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Acclimation of Photosynthetic Light Reactions during Induction of Inorganic Carbon Accumulation in the Green Alga *Chlamydomonas reinhardtii*[1,2]

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ABSTRACT

Cells of the unicellular green algae *Chlamydomonas reinhardtii* were grown in high dissolved inorganic carbon (DIC) concentrations (supplied with 50 milliliters per liter CO_2[g]) and transferred to low DIC concentrations (supplied with ≤100 microliters per liter CO_2[g]). Immediately after transfer from high to low DIC the emission of photosystem II related chlorophyll *a* fluorescence was substantially quenched. It is hypothesized that the suddenly induced inorganic carbon limitation of photosynthesis resulted in a phosphorylation of LHCII, leading to the subsequent state 1 to state 2 transition. After 2 hours of low-DIC acclimation, 77 K fluorescence measurements revealed an increase in the fluorescence emitted from photosystem I, due to direct excitation, suggesting a change in photosystem II/photosystem I stoichiometry or an increased light harvesting capacity of photosystem I. After 5 to 6 hours of acclimation a considerable increase in spillover from photosystem II to photosystem I was observed. These adjustments of the photosynthetic light reactions reached steady-state after about 12 hours of low DIC treatment. The quencher of fluorescence could be removed by 5 minutes of dark treatment followed by 5 minutes of weak light treatment, of any of four different light qualities. It is hypothesized that this restoration of fluorescence was due to a state 2 to state 1 transition in low-DIC acclimated cells. A decreased ratio of violaxanthin to zeaxanthin was also observed in 12 hour low DIC treated cells, compared with high DIC grown cells. This ratio was not coupled to the level of fluorescence quenching. The role of different processes during the induction of a DIC accumulating mechanism is discussed.

Green algae and cyanobacteria experiencing low concentrations of DIC[3] during growth have the ability to induce a DIC-accumulating mechanism (2, 4). This is an energy and light requiring mechanism and calculations by Raven and Lucas (20) indicate that 1 mol of ATP is consumed per 1 mol of carbon transported. It has been shown that photosynthetically transduced energy is used to meet this additional demand for ATP (24, 26). These observations suggest that transfer of algal cells from high-DIC to low-DIC concentrations could lead to alterations of the photosynthetic light reactions to adjust the relative production of NADPH *versus* ATP. There are several possible ways by which such adaptive alterations can occur; state transitions and related changes in spillover of excitation energy from PSII to PSI or changes in photosystem stoichiometry will all affect the relative distribution of absorbed excitation energy between the two photosystems and the relative production of NADPH *versus* ATP.

State transitions are believed to be triggered by changes in the redox state of the PQ pool. Excess PSII light reduces the PQ pool and activates a kinase which phosphorylates LHCII, resulting in a state 1 to state 2 transition. Excess PSI light on the other hand, oxidizes the PQ pool, which results in a dephosphorylation of LHCII and a consequent state 2 to state 1 transition (1, 3, 25, 29). Moreover, it has recently been suggested that in addition to the phosphorylation/dephosphorylation of LHCII, other structural and/or functional modifications of the photosynthetic thylakoid recations may also be regarded as state transitions, such as α to β conversion of PSII centers and formation of 'high spillover complexes' (28).

Changes in spillover is also coupled to the phosphorylation of LHCII, since the degree of phosphorylation will affect the distribution of LHCII in the grana and stroma lamellae of the thylakoids. Phosphorylation is thought to introduce additional negative charges into LHCII, which results in mutual repulsion of phosphorylated membrane surfaces, leading to a partial unstacking of the grana, and the subsequent migration of LHCII from the appressed to the nonappressed regions of the thylakoid membrane (29). If this migration involves whole PSII light-harvesting units an increase in spillover from PSII to PSI would be expected. If, instead, only the PSII antennae complexes undergo migration, changes in spillover can be

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[2] This paper was written in memory of Professor Beatrice Sweeney, who was a close friend to one of the authors, G. Samuelsson.

[3] Abbreviations: DIC, dissolved inorganic carbon; F_o, minimum fluorescence; F_m, maximum fluorescence; F_v, variable fluorescence (F_m-F_o); k_D, rate constant for radiationless decay; k_F, rate constant for fluorescence emission; k_p, rate constant for photochemistry of PSII; $k_{T(II-I)}$, rate constant for energy transfer from PSII to PSI; LHCII, light harvesting chl *a*/*b* protein complex II.

explained by the model of Berens *et al.* (29). The model is based on the idea introduced by Melis and Homann (15) that PSII light-harvesting units can exist in two forms, referred to as α- and β-centers. According to the model, phosphorylated LHCII migrates from α-centers, located in the appressed granal membranes, to β-centers, located in the nonappressed stromal membranes resulting in increased spillover from PSIIβ to PSI (29).

Both state transitions per se and the related changes in spillover have characteristic effects on the emission of Chl *a* fluorescence at both room temperature and 77 K (3, 8, 12, 29). The state 1 to state 2 transition results in quenching of fluorescence emission at room temperature due to detachment of phosphorylated LHCII units. The reverse state 2 to state 1 transition results in the restoration of fluorescence quenching (29). According to the bipartite model presented by Kitajima and Butler (12), an increased spillover of energy from PSII to PSI will decrease variable, 77 K, fluorescence emanating from PSII, while fluorescence emission emanating from PSI will increase. At room temperature variable fluorescence will be quenched by increased spillover.

Furthermore, the photosynthetic apparatus is also capable of long-term changes in PSII/PSI stoichiometry in order to balance the excitation energy distribution between the two photoreactions (1, 8, 29), which has also been reported to occur in response to decreased inorganic carbon availability in cultures of the cyanobacteria *Anacystis nidulans* (14).

In addition to the above described alterations of the photosynthetic light reactions, other changes in the thylakoids can also occur upon transfer of algal cells from high to low DIC. Formation of zeaxanthin, by deepoxidation of violaxanthin in the so called xantophyll cycle, present in thylakoid membranes, has been shown to be fine-tuned to respond to the balance of electron transport reactions and CO_2 uptake (10). It has been proposed that the formation of zeaxanthin could prevent light induced damages of photosynthesis by increasing the rate constant of k_D (6, 7). According to the model of Kitajima and Butler (12), an increase in k_D will also lead to fluorescence quenching. The xantophyll cycle has also been proposed to be important for the assembly of LHCII, since it has been shown that lutein, neoxanthin, and violaxanthin are all necessary for maximum yields of LHCII and PSII activity (11, 19).

Transfer of algal cells from high to low DIC concentrations creates an initial stress situation where the low concentration of inorganic carbon is limiting photosynthesis. The resulting decrease in photosynthesis slows down the reoxidation of NADPH and hence causes accumulation of reduced PQ (26). Referring to the hypothesis that reduced PQ 'triggers' a state 1 to state 2 transition, due to phosphorylation of LHCII, it could be assumed that this should occur rather immediately after transfer from high to low DIC. During induction of DIC-accumulation the inorganic carbon concentration will become less limiting and a more optimized acclimation of the photosynthetic light reactions, to better fit the energy demands from the mechanism, could be expected.

In the present study we have applied both room temperature and low temperature (77°K) fluorescence techniques to investigate the acclimation of the photosynthetic light reactions during low-DIC acclimation in *Chlamydomonas reinhardtii*. The possible role of zeaxanthin, acting as a quencher of fluorescence has also been investigated.

MATERIALS AND METHODS

Algal Material and Culturing Conditions

The green alga *Chlamydomonas reinhardtii* c137$^+$ (wild type) was grown in continuous light, with an incident PPFD of 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ obtained from fluorescent tubes (Philips TL 40W/55S), in a phosphate buffered medium (pH 7.5). The major components of the medium was as in Solter and Gibor (23) and the trace elements as in Surzycki (27).

High-DIC grown algae were supplied with 50 mL/L (5%) CO_2(g) (AGA Specialgas AB, Skellefteå, Sweden), at 27°C, corresponding to 1.58 mM CO_2(aq) and 56.2 mM HCO_3^- at equilibrium. The cultures were grown in 500 mL glass vials and were diluted daily from ≈4 to 5 $\mu gChl \cdot mL^{-1}$ to <0.5 $\mu gChl \cdot mL^{-1}$ to keep the culture in exponential growth and to reduce self-shading. The cultures were stirred by magnetic stirrers to avoid differences in light environment between single algal cells. Light was measured with a quantum meter (Li-Cor). Cultures were inoculated using algae axenically grown on agar plates.

Air-grown cells were continuously bubbled with air containing 350 to 400 $\mu L/L$ CO_2(g). The gas flow was kept at a high rate to obtain equilibrium concentrations of DIC ($[CO_2(aq)] \approx 12$ μM and $[HCO_3^-] \approx 250$ μM). The Chl concentration was held below 2 $\mu gChl \cdot mL^{-1}$.

Acclimation to Low DIC

High-DIC grown cells were harvested during exponential growth at a Chl concentration of 2 to 3 $\mu g \cdot mL^{-1}$ by a light centrifugation (1000*g* for 2–3 min) and resuspended to the same Chl concentration in a low-DIC equilibrated growth medium (bubbled with air containing ≤100 $\mu L/L$ CO_2(g)). The gas flow was kept at a high rate to favor equilibrium concentrations of DIC, ($[CO_2(aq)] \approx 2$ μM and $[HCO_3^-] \approx 50$ μM), and to prevent O_2 accumulation in the medium. Air containing ≤100 $\mu L/L$ CO_2(g) was obtained by mixing two streams of air, one with ambient air (350–400 $\mu L/L$ CO_2) and one with CO_2-free air in a ratio of 1:3.

For experiments, cells were withdrawn after different times of acclimation (0–24 h), as described in the following and in figure legends.

DIC Concentration during Fluorescence Measurements

High-DIC grown algae were supplied with 1 mM HCO_3^- prior to dark adaptation. All results obtained from low-DIC acclimating algae were the same, with or without addition of 1 mM HCO_3^- prior to measurements.

Chl Concentration

Multiple (3–4) 20 mL algal samples were harvested by centrifugation (2000*g* for 5–10 min) and Chl was extracted in hot methanol and measured with a spectrophotometer (Schi-

madzu UV-120-2) and calculated using the absorption coefficients of MacKinney (13).

Measurements of Inorganic Carbon Uptake

Uptake of inorganic carbon was calculated from high precision pH measurements as previously described (18). For these experiments algal cells were transferred from their growth medium to the 220 mL experimental cuvette by a light centrifugation, 1000*g* for 2 min, and washed twice in the experimental medium. The experimental nutrient solution was equilibrated with 367 μL/L CO_2(g) and buffered with CO_3^{2-} to an alkalinity of 130 μM, resulting in a medium with known alkalinity, pH and $[DIC]_{tot}$.

For measurements of CO_2-response curves between 20-0 μM CO_2(aq), algae were added at a CO_2(aq) concentration of 20 μM and allowed to consume CO_2(aq) below 1 μM. The procedure was repeated 3–4 times by new additions of concentrate CO_2(aq) (18).

Chl concentration during experiments was 1 $\mu g \cdot mL^{-1}$. Saturating white light (290 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was supplied by three metal halogen projector lamps (Atlas 250 W, 24 V) heading from three directions.

Measurements of Room Temperature Chl *a* Fluorescence

Measurements were performed on algal samples either after 5 min dark adaptation or 5 min dark adaptation + 5 min weak light treatment (see below). Prior to dark adaptation, a 1 mL algal sample was withdrawn from the cultures and placed in 1.5 mL centrifuge tubes without dilution or concentration, yielding a Chl concentration of 2 to 3 $\mu g \cdot mL^{-1}$.

Fluorescence was measured using a fiber-optic-based instrument (PSM, Biomonitor, Umeå, Sweden) designed and built at the department (17), allowing resolution of F_o. The signal was A/D converted and stored by an IBM-compatible personal computer equipped with a data acquisition card (Imtec, Backnang, West-Germany) allowing oscilloscope reading of the signal. The excitation light (330–660 nm, peaking at 500 nm; PPFD = 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and the fluorescence from the sample, was guided through optical fibers put on top of the centrifuge tubes. Prior to measurements the samples were gently stirred to avoid unequal distribution of algae. Duration of excitation was 5 s.

Measurements of Modulated Chl *a* Fluorescence

A pulse amplitude modulation fluorometer (PAM 101 and 103 with Schott lamp, Heinz Walz, Effeltrich, FRG) was used for measurements of fluorescence induction for prolonged times (5–10 min). For more details concerning this method see Schreiber *et al.* (21). The F_o was taken as the average signal during the first 5 s after the measuring light (0.03 $\mu mol \cdot m^{-2} \cdot s^{-1}$, modulated at 1.6 kHz) had been switched on. A saturation pulse (5600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 1 s length) was then applied to induce the F_m. The pulse was thereafter given every 10th second during the 5 to 10 minutes of measurements.

Low Temperature (77K) Fluorescence

Chl *a* fluorescence emission spectra and induction kinetics were measured at 77 K using a 3-fiber optic based spectrofluorometer as described elsewhere (16).

For measurements of emission spectra (650–750 nm) the half-bandwidth of the emission monochromator (Bausch & Lomb) was 3.2 nm. Prior to measurements the algae were concentrated, by centifugation, to a final Chl concentration of 20 $\mu g \cdot mL^{-1}$ and kept in darkness for 5 min before freezing.

The sample (200 μL) was laid on an aluminium platform coated with black tape attached to a plexiglass rod. The rod with the attached sample was frozen in liquid nitrogen in darkness in a Dewar vessel. The fiber was put on top of the rod and fluorescence emission spectra was measured after 3 min in light to obtain maximal fluorescence, F_m. The broadband excitation light (390–560 nm) reached a PPFD of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the end of the fiber.

The time courses of the increase in fluorescence from PSII, measured at 685 nm (Bausch & Lomb grating monochromator; half-bandwidth 13 nm), and PSI measured at 722 nm (Schott interference filter; 13 nm half-bandwidth), were recorded simultaneously. For this purpose two of the optic fibers were connected to separate photomultipliers (Hamamatsu R 1017) measuring 685 and 722 nm fluorescence, respectively. The wavelength positions were chosen from the peaks revealed by the fluorescence emission spectra. The third fiber was used to transmit the 390 to 560 nm broadband excitation light. The PPFD at the end of the fiber was 4.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$. 200 μL algal sample with a Chl concentration of 20 $\mu g \cdot mL^{-1}$ was laid under a plexiglass rod and frozen in liquid nitrogen, as described above. Before freezing the algae were either dark adapted for 5 min, or first dark adapted for 5 min followed by 5 min weak light treatment. The fluorescence transients, from the initial level (F_o) to the maximum steady-state level (F_m) were slow enough (2–3 min) to be resolved on a two-pen chart recorder. The transients at 685 and 722 nm were monitored in parallel and from the two traces, 10 to 12 pairs of values were taken at regular intervals from F_o to F_m. The relative fluorescence intensity at 722 nm was plotted against the relative fluorescence intensity at 685 nm.

Weak Light Treatment

Weak light (PPFD; 1 ± 0.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was provided for 5 min, after 5 min dark adaptation, with either of the following four light qualities. White light (Philips, metal halogen H3, 55W/12V), was led through Schott interference filters to create; green light, 552 nm (half-bandwidth 12 nm); orange light, 616 nm (half-bandwidth, 24 nm); and far-red light, 722 nm (half-bandwidth 12 nm). Illumination with red light, 650 nm (half-bandwidth 12 nm), was provided by using the PAM 101 measuring light beam.

Measurements of Carotenoids

Algae were high-DIC grown and low-DIC acclimated for 12 h as described above. Cells were harvested and concentrated to a final Chl concentration of ≈100 $\mu g \cdot mL^{-1}$. Prior to

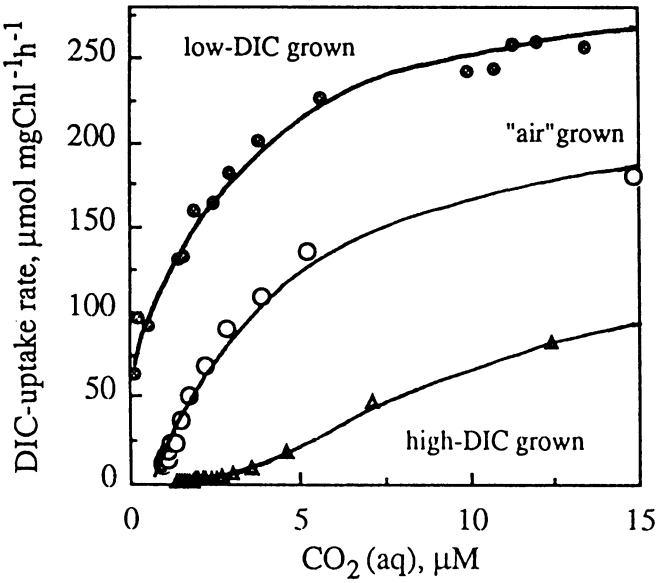

Figure 1. Rate of photosynthetic inorganic carbon uptake as a function of the external CO_2 concentration for algal cells supplied with different DIC concentrations for 12 to 15 h prior to experimental use. High-DIC grown cultures (△) were bubbled with air enriched with 50 mL/L CO_2(g). 'Air'-grown cultures (○) were intensively bubbled with ordinary air, 350 to 400 μL/L CO_2(g), and low-DIC grown cultures (●) were bubbled with ≤100 μL/L CO_2(g). The Chl concentration was 1 μg·mL^{-1} and the PPFD was 290 μmol·m^{-2}·s^{-1}.

extraction of pigments the samples were either pretreated for 5 min in darkness or for 5 min in darkness followed by 5 min weak light (650 nm). The algal pellet was extracted with 0.5 mL of DMF (dimethylformamide), sonicated for 2 min in a cooled Brasonic ultrasonic bath, and centrifuged at 15000*g* for 5 min. The pellet was resuspended with another 0.25 mL DMF and the extraction procedure was repeated. The supernatants were pooled and filtrated with a 0.22 μm Ultrafree-MC unit (Millipore) and stored in liquid N_2 (−80°C) before analysis. Thirty microliters of sample were injected to a high-performance liquid chromatograph (LKB Sweden) and the pigments detected at 440 nm using a spectrophotometer (Waters, model 481, Eschborn, F.R.G.) The liquid chromatographic conditions was as described by Siefermann-Harms (22). Peaks were identified by standard methods using ethanol and chloroform as solvents and the specific absorbance coefficients for ethanol were used to quantify the different carotenoids (5). For calibration of carotenoid concentrations and for peak identification, commercial lutein from Sigma was used. Chl *a* was determined using Chl *a* standard (Sigma).

RESULTS

The affinity for CO_2(aq) was compared between three different cultures of *Chlamydomonas reinhardtii* supplied with either 50 mL/L CO_2(g), 350 to 400 μL/L CO_2(g) (ambient air), or ≤100 μL/L CO_2(g) during growth. Both high-DIC grown and air-grown cells had a lower affinity for CO_2(aq) than those grown at the lowest DIC concentration (Fig. 1). From these results it was concluded that in air-grown cells (grown as defined in "Materials and Methods") the DIC-accumulating mechanism was not expressed to its potential maximal capacity. In the present study, we decided to transfer high-DIC grown cells to ≤100 μL/L CO_2(g) to obtain maximum low DIC acclimation. During this acclimation different characteristics of Chl *a* fluorescence emission, both at room temperature and 77°K, were measured.

The induction of Chl *a* fluorescence emission at room temperature, after 5 min dark pretreatment, was followed for 24 h after transfer of algal cells from high- to low-DIC growth conditions. The induction of Chl *a* fluorescence and values of F_o, F_m, and F_v/F_m were compared between high-DIC and 12 h low-DIC grown cells (Fig. 2; Table I). Evidently the variable component of the fluorescence induction was decreased after 12 h of low-DIC treatment. This decrease appeared immediately after transfer to low-DIC, but after 4 to 5 h of acclimation a slight increase was observed before a second decrease. The final steady state level, about 60% lower F_v, was reached after 12 h of acclimation (Fig. 3). The initial level of F_o also immediately decreased upon transfer to low-DIC, followed by an increase after 6 to 7 h of acclimation, reaching a steady state level after 12 h, of about 110% of the high-DIC grown control (Fig. 3). Values of F_o, F_v, and F_m were used to calculate the ratio of F_v/F_m, which decreased by 25 to 40% after 12 h low-DIC acclimation. This decrease was further verified when comparing the three different fluorescence methods (room-temperature, modulated, and low-temperature fluorescence) (Tables I and II). However, the quenching of F_v/F_m was also dependent on the cell density of the

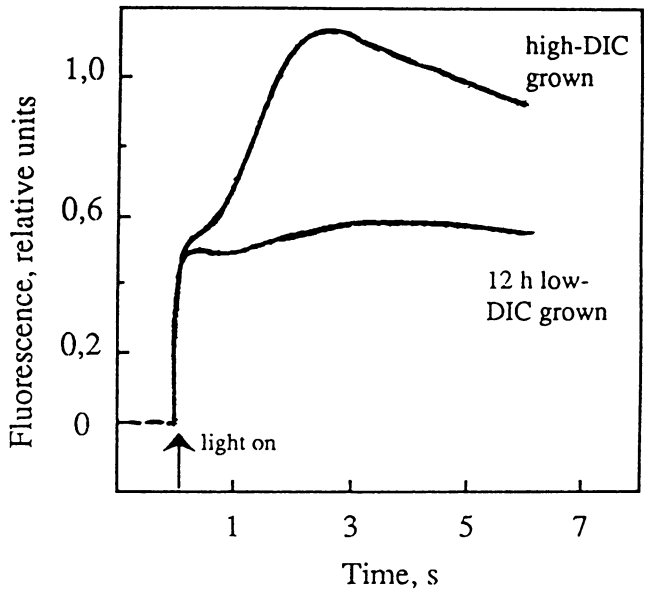

Figure 2. Induction of Chl *a* fluorescence emission at 690 nm (half-band width 12 nm) at room temperature, for high-DIC grown (50 mL/L CO_2[g]) algae and for the same culture after 12 h low-DIC treatment (≤100 μL/L CO_2[g]). The samples were dark adapted for 5 min prior to measurements. PPFD of excitation light = 400 μmol·m^{-2}·s^{-1} (330–660 nm, peaking at 500 nm). Associating mean values are presented in Table I. The Chl concentration was 3 μg·mL^{-1}.

culture. For example, if algae were grown to a Chl concentration of 10 to 15 $\mu g \cdot mL^{-1}$ at low-DIC, but with the same external PPFD, there was almost no reduction in F_v/F_m (data not shown). This implicates a decreased light 'stress,' due to mutual shading in the denser algal culture, also leading to a relatively lower CO_2 limitation.

According to the bipartite model of Butler and Kitajima (12) a decrease in the ratio of F_v/F_m of PSII, as observed by us, could either reflect a reduced rate constant for photochemistry (k_P) of PSII, an increased rate constant for transfer of energy from PSII to PSI ($k_{T[II-I]}$), or an increased rate constant for radiationless decay, (k_D).

Measurements of fluorescence emission spectra at 77 K suggested that there was an increase in $k_{T(II-I)}$ during low DIC acclimation, as indicated by an increased emission peak at 715 nm, known to originate from PSI (9, 12) (Fig. 4). This was further substantiated by kinetic measurements where it was possible to obtain a quantitative determination of the distribution of excitation energy between the two photosystems, by simultaneously measuring the induction of fluorescence emitted from PSII and PSI (Table II; Fig. 5). The method is based on the bipartite model presented by Kitajima and Butler (12) and was fully described by Ögren and Öqvist (16). When fluorescence from PSI (Y axis) was plotted against fluorescence from PSII (X axis) a straight line was obtained (Fig. 5). According to the model, the slope of the line is proportional to $k_{T(II-I)}$ whereas the value of the intercept, obtained when the line is extrapolated back to the Y axis, constitutes a measure of the absorption cross-section of PSI (α) (16).

Acclimation for 12 h to low DIC caused an 83% increase in the slope and a 33% increase in the intercept (Table II). This implies that acclimation to low DIC results in an increased distribution of energy from PSII to PSI, both in terms of the fraction of quanta initially delivered to PSI (α) and in an increase in $k_{T(II-I)}$. The increase in α could either indicate an increase in the light harvesting antenna of PSI and/or an increase in the PSI:PSII ratio after transfer from high to low DIC, while the increase in $k_{T(II-I)}$ implies a change in spillover of energy from PSII to PSI.

Transition from state 1 to state 2 and vice versa has previously been shown to occur upon illumination with light of different qualities (1, 3, 29). State 2 can be reversed to state 1 by illumination with light mainly absorbed by PSI (*i.e.* light with wavelengths longer than 700 nm), and state 1 can be reversed to state 2 by illumination with PSII light (≈650 nm). To test the hypothesis of a state transition during low-DIC acclimation, algae from both 'acclimation states' were illuminated with green, orange, red, or far-red light with a PPFD of 1 ± 0.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 5 min. Prior to weak light treatment the samples were dark adapted for 5 min. With either of the four light qualities, F_v increased by 100 to 110% at room temperature in 12 h low-DIC grown algae (Fig. 6; Table III). The presence of chloramphenicol (10 $\mu g/mL$) during the treatment did not affect these results (data not shown). At 77 K, the weak light treatment decreased α to a value close to that for the high-DIC grown control, the rate constant for spillover ($k_{T[II-I]}$) decreased with 26%, compared to 'untreated' 12 h low-DIC grown algae (Fig. 7; Table II). High-DIC grown algae were more or less uneffected by the weak light treatment (not shown).

Phosphorylation and dephosphorylation of LHC has been suggested to play a major role in state transitions (3, 25, 29). The observed increase in fluorescence emission could hence indicate a dephosphorylation of LHC during the weak light treatment. Therefore, the effect of NaF, an inhibitor of dephosphorylation, was investigated on 12 h low-DIC grown cells, during weak light treatment. In Table IV it is shown that NaF resulted in 50% inhibition of the increase in F_v at a concentration of 70 mM.

The so-called xanthophyll cycle in the thylakoid mem-

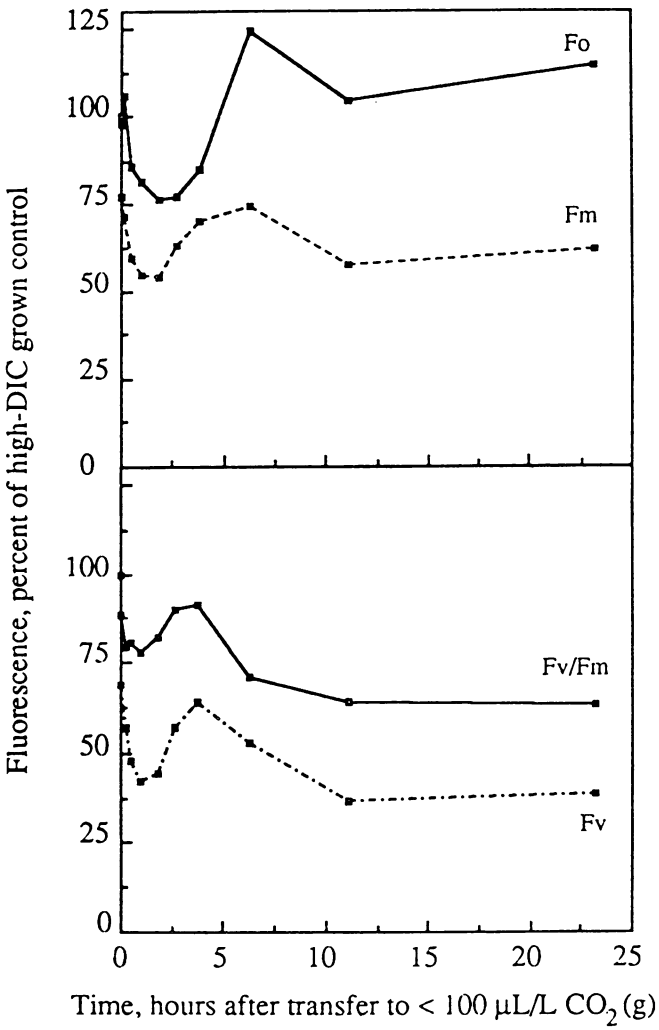

Figure 3. Induction of Chl *a* fluorescence emission at 690 nm (half-bandwidth 12 nm) at room temperature, followed for 24 h after transfer from high-DIC (50 mL/L CO_2[g]) to low-DIC (≤100 µL/L CO_2[g]) growth conditions. Values from high-DIC grown cells were set as control, at time 0. Values are given as percentage of control. F_o = minimum fluorescence; F_m = fluorescence at the peak (F_p) of the induction curve; F_v = variable fluorescence (F_m-F_o). The samples were dark adapted for 5 min before measurements. PPFD of excitation light = 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (330–660 nm, peaking at 500 nm). The Chl concentration was 3 $\mu g \cdot mL^{-1}$.

Table I. *Low-DIC Induced Quenching of Chl a Fluorescence Measured at Room-Temperature, from* C. reinhardtii *Cells*

Induction of Chl fluorescence emission at 690 nm (half-bandwidth 12 nm) at room temperature, for high-DIC grown (50 mL/L CO_2[g]) algae and for the same culture after 12 h low-DIC treatment (≤100 μL/L CO_2[g]). The samples were dark adapted for 5 min prior to measurements. PPFD of excitation light = 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (330–660 nm, peaking at 500 nm). The Chl concentration was 3 $\mu g \cdot mL^{-1}$. SD is given for n = 12, pooled from two independent experimental series.

Fluorescence Parameter	High-DIC Grown Algae	12 h Low-DIC Grown Algae	Change*
			%
F_o	0.31 ± 0.03	0.33 ± 0.01	+6
F_m	1.15 ± 0.06	0.59 ± 0.02	−49
F_v	0.84 ± 0.03	0.26 ± 0.01	−70
F_v/F_m	0.73 ± 0.01	0.45 ± 0.01	−38

[a] The percentage changes of the fluorescence parameters were identical using a PAM fluorometer at room temperature where the F_o was taken as the average signal during the first 5 s after the measuring light had been switched on (0.03 $\mu mol \cdot m^{-2} \cdot s^{-1}$, modulated at 1.6 kHz). A saturation pulse (5600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 1 s length) was then applied to induce the F_m.

Table II. *Low-DIC Induced Changes of PS_{II} Chl a Fluorescence and Associated Changes of Spillover and Absorption Cross-Section of PS_I from* C. reinhardtii *Cells, Measured at 77°K*

Low temperature (77°K) fluorescence values of F_{mI} and F_{mII} in relative units and of the slope and intercept (on the Y axis) of the line F_{722} plotted against F_{685} when excited with 390 to 560 nm; PPFD = 4.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$, during the time course of acclimation of high-DIC grown algae (50 mL/L CO_2(g) to low-DIC culturing conditions (≤100 μL/L CO_2[g]). SD is given for: 6 (high-DIC grown control), 7 (10–13 h low-DIC grown), and 5 (12 h+LL), replicate lines, pooled from three independent experiments. Algal samples were dark adapted for 5 min before freezing in all cases except for 12 h + LL, which refers to samples treated for 5 min in dark followed by 5 min in 650 nm weak light (1 ± 0.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The algae were concentrated to a Chl concentration of 20 $\mu g \cdot mL^{-1}$ by a light centrifugation before pretreatment and freezing.

Parameter	High-DIC Grown	Low-DIC Grown			
		2 h	5 h	12 h	12 h+LL[a]
F_{vII}/F_{mII}	0.81	0.79	0.77	0.61	0.65
Change (%)		−2.5	−5	−25	+6.5[b]
F_{mII}	57 ± 3	51	42	39 ± 4	41 ± 5
Change (%)		−11	−26	−32	+5[b]
F_{mI}	88 ± 3	95	110	114 ± 5	88 ± 6
Change (%)		+8	+25	+30	−30[b]
Slope	0.83 ± 0.05	0.91	1.29	1.52 ± 0.09	1.13 ± 0.16
Change (%)		+10	+55	+83	−26[b]
Intercept	44.5 ± 1.9	51.4	55.4	59.4 ± 10	44.8 ± 6
Change (%)		+16	+24	+33	−24[b]

[a] The effect of weak light treatment was the same using either of four different weak lights (552, 616, 650, or 722 nm). [b] Value compared with 5 min dark adapted, 12 h low-DIC grown algae.

branes has been suggested to act as a quencher of fluorescence. Demmig *et al.* (6, 7) suggested that the formation of zeaxanthin from violaxanthin could decrease the yield of fluorescence through an increase in radiationless decay k_D.

As shown in Table V the ratio of violaxanthin to zeaxanthin decreased during low-DIC acclimation. It was also found that the 5 min weak light treatment decreased this ratio further, even though the variable component of room-temperature fluorescence was totally restored (Tables III and V).

DISCUSSION

The results establish that not only carbon metabolism but also the photochemical properties of *Chlamydomonas reinhardtii* cells are influenced by the DIC concentration during growth. Evidence for this has also previously been presented (24, 26).

In the present study we show that transfer of high-DIC grown algae to low-DIC concentrations is accompanied by a substantial quenching of room-temperature Chl *a* fluorescence. To evaluate the origin of this quenching we have considered four different possible quenching mechanisms: (a) An increase in the rate constant for spillover of excitation energy from PSII to PSI (12, 16), due to alterations of the PSI to PSII ratio (14) and/or formation of 'high-spillover' complexes consisting of PSIIβ-LHC-P-PSI (28); (b) phosphorylation of LHCII and the subsequent state 1-state 2 transition 'triggered' by reduced PQ (1, 3, 8, 29); (c) quenching due to

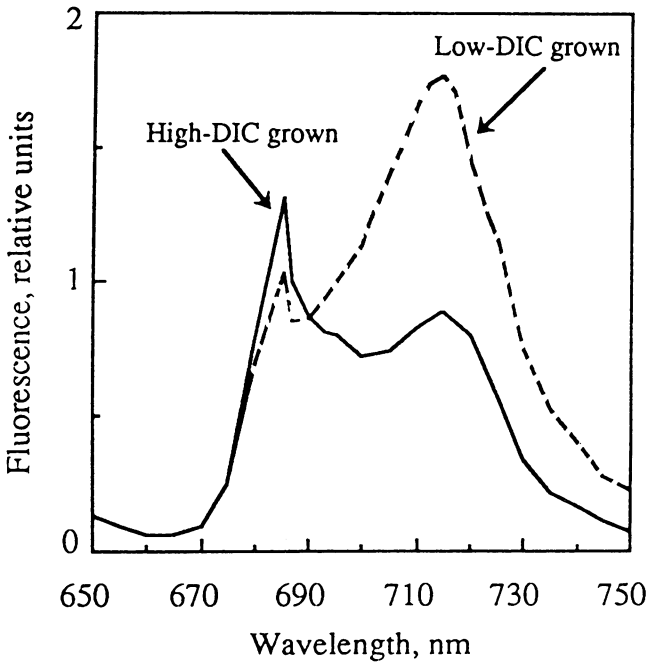

Figure 4. 77 K Chl emission at 650 to 750 nm (half-bandwidth 3.2 nm) for high-DIC grown and 12 h low-DIC grown algal cells. The algae were concentrated to a Chl concentration of 20 $\mu g \cdot mL^{-1}$ by a light centrifugation and dark adapted for 5 min before freezing. Fluorescence emission was measured at F_m. PPFD of broadband excitation light (390–560 nm) = 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

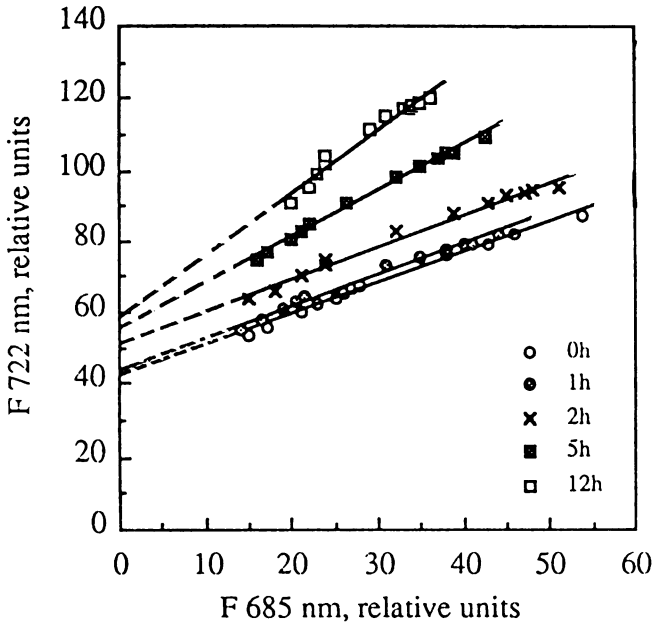

Figure 5. Time course for F_{722} *versus* F_{685} at 77 K, after transfer of high-DIC grown cells to low-DIC culturing conditions. Cells were transferred from 50 mL/L CO_2(g) to ≤100 μL/L CO_2(g), at time 0. The algae were concentrated to a Chl concentration of 20 μg·mL^{-1} by a light centrifugation and dark adapted for 5 min before freezing. The data were fitted to the straight lines by the least squares method. For every line the correlation coefficient of $r \geq 0.99$ was obtained, with one exception ($r = 0.986$) at 12 h. PPFD of broadband excitation light (390–560 nm) = 4.3 μmol·m^{-2}·s^{-1}. See also Table II.

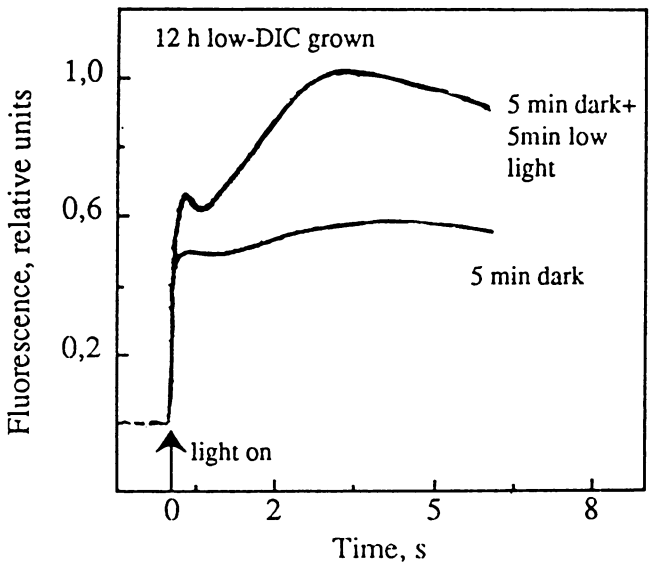

Figure 6. Induction of Chl *a* fluorescence emission at 690 nm (half-bandwidth 12 nm) at room temperature for 12 h low-DIC grown algae (≤100 μL/L CO_2[g]). The samples were either dark adapted for 5 min or first dark adapted for 5 min followed by 5 min weak light treatment. The same effect was obtained by using four different weak lights (522, 616, 650, and 722 nm, PPFD; 1 ± 0.5 μmol·m^{-2}·s^{-1}), which is summarized in Table III. PPFD of excitation light = 400 μmol·m^{-2}·s^{-1} (330–660 nm, peaking at 500 nm). The Chl concentration was 2.5 μg·mL^{-1}.

photoinhibition (12, 16); or (4) formation of zeaxanthin via deepoxidation of violaxanthin (6, 7).

The possibility of photoinhibition was ruled out by the fact that 5 min weak light treatment, in the presence of chloramphenicol, fully restored variable fluorescence (Table III; Fig. 6).

Recently, Demmig-Adams *et al.* (7) showed a correlation between quenching of fluorescence (induced by photoinhibition), zeaxanthin formation, and an increase in k_D. In 12 h low-DIC grown algae (Table V) zeaxanthin was present in amounts corresponding to what was found in Hedera helix and Monstera deliciosa (7). Therefore, we could not at first exclude the possibility that fluorescence in low-DIC grown algae was quenched by an increase in k_D, related to the zeaxanthin-cycle. However, the quenching of fluorescence was completely abolished by 5 min weak light treatment (Table III; Fig. 6), without any increased violaxanthin:zeaxanthin ratio (Table V). It was therefore concluded that the observed quenching of fluorescence was not related to the xanthophyll cycle.

After omitting photoinhibition and zeaxanthin formation from acting as quenchers of fluorescence during low-DIC acclimation, our conclusion is that both state transitions and increased spillover are the most likely candidates to explain the observed changes in fluorescence characteristics. They may operate independently of each other and/or on different time scales and their effects can also be superimposed. In the following discussion, it is therefore important to keep this complexity in mind when interpreting the fluorescence data.

Immediately upon transfer from high- to low-DIC there was a substantial quenching of F_v and to a lesser degree quenching of F_o, at room temperature (Fig. 3). We interpret this quenching as being due to phosphorylation of LHCII, triggered by reduced PQ. The observed immediate quenching of PSII related fluorescence, was not accompanied by any substantial increase in the absorption cross-section of PSI (α)

Table III. *Weak Light Induced Increase in Room-Temperature Chl* a *Fluorescence from 12 h low-DIC Grown* C. reinhardtii *Cells*

Weak light effect on induction of Chl fluorescence emission at 690 nm (half-bandwidth 12 nm) at room temperature, for 12 h low-DIC grown algae (≤100 μL/L CO_2[g]). The samples were either dark adapted for 5 min or first dark adapted for 5 min followed by 5 min weak light treatment. PPFD of excitation light = 400 μmol·m^{-2}·s^{-1} (330–660 nm, peaking at 500 nm). The Chl concentration was 2.5 μg·mL^{-1}. $n = 5$ for dark treated samples and $n = 2$ for each of the weak lights.

Fluorescence Parameter	Treatment					
	5 min dark	5 min dark + 5 min weak light				Average change of all four
		522	616	650	722	
		nm				%
F_o	0.26	0.31	0.30	0.32	0.30	+20
F_m	0.49	0.83	0.80	0.82	0.72	+62
F_v	0.23	0.52	0.50	0.50	0.38	+106
F_v/F_m	0.49	0.63	0.63	0.61	0.56	+24

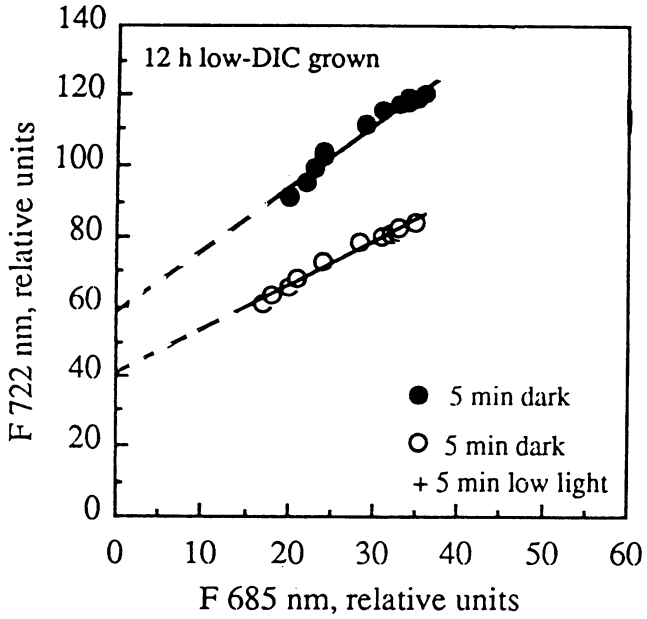

Figure 7. Average plot of F_{722} *versus* F_{685} at 77 K for 12 h low-DIC grown algae (≤100 μL/L CO_2[g]) for 5 min dark adapted cells (●) and for 5 min dark adapted + 5 min 650 nm weak light (1 ± 0.5 μmol·m^{-2}·s^{-1}) treated cells (○). The algae were concentrated to a Chl concentration of 20 μgChl·mL^{-1} by a light centrifugation before pretreatment and freezing. Slopes, intercepts and the effect of different weak light qualities are summarized in Table II.

(Fig. 5), suggesting that phosphorylated LHCII was not a functional part of the PSI antennae. This is in agreement with Deng and Melis (8), who concluded that reduced PQ induced phosphorylation and loss of light harvesting capacity of PSII without any corresponding gain in light harvesting capacity of PSI.

However, after 2 h of low-DIC acclimation an increase in the absorption cross-section of PSI (α) was observed, indicating either an increased light harvesting capacity of PSI or a decreased PSII:PSI ratio (Fig. 5; Table II). At the same time, both F_o and F_v started to increase (Fig. 3). From previous work it is known that full physiological acclimation to low-DIC is observed after 2 to 3 h (18). We therefore interpret this recovery of PSII fluorescence, as an indication of decreased 'low-DIC stress' due to induction of the DIC-accumulating mechanism. It is, however, obvious that the photosynthetic reactions continued to undergo adaptive changes during the following 10 to 12 h, since none of the measured fluorescence parameters reached steady-state values before 12 h of low DIC acclimation (Figs. 3 and 5; Table II).

After 5 h in low-DIC and onward there was a significant increase in spillover (Table II; Fig. 5). This increase did not appear gradually but appeared as a sudden shift, suggesting a more complex reorganization of the thylakoid membrane before any spillover could occur. It can be hypothesized that this reorganization involves both an increased PSI to PSII ratio and an a-to-b conversion of PSII centers together with phosphorylation of LHCII, creating the high-spillover 'PSIIβ-LHC-P-PSI' complexes that has been suggested by Timmerhaus and Weis (28). From this time of acclimation (>5 h) a pronounced and significant effect of the weak-light treatment, was also observed (Figs. 6 and 7; Tables II–IV).

Table IV. *NaF-Inhibition of Weak Light Increase in Room Temperature Fluorescence from 12 h low-DIC Grown* C. reinhardtii *Cells*

Effect of NaF on room temperature fluorescence emission at 690 nm (half-bandwidth 12 nm), measured on 12 h low-DIC (≤100 μL/L CO_2[g]) grown cells, dark adapted for 5 min followed by 5 min weak light (650 nm; PPFD = 1 ± 0.5 μmol·m^{-2}·s^{-1}) treatment prior to measurements. PPFD of excitation light = 400 μmol·m^{-2}·s^{-1} (330–660 nm, peaking at 500 nm). Values are given as percentage of change in fluorescence parameter compared with 5 min dark + 5 min weak light treated samples. NaF (20–100 mM) was added after the dark treatment. The Chl concentration was 2.5 μg·mL^{-1}.

Parameter	0	20	50	70	100 NaF
			nM		
F_o	0	−47	−48	−100	−100
F_m	0	−36	−42	−64	−70
F_v	0	−36	−42	−50	−64
F_v/F_m	0	−8	−17	+12	−25

At present we are not able to explain the mechanism behind the 'weak-light' induced recovery of fluorescence. We can only conclude that 5 min of any quality of weak light fully restored variable fluorescence (Tables II–III; Figs. 6 and 7). This restoration was partly inhibited by NaF (Table IV) suggesting the involvement of dephosphorylation of LHCII. The half-time for dephosphorylation of LHCII have been reported to occur within the same range as the observed fluorescence recovery (5–8 min) and is the most rapidly dephosphorylated protein in green algal and higher plant thylakoids (3). The enzyme responsible for this reaction is thought to be a membrane bound phosphatase, inhibited by NaF (3). Hence, our conclusion is that treatment with weak light following 5 min dark adaptation, in low-DIC acclimated algae, resulted in a state 2 to state 1 transition.

In this context, it should also be noted that measurements of modulated fluorescence also seemed to induce a state 2 to state 1 transition in low-DIC acclimated algae (>6 h in low-DIC). Low-DIC acclimated cells had a significantly lower initial F_m than high-DIC grown cells, but showed a gradual

Table V. *Low-DIC Induced Changes in the Xanthophyll Cycle in* C. reinhardtii *Cells*

Changes of carotenoids in the xanthophyll cycle for algae grown with different DIC concentrations. The algae were dark adapted for 5 min prior to extraction (^{D}) for 5 min dark + 5 min weak light (^{WL}) treated prior to extraction. Details of extraction and detection methods are given in "Materials and Methods." The values are given as the mean of two experiments ± SD.

Treatment	Violaxanthin	Antheraxanthin	Zeaxanthin	F_v/F_m
	mmol pigment/mol Chl a			
High-DIC^D	104 ± 6	1.4 ± 0.2	ND[a]	0.73
High-DIC^{WL}	112 ± 2	1.6 ± 0.4	ND	0.75
12 h Low-DIC^D	73 ± 5	21.2 ± 1.1	10.5 ± 1.6	0.49
12 h Low-DIC^{WL}	69 ± 4	13.5 ± 1.4	14.9 ± 1.8	0.61

[a] Not detectable.

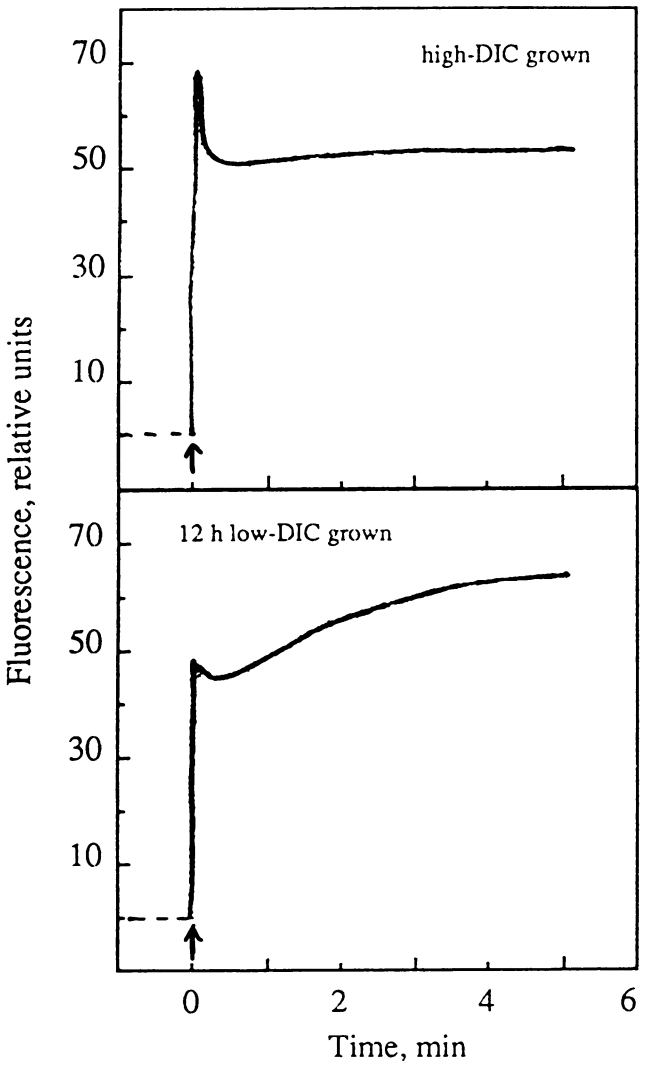

Figure 8. Induction of room temperature fluorescence emission, measured for 5 min with a modulated fluorescence PAM fluorometer (Heinz Walz) for high-DIC grown (50 mL/L CO_2[g]) algae and for 12 h low-DIC grown (≤100 μL/L CO_2[g]) algae. F_o was taken as the average signal during the first 5 s after the measuring light had been switched on (0.03 $\mu mol \cdot m^{-2} \cdot s^{-1}$, modulated at 1.6 kHz). A saturation pulse (5600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 1 s length) was then applied to induce the maximal fluorescence level, F_m. A new pulse was given every tenth second and after the second pulse the actinic white light was switched on (400 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The fluorescence signal obtained by the strong light pulse is presented in the figure.

increase in maximal fluorescence during 5 to 10 min of measurements (Fig. 8). The observations are very similar to the slow light-induced fluorescence changes observed in DCMU-poisoned algae adapted to state 2 (9, 29). In terms of the 'LHCII phosphorylation hypothesis' such fluorescence increases could be interpreted as reflecting the dephosphorylation of LHCII under conditions in which the PQ pool is oxidized (29).

In conclusion, the acclimation of high-DIC grown *C. reinhardtii* to low-DIC conditions resulted in changes of the photochemical properties of the algal cells. The initial low-DIC stress caused a state 1 to state 2 transition, probably due to phosphorylation of LHCII, while prolonged times of low-DIC acclimation caused an increase in spillover of excitation energy from PSII to PSI. The absorption cross-section of PSI was also increased during low-DIC acclimation, suggesting an increased ratio of PSI to PSII and/or an increased light harvesting capacity of existing PSI centers. We suggest that the observed alterations of the photosynthetic light reactions could either act as protection from overexcitation of PSII, or more likely, provide the cells with extra ATP for DIC-accumulation, by an increased cyclic electron flow around PSI. It was also observed that a state 2 to state 1 transition occurred during 5 min weak light treatment, with any of four different light qualities, in low-DIC acclimated cells.

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