The Effect of Glyoxylate on Photosynthesis and Photorespiration by Isolated Soybean Mesophyll Cells

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ABSTRACT

Incubating isolated soybean leaf mesophyll cells with glyoxylate increased the rates of CO₂ fixation by as much as 150%. In order to cause this stimulation, the glyoxylate must be presented to the cells before the NaHCO₃. Significant stimulation was observed 15 seconds after beginning the glyoxylate treatment. The glyoxylate-dependent stimulation was increased by high O₂ concentrations and decreased by high CO₂ concentrations. Glyoxylate treatment resulted in a 71% inhibition in the rate of CO₂ incorporation into glycine and glycine. Glyoxylate may be stimulating net photosynthesis solely by decreasing photorespiration or it may be increasing the amount of CO₂ fixed by both decreasing photorespiration and increasing gross photosynthesis. Ribulose bisphosphate carboxylase, when preactivated and assayed in situ, was unaffected by the glyoxylate treatment.

In all plants where the primary carboxylation is catalyzed by RuBP carboxylase, large amounts of glycolic acid are synthesized in the light. As this glycolate is metabolized through the glycolate pathway, 25–100% of the carbon contained in the glycolate is lost as CO₂ by the process of photorespiration (12). Could this carbon be conserved, large increases in dry matter accumulation should be possible (25). Blocking the metabolism of glycolate may cause transient increases in net photosynthesis (16, 23) but soon results in the inhibition of photosynthesis (9, 18, 19).

A more promising approach is to alter the rate of glycolate synthesis and thereby decrease photorespiration. Photorespiration is regulated by O₂ and CO₂ concentrations in the atmosphere. At atmospheric CO₂ concentrations, glycolate synthesis increased with increasing O₂ concentrations up to about 60% O₂. At 21% O₂, glycolate synthesis increased as the CO₂ level was raised from zero to approximately atmospheric levels and decreased as the CO₂ level was elevated further (11). Reducing glycolate synthesis by lowering the partial pressure of O₂ or inhibiting glycolate synthesis and stimulating gross photosynthesis by increasing the CO₂ concentration results in an increased rate of dry matter accumulation in C₃ plants (3, 25).

Glycolate synthesis, and thereby photorespiration in tobacco, can be altered by changing the intracellular concentration of certain key metabolites including glutamate, aspartate (15), and glyoxylate (10, 11, 14). Glyoxylate treatment also inhibits photorespiration in isolated spinach cells (5). In this paper, glyoxylate is shown to stimulate net photosynthetic CO₂ fixation by isolated soybean leaf mesophyll cells. The stimulation is affected by the concentration of O₂ in solution and decreased by high CO₂ concentrations. Addition of glyoxylate also results in an inhibition of glycolate and glycine synthesis and a reduction in the O₂ sensitivity of net photosynthesis.

MATERIALS AND METHODS

Mesophyll cells were isolated from 6- to 8-week-old soybean leaves by a mechanical grinding technique (13). Detailed analyses of the activities of isolated soybean leaf cells have been presented (13, 20). Photosynthesis was assayed in stoppered 10-ml Fernbach flasks in a reaction mixture that contained 0.3 mM sorbitol, 50 mM Tris-HCl (pH 7.8), 2 mM NaNO₃, 2 mM EDTA, 1 mM MnCl₂, 1 mM MgCl₂, 0.5 mM K₂HPO₄, and 2 mM DTT (added fresh daily). The isolated soybean leaf cells, containing 5–30 μg Chl and suspended in the same solution, were generally added to previously equilibrated flasks in a 25 C waterbath and illuminated for 15 s to 20 min in the absence of added NaHCO₃. Next the NaH¹⁴CO₃ was added and the illumination continued for an additional 5 min before the reaction was stopped by adding 0.1 ml 3 N HCl. The amount of acid-stable radioactivity was determined by liquid scintillation counting of 0.1- or 0.2-ml aliquots of the acidified reaction mixture.

All reactions were in equilibrium with air unless otherwise indicated. In some experiments the reaction vessels were equilibrated with CO₂-free gases by blowing the gas vigorously against the liquid surface for 10 min before the cells were added. The activity of the RuBP carboxylase in the cells was assayed by first making the membrane more permeable with toluene (7) and then adding the carboxylation substrates RuBP and NaH¹⁴CO₃. The cells were preilluminated and assayed in the same solution that was used in the photosynthetic studies except that the MgCl₂ concentration was increased to 25 mM (1). After the cells were illuminated at 25 C for 5 min, 25-μl samples of the cell mixture were transferred to the reaction flasks. Thirty s later the reaction was terminated by adding 0.1 ml 3 N HCl. The reaction flasks contained 50 μl of a 4:1 (v/v) ethanol to toluene solution, 0.35 mM RuBP, the indicated amount of NaH¹⁴CO₃, (2–4 μCi/μmol), and the assay solution above with a final volume of 1.0 ml.

Over 97% of the CO₂ fixed was dependent on the addition of RuBP and the rate of CO₂ fixation was constant for 50 s. After that time, the rate of fixation dropped off substantially. When glyoxylate was included in the reaction, 50 μg of catalase was added to destroy any H₂O₂ produced by the toluene-disrupted cells.

RESULTS AND DISCUSSION

Glyoxylate treatment greatly stimulated the rate of net photosynthetic CO₂ fixation by isolated soybean mesophyll cells. Gly-
Glyoxylate treatment did not increase the rate of dark CO₂ fixation (unpublished results). The photosynthetic rate increased in a sigmoidal fashion with increasing glyoxylate concentration to a maximum at 40–50 mM (Fig. 1). In the experiment shown, increasing the glyoxylate concentration in the media surrounding the cells from 0 to 20 mM stimulated CO₂ fixation 5.2 µmol/mg Chl-h (from 32.5 to 37.7 µmol/mg Chl-h). Increasing the glyoxylate concentration an additional 20 mM from 20 to 40 mM resulted in an additional increase of 22.6 µmol/mg Chl-h (from 37.7 to 60.3 µmol/mg Chl-h). High glyoxylate concentrations were probably needed for maximum stimulation because of the limited permeability of the anion at the pH (7.8) of the assay. At concentrations above 50 mM, glyoxylate inhibited CO₂ fixation.

In order for glyoxylate to stimulate net CO₂ fixation, it must have been presented to the cells before the ¹⁴CO₂. When the glyoxylate was added either together with or after the CO₂, photosynthesis was not increased (Fig. 2). If the cells were treated with glyoxylate for as little as 15 s before the CO₂ was added, the rate of net CO₂ fixation was stimulated by the glyoxylate treatment (Fig. 2). As has been observed with soybean cells earlier (13) the rate of CO₂ fixation following a preillumination period increased with the time of the period up to about 5–10 min. If 30 mM glyoxylate was provided during that preillumination period, the rate of CO₂ fixation in the subsequent assay period was increased. The per cent stimulation caused by the glyoxylate treatment generally increased with the preillumination time up to about 10 min. With tobacco leaf discs, the stimulation continues for at least 3 h (14).

In order for glyoxylate to stimulate photosynthesis greatly in isolated soybean cells, the cells need not be illuminated during the period of glyoxylate treatment before CO₂ fixation was measured (Fig. 3). In this experiment, cells preincubated for 5 min with 40 mM glyoxylate in the light and the dark were stimulated 55 and 44%, respectively. Preincubation in the light increased the photosynthetic rate as noted above.

Glyoxylate markedly stimulated net CO₂ fixation only at CO₂ concentrations below saturation (Fig. 4). At a NaHCO₃ concentration of 0.2 mM in an atmosphere of 21% O₂/79% N₂, 30 mM glyoxylate stimulated CO₂ fixation 83% from 24.0 to 43.8 µmol/mg Chl-h. When the NaHCO₃ concentration was increased to 5
with 30 mM glyoxylate decreased the Warburg effect to 17% from 43.8 μmol/mg Chl·h in air to 51.2 μmol/mg Chl·h in N₂ (Fig. 4).

Glyoxylate treatment does not affect the maximal velocity of CO₂ fixation in air by isolated soybean cells, but does reduce the concentration of CO₂ needed for half-maximal activity. The limiting factor for CO₂ fixation at saturating CO₂ concentrations has not been altered by the glyoxylate treatment. These limitations were probably caused by electron transport or photophosphorylation (8). Glyoxylate increased the availability of CO₂ to the carboxylating reaction at limiting CO₂ concentrations or inhibited a respiratory process that occurred at low CO₂ concentrations but not at high.

The glyoxylate effect on net photosynthesis was blocked at saturating CO₂ concentrations and was dependent on high O₂ concentrations. These parameters of the glyoxylate effect are identical to those of photorespiration. To confirm the possibility that glyoxylate was affecting photosynthesis directly, the effect of glyoxylate on glycine synthase by soybean cells was investigated. The incorporation of radiocarbon into glycine from 14CO₂ was substantially decreased by the glyoxylate treatment (Table 1). This inhibition resulted when glycolate oxidase activity was inhibited by either MHB (6) or HPMS (23). The sulfonate experiments were run at pH 4.5 to allow penetration into the cells. Exposure of the cells to this low pH caused severe inhibition of photosynthesis. Glyoxylate treatment has been shown to inhibit glycine synthesis in tobacco leaf discs (10, 11, 14). Similarly, glycine synthesis was inhibited by glyoxylate treatment in cells where glycine decarboxylation was blocked by isonicotinic acid hydrazide (17) (Table 1).

Glyoxylate reacts rapidly with isonicotinic acid hydrazide in solution (I. Zelitch, personal communication). The inhibition of the glycine to serine plus CO₂ reaction was complete following the 30-min preincubation period and the inhibition was not reversed during the subsequent assay period (15). The reaction between glyoxylate and INH, therefore, can not explain the reduction in glycine accumulation caused by glyoxylate. The stimulation in net photosynthesis caused by glyoxylate treatment resulted, at least in part, from the decrease in the rate of photorespiration caused by reduced amounts of glycolate synthesis.

Glyoxylate had been shown earlier to have no effect on the rate of carboxylation or the O₂ sensitivity of CO₂ fixation by activated

<table>
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<th>Treatment</th>
<th>Rate of CO₂ Fixation</th>
<th>Rate of Glycolate Synthesis</th>
<th>Rate of Glycine Synthesis</th>
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<tr>
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<td>1.2</td>
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purified RuBP carboxylase. The soybean cell system allowed an analysis of the effect of glyoxylate on activated RuBP carboxylase in situ. Cells were preilluminated with or without glyoxylate for 5 min.

The plasmalemma and the outer chloroplast membrane were made permeable with toluene and RuBP and NaH\(^{14}\)CO\(_3\) were added. The residual photosynthetic rate after the toluene treatment was less than 3% of the untreated rate and cells in the dark supplied with NaHCO\(_3\) and RuBP fixed less than 2% as much CO\(_2\) without the toluene treatment as with it. The carboxylase was apparently fully activated by this treatment and routinely gave a \(K_m\) for NaHCO\(_3\) of 1.0–1.2 mm (Fig. 6). This gave a calculated \(K_m\) for CO\(_2\) of 20–26 \(\mu\)M which was essentially the same as the low \(K_m\) form of the carboxylase measured with isolated chloroplasts using a similar technique (1).

When the enzyme was assayed in this manner, glyoxylate had no effect on the affinity of the carboxylase for CO\(_2\) or O\(_2\) or on the rate of the carboxylation reaction. The apparent \(K_m\) for HCO\(_3\)- was 1.2 and 1.1 mm in the presence and absence of glyoxylate. The \(V_{max}\) was 90.0 \(\mu\)mol/mg Chl-h in the control and 87.0 \(\mu\)mol/mg Chl-h when glyoxylate was included during the preincubation and assay periods (Fig. 6).

Glyoxylate did not affect purified RuBP carboxylase (10) or the enzyme in situ. These results may not exclude the possibility that a metabolite of glyoxylate was affecting the carboxylase. Analysis of the products of [\(1-{\text{\textsuperscript{14}}}\)C]glyoxylate metabolism in the light for 60 s either with or without added NaHCO\(_3\) revealed no difference in the distribution of major metabolites (unpublished results). When the CO\(_2\) was excluded from the preincubation step, net CO\(_2\) fixation during a subsequent 2-min period in this experiment was 54% greater than when the glyoxylate effect was blocked by adding the NaHCO\(_3\) with the glyoxylate. Apparently no metabolite of glyoxylate was causing the glyoxylate effect.

**CONCLUSIONS**

Glyoxylate treatment increased net CO\(_2\) fixation in isolated soybean leaf mesophyll cells as it did in tobacco leaf discs (14). This stimulation was decreased at high CO\(_2\) concentrations and increased at high O\(_2\) concentrations. Glyoxylate treatment decreased the rate of glycolate and glycine synthesis and reduced the O\(_2\) sensitivity of net photosynthesis (the Warburg effect) by the cells. Together these facts provide strong evidence that, as with the tobacco leaf disc system (10, 11, 14), a portion of the stimulation of CO\(_2\) fixation observed resulted from the inhibition of photorespiration.

Glyoxylate treatment (Fig. 2) increased net photosynthesis in isolated soybean leaf cells by as much as 150% (2.5 times the control rate). Photosynthetic CO\(_2\) fixation in tobacco was stimulated over 125% (2.25 times the control value) by the glyoxylate treatment (14). Can these large increases in the CO\(_2\) fixation rate be ascribed solely to an inhibition of photorespiration? Similarly, was enough photorespiration occurring in N\(_2\) to account for the glyoxylate-dependent stimulations in photosynthesis observed (Fig. 4) if the only effect of glyoxylate was inhibition of glycolate synthesis?

Photorespiration has been measured to be at least 50% of net photosynthesis (24). All measurements of photorespiration based on CO\(_2\) exchange measurements underestimate the amount of photorespiratory CO\(_2\) release because they can not correct for the amount of CO\(_2\) that is photosynthetically fixed before being released from the leaf.

Measurements of the rate of glycolate synthesis indicate that glycolate is synthesized in these systems at rates which are approximately equal to or greater than net photosynthesis (11, 15, 21). These measurements also are underestimates in that they assume, for example, that the time between addition of the inhibitor and complete blockage of glycolate oxidation is negligible. Uncertainties in the mechanism of glycolate metabolism prevent precise analysis of the magnitude of photorespiration from estimates of glycolate synthesis, but 25% to 50% of the carbon in glycolate is probably lost under most conditions (12). Photorespiratory CO\(_2\) release, therefore, could range from a minimum of 50% to over 100% of net photosynthesis. Glyoxylate treatment might be able to cause the large increases in CO\(_2\) fixation observed in 21% O\(_2\) or 5 mM NaHCO\(_3\) by inhibiting photorespiration alone, but uncertainties in the measurements involved do not permit an unequivocal conclusion.

At subsaturating CO\(_2\) concentrations, glyoxylate stimulated CO\(_2\) fixation in N\(_2\) (Fig. 4) where very limited glycolate synthesis and photorespiration should occur. The glyoxylate-dependent stimulation in net photosynthesis measured in N\(_2\) and 0.5 mM NaHCO\(_3\) was 26% (from 81.5 to 102.5 \(\mu\)mol CO\(_2\) fixed/mg Chl-h).

Glyoxylate treatment may have had a dual effect on net photosynthetic CO\(_2\) fixation. The large effect was an inhibition of glycolate synthesis and photorespiration with a resulting increase in photosynthesis. In addition, glyoxylate may have stimulated gross photosynthesis. This could be the reason for the increase in CO\(_2\) fixation in N\(_2\). It is possible, however, that photorespiration in N\(_2\) was 26% of net photosynthesis and that glyoxylate inhibited this reaction.

Irrespective of whether glyoxylate treatment is just inhibiting photorespiration or both inhibiting photorespiration and stimulating gross photosynthesis, it is not increasing the total capacity for photosynthesis (the rate at saturating CO\(_2\)) but is decreasing the concentration of CO\(_2\) needed for half maximal activity.

Glyoxylate would cause the observed results if it facilitated the uptake of CO\(_2\) or bicarbonate into the chloroplast stromal compartment. By increasing the concentration of CO\(_2\) at the site of the carboxylating reaction, CO\(_2\) fixation at subsaturating CO\(_2\) concentrations would be increased. The elevated CO\(_2\) concentrations would also decrease glycolate synthesis and therefore photorespi-
glyoxylate metabolized by the chloroplast (22) and the large amounts of CO₂ fixed per glyoxylate metabolized (14), however, do not support this mechanism.

A detailed explanation of the mechanism by which glyoxylate inhibits glycolate synthesis and photorespiration and stimulates net photosynthesis must await the results of further study.

Recently, these results have been partially confirmed by Hunt and Ogren (4) who have repeated the glyoxylate-dependent stimulation in net photosynthesis and partial reduction in the Warburg effect with isolated soybean leaf cells. Baumann and Gunther (2) were able to show that the glyoxylate treatment strongly inhibited glycine and serine synthesis in isolated Chenopodium album cells. They were not, however, able to see any increase in net photosynthesis under the conditions employed.

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