Photorespiratory Glycine Metabolism in Corn Leaf Discs

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ABSTRACT

The total glycine pool in Zea mays L. Mo17×B73 leaf discs was measured after steady state photosynthesis in 50%, 21% and 1% O2. The glycine pool was a function of O2 concentration; it was largest in 50% O2 and smallest in 1% O2. Incubation of discs with methyl hydroxybutynoic acid in 21% O2 in the light caused an accumulation of carbon in glycinate. This accumulation was O2 sensitive, as subsequent photosynthetic periods in 50%, 21%, and 1% O2 resulted in the largest glycinate pool in 50% O2 and the smallest in 1% O2. At the same time, the O2-dependent increase in the glycine pool was eliminated. After untreated leaf discs reached steady state photosynthesis in 21% O2, measurements made subsequently in darkness, or in 1% O2 in the light, showed that the glycine pool decreased. On the basis of these results, we conclude that a major portion of the total glycine pool in corn is an intermediate in the photorespiratory glycine pathway. Considering both the rate of decay of the glycine pool in the dark and the rate of decay of the glycine pool after changing from 21% to 1% O2, we conclude that this glycine pool is turning over slowly.

C4 plants do not show external manifestations of photorespiration (3). Photorespiration is represented biochemically as carbon flow through the glycolate pathway, and flow into the pathway is initiated by the oxygenase activity of RubiscoO2. The enzymes of the glycolate pathway are present in bundle sheath cells of C4 plants (14), and labeling studies with 14CO2 clearly show an O2-sensitive labeling of glycolate pathway intermediates in the C4 plant corn (12, 13). However, the failure of labeled carbon to chase out of the intermediates in corn as rapidly as in C3 plants (11) has led to the suggestion that photorespiration, as it occurs in C3 plants, does not occur in corn (3, 12).

It was previously observed that the steady state level of glycine in photosynthesizing soybean leaves was proportional to O2 levels and thus correlated with photorespiratory rate (7). We expected to find low levels of glycine in photosynthesizing corn leaf discs; however, we measured a larger total pool of glycine in corn leaf discs than in soybean leaves and leaf discs. The experiments reported in this paper were conducted to determine whether or not this large glycine pool in corn leaf discs was involved in the photorespiratory pathway.


2 Abbreviations: Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; MeHBA, methylhydroxybutynoic acid; HBA, hydroxybutynoic acid; PGA, phosphoglycerate; RuBP, ribulose 1,5-bisphosphate.

MATERIALS AND METHODS

Plant Material. Corn (Zea mays L. Mo17×B73) was grown in growth chambers under mixed incandescent and cool white fluorescent lights. Light intensity at pot surface at germination was approximately 1000 μE m–2 s−1. Daylength was 16 h and day/night temperatures were 27°C/22°C. Plants were watered three times weekly with double strength modified Hoagland solution (10) in which iron was supplied as the chelate Sequin-trene 138 Fe at the rate of 16.7 mg l−1. The third leaf from the top of three 21- to 28-day-old corn plants was used in each experiment. Six leaf discs were used for each experimental treatment, and there were four replications of each treatment per experiment.

Gas Exchange. Gas exchange was measured in the system described by Creach and Stewart (4). Leaf discs were allowed to reach steady state photosynthesis (15-20 min) in 1%, 21%, or 50% O2 and then either frozen in liquid N2 or darkened for various times and then frozen. In some experiments, after steady state photosynthesis in 21% O2 was reached, the O2 concentration was changed to 1% and the discs were allowed to photosynthesize for 4 to 60 min before being frozen. In the experiments using MeHBA (gift of Shell Research, U. K.; see “Acknowledgment”), discs were floated either on a 1 mM solution of MeHBA or on distilled H2O for 1 h in the growth chamber before being put into the sample chamber to reach steady state photosynthesis. The leaf discs were kept in liquid N2 until the conclusion of the experiment, at which time they were freeze dried overnight.

Metabolite and Chl Determinations. Extraction and purification of extracts, as well as glycine and glycolate determinations, were done as described previously (4). Glycolate oxidase activity was assayed in 20 mg of ground leaf disc tissue as described by Hitz (7). To measure Chl, approximately 2% of the freeze dried leaf disc powder obtained during the extraction procedure was saved and extracted with 80% acetone, and A652 was measured according to Arnon (1).

RESULTS

The total pool of glycine in corn leaf discs during steady state photosynthesis in 21% O2 [1.1 μmol (mg Chl)−1; Fig. 1] was 3 to 5 times larger than the total pool in leaf discs of the C3 plant soybean [0.4 μmol (mg Chl)−1; Ref. 4]. The total glycine pool in corn was sensitive to O2 concentration (Fig. 1), although O2 had little effect on the net photosynthetic rates (Fig. 2) and dark respiration rates (data not shown). The glycine pool was smallest in 1% O2 and largest in 50% O2. This O2 dependence suggested that flux of carbon through glycine was due to flux of carbon through the glycolate pathway.

The total pool of glycinate was also a function of O2 concentration (Fig. 3). HBA is an irreversible inhibitor of glycolate oxidase (9), and after incubation of tissue with HBA, carbon accumulates in the pathway intermediate glycinate both in C3 species (e.g. see 9) and in a C4 species (8). In this research we
used the methyl ester of HBA (MeHBA), and we observed 90% inhibition of glycolate oxidase activity in leaf discs incubated for 60 min in MeHBA in the light (data not shown). As expected, the glycolate pool increased dramatically in these leaf discs (Fig. 3). Increased pool size was observed at all O_2 concentrations because preincubation with MeHBA took place in 21% O_2 in the light. More importantly, in the presence of MeHBA the glycolate pool size was still sensitive to O_2 concentration, and the largest glycolate pool was observed in 50% O_2. In contrast, the O_2 sensitivity of the total glycine pool was eliminated (Fig. 1), indicating that the differences observed in the total pool levels at different O_2 concentrations were indeed due to differential carbon flow in the glycolate pathway.

MeHBA did have a pronounced effect on net CO_2 exchange at all O_2 concentrations (Fig. 2). Inhibition of photosynthesis by HBA and its various esters has been reported in most research using this compound (e.g. see 16).

The total glycine pool was also affected by darkness (Fig. 4). In 21% O_2 after a 60-min dark period, the glycine pool was approximately 30% of the light level. The most rapid decrease occurred during the first 4 min of darkness, at which time the pool was approximately 85% of the light level. This decay rate was much slower than that observed in soybean leaf discs in which the total pool was approximately 63% of the light level after 15 s (6). A decrease in the glycine pool is expected if glycine is synthesized by the glycolate pathway, and flow into this pathway ceases when darkness stops the production of the precursor RuBP.

The rate of glycine loss during the first 4 min in darkness should indicate a minimum rate of synthesis required to maintain the glycine pool during steady state photosynthesis. The rate of glycine loss gives an estimate of 2.4 μmol (mg Chl h)^{-1} for the minimum rate of glycine synthesis in the glycolate pathway. This rate would correspond to a photosynthetic CO_2 evolution of 1.2 μmol (mg Chl h)^{-1} or a rate of carbon flow through glycine of 4.8 μg atoms (mg Chl)^{-1}. With an average net photosynthetic rate in 21% O_2 of 77 μmol CO_2 (mg Chl h)^{-1} (Fig. 2), photosynthesis obviously reflects a very small percentage of the total carbon flow in corn.

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**Fig. 1.** Total glycine pool in the presence (●) or absence (○) of MeHBA after 20 min of photosynthesis in 1%, 21%, or 50% O_2.

**Fig. 2.** Net CO_2 exchange rate in the presence (●) or absence (○) of MeHBA after 20 min of photosynthesis in 1%, 21%, or 50% O_2.

**Fig. 3.** Total glycolate pool in the presence (●) or absence (○) of MeHBA after 20 min of photosynthesis in 1%, 21%, or 50% O_2.

**Fig. 4.** Decrease in total glycine pool after 4, 20, or 60 min in 21% O_2 in darkness (●) or 1% O_2 in the light (○), after steady state photosynthesis in 21% O_2.
A similar pattern in the decay of the total glycine pool was observed when the O₂ concentration was changed from 21% O₂ to 1% O₂ after steady state photosynthesis in 21% O₂ (Fig. 4). After 4 min of photosynthesis in 1% O₂, the glycine pool was approximately 80% of the level in 21% O₂; after 60 min in 1% O₂, the pool was 34% of the 21% O₂ level. Again, the glycine pool would be expected to decrease if flow into the glycolate pathway had been stopped by suppression of the oxygenase activity of Rubisco.

**DISCUSSION**

Our results clearly show that a relatively large pool of glycine is present in photosynthesizing corn leaf disc and that a major portion of this function as an intermediate in the photosynthetic glycolate pathway. This conclusion is based on the observation that glycine levels are O₂ dependent, decreased by MeHBA, and decreased when leaf discs are darkened or when the O₂ concentration is changed from 21% to 1% in the light. Thus, there is some flow of carbon through the photosynthetic pathway even though no photosynthetic CO₂ evolution can be measured in C₄ plants. Flow of carbon into the glycolate pathway in corn has also been suggested by labeling studies with ¹⁴CO₂ that show O₂-sensitive incorporation of label into glycine (12, 13).

The inhibition of net photosynthesis by MeHBA supports the contention (see 4 for references) that once P-glycophosphate is synthesized it must be metabolized through the complete photosynthetic pathway to avoid detrimental effects on photosynthesis. The fact that MeHBA inhibited net photosynthesis at 1% O₂ suggests that the MeHBA had effects in addition to inhibiting glycine oxidase. The observations that glycine accumulated in the presence of MeHBA and that glycine was at its dark level support the interpretation that glycine was derived from glycine in corn leaf disc.

Catabolism of glycine occurs in the dark (Fig. 4; Ref. 15). Similar decay patterns of the total glycine pool in 21% O₂ in darkness, and in 1% O₂ in the light after decreasing the O₂ concentration from 21%, suggest that glycine catabolism is a similar process in the light and in the dark. The decay occurs at an increasingly slower rate and is at times much slower than the rate of decay seen in the C₃ plant soybean (6). The slower rate is consistent with the lower in vitro activity of glycinate pathway enzymes from C₄ plants observed by Osmond and Harris (14). In addition, C₄ leaf slices catabolize exogenous glycine in the dark at slower rates than C₃ leaf slices (15).

The initial rapid decrease in the total glycine pool in the dark is due to the cessation of synthesis, and it should reflect a minimum rate of synthesis necessary to maintain the steady state level of glycine during steady state photosynthesis. A rate calculated on this basis indicates that carbon flow into the glycolate pathway is no more than 3% of the carbon flow in photosynthesis; thus, the photosynthetic glycine pool is turning over slowly. This is a much lower rate than any calculated for a C₃ plant (3). The conclusion that carbon flows more slowly in the glycolate pathway in C₄ than in C₃ plants is supported by labeling studies done with corn (12) and sunflower, a C₃ plant (11). The glycine pool in corn was labeled more slowly and to a lesser extent than in sunflower, but glycine labeling was O₂ dependent in both species. After photosynthesis in ¹³CO₂, radioactivity decreased in glycine during a subsequent chase period in ¹³CO₂ much faster in sunflower than in corn (12, 13). Label chases out of PGA at similar rates for the two kinds of plants.

It is obvious that carbon flow into the glycolate pathway does occur in corn; it is equally obvious that the flow is very slow relative to flow in C₃ species, and this could account for the observed differences in labeling patterns. There is little difference in the O₂ sensitivity of Rubisco isolated from soybean and from the bundle sheath cells of corn (2), suggesting that the slower rate of activity of the glycolate pathway must be due to the CO₂ concentrating mechanism described by Hatch and Osmond (5).

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**LITERATURE CITED**


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