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Sequence of Formation of Phosphoglycolate and Glycolate in Photosynthesizing *Chlorella pyrenoidosa*[1]

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ABSTRACT

In *Chlorella pyrenoidosa* which have been photosynthesizing in either 1.5% $^{14}CO_2$ or 0.05% $^{14}CO_2$ in air, gassing with 100% O_2 results in rapid formation of phosphoglycolate which is apparently converted to glycolate. However, only about one-third to one-half of the rate of glycolate formation can be accounted for by this route. The remaining glycolate formation may be the result of the oxidation of sugar monophosphates. The rates of formation of both glycolate and phosphoglycolate are about four times greater with algae that have been photosynthesizing in 1.5% $^{14}CO_2$ than with algae which have been photosynthesizing with air, when the algae are then gassed with 100% O_2.

When some species of green plants photosynthesize with $^{14}CO_2$, and the total CO_2 concentration is not higher than in air, glycolate is prominent among the early labeled products (5). However, its formation occurs later than the formation of PGA[2] and sugar phosphates (7), which are the first products of CO_2 fixation in photosynthesis and are intermediates of the reductive pentose phosphate cycle of photosynthesis (1). This is also true when ^{14}C-labeled glycolate is formed by isolated spinach chloroplasts photosynthesizing with $^{14}CO_2$ (8). Glycolate formation during photosynthesis is favored by low CO_2 concentration (21), high oxygen concentration (4), and high light intensity (18).

Once formed, glycolate may be metabolized via a variety of pathways (for review, see Tolbert [19], including paths which lead to photorespiratory CO_2 in higher plants. In some unicellular algae, such as *Chlorella pyrenoidosa,* glycolate may be excreted into the suspending medium.

The mechanism whereby glycolate is formed from the intermediate compounds of the photosynthetic carbon reduction cycle remains in dispute. Wilson and Calvin (21) suggested that the glycolyl moiety transferred in the transketolase-mediated reactions of the cycle might be oxidized to glycolic acid. Dihydroxyethylthiamine pyrophosphate, the intermediate in the transketolase-catalyzed reaction, can be converted to glycolate with ferricyanide (6). Shain and Gibbs (17) described a reconstituted preparation containing fragments of spinach chloroplasts, transketolase, and cofactors which was capable of rapid conversion of fructose-6-P or dihydroxyethylthiamine pyrophosphate to glycolate in the light.

Since phosphoglycolate is also seen upon the increase of concentration of oxygen with photosynthesizing *Chlorella pyrenoidosa,* Bassham and Kirk (4) suggested that phosphoglycolate could be formed *in vivo* by the oxidation of RuDP to phosphoglycolate. This would be followed by the conversion of phosphoglycolate to glycolate, which could be mediated by the active P-glycolate phosphatase found in chloroplasts (16). However, the amount of phosphoglycolate which appeared after addition of O_2 was much less than the amount of glycolate formed in response to this O_2; also the percentage increase with increasing O_2 was less for phosphoglycolate.

Recent findings that molecular oxygen competitively inhibits ribulose diphosphate carboxylase, the carboxylation enzyme of the photosynthetic reductive pentose phosphate cycle (13), led to proposals that RuDP is oxidatively split on the enzyme RuDP carboxylase by oxygen, giving phosphoglycolate and phosphoglycerate, instead of two molecules of phosphoglycerate normally formed by the carboxylation reaction.

There is considerable evidence that glycolate is formed oxidatively from sugar phosphates (10, 11, 15, 17), by one or the other of these paths, although there is also a question of whether the oxidant is oxygen, a peroxide formed by reaction of oxygen with a primary reductant, such as reduced ferredoxin, or an intermediate in the oxidation of water formed by the photosystem 2 of the photoelectron transport path (17).

The present study was undertaken to assess the importance *in vivo* of phosphoglycolate as an intermediate in glycolate formation. The kinetic curves for the appearance and disappearance of RuDP, phosphoglycolate, and glycolate following the introduction of 100% O_2 to *Chlorella pyrenoidosa* previously photosynthesizing with $^{14}CO_2$ have been determined.

MATERIALS AND METHODS

In some experiments, *Chlorella pyrenoidosa* were grown in a continuous culture apparatus aerated with 4% CO_2 in air (2). A 0.3% suspension of these algae was withdrawn, the algae were centrifuged from the culture medium and resuspended in 0.1 mM KH_2PO_4 plus 1 mM KNO_3 to a concentration of 1% (v/v). Sixty ml of this suspension were transferred to the algae vessel of the steady state apparatus (3). The pH was adjusted to 5.5 with 0.1 M HNO_3 and maintained by periodic automatic addition of 0.1 M HNO_3. The pH of the suspension in the steady state apparatus is more rapidly and accurately controlled at pH 5.5 than at higher pH values where there is a larger buffering effect of bicarbonate. This is par-

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[2] Abbreviations: PGA: 3-phosphoglycerate; PG: 2-phosphoglycolate; RuDP: ribulose-1,5-diphosphate; HMP: glucose-6-phosphate plus sedoheptulose-7-phosphate.

Table I. *Rate of Appearance of Glycolate in O_2 Compared with Rate of Disappearance of Phosphoglycolate in N_2*

In experiment 2, rate of glycolate appearance was measured at 11.3 min, while rate of phosphoglycolate disappearance was at 13.4 min (see Fig. 4). Comparable measurements were made in experiment 3. In experiments 4 and 5, O_2 was administered for 2 min instead of 3 min. Rates were again measured at points on the curve where phosphoglycolate concentration was the same in O_2 and in N_2. In all cases rate of disappearance of phosphoglycolate was measured where it was maximum. Steady state rates of CO_2 uptake, prior to O_2 flushing, were 18 to 23 μmoles CO_2/min·cm³ algae, or about 200 μmoles CO_2/hr·mg Chl. See text for discussion of k.

Experiment	d(Glycolate)/dt	d[PG]/dt	$k = \frac{d[PG]}{dt}/[PG]$
	μg atoms ^{14}C/min·cm² algae		
2 (Fig. 4)	+2.5	−0.87	2.5
3	+2.7	−0.40	2.0
4	+3.2	−0.90	2.7
5	+3.0	−0.75	2.3

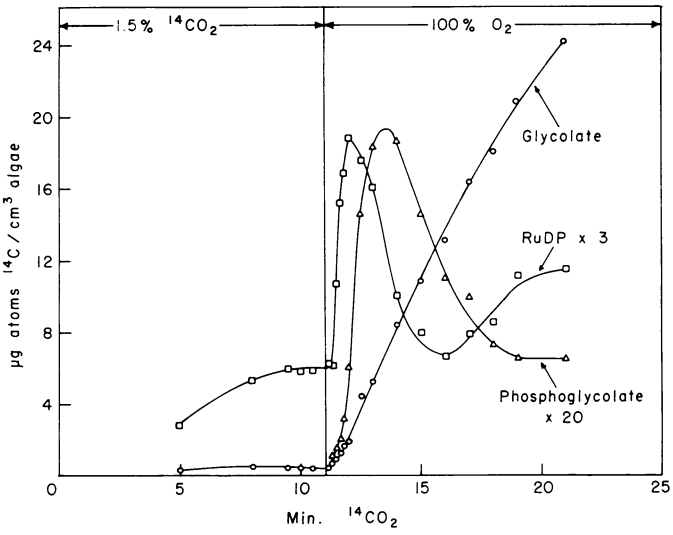

FIG. 1. Effect of 100% O_2 on levels of labeled RuDP, phosphoglycolate and glycolate in *Chlorella pyrenoidosa* after photosynthesis with 1.5% $^{14}CO_2$.

ticularly important in experiments where the gas phase in equilibrium with the algae suspension is suddenly depleted of CO_2. The temperature of the algae was kept at 20 C. CO_2 (1.5–2%) in air was bubbled through the suspension, and the lights were turned on. Cells were preilluminated in this way for about 30 min. During this time the photosynthesis rate was tested and found to be about 18 to 22 μg atoms of carbon assimilated/min·cm³ wet-packed algae (3).

The gas flow system was closed, and $^{14}CO_2$ was added so that the concentration of CO_2 was about 1.5% with a specific radioactivity of 15 μc/μmole. The cells were allowed to photosynthesize normally for 11 min, by which time the cycle intermediates had time to become "saturated" with $^{14}CO_2$. After 11 min the system was quickly flushed with 100% O_2. Samples were taken before the flushing with O_2 and were removed at short intervals after the oxygen flush began.

These samples were killed in methanol and were analyzed by paper chromatography and radioautography (14). Because of the volatility of glycolic acid from paper chromatograms developed with acidic solvents, duplicate samples of the algae were chromatographed in a second pair of chromatographic solvents. The first of these was made up of 754 ml of aqueous phenol (approximately 85% by weight), 224 ml H_2O, 22.4 ml of concentrated NH_4OH, and 2 ml of 0.5 M EDTA. This solvent is similar to one previously reported (20), but with the EDTA salt added. The second solvent system consisted of 600 ml of l-propanol, 300 ml of concentrated NH_4OH, and 100 ml of H_2O (12). The papers were developed for 30 hr in the first solvent and, after drying, for 24 hr in the second solvent. Identification of the major labeled compounds was made by cochromatography with unlabeled or ^{14}C-labeled compounds. A ^{14}C-glycolate spot cut from the chromatogram (with these alkaline solvents) did not lose any ^{14}C activity during 3 days' hanging in the fume hood, whereas a glycolate spot cut from our conventional acidic chromatogram loses up to 80% of its activity in 3 days. Separation of glycolic acid from other compounds was good, but the general separation of products of photosynthesis from one another was poor, so that all other compounds were analyzed in our usual system (14).

Although phosphoglycolate separates only a small distance from PGA in our usual system, development for 48 hr in each direction gave sufficient separation to allow quantitative results. In those few cases where good separation was not achieved initially, chromatography was repeated using another aliquot portion of the original mixture, and separation was sufficient.

Radioactivity in each compound was determined using an automatic gas-flow Geiger counter (14). The amounts of ^{14}C in each compound are expressed as μg atoms ^{14}C/cm³ algae (wet-packed volume of algae after harvesting and centrifugation). One cm³ of these packed algae contains about 6 mg of chlorophyll. Thus a typical photosynthesis rate of 20 μmoles CO_2 fixed/cm³ algae·min (Table I) is equal to $20 \times 60/6 = 200$ μmoles CO_2 fixed/mg chlorophyll·hr.

In four experiments, the period of flushing with O_2 was followed by a period of flushing with N_2, with times as shown in the figures. In one of these experiments, alternate samples were killed in 80% methanol as usual, while the remaining samples were killed in 80% methanol to which 100 μl of 12 N HCl (per 4 ml methanol) had been added. In another of these experiments, alternate samples were killed in 50% methanol, 27% formic acid, and 23% water.

A second series of experiments were performed using *Chlorella pyrenoidosa* grown on air not enriched with CO_2. For this purpose the algae are grown in a low form culture flask on a shaking apparatus in a temperature-controlled bath with illumination through the transparent bottom of the flask (2). A sintered glass bubbler provides for better aeration. Other conditions for culturing and for the exposure to $^{14}CO_2$ in the steady state apparatus were as described above for the algae grown in 1.5 to 2% CO_2. Gassing times and regimes are described under "Results."

RESULTS

A comparison of the changes in levels of RuDP, phosphoglycolate, and glycolate after the introduction of O_2 (Fig. 1) shows that the RuDP pool and the phosphoglycolate pool each increase rapidly in amount and then fall. The phosphoglycolate pool level tends to follow the level of the RuDP pool, and peaks about 90 sec after the peaking of the RuDP pool. This is clearly consistent with the idea that phosphoglycolate formation depends on O_2 and RuDP concentration. Since the only likely fate of phosphoglycolate is its conversion to glycolate, this suggests that some of the glycolate is formed by the

sequence RuDP → phosphoglycolate → glycolate. From the slope of the glycolate curve compared with the level of phosphoglycolate at various times, it is apparent that the rate of formation of glycolate only partly depends on the level of phosphoglycolate.

Among other effects of the addition of O_2 (and removal of CO_2) were the following: The levels of PGA dropped sharply while the levels of HMP, UDP-glucose, and of fructose-6-P (not shown) first dropped and then rose (Fig. 2). The level of sucrose begins to decline soon after the addition of O_2, suggesting that this reserve sugar is mobilized to supply carbon to the cells' metabolism. The later rise in levels of hexose monophosphates, following the initial drop, also suggests that carbon flows into the reductive pentose phosphate cycle from endogenous sugars. This appears to be some kind of compensatory regulatory mechanism to keep the chloroplasts "primed" for the return of CO_2. This mechanism keeps the RuDP level from dropping below its steady state level during the time of the experiment, in spite of the conversion of RuDP to phosphoglycolate, which will continue in the presence of O_2.

The levels of several amino acids including alanine dropped, while the level of glycine rose (Fig. 3). This rise in glycine level may indicate that some of the glycolate is metabolized via glyoxylate to give glycine.

An analysis of these kinetic curves for phosphoglycolate, RuDP, and glycolate concentrations as shown in Figure 1 suggested that the rate of glycolate formation changes substantially during the first 2 min of O_2 gassing. Therefore, we decided to attempt to evaluate the rate of phosphoglycolate hydrolysis under a set of conditions in which phosphoglycolate formation should have stopped. The sequence of gas changes was altered to include a second change from 100% O_2 to 100% N_2 after 3 min, when phosphoglycolate is still at a high level. Since O_2 is required for the formation of phosphoglycolate from RuDP, we reasoned that the disappearance rate of phosphoglycolate after a few seconds of N_2 flushing would represent the absolute rate of phosphoglycolate hydrolysis. Presumably this rate should be dependent on phosphoglycolate concentration. Provided that the rate constant for the hydrolysis of phosphoglycolate remains constant during the time the algae are with O_2 and the time they are with N_2, the rate of hydrolysis of phosphoglycolate at a given concentration of phosphoglycolate during the N_2 regime should be the same as the rate of hydrolysis during the O_2 regime. It might be argued that there is still appreciable phosphoglycolate formation when the algae are being flushed with N_2. The presumption in such an argument would be that O_2 evolution is still occurring (since the light is still on), and that even with N_2 flushing, the locally generated O_2 causes some oxidation of RuDP to give phosphoglycolate. However, with N_2 flushing there can be no net CO_2 uptake and hence no net O_2 evolution (since the electron acceptors, ferredoxin and NADP, would not be able to accept electrons without reoxidation). Further evidence that the rate of phosphoglycolate production becomes small or zero under N_2 flushing is the fact that the glycolate level (which is affected by glycolate formation by all pathways including phosphoglycolate hydrolysis) no longer increases after about 1 min. The increase during the 1st min of N_2 flushing is in part the consequence of phosphoglycolate hydrolysis, and perhaps in part due to sugar monophosphate oxidation.

The concentration of phosphoglycolate in this experiment (experiment 2, Fig. 4 and Table I) was the same (0.37 μg atoms $^{14}C/cm^3$ algae) at 11.3 min as it was at 13.4 min, and the rates of phosphoglycolate disappearance at 13.4 min thus can be compared with the rate of glycolate formation at 11.3 min. The rate of disappearance of phosphoglycolate at 13.4

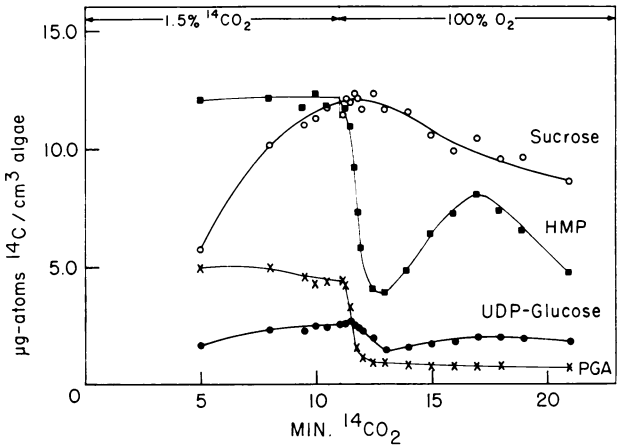

FIG. 2. Effect of 100% O_2 on levels of 3-phosphoglycerate (PGA), sugar monophosphates, sucrose, and uridine diphosphoglucose (UDP-Glucose) in *Chlorella pyrenoidosa* after photosynthesis with 1.5% $^{14}CO_2$. Sugar monophosphates include glucose-6-phosphate and sedoheptulose-7-phosphate.

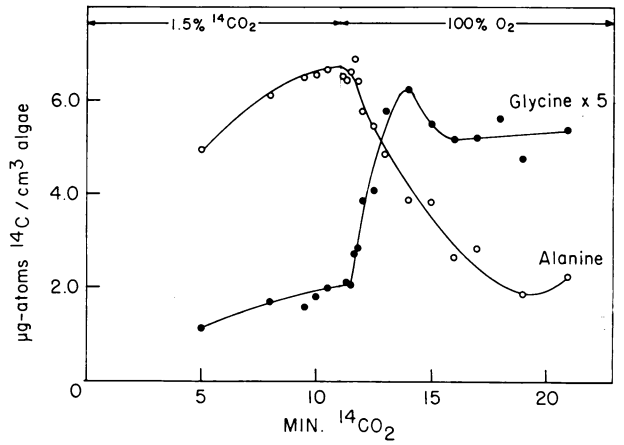

FIG. 3. Effect of 100% O_2 on levels of alanine and glycine in *Chlorella pyrenoidosa* after photosynthesis with 1.5% $^{14}CO_2$.

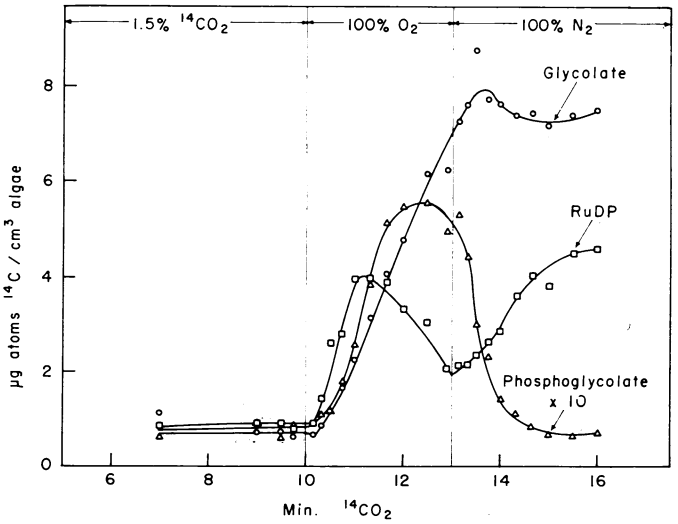

FIG. 4. Effect of 100% O_2 for 3 min followed by 100% N_2 on levels of labeled RuDP, phosphoglycolate, and glycolate in *Chlorella pyrenoidosa* after photosynthesis with 1.5% $^{14}CO_2$.

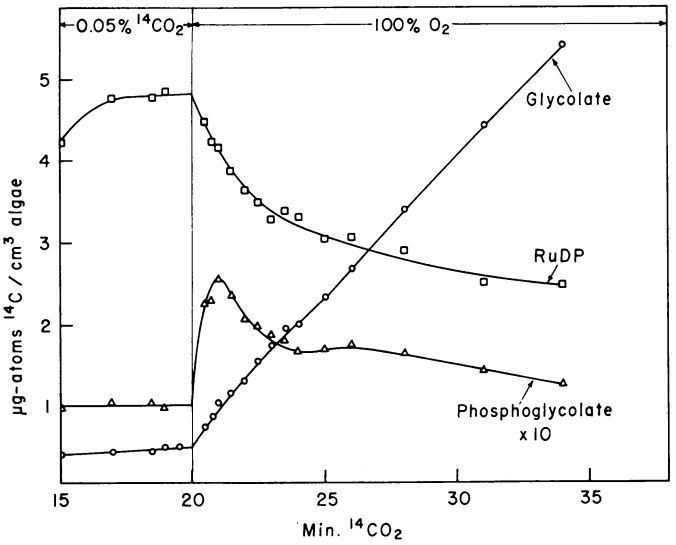

FIG. 5. Effect of 100% O_2 on levels of RuDP, phosphoglycolate, and glycolate in *Chlorella pyrenoidosa* after photosynthesis with 0.05% $^{14}CO_2$.

Table II. *Rate of Appearance of Glycolate Compared with Level of Phosphoglycolate*

Rates are calculated from slopes of curve shown in Figure 5 at the indicated times.

Time	Condition	Phosphoglycolate	$\frac{d(Glycolate)}{dt}$
min		*μg atom $^{14}C/cm^2$ algae*	*μg atom ^{14}C/ min·cm^2 algae*
17	0.05% $^{14}CO_2$ in air	0.100	0.0115
21	100% O_2	0.256	0.434
34	100% O_2	0.125	0.324

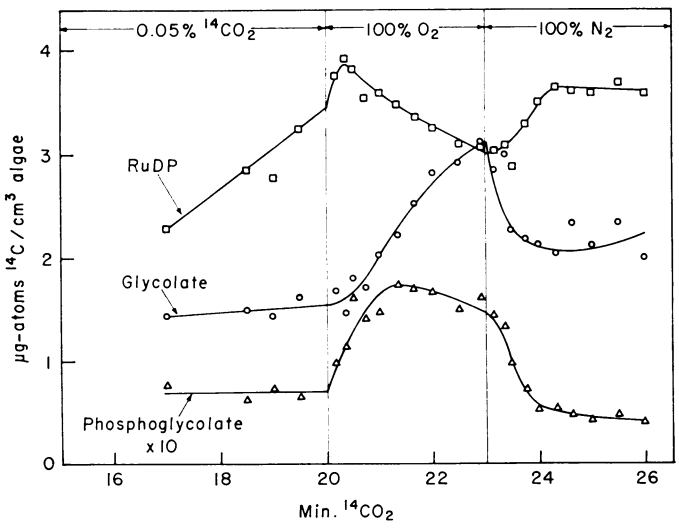

FIG. 6. Effect of 100% O_2 for 3 min followed by 100% N_2 on levels of labeled RuDP, phosphoglycolate, and glycolate in *Chlorella pyrenoidosa* after photosynthesis with 0.05% $^{14}CO_2$.

min was 0.87 μg atoms/min·cm^3 algae (Table I). The rate of glycolate appearance at 11.3 min was 2.5 μg atoms/min and represents a lower limit for the rate of formation of glycolate at that time, since there is apparently some metabolism of glycolate, though at a much slower rate than its formation (Fig. 6).

In another experiment, with similar conditions to those just described (experiment 3, Table I), the rate of appearance of glycolate at 11.3 min and of disappearance of phosphoglycolate at 13.4 min were comparable to the values just given. In experiments 4 and 5 (Table I), the O_2 was replaced by N_2 after only 2 min. In experiment 4, the rate of disappearance of phosphoglycolate was somewhat higher, but still well below the rate of appearance of glycolate at 11.3 min, the time of the same phosphoglycolate concentration during O_2 gassing. In each case we are assuming that the rate of hydrolysis of phosphoglycolate is the same at a given concentration of phosphoglycolate during the O_2 flushing as during the N_2 flushing. No additional PG was detected in samples killed with methanol plus HCl or with methanol and formic acid (27%).

With air-adapted algae, photosynthesizing in 0.05% $^{14}CO_2$, there is a measurable rate of appearance of glycolate and a measurable steady state level of phosphoglycolate before the addition of 100% O_2 (Fig. 5). After the air and $^{14}CO_2$ is replaced by 100% O_2, the rate of appearance of both phosphoglycolate and glycolate increases. However, the rate of appearance of glycolate is much less than it was in the algae switched from 1.5% $^{14}CO_2$ to 100% O_2. The rate of appearance of glycolate after the switch to 100% O_2 is much greater in relation to phosphoglycolate concentration than before (Table II), but this may be misleading, since the rate of both formation and utilization of glycolate may change when 0.05% $^{14}CO_2$ in air is replaced by O_2. The data suggest again that phosphoglycolate concentration is not very dependent on glycolate concentration.

When algae photosynthesizing in 0.05% $^{14}CO_2$ are switched first to O_2, and then after 3 min to N_2 (Fig. 6), it can be seen that the rate of glycolate utilization is in this case significant compared to rate of appearance of glycolate. Thus in O_2, glycolate increased at a rate of +0.26 μg atoms/min·cm^3 algae. During the 1st min in N_2, the glycolate concentration dropped at a rate of 0.60 μg atoms/min. If we assume that this rate of decrease in glycolate during the 1st min in N_2 represents the approximate rate of glycolate utilization in O_2, then the total rate of formation of glycolate in O_2 was at least 0.26 −(−0.60) = +0.86 μg atoms/min·cm^3 algae. This is to be compared with a rate of disapperance of phosphoglycolate in the 1st minute in N_2 of 0.22 μg atoms/min·cm^3 algae.

DISCUSSION

Although the rise and fall in phosphoglycolate concentration follows closely after the rise and fall in RuDP concentration (Fig. 1), it is not likely that the rate of phosphoglycolate formation is linearly dependent on RuDP concentration if the enzyme ribulose diphosphate carboxylase is catalyzing the conversion of RuPD to phosphoglycolate in the presence of O_2. The enzyme is normally saturated with respect to RuDP under conditions of normal *in vivo* photosynthesis, and the level of RuDP goes still higher when the O_2 gassing commences, due to removal of CO_2. Thus the rise and fall in phosphoglycolate concentration is taken as an indication of a changing rate of conversion of enzyme-RuDP complex to PGA and phosphoglycolate in the presence of O_2.

The increase in rate after the onset of O_2 gassing is easily explainable as being the result of several seconds being required for the effective dissolved concentration of O_2 to rise as a result of the switch from atmospheric O_2 to 100% O_2. We attribute the subsequent fall in the level of phosphoglycolate to a decreased rate of formation of phosphoglycolate resulting

from some change in the activity of the enzyme. Chu and Bassham (9) found that the carboxylation activity of the enzyme greatly decreases (presumably as the result of conformational change), when the enzyme is presented with RuDP in the absence of CO_2. We suspect that this change, which must occur when the O_2 gassing takes place, also inactivates the enzyme with respect to the oxidative reaction.

Given the low concentration of phosphoglycolate and the high reported activity of the enzyme phosphoglycolate phosphatase (16), it seems reasonable to suppose that the rate of hydrolysis of phosphoglycolate exhibits first order dependence on phosphoglycolate concentration under the conditions of our experiments. This assumption is implicit in our comparison of the rate of disappearance of phosphoglycolate under N_2 in experiments 2 to 5 with the maximum rates of appearance of glycolate in those experiments. We wish to use this assumption also in the analysis of the phosphoglycolate and glycolate curves in experiment 1 (Fig. 1).

There are two points in experiment 1, Figure 1, at which the rate of change of phosphoglycolate concentration is at least momentarily zero. These are at 13.5 min, when the phosphoglycolate concentration is 0.98 μg atoms of ^{14}C, and again at 20 min, when the phosphoglycolate concentration is 0.33 μg atoms of ^{14}C. We can assume that at each of these two points the rate of hydrolysis of phosphoglycolate (equal to its rate of formation) is given by k(PG). The second assumption can be that glycolate formation is given by K + k(PG). This rate experimentally is +3.00 μg atoms $^{14}C/min^{-1}$ and +1.9 μg atoms $^{14}C/min^{-1}$ at 13.5 min and at 20 min, respectively. Solving the two simultaneous equations for these two times, we get K = 1.34 μg atoms $^{14}C/min^{-1}$ and k = 1.7 min^{-1}. In other words, rate of glycolate formation = 1.34 + 1.7 [PG]. Thus there would be formation of glycolate by a pathway independent of phosphoglycolate at a rate of 1.34 μg atoms/min^{-1} and formation as the result of phosphoglycolate formation and hydrolysis at rates varying from 1.7 μg atoms/min^{-1} when phosphoglycolate formation is maximal to 0.56 μg atoms $^{14}C/min^{-1}$ at 20 min.

These values may be compared with the results for experiments 2 through 5 where the measured rates of phosphoglycolate disappearance varied from 0.4 to 0.9 μg atoms/min^{-1}, and the calculated values for k varied from 2.0 to 2.6 (Table I). Considering these measurements and calculations for experiments 1 through 5, it appears that under the conditions of O_2 gassing of *Chlorella* previously photosynthesizing in 1.5% CO_2, glycolate formation takes place both by way of phosphoglycolate (presumed to be formed by oxidation of RuDP) and by an independent pathway, which may be the oxidation of sugar monophosphates. During the first 90 sec of O_2 gassing, the pathway via phosphoglycolate may account for one-half or more of the glycolate formation, but during a subsequent period the rate of formation of the phosphoglycolate decreases, and this route to glycolate accounts for only about a third of the glycolate formation.

It will be noted that no allowance has been made for the conversion of glycolate to other metabolic products, despite the evidence (glycine formation, Fig. 3) that such conversion does occur. The actual rate of formation of glycolate is the sum of the rate of its appearance plus the rate of its conversion, so that even less of the total glycolate production can be accounted for in terms of phosphoglycolate hydrolysis.

To summarize, it appears that with *Chlorella*, photosynthesizing either in 1.5% CO_2 or in air, addition of 100% O_2 results in a rapid formation of phosphoglycolate which is converted to glycolate, but that only about one-third to one-half of the glycolate formed as a result of O_2 addition is formed by this route. The remaining glycolate formation may be the result of the oxidation of sugar monophosphates. The rates of formation of both phosphoglycolate and glycolate appear to be about four times greater when the algae had been photosynthesizing in 1.5% CO_2 than when they had been photosynthesizing with air.

These conclusions apply to a transient state imposed by removing CO_2 and increasing O_2 to 100%. This provides strong evidence that some, but not all, glycolate formed *in vivo* in *Chlorella pyrenoidosa* may be formed by the sequence RuDP → phosphoglycolate → glycolate. The relative amounts formed by this pathway (4, 13, 16) compared to oxidation of sugar monophosphate (6, 17, 21) under "normal" physiological conditions (*i.e.* in air) need not be the same as found in this study.

LITERATURE CITED

1. 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 the carbon dioxide acceptor. J. Amer. Chem. Soc. 76: 1760–1770.
2. Bassham, J. A. and M. Calvin. 1957. The path of carbon in photosynthesis. Prentice-Hall, Inc., Englewood Cliffs, N.J. pp. 1–107.
3. Bassham, J. A. and M. R. Kirk. 1960. Dynamics of the photosynthesis of carbon compounds. I. Carboxylation reactions. Biochim. Biophys. Acta 43: 447–464.
4. Bassham, J. A. and M. R. Kirk. 1962. The effect of oxygen on the reduction of CO_2 to glycolic acid and other products during photosynthesis by *Chlorella*. Biochem. Biophys. Res. Commun. 9: 376–380.
5. Benson, A. A. and M. Calvin. 1950. The path of carbon in photosynthesis. J. Exp. Bot. 1: 63–68.
6. Bradbeer, J. W. and E. Racker. 1961. Glycolate formation from fructose-6-phosphate by cell-free preparations. Fed. Proc. 20: 88.
7. Calvin, M., J. A. Bassham, A. A. Benson, V. H. Lynch, C. Ouellet, L. Schou, W. Stepka, and N. E. Tolbert. 1951. Carbon dioxide assimilation in plants. Symposia of the Society for Experimental Biology. 5: 284–305.
8. 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.
9. Chu, D. and J. A. Bassham. 1973. Activation and inactivation of ribulose 1,5-diphosphate by 6-phosphogluconate. Plant Physiol. In press.
10. Coombe, J. and C. P. Whittingham. 1966. The mechanism of inhibition of photosynthesis by high partial pressures of oxygen in *Chlorella*. Proc. Roy. Soc. Ser. B. Biol. Sci. 164: 511–520.
11. Ellyard, P. W. and M. Gibbs. 1969. Inhibition of photosynthesis by oxygen in isolated spinach chloroplasts. Plant Physiol. 44: 1115–1121.
12. Haynes, C. S. and F. A. Isherwood. 1949. Separation of the phosphoric esters on the filter paper chromatograph. Nature 164: 1107–1112.
13. Ogren, W. L. and G. Bowes. 1971. Ribulose diphosphate carboxylase regulates soybean photorespiration. Nature New Biol. 230: 159–160.
14. Pedersen, T. A., Martha Kirk, and J. A. Bassham. 1966. Light-dark transients in levels of intermediate compounds during photosynthesis in air-adapted *Chlorella*. Physiol. Plant. 19: 219–231.
15. Plaut, Z. and M. Gibbs. 1970. Glycolate formation in intact spinach chloroplasts. Plant Physiol. 45: 470–474.
16. Richardson, K. E. and N. E. Tolbert. 1961. Phosphoglycolic acid phosphatase. J. Biol. Chem. 236: 1285–1291.
17. Shain, Y. and M. Gibbs. 1971. Formation of glycolates by a reconstituted spinach chloroplast preparation. Plant Physiol. 48: 325–330.
18. Tolbert, N. E. 1963. Glycolate pathway in photosynthetic mechanisms in green plants. NSF-NRC Publication 1145. pp. 648–662.
19. Tolbert, N. E. 1971. Microbodies, peroxysomes and glyoxysomes. Annu. Rev. Plant Physiol. 22: 45–74.
20. Underwood, J. C. and L. B. Rockland. 1954. The small-scale filter paper chromatography—factors affecting the separation and sequence of amino acids. Anal. Chem. 26: 1553–1557.
21. Wilson, A. T. and M. Calvin. 1955. The photosynthetic cycle CO_2-dependent transients. J. Amer. Chem. Soc. 77: 5948–5957.