

Glycolate Metabolism and Excretion by *Chlamydomonas reinhardtii*¹

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ABSTRACT

The flux of glycolate through the C₂ pathway in *Chlamydomonas reinhardtii* was estimated after inhibition of the pathway with aminoxyacetate (AOA) or aminoacetonitrile (AAN) by measurement of the accumulation of glycolate and glycine. Cells grown photoautotrophically in air excreted little glycolate except in the presence of 2 mM AOA when they excreted 5 micromoles glycolate per hour per milligram chlorophyll. Cells grown on high CO₂ (1–5%) when transferred to air produced three times as much glycolate, with half of the glycolate metabolized and half excreted. The lower amount of glycolate produced by the air-grown cells reflects the presence of a CO₂ concentrating mechanism which raises the internal CO₂ level and decreases the ribulose-1,5-bisP oxygenase reaction for glycolate production. Despite the presence of the CO₂ concentrating mechanism, there was still a significant amount of glycolate produced and metabolized by air-grown *Chlamydomonas*. The capacity of these cells to metabolize between 5 and 10 micromoles of glycolate per hour per milligram chlorophyll was confirmed by measuring the biphasic uptake of added labeled glycolate. The initial rapid (<10 seconds) phase represented uptake of glycolate; the slow phase represented the metabolism of glycolate. The rates of glycolate metabolism were in agreement with those determined using the C₂-cycle inhibitors during CO₂ fixation.

the oxidative photosynthetic carbon cycle (C₂ cycle) may be different or absent in this alga (12, 20). When *Chlamydomonas* cells are grown with limiting CO₂ for at least 3 h they induce a CO₂ concentrating system (1, 3), which includes one or more isozymes of carbonic anhydrase (7). This CO₂ concentrating system seems to increase the effective CO₂ concentration at the site of ribulose-1,5-bisP carboxylase/oxygenase to favor the carboxylation reaction over the oxygenation reaction and reduce P-glycolate biosynthesis. This would reduce the loss of CO₂ from the decarboxylation of glycine which would reduce the CO₂ compensation point. However, when inhibitors of the C₂ cycle are added to air adapted cells illuminated in the presence of 21% O₂ and 330 μL/L CO₂, a large amount of glycolate was excreted indicating that a significant flux through the C₂ pathway did exist (27). As in a C₄ plant, the low CO₂ compensation point seen in air-grown algal cells probably reflects their ability to trap and efficiently refix the CO₂ from photorespiration.

In this work we have estimated the flux of carbon through the C₂ cycle by measuring the amount of glycolate excreted and by looking at the metabolism of externally added glycolate. While the CO₂ concentrating mechanism in air-grown cells does decrease the ribulose-1,5-bisP oxygenation reaction, it does not eliminate the biosynthesis of glycolate. In addition, while some of the enzymes of the C₂ cycle in green algae are different from those in higher plants, the flow of carbon from glycolate to glyoxylate, glycine and serine (11) is apparently the same.

Chlamydomonas reinhardtii exhibits a higher affinity for C₃ when grown phototrophically with air levels of CO₂ (0.03–0.04%) than when grown with air supplemented with 1 to 5% CO₂ (3). In addition, the air-grown algal cells, like C₄ plants, appear to lack photorespiration, in that they have a low CO₂ compensation point (<1 μM CO₂) and there is no significant O₂ inhibition of net CO₂ fixation (13, 20, 24). Air-adapted *Chlamydomonas* normally do not excrete glycolate into the media (19, 27), in contrast to the high rates of glycolate excretion by cells grown in the presence of high levels of CO₂ and then exposed to low CO₂ conditions. Both cell types have only small pools of glycine and serine even when illuminated under photorespiratory conditions (low CO₂ and/or high O₂).

Since the air-adapted cells exhibit such low CO₂ compensation points and low O₂ inhibition of CO₂ fixation and produce only small amounts of glycine and serine, it has been proposed that

MATERIALS AND METHODS

Chlamydomonas reinhardtii, strains 137+ from Dr. R. K. Togasaki and 2137+ from Dr. M. H. Spalding were grown phototrophically in minimal media (26). Cultures of 1 L were grown in Fernbach flasks with continuous mixing on an Eberbach shaker. During illumination (100 μE m⁻² s⁻¹ at 20–25°C) cultures were aerated with either air or air supplemented with 3 to 5% CO₂. Cells were harvested in the middle part of the log phase of growth by centrifugation at 1,000g for 5 min at 4°C. The cells were washed once by resuspending the pellet in 25 mM Hepes-KOH (pH 7.2) and recentrifuged at 10,000g for 5 min. The cells were then resuspended in the same buffer at a concentration of about 0.4 mg Chl·ml⁻¹ and stored on ice until use within 2 h.

Photosynthetic CO₂ assimilation was measured by determining the acid-stable ¹⁴C present after illumination in the presence of NaH¹⁴CO₃ (50 mCi/mmol). Harvested cells were diluted from the concentrated suspension to a final concentration in the buffers as indicated in the table and figure legends. Buffers were prepared daily and adjusted to the indicated pH with KOH and then bubbled with N₂ to lower both the dissolved CO₂ and O₂ prior to the addition of cells. Experiments were performed in a Rank Brothers O₂ electrode so that the O₂ concentration could be monitored throughout the experiment. To deplete the diluted cell suspension of the endogenous CO₂, the suspension was

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³ Abbreviations: C_i, inorganic carbon (CO₂ + HCO₃⁻); AOA, aminoxyacetate; AAN, aminoacetonitrile; K_{0.5} (CO₂), the CO₂ concentration when oxygen evolution is half-maximal.

illuminated until O₂ evolution ceased. At this point the O₂ concentration was still below the normal air level of 21% and could be increased by exposing the suspension to air. The suspension was then illuminated for one additional minute in the presence or absence of inhibitor(s) before the addition of [¹⁴C] bicarbonate to the concentration indicated in the table and figure legends. In some short-term experiments the C_i concentration was maintained at 60 μM by the addition of NaH¹⁴CO₃ whenever more than 10% of the added bicarbonate was depleted, as determined by the extent of O₂ evolution (3, 17).

For experiments with a constant concentration of CO₂ and O₂, air in a 20 L sealed container was circulated through the cell culture. The air had been depleted of CO₂ by recycling through a 2 N KOH solution for 3 h, and then ¹⁴CO₂ equivalent to 310 μl/L was added to the reservoir by the addition of NaH¹⁴CO₃ to a 2 N HCl solution in the container. The cell suspensions, previously depleted of endogenous CO₂, were illuminated and aerated with 300 ml·min⁻¹ of the 310 μl/L ¹⁴CO₂ air. Thus glycolate excretion and CO₂ fixation were measured in the same experiment. Excreted glycolate was assayed by the Calkins (5) procedure after sedimenting the cells. In some cases the supernatant was first concentrated by partial removal of the solvent under reduced pressure. The temperature was held at 30°C and the loss of glycolate was less than 5%.

For identification of the labeled C₂ cycle intermediates, air-grown cells or 5% CO₂-grown cells (25 mg Chl·ml⁻¹) were incubated in the light for 3 or 4 min in the presence of 10 μM NaH¹⁴CO₃. The incubation was stopped by the addition of an equal volume of hot methanol. Aliquots of the soluble labeled metabolites were chromatographed on Whatman No. 1 paper for product identification (2). Before the paper was completely dry, it was sprayed with a 1 M NaHCO₃ solution to prevent volatilization of glycolic acid. The radioactive compounds were located by exposure to x-ray film for about 2 weeks, and the spots were cut out and counted in scintillation vials. Compound identification was based upon a standard chromatographic map of R_F values (2).

For glycolate uptake experiments, air-grown or 5% CO₂-grown cells (50 μg Chl·ml⁻¹) were incubated in 25 mM Hepes-KOH (pH 7.5) with 10 mM [2-¹⁴C]glycolate or [1-¹⁴C]glycolate for the indicated times. Aliquots of 200 μL were centrifuged through silicone oil in 400 μL microfuge tubes (1, 16) and the amount of label in the pellet was determined. The amount of label in the pellet was corrected for internal volume by doing parallel uptake studies with [¹⁴C]sorbitol and ³H₂O as previously described (1, 17).

AOA, AAN, and acetazolamide were from Sigma. NaH¹⁴CO₃ was from New England Nuclear. [2-¹⁴C]Glycolate was purchased from ICN Biomedicals, Inc. The [2-¹⁴C]glycolate was found to have a small contamination of H¹⁴CO₃⁻, which was highly significant if uptake of ¹⁴C label was followed. Therefore, the labeled glycolate was purified by acidification overnight with 200 mM HCl. This acidified stock was then diluted with unlabeled glycolate to a specific radioactivity of 25 μCi·mmol⁻¹.

RESULTS

Amount of Glycolate Metabolism or Excretion. When *Chlamydomonas* cells were illuminated in the presence of low concentrations of CO₂ and 2 mM AOA, they excreted glycolate (27). The extent of glycolate excretion by air-grown or 5% CO₂-grown *Chlamydomonas* cells is shown in Table I. During the experiment, 21% of O₂ and 310 μl/L ¹⁴CO₂ were kept constant by recirculating from a large reservoir. Both glycolate excretion and ¹⁴CO₂ fixation were measured at the same time. At this low concentration of CO₂, the rate of CO₂ fixation was 2 to 3 times higher in the air-grown cells than in the 5% CO₂-grown cells due to the presence of the C_i concentrating system in the air-grown

Table I. Glycolate Excretion by Air or High CO₂-Grown *Chlamydomonas* Bubbled with Air in the Presence or Absence of Aminooxyacetate

Cells were illuminated for 30 min in 25 mM Hepes-KOH (pH 7.2).

Growth Conditions	2 mM AOA	Glycolate Excretion	CO ₂ Fixation	Glycolate Excretion as Percent of CO ₂ Fixed
		<i>μmol·h⁻¹·mg⁻¹Chl</i>		%
Air	-	0.1	77	0.1
Air	+	5.1	70	7.3
High CO ₂	-	8.0	33	24
High CO ₂	+	14.8	27	55

cells. These data are consistent with the previously measured K_{0.5}(CO₂) for air-grown or 5% CO₂-grown cells (1, 17). In aqueous solutions equilibrated with air levels of CO₂, the CO₂ concentration (11 μM) is higher than the K_{0.5}(CO₂) of 0.5 to 3 μM for the air-grown cells, but less than the K_{0.5}(CO₂) of 25 μM for the 5% CO₂-grown cells (1, 17). The air-grown cells excreted only traces of glycolate in the absence of inhibitor. However, in the presence of AOA, air-grown cells excreted about 5 μmol glycolate·h⁻¹·mg⁻¹Chl which was about one-third the rate of the high CO₂-grown cells (Table I). While this rate was lower than the rate of glycolate excretion in CO₂-grown cells, it generally was between 5 and 10% of the CO₂ fixation rate. The results indicate that there was a significant amount of glycolate production and metabolism despite the presence of the CO₂ concentrating system and the absence of excreted glycolate.

Experiments were performed to determine whether AOA was increasing glycolate biosynthesis or blocking its metabolism. Previous work has indicated that the glycolate excreted from *Chlorella* (15) or *Chlamydomonas* (28) was formed by the ribulose-1,5-bisP oxygenase and P-glycolate phosphatase activity of the C₂ cycle. Since AOA is an inhibitor of the glyoxylate-serine aminotransferase (23), it is likely that the increase in glycolate excretion was due to the blockage of glycolate metabolism. However, because the increase in glycolate excretion was large when AOA was added, and for air-grown cells somewhat unexpected, it was possible that AOA may have other effects (27).

One possible explanation for the increase in glycolate excretion by AOA treated air-grown cells was that AOA inhibited the CO₂ concentrating mechanism. This was not the case because AOA did not change the measured K_{0.5}(CO₂) of air-grown cells, which remained very low at about 1 μM. If AOA were inhibiting the CO₂ concentrating mechanism, the K_{0.5}(CO₂) would be expected to increase. In addition, air-grown cells accumulated C_i to the same extent (to 1.5 mM when incubated with 200 μM NaH¹⁴CO₃ at pH 7.2) in the presence or absence of AOA, which also indicated that the C_i concentrating mechanism was unaffected. AAN, an inhibitor of glycine to serine interconversion in higher plants (22, 29), also had no effect on the K_{0.5}(CO₂) for O₂ evolution or C_i accumulation.

Another way to test whether AOA or AAN altered the CO₂ concentration mechanism in *Chlamydomonas* was to measure CO₂ fixation rates at low concentrations of CO₂ where high rates of CO₂ fixation are most dependent on an operational C_i accumulating mechanism. AAN did not inhibit CO₂ fixation by *Chlamydomonas* at low CO₂ concentrations, and AOA caused, at most, a 10 to 18% inhibition. In contrast, when acetazolamide was used to inhibit the periplasmic carbonic anhydrase which is a component of the C_i concentrating mechanism of *Chlamydomonas* (16), there was an increase in the K_{0.5}(CO₂), and a 71% decrease in the rate of CO₂ assimilation (16). These results are consistent with the hypothesis that AOA and AAN were not affecting C_i accumulation and that the increased label in glycolate and glycine reflect the inhibition of the normal metabolism of

these C₂ cycle intermediates.

Products of the C₂ Cycle. To measure the inhibition of the C₂ cycle by AOA or AAN, NaH¹⁴CO₃ of high specific activity was given to air-grown or 5% CO₂-grown cells for short periods of time (3 or 4 min), and the per cent ¹⁴C in glycolate, glycine and serine was determined (Table II). These experiments were done at air levels of O₂ with the CO₂ concentration maintained at 10 μM by additions of [¹⁴C]bicarbonate (see legend to Table II). In agreement with the results in Table I, a rate of 30 μmol CO₂ fixation·h⁻¹·mg⁻¹Chl by 5% CO₂-grown cells was about one-third that of air-grown cells at this low CO₂ concentration. However, the maximum rates of CO₂ fixation for both cell types was about the same, averaging 110 μmol·h⁻¹·mg⁻¹Chl. When 2 mM AOA was added to air-grown cells a large increase in labeled glycolate was observed. When AAN was added to air-grown cells, the amount of label in glycine increased but the label in glycolate did not. In the presence of both inhibitors only labeled glycolate accumulated, as expected, since the pathway should be blocked by AOA before glycine formation. These data indicate that AOA was effectively blocking the C₂ pathway between glycolate and glycine, because no glycine accumulated in the presence of both inhibitors. The same pattern was observed in 5% CO₂-grown cells except that the percentage of label was higher in the C₂ cycle intermediates. This increased percentage reflects both the higher rate of glycolate production in these cells without the C₁ pump and the lower rate of CO₂ fixation. With AOA present, the label in glycolate represented 33% of the fixed CO₂, after only 4 min. With the C₂ pathway blocked, glycolate was an end product that the algal cells excreted.

Amount and Specific Activity of Excreted Glycolate. Since metabolism of the C₂ intermediates could be effectively blocked by AOA or AAN, the buildup of label in these compounds, specifically glycolate, could give an estimation of the total carbon flux through the C₂ pathway, if the specific activity of the C₂ cycle intermediates were known. The specific radioactivity of glycolate formed after the addition of ¹⁴CO₂ is less than that of the added NaH¹⁴CO₃ at short times because it arises also from the unlabeled sugar phosphate reserves of the C₃ cycle (28). To calculate the specific radioactivity of the excreted glycolate, both the total amount as measured by the Calkins method and the amount of ¹⁴C in glycolate were determined from both air-grown

Table II. ¹⁴C Incorporation into Glycolate, Glycine, and Serine by Air-Grown or 5% CO₂-Grown *Chlamydomonas*

Cells (25 μg Chl·ml⁻¹) in 25 mM HEPES-KOH (pH 7.0) were illuminated in an O₂ electrode chamber until the endogenous CO₂ was depleted. Where indicated, the inhibitors were added and the cells illuminated for an additional minute. NaH¹⁴CO₃ was then added to 60 μM and maintained at that concentration by additions of 10 μM NaH¹⁴CO₃ whenever 10 μM O₂ was evolved during the course of the experiment. The reaction was terminated by the addition of methanol to 50% after 3 min for the air-grown cells and after 4 min for the 5% CO₂-grown cells.

Cell Growth Conditions and Additions (2 mM)	Glycolate	Glycine	Serine
	% total ¹⁴ C fixed		
Air-grown			
None	2.0	0.8	4.1
AOA	14.5	0.4	1.6
AAN	1.8	9.0	2.9
AOA + AAN	14.6	0.7	1.8
5% CO ₂ -grown			
None	10.8	1.5	5.3
AOA	33.2	0.3	1.6
AAN	12.3	23.5	3.1
AOA + AAN	31.9	1.1	0.2

and 5% CO₂-grown cells. In the average of 3 experiments the specific radioactivity of the excreted glycolate was 37% of the added [¹⁴C]bicarbonate in the case of 5% CO₂-grown cells, and 78% in the case of air-grown cells. The lower specific radioactivity of the glycolate in the high CO₂-grown cells is probably due to the utilization of carbon reserves by these cells under the low CO₂ conditions of these experiments. When these cells, without the C₁ concentration mechanism, are illuminated in the presence of limiting CO₂, the rate of ribulose-1,5-bisP oxygenation could deplete the chloroplast of its sugar phosphate reserves. This is especially true when the C₂ pathway is blocked and the carbon in glycolate cannot be returned to the chloroplast as CO₂ and glycerate. Under these conditions more of the ribulose-1,5-bisP seems to come from unlabeled carbon reserves within the cell instead of from newly labeled PGA from ribulose-1,5-bisP carboxylation and the C₃ cycle. Using these figures in short-term labeling experiments (such as Table II), the flux of glycolate through the C₂ cycle was calculated to be 4.5 μmol glycolate h⁻¹·mg⁻¹Chl for air-grown cells under low CO₂ conditions, while a somewhat higher production of about 10 μmol glycolate·h⁻¹·mg⁻¹Chl was found for 5% CO₂-grown cells. These results are similar to the rate of glycolate excretion for the 30 min experiment reported in Table I.

Glycolate Uptake and Metabolism. The ability of air and CO₂-grown cells to utilize glycolate was estimated by feeding ¹⁴C-labeled glycolate. Spencer and Togasaki (25) have shown that *Chlamydomonas* can utilize exogenously added glycolate and that under low CO₂ conditions the added glycolate stimulated algal growth. The added external glycolate pool equilibrated with the internal glycolate pool in less than 10 s or by the time the first point in Figure 1. The rate of glycolate incorporation into other products in the algal cells after the first 10 s was a measure of the rate of glycolate metabolism (Fig. 1). An external glycolate concentration of 10 mM was required to saturate the rate of glycolate metabolism (data not shown). Glycolate (10 mM) also saturated the growth stimulation by glycolate previously reported (25). After feeding [¹⁴C]glycolate, the first products are glycine and serine, as reported by Lord and Merrett (14) with *Chlorella*.

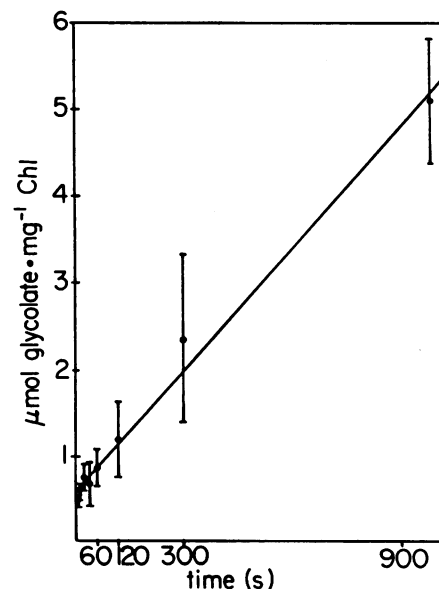


FIG. 1. Time course of the uptake of glycolate by *Chlamydomonas*. Air-grown cells were incubated at room temperature in the light with 10 mM [¹⁴C]glycolate. Cells were separated from the media by centrifuging through a layer of silicone oil. Glycolate uptake was determined by measuring the radioactivity in the pellet. Each point is the average of 6 determinations.

The rates of metabolism of added glycolate by air-grown or 3% CO₂-grown cells are shown in Figure 2. For air-grown cells glycolate metabolism was between 6 and 13 $\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{mg}^{-1}\text{Chl}$ while for CO₂-grown cells the rate was generally lower, about 3 to 8 $\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{mg}^{-1}\text{Chl}$. This difference in glycolate metabolism between the cell types probably reflects the increased levels of glycolate dehydrogenase reported in air-grown cells (19). These rates of glycolate metabolism were almost completely inhibited by the addition of AOA (Fig. 3). AAN, on the other hand, only slightly inhibited the utilization of glycolate which could still be converted to glycine. Neither inhibitor had any effect on the ability of the glycolate to enter the cells (data not shown). The nearly complete inhibition of the metabolism of added glycolate by AOA supports the contention that AOA is an effective inhibitor of glycolate metabolism, and the amount of glycolate ex-

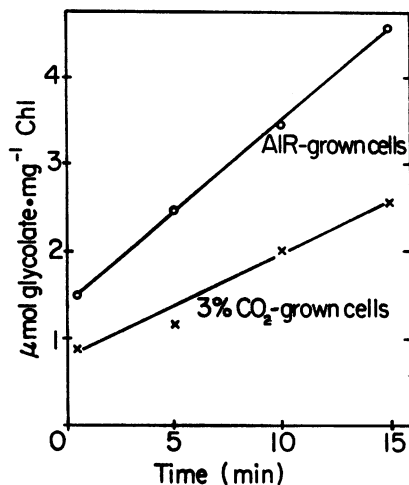


FIG. 2. Glycolate uptake by CO₂-grown or air-grown *Chlamydomonas*. Cells were incubated in the light with 10 mM [1-¹⁴C]glycolate in 25 mM Hepes-KOH (pH 7.5). At the indicated times the amount of glycolate uptake was measured after separation of the cells from the media by centrifugation through a silicone oil layer. The rate of incorporation of glycolate was 12 $\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{mg}^{-1}\text{Chl}$ in the air-grown cells and 6.4 $\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{mg}^{-1}\text{Chl}$ in the 3% CO₂-grown cells.

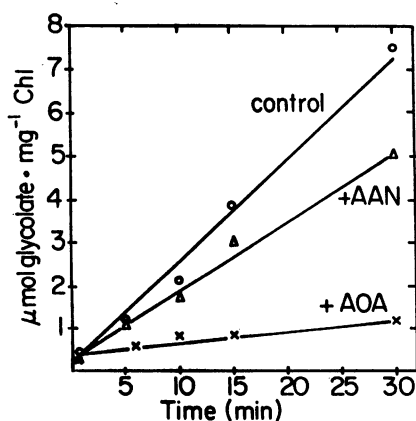


FIG. 3. Effect of aminoxyacetate and aminoacetonitrile on glycolate uptake by air-grown *Chlamydomonas* cells. Cells were incubated with 10 mM [2-¹⁴C]glycolate in 25 mM Hepes-KOH (pH 7.5) in the light at 25°C. Where indicated, either 2 mM AOA or 2 mM AAN were added to the cells 2 min before the addition of the labeled glycolate. Cells were separated from the media by centrifuging them through a layer of silicone oil. Glycolate incorporation was determined by measuring the radioactivity in the pellet.

creted in the presence of AOA reflects and amount of glycolate produced by those cells at the CO₂ and O₂ concentrations used.

DISCUSSION

The excretion of glycolate when air-grown cells were illuminated in the presence of AOA indicated that a significant amount of glycolate was being synthesized despite the operation of the CO₂ concentrating system. The glycolate excreted by *Chlamydomonas* in the presence of AOA is the result of ribulose-1,5-bisP oxygenase activity because this excretion requires O₂ and is inhibited by high concentrations of CO₂ (28). Furthermore, it is unlikely that AOA is causing an increase in glycolate biosynthesis since AOA has no effect on purified ribulose-1,5-bisP carboxylase/oxygenase; specifically, it does not alter the ratio of carboxylase to oxygenase activities (18) and has no effect on the CO₂ concentrating system. Thus the glycolate excreted by these cells in the presence of AOA was due to the blocking of glycolate metabolism. In support of this hypothesis, a second inhibitor, AAN, which blocks the glycine/serine interconversion (22, 29) caused in air-grown cells, a buildup of glycine comparable to the AOA induced glycolate build-up (Table II). These results indicate that there was a flux of about 5 $\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{mg}^{-1}\text{Chl}$ of glycolate in air-grown *Chlamydomonas* when they are illuminated in the presence of air levels of CO₂ and O₂.

The observation that glycine does not accumulate when both AOA and AAN are present indicates that AOA effectively blocks the C₂ pathway and that labeled glycine is arising from glycolate via glyoxylate as in higher plants (11). Since AOA is an excellent inhibitor of the C₂ cycle, the amount of glycolate excreted by cells in the presence of AOA can be used to determine the amount of ribulose-1,5-bisP oxygenase activity in these cells. It was noted that AOA did not cause glyoxylate accumulation or excretion, even though the inhibition of glyoxylate aminotransferase by AOA should have resulted in glyoxylate accumulation. We speculate that the glyoxylate was reduced to glycolate which was the specific compound excreted. If part of the glyoxylate was utilized in some other manner, such as oxidation or metabolism by a glyoxylate cycle, then our estimation with the AOA inhibition for the total carbon flow through the C₂ cycle is too low. However, similar values were obtained for glycine accumulation in the presence of AAN which suggest that the glyoxylate that was formed in the presence of AOA was mostly reduced back to glycolate.

A comparison of ribulose-1,5-bisP carboxylase activity (CO₂ fixation) and oxygenase activity (glycolate excretion) by air-grown or CO₂-grown cells is presented in the last column of Table I. The results point out the adaptive significance of the CO₂ concentrating system. In high CO₂-grown cells, without the CO₂ concentrating system, the rate of glycolate excretion was about 50% of the CO₂ fixation rate when the external CO₂ was at air level. This is similar to the estimates of the carboxylation to oxygenation rates in higher plants (30). In the air-adapted cells, the rate of glycolate excretion was only 7% of the CO₂ fixation rate. This is due both to a higher rate of CO₂ fixation by these cells compared to high CO₂-grown cells, and to a lowering of the rate of glycolate biosynthesis. These observations are consistent with the operation of a CO₂ concentrating system in these cells which would increase the CO₂ level thereby increasing the ribulose-1,5-bisP carboxylation reaction while decreasing the competing oxygenation reaction.

The significance of the C₂ pathway in green algae has been the subject of debate for years. The fact that algae excrete glycolate under certain conditions (12, 19, 27), utilize glycolate rather slowly (25), and contain only low levels of certain enzymes of the pathway, most notably glycolate dehydrogenase (4, 19) and glycine oxidase (4), have led to postulates that these cells have only a limited capacity to metabolize glycolate. Our results

support the idea that *Chlamydomonas* cells have a limited ability to metabolize glycolate, but in conjunction with the CO₂ concentrating system, they have enough enzymic capacity to handle the amount of glycolate normally made when cells are photosynthesizing with air levels of CO₂ and O₂.

Assuming that the total glycolate production is the same with or without AOA then the difference between the amount of glycolate excreted in the presence or absence of AOA represents the amount of glycolate normally metabolized. In the case of 5% CO₂-grown cells about half of the glycolate (approximately 5–10 μmol·h⁻¹·mg⁻¹Chl) was excreted and about half metabolized (Table I). This implies that these cells without the C_i pump do not have the enzymic capacity to handle a larger amount of glycolate produced when suddenly placed in a photorespiratory environment. The very low levels of glycolate dehydrogenase found in these cells support this argument (4, 19, 28). For the air-grown cells with their CO₂ concentrating mechanism and higher levels of glycolate dehydrogenase (19), the total glycolate production measured in the presence of AOA did not exceed 5 to 10 μmol·h⁻¹·mg⁻¹Chl. Since no glycolate was excreted, these cells can apparently metabolize this amount of glycolate by the C₂ pathway. This contention is supported by the glycolate feeding studies. When labeled glycolate was added to algal cells there was an initial rapid uptake followed by a slower incorporation of the label into C₂ cycle intermediates. The rapid uptake of glycolate by algae is similar to the rapid uptake by intact chloroplasts (9, 10). The slower incorporation of label by the algae which takes place at times greater than 10 s represents the metabolism of the added glycolate, as has been reported in *Chlorella* (14). Reports on uptake of small organic molecules such as glycolate or acetate by unicellular algae with data collected over time points greater than 10 s should be interpreted as the rate of metabolism of those compounds, rather than the rate of uptake. The rates of added glycolate utilization by *Chlamydomonas* cells (5–10 μmol·h⁻¹·mg⁻¹Chl) is close to the additional glycolate excretion seen when AOA is added to CO₂-grown cells (Table I). In other words, the extra glycolate excreted when AOA is added to block glycolate metabolism represents the amount of glycolate these cells are capable of metabolizing. In addition, the similarity between the rate of added glycolate metabolism and the glycolate excretion data indicates that the AOA and AAN sensitive pathway of glycolate metabolism is the major way these cells utilize glycolate. The estimation of the flux of glycolate through the C₂ cycle (between 5 and 10 μmol·h⁻¹·mg⁻¹Chl) is also supported by the observation that methionine sulfoximine-treated *Chlamydomonas* cells excreted 3.5 μmol of NH₃ per h⁻¹·mg⁻¹Chl when illuminated at very low CO₂ concentrations (21). This NH₃ excretion was also light dependent and inhibited by high CO₂ concentrations as is glycolate excretion. Since 1 NH₃ is released by glycine decarboxylation for every 2 glycines produced, these results indicate a flux of approximately 7 μmol glycine·h⁻¹·mg⁻¹Chl in these cells.

Other pathways for glycolate metabolism have been suggested. In the tartronic semialdehyde pathway two glyoxylates condense to form glycerate (6). If this pathway were important in *Chlamydomonas*, then glycine would not be expected to build up in the presence of AAN, and NH₃ would not be released in the presence of methionine sulfoximine in contrast to the observed results. Another proposed pathway for glycolate metabolism is the conversion of glyoxylate to CO₂ and formate (8). Activity of formate dehydrogenase was not detected (L Dahl, NE Tolbert, unpublished data) and formate was poorly utilized by *Chlamydomonas* cells (<1.5 μmol·h⁻¹·mg⁻¹Chl) (data not shown). Since [2-¹⁴C]glycolate was incorporated into the cell at a rate of 10 μmol·h⁻¹·mg⁻¹Chl in air-grown cells, the labeled formate should have been metabolized at a comparable rate if that pathway was important.

In conclusion, glycolate is produced in significant quantities during photosynthesis by both air-grown or CO₂-grown cells. This glycolate is metabolized to glyoxylate and glycine, as in higher plants, although the enzymes catalyzing these reactions are located solely in the mitochondria instead of both the mitochondria and the peroxisomes (11). This difference in subcellular location of the C₂ cycle enzymes may explain the reason for the small intracellular pools of glycine and serine in *Chlamydomonas* as compared to higher plants. In higher plants, glycine must be exported by the peroxisomes for metabolism in the mitochondria, and serine in turn must move from the mitochondria to the peroxisomes. This results in the large buildup of these metabolites. Since glycine and serine are produced and metabolized within the same organelle in *Chlamydomonas*, there may be no such intracellular pools in this alga. However, the accumulation of glycine observed when AAN is added to algal cells indicates that normally these small pools of glycine and serine are turning over rapidly.

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