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CONSUMPTION OF O_2 IN THE LIGHT BY *CHLORELLA PYRENOIDOSA* AND *CHLAMYDOMONAS REINHARDTII**

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SUMMARY

A mass spectrometer has been used to measure O_2 production and consumption and net CO_2 exchange near the CO_2 compensation point in *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* grown in air and in 2% (v/v) and 5% (v/v) CO_2 in air. Some experiments were run in presence of α-hydroxy-2-pyridinemethanesulfonic acid and, for others, data are presented from parallel assays for enzymic glycolate oxidation. Although the glycolate-oxidising enzyme was present in cells grown with and without CO_2 enrichment, the activities measured were not compatible with direct measurements of O_2 consumption in the light. This and other evidence argues against glycolate being the primary substrate for light-induced O_2 consumption by these organisms.

INTRODUCTION

After early and equivocal evidence, BROWN AND WEIS[1] published data from mass spectrometry to show that, in Ankistrodesmus, CO_2 evolution was reduced in the light, largely independent of intensity, while O_2 consumption was unaffected at low but increased at high intensities. HOCH AND KOK[2] made design modifications to their mass spectrometer which made possible the direct sampling of exchange gases without the need for a headspace. With this system, HOCH *et al.*[3] examined O_2 exchange in Anacystis and in Scenedesmus and found O_2 consumption inhibited at low and stimulated at higher intensities. HOCH *et al.*[3] suggested that the increased O_2 consumption in the light could be linked with ATP formation.

ARNON *et al.*[4] and FORTI AND JAGENDORF[5] have shown a coupling of ATP formation and O_2 uptake with water as electron donor in the light. KRALL AND BASS[6] observed that O_2 is needed for light-dependent conversion of glucose to starch in leaf discs. The O_2 requirement, however, was low. This was the case also in an O_2 consuming reaction involving photosystem I studied by HEBER AND FRENCH[7]. Although the increased O_2 consumption recorded by HOCH *et al.*[3] may reflect ATP formation, the matter is not resolved and it seems possible that at least part of the increased consumption may be traceable to simple back reactions with one or

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more reductants produced in photosynthesis as demonstrated in chloroplasts by MEHLER[8].

OZBUN *et al.*[9], working with bean leaves, obtained data which confirmed those of HOCH *et al.*[3]. In addition, they noted a depression in CO_2 evolution in the light well before photosynthesis was saturated and considered O_2-dependent ATP formation and glyoxylate formation *via* glycolate, without CO_2 release, as alternative mechanisms to explain their observations. GOLDSWORTHY[10] also obtained indications with tobacco that the substrate for CO_2 production changes during photosynthesis. However, he and a number of other investigators including DECKER[11], HEW AND KROTKOV[12], JOLLIFFE AND TREGUNNA[13], MOSS[14], POSKUTA[15], TREGUNNA *et al.*[16] and ZELITCH[17] have presented evidence based on measurements of CO_2 exchange to show that another process, referred to as photorespiration, results in markedly increased CO_2 release in the light in a variety of higher plants. Criteria for photorespiration include a post-illumination decline in CO_2 release to the normal dark rate and an elevation of the CO_2 compensation point with increasing concentrations of O_2. Depression of apparent photosynthesis by O_2 has been attributed to this process, which is favored by low concentrations of CO_2 and high light intensities.

Work, notably by ZELITCH[17] has led to the conclusion that the substrate of photorespiration is glycolic acid. Although glycolic acid oxidase has been demonstrated in a number of higher plants and in some algae including Chlorella[18], FOCK AND EGLE[19] observed that CO_2 formation by this alga in the light was relatively much weaker than in higher plants. VOZNESENSKII[20] has reported a zero CO_2 compensation point in *Chlorella pyrenoidosa* and BJORKMAN[21] found that net CO_2 fixation by Chlorella and Ulva, unlike higher plants, was not susceptible to O_2 concentration. Using Solidago and Mimulus, in which net CO_2 fixation is reduced by O_2, BJORKMAN[21], moreover, was unable to substantiate glycolic acid as the substrate in photorespiration.

It is clear that several alternatives present themselves for the interpretation of data on photosynthesis based on gas exchange[22] and that each one may be operative to some extent according to the species under study and environmental conditions. We have undertaken mass spectrometer studies addressed to this problem

MATERIALS AND METHODS

The work was initiated at RIAS*, Baltimore and continued in this laboratory. Methods at RIAS for culturing, harvesting and preparing the experimental organism *Chlamydomonas reinhardtii* have been described[23]. Here, it need be noted only that cultures, provided by Dr. B. Kok, were grown at pH 4.2 and gassed with 2% (v/v) CO_2 in air.

Measurements of gas exchange were made at 30° with a mass spectrometer equipped essentially as described by HOCH AND KOK[2]. The method of illumination was identical. In addition to following signals for O_2 of masses 32 and 36, net exchange of CO_2 was also monitored. In all cases, light intensities were adequate for full saturation of photosynthesis although a filter which blocked wavelengths < 460 nm was used routinely. This procedure was found necessary to avoid a very rapid photooxidation of the inhibitor α-hydroxy-2-pyridinemethanesulfonic acid which was used in some of the experiments. Use of the filter did not affect the course of O_2 exchange.

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From this laboratory, data are presented for two organisms: *Chlorella pyrenoidosa*, strain 211/8P, kindly provided by Dr. M. J. Merrett and *Chlamydomonas reinhardtii*, strain 137c mt$^+$ for which we thank Drs. I. Zelitch and P. R. Day. Both species were grown in LEVINE AND EBERSOLD'S[24] medium at an incident intensity of 1000 ft candles and gassed either with air or 5% (v/v) CO_2 in air. Incubation temperature was 24°. The mass spectrometer used had similar characteristics to the instrument at RIAS and the sample vessel serving as inlet system was basically identical. Measurements were made at 24°.

RESULTS AND DISCUSSION

Figs. 1 and 2 are from data collected at RIAS and show the time courses of rates of O_2 production and consumption as well as net CO_2 exchange before, during and immediately after illumination with saturating light. In order to monitor changes in rates of CO_2 exchange after illumination, it was necessary to forego monitoring O_2 over the same interval. The results shown in Fig. 1 were obtained with high, and those

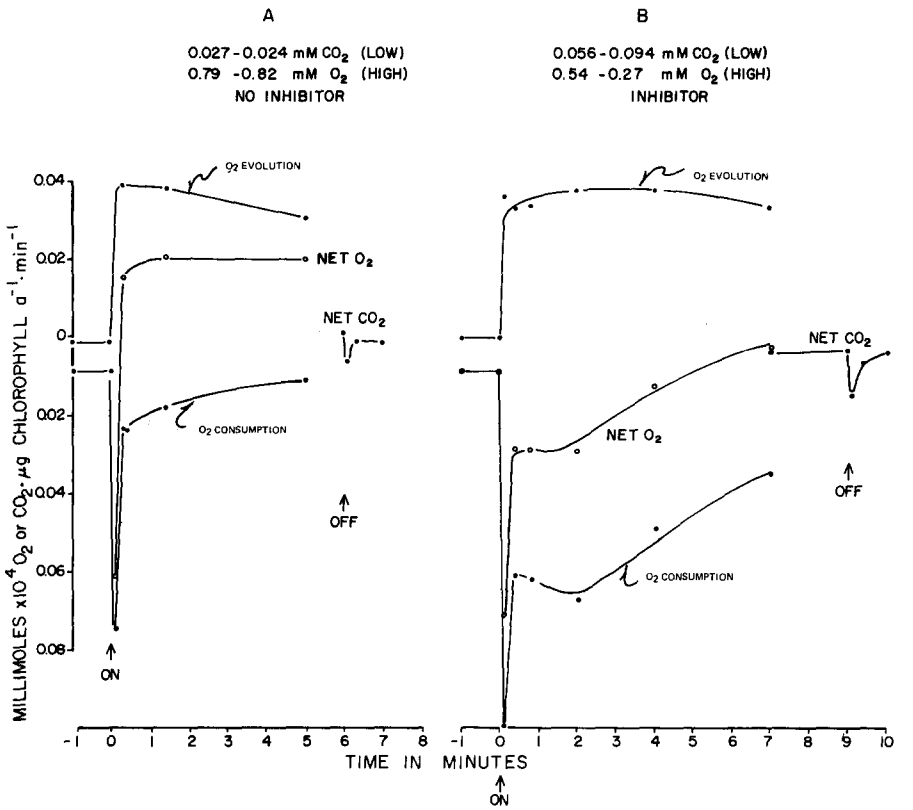

Fig. 1. Time-course of O_2 production and consumption, net O_2 exchange and net CO_2 exchange during a dark, light, dark cycle in Chlamydomonas. Low level CO_2 and high level O_2. The inhibitor was α-hydroxy-2-pyridine methanesulfonic acid ($8.8 \cdot 10^{-3}$ M). Note that rates of CO_2 exchange have been inverted to simplify comparison with the data for O_2. Data points were calculated at appropriate points from continuous recorder traces.

in Fig. 2 with low levels of dissolved O_2 relative to water in equilibrium with air at 30° (close to 0.24 mM O_2). Concentrations are shown in the figures together with comparable information for CO_2 which was held at relatively low levels throughout: close to but not reaching the CO_2 compensation point. In each figure, two runs have been included; one without and one with α-hydroxy-2-pyridine methanesulfonic acid which inhibits photorespiration. Ignoring the brief but pronounced "gulps" of O_2 shown in Fig. 1, not yet studied in any detail, we wish to draw attention to the following features. It will be seen that rates of O_2 consumption were higher in the light than in the dark. These rates did not increase with time as the concentration of CO_2 dropped, but declined. Furthermore, addition of the sulfonate resulted in increased rates of O_2 consumption in the light and did not eliminate or reduce the post-illumination decline in CO_2 production as would be expected were this phenomenon attributable solely to glycolate photorespiration. Effects of varying the O_2 concentration were not obvious although these may have been masked or offset by inevitable variations in CO_2 concentration from experiment to experiment. The dramatic reversal in O_2 consumption shown in Fig. 2 in presence of sulfonate is attributed to severe depletion of the reservoir of dissolved O_2 in the reaction vessel with the added complication that O_2 production was halted almost completely with the approach to anaerobic conditions.

While these results argued against the observed O_2 consumption reflecting a glycolate-dependent photorespiration, the possibility remained that glycolate oxidase might have been suppressed by culturing the algae in 2% CO_2. Accordingly, the opportunity was taken to continue the experiments in this laboratory.

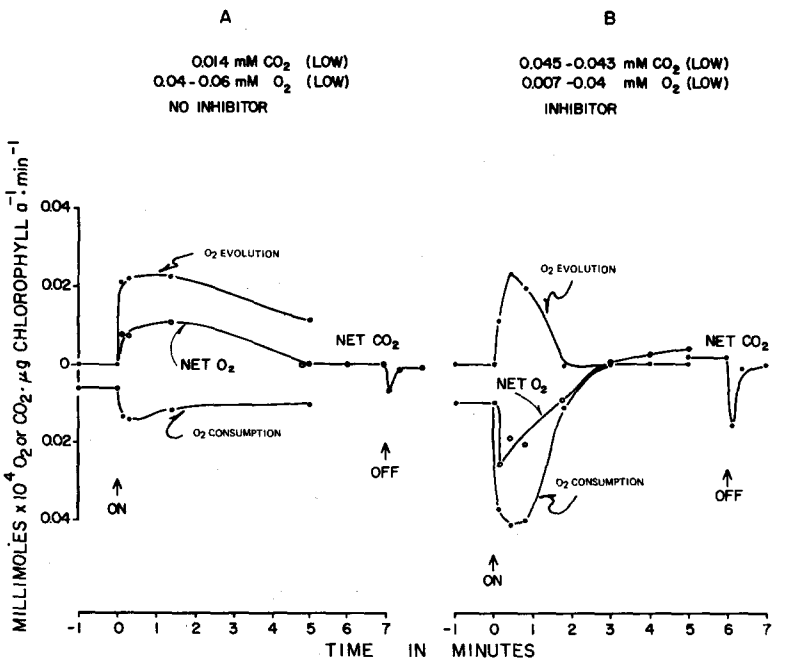

Fig. 2. Time-course of O_2 production and consumption, net O_2 exchange and net CO_2 exchange during a dark, light, dark cycle in Chlamydomonas. Low level CO_2 and low level O_2. Other details as in Fig. 1.

TABLE I

O_2 EXCHANGE AT CO_2 COMPENSATION POINT

Culture	*mmoles* × 10^4 O_2 · *μg chlorophyll* a^{-1} · min^{-1}			*O_2 consumption equivalent to glycolate–dichlorophenol-indophenol oxidoreductase* activity*
	O_2 consumption		*O_2 production*	
	Dark	*Light*		
Chlamydomonas				
0.03% CO_2	0.003	0.020	0.016	0.00030
5% CO_2	0.003	0.006	0.009	0.00002
Chorella				
2 day culture				
0.03% CO_2	0.001	0.004	0.005	0.00040
5% CO_2	0.001	0.006	0.006	0.00012
3 day culture				
0.03% CO_2	0.001	0.007	0.005	0.00033
5% CO_2	0.001	0.003	0.003	0.00008

* Cooksey, MS.

Data for observed rates of O_2 exchange by C. *reinhardtii* and *C. pyrenoidosa* at the onset of CO_2 compensation have been set out in Table I. Comparative data have been listed for cells grown in 5% (v/v) CO_2 in air and in air without CO_2 enrichment, in the case of Chlorella using cells from 2 day (log phase) and 3 day (late log phase) cultures. Air-grown Chlamydomonas was in log phase and the CO_2-grown cells were approaching the stationary phase. Also shown are rates of photorespiratory O_2 consumption that would be predicted from parallel *in vitro* glycolate-oxidising activities obtained by Dr. K. Cooksey using the same material for an associated study (Cooksey, MS).

Notice that O_2 consumption equivalent to glycolate oxidase activity *in vitro* was consistently higher in air-grown than in CO_2-grown cells. Observed O_2 consumption in the light was relatively higher in air-grown Chlamydomonas and in the older air-grown Chlorella culture. However, there was a wide disparity between predicted and observed O_2 consumption in the light. Furthermore, the ratios of predicted and observed uptakes in each case were in disagreement; in the 2-day cultures of Chlorella, where O_2 consumption in the light was slightly higher in CO_2-grown than in air-grown cells, the difference in predicted activity persisted, and, for the air-grown cells, was clearly higher than in Chlamydomonas.

Demonstration of glycolate oxidation *in vitro* has been taken as evidence for photorespiration in whole cells or organisms. However, the present study failed to demonstrate agreement between enzyme activity using an artificial electron acceptor and observed O_2 consumption in the light. In fact, CODD *et al.*[25] indicate that, in the same algae, O_2 consumption is not associated with the oxidation of glycolate. If this is true, it would follow that the O_2 consumption observed in the light in these experiments is not caused by glycolate oxidation. Whatever the process, it persists in cells grown with high levels of CO_2 and is stimulated, not inhibited, by α-hydroxy-2-pyridine methanesulfonic acid. Other authors (*e.g.* ASADA AND KASAI[26]) have suggested that sulfonates are not specific for the enzyme responsible for glycolate oxidation.

Finally, it should be noted that the rates of O_2 exchange measured by the mass spectrometer technique are possibly conservative. Although the rate of consumption of both isotopes of O_2 in the dark is proportional to their individual concentrations, it is possible that mass 32 O_2 produced during photosynthesis is preferentially consumed before an equilibrium can be established with isotope of mass 36. Such an effect would serve only to reflect greater rates of O_2 production and consumption than we have reported but would not alter the character of the observations.

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