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Oxygen Effect on Photosynthetic and Glycolate Pathways in Young Maize Leaves

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ABSTRACT

To study the effect of O_2 on the photosynthetic and glycolate pathways, maize leaves were exposed to $^{14}CO_2$ during steady-state photosynthesis in 21 or 1% O_2. At the two O_2 concentrations after a $^{14}CO_2$ pulse (4 seconds) followed by a $^{12}CO_2$ chase, there was a slight difference in CO_2 uptake and in the total amount of ^{14}C fixed, but there were marked changes in ^{14}C distribution especially in phosphoglycerate, ribulose bisphosphate, glycine, and serine. The kinetics of ^{14}C incorporation into glycine and serine indicated that the glycolate pathway is inhibited at low O_2 concentrations. In 1% O_2, labeling of glycine was reduced by 90% and that of serine was reduced by 70%, relative to the control in 21% O_2. A similar effect has been observed in C_3 plants, except that, in maize leaves, only 5 to 6% of the total ^{14}C fixed under 21% O_2 was found in glycolate pathway intermediates after 60 seconds chase. This figure is 20% in C_3 plants. Isonicotinyl hydrazide did not completely block the conversion of glycine to serine in 21% O_2, and the first carbon atom of serine was preferentially labeled during the first seconds of the chase. These results supported the hypothesis that the labeled serine not only derives from glycine but also could be formed from phosphoglycerate, labeled in the first carbon atom during the first seconds of photosynthesis.

Another noticeable O_2 effect concerned differential labeling of phosphoglycerate and ribulose bisphosphate. Phosphoglycerate is more labeled than ribulose bisphosphate in air; the reverse is observed in 1% O_2. Changes in ribulose bisphosphate and phosphoglycerate pools exhibit similar trends. To understand the effect of O_2 on the distribution of ^{14}C in these two intermediates, it was postulated that, in air, there remains an oxygenase function which produces additional phosphoglycerate at the expense of ribulose bisphosphate.

In contrast to C_3 species, leaves of maize, a typical C_4 plant, do not show a significant enhancement of net photosynthesis under low O_2 concentration, release little or no CO_2 when illuminated in a CO_2-free atmosphere, fail to manifest a CO_2 postillumination burst upon transfer from light to darkness, and have a CO_2 compensation point close to zero, which is insensitive to O_2 concentration and temperature (7). No difference between $^{14}CO_2$ and $^{12}CO_2$ uptake can be shown over a range of O_2 concentrations from 2 to 60%, and no change in quantum yield is observed in maize leaves as a result of changing the O_2 concentration (7). Thus, intact maize leaves exhibit many features which seem to indicate a lack of photorespiration. However, a measurable O_2-stimulated consumption of $^{18}O_2$ is detected during maize photosynthesis (11, 26).

Whole maize leaves, and particularly isolated bundle-sheath cells, can synthesize and transform glycolate, the substrate of the photorespiration pathway, via a O_2-sensitive route but at a lower rate than in C_3 plants. In addition, maize bundle-sheath cells contain numerous peroxisomes, mitochondria, and photorespiratory enzymes. However, the observed activities of the photorespiratory enzymes measured *in vitro* appeared to be considerably lower than in C_3 plants. Although no external manifestations of photorespiration occur in intact corn leaves, maize bundle-sheath cells possess all the potential for metabolizing glycolate via the glycolate pathway (7, 8, 28).

When maize leaves are supplied with $^{14}CO_2$ in normal air, a substantial labeling of glycine and serine, two intermediates of the glycolate pathway, has been reported. By decreasing O_2 concentration from 21 to 1%, the amount of radioactivity incorporated into these compounds decreases substantially. Such behavior is consistent with a rapid flow of carbon through a glycolate pathway, sensitive to O_2 concentration (15, 17-19). But the metabolism of serine has not been found to be stoichiometrically related to glycine production in maize, so that a route, other than the glycolate pathway, must be used for serine synthesis (17, 19). Since these data can be interpreted either way, there is at present no experimental evidence for the occurrence of a typical photorespiratory process in maize leaves.

Here, we describe several biochemical features of photosynthetic and photorespiratory metabolism in illuminated maize leaves, exposed to a gas mixture which simulated normal air (350 μl/l CO_2 and 21% O_2 in N_2), the favorable conditions for photorespiration. Very short exposures to $^{14}CO_2$ (4 s), followed by a chase with $^{12}CO_2$, were used to follow the successive and rapid steps of the processes of photosynthesis and photorespiration. We focused our attention on the kinetics of the appearance of ^{14}C among the reductive pentose phosphate cycle intermediates and amino acids involved in the glycolate pathway during the chase. Some experiments were performed to determine the dependence of the ^{14}C-labeling process on O_2 and CO_2 in conditions which were known to inhibit or stimulate photorespiration: low O_2 concentration (1 and 0% O_2 instead of 21%) and CO_2-free chase. In addition, treatment of leaves with INH,[1] an inhibitor of glycine conversion to serine, was used to investigate the pathways of serine synthesis.

[1] Abbreviations: INH, isonicotinyl hydrazide; PGA, phosphoglycerate; RuBP, ribulose bisphosphate.

MATERIALS AND METHODS

Plant Material. The seedlings of *Zea mays* L. cv. Wisconsin strain W64A were cultivated for 16 days in the Centre National de la Recherche Scientifique Phytotron [at], Gif-sur-Yvette (France), under a 16-h photoperiod with approximately 300 μE/m^2·s irradiance (400–700 nm), at 27 C during the first 9 h and at 17 C during the following 7 h. During the 8-h night, the temperature was 17 C. The RH was kept constant (70%) day and night. The vermiculite in which the plants were grown was infiltrated daily with the following nutrient solution: 2.7 mM KNO_3, 1 mM KH_2PO_4, 1.1 mM $MgSO_4 \cdot 7H_2O$, 1 mM $(NH_4)_2SO_4$, 4.6 mM $Ca(NO_3)_2 \cdot 4H_2O$, and trace elements (20, 21).

$^{14}CO_2$ Pulse-$^{12}CO_2$ Chase Experiments under Steady-state Conditions. The leaves (0.2 to 0.3 g fresh weight) were enclosed in a thermostated (30 C) copper-Perspex assimilation chamber (12 cm^3 capacity) similar to that described by Galmiche (12) and irradiated with an incandescent lamp. Irradiance (400–700 nm) at the leaf surface was 1100 μE/m^2·s. The gas mixture saturated with water containing CO_2 (350 μl/l) with 21, 1, or 0% O_2 in N_2, was supplied to the assimilation chamber at a constant flow rate of 13 liters/h in an open circuit. The $^{12}CO_2$ concentration was continuously monitored with an IR analyzer (Onera). When the photosynthetic rate became constant (after about 10 min), the $^{14}CO_2$ (80 μCi), enclosed in a tube, was introduced into the assimilation chamber by means of a gas stream (containing 350 μl/l CO_2 with 21, 1, or 0% O_2 in N_2) at a flow rate of 240 liters/h. The leaves were exposed to $^{14}CO_2$ for 4 s (pulse experiments) and chased over a time course of 3 to 160 s with $^{12}CO_2$ (350 μl/l) and 21, 1, or 0% O_2 at a flow rate of 240 liters/h for the first 10 s of the chase and then at a flow rate of 13 liters/h for the rest of the chase. The concentration of O_2 and CO_2 in the gas mixtures were similar during the pulse and chase periods, except for one experiment where the chase was performed in a CO_2-free atmosphere with 21% O_2 in N_2. In addition, certain experiments involved pretreatment with INH, the inhibitor of glycine conversion to serine; maize leaves were enclosed in a glass chamber and illuminated with their cut bases standing to a depth of 2 mm in a 146 mM aqueous solution of INH for 45 min before exposure to $^{14}CO_2$ (5). At the end of the chase, the leaves were dropped in liquid N_2 by opening a magnetic trapdoor.

Analysis of ^{14}C-labeled Products. Liquid N_2 frozen samples were ground in melting isopentane and lyophilized, and the powder was mixed with 1 ml of formamide as previously described (12).

The main part of the extract was subjected directly to high-voltage preparative paper electrophoresis at pH 4.5 (12, 20). After autoradiography, the various bands were eluted with H_2O overnight at 4 C, and their radioactivity was determined by liquid scintillation.

The neutral amino acids, sugars, and starch remaining at the origin were recovered after extracting formamide with diethylether and then eluting the neutral soluble compounds with H_2O for 12 h at 4 C. The amino acids thus obtained were separated by ion-exchange chromatography on a column of cationic resin (Hamilton HPAN 90 Li) and eluted with citrate lithium buffer as described by Benson *et al.* (3). The eluate was passed through an anthracene filled Plexiglas cell and the profile of radioactivity was continuously recorded on a potentiometric recorder. Starch was assayed by digesting the insoluble material at the origin with a mixture of α- and β-amylases (Sigma) at 37 C for 24 h. Soluble radioactivity appearing in the incubation medium was determined by liquid scintillation.

An aliquot of the powdered extract in formamide was subjected to analytical paper electrophoresis in the first dimension and then chromatography in the second dimension (20). After autoradiography, the various spots were counted in a scintillation counter.

Degradation Procedure. After separation by column chromatography, the labeled glycine and serine were degraded by ninhydrin to assay for radioactivity in the carboxyl group. The CO_2 formed in the reaction was trapped in ethanol phenylethylamine solution and the radioactivity was determined by liquid scintillation.

Determination of PGA and RuBP Pool Sizes. The pools of RuBP and PGA were measured in samples of leaves which had been submitted to a 25-s chase in 21 or 1% O_2 containing 350 μl/l CO_2. At this period of the chase, radioactivity in the components was high with complete randomization of label between the different carbon atoms of PGA and RuBP (20). ^{14}C-labeled PGA and RuBP were separated by preparative electrophoresis. Spectrophotometric assays of PGA were directly performed on an aliquot of [^{14}C]PGA eluate according to the method of Czok and Eckert (10). RuBP pool sizes were measured using an isotopic assay similar to that of Latzko and Gibbs (16) with the following modifications. An aliquot of the [^{14}C]RuBP eluate was incubated with [^{12}C]bicarbonate and low amounts of RuBP carboxylase prepared according to Wildner and Henkel (27). The rate of [^{14}C]PGA formation was determined after a 30-s incubation period. The reaction was found to be linear for the first min of incubation period and the enzyme was present in limiting amounts. [^{14}C]PGA was separated from the remaining [^{14}C]RuBP by high-voltage paper electrophoresis. With complete randomization of ^{14}C into RuBP, the specific radioactivity of the PGA formed is 5/6 that of the RuBP transformed. The specific radioactivity of RuBP in aliquots was lowered by adding known amounts of unlabeled RuBP (usually 30 nmol), resulting in a decrease in the [^{14}C] PGA formed in 30 s. It results that m (the ratio of [^{14}C] PGA formed in the presence and in the absence of unlabeled RuBP) is equal to the ratio of specific radioactivities of RuBP in aliquots and then equal to the reverse of the ratio of RuBP concentration in them:

$$m = \frac{x}{x + 30} \quad m < 1$$

where x is the total amount of RuBP in the aliquot and 30 nmol is the amount of unlabeled RuBP added. We then deduce:

$$x = \frac{30m}{1 - m}.$$

RESULTS

RATE OF CO_2 UPTAKE DURING STEADY STATE PHOTOSYNTHESIS AT TWO OXYGEN CONCENTRATIONS (21 AND 1%)

Photosynthetic rates were determined during steady-state photosynthesis in leaves exposed to two O_2 concentrations (21 and 1%), either as the rate of CO_2 uptake or as total radioactivity in stable products after a $^{14}CO_2$ pulse-chase (Table I). When the leaves were flushed with 350 μl/l CO_2 and low O_2 (1% instead of 21%), the photosynthetic rates were slightly depressed (by 5–7%), compared to normal air. There was a similar reduction in total $^{14}CO_2$ fixation at the two O_2 concentrations (Table I). Moreover, we observed that the total amount of label fixed remained constant over the chase (data not shown).

^{14}C DISTRIBUTION AMONG C_4 ACIDS, PHOSPHORYLATED INTERMEDIATES, AND END PRODUCTS OF PHOTOSYNTHESIS AT TWO O_2 CONCENTRATIONS (21 AND 1%)

At both O_2 concentrations, in the presence of 350 μl/l CO_2, maize leaves exhibited a general labeling pattern during the chase period characteristic of a C_4 photosynthetic process as previously observed (20). There were important differences in the distribution of radioactivity between several photosynthetic compounds when the O_2 concentration was changed.

Table I. *Effects of O_2 Concentration on Rates of Net CO_2 Assimilation*

Photosynthetic rates were determined during steady-state photosynthesis in maize leaves exposed to different O_2 concentrations (21, 1, and 0%) either as the rate of CO_2 uptake or as total radioactivity in stable products after a 4-s pulse in $^{14}CO_2$. The irradiance was 1100 μE/m^2·s. The temperature was 34 C and the CO_2 concentration was kept constant at 350 μl/l CO_2, except for the chase in experiment 6, achieved without CO_2. In experiments 2 and 4, the leaves were pretreated with 146 mM INH for 45 min. All values are averages of five measurements ± SD in experiments 2, 4, 5, 6 and averages of 30 ± SD in experiments 1 and 3.

Experiment No.	Conditions	Rate of CO_2 Uptake	Total Radioactivity
		nmol/s·g fresh wt	*10^6 dpm/g fresh wt*
1	21% O_2	127 ± 12	40 ± 7
2	21% O_2 + INH	100 ± 20	32 ± 8
3	1% O_2	120 ± 10	38 ± 6
4	1% O_2 + INH	90 ± 15	28 ± 9
5	0% O_2	80 ± 18	25 ± 10
6	21% O_2, $[CO_2] \rightarrow 0$	124 ± 15	39 ± 7

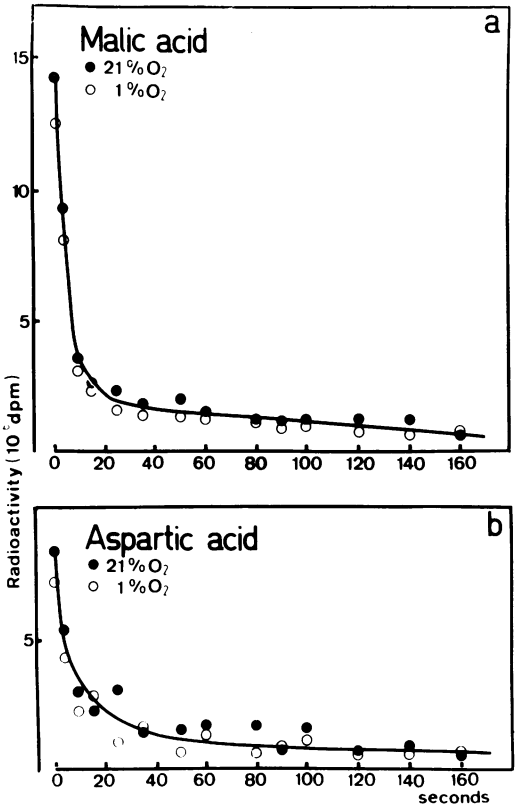

FIG. 1. Changes in radioactivity in malate (a) and aspartate (b) of maize leaves during a chase in $^{12}CO_2$ after a pulse of 4 s in $^{14}CO_2$. Atmosphere was air containing 350 μl/l CO_2 with either 21 or 1% O_2 in N_2. Irradiance was 1100 μE m^{-2}·s^{-1} and the temperature was 34 C. Data are expressed as 10^6 dpm/g fresh weight.

C_4 Acids. After a 4-s exposure to air and $^{14}CO_2$, the initial predominant label in malate and aspartate (70% of total radioactivity) strongly decreased (by as much as 75%) during the first 10 s of the chase period, reaching 3 to 5% of the total radioactivity incorporated into stable compounds after 160 s in $^{12}CO_2$ (Fig. 1). Changing the O_2 concentration had no marked effect on the ^{14}C distribution between the malate and aspartate or on the kinetics of ^{14}C disappearance in these compounds during the chase.

Phosphorylated Intermediates. At both O_2 concentrations, the radioactivity fixed into PGA, RuBP, and other phosphorylated compounds (pentose and hexose monophosphates) increased rapidly at the beginning of the chase period to reach a maximum at 4, 10, and 15 s, respectively, and then decreased (Figs. 2 and 3a). When the O_2 concentration was changed from 21 to 1%, PGA labeling was depressed by 35 to 40% and, in contrast, the ^{14}C incorporation into RuBP was increased by 40 to 45% (Fig. 2; Table II). At 34 C after 15 s chase, the [^{14}C]PGA/[^{14}C]RuBP ratio was 1.8 in 21% O_2, compared to 0.6 in 1% O_2, and, after 60 s, the ratio was 1.1 in 21% O_2, compared to 0.4 in 1% O_2. As far as the other phosphorylated compounds were concerned, a reduction in the label was also observed with reduced O_2 concentration (Fig. 3a).

RuBP and PGA pool sizes were determined after a 25-s chase period for leaves exposed to either 21 or 1% O_2. The RuBP pools appeared to be 1.5 to 2 times higher in the poorly oxygenated medium than in 21% O_2 atmosphere (Table III). In contrast, the PGA pool sizes exhibit changes in the opposite direction. However, rather high levels of PGA were seen in some samples of leaves exposed to 1% O_2 (see experiment b, Table III), which seem to indicate that some PGA originates from an O_2-insensitive process other than RuBP carboxylase, the activity of which varies from sample to sample.

Other Products. Label in neutral sugars (glucose, fructose, sucrose), starch (data not shown), glycine, serine, and alanine increased continuously during the chase, the latter three reaching a plateau after 100 to 160 s (Figs. 3b, 4, and 5). Neutral sugars were slightly more heavily labeled in 21% than in 1% O_2, whereas no differences were observed in the labeling of starch at either O_2 concentration (data not shown). The most obvious effect of lowering O_2 concentration was the considerably decreased incorporation of radioactivity into glycine (10-fold) and serine (3-fold) (Fig. 4). There was a concomitant increase in ^{14}C incorporation into alanine (Fig. 5). The same effects were emphasized at 0% O_2

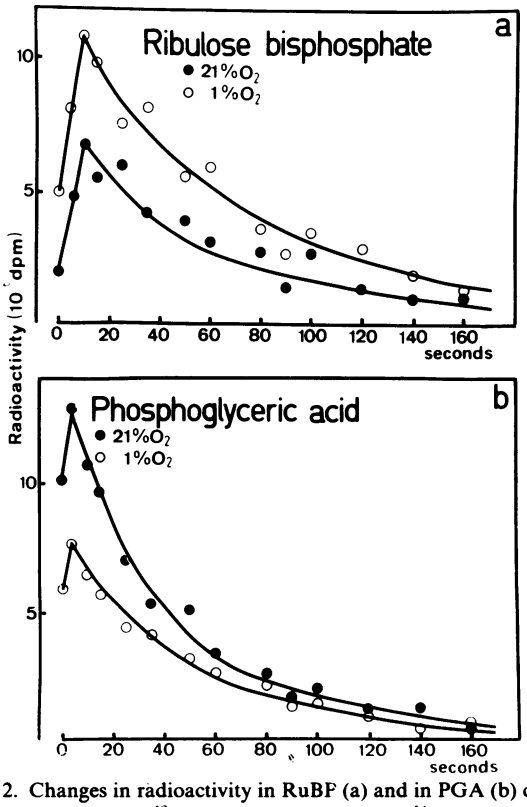

FIG. 2. Changes in radioactivity in RuBP (a) and in PGA (b) of maize leaves during a chase in $^{12}CO_2$ after a pulse of 4 s in $^{14}CO_2$. Experimental conditions as for Figure 1.

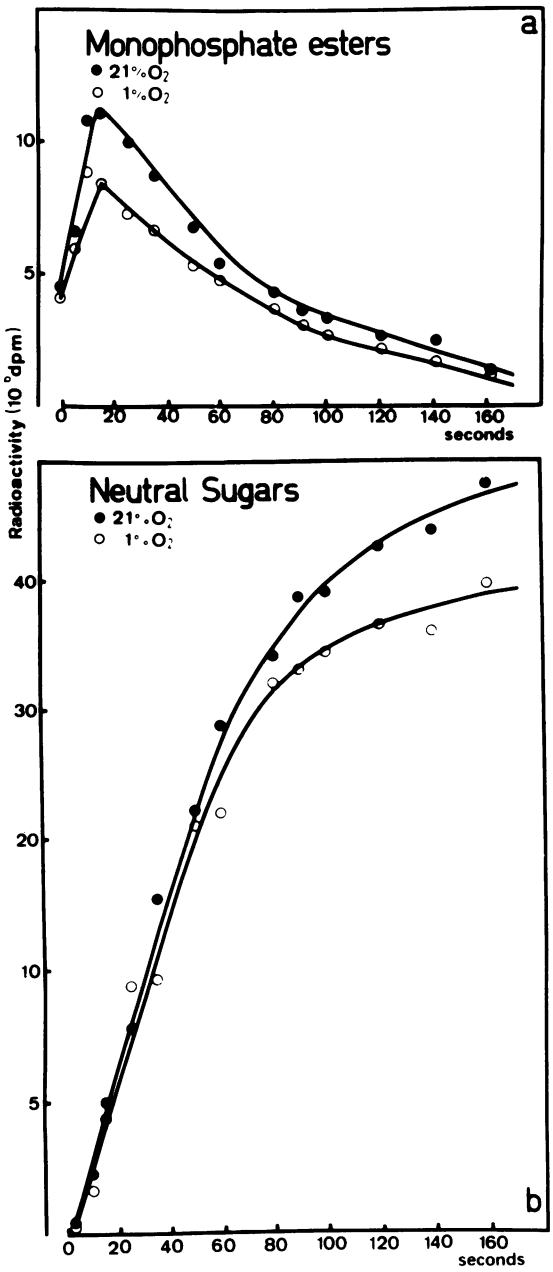

FIG. 3. Changes in radioactivity in pentose and hexose monophosphates (a) and neutral sugars (b) of maize leaves during a chase in $^{12}CO_2$ after a pulse of 4 s in $^{14}CO_2$. Experimental conditions as for Figure 1.

accompanied by a 35 to 40% decrease in CO_2 uptake (Tables I and IV). [^{14}C]Glycolate has been detected only as traces in experiments at 21% O_2 (21). The study of this important intermediate using appropriate technics is in progress.

EFFECTS OF CO_2 DEPLETION AND INH ON ^{14}C DISTRIBUTION AT TWO O_2 CONCENTRATIONS (21 AND 1%)

Effect of CO_2 Depletion and 21% O_2 on ^{14}C Distribution among Photosynthetic Products. Under conditions which should be favorable to the operation of the photorespiratory process, *i.e.* low CO_2 and high O_2 concentrations, a 90 to 110% enhancement of glycine and serine labeling occurred during the chase period, accompanied by an 80% decrease in the labeling of α-alanine (relative to the control in 350 μl/l CO_2) (Table IV).

INH Effect on Serine and Glycine Synthesis. Similar pulse-chase experiments were performed on INH-treated leaves (see "Materials and Methods"). Controls were treated with distilled H_2O. In 21% O_2 atmosphere containing 350 μl/l CO_2, INH depressed the rate of CO_2 fixation at steady-state photosynthesis by up to 20 to 25% (Table I) and simultaneously decreased the incorporation of ^{14}C into serine by 70% with a slight accumulation of label (data expressed in percentage) into glycine (Table IV). In contrast, when leaves were exposed to a 1% O_2-350 μl/l CO_2 atmosphere, INH did not significantly affect serine labeling, compared to the untreated control (Table IV).

Table II. *Changes in Radioactivity in PGA and RuBP as a Function of O_2 Concentration*

Maize leaves were submitted to 15- and 60-s chases in $^{12}CO_2$ following a 4-s pulse in $^{14}CO_2$. In all cases, the CO_2 concentration was 350 μl/l and the temperature was 34 C. O_2 concentration was as indicated. All values are averages of five measurements.

Experiment No.	Conditions	PGA		RuBP		[^{14}C]PGA/[^{14}C]RuBP	
		15 s	60 s	15 s	60 s	15 s	60 s
		% total dpm fixed				*ratio*	
1	21% O_2	24.3	8.5	13.7	7.7	1.8	1.1
3	1% O_2	16.2	7.4	28.1	16.9	0.6	0.4

Table III. *PGA and RuBP Pool Sizes as a Function of O_2 Concentration*

After a 4-s pulse in $^{14}CO_2$, the maize leaves were submitted to a 25-s chase in air with 21 or 1% O_2. The temperature was 34 C and the CO_2 concentration was kept constant at 350 μl/l CO_2.

Experiment	PGA		RuBP	
	21% O_2	1% O_2	21% O_2	1% O_2
	nmol/g fresh wt			
a	534	218	370	560
b	453	390	330	700
c	559	347	390	645

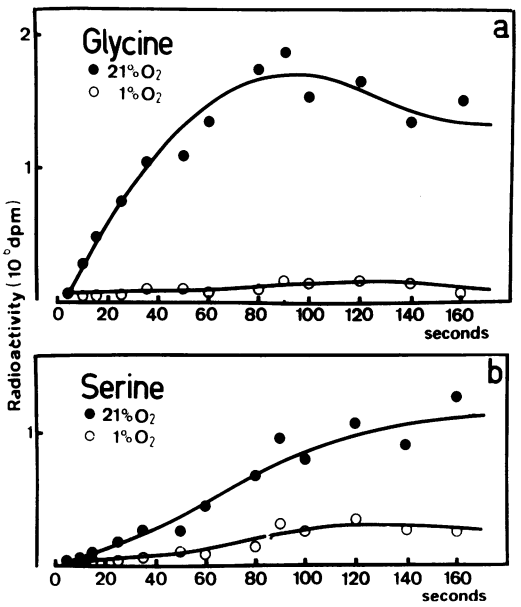

FIG. 4. Changes in radioactivity in glycine (a) and serine (b) of maize leaves during a chase in $^{12}CO_2$ after a pulse of 4 s in $^{14}CO_2$. Experimental conditions as for Figure 1.

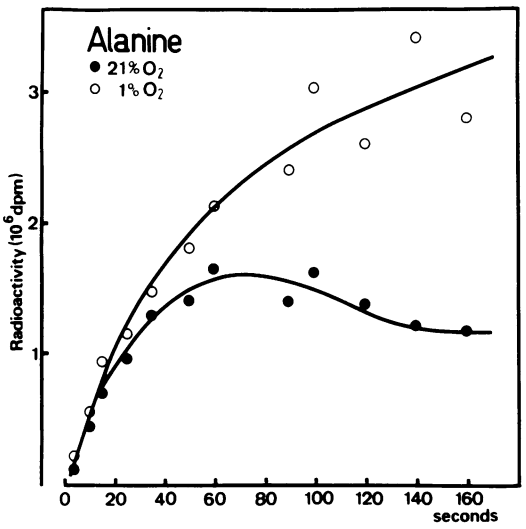

FIG. 5. Changes in radioactivity in α-alanine of maize leaves during a chase in $^{12}CO_2$ after a pulse of 4 s in $^{14}CO_2$. Experimental conditions as for Figure 1.

Table IV. *Changes in Radioactivity in Glycine, Serine, and α-Alanine as a Function of O_2 Concentration*

Maize leaves were submitted to pulse-chase experiments: pulse was 4 s; chase was 60 and 120 s; CO_2 concentration was kept constant at 350 μl/l CO_2 except for the chase in experiment 6 achieved without CO_2. In experiments 2 and 4, the leaves were pretreated with INH for 45 min. All values are averages of five measurements.

Experiment No.	Conditions	Glycine		Serine		Alanine	
		60 s	120 s	60 s	120 s	60 s	120 s
		% total dpm fixed					
1	21% O_2	3.0	4.2	1.1	2.7	4.0	3.4
2	21% O_2 + INH	3.2	5.4	0.3	0.8	2.1	4.2
3	1% O_2	0.2	0.5	0.3	1.0	5.8	6.2
4	1% O_2 + INH		1.1		0.7		8.2
5	0% O_2	0.1		0.2		8.8	
6	21% O_2, $[CO_2] \rightarrow 0$	6.3	10.2	2.2	4.5	0.8	1.1

INTRAMOLECULAR ^{14}C DISTRIBUTION IN GLYCINE AND SERINE

The distribution of ^{14}C among the different carbon atoms of glycine and serine was investigated in order to obtain information on their origin. A nearly uniform distribution of label between carbons 1 and 2 of glycine was observed during the chase, whatever the O_2 concentration (Table V). In contrast, carbon atom 1 of serine was more heavily labeled than carbon atoms 2 and 3 after 3 and 10 s chase. In long chase periods, radioactivity was nearly uniformly distributed among the 3 carbon atoms of serine, regardless of the O_2 concentrations (Table V) (data for 1% are not shown).

DISCUSSION

These experiments provide further evidence that maize leaves exposed to air containing low O_2 levels exhibit no enhancement of photosynthesis but show a slight decrease in the apparent photosynthetic rate as observed previously by Hickman and Keys (15). However, there have been reports in the literature that the depletion of O_2 during photosynthesis caused either no effect or a slight enhancement of CO_2 fixation in maize and other C_4 plants (17–19, 22).

Although no differences in the total amount of fixed ^{14}C were seen in maize leaves exposed to atmospheres containing either 21 or 1% O_2, marked changes were observed in the ^{14}C distribution in a variety of photosynthetic products. The most striking effects of lowering the O_2 tension from 21 to 1% were, on one hand, decreased glycine and serine labeling and, on the other hand, increased labeling of RuBP, associated with a concomitant decrease in PGA.

Evidence for Operation of a Glycolate Pathway. In 21% O_2, the appearance of ^{14}C into glycine and serine, two intermediates of the glycolate pathway, occurred after 3 to 10 s of $^{12}CO_2$ chase and accounted for 4 to 5% of the total ^{14}C after 60 s. In C_3 plants, such as spinach and tobacco, under the same conditions, the percentage of ^{14}C recovered into glycine and serine was 20 to 30% of the total fixed ^{14}C (unpublished data). At 1% O_2 in maize, as well as in spinach and tobacco, the labeling of glycine and serine was dramatically decreased (Fig. 4). These results clearly indicate the existence of an O_2 effect on glycine and serine labeling in maize leaves, similar to that observed in C_3 plants.

The rapid and nearly uniform labeling of carbon atoms in glycine molecules over the entire chase is consistent with the production of these amino acids from early, uniformly labeled, photosynthetic intermediate(s), belonging to the reductive pentose phosphate pathway. It is currently assumed that glycolate, the precursor of glycine, is formed either from a two-carbon fragment derived from a sugar monophosphate of the Calvin-Benson cycle (13, 25, 28) or from RuBP oxidized by the RuBP oxygenase to phosphoglycolate and PGA (1, 2, 23). Such glycine production in maize leaves by this latter route is consistent with the fact that isolated maize bundle-sheath strands, which display a reversible O_2 inhibition of photosynthesis, are capable of rapidly producing and transforming glycolate under 21% O_2 (8). However, glycolate production is always lower in maize than in C_3 leaves (28), probably because of a CO_2 concentrating mechanism in bundle-sheath cells (4, 12, 14) which must reduce the oxygenase activity of the RuBP carboxylase-oxygenase and simultaneously increase the carboxylase activity (see below). In contrast, when maize leaves were exposed to a 21% O_2 atmosphere free from CO_2 during the chase, a progressive decrease in the internal CO_2 concentration stimulated the oxygenase activity (see below) so that synthesis of glycolate, and consequently of glycine and serine, was activated (Table IV). Last, by removing O_2 from the ambient gas mixture, the RuBP oxygenase activity is abolished, which is consistent with the observation of a vestigial labeling of glycine (Table IV).

Since a good deal of evidence indicates that the main pool of [^{14}C]glycine originates from glycolate, it seems possible that mul-

Table V. *Distribution of ^{14}C in First Carbon Atom of Glycine and Serine as a Function of Chase Time*

Data were expressed in per cent of radioactivity fixed in glycine and serine. Conditions were CO_2 at 350 μl/l and O_2 at 21%.

Compounds	Radioactivity Fixed in Carbon Atom 1 of Molecule after Chase of								
	3 s	10 s	15 s	25 s	35 s	60 s	100 s	120 s	140 s
					% dpm				
Glycine	53	38	36	37	39	42	47	55	47
Serine	80	53	34	31	28	48	32	36	33

tiple pathways for serine synthesis exist in maize leaves (17, 19). Indeed, by lowering O_2 pressure, the serine labeling is decreased by 66%, compared to 90% for glycine, and INH does not completely block the conversion of glycine to serine under 21% O_2 (Table IV). Moreover, the labeled serine molecules formed in the first 15 s of the chase exhibit predominant incorporation in the first carbon atom (Table V). Consequently, our results are consistent with the view that a part of the [^{14}C]serine pool could derive from an intermediate other than [^{14}C]glycine. A possible route is from PGA via glycerate and 3-hydroxypyruvate (6, 23) since the first carbon atom of PGA appears heavily labeled after a few s of chase, following a 3- to 4-s pulse in $^{14}CO_2$ (12, 20). Labeling of serine during the first 20 s of the chase may result from the interconversion between highly labeled PGA and unlabeled serine since the two components are involved in reversible reactions via two intermediates, glycerate and 3-hydroxypyruvate. Thus, the labeling of serine could reflect an exchange of ^{14}C between components rather than a net flux of carbon.

Finally, there is an inverse relationship between the labeling of serine, glycine and alanine, as observed previously by Hickman and Keys (15) and Lawlor and Fock (17), probably indicating a competition between glyoxylate and pyruvate for amino groups required for transamination.

Equilibrium between RuBP and PGA Labeling. A marked and very characteristic effect of changing O_2 pressure on the labeling of the acceptor and the primary product of RuBP carboxylase-oxygenase is observed in 350 μl/l CO_2 at 34 C (Table II). The labeling of PGA is reduced, whereas there is a higher incorporation of ^{14}C into RuBP in a poorly oxygenated atmosphere (1% O_2). This could be interpreted as a higher rate of RuBP formation or as a lower rate of RuBP consumption, associated with a lower rate of PGA synthesis (Fig. 2; Table II). The latter interpretation is confirmed by the observation of changes in the total RuBP and PGA pools nearly parallel to those of the labeled pools (Table III; Fig. 2). A similar O_2 effect on the RuBP pool sizes has already been observed in photosynthesizing C_3 plants (spinach) and algae, especially at low CO_2 concentration (9).

How can changes in O_2 concentration affect the rates of RuBP and PGA synthesis since O_2 does not change the rate of CO_2 uptake in 350 μl/l CO_2? A simple interpretation of the O_2 effect on RuBP and PGA is to postulate that, at steady-state photosynthesis in air, there is substantial photorespiration in bundle-sheath cells of maize. This photorespiratory process results in a greater production of PGA and glycolate, decreasing the RuBP pool compared to leaves treated with 1% O_2 (Table III). Such a low oxygenase activity in air is predicted by the model of C_4 photosynthesis proposed by Berry and Farquhar (4) and is believed to be due to the presence of a high internal O_2 in bundle-sheath cells. Moreover, this model predicts that increasing O_2 pressure from 1 to 21% O_2 slightly increases the CO_2 pressure in bundle-sheath cells, which could stimulate the RuBP carboxylase (working near its V_{max}) with higher production of PGA. It is possible, using this model, to explain why there is no O_2 effect on net CO_2 assimilation by O_2 concentrations. When lowering the CO_2 pressure to nearly zero in 21% O_2, the model predicts a decrease of both CO_2 and O_2 pressure in bundle-sheath cells but to a much lesser extent for the latter than for the former. Such a situation would permit a higher RuBP oxygenase activity than in ambient CO_2 concentration, responsible for the relatively high glycine and serine production as it is observed in Table IV. Last, in maize no external manifestation of CO_2 release can be detected during a chase, even under CO_2-free conditions (experiment 6, Table I), since the CO_2 derived from the glycolate pathway is immediately refixed and metabolized (24).

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