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Influence of carbon dioxide concentration during growth on fluorescence induction characteristics of the Green Alga *Chlamydomonas reinhardii*

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Abstract. Carbon dioxide concentration during growth is commonly not considered to be a factor influencing the photochemical properties of plants. It was observed that fluorescence induction in *Chlamydomonas reinhardii* cells grown at air levels of CO_2 was both qualitatively and quantitatively different from that of cells grown at 5% CO_2. In the two cell types, measured at equivalent chlorophyll and irradiance levels, the fluorescence intensity and the ratio of the levels of peak fluorescence (F_p) to that of the initial fluorescence (F_o) were much lower in the air-adapted than in the 5% CO_2 adapted cells. The maximum fluorescence (F_{max}) in the presence of diuron was also lower for air-adapted cells. Roughly twice the light input was required for the air-adapted cells to give a fluorescence induction transient and intensity equivalent to that of the 5% CO_2-adapted cells. Similar properties were observed in several other unicellular green algae and in cyanobacteria. *Chlamydomonas* grown under variable CO_2 concentrations exhibit significant differences in photosynthetic carbon metabolism and are presumed to have altered energy requirements. The observed variation in fluorescence induction may be due to changes in the properties of the thylakoid reactions (e.g. cyclic electron flow) of *Chlamydomonas* cells, which may, in turn, be due to a response to the altered energy requirements.

Introduction

Certain unicellular green algae and cyanobacteria exhibit marked differences in photosynthetic carbon metabolism dependent on the CO_2 concentration during growth [1, 11]. Using room temperature chlorophyll *a* fluorescence as a monitor of photochemical activity, we have observed that the concentration of CO_2 during growth of the green alga *Chlamydomonas reinhardii* influences not only the carbon metabolism but also the photochemical properties of the cells.

Chlorophyll *a* fluorescence is an intrinsic property of chloroplast thylakoids which monitors the structural and functional changes accompanying photosynthetic reactions [6, 12, 18]. In dark-adapted photosynthetic systems, including isolated chloroplasts, cells, intact leaves or green algae, chlorophyll *a* fluoresence intensity undergoes characteristic changes over

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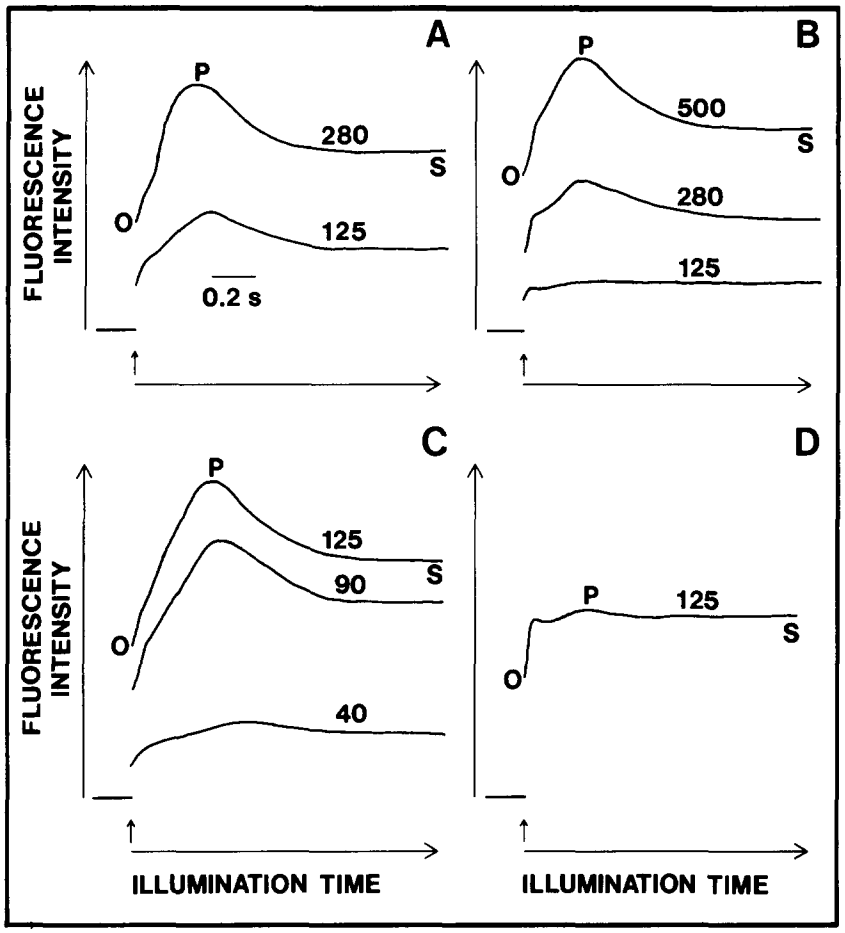

Figure 1. Kinetics of Chl *a* fluorescence at room temperature as a function of quantum flux density in *Chlamydomonas reinhardii* cells grown at (A and C) 5% or (B and D) 0.03–0.04% CO_2 (air). Traces in C and D were measured at higher sensitivity than A and B. Numbers on transients refer to quantum flux densities in $\mu E\ m^{-2}\ s^{-1}$. [Chl] was $10\ \mu g\ ml^{-1}$ suspension in a total volume of 2 ml of 50 mM MOPS-KOH, pH 7.0. The letters O, P, and S refer to characteristic points on the fluorescence transient [6].

time, a phenomenom called fluorescence induction or the Kautsky effect [6, 18]. It represents a superposition of variable fluorescence (F_v) over a constant fluorescence background (F_o), and instantaneously monitors the balance of all the competing processes. The peak level of variable fluorescence at approximately 0.5 sec is designated at F_p (Figure 1). The maximum fluorescence observed in the presence of diuron is referred to as F_{max}. At room temperature, most fluorescence is derived from chlorophyll *a* associated with photosystem II (PS II) [6, 18].

Methods

Chlamydomonas reinhardii strain 2137 *mt+* was cultured photoautotrophically [20], aerated either with air (air-adapted) or 5% CO_2 in air (5% CO_2-adapted). Photosynthetic O_2 exchange was measured in intact cells as previously described [20].

Kinetics of chlorophyll *a* fluorescence at room temperature were followed using the apparatus described by Munday and Govindjee [17]. Excitation was with broad-band blue light (Corning filters CS 4-76 and 3-73), and fluorescence was detected through a monochromator set at 688 nm (band width 9.9 nm) and a Corning CS 2-64 filter. The transients were photographed from an oscilloscope screen.

P_{700} was estimated with an Aminco DW-2 spectrophotometer from the light-induced absorbance change at 702 nm in isolated thylakoids. The assay medium for P_{700} measurement contained 1% (v/v) Triton X-100, 1 mM methyl viologen, 1 mM ascorbate and 50 mM MOPS-KOH (pH 7.0). Chlorophyll content and chlorophyll *a*/*b* ratio were determined in 96% (v/v) ethanol according to Wintermans and De Mots [24].

Relative amounts of the PS II electron acceptor Q_A were estimated from room temperature fluorescence induction transients in the presence of diuron according to Malkin et al. [13] using the following equation:

$$\frac{Q_A}{Chl} \propto \left(1 - \frac{F_o}{F_{max}}\right) \alpha_2 \epsilon_i I_o \bar{\Upsilon} \tag{1}$$

where $(1 - F_o/F_{max})$ is the maximum quantum yield of excitation trapping when all the reaction centers are open, ϵ_i is the extinction coefficient for Chl *in vivo*, I_o is the incident light intensity, Υ is the average induction time (given by the area above the induction curve when F_{max} is normalized to unity), and α_2 is a factor describing light distribution to PS II. The value for α_2 was estimated as 0.5 as in Malkin et al. [13], but see the caution below in the discussion of these results. For the measurements made in this study it was assumed that ϵ_i and I_o were the same for both cell types, so relative values for Q_A/Chl can be calculated from the following equation:

$$(Q_A/Chl)_{rel} = (1 - F_o/F_{max})\alpha_2 \bar{\Upsilon} \tag{2}$$

Results and discussion

Fluorescence induction in *C. reinhardii* cells grown photoautotrophically at air levels CO_2 (0.03–0.04%) (Figure 1B, D) was qualitatively and quantitatively different from that of cells cultured at 5% CO_2 (Figure 1A, C). In the two cell types, measured at equivalent chlorophyll and irradiance levels, the fluorescence intensity (Figure 1), measured as described earlier [17], and the ratio of the peak fluorescence (F_p) to that of the initial fluorescence (F_o)

Table 1. Photosynthetic properties of *Chlamydomonas reinhardii* grown at two different CO_2 concentrations

Measurement	5% CO_2 Grown	Air Grown
F_p/F_o		
125 $\mu E\ m^{-2}s^{-1}$	2.1 ± 0.3	1.5 ± 0.2
280 $\mu E\ m^{-2}s^{-1}$	2.1 ± 0.2	1.6 ± 0.1
500 $\mu E\ m^{-2}s^{-1}$	–	1.6 ± 0.1
Chl/P_{700}	480 ± 14	300 ± 35
$(Chl/Q_A)_{rel}$	350	338
Chl *a*/*b*	2.5 ± 0.1	2.6 ± 0.1
Maximum photosynthetic rate		
$H_2O \rightarrow CO_2$	197	161
$H_2O \rightarrow MV$	247	243

Photosynthetic rate was measured as O_2 exchange of intact cells and is reported as μmoles O_2 (mg Chl)$^{-1}h^{-1}$. Measurements were made at 25 C and pH 7.0 using either 2.5 mM $NaHCO_3$ or 1 mM methyl viologen (plus 1 μM nigericin as uncoupler) as electron acceptor.

(Table 1) were much lower in the air-adapted than in the 5% CO_2-adapted cells. These fluorescence properties were unaffected by the CO_2 concentration present during the measurement (data not shown).

The quantitative and qualitative differences in fluorescence between the cell types were consistently observed at light levels from 40 to 500 $\mu E\ m^{-2}s^{-1}$ (Figure 1). Roughly twice the light input was required in the air-adapted cells to give a fluorescence induction transient and yield equivalent to that of the 5% CO_2-adapted cells. However, the ratio of F_p to F_o remained constant over a range of quantum flux densities (Table 1).

Even in the presence of diuron [DCMU ≡ 3-(3,4-dichlorophenyl)-1,1-dimethyl urea], which inhibits photosynthetic electron transport between PS II and PS I, the difference in fluorescence yield was maintained. This observation indicates that the biochemical alteration responsible for the fluorescence yield difference occurs prior to the site of diuron inhibition and must, therefore, lie in the light absorption, excitation energy transfer, or electron transport properties of PS II.

The above phenomena are of general occurrence since similar changes in fluorescence properties, dependent on the CO_2 concentration during growth, were observed in the unicellular green algae *Chlamydomonas eugamytos*, *Dunaliella salina*, *Dunaliella parva*, and *Chlorella vulgaris*, and in the cyanobacteria *Coccochloris peniocystis*, *Synechococcus leopoliensis* (≡*Anacystis nidulans*), and *Plectonema boryanum* (data not shown). Similar observations have also been made by Govindjee and T. Ogawa (unpublished data) with the filamentous cyanobacterium *Anabaena variabilis*.

A decreased fluorescence yield in air-adapted, as compared to CO_2-grown, cells is apparently due to a lowered $[Q_A^-]$. There could be four possible

explanations: (1) an increased electron flow from Q_A^- to PS I; (2) a decreased electron flow to Q_A from PS II; (3) an increased back flow of electrons from Q_A^- to the water side in PS II or to unknown intermediates; and (4) an increased distribution (or redistribution) of excitation energy to the weakly fluorescent PSI. The first possibility can be immediately eliminated because the decreased fluorescence yield persists even in the presence of diuron which blocks electron flow between PS II and PS I. The second possibility can also be discarded because this should have resulted in a slower fluorescence rise, but ultimately the same fluorescence yield maximum. The absence of differences in water to methylviologen reaction rates, at high light intensities, in the two type of cells (Table 1) suggests that no new rate-limiting reaction in noncyclic electron flow was created in air-adapted cells. The third possibility cannot be eliminated and will be discussed below. The fourth possibility can also be eliminated but it requires further discussion (see below).

The third possible explanation for the fluorescence difference was suggested to lead to increase in air-adapted *Chlamydomonas* of cyclic electron flow or back-reaction in PS II. Such a cyclic electron flow has been suggested to occur in photoheterotrophically-grown but not photoautotrophically-grown (high CO_2) *Chlamydobotrys stellata* [15, 16]. Cyclic PS II electron flow or back-reaction would reduce the apparent concentration of reduced Q_A, thus reducing relative fluorescence yield. It is interesting to note that Horton and Lee [10] have recently related an increased back-reaction in PS II with a transition from state I (highly fluorescent) to state II (weakly fluorescent) following phosphorylation of proteins in isolated thylakoids. Thus, this explanation remains a valid one.

The fourth possible explanation of increased distribution of energy to PS I requires further discussion. A decrease in fluorescence yield in air-adapted cells may imply an increased antenna size of weakly fluorescent PS I with or without a decreased antenna size of strongly fluorescent PS II; the same result can be obtained by an increased excitation energy transfer from PS II to PS I. Differences between sun and shade leaves have indeed been explained by differences in the ratio of PS I to PS II sizes [4, 14; also see refs. 5, 22]. Data on the ratio of total Chlorophyll/P_{700} (Table 1), however, suggests that PS I size in air-adapted cells may even be smaller than in CO_2-grown cells in contrast to our expectation. Furthermore, normalized fluorescence transients in the presence of diuron (Figure 3) do not suggest any significant change in PS II size in air-adapted cells (compare area over the two fluorescence curves). The slightly longer ($\simeq 20\%$) half-rise time is apparently not enough to explain the almost two-fold decrease in fluorescence yield maximum in air-adapted cells (Figure 2). If values of 0.4 and 0.6 are used for α_2 in equation 2 for air-adapted and CO_2-adapted cells, respectively, the calculated ratios of PS I/PS II become similar for the two cell types. Furthermore, an alteration of the antenna PS I/PS II ratio might be expected to result in an altered ratio of Chl *a*/*b*, but this ratio was also found to be quite similar for both cell types.

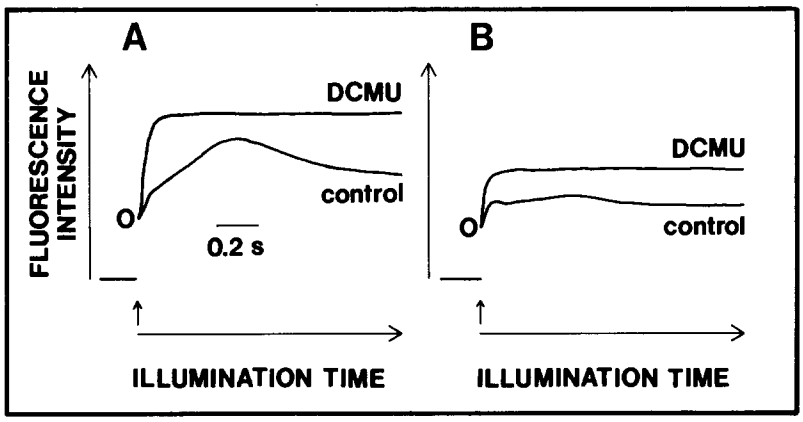

Figure 2. Chl *a* fluorescence transients of *Chlamydomonas reinhardii* cells adapted to (A) 5% or (B) 0.03–0.4% CO_2 (air) in the absence or presence of 10 μM diuron (DCMU). [Chl], 10 μg ml^{-1} suspension; quantum flux density, 125 $\mu E\ m^{-2}s^{-1}$. Other conditions as in Figure 1.

Thus, a change in PS I antenna size, PS II antenna size, or their ratios does not appear to explain the lowered fluorescence yield in air-adapted cells. However, explanations involving a contribution of state changes from fluorescence State I to a weakly fluorescent State II [8, 9, 12, 19, 23] require further experimentation.

An explanation of quenching of fluorescence other than that based on Q_A

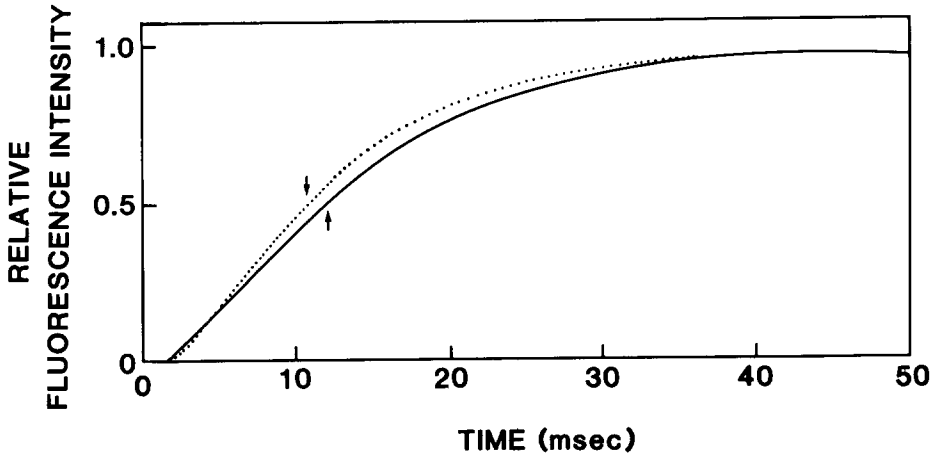

Figure 3. Variable Chl *a* fluorescence transients of *Chlamydomonas reinhardii* cells adapted to (. . .) 5% CO_2 or (——) 0.03–0.4% CO_2 (air) in the presence of 10 μM diuron (DCMU) and with $F_{max} - F_o$ normalized to unity. Arrows indicate half-rise times of variable fluoresence for each curve. [Chl], 5.5 μg ml^{-1} suspension; quantum flux density, 500 $\mu E\ m^{-2}s^{-1}$. The ratio of $F_{max} - F_o$ (with diuron) for CO_2-grown and air-adapted cells was $\simeq$ 2.0. Other conditions as in Figure 1.

has also been suggested. This is quenching by the so-called high-energy state [3, 18]. The ratio of P_{700}/total chlorophyll *a* is about 60% higher in air-adapted cells (Table 1). This could imply that the concentration of reaction center of PS I (P_{700}) is higher in air-adapted cells. Air-adapted *C. reinhardii* require a greater ATP to NADPH ratio for CO_2 assimilation than 5% CO_2-adapted cells (see below) and may therefore have increased cyclic photophosphorylation. A higher ratio of PS I reaction center to PS II reaction center in air-adapted cells may indeed reflect a higher capacity for PS I cyclic electron flow to supply the additional ATP. Increased PSI cyclic electron flow could result in greater high-energy state quenching both in the presence and the absence of diuron.

In summary, the low fluorescence yield of air-adapted cells is best explained here by an increased cyclic electron flow around PS I and PS II. Whatever the actual biochemical difference(s) between the two *Chlamydomonas* cell types, there is a change in the properties of the thylakoid reactions of the cells which is dependent on the CO_2 concentration present during cell growth. Its relation to the bicarbonate effect in thylakoid membranes [7, 21] is not yet clear.

Chlamydomonas cells grown at air levels of CO_2 possess a CO_2 concentrating mechanism not present in cells grown at 5% CO_2 [1]. This system involves active bicarbonate transport and therefore requires an increased energy (ATP) input. The relative quantum efficiency for CO_2-dependent O_2 evolution at limiting light and saturating CO_2 was found to be approximately 25% less in air-adapted compared to 5% CO_2-adapted *Chlamydomonas* (data not shown). In addition, Badger and Andrews [2] have reported a two-fold decrease in the relative quantum efficiency of CO_2 reduction in air-adapted compared to high CO_2-adapted *Synechococcus* cells. Increased ATP demand for active bicarbonate transport, leading to increased PS I cyclic photophosphorylation, would reduce the apparent quantum efficiency in air-adapted cells of both species when measured as CO_2-dependent O_2 evolution. It is therefore possible that the fluorescence differences reported here reflect changes in the properties of the air-adapted *Chlamydomonas* which are necessary to meet the increased energy requirements of active bicarbonate transport.

Acknowledgments

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