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Glycolate Pathway in Green Algae[1]

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ABSTRACT

By three criteria, the glycolate pathway of metabolism is present in unicellular green algae. Exogenous glycolate-1-^{14}C was assimilated and metabolized to glycine-1-^{14}C and serine-1-^{14}C. During photosynthetic $^{14}CO_2$ fixation the distributions of ^{14}C in glycolate and glycine were similar enough to suggest a product-precursor relationship. Five enzymes associated with the glycolate pathway were present in algae grown on air. These were P-glycolate phosphatase, glycolate dehydrogenase (glycolate:dichloroindophenol oxidoreductase), L-glutamate:glyoxylate aminotransferase, serine hydroxymethylase, and glycerate dehydrogenase. Properties of glycerate dehydrogenase and the aminotransferase were similar to those from leaf peroxisomes. The specific activity of glycolate dehydrogenase and serine hydroxymethylase in algae was 1/5 to 1/10 that of the other enzymes, and both these enzymes appear rate-limiting for the glycolate pathway.

Labeling patterns for products of the glycolate pathway during $^{14}CO_2$ fixation are not the same as those obtained with higher plants. In higher plants glycolate, glycine, and serine are uniformly labeled at shortest time periods. In algae, serine was predominately carboxyl-labeled, similarly to 3-phosphoglycerate. This result, plus the lower specific activity of serine hydroxymethylase, indicates that the glycine-serine interconversion in algae is slower than in plants. Initially (2 to 4 seconds) glycolate and glycine were more C-2 labeled. They rapidly became uniformly labeled, with glycine becoming uniformly labeled first. In the presence of isonicotinylhydrazide, labeled glycolate and glycine accumulated, and only a trace of serine-^{14}C was detected. Then glycolate and glycine were initially carboxyl-labeled, and glycolate became uniformly labeled almost immediately and before glycine. These results suggest rapid metabolism of glycolate and glycine, in addition to the glycolate pathway.

Glycolic acid formed from $^{14}CO_2$ during photosynthesis by higher plants is uniformly labeled at times as short as 4 sec (10, 25, 27, 38). Likewise the glycine and serine, formed in the same time period by many higher plants, appear nearly uniformly labeled (24). These labeling patterns are compatible with the proposed reactions of the glycolate pathway, shown in Figure 1. Two molecules of glycolate are oxidized to glyoxylate and subsequently transaminated to glycine; two glycine molecules are then converted to one molecule each of serine and CO_2, and the serine is further converted to glycerate.

The details of this pathway in algae are less certain, although the algae rapidly produce labeled glycolate, glycine, and serine during $^{14}CO_2$ fixation (20). When Schou *et al.* (25) first fed ^{14}C-labeled glycolate to *Scenedesmus*, the labeling patterns in glycerate were consistent with the proposal later for the glycolate pathway (27). The existence of the glycolate pathway in algae became less certain after the discovery of glycolate excretion by algae (31) and a general inability of research workers to measure exogenous glycolate assimilation (6, 11, 16). More recently it was recognized that glycolate oxidase is not present in green algae (11). Reports of the oxidase in algae (14, 39) were due to measurements of an algal glycolate dehydrogenase, which does not link to oxygen and which is repressed when the algae are grown on 1% CO_2 (17, 18). Distribution of ^{14}C in intermediates of the glycolate pathway after photosynthetic fixation of $^{14}CO_2$ differ between algae and plants. Glycolate in algae is not uniformly labeled at very short times as it is in leaves (11), but rather the C-2 carbon is initially more labeled. Nevertheless, glycine, which should arise from the glycolate, was found to be uniformly labeled by this laboratory (11), whereas Zak and Nichiporovich (36) found that glycine from *Chlorella* was initially carboxyl-labeled and then rapidly became C-2-labeled. Serine formed by green algae was primarily carboxyl-labeled, while serine formed by plants was initially uniformly labeled (11).

We have re-evaluated the existence of the glycolate pathway in green algae by three criteria: (*a*) labeling patterns in glycolate, glycine, and serine from very short periods of $^{14}CO_2$ photosynthetic fixation; (*b*) labeling in products formed from added glycolate-1-^{14}C; and (*c*) enzymes of the glycolate pathway. Although the data demonstrate the existence of the glycolate pathway in green algae, the results also suggest synthesis and metabolism of glycolate by other pathways which have not yet been recognized.

METHODS AND MATERIALS

Plants. Synchronized *Scenedesmus obliquus* cells were cultured on a 16-hr light 8-hr dark cycle with CO_2-enriched air as previously described (8, 19). *Chlamydomonas reinhardtii* Dangeard (−) were grown in continuous culture on a high phosphate medium (20). Cultures of *Chlorella pyrenoidosa* Warburg were grown as previously described (19). The algae cultures were grown in Fernbach flasks containing 1.2 liters of medium. Flasks were shaken continuously on reciprocating shakers under light of about 1,200 ft-c in a growth chamber at 22 to 23 C. *Scenedesmus* and *Chlorella* were aerated with 0.3 to 0.5% CO_2-enriched air and *Chlamydomonas* with air (0.03% CO_2).

Pea beans (*Phaseolus vulgaris*, var. Sanilac) were grown in the greenhouse in muck-type soil. A fertilizer application of 300

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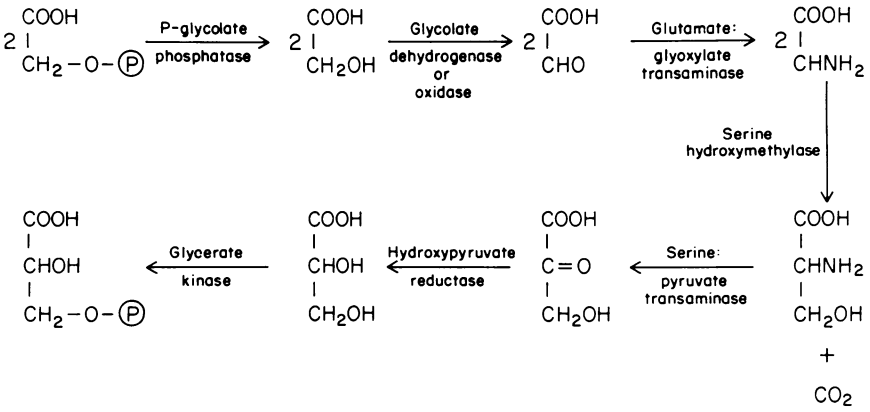

FIG. 1. Glycolate pathway.

μg/g N, 250 μg/g P, and 400 μg/g K was mixed with the soil in each pot 1 day prior to planting. A micronutrient mixture was added in a band 1 inch below the seeds at the time of planting.

Isotopic Procedures. *Chlorella* cells were harvested by centrifugation and suspended in sufficient 0.001 M phosphate buffer to give a 2% (v/v) suspension. Short time $^{14}CO_2$ fixations were carried out in two ways. In one the algal suspension and radioactive sodium bicarbonate solution were mixed rapidly by flowing together in an illuminated (3000 ft-c) vertical glass tube. The flow rate was adjusted by a pinch clamp at the lower end of the tube. The mixture dropped directly into hot methanol, which was boiled immediately. The reservoir containing the algal suspension was also illuminated and aerated with air for about an hour so that approximately steady state conditions prevailed. A more detailed description of this apparatus can be obtained in Reference 4. The length of ^{14}C fixation period was the time required for the algae to flow from the point of mixing with the radioactive bicarbonate to the end of the glass tubing. This time was determined in blank runs by dyes. Routinely 45 ml of cells and 50 μc of sodium bicarbonate in 10 ml of water were used for each time period.

In an alternate method of $^{14}CO_2$ fixation, 25 ml of a 1% suspension of cells were placed in a 400-ml beaker and were illuminated from above and from the sides with incandescent flood lamps. The suspension was vigorously stirred with a magnetic stirrer. A 0.5-ml aliquot of sodium bicarbonate containing 50 μc of radioactivity was injected into the suspension, and fixation was stopped by adding sufficient methanol to give an 80% solution. The mixture was immediately brought to a boil.

In experiments with 0.01 M INH[4] the suspension medium was prepared with both phosphate buffer and INH. The cells were incubated in this medium for 45 min in light with aeration to assure uptake of the inhibitor before the $^{14}CO_2$ fixation periods.

After $^{14}CO_2$ fixation, solutions were counted for total nonvolatile radioactivity; products were separated by paper chromatography (3) and located by radioautography. For preparation of larger amounts of glycolate, glycine, and serine for degradation, a gross separation of amino acids from other compounds was carried out by means of a Dowex 50-H^+ column (22) The glycolate remained in the effluent while the amino acids were retained on the column and were eluted by 0.4 N NH_4OH in 80% (v/v) ethanol. Large scale initial separations were achieved by one-dimensional paper chromatography using phenol-water for the amino acids and *n*-butanol-propionic acid-water for glycolate. The compounds were eluted from the strips and rechromatographed in the usual two-dimensional system (3) before degradation.

For $^{14}CO_2$ fixation by pea beans, single leaflets of similar size were exposed to 200 μc of $^{14}CO_2$ as previously described for tobacco leaves (10). Experiments were run out-of-doors in full sunlight in a Plexiglass chamber of 125-ml volume. At the end of a 6-sec period of photosynthesis, the tissue was killed in approximately 1 sec by addition of methanol.

Degradations. A more complete description of the degradation procedure has been published (4, 7, 11). Glycolate was degraded by enzymatic oxidation to glyoxylate, and the glyoxylate was oxidized to CO_2 and HCOOH by ceric sulfate (38). Glycine was degraded by ninhydrin and serine by periodate. $^{14}CO_2$ was trapped in a scintillation fluid containing phenethylamine (35). The $^{14}CO_2$ from glycine decarboxylation was trapped as $BaCO_3$ and the specific radioactivity was determined on a low background gas flow counter according to procedures described by Van Slyke *et al.* (32). In all cases the procedures were proven within 2 to 5% error by degradation of commercial samples of specifically labeled compounds. Glycolate-1-^{14}C or -2-^{14}C was from Orlando Chemicals, serine-1-^{14}C or -3-^{14}C from Sigma, and glycolate-1,2-^{14}C and glycine-1-^{14}C or -2-^{14}C from Calbiochem.

Metabolism Of Glycolate-1-^{14}C By *Scenedesmus*. For glycolate uptake and metabolism, synchronous cultures of *Scenedesmus* were harvested at the 4th to 6th hr of the dark period by centrifugation at 1000*g*. At this stage they were small daughter cells just after cell division (8, 19). The cells were resuspended in 0.01 M phosphate, pH 6.6, as a 2% (v/v) mixture at 25 C. Glycolate was added to give an initial concentration of 5.5×10^{-4} M. At intervals aliquots were removed, cells were sedimented by centrifugation, and glycolate was determined (5) in the supernatant fluid. For radioactive feeding experiments 2.5-ml cell aliquots were mixed with 0.5 ml of 0.01 M glycolate-1-^{14}C, Na^+ salt (0.58 μc/mmole). After incubation the suspension was transferred into the amount of hot absolute ethanol necessary to give an 80% solution. The 80% ethanol-soluble products were then separated by two-dimensional paper chromatography (3).

Enzyme Assays. Cells were harvested by centrifuging at 1,000*g*, washed once in distilled H_2O, and then resuspended in buffers to approximately a 20 to 30% (v/v) cell suspension. The cell suspensions were passed three times through a precooled French pressure cell at 10,000 to 14,000 psi. Assays were made of the supernatant obtained after centrifuging the homogenate at 29,000*g*.

Glycolate dehydrogenase (glycolate-dichloroindophenol oxidoreductase) was assayed at pH 8.7 by following dichlorindophenol reduction at 600 nm (18). L-Glutamate-glyoxylate aminotransferase (EC 2.6.1.4) was assayed at 25 C by the formation of ^{14}C-

[4] Abbreviation: INH: isonicotinylhydrazide.

Table I. *Distribution of ^{14}C in Glycolate and Glycine after Photosynthetic $^{14}CO_2$ Fixation by Chlorella*

	Distribution							
	2 sec	4 sec	6 sec	10 sec	12 sec	15 sec	35 sec	60 sec
	%	%	%	%	%	%	%	%
Photosynthesis in phosphate buffer								
Glycolate								
C-1		15		31		46		51
C-2		85		69		54		49
Glycine								
C-1	31		42	55		55	49	
C-2	69		58	45		45	51	
Photosynthesis in phosphate buffer and INH								
Glycolate								
C-1	58		50		50			
C-2	42		50		50			
Glycine								
C-1	76		60		64		57	
C-2	24		40		36		43	

glycine from glycolate (13). This assay was linear with time and protein concentration, and the rates were corrected for nonenzymatic glycine formation from glyoxylate. The P-glycolate phosphatase (EC 3.1.3.19) assay has been described (1). Glycerate dehydrogenase (EC 1.1.1.29) and catalase (EC 1.11.1.6) assays were similar to those used for leaf extracts (28, 37).

The serine hydroxymethylase (EC 2.1.2.1) assay was based on formaldehyde formation and involved minor modifications of the procedure described by Taylor and Weissbach (26). The final mixture of 0.5 ml contained 7.5 μmoles of phosphate buffer at pH 7.5; 0.25 μmole of pyridoxal phosphate; 0.05 μmole of tetrahydrofolate in 0.03% mercaptoethanol; and enzyme. Cofactors were adjusted to pH 7.5 before use. The reaction was initiated by adding 0.8 μmole of serine-3-^{14}C containing about 200,000 cpm of radioactivity. After 10 min the reaction was stopped by the addition of 0.5 ml of 1.0 M sodium acetate, pH 4.5. For isolation of the radioactive formaldehyde product (in the form of methylene tetrahydrofolate), 0.2 ml of 0.1 M formaldehyde as a carrier was added, followed by 0.3 ml of 0.4 M dimedon in 50% ethanol. The solutions were mixed and placed in a boiling water bath for 5 min. After cooling the dimedon derivative was extracted into 5.0 ml of toluene. The upper layer of toluene solution was removed and a 3.0-ml aliquot was counted in 10 ml of scintillation fluid made of 5.0 g of 2,5-diphenyloxazole (PPO) and 100 mg of *p*-bis[2-(5-phenyloxazolyl)]benzene (POPOP) in 1 liter of toluene. A correction in observed cpm for quenching by chlorophyll was determined by means of external standardization on the Packard Tri-Carb scintillation spectrometer, model 3310.

Protein was determined by the method of Lowry *et al.* (15).

RESULTS

Labeling Patterns in Glycolate and Glycine after $^{14}CO_2$ Fixation. The distribution of ^{14}C label in glycolate (Table I) formed photosynthetically by *Chlorella* was in good agreement with the results of Hess and Tolbert (11). In times up to 10 sec the glycolate was C-2-labeled, but by 15 to 60 sec the glycolate became uniformly labeled. Glycine showed a skewed labeling pattern nearly similar to that of glycolate, except that glycine became uniformly labeled more rapidly than did glycolate. Previously we had observed that the glycine was initially uniformly labeled, when the glycolate was still C_2-labeled (11). After 2 sec, 69% of the label in glycine was in the C-2 position; after as short a time as 10 sec the molecule was nearly uniformly labeled. It is not known why the glycine should become uniformly labeled faster than glycolate.

Table II. *Distribution of ^{14}C in Serine after Photosynthetic $^{14}CO_2$ Fixation*

	Distribution		
	6 sec	10 sec	15 sec
	%	%	%
Chlorella			
C-1	50.9	48.5	63.0
C-2	21.2	23.4	18.3
C-3	27.9	28.1	18.7
Phaseolus vulgaris			
C-1	31		
C-2	33		
C-3	36		

In the presence of INH, which blocks the glycolate pathway between glycine and serine, total ^{14}C accumulation in glycolate and glycine was greater than in untreated controls, as previousy reported (23). However, the labeling patterns of glycolate and glycine in the presence of INH were distinctly opposite from those of the uninhibited controls. Glycolate and particularly glycine were initially (2 sec) carboxyl-labeled. Glycolate became uniformly labeled very rapidly. The glycine produced in the presence of INH remained carboxyl-labeled for at least 35 sec. This difference induced by INH has not been explainable.

Labeling Pattern Of Serine. Only serine formed by untreated *Chlorella* was degraded (Table II), since serine formation in the presence of INH was too small to be detected at these short times. The serine was distinctly C-1-labeled as previously reported (11) and remained carboxyl-labeled. This result is in direct contrast to the initial C-2 labeling pattern in glycolate and glycine from the same experiment with these algae, and the uniform labeling in glycolate and glycine after 10 to 15 sec. Also the carboxyl label in serine from *Chlorella* is different from the uniformly labeled serine produced at 6 sec in leaves of the pea bean (Table II). In general serine is initially uniformly labeled when formed during $^{14}CO_2$ fixation by leaves for short periods when 3-P-glycerate is still heavily carboxyl-labeled (24). However, an initial carboxyl labeling pattern for serine has been reported for corn leaves (24) and for soybean leaves (33). As previously proposed (11, 34), the most likely source of the carboxyl-labeled serine is 3-P-glycerate. The retention of asymmetric labeling in the carboxyl group of serine for relatively long periods of time by *Chlorella*, while the glycine was uniformly labeled, suggests that the glycine-serine interconversion is slow. Carbon flow into these two related amino acids appeared to occur by two pathways: from glycolate to glycine and from 3-phosphoglycerate to serine.

Assimilation Of Glycolate By *Scenedesmus*. In order to demonstrate direct conversion of glycolate into glycine and then to serine via the glycolate pathway, it was necessary to develop reproducible conditions for the metabolic uptake of exogenous glycolate. This was accomplished only with synchronous cultures of *Scenedesmus obliquus*. The criterion for uptake was metabolism of the glycolate or retention of glycolate-^{14}C within the cells, after they had been removed from the suspension medium by centrifugation or filtration on a Millipore filter. Glycolate assimilation was maximal during the dark phases of cell division and was negligible during most of the light stages of cell growth (Fig. 2). The synchrony for glycolate excretion by *Scenedesmus* and

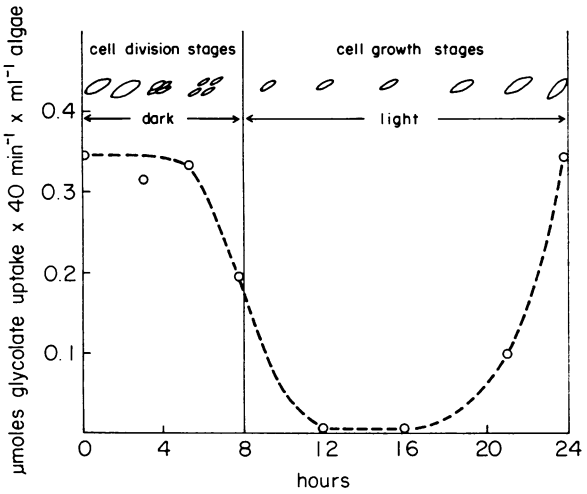

FIG. 2. Assimilation of glycolate by synchronized *Scenedesmus*

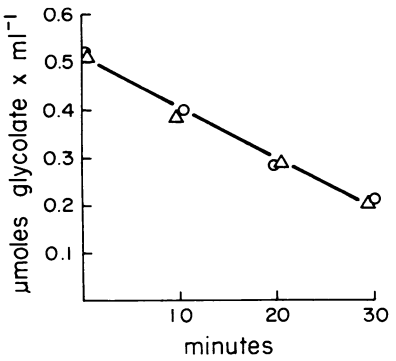

FIG. 3. Rate of assimilation of glycolate by *Scenedesmus*. Synchronized cells were harvested after 6 hr of darkness. ○: Dark; △: light with or without 3-(*p*-chlorophenyl)-1,1-dimethylurea.

Ankistrodesmus is the opposite: excreted in the light stages and not synthesized or excreted in the dark cell division stages (8, 9). Thus, it appears that the cells can readily assimilate glycolate at cell division stages when they are not capable of its synthesis and excretion. For subsequent studies of glycolate metabolism by *Scenedesmus*, we routinely used cells 4 to 6 hr after the beginning of the dark phase, when cell division was essentially complete. At this stage uptake of glycolate was linear in both light and dark for at least 30 min (Fig. 3). 3-(*p*-Chlorophenyl)-1,1-dimethylurea did not inhibit uptake in the light.

Labeling in Glycine and Serine from Glycolate-1-^{14}C. *Scenedesmus* cells right after the dark stage of cell division were fed glycolate-1-^{14}C, and labeled glycine and serine were isolated and degraded. The glycine was entirely C-1-labeled, and 70% of the label in serine was in the carboxyl group (Table III). The data are consistent with metabolism of glycolate via glycine to serine.

Enzymes for the Glycolate Pathway. Six enzymes, associated with the glycolate pathway in leaves, are present in green algae (Table IV). The specific activities are for cells grown on air, except for glycerate dehydrogenase, which was from cells grown on 1% CO_2. Growth on air increases the specific activity of glycolate dehydrogenase 3-fold (17, 18), but it has no effect on P-glycolate phosphatase. The effect of CO_2 concentration on the specific activity of the other enzymes of the glycolate pathway has not been determined.

P-glycolate phosphatase is present in green algae (Table IV), and its properties (D. D. Randall and N. E. Tolbert, unpublished) are similar to those of the enzyme from spinach leaves (1). Glycolate dehydrogenase in green algae catalyzes glycolate oxidation, and this enzyme functions in lieu of glycolate oxidase in higher plants (17, 18). The specific activity of catalase in extracts of *Chlamydomonas* was only 2.4 × 10^4 nmoles per min per mg of protein, whereas in leaves, catalase has a specific activity of 1 to 2 × 10^5 (29). Large amounts of catalase in the leaf are located in the peroxisomal particle with glycolate oxidase. The relatively low level of catalase activity in algae in comparison with leaves, and its relationship, if any, to the glycolate pathway are not clear, since the algal oxidation of glycolate to glyoxylate does not involve oxygen and H_2O_2 production.

The algal L-glutamate-glyoxylate aminotransferase has an amino acid specificity (Table V) similar to that of the higher

Table III. *Radioactivity in Glycine and Serine after Feeding Glycolate-1-^{14}C to Scenedesmus*

	Distribution
	%
Glycine	
C-1	100
C-2	0
Serine	
C-1	70
C-2 plus C-3	30

Table IV. *Some Enzymes of the Glycolate Pathway in Algae*

Enzyme	Algae Examined	Specific Activity of Extract
		nmoles/min·mg protein
P-Glycolate phosphatase	*Chlorella*, *Scenedesmus*, *Chlamydomonas*	30–60
Glycolate dehydrogenase	*Chlorella*, *Scenedesmus*, *Chlamydomonas*	3–11
Glycerate dehydrogenase[1]	*Chlorella*	150–500
L-Glutamate-glyoxylate aminotransferase	*Chlamydomonas*, *Chlorella*	50–100
Serine hydroxymethylase[2]	*Chlamydomonas*, *Chlorella*	3–6
Catalase	*Chlamydomonas*	2.4 × 10^4

[1] Assayed with hydroxypyruvate and NADH.
[2] Exact values were 2.6 for *Chlamydomonas* and 6.1 for *Chlorella*.

Table V. *Specificity of Amino Group Donor for Glycine Formation by Crude Extracts of Chlamydomonas reinhardtii*

Relative rates of glycine formation with L-glutamate taken as 1000.

L-Glutamate	1000
L-Alanine	1000–1200
DL-Serine	490
DL-Asparagine	400
L-Ornithine	350
L-Arginine	220
DL-Glutamine	199
D-Alanine	112
DL-Aspartate	104
DL-γ-Aminobutyrate	94
DL-Tryptophan	70
DL-Valine	44
L-Leucine	42
β-Alanine	35

TABLE VI. *Specificity of Glycerate Dehydrogenase from Chlorella*[1]

Fraction	Total Protein	Activity Recovered	Hydroxypyruvate Reduction		Glyoxylate Reduction		Ratio with NADH of Hydroxypyruvate to Glyoxylate
			NADH	NADPH	NADH	NADPH	
	mg	*%*	*μmoles oxidized/min·mg protein*				
Crude	682	100	0.15	0.036	0.048	0.008	3.1
45–60% $(NH_4)_2SO_4$	42	53	0.89	0.120	0.300	0.033	3.0

[1] *Chlorella* had been grown on 1% CO_2 in air.

plant peroxisomal enzyme (12). Addition of pyridoxal phosphate to algal extracts was generally not necessary in the assay, although activity was sometimes stimulated by addition of the cofactor. Both L-glutamate and L-alanine are the optimal amino donors. The specific activity of this transaminase in crude algal extracts is relatively high in relationship to glycolate dehydrogenase, whereas in leaves the activity of this transaminase is much less than that of glycolate oxidase (13, 30).

Serine hydroxymethylase, which is located in the mitochondria of higher plants (4, 13), was present in the algae at about the same level of activity as glycolate dehydrogenase (Table IV).

Glycerate dehydrogenase, also called glyoxylate reductase or hydroxy-pyruvate reductase, was the most active of the enzymes of the glycolate pathway in *Chlorella* extracts (Table IV). Much of the activity was precipitated between 45 and 60% $(NH_4)_2SO_4$ (Table VI). The enzyme was approximately three times more active with hydroxypyruvate than with glyoxylate (Table VI), as is the leaf peroxisomal enzyme. Pyridine nucleotide specificity of the crude preparations favored NADH (Table VI), which may reflect the presence of both a NADH- and a NADPH-specific enzyme in algae, as is the case in higher plants. Optimal activity was at approximately pH 5.5 (data not shown). These properties are similar to those for glycerate dehydrogenase from leaf peroxisomes except that the pH optimum is nearly 1 unit lower (30, 37).

DISCUSSION

By three criteria the glycolate pathway (Fig. 1) in unicellular green algae is complete. Assimilation of exogenous glycolate-1-^{14}C produced glycine and serine labeled in the carboxyl carbon atom as predicted. During photosynthetic CO_2 fixation the labeling patterns for glycolate and glycine are somewhat similar, as if they represented a substrate-product relationship shown in the pathway. Five enzymes of the pathway, catalyzing the reactions shown in Figure 1, are in algal extracts with specific activities reported in Table IV. Algae also contain catalase, but about 1/10 as much as in leaves. Although the present data indicate that carbon can flow through a glycolate pathway in algae, the extent of this metabolism may vary depending on physiological conditions. When glycolate dehydrogenase is repressed by high CO_2 levels, glycolate, as it is formed photosynthetically, is excreted. The algae are able only to reassimilate exogenous glycolate during cell division. Exogenous glycolate is converted into glycine, serine, and glycerate. However, the labeling pattern in serine produced photosynthetically from $^{14}CO_2$ indicates that the glycine to serine interconversion is not rapid.

Part of the algal data suggests some differences from the operation of glycolate pathway as it occurs in higher plants. It was confirmed that the serine produced from photosynthetic $^{14}CO_2$ fixation was carboxyl-labeled and remained so, while glycolate and glycine were uniformly labeled (11, 21, 36). If the glycolate pathway were very active, as in higher plants such as bean leaves, the serine should have been uniformly labeled. As this was not the case in *Chlorella*, the glycine to serine reaction must be proceeding slowly. The labeling pattern in carboxyl-labeled serine was similar to that in 3-P-glycerate. Thus a relatively large flow of carbon into serine and perhaps glycine during photosynthesis by algae occurs both from glycolate and from 3-P-glycerate. Since exogenous glycolate is converted to glycine, serine, and glycerate according to the predictions by the glycolate pathway, carbon can also flow one way through the pathway to glycerate in algae.

After 10 to 15 sec of $^{14}CO_2$ fixation, glycolate and glycine were uniformly labeled, but at shorter times of 2 to 10 sec the labeling patterns of these compounds were different. Initially both compounds were C-2-labeled, with glycine becoming uniformly labeled faster than glycolate. If the labeled glycine came only from glycolate, glycolate should have been first to become uniformly labeled. Perhaps there was some conversion of carboxyl-labeled serine to carboxyl-labeled glycine so that the combined glycine sample appeared more uniformly labeled. The formation of C-2-labeled glycolate and glycine does not seem to be consistent with glycolate biosynthesis by direct CO_2 fixation into the carboxyl group. The labeling pattern in the glycolate is consistent with the hypothesis that glycolate is oxidatively formed from the terminal two carbons of a sugar phosphate of the photosynthetic carbon cycle. Kandler and Gibbs (12) have shown that C-1 or C-6 of sugars, which would become C-2 of glycolate, initially during $^{14}CO_2$ fixation have higher ^{14}C content than C-2 or C-5, respectively, which would become the carboxyl group of glycolate. Also in sedoheptulose, C-3 has been reported in some cases to contain more ^{14}C than C-4 (2), and these carbons would become C-1 and C-2 of pentose and would give rise to C-2 and C-1 of glycolate.

The labeling pattern in glycolate and glycine in the presence of INH cannot be understood by current knowledge of glycolate metabolism. This inhibitor caused the accumulation of glycolate and glycine during $^{14}CO_2$ fixation (16) and reduced the labeling in serine. Because of a structural similarity to pyridoxal, INH was thought to inhibit the glycolate pathway by inhibition of transaminases. If the serine arises from 3-P-glycerate, this inhibitor should prevent serine formation. The question arises then, why does INH not inhibit the formation of glycine also? Furthermore, the accumulated compounds were carboxyl-labeled in the presence of INH rather than C-2 labeled as in the absence of the inhibitor. The totally carboxyl-labeled glycine reported by Zak and Nichiporovich (36) was not observed by us without INH. These labeling patterns cannot be explained, and one can only speculate that the two patterns reflect different biosynthetic or metabolic pathways for glycolate. The rapid randomization of the label in glycolate and glycine in the presence or absence of INH suggests that pools of both compounds are rapidly saturated by newly fixed ^{14}C in a cyclic process other than the complete glycolate pathway. It is unlikely that formation of carboxyl-labeled glycolate in the presence of INH could have been from carboxyl-labeled serine or 3-P-glycerate. Only trace amounts of serine are formed in the presence of INH. Decarboxylation of 3-P-glycerate-1-^{14}C would not produce glycolate-1-^{14}C.

Glycolate Assimilation. The assimilation of exogenous glycolate occurred rapidly and at a linear rate only by synchronized cultures

of *Scenedesmus* in the dark phases of cell division and immediately after cell division. Then the glycolate was metabolized by the glycolate pathway. Glycolate also stimulated respiration and oxygen evolution at this stage of the algal life cycle (19), perhaps because this is the only stage during which glycolate is absorbed by the cells. In the light stages of cell growth glycolate is biosynthesized and excreted (8, 9), but exogenous glycolate is not then assimilated. A random culture of *Scenedesmus* growing logarithmically in continuous light should contain a portion of cells at all stages of division. Thus the whole culture should be able to assimilate glycolate at a rate proportional to the number of cells at these stages of division. This, however, is not observed as random cultures of *Scenedesmus* growing in continuous light will not assimilate a significant amount of glycolate.

Enzymes of the Glycolate Pathway. The conversion *in vivo* of glycolate-^{14}C to labeled glycine, serine, and glycerate was substantiated by the presence of glycolate dehydrogenase, glutamate-glyoxylate aminotransferase, serine hydroxymethylase, and glycerate dehydrogenase in algal extracts. Glycolate dehydrogenase and serine hydroxymethylase had approximately the same specific activity. The glutamate-glyoxylate aminotransferase was 5- to 10-fold more active. Of the enzymes for glycolate metabolism in the peroxisome of higher plants, this aminotransferase was the least active (30). Consequently, the peroxisomal glycolate pathway may be regulated by this limiting aminotransferase, which may be at a metabolic branch point (30). In the algae, glycolate dehydrogenase, the level of activity of which is regulated by CO_2 availability, may be the rate-limiting enzyme, while the aminotransferase appears to be in excess.

The slow interconversion of uniformly labeled glycine and carboxyl-labeled serine that is formed during $^{14}CO_2$ fixation may be explained by the relatively low specific activity of serine hydroxymethylase that was found in the algal extract. If this activity is lower than in higher plants, more carbon would flow into the serine pool via 3-P-glycerate than from glycine. Since the glycine to serine conversion is considered to be the source of CO_2 evolution during photorespiration, CO_2 loss by algae in light in CO_2-free air should occur in algae, but to a limited extent, as has been observed (38).

LITERATURE CITED

1. ANDERSON, D. E. AND N. E. TOLBERT. 1966. Phosphoglycolate phosphatase. *In:* W. A. Wood, ed., Methods of Enzymology, Vol. IX. Academic Press, New York. pp. 646–650.
2. BASSHAM, J. A., A. A. BENSON, L. D. KAY, A. Z. HARRIS, A. T. WILSON, AND M. CALVIN. 1954. The path of carbon in photosynthesis. XXI. The cyclic regeneration of carbon dioxide acceptor. J. Amer. Chem. Soc. 76: 1760–1770.
3. BENSON, A. A., J. A. BASSHAM, M. CALVIN, T. C. GOODALE, W. A. HAAS, AND W. STEPKA. 1950 The path of carbon in photosynthesis. V. Paper chromatography and radioautography of the products. J. Amer. Chem. Soc. 72: 1710–1718.
4. BRUIN, W. J. 1969. An examination of glycolate metabolism in plants. Ph.D. thesis. Michigan State University, East Lansing.
5. CALKINS, V. P. 1943. Micro-determination of glycolic and oxalic acids. Ind. Chem. Anal. Ed. 15: 762–763.
6. CHAN, H. W.-S. AND J. A. BASSHAM. 1967. Metabolism of ^{14}C-labeled glycolic acid by isolated spinach chloroplasts. Biochim. Biophys. Acta 141: 426–429.
7. CHANG, W.-H. AND N. E. TOLBERT. 1965. Distribution of ^{14}C in serine and glycine after $^{14}CO_2$ photosynthesis by isolated chloroplasts. Modification of serine-^{14}C degradation. Plant Physiol. 40: 1048–1052.
8. CHANG, W.-H. AND N. E. TOLBERT. 1970. Excretion of glycolate, mesotartrate, and isocitrate lactone by synchronized cultures of *Ankistrodesmus braunii*. Plant Physiol. 46: 377–385.
9. GIMMLER, H., W. ULLRICH, J. DOMANSKI-KADEN, AND W. URBACH. 1969. Excretion of glycolate during synchronous cultures of *Ankistrodesmus braunii* in the presence of disalicylidenepropanediamine or hydroxypyridinemethanesulfonate. Plant Cell Physiol. 10: 103–112.
10. HESS, J. L. AND N. E. TOLBERT. 1966. Glycolate, glycine, serine, and glycerate formation during photosynthesis by tobacco leaves. J. Biol. Chem. 241: 5705–5711.
11. HESS, J. L. AND N. E. TOLBERT. 1967. Glycolate pathway in algae. Plant Physiol. 42: 371–379.
12. KANDLER, O. AND M. GIBBS. 1954. Asymmetric distribution of C^{14} in the glucose phosphates formed during photosynthesis. Plant Physiol. 31: 411–412.
13. KISAKI, T. AND N. E. TOLBERT. 1969. Glycolate and glyoxylate metabolism by isolated peroxisomes or chloroplasts. Plant Physiol. 44: 242–250.
14. LORD, M. J. AND M. J. MERRETT. 1968. Glycolate oxidase in *Chlorella pyrenoidosa*. Biochim. Biophys. Acta 150: 543–544.
15. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.
16. MARKER, A. F. H. AND C. P. WITTINGHAM. 1968. The photoassimilation of glucose in *Chlorella* with reference to the role of glycolic acid. Proc. Roy. Soc. Ser. B. Biol. Sci. 165: 473–485.
17. NELSON, E. B. AND N. E. TOLBERT. 1969. Regulation of glycolate metabolism in *Chlamydomonas reinhardtii*. Biochim. Biophys. Acta 184: 263–270.
18. NELSON, E. B. AND N. E. TOLBERT. 1970. Glycolate dehydrogenase in green algae. Arch. Biochem. Biophys. In press.
19. NELSON, E. B., N. E. TOLBERT, AND J. L. HESS. 1969. Glycolate stimulation of oxygen evolution during photosynthesis. Plant Physiol. 44: 55–59.
20. ORTH, G. M., N. E. TOLBERT, AND E. JIMENEZ. 1966. Rate of glycolate formation during photosynthesis at high pH. Plant Physiol. 41: 143–147.
21. PASSERA, C., G. FERRARI, AND F. GORI. 1966. Genesi di alania, serina, e glicia in *Chlorella vulgaria*. Agrochimica 10: 251–258.
22. PLAISTED, P. H. 1968. Clearing free amino acid solutions of plant extracts for paper chromatography. Contrib. Boyce Thompson Inst. Plant Res. 19: 231–244.
23. PRITCHARD, G. G., C. P. WITTINGHAM, AND W. J. GRIFFIN. 1961. Effect of isonicotinylhydrazide on the path of carbon in photosynthesis. Nature 190: 553–554.
24. RABSON, R., N. E. TOLBERT, AND P. C. KEARNEY. 1962. Formation of serine and glycine by the glycolate pathway. Arch. Biochem. Biophys. 98: 154–163.
25. SCHOU, L., A. A. BENSON, J. A. BASSHAM, AND M. CALVIN. 1950. The path of carbon in photosynthesis. XI. The role of glycolic acid. Physiol. Plant. 3: 487–495.
26. TAYLOR, R. T. AND H. WEISSBACH. 1965. Radioactive assay for serine transhydroxymethylase. Anal. Biochem. 13: 80–84.
27. TOLBERT, N. E. 1963. Glycolate pathway. Nat. Acad. Sci. Nat. Res. Counc. Publ. No. 1145.
28. TOLBERT, N. E., A. OESER, T. KISAKI, R. H. HAGEMAN, AND R. K. YAMAZAKI. 1968. Peroxisomes from spinach leaves containing enzymes related to glycolate metabolism. J. Biol. Chem. 243: 5179–5184.
29. TOLBERT, N. E., A. OESER, R. K. YAMAZAKI, R. H. HAGEMAN, AND T. KISAKI. 1969. A survey of plants for leaf peroxisomes. Plant Physiol. 44: 135–147.
30. TOLBERT, N. E. AND R. K. YAMAZAKI. 1969. Leaf peroxisomes and their relation to photorespiration and photosynthesis. Ann. N. Y. Acad. Sci. 168: 325–34
31. TOLBERT, N. E. AND L. P. ZILL. 1956. Excretion of glycolic acid by algae during photosynthesis. J. Biol. Chem. 222: 895–906.
32. VAN SLYKE, D. D., R. STELLE, AND J. BLAZIN. 1951. Determination of total carbon and its radioactivity. J. Biol. Chem. 192: 769–805.
33. VERNON, L. P. AND S. ARONOFF. 1950. Metabolism of soybean leaves. II. Amino acids formed during short-term photosynthesis. Arch. Biochem. 29: 179–186.
34. WILLIS, J. E. AND H. J. SALLACH. 1963. Serine biosynthesis from hydroxypyruvate in plants. Phytochemistry 2: 23–28.
35. WOELLER, F. H. 1961. Liquid scintillation counting of $^{14}CO_2$ with phenethylamine. Anal. Biochem. 2: 508–511.
36. ZAK, E. G. AND A. A. NICHIPOROVICH. 1964. Formation of amino acids during photosynthesis. Fiziol. Rast. 11: 945.
37. ZELITCH, I. 1955. The isolation and action of crystalline glyoxylate reductase from tobacco leaves. J. Biol. Chem. 216: 553–575.
38. ZELITCH, I. 1965. The relation of glycolic acid synthesis to the primary photosynthetic carboxylation reaction in leaves. J. Biol. Chem. 240: 1869–1876.
39. ZELITCH, I. AND P. R. DAY. 1968. Glycolate oxidase activity in algae. Plant Physiol. 43: 289–291.
40. ZELITCH, I. AND P. R. DAY. 1968. Variation in photorespiration. The effect of genetic differences in photorespiration on net photosynthesis in tobacco. Plant Physiol. 43: 1838–1844.