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Photosynthetic O_2 Exchange Kinetics in Isolated Soybean Cells[1]

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ABSTRACT

Light-dependent O_2 exchange was measured in intact, isolated soybean (*Glycine max.* var. Williams) cells using isotopically labeled O_2 and a mass spectrometer. The dependence of O_2 exchange on O_2 and CO_2 was investigated at high light in coupled and uncoupled cells. With coupled cells at high O_2, O_2 evolution followed similar kinetics at high and low CO_2. Steady-state rates of O_2 uptake were insignificant at high CO_2, but progressively increased with decreasing CO_2. At low CO_2, steady-state rates of O_2 uptake were 50% to 70% of the maximum CO_2-supported rates of O_2 evolution. These high rates of O_2 uptake exceeded the maximum rate of O_2 reduction determined in uncoupled cells, suggesting the occurrence of another light-induced O_2-uptake process (*i.e.* photorespiration).

Rates of O_2 exchange in uncoupled cells were half-saturated at 7% to 8% O_2. Initial rates (during induction) of O_2 exchange in uninhibited cells were also half-saturated at 7% to 8% O_2. In contrast, steady-state rates of O_2 evolution and O_2 uptake (at low CO_2) were half-saturated at 18% to 20% O_2. O_2 uptake was significantly suppressed in the presence of nitrate, suggesting that nitrate and/or nitrite can compete with O_2 for photoreductant.

These results suggest that two mechanisms (O_2 reduction and photorespiration) are responsible for the light-dependent O_2 uptake observed in uninhibited cells under CO_2-limiting conditions. The relative contribution of each process to the rate of O_2 uptake appears to be dependent on the O_2 level. At high O_2 concentrations (≥40%), photorespiration is the major O_2-consuming process. At lower (ambient) O_2 concentrations (≤20%), O_2 reduction accounts for a significant portion of the total light-dependent O_2 uptake.

It is generally recognized that one of the important factors limiting net photosynthesis in higher C_3 plants under ambient conditions is the prevailing CO_2 and O_2 tensions (13, 31, 32). Decreasing CO_2 or increasing O_2 concentrations (from ambient) leads to significant decreases in the net rate of photosynthesis. Conversely, the net rate of photosynthesis can be increased by increasing the CO_2 or decreasing the O_2 concentrations. These observations have been interpreted as a reflection of the direct and/or indirect competition between CO_2 and O_2 for photosynthetic-reducing equivalents. Three principal O_2-consuming processes have been shown to occur in higher C_3 plants: mitochondrial respiration, O_2 reduction (pseudocyclic electron transport or Mehler reaction), and photorespiration. In strong light, the rate of mitochondrial O_2 consumption is generally thought to represent a relatively minor component of the total O_2 uptake (see, however, 9, 10) and most attention has focused on the light-dependent processes of O_2 reduction and photorespiration. The extent to which each process occurs *in vivo* under different photosynthetic conditions is unresolved.

Endogenous O_2 reduction has been shown to occur in a variety of photosynthetic systems (2, 8, 11, 17, 21, 29). This light-dependent process is eliminated by DCMU, is absent in PSI-deficient algal mutants (24), and is not directly dependent on reactions of the carbon reduction cycle. Inhibition of O_2 reduction by CO_2 presumably reflects the competition between O_2 and $NADP^+$ for photosynthetic reducing equivalents. O_2 reduction generally occurs at lower rates in washed, broken chloroplast preparations compared with intact chloroplasts and/or cell preparations (12, 17). Collectively these observations suggest that O_2 reduction involves the direct photoreduction of O_2 by some soluble component operating on the reducing side of PSI.

Photorespiration has been extensively investigated and has been suggested to occur at substantial rates in C_3 plants. Ribulose bisPcarboxylase/oxygenase is generally thought to be the pivotal point between the photosynthetic carbon-reduction cycle and the photorespiratory cycle (6, 15, 31; see, however, 20). The relative magnitude of carboxylation (photosynthesis) or oxygenation (photorespiration) is dependent on direct competition between CO_2 and O_2 (6, 15).

We previously reported (17) the light-dependent O_2 exchange characteristics of isolated, intact chloroplasts and cells from spinach under conditions of strong light, high O_2, and saturating CO_2 concentrations. Under these conditions, significant rates of O_2 uptake were found only during induction or when CO_2 fixation was inhibited. The O_2 uptake that was observed under these conditions was attributed to O_2 reduction. The effects of varying O_2 and CO_2 concentrations on steady-state rates of O_2 exchange have subsequently been examined in whole leaves using similar stable isotope techniques (5, 7, 9; see also 13, 16, 18). These studies suggest a complex relationship between photosynthetic O_2-exchange processes, light intensity, and prevailing O_2/CO_2 concentrations. In this communication, we report the effects of varying O_2 and CO_2 concentrations on rates of light-dependent O_2 exchange in intact, isolated soybean cells. The use of intact cell preparations should potentially eliminate some of the inherent gas diffusion problems encountered in studies with whole leaves. Unlike isolated, intact chloroplasts, cell preparations also contain a complete photorespiratory cycle. A preliminary report of a portion of this work has already appeared (4).

MATERIALS AND METHODS

Soybean (*Glycine max.* var. Williams) plants were grown in a greenhouse in soil and fertilized weekly with 20-20-20. Intact cells were isolated from young, not fully expanded leaves.

Cells were isolated from soybean as previously described for spinach (17), except for differences in the composition of the

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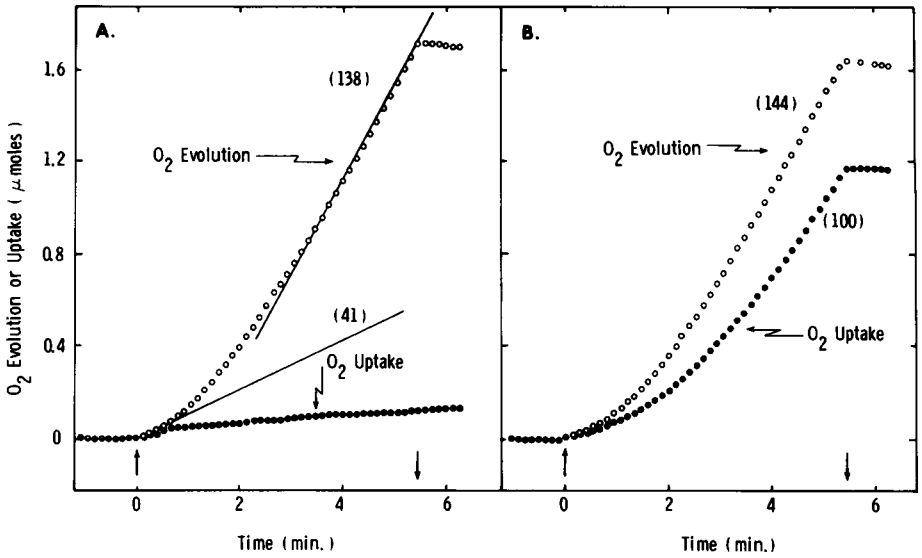

FIG. 1. Time course of light-induced O_2 evolution and O_2 uptake in intact isolated soybean cells. A, Initial O_2 and CO_2 concentrations were 48% (0.565 mM) and 298 μM, respectively. B, Initial O_2 concentration was 46% (0.548 mM). Initial and final CO_2 concentrations were 10 and 8 μM, respectively. Numbers in parentheses refer to rates of O_2 evolution or uptake expressed as μmol · mg Chl^{-1} · h^{-1}. Actinic light on (↑) and off (↓). Total Chl concentration was 75 μg/ml.

media. Leaves were sliced into small strips (0.5 × 2 cm) and vacuum infiltrated with 20 ml of media containing 20 mM MES-KOH buffer (pH 5.6), 12.5 mM K_2SO_4, 2% (w/v) PVP, and 0.75% (w/v) Macerase (CalBiochem). Leaf strips were subsequently digested in infiltration media containing 0.3 M sorbitol as previously described (27, 28). Released cells were pelleted (100*g*; 5 min; 4°C); washed twice with media containing 50 mM Hepes-KOH (pH 7.8), 0.2 M sorbitol, and 1 mM $MgCl_2$; and finally resuspended in wash medium containing 5 mM DDT. Cell preparations were greater than 95% intact as judged by staining with 2.5% (w/v) Evans Blue in 0.2 M sorbitol. Except as noted, cells were assayed in a medium consisting of 0.2 M sorbitol, 50 mM Hepes-KOH (pH 7.8), 1 mM $MgCl_2$, and 50 units/ml of carbonic anhydrase (Sigma). In the presence of a saturating amount of $NaHCO_3$, CO_2-dependent rates of net O_2 evolution were typically ≥100 μmol O_2 evolved · mg Chl^{-1}·h^{-1}. Chl concentrations were determined spectrophotometrically as described by Arnon (1).

O_2 exchange was measured polarographically (net), or with a quadrupole mass spectrometer, using isotopically labeled O_2 (99 atom % [$^{18}O_2$], Bio-Rad Labs). The instrumentation and methods for data analysis and calibration have been described previously (23). All experiments were done using broad-band saturating, orange-red light (Schott filter OG 530 and appropriate heat filters) at room temperature (20–25°C). The initial O_2 and CO_2 concentrations used in each experiment are given in the figure legends. It should be noted that at the pH used in these experiments (pH 7.8) most of the 'total CO_2'[2] was present in the form of HCO_3^-. Carbonic anhydrase was added to ensure rapid equilibration between CO_2 and HCO_3^- and thus minimize major changes in CO_2 concentration during the course of an experiment.

RESULTS

O_2 Exchange Characteristics at High and Low CO_2 Concentrations. The contrasting effects of high (saturating) and low (near ambient) CO_2 concentrations on the kinetics of light-dependent O_2 exchange in intact isolated soybean cells are illustrated in Figure 1. As reported previously (17), in the presence of saturating amounts of CO_2, O_2 evolution begins immediately upon illumination (Fig. 1A). Concomitantly, there is an immediate light-dependent increase in the rate of O_2 consumption. After an induction period (~1 min), the rate of O_2 evolution increases severalfold, whereas the rate of light-dependent O_2 uptake becomes essentially negligible.

The comparable experiment, done in the presence of approximately ambient CO_2 (~10 μM) is shown in Figure 1B. The kinetics of O_2 evolution are virtually identical to those observed in the presence of saturating amounts of CO_2. In contrast to conditions in which CO_2 is saturating, the rate of light-dependent O_2 uptake increases dramatically following induction. After several minutes of illumination, O_2-uptake processes account for a significant proportion (60–70%) of the O_2 evolved, *i.e.* net rates of O_2 evolution are markedly decreased at low compared with saturating CO_2.

As discussed previously (17), our interpretation of the O_2-exchange kinetics observed under the high CO_2 conditions of Figure 1A is that the initial rate of O_2 uptake corresponds to O_2 reduction. The low steady-state rate of O_2 uptake indicates that high levels of CO_2 inhibit O_2 uptake associated with O_2 reduction. At ambient CO_2 concentrations, steady-state rates of O_2 consumption are greatly stimulated. This light-dependent O_2 uptake could be associated with O_2 reduction and/or photorespiratory O_2-uptake processes. The direct photosynthetic reduction of O_2 has been shown to occur under conditions where CO_2 fixation is inhibited (17) and it has been widely reported (12, 31, 32) that photorespiration is markedly stimulated by low CO_2 and high O_2 concentrations.

O_2 Exchange Characteristics of Uncoupled Cells. A potential means of determining whether the high rates of light-dependent O_2 uptake observed in Figure 1B are associated with photorespiratory and/or O_2-reduction processes would be to establish the dependence of light-induced O_2 uptake on O_2 concentration. Although widely varied estimates of the apparent affinity of photorespiratory O_2 uptake for O_2 have been reported, most studies (3, 7, 14) suggest a relatively low affinity for O_2 (>20%). In contrast, measurements of O_2 uptake associated with O_2 reduction suggest that this process has a higher affinity for O_2 (≤8–10%) (2, 17, 22, 26).

The apparent affinity of the O_2-reduction process for O_2 was determined using intact, isolated soybean cells (Fig. 2). Experi-

[2] Abbreviations: 'total CO_2', = CO_2 + HCO_3^-; CCCP, carbonyl cyanide, *m*-chlorophenyl hydrazone

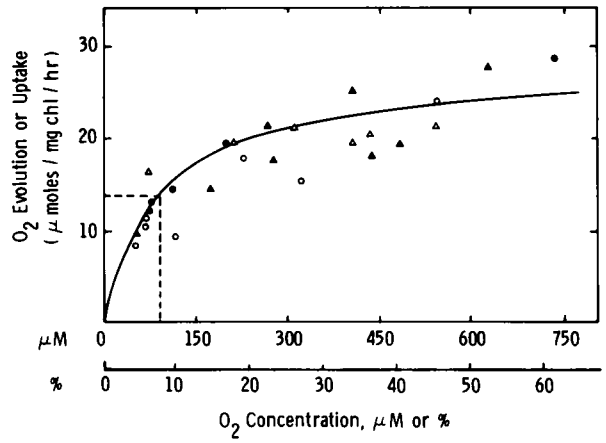

FIG. 2. Rates of O_2 exchange as a function of O_2 concentration assayed in presence of 4 μM CCCP and 5 mM iodoacetamide. Each data point represents averaged rate of O_2 evolution and O_2 uptake observed. Unnormalized data (different symbols) obtained from four cell preparations. CO_2-saturated rates of net photosynthesis in uninhibited cells were 84 (○), 121 (●), 139 (△), 190 (▲), expressed as μmol O_2 evolved · mg Chl^{-1} · h^{-1}. Curve is a rectangular hyperbola fitted to data assuming a maximum rate of 27.5 μmol · mg Chl^{-1} · h^{-1} and an O_2 concentration of 7.5% (80–95 μM) for half saturation.

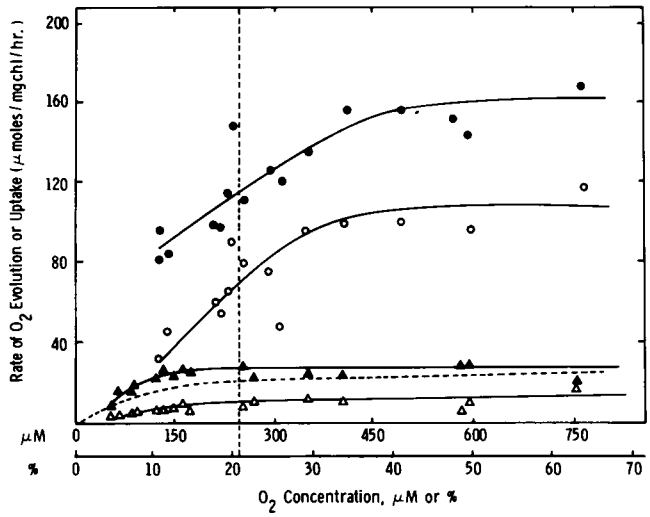

FIG. 3. Effect of O_2 concentration on initial and steady-state rates of light-dependent O_2 evolution and O_2 uptake. Initial CO_2 concentrations were 8 to 12 μM. Initial rates of O_2 exchange were plotted as a function of O_2 concentration at onset of illumination. Steady-state rates were plotted as a function of O_2 concentration observed following attainment of a steady-state since net O_2 was evolved during course of these experiments. "O_2 reduction" curve (---) was redrawn from Figure 2 assuming a V_{max} of 27.5 μmol O_2 consumed · mg Chl^{-1} · h^{-1} and half-saturation at 7.5% O_2. Vertical dashed line illustrating 21% O_2. Steady-state (but not initial) rates from four different cell preparations were normalized to the highest observed CO_2-saturated rate of O_2 evolution (see "Discussion"). △, Initial rate of O_2 uptake; ▲, initial rate of O_2 evolution; ○, steady-state rate of O_2 uptake; ●, steady-state rate of O_2 evolution.

ments were done at varied O_2 concentrations in the presence of a Calvin cycle inhibitor (iodoacetamide) and an uncoupler (CCCP). The sustained light-dependent O_2 uptake (and comparable rates of O_2 evolution) observed under these conditions reflects the O_2-reduction process (17) unemcumbered by coupling to photophosphorylation. Note that rates of O_2 exchange increase with increasing O_2 concentration with an apparent K_m (O_2) of approximately 7% to 8% O_2 or 80 to 95 μM O_2 and that the maximum capacity of the system to photoreduce O_2 is approximately 20 to 30 μmol O_2 · mg Chl^{-1} · h^{-1}. (These values are minimum estimates since we assume O_2 isotope equilibrium between the inside and outside of the cells [30].) This O_2 affinity is similar to that previously determined for algae (22, 26), but considerably lower than that reported from experiments with broken chloroplasts (2, 12). The inherent rate of O_2 reduction in soybean cells is considerably lower than some algae, *e.g. Scenedesmus* (21), and is relatively independent of the photosynthetic CO_2-fixation capacity of the cells (see legend of Fig. 2).

O_2 Exchange Characteristics of Coupled, CO_2-Limited Cells. Experiments similar to those of Figure 2 were conducted with coupled (uninhibited) cells in the presence of limiting (8 to 12 μM) CO_2 concentrations. Figure 3 shows the initial (*i.e.* during induction) and steady-state rates of O_2 evolution and O_2 uptake (Fig. 1) as a function of O_2 concentration. The O_2 response curves for these initial rates of O_2 exchange were similar to those obtained with uncoupled cells, *i.e.* K_m (O_2)≈8%, suggesting that the initial O_2 exchange corresponds to O_2 reduction. On the other hand, steady-state rates of O_2 evolution and O_2 uptake showed a much higher half-saturation value.

Several features can be noted regarding the steady-state rates of O_2 exchange (Fig. 3). The maximum rate of O_2 uptake observed in these experiments was approximately 100 μmol O_2 · mg Chl^{-1} · h^{-1}, which greatly exceeds the apparent capacity for O_2 reduction (Fig. 2 and dashed curve of Fig. 3). This implies that another light-dependent O_2-uptake process, presumably photorespiration, is occurring. Steady-state rates of O_2 uptake were half-saturated at approximately 18% to 20% O_2 or 210 to 240 μM O_2. This is similar to some previously reported values for the apparent K_m (O_2) for photorespiration (3, 7). Steady-state rates of gross O_2 evolution also increased with increasing O_2 concentrations with a response similar to that observed for steady-state O_2 uptake.

The contrasting effects of different combinations of high (~50%) or low (~10%) O_2 concentrations and high (>240 μM) or low (~9 μM) CO_2 concentrations on initial and steady-state rates of O_2 exchange are summarized in Table I. Rates of O_2 exchange in these cell preparations were also measured in the presence of CCCP and iodoacetamide (trial a). As noted earlier, rates of O_2 exchange in uncoupled, Calvin cycle-inhibited cells generally are 20 to 30 μmol O_2 · mg Chl^{-1} · h^{-1} when measured at high O_2 concentrations. Rates of O_2 evolution and O_2 uptake are sustained in the light and are approximately equivalent, resulting in little net O_2 evolution.

Initial and steady-state rates of O_2 evolution were comparable at both low and high CO_2 concentrations provided experiments were done at high O_2 tensions (trial b *versus* c). At low O_2 concentrations, initial rates of O_2 evolution were decreased (relative to high O_2) irrespective of CO_2 concentration, whereas steady-state O_2 evolution was suppressed by low CO_2 (trials b and c *versus* d and e).

Rates of light-dependent O_2 uptake recorded simultaneously in the experiments of Table I essentially follow previously described characteristics. For example, initial and steady-state rates of O_2 consumption (at low CO_2) were dependent on O_2 concentration (trial c *versus* e), whereas steady-state rates of O_2 uptake were sensitive to CO_2 concentration both at high O_2 tensions (trial b *versus* c) and at low O_2 tensions (trial d *versus* e).

One feature that should be noted regarding the data in Table I is that with the exception of uncoupled, Calvin cycle-inhibited cells (trial a), there is a clear discrepancy between initial rates of O_2 evolution and rates of O_2 uptake such that some net O_2 is evolved at the onset of illumination. Although the degree of imbalance can vary, we routinely observe higher initial rates of O_2 evolution than O_2 consumption in our cell preparations (see also Figs. 1 and 3 and Ref. 17). This imbalance does not appear to be an experimental artefact since similar initial rates of net O_2

Table I. *Effects of Varied* O_2 *and* CO_2 *Concentrations on Rates of* O_2 *Exchange*

Experiments 1 and 2 were done with different cell preparations assayed as described in "Materials and Methods." Total Chl concentration was 75 $\mu g\ ml^{-1}$.

Experiment	Trial	$[O_2]^a$	$[CO_2]^a$	Rate of O_2 Evolution		Rate of O_2 Uptake		Net Rate of O_2 Evolution
				Initial	Steady-State	Initial	Steady-State	
		%	*M*	*mol·mg Chl*$^{-1}$ *h*$^{-1}$				
1	a[b]	45	11	22	22	20	20	2
	b	48	241	25	95	9	<1	95
	c	48	9	29	91	19	53	38
	d	9	252	16	93	7	<1	93
	e	12	7	16	59	7	39	20
2	a[b]	48	11	31	31	30	30	<1
	b	53	322	38	133	10	<1	133
	c	53	10	42	134	18	81	52
	d	10	295	26	129	7	<1	129
	e	12	9	22	92	11	65	27

[a] Refer to O_2 and CO_2 concentrations at the onset of illumination.

[b] Assay medium contained iodacetamide (5 mM) and CCCP (4 μM).

evolution can be observed under these conditions with the mass spectrometer (in the absence of isotopic O_2) or polarographically (17). One explanation for this discrepancy would be that CO_2-fixation reactions are not completely inactivated in our dark-adapted cell preparations. The observed effect of CO_2 concentration on initial rates of O_2 uptake *e.g.* trial b *versus* c, would support this idea. However, even at low CO_2 concentrations, initial rates of O_2 evolution and uptake still do not balance. As subsequently presented, it is possible that other substances may compete for recently generated photoreductant.

Effect of CO_2 Concentration on Ratio of O_2 Exchange. The effect of CO_2 concentration on steady state rates of O_2 evolution and uptake is shown in Figure 4. Data from four different cell preparations, all obtained in the presence of high O_2 concentrations (50–80%) and strong light, are presented. Steady-state rates of O_2 evolution (●) were relatively constant over a wide range of CO_2 concentrations. Rates of evolution were suppressed, however, approximately 25% at the lowest CO_2 concentrations. In contrast, steady-state rates of light-dependent O_2 uptake (○) progressively decreased from approximately 90 μmol O_2 consumed·mg Chl^{-1}·h^{-1} at the lowest O_2 concentrations tried, to essentially zero at high

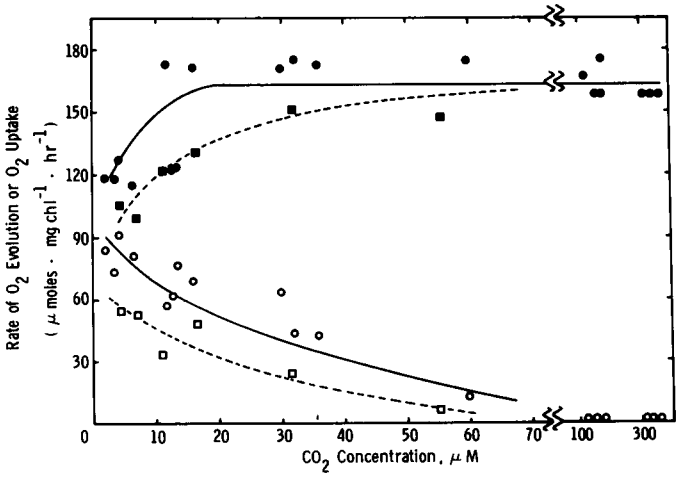

FIG. 4. Effect of CO_2 on steady-state rates of O_2 evolution and uptake. O_2 concentrations at steady-state were between 50% and 80% (0.595–0.953 mM) (○ ●) or 15% and 24% (0.179–0.285 mM) (□ ■). ●, ■, O_2 Evolution ○, □, O_2 uptake. Data were compiled from five different cell preparations normalized as in Figure 3 to correct for differences in photosynthetic activity between preparations.

(>70 μM) CO_2 concentrations.

Similar CO_2-response experiments were also conducted in the presence of lower O_2 tensions. Measurements at low O_2 concentrations (<15%) proved impractical since O_2 levels in the reaction vessel changed significantly during the course of illumination, particularly as CO_2 concentrations were increased. However, steady-state rates of O_2 evolution (■) and O_2 uptake (□) were lower (relative to high O_2) at all CO_2-limiting concentrations when measured in the presence of moderate O_2 levels (15–24%) (Fig. 4). Increasing CO_2 tensions progressively stimulated rates of O_2 evolution and correspondingly inhibited rates of light-dependent O_2 consumption.

Although the scatter in the data of Figure 4 does not allow a precise kinetic description of the stimulation of O_2 evolution or inhibition of O_2 uptake by CO_2 at higher *versus* moderate O_2 tensions, several features are evident. For example, the apparent CO_2-stimulation of O_2 evolution is sensitive to O_2 and becomes more evident as O_2 concentrations are decreased (see also Table I). Likewise, higher CO_2 concentrations are required to inhibit O_2 consumption at high compared with moderate O_2 levels. As noted earlier, O_2-uptake processes appear to support substantial rates of electron transport. At low CO_2, rates of O_2 uptake were approximately 50% to 60% and 30% to 40% of the maximum CO_2-supported rates of O_2 evolution when measured in the presence of high and moderate O_2 levels, respectively.

Effect of Nitrate on O_2 Reduction. We suggested above that O_2 and CO_2 may not be the sole competitors for recently generated photoreductant. To establish whether nitrate reduction could compete with O_2 reduction, we monitored light-dependent O_2 exchange in uncoupled, CO_2-inhibited preparations in the presence and absence of added nitrate. Gross rates of O_2 evolution were not significantly changed when cells were resuspended and assayed with or without added nitrate (Fig. 5). Rates of O_2 uptake, however, were reduced approximately 25% to 30% in the presence of added nitrate. These results would suggest that nitrate (presumably following its uptake and conversion to nitrite) (19) can compete (directly or indirectly) with O_2 for photosynthetic-reducing equivalents and thus potentially contribute to the O_2 evolution/uptake imbalances we have observed in cells at least when CO_2-fixation reactions are limiting.

DISCUSSION

Photosynthetic O_2 Reduction. To separate O_2 uptake associated with the O_2-reduction process from other light-dependent O_2-consuming processes, we examined the kinetics of O_2 exchange in

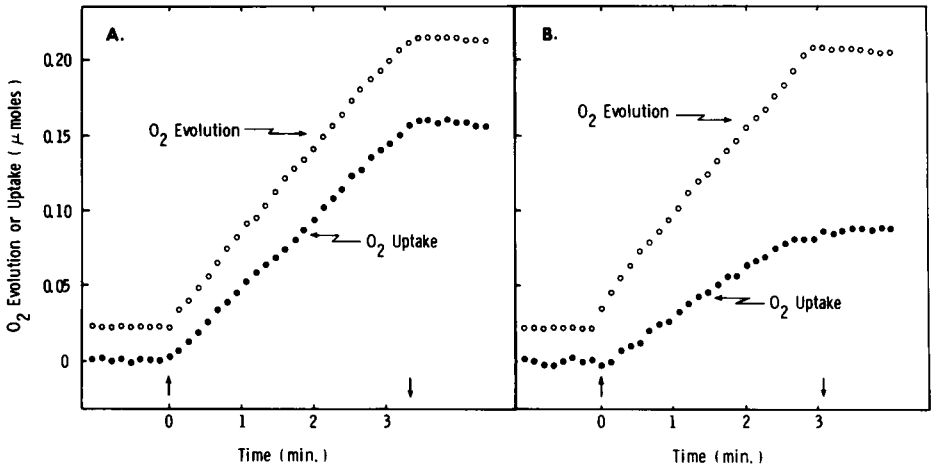

FIG. 5. Light-induced O_2 exchange in presence of 5 mM iodoacetamide and 4 μM CCCP. A, Cells resuspended and assayed in media without added nitrate. B, Cells resuspended and assayed in media containing 5 mM KNO_3 and 2 mM $Ca(NO_3)_2$. Initial O_2 concentration was 44% (0.523 mM) in A and 48% (0.570 mM) in B. Actinic light on (↑) and off (↓). Total Chl concentration was 75 μg/ml.

uncoupled, Calvin cycle-inhibited cells. The apparent maximum capacity for endogenous O_2 photoreduction under these conditions was approximately 20 to 30 μmol O_2 exchanged·mg $Chl^{-1}\cdot h^{-1}$ (Fig. 2). This, however, is a minimum estimate since we assume O_2 isotope equilibration between the inside and outside of the cell (17). Rates of O_2 reduction in different cell preparations also proved to be relatively independent of CO_2-saturated rates of photosynthesis, suggesting that photophosphorylation and/or CO_2-fixation processes were potentially more susceptible to damage than the O_2-reduction process during the course of cell isolation. Thus, expressing rates of O_2 reduction as a percentage of CO_2-fixation rates should be interpreted with caution.

The apparent affinity of O_2 reduction (K_m O_2) for O_2 in uncoupled soybean cells was found to be approximately 7% to 8% or 80 to 95 μM O_2 (Fig. 2). This suggests that O_2 reduction is not appreciably substrate limited at atmospheric O_2 concentrations.

Similar O_2-reduction kinetics have been reported in a variety of studies with intact photosynthetic preparations. For example, relatively high rates (>15 μmol·mg $Chl^{-1}\cdot h^{-1}$) of O_2 reduction have been observed in algae and in intact chloroplasts and cells isolated from spinach (17, 21, 26). The apparent O_2 affinity of O_2 reduction (7–8% O_2) is also similar to that found with intact spinach chloroplasts and algae (17, 21, 26). In contrast, there have been a number of reports (2, , 29) suggesting lower endogenous rates of O_2 reduction (<8 μmol O_2·mg $Chl^{-1}\cdot h^{-1}$) and/or higher affinities for O_2 (K_m [O_2] 0.1–0.4% O_2). In general, these studies have been done either with intact chloroplast preparations using indirect methods of assay, *e.g.* H_2O_2 formation (29), or with broken (class II) chloroplast preparations (2, 12).

We suggested earlier (17) that there may be an inherent difference between the capacity of higher plants and algae to carry out O_2 reduction. Unlike our findings with spinach and now with soybean, certain algae (21) have been shown to carry out O_2 reduction at rates approaching maximum (CO_2-saturated) rates of photosynthesis. As recently reported, however, other species of algae, *e.g. Chlorella*, appear to have much lower O_2 photoreduction capabilities (25). Whether similar variability exists among higher C_3 plants is unresolved.

Effects of CO_2 and O_2 Exchange. Our experiments on light-dependent O_2 exchange were all done at high light intensities. In the presence of saturating CO_2 concentrations, we observed that maximum steady-state rates of photosynthesis (monitored as gross O_2 evolution) were relatively constant for O_2 tensions between ~10% and 50% (Table I). Likewise, in the presence of high concentrations of O_2 (≥50%), gross rates of O_2 evolution were also relatively constant over a wide range (10 to 350 μM) of CO_2 concentrations (Table I, Figs. 1 and 4). Rates of O_2 uptake (and correspondingly net rates of photosynthesis), however, were markedly affected by CO_2 concentration. At high O_2 and low CO_2 concentrations, rates of O_2 uptake were 50% to 70% of the CO_2-saturated rates of O_2 evolution (Figs. 3 and 4). These results suggest that O_2, like CO_2, can sustain high rates of photosynthetic electron transport. This conclusion conflicts with that reached from recent studies (7) done at high light intensity with intact leaves, where it was suggested that O_2 supports only 25% to 35% of the CO_2-saturated rate of photosynthesis. The estimates from intact leaf measurements were done at low CO_2 and moderate (24–30%) concentrations of O_2. Our results (Figs. 3 and 4) suggest that all light-dependent O_2-uptake processes are not saturated at these O_2 tensions.

We also failed to observe (Fig. 4) the complex dependency of gross O_2 evolution and O_2 uptake on CO_2 concentration reported from studies with intact leaves, particularly at high light intensities (7, 9). This may simply reflect the fact that the leaf experiments were done at lower CO_2 concentrations, *i.e.* CO_2 compensation. In the present experiments, net O_2 evolution was observed even at the lowest CO_2 concentrations tried (Fig. 4). Alternatively, as mentioned above, if we assume that the reported leaf measurements were done at subsaturating O_2 concentrations, then the initial increases in O_2 evolution and O_2 uptake that were observed with increasing CO_2 concentrations may (in part) reflect increases in leaf O_2 tensions resulting from CO_2-dependent increases in the net rate of photosynthesis.

When both CO_2 and O_2 tensions are subsaturating, *e.g.* at ambient levels, steady-state rates of O_2 evolution are reduced (Figs. 3 and 4, Table I). Steady-state rates of O_2 uptake are likewise depressed under these conditions. Interestingly, at approximately ambient CO_2 tensions, increasing O_2 tensions (from ambient) stimulated gross rates of O_2 uptake and O_2 evolution about the same extent (Fig. 3). This suggests that draining photoreductant from the system via O_2-uptake processes is approximately compensated for by increases in the rate of water photooxidation. Similar results have been obtained in studies with intact leaves (7), but at low light intensities and with a slight shift in the O_2 response curve toward lower O_2 concentrations.

O_2 Uptake Mechanisms. Evidence has already been presented indicating that the light-dependent O_2 uptake observed during induction or when CO_2-fixation reactions are blocked can be principally attributed to O_2 reduction (17). Several lines of evidence indicate that the light-dependent steady-state O_2 uptake observed in soybean cells at subsaturating CO_2 is attributable to both O_2 reduction and photorespiratory processes. For example,

both O_2-uptake processes are sensitive to CO_2 concentrations. Thus, we assume that under CO_2-limiting conditions (and high light) both O_2 reduction and photorespiration will occur. It is suggested by the sustained operation of this cycle in the presence of uncouplers and CO_2 fixation blockers (Fig. 5A) that O_2 reduction is not simply a transient phenomenon (under conditions of suboptimal CO_2 fixation). Our data are also consistent with the numerous suggestions that photorespiratory O_2 uptake operates under CO_2-limiting conditions. Under appropriate conditions, we observe steady-state rates of gross O_2 uptake (Figs. 1B and 3) which greatly exceed the apparent maximum capacity for O_2 reduction in soybean cells (Fig. 2). The apparent O_2 affinity (K_m $[O_2] \simeq$ 18–20% O_2) for steady-state O_2 uptake is less than that found for O_2 reduction (Fig. 3) and similar to more recent estimates for photorespiration and for the oxygenase function of activated ribulose bisPcarboxylase/oxygenase measured *in vitro* (3, 7).

At high light and in the presence of approximately ambient CO_2 tensions, our results suggest that the relative contributions of O_2 reduction and photorespiration to total O_2 uptake are influenced by the prevailing O_2 tensions. At high O_2 concentrations (>40%), photorespiration is the major O_2-consuming process. At lower O_2 concentrations (≤20%), O_2 reduction accounts for a significant portion of the total light-dependent O_2 uptake. From the data in figure 3 we have attempted to estimate the relative contributions of O_2 reduction and photorespiration to total O_2 uptake as a function of O_2 concentration. If we assume that at steady-state, O_2 reduction occurs at near maximal rates (≃25 μmol·mg·Chl^{-1}·h^{-1}; see dashed O_2 reduction curve in Fig. 3) at ambient O_2 concentrations (21%), and that the remaining observed rate of O_2 uptake (~50 μmol·mg Chl^{-1}·h^{-1}) is due to photorespiration, then we estimate that at ambient O_2/CO_2 tensions, approximately 35% of total O_2 uptake is associated with O_2 reduction and 65% with photorespiratory uptake. At high O_2 tensions (≥50%), we infer that photorespiratory O_2 uptake accounts for approximately 77% of the total uptake and correspondingly <65% at low (<250 μM) O_2 tensions.

Our estimate of the relative magnitude of the O_2 uptake mechanisms operating under ambient O_2/CO_2 tensions agrees quite well with that derived from studies with intact leaves (7), namely that photorespiration accounts for ~60% of the total O_2 uptake. (Note, however, that leaf measurements were done at low light and at CO_2 compensation.) We have attributed the remaining light-dependent O_2 uptake principally to O_2 reduction. This appears to be in conflict with the actual rate of O_2 reduction observed during induction in the experiment of Figure 3 (△). We have assumed, however, that the rates of O_2 reduction observed during induction are depressed and that following the onset of CO_2 metabolism and ATP utilization, the system will effectively become 'uncoupled.'

It should be noted that apparent rates of photorespiratory O_2 uptake are not a direct measure of the turnover of the photorespiratory cycle. For example, from the data in Figure 3, at high (>50%) O_2 concentrations, we estimated that the rate of photorespiratory O_2 uptake was approximately 80 μmol O_2 consumed·mg Chl^{-1}·h^{-1} (the difference between the steady-state rate of O_2 uptake and the maximum rate of uncoupled O_2 reduction). If we assume that 1.75 molecules of O_2 are consumed for each molecule of glycolate synthesized and metabolized through the photorespiratory cycle (5), then an estimate (in terms of glycolate metabolized) of the actual turnover of the photorespiratory cycle under these conditions would be approximately 46 μmol glycolate·mg Chl^{-1}·h^{-1}. This is in the range of previously reported estimates of photorespiratory turnover under these conditions (5, 32).

Competition for Photosynthetic Reductant. Two observations indicate that O_2 and CO_2 are not the sole competitors for photosynthetic-reducing equivalents in isolated cell preparations within the chloroplast. In dark-adapted cells, we typically observed that initial rates of O_2 evolution exceeded initial rates of O_2 uptake during induction (Fig. 1, A and B, Fig. 3, and Table I). Furthermore, initial rates of O_2 evolution in coupled cells often exceeded uncoupled (Calvin cycle-inhibited) rates of O_2 reduction. The data in Table I suggest that a portion of this imbalance or 'excess' O_2 evolution may simply be due to incomplete dark inactivation of CO_2 processes. Evidence has been presented, however, that nitrate reduction may also compete for photoreductant (Fig. 5). It is thought that nitrate is first reduced to nitrite in the cytoplasm and subsequently moved into the chloroplast where it is reduced to ammonia (19). The apparent competition between nitrate (nitrite) and O_2 for photoreductant was observed in uncoupled, CO_2-inhibited cell preparations. This is consistent with the known ability of isolated intact, uncoupled chloroplasts to photoreduce nitrite. The extent to which nitrite or other acceptors, *e.g.* sulfate, compete with O_2 (or CO_2) in coupled cells is unknown.

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