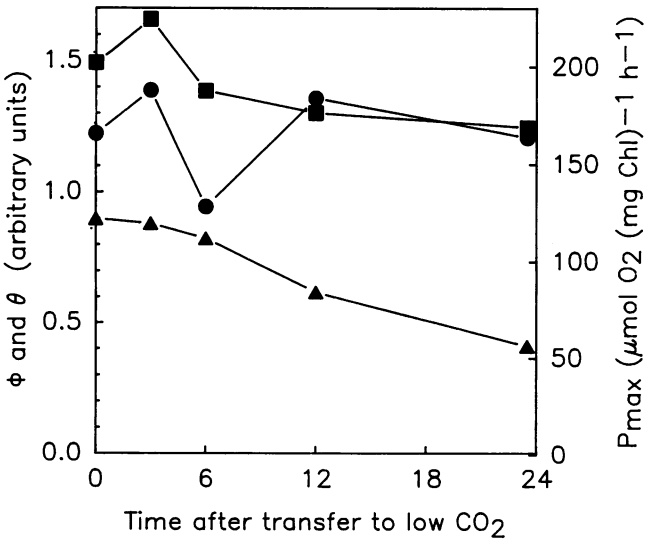

Figure 2. The modeled parameters P_{max} (■), Φ (●), and θ (▲) of the light-response curves presented in Figure 1, expressed as a function of the time of exposure to low Ci. The parameters were modeled by using Equation 1 from Leverenz et al. (16) for $n = 5$. The low-Ci treatment was started at time 0 by exchanging the gas-bubbling supply from 5 mL L^{-1} CO_2(g) to ambient air (450 μL L^{-1}).

Low-Ci-Induced Changes Within the PSII Population

According to Collatz et al. (5), the curvature parameter θ has no mechanistic basis and θ can be regarded as indicating the extent to which sequential steps colimit photosynthesis. It has recently been suggested that one cause for a reduced θ may be a decrease in the rate of turnover of closing and reopening of PSII relative to whole-chain electron transport, i.e. a decreased efficiency of the PSII centers (9, 32). A correlation between a reduced θ and changes in PSII energy transduction is also evident in the present study. Figure 3 shows that during the time course of low-Ci acclimation, there was a reduction of both the amount of PSIIα and PSIIβ Q_B-reducing centers as well as a reduction of the F_V/F_M ratio, and that the amount of PSIIβ Q_B-nonreducing centers increased. The kinetics for these changes followed the same pattern as for the decline in θ (Figs. 1 and 2), where most of the changes appeared after 6 to 12 h. The reduction in F_V/F_M could almost entirely be attributed to a decrease in F_V, whereas no significant decrease in F_0 could be observed (Fig. 4). The decrease in PSIIα and PSIIβ Q_B-reducing centers was in part counteracted by an increase in the fraction of PSIIβ Q_B-nonreducing centers, although the 30% decrease in F_V/F_M still indicates a substantial decrease in the overall PSII activity.

Low-Ci-Induced Changes of Fluorescence Quenching

The amount of q_P and q_N of Chl *a* fluorescence was followed at actinic light intensities ranging from 0 to 1750 μmol m^{-2} s^{-1} and measured after 0 to 24 h of low-Ci treatment. During the induction of the CCM, there was a progressive decrease in q_P at all light intensities above 500 μmol m^{-2} s^{-1} (Fig. 5). At a PPFD of 1250 μmol m^{-2} s^{-1}, approximately 70% of the PSII centers were reduced in high-Ci cells, whereas only approximately 40% were reduced in cells treated at low Ci for 12 to 24 h. According to Krause and Weis (15), this should be interpreted as a higher capacity to reoxidize Q_A, which implies that cells with the CCM have a higher turnover rate of Q_A reoxidation than do high-Ci-grown cells.

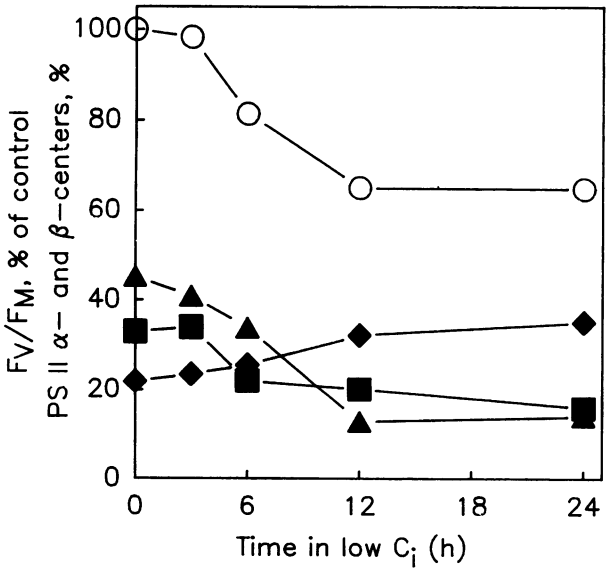

Figure 3. Changes in F_V/F_M (O), PSIIα (▲), PSIIβ Q_B-reducing (■), and PSIIβ Q_B-nonreducing (◆) centers as a function of the time of exposure to low Ci, as measured by induction of room temperature fluorescence for $n = 4$. The low-Ci treatment was started at time 0 by exchanging the gas-bubbling supply from 5 mL L^{-1} CO_2(g) to ambient air (450 μL L^{-1}). High-Ci-grown cells (control) had an F_V/F_M of 0.70.

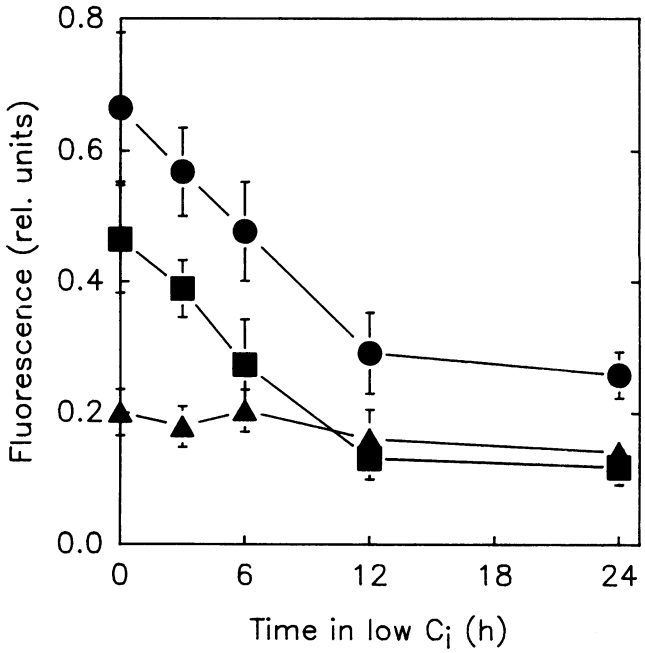

Figure 4. Changes in F_M (●), F_V (■), and F_0 (▲) as a function of the time of exposure to low Ci as measured by room temperature fluorescence induction for $n = 4$. The low-Ci treatment was started at time 0 by exchanging the gas-bubbling supply from 5 mL L^{-1} CO_2(g) to ambient air (450 μL L^{-1}).

The yield of noncyclic electron transport also decreased during the time course of low-Ci treatment (Fig. 6). The yield was lower for cells with an induced CCM compared with high-Ci grown cells at all light intensities, although the difference was most pronounced at PPFDs below 500 μmol m^{-2} s^{-1}. At the light intensity experienced during growth (125 μmol m^{-2} s^{-1}), high-Ci-grown cells revealed a 15 to 40% higher yield of noncyclic electron transport than cells with a fully induced CCM.

Figure 7 shows the change in q_N with increasing actinic light intensities. q_N was significantly lower in high-Ci cells compared with low-Ci-treated cells at all PPFDs above 71 μmol m^{-2} s^{-1}. In cells with a fully induced CCM, q_N was as high as 0.8 at light intensities above 500 μmol m^{-2} s^{-1}. At the PPFD experienced during growth (125 μmol m^{-2} s^{-1}), cells with the CCM had a 30 to 70% higher q_N than did high-Ci cells.

DISCUSSION

This paper gives evidence for a prominent and gradually increasing effect on PSII heterogeneity, PSII-related fluores-

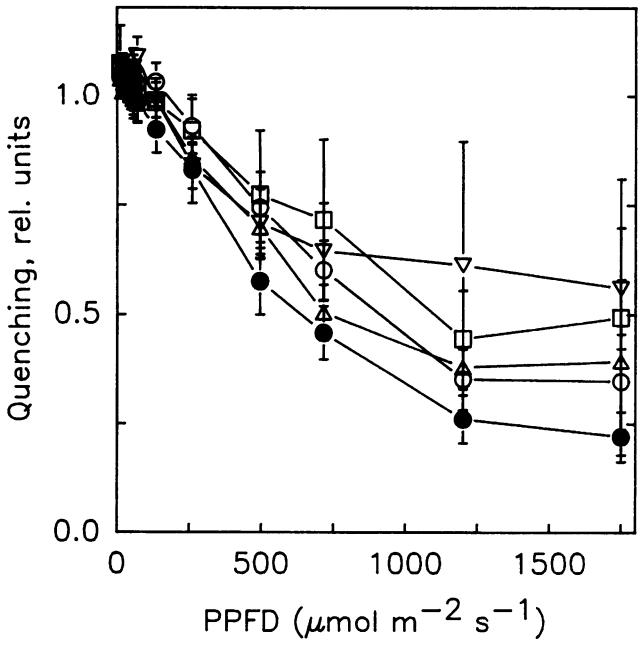

Figure 5. Changes in q_P as a function of the incident PPFD of the excitation light, measured after 0 (●), 3 (○), 6 (△), 12 (□), and 24 h (▽), respectively, of low-Ci treatment. The low-Ci treatment was started at time 0 by exchanging the gas-bubbling supply from 5 mL L^{-1} CO_2(g) to ambient air (450 μL L^{-1}). Error bars (±SE) are indicated for $n = 4$ when they exceed the symbol size.

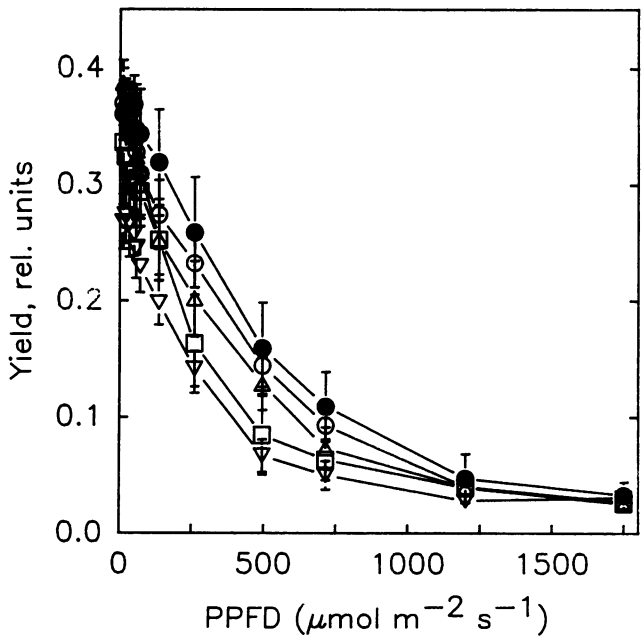

Figure 6. Changes in the yield of noncyclic electron transport as a function of the incident PPFD, measured after 0 (●), 3 (○), 6 (△), 12 (□), and 24 h (▽), respectively, of low-Ci treatment. The low-Ci treatment was started at time 0 by exchanging the gas-bubbling supply from 5 mL L^{-1} CO_2(g) to ambient air (450 μL L^{-1}). Error bars (±SE) are indicated for $n = 4$ when they exceed the symbol size.

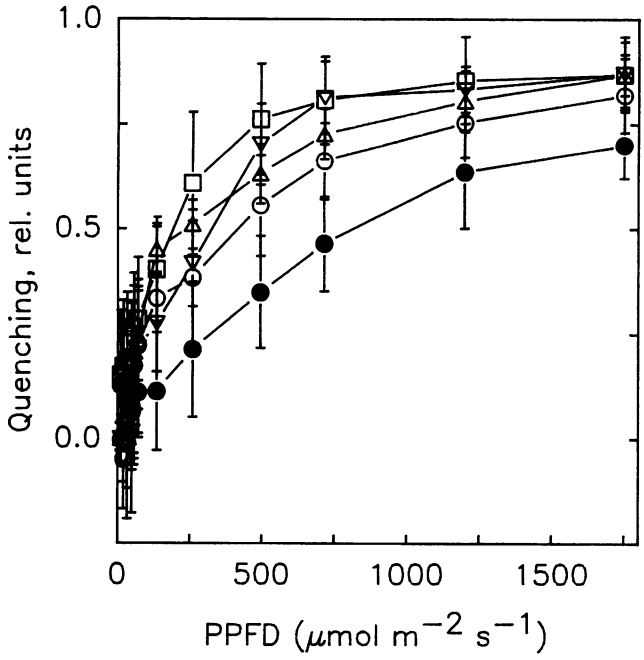

Figure 7. Changes in q_N as a function of the incident PPFD measured after 0 (●), 3 (○), 6 (△), 12 (□), and 24 h (▽), respectively, of low-Ci treatment. The low-Ci treatment was started at time 0 by exchanging the gas-bubbling supply from 5 mL L^{-1} CO_2(g) to ambient air (450 μL L^{-1}). Error bars (±SE) are indicated for $n = 4$ when they exceed the symbol size.

cence quenching, noncyclic electron transport, and photosynthetic light utilization efficiency in *C. reinhardtii* cells inducing the CCM. In several ways, these changes resemble earlier reported changes of the photosynthetic apparatus in *C. reinhardtii* cells exposed to a mild photoinhibitory treatment (6, 7, 16). In this context, it is important to emphasize that even though low-Ci cells of *C. reinhardtii* can accumulate CO_2 up to 40 times the concentration of an air-equilibrated medium (3), which has a CO_2(aq) concentration of 10 to 12 μM, this would still only represent 20 to 30% of the CO_2 concentration of a medium in equilibrium with 50 mL L^{-1} CO_2(g), which has a CO_2(aq) concentration of 1600 μM. This suggests that in *C. reinhardtii* cells with a fully induced CCM, the photosynthetic apparatus would still be exposed to a relatively higher excitation pressure compared with high-Ci-grown cells and, thus, the conditions may resemble a mild photoinhibitory treatment. As a consequence, it may still be difficult to distinguish between functional changes of the photosynthetic light reactions related to the CCM and changes due to a relative increase in the excitation pressure, even though the algae were gradually exposed to low-Ci conditions in the present investigation.

As a result of the slow introduction of low-Ci conditions, no alterations of either the photosynthetic light-response curve (Figs. 1 and 2) or the different characteristics related to PSII fluorescence (Figs. 3–7) could be observed within the first 3 h of low-Ci treatment. Thereafter, however, changes in the characteristics of all the investigated parameters occurred parallel to each other and proceeded until a new

steady state was reached after approximately 12 h (Figs. 1–7). This finding is consistent with earlier investigations that have reported a requirement for up to 12 h before the CCM has been fully induced in *C. reinhardtii* (2, 18, 22, 23). After 12 to 24 h of low-Ci treatment, the modeled parameter θ had decreased by 55% (Fig. 2) and the F_V/F_M ratio by 35% (Fig. 3). The portion of PSIIα and PSIIβ Q_B-reducing centers had decreased by 69 and 52%, respectively, and PSIIβ Q_B-nonreducing centers had increased by 60% (Fig. 3). This last observation suggests that at least a portion of the Q_B-reducing centers were transformed to the Q_B-nonreducing form in agreement with the hypothetical "PSII repair cycle" (11, 19, 20). Moreover, in cells with the CCM, the yield of noncyclic electron transport was reduced by approximately 15 to 40% at the light intensity experienced during growth (Fig. 6), which indicates a reduced efficiency and/or decreased amount in the total amount of PSII.

Taken together, these results suggest that turnover of energy in PSII may become relatively more limiting for overall electron transport and photosynthesis in cells with a fully induced CCM than in high-Ci-grown cells. For photoinhibited cells of *C. reinhardtii*, it has previously been shown that a reduced PSII activity is correlated with a decline in the curvature parameter θ of the photosynthetic light-response curve (6, 7, 16). Thus, it can be hypothesized that the low θ observed in *C. reinhardtii* cells with a fully induced CCM (Fig. 2) may be attributed in part to the apparent decline in energy turnover through PSII. The finding that the q_P was higher in these cells compared with high-Ci-grown cells (Fig. 5), which is indicative of a higher rate of Q_A and, hence, PQ reoxidation, may seem contradictory to this hypothesis. However, because it has previously been shown that the induction of the CCM also results in an increase in the activity of PSI versus PSII (24, 26), we suggest that the rate of PQ reoxidation might be enhanced by PSI cyclic electron transport. As a consequence, reoxidation of PQ may well proceed at a high rate even though the rate of energy turnover through PSII has decreased (14). Taken together, our results support the idea that increased cyclic electron transport around PSI may provide the cells with extra ATP for the CCM (24, 26, 29), but apparently at the cost of a decreased noncyclic electron transport (Fig. 6). However, under certain conditions, such as when the oxygen concentration is above air-equilibrium, it has previously been shown that pseudocyclic electron transport to oxygen is also increased during low-Ci acclimation in *C. reinhardtii* (28), which indicates a role for both cyclic and pseudocyclic ATP generation in supplying energy for the CCM.

It was also evident that q_N was higher in cells with a fully induced CCM than in high-Ci-grown cells (Fig. 7), which was most pronounced at the light intensity experienced during growth. Under physiological conditions, q_N may be caused by three major mechanisms: q_E, q_T, and q_I (15). The molecular mechanism for q_E is still unknown, although it has been shown that energization of the thylakoids, due to the build-up of a transmembrane ΔpH, may lead to quenching of up to about 90% of F_V (15). Because a high rate of cyclic electron transport around PSI would result in an increased ΔpH across the thylakoid membrane, an increase in q_E is thus a good candidate for the observed increase in q_N. However, because it has recently been suggested that the ATP requirement of photosynthetic cells may control state transitions (30), where state 2 can be regarded as a state favoring cyclic photophosphorylation around PSI, the increase in q_N may also be due to an increase in q_T. The fact that an increased amount of PSIIβ centers (Fig. 3) coincides with the earlier reported increase in spillover of excitation energy from PSII to PSI (24) also indicates that a state transition may occur during the induction of the CCM. Thus, the observed increase in q_N may be due to an increase in both q_E and q_T, which may both be related to the altered demand for ATP in cells with the CCM.

The results presented in this paper also support the idea that a decrease in the curvature parameter θ of the photosynthetic light-response curve may be explained by a decreased energy turnover through PSII (6, 7, 16). However, despite this apparent correlation, there may be other factors that contribute to the low θ. The fact that cells with the CCM utilize photosynthetically transduced energy both to provide Rubsico with substrate and to regenerate ribulose-1,5-bisphosphate suggests that the extent of colimitation between the sequential reactions of photosynthesis will be increased, compared with high-Ci cells, which would also result in a decreased θ (5, 8, 9). Finally, it may be concluded that even though *C. reinhardtii* cells with a fully induced CCM have a higher CO_2 utilization efficiency than high-Ci-grown cells (2–4, 17, 22, 23), the increase in affinity for CO_2 is apparently obtained at the cost of a reduced light utilization efficiency.

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