December, 1979

Mechanism of Decarboxylation of Glycine and Glycolate by Isolated Soybean Cells

David J. Oliver

Available at: https://works.bepress.com/david_oliver/26/
Mechanism of Decarboxylation of Glycine and Glycolate by Isolated Soybean Cells

DAVID J. OLIVER

Department of Biochemistry, The Connecticut Agricultural Experiment Station, Box 1106, New Haven, Connecticut 06504

ABSTRACT

Isolated soybean leaf mesophyll cells decarboxylated exogenously added [1,14C]glycine and [1-14C]glycolate in the dark. The rate of CO2 release from glycine was inhibited over 50% by isonicotinic acid hydrazide and about 80% by KCN, two inhibitors of the glycine to serine plus CO2 reaction. The release of CO2 from glycinate was inhibited by less than 50% under the same conditions. This indicates that about 50% of the CO2 released from glycinate occurred at a site other than the glycine to serine reaction. The sensitivity of this alternative site of CO2 release to an inhibitor of glycinate oxidase (methyl-2-hydroxy-3-butynoate) but not an inhibitor of the glutamate-glyoxylate aminotransferase (2,3-epoxypropionate) indicates that this alternative (isonicotinic acid hydrazide insensitive) site of CO2 release involved glycine. Catalase inhibited this CO2 release. Under the conditions used it is suggested that about half of the CO2 released from glycinate occurred at the conversion of glycine to serine plus CO2 while the remaining half of the CO2 loss resulted from the direct oxidation of glycine by H2O2.

The rate of glycine decarboxylation by the glycine to serine reaction was apparently controlled by the amount of NAD in the mitochondria. Mitochondrial electron transport inhibitors, KCN and actinomycin A, inhibited glycine decarboxylation while an uncoupler, 2,4-dinitrophenol, stimulated the reaction. Competition within the mitochondria between the enzymes of dark respiration and glycine decarboxylation for limiting NAD may force substantial amounts of the glycinate formed to be decarboxylated by the direct oxidation of glycine.

In plant species where the primary photosynthetic carboxylation reaction involves the formation of P-glycerate catalyzed by ribulose bisP carboxylase, glycinate is synthesized in the light at rates which are approximately equal, on a molar basis, to the net rates of photosynthetic CO2 fixation (7, 14, 16). The rate of glycinate synthesis can be controlled by the atmospheric concentrations of CO2 and O2 (8, 12) and the intracellular concentration of certain key metabolites including aspartate, glutamate, and glycylate (6, 7, 9, 10).

Glycinate is usually assumed to be metabolized by the pathway outlined by Tolbert (13). In this reaction sequence (Fig. 1), glycinate is oxidized by the enzyme glycinate oxidase to glyoxylate. This reaction in leaves is coupled to the reduction of O2 to H2O2. Glyoxylate is converted to glycine by either a glutamate- or serine-dependent glyoxylate transaminase. In the mitochondria several reactions combine to convert 2 mol of glycine to 1 mol each of serine, CO2, and NH3 (1, 5). In isolated mitochondria there is an absolute requirement of NAD for this conversion to occur. The resulting NADH is oxidized in mitochondrial preparations by the oxidative electron transport chain with the concurrent synthesis of 3 mol of ATP for each atom of oxygen taken up (1, 5). The serine thus formed is deaminated to hydroxypyruvate, which is in turn converted to P-glycerate and then glycine.

In addition to being transaminated to glycine, glyoxylate may be further oxidized directly. H2O2 produced either from the glycolate oxidase reaction (2, 3, 15) or in the light by a Mehler reaction within the chloroplast (17) can be the oxidant (Fig. 1). Such a reaction may or may not be enzymatic. The carboxyl carbon from glycolate is released as CO2. The C1 fragment from the α-carbon forms formic acid from the reaction with H2O2. Grodzinski and Grodzinski (3) have suggested that this C1 unit may supply a substrate for the hydroxymethyltransferase and react with glycine to form serine. The α-carbon could also be oxidized completely to CO2 and contribute to the photosynthetic CO2 loss.

This paper presents a series of experiments which have taken advantage of the known inhibitors of the reactions in the glycinate pathway to attempt to evaluate if two sites of CO2 release do exist in the glycinate pathway, and the relative contribution of each site. The inhibitors used were MHB, a suicide analog of glycine that irreversibly inhibits glycinate oxidase (4); INH, an inhibitor of the glycine to serine plus CO2 reaction (11); 2,3-epoxypropionate also known as glycinate, which inhibits glutamate glyoxylate aminotransferase (6), and catalase, an enzyme which decreases glyoxylate oxidation by destroying H2O2.

The existence of multiple sites of CO2 release in the glycinate pathway is clearly demonstrated. It is also proposed that the competition between dark respiration and glycine decarboxylation for NAD in the mitochondria may provide possibilities for directing the choice of the substrate for photosynthetic CO2 between glycine and glycolate.

MATERIALS AND METHODS

[1-14C]Glycinate and [1-14C]glycolate were obtained from American Corp. The glycinate was purified by high voltage paper electrophoresis and the glycolate by Dowex 1-acetate chromatography (9, 10) before use. Thymol-free catalase (Sigma) was used. The MHB was a generous gift of Dr. P. J. Jewess, and the glycinate from Dr. J. Zelitch.

Soybean mesophyll cells were isolated by a mechanical grinding technique from mature leaf tissue (8). The cells had an average photosynthetic capacity of about 200 μmol CO2 fixed/mg Chl·h (35°C, 50 μM NaHCO3, 1.400 μE/m²·s PAR and were contaminated by very few isolated chloroplasts.

The decarboxylation reactions were carried out in 10-ml Warburg flasks with centerwell containing a filter paper wick moistened with 25 μl of 5% monothanolamine. The reaction mixture

1 Current address: Department of Bacteriology and Biochemistry, University of Idaho, Moscow, Idaho 83843.

2 Abbreviations: MHB: methyl d,l-2-hydroxy-3-butynoate; INH: isonicotinic acid hydrazide; DNP: 2,4-dinitrophenol.
contained a final concentration of 0.33 mM sorbitol, 50 mM Tris-HCl (pH 7.8), 2 mM NaNO3, 2 mM EDTA, 1 mM MnCl2, 1 mM MgCl2, 0.5 mM K-phosphate, and 2 mM DTT (8). After the flasks containing the reaction mixture and any added inhibitors were equilibrated in a 25°C shaking water bath for 2 to 5 min, 50 μl of the isolated cell preparation containing 20 to 30 μg Chl was added and the water bath was covered to exclude all light. After a dark preincubation period of 30 min, 100 μl of the radioactive substrate was carefully pipetted into the reaction chamber and the flask sealed with a plastic stopper. After a 30-min dark reaction period, 100 μl of 3 N H2SO4 was added to the reaction chamber to kill the cells and release all 14CO2 formed. Following an additional 30-min period, the filter paper wicks were removed and the 14CO2 trapped was determined by liquid scintillation counting. The toluene-based scintillation cocktail included 100 μl of protosol (Amersham Corp.) to facilitate release of the 14CO2 from the filter paper strips. All values were corrected for the amount of 14CO2 released from the substrates in the absence of cells.

When the amount of [14C]glycine and [14C]glyoxylate produced was to be determined, the acidified reaction mixture was added to 2 ml of ethanol and placed in a boiling water bath for 5 min. The amino acid fraction was isolated from a Dowex 50 column after the acidic compounds were first removed by passage through a Dowex 1-acetate column (10). Glycine was then isolated by high voltage paper electrophoresis and the amount of radiocarbon determined by liquid scintillation counting (6).

RESULTS AND DISCUSSION

Isolated soybean mesophyll cells in the dark released 14CO2 from [1-14C]glycine or [1-14C]glyoxylate. Rates of CO2 release were linear for at least 60 min. Because the reactions were carried out at pH 7.8 (with over 99% of either substrate present as the anion) glycine and glyoxylate decarboxylation was limited by the amount of uptake. The rate of CO2 release from either substrate was proportional to the substrate concentration up to 0.5 mM and not saturated by 1.0 mM. Rates of CO2 release were up to 40 μmol/mg Chl·h at these very high substrate concentrations but averaged much lower at the concentrations (5–10 mM) reported here.

The amount of CO2 released from either glycine or glyoxylate was decreased by incubating the cells with INH, an inhibitor of the glycine to serine plus CO2 reaction, for 30 min before adding the substrate (Fig. 2). INH inhibited the rate of CO2 release from glycine by over 90%, and that from glyoxylate by less than 50% (Fig. 2). The differences in sensitivity of these two reactions to INH inhibition indicate that some CO2 can be released from glycolate at an alternative site which does not involve the conversion of glycine to serine plus CO2. Since only about half of the CO2 released from glycolate was sensitive to INH, under conditions where glycine to serine plus CO2 conversion was blocked, about half of the CO2 released probably arose from this alternative site.

To corroborate the conclusions from results with INH another inhibitor of the glycine to serine plus CO2 reaction, KCN, was employed. Cyanide would be expected to inhibit glycine oxidation by preventing the oxidation of NADH to NAD, an essential cofactor for the reaction (1, 5). Like INH, KCN inhibited CO2 release from both glycine and glyoxylate (Fig. 3). The oxidation of glycine to CO2 was inhibited about 75% while the metabolism of glyoxylate to CO2 was only inhibited about 25%. This again supports the existence of a substantial source of CO2 release from glycolate which is not related to the conversion of glycine to serine plus CO2. Identical results were found when another inhibitor of oxidative electron transport, antimycin A was employed (unpublished observation).

Similar results were obtained when increasing concentrations of
unlabeled glycine were added to cells which were metabolizing [14C]glycolate. As the concentration of unlabeled glycine supplied to the cells increased, the specific radioactivity of the glycine pools derived from intracellular [14C]glycolate would be expected to decrease, thus decreasing the amount of 14CO2 released. Figure 4 shows that at unlabeled glycine concentrations that are 20 to 50 times larger than the concentration of [14C]glycolate provided, the rate of 14CO2 released was decreased by only 40%. Since over half of the 14CO2 formed from [14C]glycolate was not susceptible to dilution by unlabeled glycine, the results suggest that this CO2 was released in the reaction sequence (Fig. 1) prior to glycine formation. The substrate for the alternative site of CO2 release would be glycolate or glyoxylate.

Glycidate, an inhibitor of glutamate-glyoxylate aminotransferase, inhibited CO2 release from glycolate by 40% while it decreased glycine formation from glycolate by 75% (Table I). The higher relative sensitivity of glycine synthesis as compared to CO2 release again supports the idea that a major source of CO2 release from glycolate occurs before glycine formation in the glycolate pathway (Fig. 1).

CO2 release from glycolate was highly sensitive (96% inhibited) to the glycolate oxidase inhibitor MHB. This shows that glycogonate must be oxidized to glyoxylate before any CO2 is released. The source of this alternative (INH-insensitive) site of CO2 release apparently was from glyoxylate since CO2 release from glycolate was sensitive to MHB but not glycinate (Fig. 1). Further evidence on this point comes from the finding that this alternative site of CO2 release was inhibited by catalase. As mentioned in the introduction, H2O2, possibly produced by the glycylate oxidase reaction, reacts readily with glyoxylate to produce CO2. When excess catalase was supplied to the assay media around the cells, H2O2 was destroyed and thereby the direct oxidation of glyoxylate was prevented (Table I). Catalase decreased the rate of CO2 release from glycolate by about 50%. Catalase probably did not enter the cells. H2O2 is readily diffusible, however, and can be detected in the solution around illuminated leaf tissue. The reduction of H2O2 most likely occurred outside the cells.

The addition of catalase and the resultant inhibition of the direct oxidation of glyoxylate resulted in increased glycine formation from glycolate (Table I). Similar increases in the size of the glycine pool were found when the glycine to serine plus CO2 reaction was blocked with INH. The addition of saturating amounts of both catalase and INH resulted in nearly complete inhibition of CO2 release from glycolate. The additive nature of these two inhibitors indicates that the compounds were effective at two different sites of CO2 release (Table I). This is consistent with the scheme in Figure 1.

None of the inhibitors used decreased the rate of glycolate uptake by the cells or, with the exception of MHB, the activity of glycine oxidase. Treatment with INH, KCN, and catalase did increase, however, the size of the glyoxylate pool by as much as 3-fold (data not shown).

When 25 mM [1-14C]glycolate was fed to INH-treated cells for 30 min under standard conditions 4.55 (se 0.18) pmol CO2/mg Chl was produced and 10.07 (se 0.28) pmol radioactive glycine and glyoxylate were present. If the glycine and glyoxylate pools were completely decarboxylated from glycine in the absence of INH, they should yield 5.04 pmol CO2/mg Chl. This again indicates that approximately equal amounts of CO2 were released by the glycine to serine plus CO2 reaction and by the direct oxidation of gly oxylate under these conditions. This suggests that for every 1 mol of glycolate that was oxidized to CO2 from glyoxylate, 2 mol of glycolate would be processed by the glycine to serine plus CO2 reaction.

The sensitivity of CO2 release from glycine and glycolate to cyanide (or antimycin A) suggests that under the conditions used

---


<table>
<thead>
<tr>
<th>Condition</th>
<th>14CO2 Released</th>
<th>Glycine Present after 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.31</td>
<td>0.410</td>
</tr>
<tr>
<td>+INH</td>
<td>1.30</td>
<td>1.054</td>
</tr>
<tr>
<td>+Catalase</td>
<td>1.60</td>
<td>1.033</td>
</tr>
<tr>
<td>+INH +catalase</td>
<td>0.32</td>
<td>1.303</td>
</tr>
<tr>
<td>+MHB</td>
<td>0.14</td>
<td>0.016</td>
</tr>
<tr>
<td>+glycinate</td>
<td>2.04</td>
<td>0.098</td>
</tr>
</tbody>
</table>

Soybean cells were incubated with either 10 mM INH, 1 mM MHB, 10 mM glycolate, or 100 μg catalase/ml as indicated for 30 min at 25 C. Glycolate (10 mM) was then added and the decarboxylation reaction allowed to proceed for 30 min before the reaction was stopped and any 14CO2 released by acidification.
From the methylene carbon per mol of glycolate metabolized, Grodzinski (3) suggested that this C₂ fragment may react with glycine to form serine or may be further oxidized to CO₂. Depending on which reaction predominated, 50 to 100% of the carbon in glycolate metabolized by this pathway would be released as CO₂. The stoichiometry of the glycine to serine plus CO₂ reaction releases as CO₂ 25% of the carbon from glycolate that transverses this step. The relative contributions of the two pathways of CO₂ release would control the percentage of the carbon in glycolate released as CO₂.

CO₂ released from the conversion of glycine to serine was probably tightly regulated by the amount of NAD in the mitochondria. When NADH oxidation, and presumably NAD availability, was decreased by KCN treatment, CO₂ release from glycine and glycolate was lessened (Fig. 3). Conversely, when NADH oxidation was increased by the uncoupler DNP, CO₂ release from both glycine and glycolate was stimulated.

Since there is a requirement within the mitochondria for NAD for both glycine decarboxylation and dark respiration (the Krebs tricarboxylic acid cycle), it is interesting to consider the possible interaction of these two pathways. The rate of CO₂ release by photorespiration is approximately three to five times the rate of CO₂ release by dark respiration in C₃ plants (16). The rate of oxidative phosphorylation and electron transport limit the availability of NAD within the mitochondria, as indicated by the ability of DNP to stimulate glycine decarboxylation in the dark (Fig. 5). If the amount of respiration (photorespiration and dark combined) in the light is much greater than the amount in the dark, then glycine decarboxylation and dark respiration must be in competition for sharply limiting supplies of NAD in the light.

Assuming that NADH oxidation and oxidative phosphorylation are tightly and invariably coupled and that no other major mechanisms of NADH oxidation exist in the mitochondria in the light, then the rates of glycine oxidation and dark respiration must be co-regulated through NAD. Rapid flux of metabolites through the tricarboxylic acid cycle should lessen glycine decarboxylation. If dark respiration proceeds at the same time as rapid rates of photorespiratory CO₂ loss, than a substantial portion of the CO₂ must be released by the direct oxidation of glyoxylate. Glycolate metabolized by this reaction releases two to four times as much carbon as CO₂ as does the decarboxylation of glycine. Thus, dark respiration may indirectly control photorespiration, not by regulating glycine synthesis but rather by altering the efficiency of glycolate decarboxylation.

Acknowledgments—The author wishes to thank Israel Zeitch, Arthur Lawyer, and Mary Berlym for helpful discussions and Marilyn Newman for growing the plants.

LITERATURE CITED

7. OLIVER DJ 1979 The interaction between O₂ and CO₂ concentrations on the regulation of glycolate synthesis in tobacco leaf discs. Planta 141: 35-40
15. ZELITCH I, S OCHOA 1953 Oxidation and reduction of glycolic and glyoxylic acid in plants. J Biol Chem 201: 707-718