Photorespiratory Ammonia Does Not Inhibit Photosynthesis in Glutamate Synthase Mutants of Arabidopsis

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ABSTRACT

Exposure of ferredoxin-dependent glutamate synthase (EC 1.4.7.1) mutants of Arabidopsis thaliana to photorespiratory conditions resulted in the accumulation of NH₄⁺ and the inhibition of photosynthesis. However, upon transfer from 2% O₂, 350 micromolars per liter CO₂ to 21% O₂, 350 micromolars per liter CO₂, net photosynthesis declined at a slower rate in methionine sulfoxime treated leaf discs relative to controls. The recovery of photosynthesis was also more rapid in MSO-treated leaf discs although ammonia levels were more than threefold higher. Photosynthesis in leaf discs treated with azaserine was inhibited more than controls when transferred to 21% O₂ and recovered less than controls when returned to 2% O₂ although NH₄⁺ levels were not significantly different. The results obtained are consistent with the view that the rapid inhibition of photosynthesis in the glutamate synthase mutants in photorespiratory conditions is not due to the accumulation of NH₄⁺ but rather to the depleion of amino donors for glyoxylate and the consequent effects of glyoxylate on the lack of return of carbon to the chloroplast.

The exposure of glutamate synthase mutants of Arabidopsis thaliana to normal air results in the accumulation of NH₄⁺ and glutamine and the inhibition of photosynthesis. The inhibition of photosynthesis in these mutants has been attributed to (a) a decline in the carbon skeletons to the Calvin cycle (1, 16, 18), (b) a triose phosphate limitation on photosynthesis (1), (c) a buildup of inhibitory metabolites such as glyoxylate (4) which may inactivate Rubisco (3), or (d) the accumulation of NH₄⁺ (9, 12) which may inhibit photosynthesis by coupling photophosphorylation (8).

Exposure of serine glyoxylate aminotransferase, glycine dehydrogenase or serine hydroxymethytransferase mutants of Arabidopsis to 50% O₂, 350 μL L⁻¹ CO₂ resulted in rapid inactivation of Rubisco (3). However, in GluS and dicarboxylate transport mutants (17)—both of which accumulate ammonia under photorespiratory conditions—Rubisco was only inactivated after prolonged exposure to photorespiratory conditions. Thus, the rapid decline in photosynthesis in these mutants cannot be attributed to a decline in Rubisco activity and may be directly related to the accumulation of ammonia.

In Arabidopsis, glutamine and glutamate make up more than 60% of the total soluble reduced N in the leaf and are the largest potential amino donors to the photosynthetic N cycle (12). However, the availability of glutamine as a source of amino donors to glyoxylate is limited by the low rates of NADH-GluS and Fd-GluS activity (12). Because MSO would inhibit the reassimilation of photorespiratory ammonia (14), the addition of MSO to the leaves of GluS mutants might be expected to result in a more rapid increase in ammonia levels and a more rapid inhibition of photosynthesis if photophosphorylation was uncoupled. However, by preventing the utilization of glutamate by GS, MSO might also increase the availability of glutamate as an amino donor to glyoxylate and thus help to maintain the flux of carbon through the photorespiratory pathway. This paper examines the effect of MSO and AZA, an inhibitor of glutamate synthase on the accumulation of ammonia and the inhibition of photosynthesis in the GluS mutants of Arabidopsis thaliana.

While this paper was in preparation, Grumbles (5) published a study which compared the photoresynthetic decline of glutamate synthase mutants to that in wild type plants which were fed with ammonia. Grumbles concluded that ammonia was not a factor in the inhibition of photosynthesis and suggested that the inhibition of photosynthesis was due to the inactivation of Rubisco. The results in this study demonstrate that neither ammonia accumulation nor the inactivation of Rubisco seem to be responsible for the inhibition of photosynthesis in these mutants. Rather, photosynthesis seems to be inhibited either because of the decrease in rate of carbon return to the Calvin cycle or from the inactivation of metabolic reactions by glyoxylate.

MATERIALS AND METHODS

Seeds of wild-type and GluS mutants (Cs113) of Arabidopsis thaliana (16) were kindly supplied by Dr. Chris Somerville (Michigan State University) and were grown as previously described (12). Leaf discs (6 mm) were cut from mature leaves with a cork borer and floated in 50 μM K₂HPO₃ (pH 7.2) or buffer containing 1 mM MSO or 1 mM AZA for 60 min. The discs were then placed abaxial side up on two thicknesses of wet cheesecloth which was stretched across the top of a Perpex cylinder (0.5 x 6 cm, diameter) in a gas exchange cuvette (9). Net photosynthesis was measured (after illumination) in an open gas exchange system (12) at a light intensity of 700 μE m⁻² s⁻¹ and 25 to 27°C. At the various sampling times the cuvette was covered with a dark cloth, the leaf discs were briefly removed from the cuvette in a darkened room.
RESULTS

Upon transfer to the light and 2% O2, photosynthesis in control and MSO-treated leaf discs continued to increase over a 30 min interval (Fig. 1). However, in AZA-treated leaf discs, photosynthesis was inhibited after 10 min and, after 30 min, the photosynthetic rate had declined to only 50% of that in the control and MSO-treated discs.

Following a change in gas composition from 2% O2 to 21% O2, photosynthesis declined rapidly to 54% of the original rate of photosynthesis in 2% O2 and continued to decline at a slightly slower rate so that after 5 min the photosynthetic rate in the control leaf discs was 32 ± 1.4% of the original rate in 2% O2. The rate of decline of photosynthesis in MSO-treated leaf discs was slower than in controls so that after 5 min of exposure to 21% O2, net photosynthesis had declined to only 43.6 ± 1.4% of the original rate in 2% O2. Exposure of AZA-treated leaf discs to 21% O2 resulted in a marked inhibition of photosynthesis which declined to 17 ± 2% of the maximum rate reached in 2% O2.

Upon return to 2% O2, photosynthesis in control leaf discs increased by 24% after 2 min to 56% and subsequently increased more slowly to reach 93% of the original rate at the end of a 20 min recovery period (Fig. 1; Table I). During the first 2 min of the recovery period in 2% O2, the photosynthetic rate of MSO-treated leaf discs increased 30% to 75% of the original rate and subsequently recovered to a maximum of 95% after 5 min in 2% O2 (Fig. 1; Table I). In contrast, photosynthesis in AZA-treated leaf discs recovered by only 12% during the first 2 min following a return to 2% O2 and recovered to only 52% of the original maximum rate of photosynthesis in 2% O2 at the end of the 20 min recovery period (Fig. 1; Table I).

Although the MSO treatment resulted in substantial accumulation of ammonia (Table II) the inhibition of photosynthesis in 21% O2 was less and the subsequent recovery of photosynthesis in 2% O2 was much more rapid than in control leaf discs (Fig. 1) where ammonia levels were much lower. In contrast, photosynthesis of AZA-treated leaf discs was inhibited much more rapidly in 21% O2 and recovered more slowly than in control leaf discs, although the ammonia levels in AZA-treated leaf discs were not significantly different.

DISCUSSION

The mechanism which results in the early and rapid inhibition of photosynthesis in the GluS mutants under photosynthetic conditions is clearly not related to the accumulation of ammonia. Although NH4+ levels in MSO-treated discs were more than threefold higher than controls, photosynthesis declined more slowly in 21% O2 and recovered more rapidly in 2% O2 than in controls. The rapid and almost complete

Table I. Recovery of Photosynthesis in Leaf Discs of Glutamate Synthase Mutants Following Exposure to 21% O2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>After 5 min exposure to 21% O2</th>
<th>After 2 min recovery in 2% O2</th>
<th>Relative Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32 ± 1.4</td>
<td>56 ± 3</td>
<td>175</td>
</tr>
<tr>
<td>MSO (1 mm)</td>
<td>43 ± 1.4</td>
<td>74 ± 3</td>
<td>172</td>
</tr>
<tr>
<td>AZA (1 mm)</td>
<td>18 ± 2</td>
<td>29 ± 2</td>
<td>161</td>
</tr>
</tbody>
</table>

* Value labeled as 'a' in Figure 1.  † Value labeled as 'b' in Figure 1.  ‡ Calculated as (b'/a') × 100.

Table II. Effect of Methionine Sulfoximine (MSO) and Azaserine (AZA) on NH4+ Levels in Leaf Discs of Glutamate Synthase Mutants

The data were obtained from the leaf samples used for the experimental data of Figure 1 at the end of the recovery period.

<table>
<thead>
<tr>
<th>Ammonia Levels</th>
<th>μmol/mg Chl</th>
<th>mM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.1</td>
<td>3.1</td>
</tr>
<tr>
<td>MSO</td>
<td>6.6</td>
<td>9.7</td>
</tr>
<tr>
<td>AZA</td>
<td>2.4</td>
<td>3.5</td>
</tr>
</tbody>
</table>

* Estimated concentration of NH4+ in cell sap given that the Chl concentration was 0.68 mg Chl g⁻¹ fresh weight of tissue and that NH4+ was uniformly distributed.
recovery of photosynthesis in MSO-treated leaf discs following the return to 2% O2 suggested that photophosphorylation was not uncoupled by the high levels of ammonia. Since the K_i for uncoupling of photophosphorylation was estimated (8) to be 3 x 10^{-4} M, and the average cell concentration at the end of the experiment was more than 9 x 10^{-3} M, this suggests that the photorespiratory ammonia must have been sequestered away from the chloroplast possibly being in the more acidic vacuole.

It has been suggested that exposure of photorespiratory mutants to 21% O2 might result in an inhibition of photosynthesis due to a triose phosphate limitation of the Calvin cycle (1). A characteristic of this type of photosynthetic inhibition is that photosynthesis becomes independent of the rate of oxygenation and carboxylation (15). However, the data shown in Table I indicate that the percentage increase of net photosynthesis during the first 2 min of the recovery period reflected the decrease in oxygenase activity expected by the return to 2% O2 (2). Thus, the early inhibition of photorespiratory mutants was not consistent with a triose phosphate limitation of the Calvin cycle.

The results reported here do not rule out the possibility that the long term inhibition of photosynthesis was, in part, due to the inactivation of Rubisco (5), but they do suggest that this is an unlikely reason for the rapid and short-term inhibition observed here. In vitro assays of GluS and dicarboxylate shuttle mutants suggest that Rubisco activity is inactivated slowly following exposure to photorespiratory conditions, even in comparison to other photorespiratory mutants (3). The rapidity of the inhibition of photosynthesis in these experiments (Fig. 1) is not consistent with a slow inactivation of Rubisco, nor is the rapid recovery (Table I) of photosynthesis when the leaf discs were returned to nonphotorespiratory conditions, as this would require a rapid reactivation of the enzyme. As MSO apparently does not affect any Calvin cycle enzymes (13), it is also difficult to explain why MSO should have any effect on the rate of inhibition or recovery of photosynthesis in these mutants, if such inhibition were due to the inactivation of Rubisco.

Previous studies in wheat (7, 20) have shown that the MSO-induced inhibition of photosynthesis was delayed by the addition of externally added amino acids, although this resulted in a marked increase in ammonia levels. The lower rate of decline of photosynthesis has been attributed to the increased availability of amino donors to the photosynthetic cycle and the maintenance of carbon flux through the photorespiratory pathway (19). In the GluS mutants, the pool of glutamate is depleted by the combined activity of glutamate-glyoxylate aminotransferase and GS. The lower rate of photosynthetic inhibition of MSO-treated leaf discs is consistent with the interpretation that the inhibition of GS resulted in an increased availability of glutamate as an amino donor to glyoxylate (19), thus serving to maintain the integrity of the photosynthetic cycle. In contrast, the inhibition of residual ferredoxin and NAD-GluS activity (12) by AZA would prevent the recycling of glutamine in the photosynthetic cycle and the inhibition of photosynthesis by AZA may be attributed to the more rapid depletion of glutamate. The marked inhibition by AZA in 2% O2 was unexpected but may be due to the requirement for glutamate recycling to support the lower rates of photosynthetic activity observed at 2% O2.

Because the inhibitors affect the rate of inhibition and recovery of photosynthesis and also affect the availability of amino donors for glyoxylate, it would appear the initial inhibition of photosynthesis in these mutants is due to a toxic effect of glyoxylate (4, 6) when its amination to glycine is prevented or to the restriction of carbon flow through the photorespiratory pathway because of a decline in the availability of amino donors.

LITERATURE CITED