Kinetics and Apparent $K_m$ of Oxygen Cycle under Conditions of Limiting Carbon Dioxide Fixation

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ABSTRACT

A mass spectrometer with a membrane inlet was used to monitor light-driven O$_2$ evolution, O$_2$ uptake, and CO$_2$ uptake in suspensions of algae (Scenedesmus obliquus). We observed the following. (a) The rate of O$_2$ uptake, which, in the presence of iodoacetamide, replaces the uptake of CO$_2$, showed a distinct plateau ($V_{\text{max}}$) beyond ~30% O$_2$ and was half-maximal at ~8% O$_2$. We concluded that this light-driven O$_2$ uptake process, which does not involve carbon compounds, is saturated at lower O$_2$ concentrations than are photosynthesis and glycolate formation. (b) In the absence of inhibitor, O$_2$ evolution was relatively unaffected by the presence or absence of CO$_2$. During the course of CO$_2$ depletion, electron flow to CO$_2$ was replaced by an equivalent flow to O$_2$. (c) There was a distinct delay between the cessation of CO$_2$ uptake and the increase in O$_2$ uptake. We ascribe this delay to the transient utilization of another electron acceptor—possibly bicarbonate or another bound form of CO$_2$.

RESULTS AND DISCUSSION

Substrate Affinity of O$_2$ Cycle (Apparent $K_m$). As described earlier (7), in the presence of the Calvin cycle inhibitor, iodoacetamide, O$_2$ uptake replaces CO$_2$ uptake and balances O$_2$ evolution, so that there is no net change in O$_2$ concentration. This inhibitor offers the opportunity to study the O$_2$ uptake reaction unencumbered by the varying and complicating effects of CO$_2$. Figure 1, which is a compilation of a series of experiments similar to those of Figure 2 in reference 7, shows the net rates of O$_2$ uptake (and O$_2$ evolution, the two are mirror images) by Scenedesmus D$_3$ in the presence of iodoacetamide. Note that at O$_2$ concentrations above 30% the rate is maximal and invariant within experimental error (in Fig. 1, $V_{\text{max}} = 33.5$ cell vol/hr). Half this rate is observed at an atmospheric abundance of 8% (indicated as $K_m$, Fig. 1). Five repetitions of this set of experiments (all made at room temperature and at intensities inducing greater than half-maximal rates) gave similar results.

The linear plot (v versus v/O$_2$) in the inset of Figure 1 and the dashed curve with its computed maximum rate value ($V_{\text{max}} = 40$ cell vol/hr) and half-value ($K_m = 11\%$) show an attempt to fit the data with a rectangular hyperbola. Such a plot is customarily associated with Michaelis-Menten kinetics, the mechanistic basis for the notation $K_m$. This function describes the data rather poorly, particularly the sudden transition to a well defined saturation plateau. A much better fit is obtained by an exponential function (solid line in Fig. 1). It might be interesting to note that similar saturation curves have been reported for CO$_2$ and light intensity (5). Unfortunately, however, with properly adjusted parameters, other (hyperbolic) functions can describe the data equally well and within experimental error.

The half-saturation value of 8% obtained in these experiments is significantly different from the half-saturation value(s) reported for photorespiration and glycolate formation. According to reference 10 (see also 4), the latter processes are not rate-saturated even at 100% (1 atm) of O$_2$.

Invariance of Electron Flow during Course of CO$_2$ Depletion. Figure 2 shows the kinetics of the gas exchange reactions during the final phase of CO$_2$ depletion (the light was turned on 4 min...
**FIG. 1.** Rate of O₂ uptake and evolution in the presence of 3 mM iodoacetamide as a function of O₂ concentration. Gas exchange was monitored at a rate of 9 cycles/min; during each cycle the amplitude of 5 m/e values (32, 18O₂; 36, 18O₂; 40, Ar; 44, 13CO₂; 45, 12CO₂) was monitored. Each point on the figure represents the average of 10 successive values computed from the data of a single run. O₂ concentration was varied by mixing varying amounts of O₂-equilibrated and O₂-depleted buffer. The final cell concentration in each experiment was 1% (v/v). The Chl concentration was about 11 μg of Chl/μl of cells, so that the maximum rate in this experiment of 33.5 cell vol/hr corresponds to about 120 O₂/Chl hr. (--): Empirical best fit (inset: Hofstee plot of experimental data. The y intercept of the least squares best fit line gives the maximum rate (V_max); the slope gives the half-saturation value (K_m = 11 ± 1 [se]).

Before time zero in the fig.). In this experiment we used algae grown in 12CO₂ and no CO₂ was added; the CO₂ in the reaction vessel originated from carryover from the culture medium and respiration during the preceding dark period (~10'), and therefore it consisted mainly of 18CO₂. During the first 5 min of illumination (excluding the lag), net O₂ evolution and CO₂ uptake proceeded with constant and near equal velocity; the assimilatory quotient (ΔCO₂/ΔO₂) was 0.93 to 0.98.

At the moment that the concentration of free CO₂ reached ~30 μM, the rate of CO₂ uptake began to decline, as can be seen more clearly in the bottom panel of Figure 2. At ~10 μM free CO₂ (15 μM "total CO₂") the CO₂ uptake rate had dropped to half (c.f. left dashed line). Surprisingly, this decline of the CO₂ uptake rate was not at all reflected in the O₂ uptake trace. The O₂ uptake remained

had attained a steady state. Gas exchange was monitored at the rate of 13.5 cycles (67.5 m/e values)/min (see legend of Fig. 1). Each point in the figure represents the value of O₂ evolution (E_r), O₂ uptake (U_r), and CO₂ uptake (U_c), computed as described under "Materials and Methods." The final cell concentration was 0.3% (v/v). The rate of O₂ evolution, equal to the final O₂ uptake rate, was 41 cells vol/hr. Since the Chl concentration was about 10 μg of Chl/μl of cells, this corresponds to a rate of about 170 O₂/Chl hr. Numbers in brackets representing total CO₂ were calculated from the measured concentration of free CO₂ and the pH (6) of the suspension medium. The O₂ concentration at t = 0 was 39%. Bottom panel: rate of CO₂ uptake obtained from a point-by-point differentiation of the CO₂ data shown in the middle panel. The first vertical dashed line connects the point at which CO₂ uptake is 0.5 V_max with the corresponding CO₂ concentration in the middle panel. The second vertical line connects the break in the O₂ uptake rate of the middle panel with a corresponding point in the rate of CO₂ uptake. The area bounded by the latter dashed line and the rate curve (hatched) represents the deficit E_r U_r U_c, which has the dimensions of concentration ([nmol/ml · min] × min). Top panel: cumulative deficit E_r U_r U_c obtained from data in the middle panel.
constant until the CO₂ uptake rate had practically declined to zero and the CO₂ concentration had dropped to \( \leq 10 \mu M \). At that point, O₂ uptake showed a sudden break, after which the uptake rate equaled the evolution rate. Note that O₂ evolution (and therefore electron transport) was relatively invariant during (and after) the transition from CO₂ uptake to O₂ uptake. In this instance the O₂ cycle proceeded at high rate in the absence of an inhibitor of CO₂ fixation.

The final CO₂ concentration cannot be very precisely determined in this experiment; we estimate it to be \(~0.1 \mu M\) CO₂, which corresponds to about 3 \( \mu l/1 \) CO₂ in the gas phase. This number is consistent with the findings of Brown and Tregunna (1), who showed that Scenedesmus can deplete the CO₂ concentration to a very low level.

An intriguing aspect of the experiment in Figure 2 is the temporary change in the value of the function \( (E_0 - U_0 - U_c) \). During this time O₂ was evolved without concomitant CO₂ uptake, indicating the transient utilization of an electron acceptor other than external CO₂. The top panel of Figure 2 shows the accumulated “uptake deficit” (i.e. the O₂ evolution not balanced by either CO₂ or O₂ uptake). This same pool is also shown by the hatched area in the bottom panel. In three experiments of this type we found near identical pool sizes, about 1 CO₂/Chl.

At the moment we can only speculate as to the identity of this alternate electron acceptor. A likely candidate is HCO₃⁻; there is evidence that bicarbonate is actively accumulated by some algae (e.g. 9). Such an energy-consuming process may underlie the very low CO₂ depletion values observed with these algae (cf. Fig. 2 and ref. 1), equivalent to those obtained with C₄ plants.

**LITERATURE CITED**

6. **Radmer RJ** 1977 The oxygen cycle: apparent \( K_m \) values for CO₂ and O₂. 4th Int Cong on Photosynthesis, Reading, England, Abst p 308

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2 This discrepancy, lasting about a minute, is not due to CO₂-HCO₃⁻ disequilibrium in the suspension medium; we measured a halftime of only \(~5 \) sec for this reaction.