Photoreduction of $O_2$ Primes and Replaces $CO_2$ Assimilation

Received for publication March 22, 1976 and in revised form June 7, 1976

RICHARD J. RADMER AND BESSEL KOK
Martin Marietta Laboratories, Baltimore, Maryland 21227

ABSTRACT

A mass spectrometer with a membrane inlet system was used to monitor directly gaseous components in a suspension of algae. Using labeled oxygen, we observed that during the first 20 seconds of illumination after a dark period, when no net $O_2$ evolution or $CO_2$ uptake was observed, $O_2$ evolution was normal but completely compensated by $O_2$ uptake. Similarly, when $CO_2$ uptake was totally or partially inhibited, $O_2$ evolution proceeded at a high (near maximal) rate. Under all conditions, $O_2$ uptake balanced that fraction of the $O_2$ evolution which could not be accounted for by $CO_2$ uptake.

From these observations we concluded that $O_2$ and $CO_2$ are in direct competition for photosynthetically generated reducing power, with $O_2$ being the main electron acceptor during the induction process and under other conditions in which $CO_2$ reduction cannot keep pace with $O_2$ evolution. The high rate of the $O_2$ uptake reaction observed in the presence of iodoacetamide, KCN, or carbonyl cyanide $p$-trifluoromethylphenoxypyphenylhydrazone, suggests that a special high capacity oxidase distinct from ribulose diphosphate oxygenase exists in whole cells. The rapid reduction of molecular $O_2$ after a period of darkness probably serves as a priming reaction for the photosynthetic apparatus. The high steady state rate of the $O_2$ cycle in the absence of $CO_2$ fixation suggests that the regulation of photosynthesis does not involve significant changes in the rate of photochemical electron transport.

After a period of darkness, photosynthesis has a distinct induction phase in which there is a lag (zero rate) of net $O_2$ evolution and its associated $CO_2$ uptake (14, 17, 19). It is unlikely that the lag is due to the $O_2$-evolving system; in isolated chloroplasts, $O_2$ evolution is virtually maximal from the onset of illumination. In this communication we will describe experiments in which a mass spectrometer with a special inlet and a fast mass step system was used to monitor simultaneously $O_2$ evolution, $O_2$ uptake, and $CO_2$ uptake by algae during and after the induction period. A preliminary report of some of these experiments was presented earlier (15).

MATERIALS AND METHODS

The mass spectrometer inlet system used in these experiments is similar to the one developed earlier in this laboratory (5). The reaction vessel is a 1.5-ml cylindrical cavity made of Plexiglas that can be filled completely with a suspension of algae or chloroplasts. The suspension is stirred with a rotating Teflon-covered magnetic bar. Gases are introduced into the mass spectrometer vacuum system through a circular window (~16 mm²) of 1 mil silicone rubber membrane (MEM 213, General Electric Co.) that forms part of the wall of the cavity; it transmits only dissolved gases and some water vapor from the liquid phase.

Since this sampling system does not require equilibration between a gas and a liquid phase, the mass spectrometer signals rather accurately reflect the gaseous components in the reaction vessel. The time response of this system is limited by the mass spectrometer, its associated vacuum system, and the transport characteristics of the membrane; the response time of our present system end-to-end is 2 to 3 sec.

The quadrupole mass spectrometer (Extranuclear Laboratories, Inc.) is programmed using a peak selection stepper system developed in-house. This system monitors up to eight masses, stepped cyclically. In the experiments described below we monitored the m/e values 32($^{16}O_2$), 36($^{16}O_2$), 40(Ar), 44($^{12}CO_2$), and 45($^{13}CO_2$), in the order 32-36-45-32-36-45-44, dwelling on each mass number for 1 sec. The signals were directly recorded on a fast running strip chart. Simultaneously, a PDP-8 minicomputer was used to average the signal amplitude over each 1-sec interval.

Algae suspensions were illuminated using a 1000-w projector lamp focused on the reaction vessel. To minimize heating effects, the light beam passed through a 30-cm water bath containing copper sulfate and an OG-3 (Schott) filter.

The usual assay procedure (unless stated otherwise) was as follows. Five ml of cells (cell density 0.3%, v/v) were concentrated to 0.1 ml by centrifugation and transferred to the reaction vessel using a Pasteur pipette (15 μl Scenedesmus cells, equivalent to ~150 μg Chl in 1.5 ml). This procedure was not particularly precise, but allowed the addition of a maximum amount of pre-equilibrated buffer to the vessel. The buffer contained 0.1 m phosphate, pH 6, and had been previously depleted of $O_2$ and $CO_2$ (by bubbling with argon). An aliquot was taken up in a 10-ml syringe, equilibrated with a bubble of $^{18}O_2$ by vigorous shaking, and used to fill the vessel. After the addition of a few small crystals of $^{13}$-labeled potassium carbonate, the vessel was closed, stirring initiated, and the measurement begun. During centrifugation and transfer the algae were in virtual darkness. An additional (~5 min) period of complete darkness preceded each illumination.

The values for $O_2$ uptake ($U_o$) and evolution ($E_o$) were obtained from the expressions:

$$U_o = \Delta^{18}O_2 (1 + \Delta^{18}O_2/\Delta^{16}O_2)$$
$$E_o = \Delta^{16}O_2 - \Delta^{18}O_2 (\Delta^{18}O_2/\Delta^{16}O_2)$$

Values for $CO_2$ uptake ($U_c$) were obtained from the sum of the uptakes of the added $^{18}CO_2$ and $^{12}CO_2$ (except for Fig. 2). The argon added during the course of the $O_2$-$CO_2$ depletion procedure served as an internal standard to monitor leaks, heat effects, bubble formation, etc. Gaseous $^{18}O_2$ (90.8 atom %) was obtained from Miles Laboratories. $^{13}$-Labeled potassium carbonate (91.3 atom %) was obtained from Pro-Chem, London, U. K.

Most of the data described in this communication were obtained using Scenedesmus obliquus (Gaffron strain D2). Similar experiments were also done with Chlorella vulgaris and Anacys-

1 This work was supported in part by the Energy Research and Development Administration Contract E(11-1)-3326, the National Science Foundation Grant PCM74-20736 AO1, and Grant AER73-03291. Any opinions, findings, conclusions, or recommendations expressed herein are those of the authors and do not necessarily reflect the views of the supporting agencies.
Photoreduction of O₂

**RESULTS**

Figure 1 illustrates the time course of O₂ evolution, O₂ uptake, and CO₂ uptake during the 1st min of illumination following a dark period observed in *Scenedesmus*. The strong light (~80% of saturation) induced a high rate of steady state photosynthesis (~40 cell volumes O₂ evolution/hr, equivalent to ~180 O₂/mg Chl·hr). The rate of photosynthetic O₂ evolution was high from the onset of illumination, and remained relatively constant throughout the experiment. In contrast, CO₂ uptake was negligible for the first 20 sec. During this initial induction period, the O₂ evolution was balanced by a high rate of O₂ uptake so that no net gas exchange occurred. The subsequent onset of CO₂ uptake largely suppressed this extra uptake of O₂. However, even after the maximum CO₂ uptake rate was established, O₂ uptake accounted for about 25% of the total (CO₂ + O₂) uptake. Throughout the experiment the O₂ output was balanced by the sum of the O₂ and CO₂ uptakes. This balance, plotted at the top of Figure 1 (E₀ - U₀ - U_r), was maintained within experimental error. (Because of the gradual dilution of the 18O₂ with photosynthetically produced 16O₂, the precision of this type of measurement tends to decline during the course of the experiment.) All of these gas exchange reactions were abolished by DCMU (not shown).

In the reported experiments the rate of respiration immediately before illumination was less than 5% of the O₂ evolution rate in the light (except Fig. 6). No attempt was made to correct for this “dark respiration,” since there is evidence that this process is suppressed in the light (6). During the lag there was no measurable CO₂ exchange (13CO₂ or 12CO₂) except for the negligible respiratory 13CO₂ evolution. After the lag the two isotopes were consumed in proportion to their concentrations. For this reason, Figure 1 shows the sum of the uptakes of the two isotopic species. The data of Figure 1 suggest that, although balance is maintained, the initial rate of O₂ evolution is 20 to 30% lower than it is after the onset of CO₂ uptake. Later in this paper, we will argue that this difference is not real, but due to an experimental artifact (viz. isotope exchange inside the algae).

Figure 2 shows another experiment, performed with a sample of the same suspension of *Scenedesmus* cells, in which CO₂ reduction was totally inhibited by the addition of iodoacetamide. In this case, we observed that the stoichiometric exchange of 18O₂ and 16O₂ (seen initially in Fig. 1) continued throughout at a constant high rate. Thus, reducing power was generated and consumed at a normal rate despite the absence of CO₂ metabolism (13CO₂ increased slightly at a rate corresponding to the previous dark respiration).

Figure 3 shows the same phenomena in *Anacystis nidulans*. In this organism the lag of CO₂ uptake was much more pronounced, and the gas exchange sequence took several minutes to unfold.

Figure 4 shows the results obtained when KCN was added to another sample of the *Scenedesmus* suspension used in Figures 1 and 2. Under these conditions we observed a significant amount of CO₂ uptake after the initial lag. This uptake stopped completely after about 1 min, and was replaced by O₂ uptake at a rate close to that observed during the initial lag phase. This progressive inhibition probably reflects the *in vivo* production of the cyanohydrin complex of RuDP, an inhibitor of both the RuDP carboxylase and the oxygenase function (10, 11).

Figure 5 shows the observed gas exchange kinetics in the presence of the uncoupler FCCP. Under these conditions the initial lag of CO₂ uptake and the compensating O₂ uptake were both prolonged; in the steady state the rates of O₂ reduction and

**Abbreviations:** RuDP: ribulose diphosphate; FCCP: carbonyl cyanide p-trifluoromethylphenylhydrazone.

---

**Fig. 1.** Bottom: time course of O₂ uptake, O₂ evolution, and CO₂ uptake in *Scenedesmus*. Initial concentrations (μmol): 18O₂, 0.126; 16O₂, 0.236; CO₂, 2.271. Light intensity, 32% (see Fig. 6). Top: algebraic sum of O₂ evolution (E₀), O₂ uptake (U₀), and CO₂ uptake (U_r). Scales are the same as in the lower plot.

**Fig. 2.** Bottom: time course of gas exchange by *Scenedesmus* in the presence of 1 mm iodoacetamide. CO₂ uptake represents 13CO₂ only; the small 13CO₂ release is not presented. Initial concentrations (μmol): 18O₂, 0.101; 16O₂, 0.275; CO₂, 2.801. Light intensity, 32% (see Fig. 6). Top: E₀ - U₀ - U_r; see legend of Fig. 1.
CO₂ assimilation were about equal. These observations probably reflect the partial inhibition of ATP production required for the turnover of the Calvin cycle. Again, as in the experiment shown in Figure 4, isotope dilution decreased the precision of the measurements at the end of the experiment.

We generally observed that the inhibition of CO₂ uptake resulted in a gradual increase of the balance expression \((E_u - U_e - U_i)\) which was beyond the apparent limits of experimental error.

\[ E - U_0 - U_C \]

\[ O_2 \text{ evolution} \]

\[ O_2 \text{ uptake} \]

\[ CO_2 \text{ uptake} \]

**FIG. 3.** Bottom: time course of \(O_2\) uptake, \(O_2\) evolution, and \(CO_2\) uptake in *Anacystis*. Initial concentration (mM): \(^{18}O_2, 0.196; ^{16}O_2, 0.196; CO_2, 2.520\). Final cell density 0.33\% (v/v). The light intensity was at least 90\% of saturation. Top: \(E_u - U_0 - U_i\); see legend of Fig. 1.

\[ E - U_0 - U_C \]

\[ O_2 \text{ evolution} \]

\[ O_2 \text{ uptake} \]

\[ CO_2 \text{ uptake} \]

**FIG. 4.** Bottom: time course of gas exchange by *Scenedesmus* in the presence of 1 mM KCN. Initial concentrations (mM): \(^{18}O_2, 0.139; ^{16}O_2, 0.306; CO_2, 2.412\). Light intensity, 32\% (see Fig. 6). Top: \(E_u - U_0 - U_i\); see legend of Fig. 1.

\[ E - U_0 - U_C \]

\[ O_2 \text{ evolution} \]

\[ O_2 \text{ uptake} \]

\[ CO_2 \text{ uptake} \]

**FIG. 5.** Bottom: time course of gas exchange by *Scenedesmus* in the presence of 10 \(\mu\)M FCCP. Initial concentrations (mM): \(^{18}O_2, 0.111; ^{16}O_2, 0.260; CO_2, 1.835\). Light intensity, 32\% (see Fig. 6). Top: \(E_u - U_0 - U_i\); see legend of Fig. 1.

\[ E - U_0 - U_C \]

\[ O_2 \text{ evolution} \]

\[ O_2 \text{ uptake} \]

\[ CO_2 \text{ uptake} \]

**FIG. 6.** Effect of light intensity on the amplitude of the \(^{18}O_2\) transient (△) and the subsequent steady state rate of photosynthesis (△) observed with *Scenedesmus*. The amplitude (and half-time) of the transient were calculated as shown in the inset. The initial 2- to 3-sec lag is due to instrument response.
We tentatively ascribe this uptake deficiency to NO$_3^-$ reduction because (a) the algae were grown in a nitrate-containing medium and not exhaustively washed, and (b) we have observed the formation of nitrogen oxides (m/e = 30 and 44) under these conditions. Figure 6 illustrates the light intensity dependence of the O$_2$ uptake transient in the absence of inhibitors. The amplitude of the transient (calculated as described in the inset) roughly parallels the subsequent steady state rate of CO$_2$ reduction (and net O$_2$ evolution), indicating that the initial rate of O$_2$ reduction parallels the later rate of CO$_2$ reduction. In contrast, we observed (not shown in Fig. 6) that the half-time of this transient is independent of intensity (within the somewhat crude precision of the determination), which is consistent with the generally observed intensity independence of the CO$_2$ induction lag time.

**DISCUSSION**

The light-induced uptake of O$_2$ by photosynthetic tissue has been the topic of numerous reports during the past 25 years (for extensive discussions within the context of photorespiration see refs. 7, 16, and 20). Our data imply that this O$_2$ uptake is not a spurious pathway; it can proceed in intact unpoisoned cells under conditions where CO$_2$ is not limiting (during the initial transient) and at rates approaching or equal to (cf. below) the maximum rate of photochemical electron transport. O$_2$ and CO$_2$ are in direct competition for the light-generated reducing power of system I, with O$_2$ being the principal electron acceptor during the induction process and under other conditions in which CO$_2$ reduction cannot keep pace with O$_2$ evolution. The magnitude and intensity dependence of the O$_2$ exchange reactions and their sensitivity to DCMU suggest that O$_2$ uptake (like CO$_2$ uptake) requires the co-operation of both photosystems.

The question arises whether or not the rate of O$_2$ evolution is completely unaffected by the rate of the reducing equivalents at the high O$_2$ tension used in these experiments. In the experiments shown above, the computed O$_2$ evolution rate was somewhat lower when O$_2$ rather than CO$_2$ was the electron acceptor; for instance, in Figures 1, 3, 4, and 5 the ratios of initial to final rate are ~80%. The difference is barely discernible in most experiments done with Anacystis. Since the observation of the initial rate transition is hardly affected by the (2 to 3 sec) time constant of our apparatus, this difference could possibly reflect a true rate limitation associated with the reduction of O$_2$. However, we suspect that the small changes in rate are only apparent, and reflect the incomplete equilibration of the O$_2$ isotopes between the inside and outside of the cells. This idea is supported by the near absence of a change in the computed rate of O$_2$ evolution in Anacystis (Fig. 3); the slower induction kinetics and smaller size of this alga might allow a more complete isotopic equilibration.

The fact that we observed the same phenomena in several different algae suggests that the rapid direct photo-reduction of O$_2$ is a reaction common to all photosynthetic organisms. A rapid and sustained light driven O$_2$ uptake under conditions of low CO$_2$ has been observed in Anabaena (9), bean leaves (12), and maize leaves (18). This O$_2$ uptake could also reflect the direct photo-reduction of O$_2$, although in these experiments one cannot rule out a mechanism involving carbon metabolism and a rapid cycling of CO$_2$. The lower observed rate of O$_2$ evolution in leaves (30 to 40% of the CO$_2$-supported rate) could be ascribed to isotopic disequilibrium (see above); one might expect that isotopic O$_2$ transfer from the inside to the outside of a leaf is significantly slower than transfer from an alga to its surrounding medium.

**Pathway of O$_2$ Uptake.** The O$_2$ uptake rates by whole algae reported here far exceed the rates observed in broken chloroplasts. This implies that the catalyst involved is only loosely bound to the photochemical electron transport apparatus (like the CO$_2$ reduction enzymes). However, since the O$_2$ uptake also takes place in the presence of Calvin cycle inhibitors, the pathway does not seem to involve the carbon cycle (e.g. RuDP carboxylase oxygenase) (3). One possible candidate for this terminal oxidase is ferredoxin, which has been shown to mediate the reduction of O$_2$ by illuminated spinach chloroplasts (1).

A transient burst and small steady state production of H$_2$O$_2$ has been observed in Anacystis and other blue-green algae (but not in green algae) (13). We therefore assume that, at least in blue-green algae, the O$_2$ uptake leads to the formation (and breakdown) of H$_2$O$_2$. These observations probably reflect a competition between the assay procedure and cellular "catalase" for the H$_2$O$_2$ produced. It remains to be seen whether the only difference between blue-green and green algae in this respect is a greater catalase activity of the latter organisms. Preliminary experiments (not shown) in which we added H$_2$O$_2$ to an Anacystis or Scenedesmus suspension indicated that the breakdown of H$_2$O$_2$ to H$_2$O + 1/2 O$_2$ occurred rapidly even in the presence of 1 mM KCN (cf. Fig. 3). This suggests that the observed H$_2$O$_2$ decomposition may proceed via a route other than catalase.

**Role and Regulation of Oxygen Cycle.** The O$_2$-mediated electron flow after a period of darkness allows the translocation of protons and cations and makes ATP available. Therefore, it probably serves to prime the CO$_2$ reduction system. Bassham and Jensen (2) have demonstrated that the CO$_2$ fixation cycle is inactive in the dark, and requires light for its activation. The involvement of O$_2$ reduction in the induction phase in chloroplasts has been suggested by Jennings and Forti (8) on the basis of the effects of anaerobiosis on the turnover of Cyt f.

In the steady state the ATP generated in the O$_2$ cycle probably supplements the (presumably barely adequate) ATP production linked to CO$_2$ fixation and other metabolic activities (6). Still, under conditions of high O$_2$ uptake the energy gain of the cycle must be small. For instance, a 1-min operation of the O$_2$ cycle (Fig. 2) could yield 10 ATP's/Chl, corresponding to at least 10% of the dry weight of the algae! Clearly some as yet unknown regulatory mechanism (such as an ATPase) must become operative.

**Regulation of Photosynthesis.** If our interpretation of the data is correct, an earlier concept of the rate limitation (or rather regulation) of photosynthesis needs to be amended. According to this concept low [CO$_2$] would lower the rate of CO$_2$ reduction and thus the consumption of ATP. The resulting high ATP to ADP ratio would create a backpressure, slow down the Q-P transfer (a reaction presumably coupled to proton translocation and ATP formation), and thus shut off the two photoacts (Q reduced, P oxidized). However, the experiments shown above disprove this concept; the photoacts keep operating at fully uncoupled rates regardless of whether CO$_2$ or O$_2$ is the electron acceptor, even at saturating light intensities. This constant high turnover of the photosynthetic apparatus may provide a mechanism to safely dispose of excess light.

Acknowledgment — We thank O. J. Ollinger for technical assistance and the design and construction of the peak selection stepper system.

**LITERATURE CITED**


