Role of Glycine and Glyoxylate Decarboxylation in Photorespiratory CO2 Release

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ABSTRACT

Mechanically isolated soybean leaf cells metabolized added glycolate by two mechanisms, the direct oxidation of glycolate and the decarboxylation of glycine. The rate of glyoxylate decarboxylation was dependent on the cellular glycine concentration and was linear between 0.58 and 2.66 micromoles glycine per milligram chlorophyll. The rate extrapolated to zero at a concentration of zero. The concentration and, therefore, the rate of oxidation of glyoxylate could be decreased by adding glutamate or serine to the cells. These substrates were amino donors for the transamination of glycine to glycine. In the presence of these amino acids more CO₂ was released from added glycolate via the glycine decarboxylation reaction and less by the direct oxidation of glyoxylate.

Leaves from soybean plants of various ages grown under different nitrogen regimes had glycine concentrations of about 80 to 100 nanomoles per milligram chlorophyll. Using the isolated cells as a model to determine the relationships between the glycine concentration and rate of its decarboxylation indicated that about 2.5% of the photorespiratory CO₂ would arise from this reaction. This percentage would not be expected to vary greatly with growth conditions.

During photosynthetic carbon fixation in many species, carbon is drawn from intermediates in the Calvin cycle to make glycolate. Before the carbon can reenter the cycle it must be processed through the glycolate pathway. During this processing, some part of the carbon is lost as CO₂ (18, 20). The major part of this photorespiratory CO₂ loss has been associated with the mitochondrial conversion of 2 mol of glycine to one each of serine, CO₂, and NH₃ (8, 18).

Several authors, however, have presented data which could be interpreted as suggesting that some CO₂ could result from the direct oxidation of glyoxylate. Zeilitch (21) showed that oxidants produced by illuminated chloroplasts were able to oxidize added glycolate to CO₂ and formate. In isolated peroxisomes, excess H₂O₂ produced by the glycolate oxidase reaction results in glyoxylate decarboxylation (2-4). Kinetic studies with intact leaves (1) and isolated cells (16) have suggested that the rate of glycine synthesis can readily exceed the rate of glycine synthesis, suggesting that carbon is lost (probably as CO₂) between glycine and glycine.

Inhibitor studies with mechanically isolated soybean leaf cells have shown conclusively that with these preparations added glycolate was metabolized by two mechanisms (11). The first mech-

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² Abbreviations: INH, Isonicotinic acid hydrazide; AAN, aminoaceto-nitrile.
the tip of the glass rod. The amount of NH₃ trapped was determined by the Nessler reaction (6).

Glycolate was determined colorimetrically by measuring the ferricyanide oxidized phenylhydrzone derivative (10). Leaves were plunged into liquid N₂ before detaching from plants. After the leaves were thoroughly ground, the still frozen powder was extracted with 5 ml of ether to remove Chl and denature proteins. After 1 h, 5 ml of 4 N phosphate buffer (pH 7.0) was added. Over 95% of added glycolate was found to partition into the aqueous phase.

RESULTS AND DISCUSSION

Substantial ¹⁴CO₂ was released from [¹⁻¹⁴C]glycolate under conditions where the release of ¹⁴CO₂ from [¹⁻¹⁴C]glycine was blocked. This resulted whether the glycine decarboxylation reaction was inhibited by INH (Fig. 1) or AAN, an alternate inhibitor of the reaction (19) (Table I). The sensitivity of ¹⁴CO₂ release from glycine to INH or AAN could, however, be completely restored by including 20 mM glutamate in the reaction mixture (Fig. 1 and Table I). Serine, the alternate substrate for the transamination reaction, could substitute for glutamate (Table I).

These data confirm that isolated soybean leaf cells, metabolizing added glycolate, release much of the photorespiratory CO₂ from the direct decarboxylation of glycolate. When amino donors were readily available, however, the glycolate was transaminated to glycine and glyoxylate decarboxylation was replaced by INH-sensitive glycine decarboxylation as the source of photorespiratory CO₂ loss.

The glycine decarboxylase reaction has a fixed stoichiometry of 2 mol of glycine yielding one each of CO₂, NH₃, and serine (18). The reassimilation of NH₃ was blocked by the glutamine synthase inhibitor, methionine sulfoximide (6), and cells were darkened to prevent the photosynthetic re fixation of the photorespiratory CO₂. Under these conditions, the metabolism of added glycine yielded equal rates of CO₂ and NH₃ release (Table II). The addition of glutamate did not alter this ratio. When soybean leaf cells metabolized glycolate, the rate of CO₂ release was 4 times the rate of NH₃ release. This provides additional proof that under these conditions, large amounts of CO₂ were being released from the direct oxidation of glycolate.

The addition of 20 mM glutamate to cells metabolizing added glycolate stimulated the rate of CO₂ release from 5.4 to 9.3 μmol/mg Chl-h. Ammonia release was increased from 1.3 to 8.0 μmol/mg Chl-h (Table II). Glutamate addition decreased the ratio of CO₂ release to NH₃ release from 4.15 to 1.15. In this latter case, the site of photorespiratory CO₂ release was shifted from the direct decarboxylation of glycolate to the glycine decarboxylation reaction by the addition of the substrate for the transaminase reaction, glutamate.

Glycine and serine stimulated CO₂ release from glycine in cells that were not preincubated with INH (Fig. 2). The increase in CO₂ loss was accompanied by a decrease in the tissue levels of glycolate, presumably because of its transamination to glycine (Fig. 2). If, as indicated, the movement of carbon from glycolate to glycine increases the rate of CO₂ release, then the propensity for photorespiratory CO₂ release from glycine must be greater than from glycolate.

The kinetics for the stimulation of CO₂ release from glycine by the two amino acids were different. Glutamate increased the decarboxylation rate from 1.28 to 5.39 μmol/mg Chl-h. Serine stimulated CO₂ release from a control value of 1.28 to a maximum of 3.87 μmol/mg Chl-h. Concentrations of serine above 10 mM showed a small but reproducible decrease from the maximum rate of CO₂ release (Fig. 2).

The increase in CO₂ release was half maximal at about 1.5 mM for serine and 4 to 6 mM for glutamate. The KM for glutamate of the glutamate:glyoxylate aminotransferase has been reported to be between 3.6 mM (7) and 5.7 mM (9). The KM for serine of the serine:glyoxylate aminotransferase has been reported as 1.5 mM (9). All of these values were for the tobacco enzymes.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Amino Donor</th>
<th>Conc AAN</th>
<th>Rate of ¹⁴CO₂ Release</th>
<th>μmol/mg Chl-h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>Serine</td>
<td>0</td>
<td>3.74</td>
<td>3.58</td>
</tr>
<tr>
<td></td>
<td>Glutamate</td>
<td>0</td>
<td>4.38</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>Glycolate</td>
<td>Serine</td>
<td>30</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Glutamate</td>
<td>30</td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>Glycolate</td>
<td>Serine</td>
<td>0</td>
<td>1.60</td>
<td>4.94</td>
</tr>
<tr>
<td></td>
<td>Glutamate</td>
<td>0</td>
<td>8.03</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td></td>
<td>1.09</td>
</tr>
</tbody>
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Table II. Effect of Glutamate on the Rate of CO₂ and NH₃ Release from Glycolate and Glycine by Soybean Leaf Cells

Cells were preincubated in the dark with 5 mM methionine sulfoximide for 15 min before the addition of 10 mM [¹⁻¹⁴C]glycine, 10 mM [¹⁻¹⁴C]glycolate, and 20 mM glutamate as indicated. After 30 min, the cells were killed by adding acid, and ¹⁴CO₂ release and NH₃ formation were measured.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Rate of Release</th>
<th>CO₂</th>
<th>NH₃</th>
<th>CO₂/NH₃</th>
<th>μmol/mg Chl-h</th>
<th>ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.8</td>
<td>11.0</td>
</tr>
<tr>
<td>Glycine + glutamate</td>
<td>12.5</td>
<td>12.7</td>
<td>0.98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycolate</td>
<td></td>
<td>5.4</td>
<td>1.3</td>
<td>4.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycolate + glutamate</td>
<td>9.2</td>
<td>8.0</td>
<td>1.15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CO₂ release from added glycolate was also measured in cells where the glycine decarboxylase reaction was inhibited by INH treatment. As the concentration of added glutamate or serine was increased from 0 to 20 mM, the rate of CO₂ release decreased from 1.41 to 0.64 µmol/mg Chl·h for glutamate and 0.50 µmol/mg Chl·h for serine (Fig. 3A). The simultaneous addition of 20 mM glutamate and 10 mM serine decreased the rate of INH-insensitive CO₂ release to 0.32 µmol/mg Chl·h.

The concentration of glyoxylate in the tissue closely paralleled the rate of CO₂ release from glycolate in the presence of INH. The glyoxylate concentration fell from 2.66 µmol/mg Chl in untreated cells to 1.17 and 1.43 µmol/mg Chl in the presence of 20 mM glutamate and serine, respectively. When both amino donors were added (20 mM glutamate and 10 mM serine) the glyoxylate concentration decreased to 0.58 µmol/mg Chl. Half maximal decrease in the glyoxylate pools occurred at 5 mM glutamate and 2 mM serine. These values agree with those determined from Figure 2. Illuminating the cells did not significantly decrease the steady state glyoxylate concentration or the rate of INH-insensitive CO₂ release (data not shown).

When the data from Figure 3A were replotted as the rate of CO₂ release against the cellular glyoxylate concentration, a straight line resulted (Fig. 3B). The only points which do not fall on the line are the values obtained at the three highest serine concentrations. As noted above (Fig. 2), serine concentrations above 10 mM may be inhibitory because they decrease the maximum rate of glyoxylate decarboxylation. The line generated by comparing glyoxylate concentration and CO₂ release extrapolates to an INH-insensitive glyoxylate decarboxylation rate of zero at a glyoxylate concentration of zero.

Soybean leaves were grown in sterile sand and were watered weekly with solutions containing either 0, 0.1, or 1.0 mM KNO₃ for the low, medium, and high N treatments, respectively.

### Table III. Effect of Nitrogen Deficiency on the Level of Glyoxylate in Intact Soybean Leaves

<table>
<thead>
<tr>
<th>Nitrogen Level</th>
<th>Glyoxylate Concentration (nmol/mg Chl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>82 ± 6</td>
</tr>
<tr>
<td>Medium</td>
<td>90 ± 4</td>
</tr>
<tr>
<td>High</td>
<td>85 ± 8</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Isolated soybean leaf cells decarboxylated added glycolate by two mechanisms, the glycine decarboxylase reaction and the direct oxidation of glyoxylate. Adding the amino donors serine and glutamate greatly decreased the rate of glyoxylate decarboxylation. This decrease was matched by a parallel decrease in the cellular glyoxylate concentration, and an increase in the rate of glycine decarboxylation. This suggests that the direct oxidation of glyoxylate was not limited by the availability of H₂O₂ but by the rapid transamination of glyoxylate to glycine.

The reason for the apparent amino donor deficiency in the isolated leaf cells is not immediately apparent. Several lines of study have suggested that the outer membranes of these cells are leaky (unpublished results). The cells were isolated from rapidly
growing plants that were supplied with sufficient nitrogen.

A reasonable estimation of the rate of glyoxylate decarboxylation in intact soybean leaves can be made by using an average foliar glyoxylate concentration of 100 nmol/mg Chl and the data presented in Figure 3B. This figure shows that soybean cells with a glyoxylate concentration of 100 nmol/mg Chl decarboxylate glyoxylate at about 5% the rate of control cells. From the data in Figure 1 and (11) it can be estimated that about 50% of the CO₂ lost from glycolate metabolism with untreated soybean cells comes from the direct decarboxylation of glyoxylate. Taking 5% of 50% would indicate that roughly 2.5% of the photorespiratory CO₂ might arise from glyoxylate decarboxylation.

There are, of course, many possible sources of error in this estimate. What is apparent from the data presented in Figure 3B is that there is a direct proportionality between the glyoxylate concentration in the tissue and the rate of glyoxylate decarboxylation. As long as glyoxylate is present in the tissues, some portion of it will be decarboxylated. The amount of glyoxylate decarboxylation that occurs in normal tissue, however, appears to be small when compared to the rate of photorespiratory CO₂ loss from glycine decarboxylation. It also does not appear to change with changes in the age and nutrient status of the plant.

LITERATURE CITED