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Interactions between Photosynthesis and Respiration in the Green Alga *Chlamydomonas reinhardtii*[1]

Characterization of Light-Enhanced Dark Respiration

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The rate of respiratory O_2 consumption by *Chlamydomonas reinhardtii* cell suspensions was greater after a period of photosynthesis than in the preceding dark period. This "light-enhanced dark respiration" (LEDR) was a function of both the duration of illumination and the photon fluence rate. Mass spectrometric measurements of gas exchange indicated that the rate of gross respiratory O_2 consumption increased during photosynthesis, whereas gross respiratory CO_2 production decreased in a photon fluence rate-dependent manner. The rate of postillumination O_2 consumption provided a good measure of the O_2 consumption rate in the light. LEDR was substantially decreased by the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea or glycolaldehyde, suggesting that LEDR was photosynthesis-dependent. The onset of photosynthesis resulted in an increase in the cellular levels of phosphoglycerate, malate, and phospho*enol*pyruvate, and a decrease in whole-cell ATP and citrate levels; all of these changes were rapidly reversed upon darkening. These results are consistent with a decrease in the rate of respiratory carbon flow during photosynthesis, whereas the increase in respiratory O_2 consumption during photosynthesis may be mediated by the export of photogenerated reductant from the chloroplast. We suggest that photosynthesis interacts with respiration at more than one level, simultaneously decreasing the rate of respiratory carbon flow while increasing the rate of respiratory O_2 consumption.

It is frequently observed that the rate of respiratory O_2 consumption after a period of photosynthesis is greater than the rate prior to illumination (Raghavendra et al., 1994). This phenomenon has been termed "light-enhanced dark respiration" (LEDR) and is observed in both microalgae (Stone and Ganff, 1981; Weger et al., 1989; Beardall et al., 1994) and in plants (Azcón-Bieto et al., 1983; Reddy et al., 1991; Gardeström et al., 1992; Hill and Bryce, 1992). It has been postulated that LEDR is the result of a photosynthesis-derived increase in the amount of substrate for respiration (reviewed by Raghavendra et al., 1994), and LEDR is sometimes associated with an increase in both soluble sugars and in the rate of alternative oxidase activity (Azcón-Bieto et al., 1983). Although it has been suggested that LEDR is strictly a postillumination phenomenon (Bate et al., 1988), there is evidence that the immediate postillumination rate of O_2 consumption provides a good estimate of the O_2 consumption rate in the preceding light period (Weger et al., 1989).

A significant debate exists about the effects of photosynthesis on respiratory processes (respiratory carbon flow and mitochondrial ETC activity). Various studies have presented evidence that respiratory carbon flow during photosynthesis occurs at rates 25 to 100% of the dark control (reviewed by Krömer, 1995), whereas the effects of light on O_2 consumption by the mitochondrial ETC are even more contentious. Evidence from various studies indicates that the overall rate of O_2 consumption decreases in the light, increases in the light, is due predominately to the Mehler reaction and/or is due predominately to mitochondrial ETC activity (reviewed by Turpin and Weger, 1989). Additionally, it has been suggested that stimulation of respiration by light may occur independently of photosynthesis, via a physiological response to blue light (Kowallik, 1982).

In this study we examined the nature of LEDR in the unicellular green alga *Chlamydomonas reinhardtii*, using evidence from both gas-exchange measurements and changes in the levels of respiratory metabolites during dark-to-light and light-to-dark transitions. We provide evidence that LEDR is a photosynthesis-dependent phenomenon, and that inhibitors of photosynthesis inhibit LEDR. We demonstrate that photosynthesis results in an increase in the rate of respiratory O_2 consumption during photosynthesis, and that postillumination rates of O_2 consumption represent a good estimate of the rate of respiratory O_2 consumption in the preceding light period.

MATERIALS AND METHODS

Green alga (*Chlamydomonas reinhardtii* UTEX 89) cells were grown in semi-continuous culture in water-jacketed, glass chemostat vessels at a temperature of 20°C and a PFR

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Abbreviations: ATP-PFK, ATP-dependent phosphofructokinase; Chl, chlorophyll; ETC, electron transport chain; FBP, Fru bisphosphatase; LEDR, light-enhanced dark respiration; PFR, photon fluence rate; PGA, phosphoglycerate.

of 220 μmol quanta m^{-2} s^{-1} (Torkelson et al., 1995). Cells were bubbled with a humidified stream of 1% CO_2 in air. Light was provided by a bank of cool-white, high-output fluorescent lights (F48T12/CW/HO, Philips Electronics, Scarborough, Ontario, Canada). The medium was a modification of that formulated by Hughes et al. (1958), and contained 300 μM K_2HPO_4, 3 mM NH_4NO_3, and was buffered at pH 7.0 with 15 mM Hepes-KOH. Minor elements and iron were supplied as described by Weger et al. (1996). Cells were maintained in exponential phase by continuous dilution, and draining of the culture once per day. The cell growth rate was approximately 1.3 d^{-1}, and cells were dark-adapted for 12 h prior to the experiments.

Gas Exchange

Both respiratory O_2 consumption and photosynthetic O_2 evolution were quantified using two different methods: an O_2 electrode (Hansatech, King's Lynn, UK) and a membrane-inlet mass spectrometer (MM14-80SC, VG Gas Analysis, Middlewich, UK), CO_2 exchange was quantified by MS. Illumination for both types of experiments was provided by a single tungsten halogen lamp (ENX, 360 W, Sylvania). All experiments were performed at 20°C. For experiments using the O_2 electrode, 1.0-mL aliquots of dark-adapted cells were used. Inorganic carbon was added as $KHCO_3$ to a final concentration of 2 mM. In preliminary experiments temperature changes inside the O_2 cuvette were monitored using a thermocouple sensor that consisted of very fine copper and constantan wires. At the highest PFR, temperatures inside the cuvette changed less than 0.08°C.

For experiments examining the effects of photorespiratory conditions on LEDR, cells were centrifuged and resuspended in CO_2-free buffer (15 mM Na-Hepes HCl, pH 7.0) and placed in the O_2 electrode cuvette under a stream of N_2 in the dark. Cells were briefly bubbled with 100% O_2 to allow respiratory O_2 consumption to occur. After a steady-state rate of respiration was attained, the light was turned on (PFR = 800 μmol quanta m^{-2} s^{-1}) for 15 min. Residual inorganic carbon in the cuvette was fixed by photosynthesis within approximately 5 min, and thereafter, net photosynthetic O_2 exchange equaled zero. LEDR was measured as the rate of O_2 consumption immediately after turning off the light.

For investigations on the effects of light quality, cell suspensions were illuminated for 15 min, and the O_2 consumption rate immediately postillumination was measured. Blue light was produced using a glass narrow-band-pass filter (Corning C.S. 5–75), with a peak transmittance at 458 nm and a one-half-bandwidth of 18 nm, and red light was produced with a long-pass glass filter (Corning C.S. 2–61; 50% transmittance at 630 nm, 80% at 662 nm, and <0.5% at 590 nm). The control light was produced by a tungsten halogen lamp. PFR for all light qualities was 15 μmol quanta m^{-2} s^{-1}.

On-line mass spectrometric gas exchange was monitored using a mass spectrometer (VG Gas Analysis) that was equipped with a membrane inlet inside a 6.0-mL water-jacketed glass cuvette. Dark-adapted cells (6.0 mL) were harvested by centrifugation and resuspended in O_2- and CO_2-free buffer (15 mM NaHepes- HCl, pH 7.0). Inorganic carbon was added as 99% [^{13}C]K_2CO_3 to a concentration of 2 mM. $^{18}O_2$ was added as a bubble to the algal suspension, to a concentration of approximately 500 μM; the bubble was removed prior to the experiment. To facilitate the equilibrium between CO_2 and HCO_3^-, 15 μL of 15 mg mL^{-1} carbonic anhydrase (Sigma) was added to the suspension. The gases $^{16}O_2$, $^{18}O_2$, $^{12}CO_2$, and $^{13}CO_2$ were monitored in a cycle time of approximately 7 s (Weger et al., 1988). Isotopes were from Merck, Sharpe & Dohme (Montreal, Canada). Rates of O_2 uptake and evolution were calculated according to Radmer and Kok (1976), taking into account the production of $^{16}O_2$ by photosynthesis and the consumption of $^{16}O_2$ and $^{18}O_2$ both by the cells and the mass spectrometer (Peltier and Thibault, 1985). Rates of CO_2 exchange were calculated in an analogous manner, with the exception that mass spectrometer consumption of the isotope was not considered. At pH 7.0, most of the inorganic carbon was in the form of HCO_3^-, which is not consumed by the mass spectrometer (Miller et al., 1988).

The mass spectrometer was calibrated for all isotopes that were used. The response of the mass spectrometer to $^{16}O_2$ was determined by bubbling air through 6.0 mL of distilled water in the mass spectrometer cuvette. The air equilibrium O_2 concentration at 20°C was calculated according to Green and Carritt (1967). $^{18}O_2$ was calibrated by drawing 4.0 mL of $^{18}O_2$ gas into a 50-mL syringe that contained 30 mL of N_2-sparged buffer. The syringe was shaken for 20 min to promote saturation of the buffer with $^{18}O_2$. The concentration of total O_2 in the buffer (mostly $^{18}O_2$) was quantified using an O_2 electrode. Next, 6.0 mL of the $^{18}O_2$-equilibrated buffer was placed in the mass spectrometer cuvette, which had been purged with N_2. Contaminating $^{16}O_2$ in the buffer was subtracted from the calibration that was provided by the O_2 electrode, and the response of the mass spectrometer to $^{18}O_2$ was calculated. Calibration of C isotopes was performed by injecting known amounts of $KH^{12}CO_3$ or $K_2{}^{13}CO_3$ into 6.0 mL of buffer in the presence of carbonic anhydrase to facilitate the CO_2/HCO_3^- equilibrium. Thus, monitoring of the CO_2 allowed quantification of total inorganic carbon in the medium. The Chl concentrations for all gas-exchange experiments were in the range of 19 to 21 μg mL^{-1}.

Metabolite Experiments

For metabolite experiments, dark-adapted cells (approximately 20 μg Chl mL^{-1}) were harvested by centrifugation (2000*g* for 5 min) and resuspended in medium at a Chl concentration of approximately 600 μg Chl mL^{-1}. Concentrated cell suspensions were placed in a water-jacketed reaction glass vessel (20°C), bubbled with humidified air, and gently stirred for 1 h in the dark prior to the beginning of the experiment. Inorganic carbon for photosynthesis was added as 2 mM $KHCO_3$.

During the experiment, individual 3.0-mL samples were removed and immediately put into 40-mL glass centrifuge tubes in liquid N_2. Since a concentrated cell suspension was needed for the metabolite experiments, illumination was provided by three tungsten-halogen light sources; two illuminating from the side and one illuminating from above.

Roux flasks filled with water were inserted between the light sources and the glass vessel to serve as heat sinks. Measurements with a copper/constantan thermocouple inside the vessel indicated that the temperature of the cell suspension did not increase by more than 0.2°C during illumination.

PFR inside the glass vessel was quantified using a photocell attached to a voltmeter. The photo-cell was calibrated against a quantum meter (model Li-185B, Li-Cor, Lincoln, NE). Using the above configuration of light sources, PFR in the center of an empty vessel was approximately 800 μmol quanta m^{-2} s^{-1}, and in the presence of the cell suspension, PFR was not less than 500 μmol quanta m^{-2} s^{-1} in any part of the vessel.

Samples for metabolite analysis were lyophilized for 48 h, and metabolites were extracted from lyophilized samples with 10% $HClO_4$. After 20 min, samples were transferred to microcentrifuge tubes and centrifuged for 4 min at 19,800*g*. After neutralizing with 5 N KOH/1 M triethanolamine, samples were centrifuged and stored at −80°C until analysis. All sample processing was performed on ice.

Metabolites were quantified using standard coupled enzymatic assays involving NAD(P) oxidation or reduction (Bergmeyer, 1974). Measurements were made using a diode array spectrophotometer (HP8452A, Hewlett-Packard), using wavelength integration and subtraction of background ([334–346 nm] minus [402–412 nm]). Coupling enzymes and reagents were from Boehringer Mannheim and Sigma.

Pyridine Nucleotides

NAD^+, $NADP^+$, NADH, and NADPH were determined using an enzymatic cycling procedure, modified from the protocols of Vanlerberghe et al. (1992) and Passoneau and Lowry (1993). Cells were concentrated by centrifugation (to approximately 300 μg Chl mL^{-1}), resuspended in medium, placed in a water-jacketed cuvette in the dark at 20°C, bubbled with O_2, and gently stirred for 1 h before the start of the experiment. The experiment was performed over a 10-min time course. Cell samples (200 μL) were killed by injection into a mixture of 900 μL of a kill solution (17.5 methanol:7.5 chloroform:1 [v/v] of either 1 N HCl [NAD^+, $NADP^+$] or 1 N KOH [NADH, NADPH]). The KOH samples were heated to 60°C for 5 min. All samples were neutralized using either HCl or KOH. Enzymatic cycling reagents were prepared according to the method of Vanlerberghe et al. (1992), and samples were cycled for 1 h at 38°C, followed by heating in a boiling-water bath for 3 min. The pyruvate or 6-phosphogluconate that was produced by the cycling reactions was quantified spectrophotometrically using coupled enzymatic assays (Bergmeyer, 1974). Standard curves were prepared for each set of analyses.

Other Methods

Chl was measured spectrophotometrically after extraction in 100% methanol (Porra et al., 1989). DCMU and propyl gallate were added from 0.5 M stocks in 95% ethanol. All other inhibitors were added from stocks in distilled water.

RESULTS

Gas-Exchange Measurements

Postillumination respiratory O_2 consumption by cells taken directly from the chemostat (grown in continuous light, nondark-adapted) was almost completely insensitive to a period of illumination (Fig. 1). LEDR of dark-adapted cells was a function of both the PFR and the time of illumination (Fig. 1). PFRs greater than 800 μmol quanta m^{-2} s^{-1} had no further stimulatory effect on LEDR (data not shown). When cells were placed under photorespiratory conditions for 15 min (PFR = 800 μmol quanta m^{-2} s^{-1}, no inorganic carbon), LEDR declined to 137 ± 9% of the control (n = 4), i.e. photorespiratory conditions inhibited LEDR. Preincubation of cell suspensions with inhibitors of photosynthesis (5 μM DCMU or 10 mM glycolaldehyde) also decreased the magnitude of LEDR (Table I).

Illumination with the light of a low PFR (15 μmol quanta m^{-2} s^{-1}) resulted in detectable LEDR; however, there was no detectable light-quality effect. Red, blue, and control light (tungsten halogen light source) were approximately equivalent in powering LEDR (data not shown).

The contribution of the alternative respiratory pathway to LEDR was examined by the use of the alternative oxidase inhibitor propyl gallate (Weger and Dasgupta, 1993). Addition of propyl gallate, added pre- or postillumination, had no effect on the postillumination O_2 consumption rates (data not shown).

Mass spectrometric measurement of gas exchange indicated that the onset of illumination resulted in a complex pattern of the stimulation of O_2 consumption (Fig. 2). There

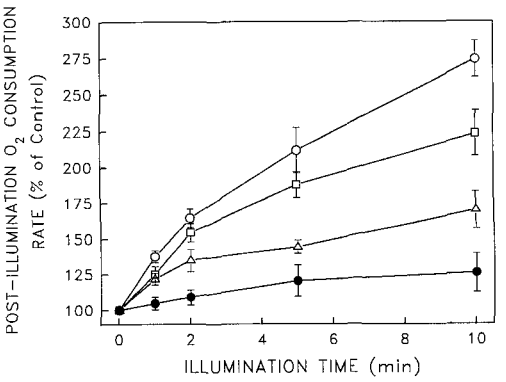

Figure 1. Postillumination O_2 consumption rates as a function of PFR and time of illumination. O_2 consumption was measured using an O_2 electrode. Open symbols, Dark-adapted cells (12 h of darkness); solid symbols, cell suspensions taken directly from the chemostat vessel. Reported rates of O_2 consumption occurred immediately upon darkening. Inorganic carbon concentration was 2 mM. For dark-adapted cells, PFR equals 50 (△), 150 (□), or 800 μmol quanta m^{-2} s^{-1} (○). PFR equals 800 μmol quanta m^{-2} s^{-1} for nondark-adapted cells (●). Bars represent SE (n = 3–5). The control rate of respiratory O_2 consumption prior to illumination was 106 ± 10 nmol O_2 mL^{-1} h^{-1} (n = 8) for dark-adapted cells and 166 ± 13 nmol O_2 mL^{-1} h^{-1} (n = 6) for cells taken directly from the chemostat.

Table I. *Effect of photosynthetic inhibitors on LEDR*

DCMU was added to a final concentration of 5 μM, and glycolaldehyde was added to 10 mM. Illumination was provided for 15 min. Rates are expressed as a percentage of the preilluminated control ± SE (n = 5).

PFR	LEDR		
	Control	+DCMU	+Glycolaldehyde
μmol quanta m^{-2} s^{-1}	% of control[a]		
50	172 ± 7	132 ± 10	ND[a]
150	222 ± 7	142 ± 7	ND
800	279 ± 3	153 ± 6	98 ± 2

[a] ND, Not determined.

was an immediate increase in the rate of O_2 consumption at PFRs of 150 or 800 μmol quanta m^{-2} s^{-1} (Fig. 2, B and C), but not at 50 μmol quanta m^{-2} s^{-1} (Fig. 2A). At 150 and 800 μmol quanta m^{-2} s^{-1}, the increase was followed by a subsequent decline in the O_2 consumption rate (but was still well above the control rate), followed by another increase (Fig. 2, B and C). Upon darkening, there was a slow decline in the O_2 consumption rate for all PFRs (Fig. 2). The rate of O_2 consumption immediately postillumination was a good estimate of the O_2 consumption rate in the light. After 5 min of postillumination darkness, the O_2 consumption rates were still much greater than the control (Fig. 2).

Two inhibitors of photosynthesis were examined for their effects on LEDR. DCMU is an inhibitor of the noncyclic electron flow after the primary electron acceptor of PSII, but cyclic electron flow around PSI is still possible in the presence of this compound (Trebst, 1980). Glycolaldehyde is a Calvin cycle inhibitor that prevents the regeneration of ribulose-1,5-bisphosphate from triose phosphate (Sicher, 1984). Inhibitors were added immediately prior to the beginning of the experiment. Addition of either DCMU or glycolaldehyde greatly reduced LEDR, but the two inhibitors had contrasting effects on O_2 consumption in the light. In the presence of 5 μM DCMU illumination with 800 μmol quanta m^{-2} s^{-1} had no measurable effect on O_2 consumption in the light (Fig. 3A). There was, however, a postillumination stimulation of O_2 consumption (Fig. 3A; Table I), which could not be inhibited by higher DCMU concentrations (data not shown).

In contrast to the results with DCMU, preincubation with 10 mM glycolaldehyde resulted in an immediate and prolonged stimulation of O_2 consumption upon the onset of illumination (Fig. 3B). O_2 consumption immediately returned to approximately control rates when the light was turned off.

Measurements of respiratory CO_2 production indicated that there were pronounced effects of PFR and of inhibitors (Figs. 4 and 5). In the absence of inhibitors, the onset of illumination resulted in a decrease in the rate of CO_2 production at all tested PFRs (Fig. 4A). The decrease in CO_2 production was a function of PFR, and at high PFR there was even an apparent negative rate of CO_2 production, beginning approximately in the middle of the light period (Fig. 4A). The onset of illumination resulted in the onset of gross photosynthetic CO_2 uptake, which ended with the imposition of darkness (Fig. 4B). At high PFR, there was a negative rate of postillumination CO_2 fixation, i.e. there was $^{13}CO_2$ production in darkness (Fig. 4B).

In the presence of glycolaldehyde, the onset of illumination (800 μmol quanta m^{-2} s^{-1}) resulted in a decrease in the rate of CO_2 production (Fig. 5), although the decline

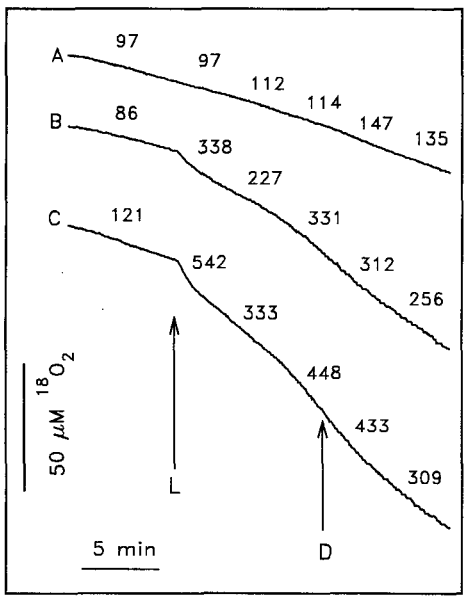

Figure 2. O_2 consumption by dark-adapted cells as affected by illumination. Gas exchange was monitored by MS, and O_2 concentration was corrected for simultaneous consumption of $^{16}O_2$ and $^{18}O_2$ and mass spectrometer consumption of gas. Numbers represent the rates of gross O_2 exchange in units of nmol O_2 mL^{-1} h^{-1}. PFR equals 50 (A), 150 (B), and 800 μmol quanta m^{-2} s^{-1} (C). L, Light; D, dark.

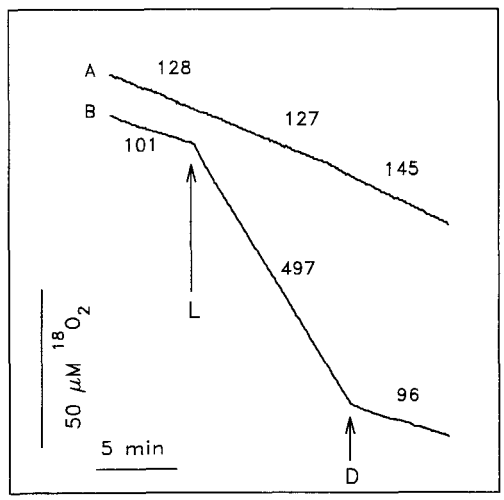

Figure 3. O_2 consumption by dark-adapted cells as affected by illumination in the presence of 5 μM DCMU (A) or 10 mM glycolaldehyde (B). Gas exchange was monitored by MS, and O_2 concentration was corrected for simultaneous consumption of $^{16}O_2$ and $^{18}O_2$ and mass spectrometer consumption of gas. Numbers represent the rates of gross O_2 exchange in units of nmol O_2 mL^{-1} h^{-1}. PFR was 800 μmol quanta m^{-2} s^{-1}. L, Light; D, dark.

was smaller than in the absence of glycolaldehyde (Fig. 4A). In the light photosynthetic CO_2 uptake was negligible in the presence of glycolaldehyde (data not shown). In contrast, illumination with the same PFR in the presence of DCMU resulted in a small decrease in CO_2 production (Fig. 5), whereas CO_2 fixation was completely abolished (data not shown).

From a plot of $^{12}CO_2$ production as a function of the $^{13}CO_2$ fixation rate, it is evident that a PFR of 50 μmol quanta m^{-2} s^{-1} resulted in a substantial decline in the rate of respiratory CO_2 production (Fig. 6). Further increases in PFR led to substantial increases in the rate of photosynthetic CO_2 fixation, but had only a small effect on the CO_2 production rate.

Metabolite Analysis

Illumination had little effect on the NAD pool; NADH and NAD^+ remained constant during dark-to-light and light-to-dark transitions (Fig. 7A). In contrast, NADPH levels declined during photosynthesis, with a recovery to control levels in the subsequent dark period (Fig. 7B).

Under control conditions, illumination resulted in large changes in the composition of the cellular adenylate pool. Cellular ATP levels declined and ADP and AMP levels increased. These changes were rapidly reversed upon darkening of the cells (Fig. 8). The onset of illumination resulted in an increase in cellular PGA levels (Fig. 9A). Steady-state PGA levels were attained approximately 10 min into the light period, and upon darkening, PGA levels rapidly declined. The effects of illumination on the malate levels (Fig. 9B) were similar to those on levels of PGA; malate levels increased upon illumination and decreased upon darkening (Fig. 9B).

Changes in whole-cell levels of other photosynthetic and respiratory metabolites upon illumination and subsequent darkening were smaller than the changes reported for PGA or malate. Triose phosphate levels changed little upon the onset of illumination, whereas Fru-6-P increased and FBP declined (Fig. 10A). Glc-6-P levels changed in parallel with Fru-6-P levels (Fig. 10); Fru-6-P and Glc-6-P declined immediately upon darkening. PEP slowly increased in the light, and pyruvate levels were mostly unaffected (Fig. 11A). Upon darkening, pyruvate exhibited a transient in-

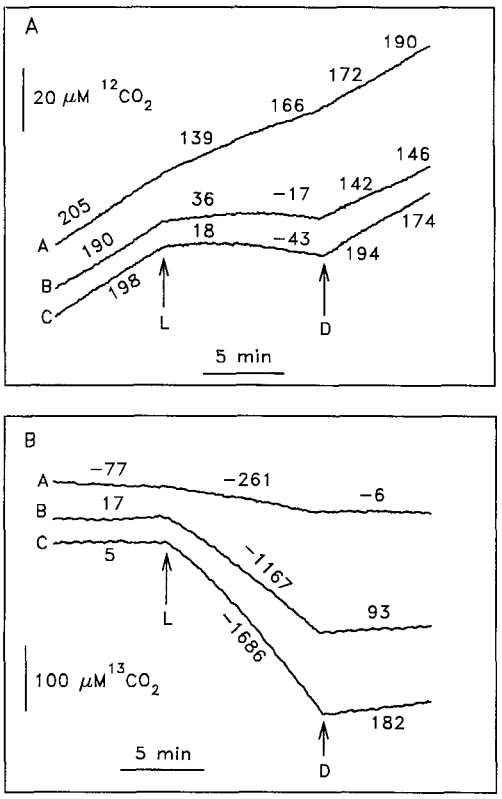

Figure 4. CO_2 exchange by dark-adapted cells as affected by illumination. A, CO_2 production; B, CO_2 fixation. Gas exchange was monitored by MS, and CO_2 concentration was corrected for simultaneous fixation of $^{12}CO_2$ and $^{13}CO_2$. Numbers represent the rates of gross CO_2 exchange in units of nmol CO_2 mL^{-1} h^{-1}. PFR equals 50 (A), 150 (B), and 800 μmol quanta m^{-2} s^{-1} (C). L, Light; D, dark.

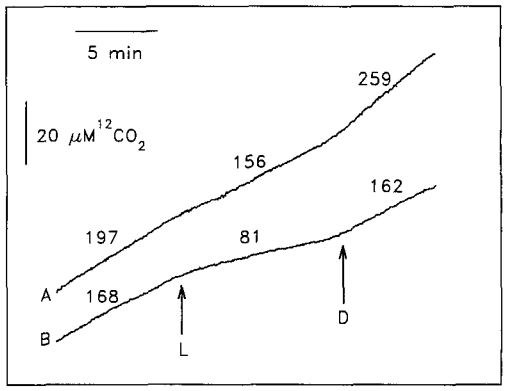

Figure 5. CO_2 exchange by dark-adapted cells as affected by illumination in the presence of 5 μM DCMU (A) or 10 mM glycolaldehyde (B). Gas exchange was monitored by MS, and CO_2 concentration was corrected for simultaneous fixation of $^{12}CO_2$ and $^{13}CO_2$. Numbers represent the rates of gross CO_2 exchange in units of nmol CO_2 mL^{-1} h^{-1}. PFR was 800 μmol quanta m^{-2} s^{-1}. Gross CO_2 fixation was negligible. L, Light; D, dark.

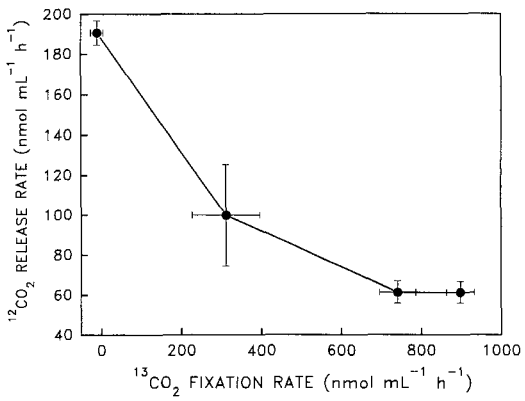

Figure 6. Rate of respiratory release of $^{12}CO_2$ by dark-adapted cells as a function of the gross photosynthetic ($^{13}CO_2$) fixation rate. Bars represent SE (n = 4–9). Gas exchange was measured by MS. Rates of gas exchange from the initial 2 min of illumination are presented.

crease (Fig. 11A). Citrate slowly decreased in the light and exhibited a transient, postillumination increase (Fig. 11B).

DISCUSSION

The postillumination rate of respiratory O_2 consumption (LEDR) by dark-adapted cells was clearly a function of both PFR and the duration of illumination (Fig. 1), suggesting a close link to photosynthesis. The lack of substantial LEDR in cells taken directly from photoautotrophic culture (Fig. 1) is correlated with the higher rates of respiratory O_2 consumption observed in those cells, i.e. these cells were already fully light-acclimated.

Similar to the data from pea mesophyll protoplasts (Reddy et al., 1991), LEDR was substantially decreased by preincubation of the cell suspension with photosynthetic inhibitors. Inhibition of the Calvin cycle with glycolaldehyde or inhibition of noncyclic photosynthetic ETC activity with DCMU substantially reduced the magnitude of LEDR (Table I). This is in contrast to the work of Hill and Bryce (1992), who reported that LEDR of barley mesophyll protoplasts was insensitive to preincubation with 20 mM glycolaldehyde. Photorespiratory conditions (high PFR, zero inorganic carbon) resulted in a decrease in the magnitude of LEDR. Taken as a whole, these results suggest that LEDR was a function of the rate of photosynthetic CO_2 fixation.

Other workers have suggested that light quality may affect respiratory metabolism. Illumination with low-PFR blue light may result in the stimulation of respiratory carbon flow, which is independent of photosynthesis (Kowallik, 1982). Although the mechanism responsible for blue-light stimulation of respiration in plants and algae is not yet clear, our results indicated that blue light did not possess any special stimulatory properties in *C. reinhardtii* (data not shown). LEDR in *C. reinhardtii* is not likely to be a manifestation of the "blue-light syndrome," and appears to be photosynthesis-dependent.

Work with wheat leaves has suggested that the increase in the postillumination rate of O_2 consumption is mediated primarily by an increase in the rate of O_2 consumption by

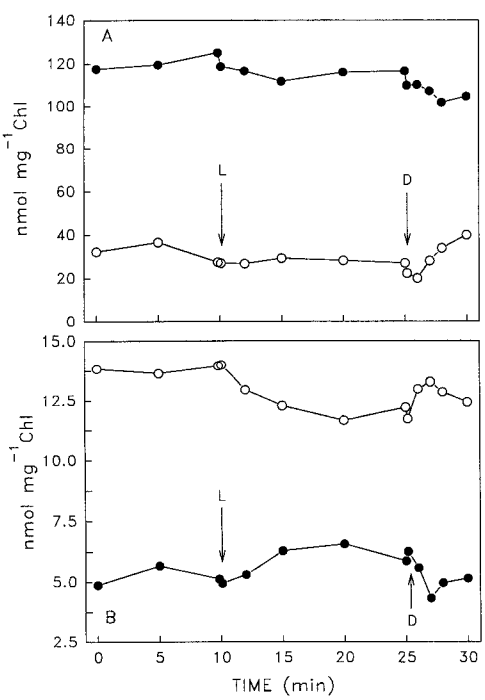

Figure 7. Cellular pyridine nucleotide levels as affected by illumination. A, NADH (○) and NAD^+ (●) (n = 6); B, NADPH (○) and $NADP^+$ (●) (n = 4). SE was less than 20% of the mean for all points. L, Light; D, dark.

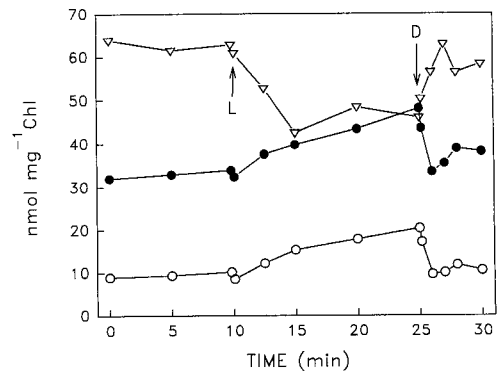

Figure 8. Cellular adenylate levels of dark-adapted cells as affected by illumination. Data are the means of at least four experiments. SE was less than 10% of the mean for all points. L, Light; D, dark. ▽, ATP; ●, ADP; ○, AMP.

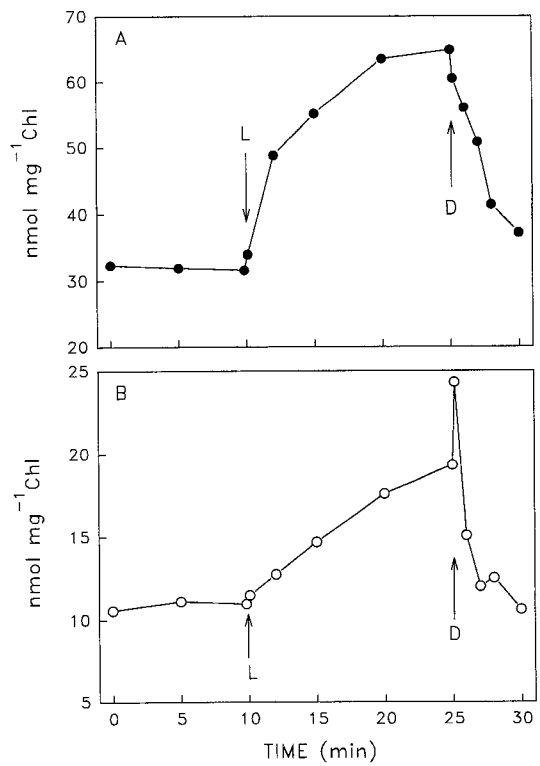

Figure 9. Cellular levels of PGA (A) and malate (B) of dark-adapted cells as affected by illumination. Data are the means of at least four experiments. SE was less than 10% of the mean for all PGA data points, and less than 20% of the mean for all malate data points. L, Light; D, dark.

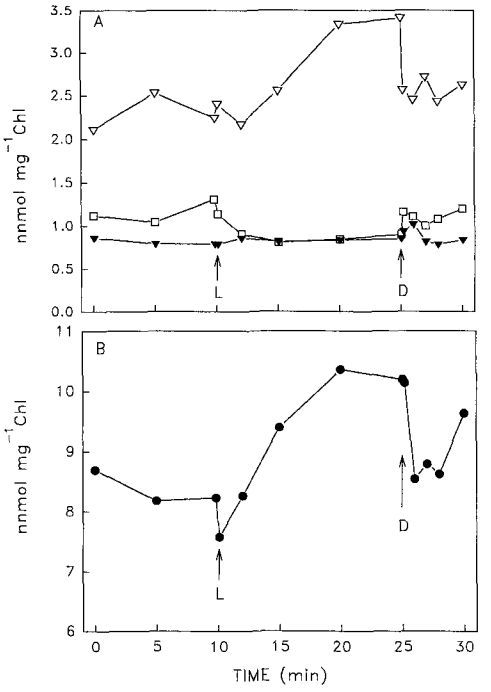

Figure 10. Cellular levels of triose phosphate (▼), FBP (□), Fru-6-P (▽), and Glc-6-P (B, ●) as affected by illumination. Experiments were run with dark-adapted cells in the absence of inhibitors. Data are the means of at least four experiments. SE was less than 15% of the means for all points. L, Light; D, dark.

the alternative oxidase (acting as an energy overflow), whereas preillumination respiratory O_2 consumption is mediated by the Cyt pathway (Azcón-Bieto et al., 1983). In contrast, addition of the alternative oxidase inhibitor propyl gallate had no measurable effect on the rate of respiratory O_2 consumption by the *C. reinhardtii* cell suspensions, either pre- or postillumination (data not shown), suggesting that the alternative oxidase was not functioning as an overflow during LEDR by *C. reinhardtii*.

Mass spectrometric measurement of O_2 consumption in the light indicated that the O_2 consumption rate immediately postillumination provided a good estimate of the O_2 consumption rate in the preceding light period (Fig. 4). Respiration rates only declined slowly after illumination. The fact that turning the light off did not result in a sudden decrease in O_2 consumption argues against a measurable contribution of the Mehler reaction (O_2 photoreduction) to O_2 consumption under steady-state conditions in the light. This is in contrast to the work of Sültemeyer et al. (1986, 1987), who concluded that at high PFR, O_2 consumption by *C. reinhardtii* was mediated to a large extent by the Mehler reaction. We suggest that steady-state O_2 consumption, even at 800 μmol quanta m^{-2} s^{-1}, was mediated primarily by mitochondrial ETC activity. Similar to our results, Peltier and Thibault (1985), using a mass spectrometer to monitor gas exchange, did not detect Mehler reaction-mediated O_2 consumption during photosynthesis by *C. reinhardtii*.

Although DCMU completely abolished the stimulation of O_2 consumption during illumination, there was still a small LEDR that could be consistently observed postillumination (Table I; Fig. 3A). This may suggest that light, independent of photosynthesis, may interact with respiration in a quantitatively minor way. Illumination of cell suspensions in the presence of gylcolaldehyde resulted in a large and sustained increase in O_2 consumption (Fig. 3B). The O_2 consumption rate immediately returned to control rates upon turning off the light, suggesting that light stimulation of O_2 consumption was mediated by the Mehler reaction. The Mehler reaction may have been responsible for the transient stimulation of O_2 consumption upon illumination (Fig. 2, B and C) (Radmer and Kok, 1976). The fact that the postillumination O_2 consumption rate was similar to the control rate in Figure 3B suggests that, in the presence of glycolaldehyde, light did not stimulate mitochondrial ETC activity. Evidence from both the O_2 electrode experiments (Table I) and mass spectrometric measurements of O_2 exchange (Figs. 2 and 3) suggest that photosynthetic CO_2 fixation is required for LEDR in *C. reinhardtii*.

Under control conditions (absence of inhibitors), or in the presence of 10 mM glycolaldehyde, we consistently observed that illumination resulted in a decrease in the rate of $^{12}CO_2$ production (Figs. 4A and 5). There has been much debate about whether mass spectrometric measurements of CO_2 release in the light are a reliable measure of respiratory CO_2 production, or whether they are biased by pho-

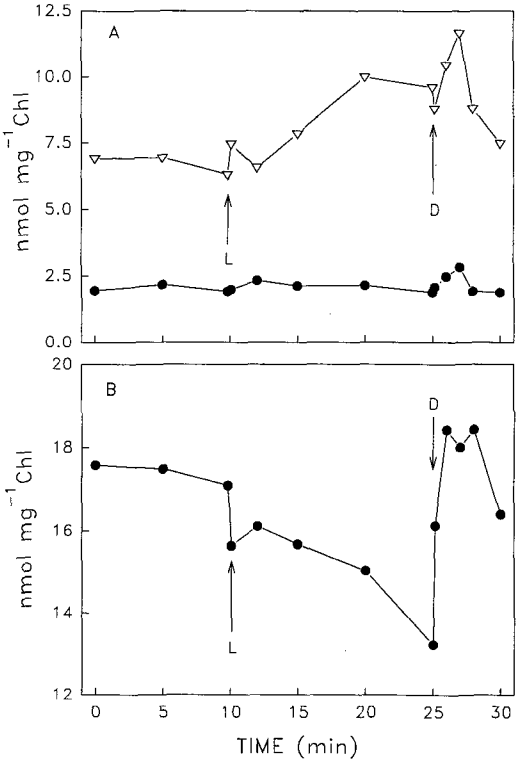

Figure 11. Cellular levels of PEP and pyruvate (A, ▽ and ●, respectively) and citrate (B) as affected by illumination. Experiments were run with dark-adapted cells in the absence of inhibitors. Data are the means of at least four experiments. SE was less than 15% of the mean for all points. L, Light; D, dark.

tosynthetic refixation of $^{12}CO_2$ produced by respiration. A photosynthesis-induced decrease in the rate of respiratory $^{12}CO_2$ release is typically observed during mass spectrometer experiments (Krömer, 1995). Avelange et al. (1991), based on a combination of mass spectrometric gas-exchange measurements and modeling, argued that refixation of respiratory CO_2 is of minor importance, and that the light-induced decline in the rate of CO_2 production is due to light inhibition of TCA-cycle activity.

Our gas-exchange results pointed to another possible concern with respect to the measurement of CO_2 production in the light: at intermediate or high PFR, there was apparent net negative respiratory CO_2 production that began at approximately 5 min after the onset of illumination. We suggest that apparent negative CO_2 production was due to the respiration of recently fixed carbon, which would have been predominately labeled with ^{13}C. The respiration of recently fixed carbon would result in both an underestimate of the rate of respiratory CO_2 production in the light and an underestimate of the rate of gross photosynthetic CO_2 fixation. Net negative respiratory CO_2 release became apparent only after a period of photosynthesis, and was a greater problem at high PFR than at low PFR (Fig. 4A). The observation of postillumination net $^{13}CO_2$ release (Fig. 4B) adds additional evidence for the respiration of recently fixed carbon. We suggest that the initial rates of CO_2 release in the light provide the best estimate of respiratory CO_2 production, because they are less biased by $^{13}CO_2$ release.

From a plot of the CO_2 production rate as a function of gross photosynthetic CO_2 fixation (Fig. 6), it is evident that the depression in the rate of respiratory CO_2 was not a linear negative function of the gross photosynthetic rate. Low PFR, resulting in low rates of gross photosynthesis, was proportionally more inhibitory than high PFR. These results suggest that photosynthesis inhibited respiratory carbon flow, although the results do not rule out the possibility that refixation of $^{12}CO_2$ was simultaneously occurring. A decline in respiratory CO_2 production during photosynthesis has also been demonstrated in higher plants using net CO_2 exchange measurements (Villar et al., 1994).

Experiments run in the presence of DCMU suggested that light per se had only a minor effect on the rate of respiratory carbon flow (Fig. 5A). Conversely, when Calvin cycle activity was inhibited by preincubation with glycolaldehyde, illumination resulted in a decrease in the measured rate of CO_2 release (Fig. 5B). In the presence of either DCMU or glycolaldehyde, gross photosynthetic CO_2 fixation equaled zero (data not shown); thus, refixation of $^{12}CO_2$ or respiration of ^{13}C-carbohydrate would not bias the results. These data are consistent with partial light-induced inhibition of respiratory carbon flow, with the inhibition being mediated at least partially by the photosynthetic light reactions.

Whatever the magnitude of respiratory CO_2 production in the light, it is clear that the source(s) of the CO_2 must be different during photosynthesis and darkness. Oxidative pentase phosphate pathway activity is rapidly decreased in the light in *C. reinhardtii* (Farr et al., 1994). Furthermore, the onset of illumination results in the thioredoxin-mediated activation of *C. reinhardtii* chloroplast FBP (Huppe and Buchanan, 1989) and a decrease in ATP-PFK activity, resulting in less respiratory breakdown of hexose-P that is produced from starch breakdown. Coupled with the observed increase in PGA (Fig. 9A), which is both a Calvin cycle and a glycolytic intermediate, this suggests that recently fixed carbon will become a potential substrate for respiration in the light.

Consistent with the O_2-exchange data, cellular NADH levels were relatively unaffected by photosynthesis (Fig. 7A). If photosynthesis had simply resulted in a decrease in the rate of glycolysis and TCA-cycle activity, without another source of reducing power being present, then we predict that NADH levels would have fallen in the light. The maintenance of NADH levels in the light suggests that respiratory reductant production was maintained in the light, although we cannot exclude the possibility that changes in the NADH level upon illumination were not detected due to the high background of bound NADH.

Dark-to-light and light-to-dark transitions resulted in marked changes in the cellular levels of many of the respiratory and photosynthetic intermediates. However, interpretation of metabolite changes in *C. reinhardtii* must take into account the compartmentation of respiratory pathways. Glycolysis is compartmented between the single chloroplast and the cytosol. Glycolytic reactions leading to triose phosphate are localized exclusively in the chloroplast, whereas the enzymes responsible for conversion of PGA to pyruvate occur predominately in the cytosol (Klein, 1986); Suc appears to be absent (Klein, 1987; X. Xue and H.G. Weger, unpublished data). ATP-PFK appears to be localized exclusively in the chloroplast in green algae (Kombrink and Wöber, 1982; Klein, 1986), and pyrophosphate-dependent PFK is absent in *C. reinhardtii* (Carnal and Black, 1983; Klein, 1986), which also lacks a plastidic isozyme of pyruvate kinase (Klein, 1986; Wu and Turpin, 1992). However, chloroplasts from *C. reinhardtii* appear to possess a higher-plant-type phosphate translocator (Klein et al., 1983).

The onset of illumination resulted in a decline in cellular ATP levels, and a corresponding increase in ADP and AMP (Fig. 8). There is consistent evidence from higher plants that the chloroplast ATP/ADP ratios increase in the light (Santarius and Heber, 1965; Hampp et al., 1982; Stitt et al., 1982) due to photophosphorylation. Part of the reason for the apparent decline in cellular ATP/ADP may be the substantial increase in activity of the plasma membrane H^+-ATPase during photosynthesis in *C. reinhardtii* (X. Xue and H.G. Weger, unpublished data).

The onset of illumination and photosynthesis was correlated with an increase in levels of PGA (Fig. 9A), which could be abolished by the presence of DCMU (data not shown). Similar to experiments with spinach leaves (Gerhardt et al., 1987) and barley protoplasts (Hill and Bryce, 1992), malate levels increased in the light (Fig. 9B). The increase in malate suggests that chloroplast oxaloacetate reduction to malate may act as an electron sink for a photo-generated reductant (Heineke et al., 1991), although

such a reductant shuttle has not yet been demonstrated for *C. reinhardtii*.

Illumination resulted in a change in the levels of various chloroplast metabolites that are effectors of ATP-PFK. Plastidic ATP-PFK from both green algae and higher plants is sensitive to inhibition by ATP, PEP, and PGA and is activated by Pi (Kombrink and Wöber, 1982; Kelly et al., 1985; Dennis and Greyson, 1987; Botha and Turpin, 1990). The onset of illumination will presumably result in an increase in the chloroplast ATP/Pi ratio, and resulted in increased PGA levels (Fig. 9A) due to Rubisco activity. These changes serve to decrease the activation of ATP-PFK. The observed increase in levels of PEP in the light (Fig. 11A) may not be physiologically relevant to ATP-PFK regulation, due to the compartmentation of PEP in the cytosol. One possible explanation for the observed increases in Fru-6-P and Glc-6-P upon illumination, and the concomitant decrease in FBP (Fig. 10), is light-activation of FBP and partial light-inhibition of ATP-PFK, which suggests that the amount of reduced carbon for respiration supplied by starch breakdown would decrease in the light.

The CO_2 exchange data (Figs. 4A, 5, and 6) are consistent with a photosynthesis-induced decline in the rate of respiratory carbon flow. As well, levels of the TCA-cycle intermediate citrate slowly declined in the light, and rapidly recovered postillumination, which is consistent with a moderate decline in glycolytic carbon flow. The increase in the PEP levels suggests an inhibition of glycolysis at the level of pyruvate kinase and/or PEP carboxylase, which might be mediated by the increased levels of PGA and malate (Fig. 9) (Peak and Peak, 1981; Schuller et al., 1990; Wu and Turpin, 1992). The onset of photosynthesis also results in a 25% decrease in pyruvate dehydrogenase activity, which is rapidly reversed upon darkening (X. Xue, D.D. Randall, and H.G. Weger, unpublished data).

The postillumination rapid decline in malate and the slower decline in PGA levels alleviate the possible inhibition of PK and/or PEP carboxylase activity. Furthermore, a decrease in stromal pH upon darkening promotes the export of PGA from the chloroplast to the cytosol, making increased levels of PGA available for respiration. Coupled with increased pyruvate dehydrogenase activity upon darkening, these changes allow for a postillumination increase in respiratory carbon flow. Consistent with this model, there was a marked increase in the rate of respiratory CO_2 production upon darkening (Fig. 4A) and a concomitant increase in the citrate levels (Fig. 11B).

CONCLUSIONS

Photosynthesis resulted in a stimulation of respiratory O_2 consumption in *C. reinhardtii*. LEDR was correlated with the photosynthetic production of PGA and malate, and was photosynthesis-dependent. The postillumination rate of respiratory O_2 consumption provided a good estimate of the O_2 consumption rate in the preceding light period. CO_2-exchange data were consistent with a photosynthesis-dependent decrease in the rate of respiratory carbon flow and a decrease in the rate of CO_2 evolution from the TCA cycle. Despite the decreased rate of respiratory carbon flow during photosynthesis, the respiratory O_2 consumption rate increased in the light.

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