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THE EFFECT OF OXYGEN ON THE REDUCTION OF CO<sub>2</sub> TO GLYCOLIC  
ACID AND OTHER PRODUCTS DURING PHOTOSYNTHESIS BY CHLORELLA

J. A. Bassham and Martha Kirk

September 1962

The Effect of Oxygen on the Reduction of CO<sub>2</sub> to Glycolic Acid and Other Products during Photosynthesis by Chlorella

J.A. Bassham and Martha Kirk

The formation of labeled glycolic acid during photosynthesis with <sup>14</sup>CO<sub>2</sub> by Chlorella and by higher plants has been studied by Benson and Calvin (1950), Schou et al. (1950), Wilson (1954), Wilson and Calvin (1955), Tolbert and Zill (1956), Tolbert (1958), Warburg (1960), and Pritchard et al. (1961, 1962). In all these studies glycolic acid formation was found to be favored by low CO<sub>2</sub> pressure. The maximum glycolic acid production in air occurs at 0.1% CO<sub>2</sub> (Pritchard et al., 1962). Tolbert and Zill reported greater formation of glycolic acid in 1% O<sub>2</sub>-99% N<sub>2</sub> than in N<sub>2</sub> under comparable conditions and therefore concluded that aerobic conditions are required for glycolic acid production. However, there appears to have been no investigation thus far of the possible correlation between glycolic acid production as a function of oxygen pressure and CO<sub>2</sub> pressure and the well known effect of oxygen inhibition of the rate of photosynthesis, which is especially pronounced at low CO<sub>2</sub> pressure. Studies of this last effect, discovered by Warburg (1920) have been reviewed recently by Turner and Brittain (1962).

It has been suggested (Wilson and Calvin, 1955) that glycolic acid produced during photosynthesis is derived from carbon atoms 1 and 2 of the pentose phosphate intermediates of the carbon reduction cycle (Bassham et al., 1954). More specifically, the oxidation of the glycolaldehyde-thiamine pyrophosphate addition compound has been proposed (Bassham and Calvin, 1962; Bassham, 1961). This glycolaldehyde thiamine

pyrophosphate addition compound appears to be the intermediate compound (Breslow, 1958) in the transketolase reaction (Racker et al., 1953; Horecker et al., 1953), and it is also involved (White and Ingraham, 1962) in the phosphoketolase reaction (Heath et al., 1958). Its proposed oxidation might involve concomitant reduction of a disulfide (such as lipoic acid) to disulfhydryl, which directly or indirectly might be reoxidized by  $O_2$ . Therefore, we have looked for effects of  $O_2$  on the formation of glycolic acid and other early products of photosynthetic  $CO_2$  reduction during photosynthesis, as well as effects on the total  $CO_2$  incorporation.

Chlorella pyrenoidosa, grown in continuous culture (Bassham and Calvin, 1957) was harvested and resuspended in  $10^{-3}$  M  $(NH_4)H_2PO_4$ , 0.5 ml packed cells in 50 ml for each of the three experiments described. In each case the algal suspension was placed in a "lollipop" illuminated from each side by General Electric DXB Photospot lamps, est. 190,000 lux incident, 5% transmitted, and allowed to photosynthesize in 1.0%  $CO_2$  in air for 10 min. Then the algal suspension, always illuminated, was flushed for two min with either  $O_2$ ,  $CO_2$ -free air, or  $N_2$ . At that point, 1.0 ml of  $NaH^{14}CO_3$  solution (60 micromoles; 1.28 millicuries) was added to the algae in the "lollipop". This vessel was immediately stoppered and shaken for 30 sec. The stopper was then removed and the suspension flushed for 90 sec with the same gas as prior to the addition of  $^{14}C$ . Thus the algae photosynthesized  $^{14}C$ -labeled compounds for 2 min in all. The cells were then killed in methanol (final suspension 80% methanol in water). The resulting mixtures were concentrated at room temperature in vacuo and an aliquot portion of each analyzed by paper chromatography and radioautography (Bassham and

Calvin, 1957). The amount of  $^{14}\text{C}$  in each compound was then determined with a thin window G.M. tube. Another aliquot sample was applied to a planchette in each case and the total non-volatile radioactivity was determined. In the killed algal suspension the pH was about 7, so that sodium glycolate was not lost from the planchette. After the paper chromatograms had been developed, the paper was somewhat acidic (second solvent: butanol-propionic acid). Consequently, a certain amount of glycolic acid was lost from the papers and the values reported represent a lower limit. We have found previously that the loss of glycolic acid from the paper is more than proportional to the amount of glycolic on the paper at the start, so that the percentage loss is less in the case of the papers with small amounts of glycolic acid.

The results of these experiments are given in Table I. The stimulation of glycolic acid production by  $\text{O}_2$  is very large, while smaller stimulations are seen for the formation of phosphoglycolic acid and glycine. In comparison with the total fixation of  $^{14}\text{CO}_2$  under  $\text{N}_2$ , the fixation under  $\text{O}_2$  is down 30%. The radioactivity in ribulose diphosphate is over 30% less in  $\text{O}_2$  and that in phosphoglyceric acid is 50% less. Marked inhibition in the formation of alanine, thought to be derived rather immediately from phosphoglyceric acid (Smith et al., 1961), is caused by  $\text{O}_2$ .

Some of the values for the  $\text{CO}_2$ -free air are intermediate between those for  $\text{O}_2$  and  $\text{N}_2$ . In other cases the levels of compounds in air are close to either the level in  $\text{N}_2$  or to that in  $\text{O}_2$ . Since the results show only the situation at the end of 2 min of extremely varying physiological conditions, such results can be expected.

By themselves, these results could be explained as consequences of some

TABLE I. Effect of Oxygen on Photosynthesis with  $^{14}\text{CO}_2$ 

	(μc $^{14}\text{C}/\text{gm}$ algae)		
	$\text{O}_2$	$\text{CO}_2$ -free air	$\text{N}_2$
Total $^{14}\text{C}$ fixed	508.0	642.7	732.9
Glycolic acid	48.2	9.2	2.0
Phosphoglycolic acid	3.4	2.1	1.7
Ribulose diphosphate	58.4	91.0	87.6
Other sugar diphosphates	0.3	0.5	1.3
Phosphoglyceric acid	20.1	25.3	40.6
Alanine	35.2	50.3	81.5
Glycine	27.6	21.7	5.4
Serine	17.1	21.9	18.0
Aspartic acid	24.3	32.8	27.6
Glutamic acid	4.5	11.6	8.6
Citric acid	1.4	2.2	1.8
Malic acid	32.5	52.4	55.6

primary effect of oxygen on the reduced cofactors formed by the light reaction. However, ribulose diphosphate is considered to be the substrate for the principal carboxylation reaction. The formation of glycolic acid may represent a metabolic path competing with the formation of ribulose diphosphate from the other sugar phosphates. The  $\text{O}_2$  and  $\text{N}_2$  data support the thesis that oxygen stimulates the oxidation of the glycolaldehyde moiety formed from carbon atoms 1 and 2 of sugar phosphates, thereby lowering the level of ribulose diphosphate and inhibiting  $\text{CO}_2$  fixation and the rate of photosynthesis.

The fact that the level of ribulose diphosphate labeling in  $\text{CO}_2$ -free air is as high as in  $\text{N}_2$  appears to be an obstacle to the conclusion just reached. The mechanism of the carboxylation of ribulose diphosphate during photosynthesis is not known. Ultimately it must involve the carboxylation of carbon atom number 2 of ribulose diphosphate which remains bonded to carbon



1 (bearing a phosphate group (Weissbach et al., 1956). This three-carbon moiety somehow receives electrons from the moiety composed of carbon atoms 3,4 and 5 of the ribulose diphosphate, so that two molecules of 3-phosphoglyceric acid are produced. It may be that at some early stage in this reaction mechanism, the moiety composed of carbon atoms 1 and 2 can be oxidized, giving rise to phosphoglycolic acid. The observed O<sub>2</sub> stimulation of phosphoglycolic acid production supports this view, particularly if one considers the rapid hydrolysis of phosphoglycolic acid in vivo in green tissue which has been reported (Richardson and Tolbert, 1961).

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