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THE EFFECTS OF CARBON DIOXIDE CONCENTRATION ON OXYGEN EVOLUTION AND FLUORESCENCE TRANSIENTS IN SYNCHRONOUS CULTURES OF *CHLORELLA PYRENOIDOSA*

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SUMMARY

1. The steady-state fluorescence yield of *Chlorella pyrenoidosa* is strongly affected by CO_2 concentration: the yield is approximately 2-fold higher in the presence than in the absence of CO_2. During induction, in the presence of saturating CO_2, accelerating oxygen evolution is paralleled by rising fluorescence (M_2–P_3 transient); in the absence of CO_2, fluorescence yield remains at the low M_2 level.

2. Both illumination and CO_2 content are important in determining the steady-state fluorescence yield: at lower illuminations, lower concentrations of CO_2 are required to obtain a maximum fluorescence yield.

3. The slow fluorescence transients are not affected directly by pH but only indirectly through the CO_2 concentration.

4. The CO_2-dependent fluorescence rise (M_2–P_3 transient) is most readily observed in cells harvested early in the light period of a synchronous culture, but it can also be elicited in cells harvested during the dark period.

5. Addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) to CO_2-deprived cells raises the fluorescence yield approximately 4-fold, that is to the same high level as cells supplied with CO_2 and DCMU.

6. The effects of CO_2 provide a new example of a marked parallelism between photosynthetic electron transport and fluorescence. To explain such parallelism, it seems necessary to postulate large changes in the de-excitation processes within Photosystem II units or in the distribution of excitation between Photosystems I and II.

INTRODUCTION

When, after several minutes darkness, a photosynthetic organism is illuminated with saturating light, a complex induction of chlorophyll fluorescence is observed. Following the description of Bannister and Rice[1], the characteristic features of induction are (1) the O–P_1 rise in fluorescence yield in the first 20 ms, discovered by Joliot[2] and probably related to changes in the distribution of S-states[3]; (2) the

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; PMS, phenazine methosulfate.

P_1–M_1–P_2 fluorescence rise reached in 0.2–0.5 s and believed to accompany the exhaustion of a pool of System II electron acceptor; (3) the P_2–M_2 decline completed in 5–30 s and believed to reflect an activation of a dark enzymatic step in electron transport which allows regeneration of System II acceptors; (4) a slow M_2–P_3 rise, lasting from about 30 s to about 2 min; and (5) the P_3–S change (often small) leading to the final steady-state fluorescence level. In the P_1–M_1–P_2 and P_2–M_2 phases, the changes in fluorescence and oxygen evolution are antiparallel or complementary. This relationship is consistent with a simple photochemical system in which fluorescence and chemistry are competing de-excitation processes. In contrast, the M_2–P_3 fluorescence rise is paralleled by an accelerating rate of oxygen evolution. In order to explain parallelism one must postulate either changes in the de-excitation steps within System II units (for example an activation of system II units[1]), or changes in the distribution of absorbed energy between Systems I and II (refs 4 and 5).

Rabinowitch[6] has reviewed early observations which point to some effect of CO_2 on fluorescence yield in the later phases of induction. McAlister and Myers[7] recorded differences in fluorescence induction in *Chlorella* resulting from variation of the CO_2 level during growth and during fluorescence measurement. In this paper, we describe a lowering of fluorescence along with oxygen evolution which occurs when CO_2 becomes limiting. The effect is a new instance of parallelism, and it shows that modifications on the System I side of System II (in this case in the carbon cycle) can lead to marked changes in the fluorescence yield from System II.

MATERIALS AND METHODS

Cultures

Chlorella pyrenoidosa was cultured at 30 °C in a medium containing 2.5 mM $CaCl_2$, 1.0 mM $MgSO_4 \cdot 7H_2O$, 0.04 mM K_2HPO_4, 1.2 mM KNO_3, and 0.5 ml/l of Hutner's trace element solution. Cultures were gassed with 1% CO_2 in air. Synchrony was maintained by a cycle of 14 h of 1000 ft candles illumination and 10 h dark, and by daily subculturing. At the end of the dark period cell numbers ranged between $3 \cdot 10^6$–9×10^6 cells per ml. Samples for experiments were usually harvested in the 2nd–3rd h of light, a stage shown by Wang[8] to give a maximum M_2–P_3 rise.

Fluorescence and O_2 evolution

Fluorescence (680 nm) and rates of O_2 evolution were measured with the apparatus described by Bannister and Rice[1]. Suspensions mounted in the rate electrode contained 5–8 μl packed cells per ml, and the electrolyte medium was growth medium *plus* 0.05 M KCl and 0.05 M NaCl. With the exception of the light curve measurements, the other experiments were performed using an electrode with an area of 2.0 mm $\times$ 12.0 mm and a 0.20-mm recess; for this, the rate of oxygen evolution ($\mu l \cdot \mu l^{-1}$ cells $\cdot h^{-1}$) is given by 48 i/d where i is the photocurrent in μA and d the cell density in $\mu l \cdot ml^{-1}$ in suspension. For the O_2 and fluorescence light curves, an electrode with the platinum recessed 0.33 mm and with a diameter of 1.0 cm was used in the measurements. The slightly deeper electrode permitted partial CO_2 inhibition to be maintained with somewhat higher (and hence move easily regulated) partial pressures of CO_2. In this case, the rate of oxygen evolution could be estimated as 8.06 i/d.

Continuous 5-min light–3-min dark cycles provided automatically with a programmed electronic shutter were used in most experiments. The actinic illumination obtained with 2 Corning 4303 filters and a KG-1 infrared filter was "broad band blue" (440–660 nm); it was varied with calibrated neutral density filters and measured with a galvanometer and thermopile. The oxygen and fluorescence signals were amplified by a Keithley 150 A Microvolt–Ammeter and a Keithley 417 Picoammeter, respectively, and recorded on a Mosely 7100 B strip chart recorder.

The electrode assembly was modified to accommodate a Fischer (E-5) microprobe combination pH electrode so the pH of the medium could be constantly monitored. Small additions of 1 M NaOH or HCl were made in order to maintain the desired pH. The assembly was held at 25 °C with a constant temperature circulator.

Gas control

The concentration of dissolved $CO_2+H_2CO_3$ in the 100-ml volume of the electrolyte medium was controlled by regulating the partial pressure of CO_2 in the gas stream which aerated and stirred the electrolyte at a fixed rate of 50 $ml \cdot min^{-1}$. Composition of the gas was adjusted as follows. Double reduction regulators on tanks of N_2, 6% CO_2 in N_2 and 50% O_2 in N_2 provided pressures in the range of 0–100 $lb/inch^2$. Gas-flow rates from the tanks were limited by the supply pressures and 4-inch lengths of stainless steel capillary tubing (0.005 inch internal diameter). The flow rates were measured with calibrated mercury (Hg) flowmeters. A flow rate of 50 $ml \cdot min^{-1}$ corresponded to 100 $lb/inch^2$ supply pressure and a 20-cm displacement between Hg menisci in the flowmeters. The flow rates could be set reproducibly to ± 0.5 $ml \cdot min^{-1}$ (± 2 mmHg). In the following experiments the gas bubbled through the electrolyte always contained 6% O_2 while the CO_2 content was varied between 0 and 5%.

The concentration of dissolved CO_2 *plus* H_2CO_3 in the aqueous solution is directly proportional to the partial pressure of CO_2 and is approximately 300 μM at $P_{CO_2}=0.01$ atm. Using the deeper electrode with this concentration of CO_2 and with saturating light, steady-state photosynthesis was about 45% saturated. Others[9,10] have shown that in well-stirred suspensions of *Chorella*, CO_2 limitation begins with concentrations of 10 μM or less. The discrepancy must be attributed to the long diffusion path (0.33 mm+dialysis membrane thickness) from the stirred and aerated external electrolyte into the cell layer on the platinum electrode.

A change in the composition of the gas supply could be completed in about 1 min. The photosynthetic response to turning off the CO_2 component in the gas, and replacing it with an equal component of N_2 to maintain a constant flow rate, was complete in about 15 min at pH 5.0–5.5. At pH >6.5 the response was much slower due to the presence of bicarbonate. During changes in the P_{CO_2}, the pH was maintained by titration with 1 M HCl or 1 M NaOH solution.

Fluoroscope

Additional fluorescence measurements were made using the fluoroscope described by Hoch and Randles[11]. A modulated 13-cycles/s measuring beam, 630 nm, (approx. 0.002 $mW \cdot cm^{-2}$) and a white actinic beam (approx. 1 $mW \cdot cm^{-2}$) modulated at 1080 cycles/s were provided. The 13 cycles/s modulated fluorescence at 696 nm

was measured. In this series of measurements, 2.5-ml aliquots of cells were taken directly from the culture tubes, placed in a cuvette and aerated with 1% CO_2 in air or air passed through a NaOH solution to remove CO_2 while the fluorescence measurements were made.

RESULTS

Dependence of M_2–P_3 rise on CO_2

Fig. 1 shows fluorescence and O_2 induction transients in *Chlorella pyrenoidosa* during the shift from 5 to 0% CO_2, the pH being maintained constant at 5.3. The cells were harvested in the 2nd h of light from a synchronous culture and gave a light- and CO_2-saturated rate of O_2 evolution of 28 $\mu l \cdot \mu l^{-1} \cdot h^{-1}$. All the induction curves exhibit typical P_2–M_2 declines of fluorescence yield, the M_2 minimum being reached after about 30 s. With 5% CO_2 (Curve a), there is a striking M_2–P_3 rise paralleled by an increasing rate of O_2 evolution. The P_3 level is reached in about 1.5 min, and thereafter the fluorescence yield changes little (*i.e.* the P_3 level and the final steady-state level S are nearly the same). This induction pattern is essentially identical to that previously described for *Chlamydomonas reinhardti*[1] and contrasts with that reported by Papageorgiou and Govindjee[12] for *Chorella*, in which a decline from P_3 to the final steady-state level S was regularly observed.

Curves b–f of Fig. 1 follow the modification of inductions as CO_2 was sparged from the electrolyte solution. Already in the first period after shutting off the CO_2 (Curve b), there is a prominent P_3–S decline in fluorescence accompanied by a parallel decline in O_2 evolution. In the next two cycles (Curves c and d), the P_3 peak and O_2 evolution are progressively depressed. By the fourth cycle (Curve e), about

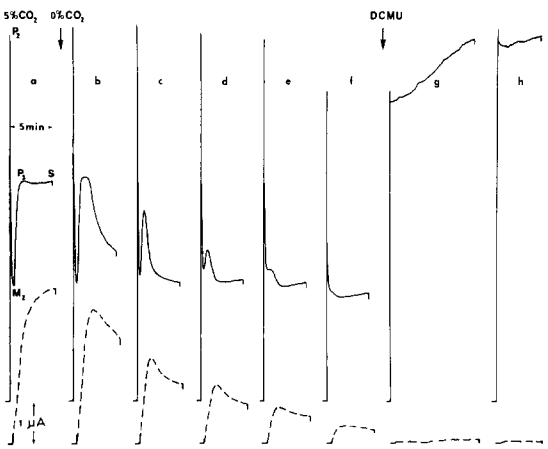

Fig. 1. Effect of CO_2 removal on O_2 and fluorescence induction. Rate of oxygen evolution (dashed line) and 680 nm fluorescence (continuous line) with "2-h" cells of *Chlorella pyrenoidosa* (6.6 μl cells per ml) during shift from 5 to 0% CO_2, at pH 5.3. Illumination cycle: 5 min light (5.25 $mW \cdot cm^{-2}$ (broad band blue (440–660 nm)) – 3 min. dark. Temperature 25 °C. Gas phase: 6% O_2 throughout, (a) 5% CO_2 and (b)–(h) 0% CO_2 with the balance in N_2. Traces (b)–(e) show induction in the first four cycles after CO_2 was cut off, while (f) was recorded after 1.5 h without CO_2. (g) and (h) are results after the addition of 10 μM DCMU to cells without CO_2.

30 min after turning off the CO_2, the P_3 peak is virtually absent, the steady-state fluorescence is half the value in CO_2, and steady-state O_2 evolution is about 75% inhibited. Further continuation of the light–dark regime up to the 12th cycle (Curve f) led to some lowering of the P_2 level and of the O_2 signal. The apparent O_2 evolution rate (about 2 μl $O_2 \cdot \mu l^{-1} \cdot h^{-1}$) may be attributable to photo-inhibition of respiration.

Effect of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)

When DCMU (10 μM) is added after removing CO_2 (Curves g and h of Fig. 1), the fluorescence rises immediately to the lower P_2 level characteristic of the CO_2-depleted state. Thereafter, there is a very slow rise of fluorescence up to the higher P_2 level characteristic of 5% CO_2. Fig. 2 records a parallel experiment with cells from the same culture in which DCMU was added in the presence of 5% CO_2. In this case, fluorescence rises quickly and remains at the higher P_2 level. Note that in Figs 1, 2 and 8, CO_2 does not affect the fluorescence level obtained with DCMU.

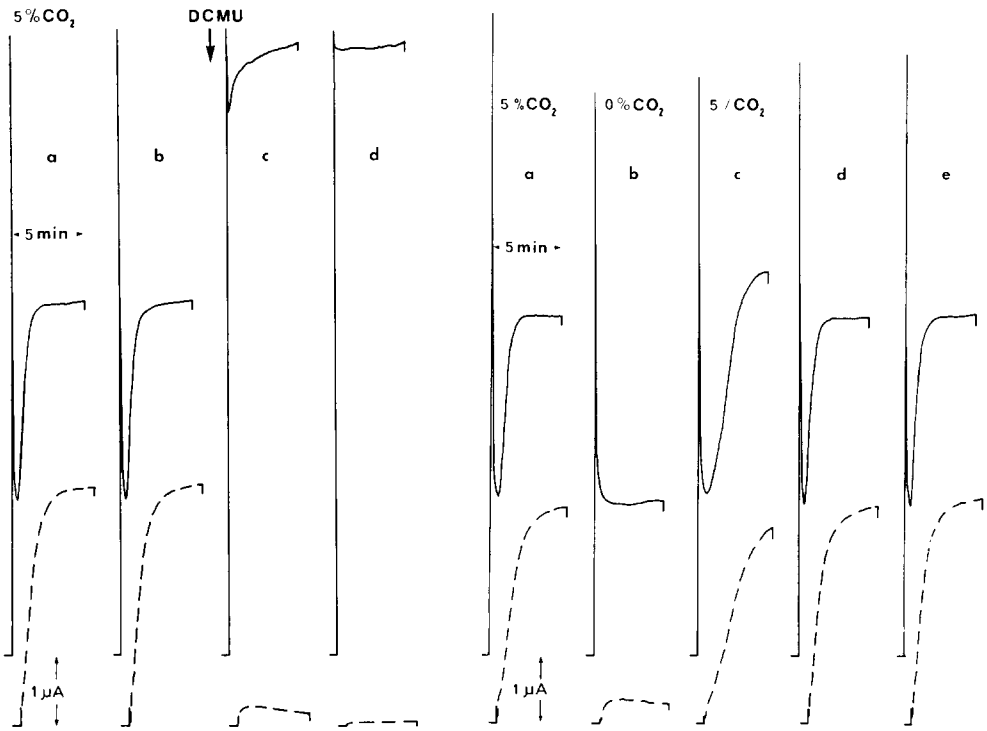

Fig. 2. Traces of oxygen evolution (dashed line) and 680 nm fluorescence (solid line) transients in *Chlorella pyrenoidosa* (6.4 μl cells per ml) with 5% CO_2 *plus* and *minus* 10 μM DCMU. Conditions same as Fig. 1 except 5% CO_2 held constant at pH 5.3. (a) and (b) two cycles without DCMU; (c) and (d) traces after the addition of 10 μM DCMU.

Fig. 3. Reversibility of CO_2 effects on oxygen evolution (dashed line) and 680 nm fluorescence transients (solid line) in *Chlorella pyrenoidosa* (6.4 μl cells per ml). Conditions identical to Fig. 1. (a) 5% CO_2 at pH 5.3; (b) 20th cycle after turning off CO_2; (c) 1st cycle after addition of 5% CO_2; (d) and (e) 3rd and 4th cycles after addition of 5% CO_2.

(In contrast with *Chlamydomonas*[1], *Chlorella* does not exhibit a clear-cut M_2–P_3 rise in the presence of DCMU in repeated light–dark cycles.)

Restoration of M_2–P_3 on adding CO_2

Fig. 3 shows that O_2 evolution, the M_2–P_3 rise, and the high steady-state fluorescence yield are quickly restored when 5% CO_2 is turned on (in this experiment, after 1.5 h of light–dark cycles in the absence of CO_2). Normal induction was seen in the 3rd cycle after readmitting CO_2. The prompt recovery shows that there is no photoautooxidative damage, the reversal of which, as is well known, requires periods of one to several hours.

Induction with "16-h" cells

Wang[8] found that the M_2–P_3 transient was most striking in *Chorella* harvested in the first hours of the light period and was absent in cells harvested 16–18 h after the beginning of light (*i.e.* in the 2nd to 4th h of the dark period). Fig. 4 shows the effects of CO_2 on induction in "16-h" cells, which were harvested and mounted on the electrode with a minimum exposure to light. In the presence of 5% CO_2 (Curves a and b), and with the normal cycle of 5 min actinic light – 3 min dark, only a slow O_2 evolution (about 4 $\mu l \cdot \mu l^{-1} \cdot h^{-1}$) was achieved by the end of the light period. At the same time, fluorescence induction was sluggish: the P_2–M_2 decline required 1 min, and thereafter only a slight, slow rise occurred. As the cycles were repeated and especially when longer illumination periods were provided (Curve c), a slow M_2–P_3 rise of normal magnitude was observed. When the CO_2 supply was turned off, the M_2–P_3 transient was abolished within three cycles, just as in "2-h" cells. In this experiment O_2 evolution remained low and essentially constant throughout. These observations, which have been repeated several times, confirm the inhibition

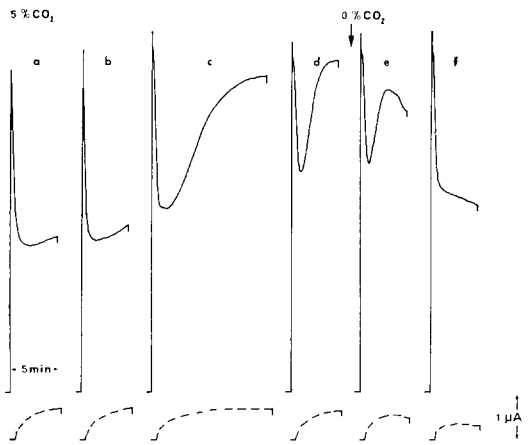

Fig. 4. The effects of CO_2 on oxygen evolution and 680 nm fluorescence in synchronous cultures of *Chlorella pyrenoidosa* (6.5 μl cells per ml) harvested after 16 h, or 2 h after cessation of the light period. Conditions again identical to Fig. 1 except measurements made at pH 5.2. (a) and (b) first two traces recorded; (c) transient recorded with 15-min illumination; (d) transient following (c) with regular 5 min illumination period; (e) and (f) traces recorded immediately following the cutoff of CO_2 to the media.

of O_2 evolution and the absence of an M_2–P_3 rise (as ordinarily measured) in 16-h cells, but it is clear that such cells can be made to exhibit the M_2–P_3 rise by long illumination.

Similarity of induction at pH 5.3 and 8.3

For *Chlorella pyrenoidosa*, Papageorgiou and Govindjee[13] claimed that slow fluorescence transients, in the 1st to 5th min of induction, are strongly dependent on pH. Fig. 5 records the results of an experiment showing that the large M_2–P_3 transient remains unchanged over the pH range 5.3–8.3, provided CO_2 is maintained, and that the transient is always absent when CO_2 is sparged. Curve a shows the typical fluorescence rise with 5% CO_2 at pH 5.3. When, simultaneously, the CO_2 is shut off and the pH shifted to 8.7 with NaOH, the total carbon concentration is maintained constant (at about 1.5 mM), but the distribution is changed from >91% dissolved CO_2 to about 95% bicarbonate. The result, as shown by Curves b and c, is the abolition of the M_2–P_3 rise and the strong inhibition of O_2 evolution already in the second cycle after the pH shift. When 5% CO_2 was provided anew and the pH maintained at 8.3 by addition of NaOH (the bicarbonate concentration

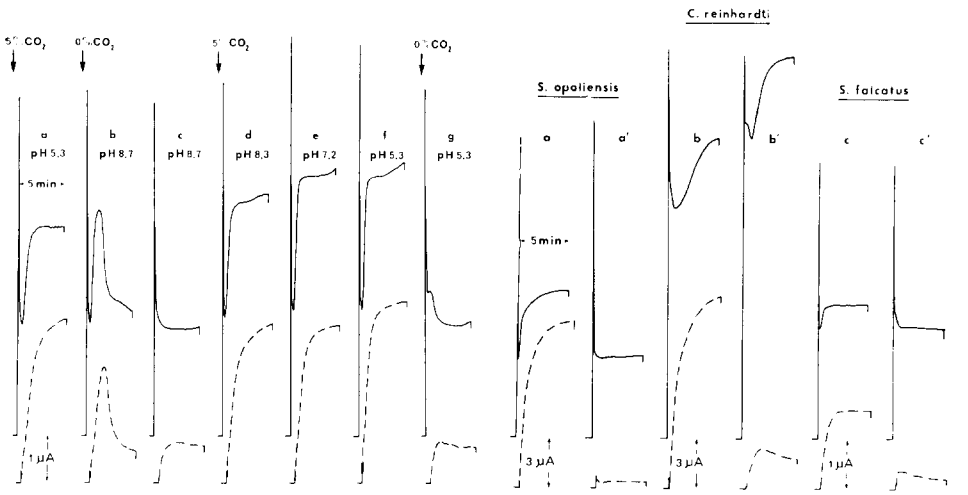

Fig. 5. The oxygen evolution (dashed line) and 680 nm fluorescence transients (solid line) in *Chlorella pyrenoidosa* (6.5 μl cells per ml) at different pH values of the suspension media. Conditions identical to Fig. 1 except light intensity was 5.35 mW·cm^{-2}. (a) The normal transient recorded with 5% CO_2 at pH 5.3; (b) the first trace obtained after CO_2 shut off and the pH simultaneously shifted to pH 8.7 with NaOH; (c) second transient obtained after procedure in (b); (d) transient at pH 8.3 with 5% CO_2 aeration; (e) transient at pH 7.2 with 5% CO_2 aeration; (f) transient at pH 5.3 with 5% CO_2 aeration; (g) transient at pH 5.3, 0.5 h after CO_2 removed.

Fig. 6. CO_2-dependent effects on fluorescence and oxygen evolution in other algal species. *Scenedesmus opoliensis* (5 μl cells per ml) on deep (0.33 mm) rate electrode with 5.7 mW·cm^{-2} illumination and (a) 5% CO_2, (á) 0% CO_2 at pH 5.2. *Chlamydomonas reinhardti* (5 μl cells per ml) on deep rate electrode at pH 5.4 with 6.1 mW·cm^{-2} illumination and (b) 5% CO_2 and (b') 0% CO_2. *Scenedesmus falcatus* (6 μl cells per ml) on shallow (0.2 mm) electrode at pH 5.3 with 5.25 mW·cm^{-2} illumination and (c) 5% CO_2 and (c') 0% CO_2.

rising to 50–100 mM), the M_2–P_3 rise and O_2 evolution were restored (Curve d). Titration with HCl to pH 7.2 and then to 5.3, while maintaining 5% CO_2, did not further change the fluorescence and O_2 inductions. Finally, when CO_2 was turned off again at pH 5.3 (Curve g), the M_2–P_3 rise was abolished and O_2 evolution inhibited just as at pH 8.7.

These observations show that pH *per se* has little or no effect on the M_2–P_3 rise, nor (so far as we can tell) on the P_3–S phase of induction. On the other hand, the decisive role of dissolved CO_2 at both pH 5.3 and 8.3 is obvious. It is also evident that bicarbonate is a poor carbon source for *Chlorella* photosynthesis.

Similar inductions in other species

Fig. 6 shows that the M_2–P_3 rise and the maintenance of a high fluorescence yield at the end of a 5-min light period depend on the presence of CO_2 also in *Scenedesmus opolienses* and *Scenedesmus falcatus* (species isolated by us in pure culture from local waters). In these two cases, the extent of the M_2–P_3 rise was smaller than in "2-h" *Chlorella* (we did not grow these species synchronously), but the qualitative results are the same as for *Chlorella*. On the other hand, *Chlamydomonas reinhardti*, the species studied by Bannister and Rice[1], behaves differently. It exhibits a marked M_2–P_3 rise, both in the presence and absence of CO_2. Moreover, when CO_2 is cut off, there are complementary effects: steady-state fluorescence rises while steady-state O_2 evolution is inhibited. Rice[14] has observed the same effect. In this connection, we recall that the M_2–P_3 rise is eliminated by DCMU in *Chlorella*, but not in *Chlamydomonas*.

Effect of CO_2 on light curves of steady-state O_2 evolution and fluorescence

Fig. 7 shows results which have been obtained several times with "2-h" *Chlorella*. With 5% CO_2, the fluorescence and O_2 light curves exhibit the well-known relationship (see, for example ref. 5). The fluorescence light curve consists of two linear portions: a low-yield portion over a low-illumination range and a high-yield portion at higher illumination. The transition between low and high yield occurs approximately at the illumination at which the O_2 light curve first becomes non-linear. As expected, when the dissolved CO_2 concentration is lowered, the yield of O_2 evolution at low light remains unchanged but the light-saturated rate declines. What is especially interesting is that, at a somewhat reduced CO_2 level (2.4%), the fluorescence light curve follows the corresponding 5% CO_2 curve throughout the low-yield portion and into the high-yield region, but then, with further increase of illumination, the curve crosses back to the low-yield condition. The lower the CO_2 level, the lower the illumination for "crossing back".

In Fig. 7, the steady-state fluorescence S (at the end of the 5-min light period) has been plotted. When an intermediate CO_2 level is maintained and strong enough actinic light provided, then in each successive light period fluorescence induction reproducibly exhibits an M_2–P_3 rise followed by a rapid P_3–S decline. (A similar induction was seen in Curves b and c of Fig. 1, where the phenomenon was a transient one following a change from 5 to 0% CO_2). Thus, the fluorescence light curves of Fig. 7 indicate not only the fluorescence level after 5 min but also the limits approached by the M_2 and P_3 levels earlier in induction. For example, during induction at a

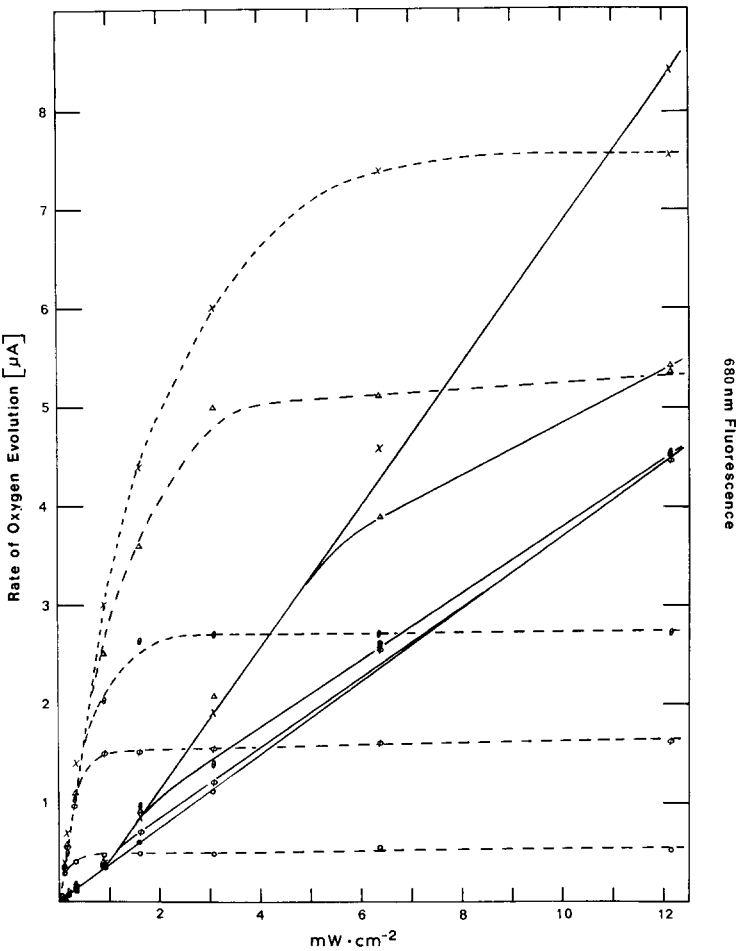

Fig. 7. Light curves for 680 nm fluorescence (solid lines) and oxygen evolution (dashed lines) at various CO_2 concentrations. *Chlorella pyrenoidosa* (8 μl cells per ml) in deeply recessed (0.33 mm) platinum electrode at constant pH 5.2. CO_2 partial pressures: ×, 5% CO_2; △, 2.4% CO_2, θ, 1% CO_2: φ, 0.5% CO_2: O, 0% CO_2.

given illumination and CO_2 concentration the M_2 level approaches the low yield limit (0% CO_2 line) and the P_3 level tends toward the high-yield limit (5% CO_2 line).

CO_2 effect on fluorescence induction measured in a fluoroscope

The possibility that induction in cells mounted on the platinum rate electrode, might be affected by changes of the CO_2 diffusion gradient inherent in electrode geometry, led to experiments with cell suspensions. In the apparatus described previously[11], an algal suspension was contained in a cuvette, and the gas phase was adjusted by bubbling prior to and during the measurement. The relative fluorescence yield, exerted by a very weak (0.002 mW·cm^{-2}) modulated exciting beam, could be measured both in the presence and absence of the modulated actinic beam

(1.0 $mW \cdot cm^{-2}$). Fig. 8 records a typical experiment. In the presence but not in the absence of 1% CO_2, there is a strong M_2–P_3 rise. This observation proves that the CO_2 dependence of the M_2–P_3 rise is a cellular phenomenon (not an artifact associated with the platinum electrode).

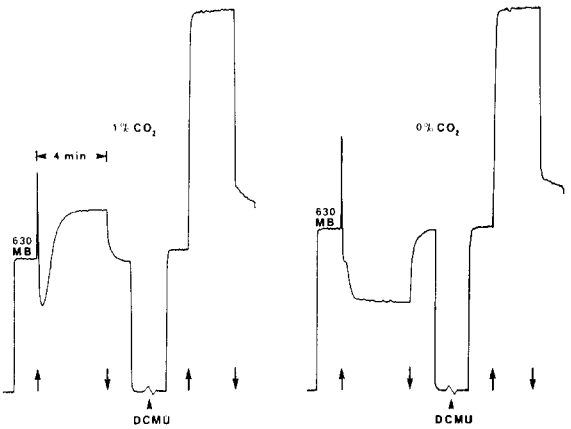

Fig. 8. Fluorescence induction transients of *Chlorella pyrenoidosa* (approx. $6 \cdot 10^6$ cells per ml) suspended in 2.5 ml medium. MB is the 696-nm fluorescence elicited by the weak modulated measuring beam as described in Methods. Arrows (↑ on, ↓ off) indicate the addition of white actinic light to the sample. First trace obtained with 1% CO_2 aeration and second trace with CO_2-free air. DCMU (4 μM) added in dark before second cycle.

Especially noteworthy is that the M_2 level, whether reached transiently in the presence of CO_2 or maintained in the absence of CO_2, is substantially lower than the fluorescence yield in "darkness" (*i.e.* with the actinic light off). Moreover, actinic light is required to reach the minimum-yield condition. A similar lowering of the fluorescence yield by actinic light was reported by Hoch and Randles[11], for *Porphyridium* in the presence of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) and under anaerobic conditions, and by Vredenburg[18] in *Nitella*.

It should be mentioned that the absence of CO_2 appears to increase the dark-level fluorescence 10% in Fig. 8. This result could not be definitely confirmed in the experiments establishing the light curves of oxygen evolution and fluorescence in Fig. 7 because some slow drift in the signals over the long duration of the measurements, along with their limited precision in the low-illumination range, may have obscured this effect.

DISCUSSION

Our results add a new example of parallelism of electron transport and fluorescence. In this case, parallelism is manifested not only in the steady state (fluorescence and oxygen evolution declining together as CO_2 concentration falls), but also during induction (both oxygen evolution and fluorescence first increasing in the M_2–P_3 phase, then falling during the P_3–S phase). A number of other instances of parallelism have been reported. Among intact algae, parallelism has been observed (1) when *Chlorella* is poisoned with the uncoupler FCCP[15], (2) during induction

of *Chlamydomonas*[1,14], and (3) in the Bonaventura effect in *Chlorella* in response to wavelength shifts. Recently parallelism has also been observed in chloroplasts. Rurainski *et al.*[16] and Rurainski and Hoch[17] have shown that addition of Mg^{2+} (2.5 mM) increases $NADP^+$ reduction and fluorescence 2-fold or more, while at the same time P_{700} turn-over is 80–90% inhibited.

Of the two explanations for parallelism, (i) changes in de-excitation processes and (ii) changes in α (*i.e.* in energy distribution between photosystems), present information provides no adequate test. Currently, the α-change hypothesis is the more popular, based on the work of Bonaventura and Myers[5] and on the report of Duysens[25] that the oxygen flash yield is constant during the transition from State 2 to State 1. The latter observation would rule out the earlier Q′ hypothesis[26] of inactivation of System II units.

On the other hand, the α-change hypothesis was conceived of to explain rather small fluorescence changes, which Bonaventura and Myers[5] calculated could be accounted for by about a 10% change in α. Our work shows that when CO_2 is removed, steady-state fluorescence falls 2-fold, and presumably the fraction of open System II traps also declines. The α-value would therefore have to fall more than 2-fold. An even more extreme α-change would be required to explain the effects of Mg^{2+} addition to chloroplasts, for example, the 2-fold increases in fluorescence and in rate of $NADP^+$ reduction, and an 80–90% inhibition of P_{700} flux[17]. Since Mg^{2+} increases the light-saturated rate of $NADP^+$ reduction, but does not alter the shape of the light curve, nor the values of the time constants for relaxation of P_{700} or the modulated component of fluorescence, Rurainski and Hoch [17] concluded that Mg^{2+} increases the number of fluorescent and chemically active System II units. Thus they adopted a unit activation rather than α-change explanation. While available evidence does not allow a final choice between unit activation and α-changes, the large magnitude of the parallel changes in fluorescence and electron transport, both in whole cells and chloroplasts, lead us to favor the unit activation hypothesis.

Papageorgiou and Govindjee[12] and Vredenburg[18] have offered evidence that fluorescence yield changes are correlated with ion-transport phenomena in whole cells. Thus far, no firm causal relationship has been established; should it be established in the future, it will still be necessary to show whether ion transport leads to fluorescence changes by activation of System II units or by α-changes (*i.e.* by altering energy distribution between System I and II units).

Murata and Sugahara[27] have explored the possible relation between fluorescence yield and the "energy state" (presumably determined by the magnitude of ion gradients) in chloroplasts. They showed that the high fluorescence yield, in strong light and in the presence of DCMU, declined about 15% when phenazine methosulfate (PMS) was added, and later was restored when an uncoupler was added. They proposed that the addition of PMS led to cyclic electron transport, and to increased ion gradients, which depress the fluorescence yield. For us this hypothesis raises some difficulties. First, the low fluorescence yield of chloroplasts in darkness apparently must be attributed to the maintenance of steep ion gradients in the dark. Second, in strongly illuminated algae, with or without DCMU, the uncoupler FCCP always reduces the fluorescence yield[15,11]. Thirdly, the removal of CO_2 lowers the fluorescence of unpoisoned algae but not that of DCMU-poisoned cells; thus removal of CO_2 does not have the same effect as adding PMS to chloroplasts.

At present, the results of Murata and Sugahara[27] with poisoned chloroplasts are difficult to relate to our observations mainly with unpoisoned whole cells.

Finally, the important role CO_2 plays in determining the fluorescence and photochemical state of the photosynthetic apparatus has obvious bearing on a number of previously reported observations. The bicarbonate requirement for the Hill reaction is one[19,20]. Another is the oscillations in oxygen evolution[21,22] and fluorescence[23] which may reflect an unstable fluctuation between active and inactive units (or between α-states) (Recent work[24] shows that ATP also oscillates but always tends to return to the original level.) Thirdly, the observation of a P_3–S fluorescence decline in ungassed algal suspensions[12] ought to be considered a sign of CO_2 depletion.

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