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Glycolate Excretion and the Oxygen to Carbon Dioxide Net Exchange Ratio during Photosynthesis in *Chlamydomonas reinhardtii*[1]

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ABSTRACT

Chlamydomonas reinhardtii **cells were grown in high (5% v/v) or low (0.03% v/v) CO_2 concentration in air. O_2 evolution, HCO_3^- assimilation, and glycolate excretion were measured in response to O_2 and CO_2 concentration. Both low- and high-CO_2-grown cells excrete glycolate. In low-CO_2-grown cells, however, glycolate excretion is observed only at much lower CO_2 concentrations in the medium, as compared with high-CO_2-adapted cells. It is postulated that the activity of the CO_2-concentrating mechanism in low-CO_2-grown cells is responsible for the different dependence of glycolate excretion on external CO_2 concentration in low- *versus* high-CO_2-adapted cells.**

The O_2/CO_2 net exchange ratio is dependent on the CO_2 concentration in the medium and is linearly dependent on the fraction of glycolate excreted per CO_2 taken up. Glycolate excretion, however, is too low to account for the deviation of the O_2/CO_2 net exchange ratio from unity.

The rate of glycolate excretion by green and blue-green algae is strongly affected by the CO_2 concentration experienced by the cells during growth. Grown at high (0.2 to 5% v/v) and transferred to low (0.03% v/v) concentrations of CO_2, algal cells excrete large quantities of glycolate (12, 17, 18, 22, 29). The rate of glycolate excretion under these conditions decays to zero within several h. This cessation of glycolate excretion during the course of adaptation of algae from high- to low-CO_2 conditions was attributed to the induced activity of glycolate dehydrogenase (glycolate dichloroindophenol oxidoreductase) which enables low-CO_2-adapted cells to metabolize glycolate (8, 11, 27).

There are different reports on the effect of CO_2 concentration on the rate of glycolate excretion and the fraction of fixed carbon which is excreted as glycolate (3 to 90% in different reports (*e.g.* refs. 7, 16, 17, and 28). If glycolate is mainly produced by the RuBP[3] oxygenase reaction (20, 21), it is expected that changing the turnover rate of the Calvin cycle without affecting the ratio of oxygenase to carboxylase activities will result in a constant ratio of glycolate excreted to CO_2 taken up. This expectation has been confirmed when glycolate excretion and photosynthesis were measured as a function of light intensity in *Chlamydomonas* (7). Changing the relative activities of RuBP oxygenase and RuBP carboxylase, however, by raising or lowering O_2 or CO_2 concentration should affect the relative amount of glycolate excreted to CO_2 fixed. O_2 and CO_2 concentrations affect both the turnover rate of the Calvin cycle and the relative activity of RuBP carboxylase/oxygenase. CO_2 and O_2 concentrations must, therefore, be kept constant during the experiment, which is fairly difficult to achieve, especially at subsaturating concentration of CO_2.

Inasmuch as glycolate is a relatively oxidized end product of photosynthesis, its excretion in large quantities should result in an O_2/CO_2 net exchange ratio lower than unity. The O_2/CO_2 net exchange ratio in *Chlorella* was affected by O_2 concentration (15), probably due to the effect of O_2 concentration on glycolate production and excretion (16). CO_2 concentration, however, had a very small effect on the O_2/CO_2 net exchange ratio in *Chlorella* (15). Inasmuch as the deviation of the O_2/CO_2 exchange ratio from unity must be explained by the accumulation of a relatively oxidized end product of photosynthesis (6, 19), it is difficult to interpret data showing no effect of CO_2 concentration on this ratio, although glycolate excretion (which was not measured) should have been affected. It is also not known to what extent glycolate excretion can explain the deviation of the observed O_2/CO_2 ratio from unity.

Here, glycolate excretion and the O_2/CO_2 net exchange ratio of high- and low-CO_2-adapted *Chlamydomonas reinhardtii*, as affected by CO_2 concentration, is reported.

MATERIALS AND METHODS

C. reinhardtii cells (haploid strain 137C, stock GB-126) were grown phototropically in 300-ml shake cultures of HS culture media (25) aerated with air (low-CO_2-grown) or 5% (v/v) CO_2 in air (high-CO_2-grown), as described by Berry *et al.* (4).

O_2 evolution was measured in a closed electrode chamber (Rank Brothers, Bottisham, Cambridge, England), modified for increased sensitivity as described by Berry and Bowes (3). After depletion of endogenous CO_2 (in the media or as a pool within the cells), O_2 evolution was completely dependent upon the addition of $NaHCO_3$. $NaHCO_3$ was injected at a constant rate (using a syringe pump) which permitted the maintenance of a constant rate of O_2 evolution. The latter was completely dependent on the rate of HCO_3^- injection. Raising the rate of injection resulted in a faster rate of O_2 evolution. The steady-state HCO_3^- concentration within the O_2 electrode chamber could not be determined directly. It was estimated, however, by comparing the rate of O_2 evolution during the continuous injection of HCO_3^- with that observed following the addition of known amounts of HCO_3^- to cells which had previously depleted the inorganic carbon in the medium (as indicated by O_2 compensation point). The above procedure allowed construction of a "calibration curve" relating the rate of O_2 evolution to the HCO_3^- concentration in the same type of cells under the same experimental conditions. The concentration of inorganic carbon in the medium was also deduced from the

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[3] Abbreviation: RuBP, ribulose 1,5-bisphosphate.

amount of acid labile ^{14}C detected following the injection of $NaH^{14}CO_3$, using the same concentration and the same experimental conditions as in the unlabeled experiments. There was good agreement between the radioactive method and the one described above.

Cells were washed with the growth media (excluding the microelements which were found to interfere with the glycolate determination) transferred to 50 mM Hepes buffer (pH 7.0), counted, and then placed in the O_2 electrode chamber (4 ml cell suspension, 5×10^6 cells/ml, 20 C, 35 nE cm^{-2} s^{-1}, 400–700 nm) and allowed to use up the CO_2 in the media. Injection of $NaHCO_3$ started when O_2 compensation point was reached. To measure the amount of glycolate present in the solution, a 0.5-ml cell suspension sample was taken at the same time. Injection of HCO_3^- and O_2 evolution were allowed to proceed for at least 20 min inasmuch as it was found that the rate of glycolate excretion remains constant during that period (not shown; see ref. 17). Cells were separated from the solution by filtration, and the glycolate concentration in the medium was measured by the method of Calkins (9).

The rate of O_2 evolution was calculated from the signal of the O_2 electrode. The rate of HCO_3^- uptake was calculated from the concentration of HCO_3^- injected and the rate of injection. It should be emphasized that the rate of HCO_3^- assimilation may be overestimated, especially at the higher range of HCO_3^- concentration, as HCO_3^- could accumulate in the O_2 electrode chamber (see below).

RESULTS AND DISCUSSION

In both high- and low-CO_2-grown *Chlamydomonas*, the rates of glycolate excretion decrease and O_2 evolution increases with elevated concentration of HCO_3^- in the medium (Fig. 1). Maximum rate of glycolate excretion is obtained at the lowest HCO_3^- concentration. Ingle and Colman (18) found a similar effect of CO_2 concentration on the rate of glycolate excretion in *Coccochloris peniocystis*. Hess *et al.* (17) reported a larger rate of glycolate excretion in *Scenedesmus* and *Chlorella* exposed to 10 mM HCO_3^-, as compared with no addition of HCO_3^-. Their experiments however, were conducted in an O_2 atmosphere. In the experiments reported here, O_2 and CO_2 concentrations were kept constant (within ±10%) even at subsaturating concentrations of CO_2, which is of paramount importance in this type of experiment. It is possible that, under different conditions, such as higher O_2 concentrations, glycolate excretion rate will initially increase with elevated CO_2 concentration (7).

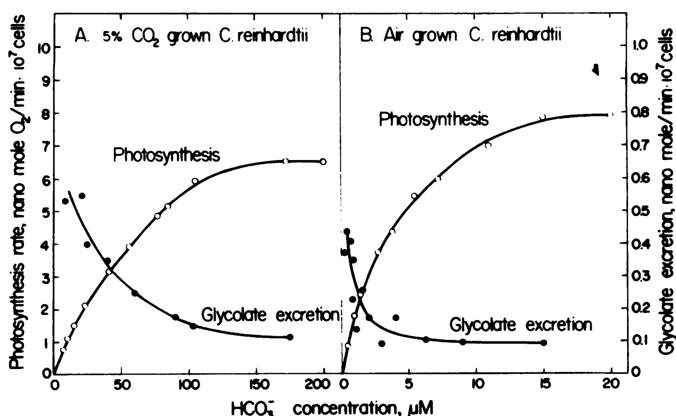

FIG. 1. Response of glycolate excretion and O_2 evolution to HCO_3^- concentration in the medium in high- (A) and low- (B) CO_2-grown *C. reinhardtii.* Chl content was 5.3 and 6.6 μg/10^7 cells of air-grown and 5% CO_2-grown, respectively. Four ml cell suspension, 5×10^6 cells/ml, 20 C, 50 mM Hepes buffer (pH 7.0), 35 nE cm^{-2} s^{-1} (400–700 nm), 20 to 22% O_2. Note: 10-fold difference in scale of HCO_3^- concentration.

It has been reported that low-CO_2-grown algae do not excrete glycolate. This has been attributed to the induction of glycolate dehydrogenase activity by low CO_2 conditions (22). Data presented here (Fig. 1) clearly demonstrate that glycolate excretion by low-CO_2 cells can only be detected at very low CO_2 concentration. The activity of glycolate dehydrogenase may explain the lower rate of glycolate excretion in low-CO_2 cells as compared with high-CO_2-grown cells (Fig. 1). Glycolate dehydrogenase activity, however, cannot explain the very different dependence of the rates of O_2 evolution and glycolate excretion upon HCO_3^- concentration in high- *versus* low-CO_2-grown cells. Further, experiments in which the incorporation of [^{14}C]glycolate and the activity of glycolate dehydrogenase were measured showed that the difference in glycolate excretion between high- and low-CO_2-grown algae cannot be attributed to glycolate being metabolized (12, 26).

The different apparent photosynthetic affinity to CO_2 in high- as compared with low-CO_2-grown *Chlamydomonas* (ref. 4 and Fig. 1) is attributed to the activity of the CO_2-concentrating mechanism. Low-CO_2-grown *Chlamydomonas* are capable of concentrating CO_2 inside the cells up to 40 times its concentration in the medium, whereas high-CO_2-grown cells exhibit very limited capability of concentrating CO_2 (1, 2). It has been suggested that CO_2 and O_2 compete for the RuBP carboxylase/oxygenase reaction which lead to the formation of glycolate (20, 21). Hence, the elevated internal CO_2 concentration in low-CO_2-grown cells may inhibit the rate of RuBP oxygenase, resulting in a lower rate of glycolate formation. The kinetics of inhibition of glycolate excretion by CO_2 is presented as a Dixon plot (24) in Figure 2. CO_2 is for this purpose treated as the inhibitor (since it competitively inhibits the RuBP oxygenase). O_2 is regarded as the substrate and V is the rate of glycolate excretion. In both high- and low-CO_2-grown cells, we could not detect glycolate excretion at O_2 concentration of 3%, regardless of the CO_2 concentration in the medium (not shown). The lack of glycolate excretion at low O_2 concentration is presumably due to the effect of O_2 concentration on the rate of glycolate formation by the RuBP oxygenase. In the case of high-CO_2-grown cells, linear correlation between $1/v$ and the concentration of CO_2 in the medium is obtained in both 20 to 22% O_2 (Fig. 2, line A) and 40 to 44% O_2 (Fig. 2, line B). The expected rate of glycolate excretion at zero CO_2 (the intersection with the

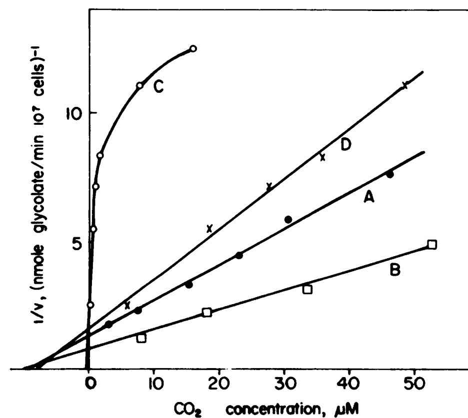

FIG. 2. The $1/v$ (v = rate of glycolate excretion) as a function of CO_2 concentration in *C. reinhardtii.* A, high-CO_2-grown cells, 20 to 22% O_2; B, high-CO_2-grown cells, 40 to 44% O_2; C, low-CO_2-grown cells, 20% O_2, $1/v$ plotted against external CO_2 concentration; D, data of line C plotted against the internal CO_2 concentration [calculated from Badger *et al* (1, 2)]; other conditions were as for Figure 1. Note: the lowest concentration of CO_2 in line C is 0.2 μM. Data presented in line C are those for which we have the dependence of the internal CO_2 concentration on the external one.

y axis) is 0.77 and 1.25 nmol min^{-1} 10^{-7} cells for the 20 and 40% O_2 concentrations, respectively. The two lines (Fig. 2, A and B) intersect at K_i = 8 μM CO_2. In low-CO_2-grown cells, on the other hand, a curved line is obtained when $1/v$ is plotted against the external CO_2 concentration (Fig. 2, line C). However, if $1/v$ is plotted against the internal CO_2 concentration (calculated from the dependence of the internal CO_2 concentration on the external one; *e.g.* ref. 2), a linear correlation is observed (Fig. 2, line D). Line D (in Fig. 2) intersects with the *y* axis at V = 0.67 nmol min^{-1} 10^{-7} cells. In the case of low-CO_2-grown cells, K_i cannot be calculated since the dependence of the internal CO_2 concentration on the external one was not determined at O_2 concentration other than 20%. Considering the similar K_m (CO_2) of RuBP carboxylase and the similar activity of RuBP oxygenase in high- and low-CO_2-grown *Chlamydomonas* (4) and the nature of the data presented in Figure 2, it is very likely that K_i in low-CO_2-grown cells is very similar to that obtained in high-CO_2 cells. The lower V indicated by the intersection of line D (Fig. 2) with the *y* axis (*i.e.* at zero CO_2) in low-CO_2 cells (0.67 nmol min^{-1} 10^{-7} cell as compared with 0.77 in high-CO_2-grown cells) may be due to glycolate dehydrogenase activity in low-CO_2 cells.

The data presented in Figure 2 (lines A and B) clearly indicate that the mode of inhibition of glycolate excretion by CO_2 is competitive (24). The fact that, in the long run, CO_2 is also the substrate for the formation of RuBP which is the precursor for glycolate does not seem to affect the competitive inhibition kinetics observed, at least not within the experimental period investigated here. The data in Figure 2 also suggest that lower rate of glycolate formation and not faster rate of glycolate metabolism is responsible for the decay of glycolate excretion during adaptation of *Chlamydomonas* from high to low CO_2 concentration.

The effect of HCO_2^- concentration on the O_2/CO_2 net exchange ratio and the glycolate excreted/HCO_3^- assimilated is shown in Figure 3, A and B, for high- and low-CO_2 cells, respectively. O_2/HCO_3^- net exchange ratio in both types of cells increased with elevated HCO_3^- concentration and would probably have reached a value very close to 1, as glycolate excretion decreased (Fig. 1). However, as a result of the technique used, when photosynthetic rate deviated from being linearly dependent on HCO_3^- concentration, HCO_3^- could accumulate in the reaction chamber. This resulted in a decrease (not shown) in O_2/HCO_3^- net exchange ratio after reaching the plateau shown in Figure 3. The deviation of the O_2/HCO_3^- exchange ratio from unity at the lower range of HCO_3^- concentration cannot be attributed to accumulation of HCO_3^-, as such accumulation should have affected the rate of O_2 evolution. The latter however, remained constant during the experiment even at subsaturating concentration of HCO_3^-.

As expected, the glycolate/HCO_3^- ratio (presented on a molar basis) decreased in both types of cell with elevated concentrations of HCO_3^-. This reflects the decreased rate of glycolate excretion and the increased rate of HCO_3^- uptake with elevated concentrations of HCO_3^-. The ratio of glycolate excreted/HCO_3^- assimilated represents the drain on the carbon pool imposed by glycolate excretion. At low CO_2 concentrations, the amount of carbon excreted as glycolate may even exceed the amount of carbon fixed, presumably on the expense of reserve carbohydrates used as substrates for glycolate formation (see Fig. 3 and refs 16 and 17). It is difficult to estimate the drain of carbon imposed by glycolate excretion, as the amount of carbon cycled in dark respiration processes was not measured (5, 10, 16), It is clear, however, that this drain of carbon due to glycolate excretion is very dependent on the CO_2 and O_2 concentration.

O_2/HCO_3^- net exchange ratio in *Chlamydomonas* also depends on CO_2 and O_2 concentration. A ratio of 1.0 is obtained at low (~ 3%) O_2 concentration (data now shown). The O_2/CO_2 net exchange ratio observed here indicates that a product which is relatively oxidized should have accumulated during the course of our experiment (6, 19). If glycolate is the only relatively oxidized product which accumulates, its production should quantitatively explain the observed O_2/HCO_3^- ratio. The expected line shown in Figure 4 was, therefore, calculated on the basis that, if all the CO_2 taken up in photosynthesis is recovered in glycolate, glycolate/HCO_3^- (molar basis) would equal 0.5 and O_2/HCO_3 = 0.75. O_2/HCO_3^- is linearly dependent upon glycolate/HCO_3^- (correlation coefficient = 0.88). The experimental data, however, deviates from the expected line. It is concluded that the excretion of glycolate is not large enough to account for the deviation of the O_2/CO_2 ratio from unity. The O_2/CO_2 ratio may be the result of an increasing pool of glycolate which was not excreted (13) and which was dependent upon the rate of glycolate formation. CO_2 evolution in the light in high-CO_2-adapted cells (14) could also contribute to the observed deviation. The exact contribution, however, is not known, as CO_2 evolution was not measured.

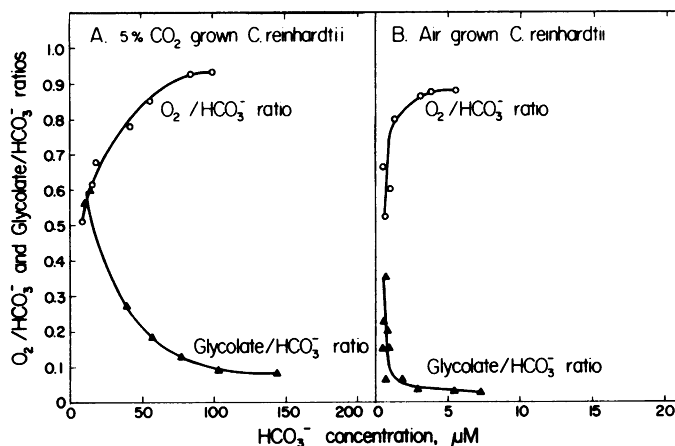

FIG. 3. Glycolate/HCO_3^- and O_2/HCO_3^- net exchange ratios in response to HCO_3^- concentration in the medium of high- (A) and low- (B) CO_2-grown *C. reinhardtii*. Experimental conditions were as for Figure 1.

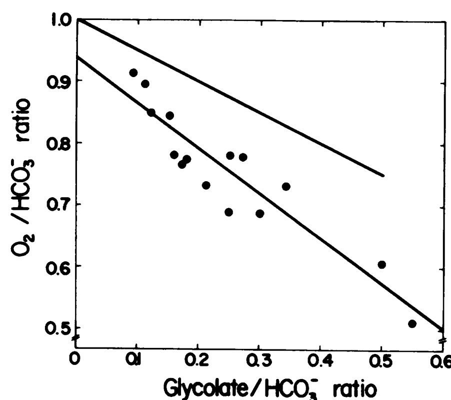

FIG. 4. O_2/HCO_3^- as a function of glycolate/HCO_3^- in high-CO_2-grown *C. reinhardtii*. The upper line presents the expected results if all HCO_3^- taken up would have been recovered in the excreted glycolate (O_2/HCO_3^- = 0.75, glycolate/HCO_3^- = 0.5). Data are replotted from Figure 3.

O_2 evolution is often used as a criterion for CO_2 fixation by algae. Because of the dependence of O_2/CO_2 net exchange ratio on CO_2 and O_2 concentration reported here, it is suggested that O_2 evolution should not be used to assess the kinetics of CO_2 assimilation without monitoring the O_2/CO_2 net exchange ratio (23).

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