Short Communication

Sulfate Assimilation in C₄ Plants

INTERCELLULAR AND INTRACELLULAR LOCATION OF ATP SULFURYLASE, CYSTEINE SYNTHASE, AND CYSTATHIONINE β-LYASE IN MAIZE LEAVES

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ABSTRACT

The activity of ATP sulfurylase, cysteine synthase, and cystathionine β-lyase was measured in crude leaf extracts, bundle sheath strands, and mesophyll and bundle sheath chloroplasts to determine the location of sulfate assimilation of C₄ plant leaves. Almost all the ATP sulfurylase activity was located in the bundle sheath chloroplasts while cysteine synthase and cystathionine β-lyase activity was located, in different proportions, in both chloroplast types.

A new spectrophotometric assay for measuring ATP sulfurylase activity is also described.

The C₄ pathway of CO₂ assimilation is compartmentalized into specific cell types with atmospheric CO₂ initially being fixed into organic acids in the mesophyll cells of C₄ plants and then transported to the bundle sheath cells. Within the two types of cells the reactions are compartmentalized into chloroplasts, cytoplasm and mitochondria. The intercellular compartmentation of sulfur metabolism is less well defined.

The intercellular compartmentation of ATP sulfurylase (EC 2.7.7.4) has been reported in a wide variety of C₄ plants (8, 9). An hypothesis for the pathway of sulfur assimilation in leaves of C₄ plants has been described based upon the location of ATP sulfurylase and, to a lesser extent, thiosulfonate reductase/sulfite reductase (8). Since ATP sulfurylase and sulfite reductase are involved in only the initial stages of assimilatory sulfate pathway in plants, the location of enzymes involved in the synthesis of cysteine and methionine remains unresolved.

Although it is generally agreed that in C₄ plants, chloroplasts are the main site of SO₄²⁻ assimilation (activation and reduction to S⁰) (1) the subcellular distribution of cysteine synthase (EC 4.2.99.8) appears to differ between plant species. In wheat and rape leaves and kidney bean seedlings, the enzyme is apparently cytoplasmic (3, 14, 16) whereas in spinach, white clover, and pea leaves the enzyme is associated with chloroplasts (7, 15). A recent report suggests that cystathionine β-lyase (EC 4.4.1.8) in barley leaves is at least partially plastidic (17).

This paper reports on the inter- and intracellular location of ATP sulfurylase, cysteine synthase and cystathionine β-lyase in Zea mays. A new spectrophotometric assay for ATP sulfurylase is also described.

MATERIALS AND METHODS

Maize (Zea mays, cv Dekalb XL81) was grown in sterile soil in a naturally illuminated glasshouse between 22 to 30°C and was used 18 to 20 d after germination. All biochemicals were of analytical reagent grade and were purchased from either Sigma Chemical Co. or Boehringer-Mannheim, Australia. PDRP was partially purified as described previously (4).

Mesophyll chloroplasts were isolated and purified as described previously (13) except that 2 mm DTT was included in the extraction, washing and Percoll density buffers. Bundle sheath strands and chloroplasts were prepared as described by Jenkins and Boag (12). Crude extracts were prepared as described previously (13). Chl was determined as described previously (2).

Pyruvate, Pi dikinase was activated as described previously (13) except that 0.3 units partially purified PDRP was added. Pyruvate, Pi dikinase (EC 2.7.9.1) and PEP carboxylase (EC 4.1.1.31) activities were measured as described previously (6, 11), and NADP-malic enzyme (EC 1.1.1.40) activity was measured as described previously (12).

ATP sulfurylase activity was measured spectrophotometrically by coupling the reverse reaction catalyzed by ATP sulfurylase with hexokinase and glucose-6-P dehydrogenase and measuring the APS- or Pi-dependent reduction of NADP. Reaction mixtures contained in a volume of 1 ml: 1 μmol APS, 1 μmol Pi, 5 μmol MgCl₂, 5 μmol glucose, 0.3 μmol NADP, 5 units hexokinase, 5 units glucose-6-P dehydrogenase, and 50 μmol Tris-HCl, pH 8.0. Cysteine synthase and cystathionine β-lyase were assayed by the methods described previously (5).

All enzyme assays were run at 25°C in the presence of 0.1% (v/v) Triton X-100 added to ensure organelle breakage.

RESULTS

NADP-malic enzyme was assayed as a marker for bundle sheath chloroplasts, pyruvate, Pi dikinase as a marker for mesophyll chloroplasts and PEP carboxylase to determine the level of contamination by mesophyll cell cytoplasm. The activities of the marker enzymes indicated that there was little cross-contamination of the two types of chloroplast extracts (Table I). In addition, the low level of PEP carboxylase activity in either chloroplast preparation indicated little mesophyll cell cytoplasm contamination of either chloroplast preparation.

When initially measured, the pyruvate, Pi dikinase activity in both crude extracts and mesophyll chloroplasts was low and extended incubation at 25°C in the presence of Pi did not activate the enzyme. However, the enzyme was activated when mesophyll chloroplast and crude extracts were incubated with both PDRP and Pi (Table II). This result suggests that either the PDRP in both crude and mesophyll extracts was inactivated during prep-
chloroplasts.

Table 1. Localization of Enzymes in Chloroplasts Isolated from Maize Leaves

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Crude Extract</th>
<th>Mesophyll Chloroplasts</th>
<th>Bundle Sheath Chloroplasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADP-malic enzyme</td>
<td>12.2</td>
<td>3.05 (2.01)*</td>
<td>34.1 (11.6)</td>
</tr>
<tr>
<td>Pyruvate, Pi dikinase</td>
<td>6.3</td>
<td>10.9 (7.19)</td>
<td>0.32 (0.11)</td>
</tr>
<tr>
<td>PEP carboxylase</td>
<td>22.2</td>
<td>0.07 (0.05)</td>
<td>0.35 (0.12)</td>
</tr>
<tr>
<td>ATP sulfurylase</td>
<td>0.69</td>
<td>0.06 (0.04)</td>
<td>1.64 (0.56)</td>
</tr>
<tr>
<td>Cysteine synthase</td>
<td>1.58</td>
<td>1.45 (0.96)</td>
<td>1.72 (0.38)</td>
</tr>
<tr>
<td>Cystathionine β-lyase</td>
<td>0.15</td>
<td>0.18 (0.12)</td>
<td>0.06 (0.02)</td>
</tr>
</tbody>
</table>

* Values in parentheses are the specific activities of the enzymes after correcting for the relative distribution of Chl in the mesophyll (66%) and bundle sheath (34%) chloroplasts.

Table II. Effect of Pyruvate, Pi Dikinase Regulatory Protein and Pi upon Pyruvate, Pi Dikinase Activity in Crude and Chloroplast Extracts of Maize Leaves

<table>
<thead>
<tr>
<th>Pyruvate, Pi Dikinase Activity</th>
<th>Crude Extract</th>
<th>Mesophyll Chloroplasts</th>
<th>Bundle Sheath Chloroplasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/min·mg Chl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No addition</td>
<td>4.9</td>
<td>3.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Plus PDRP*</td>
<td>5.9</td>
<td>4.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Plus Pi</td>
<td>4.8</td>
<td>3.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Plus PDRP and Pi</td>
<td>6.3</td>
<td>10.9</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Extracts were incubated in the presence or absence of partially purified pyruvate, Pi dikinase regulatory protein (PDRP) (0.7 mg) and Pi (5 mM) at 25°C. Pyruvate, Pi dikinase was measured after 30 and 60 min, no increase in activity as detected after 30 min.

From 0.05 to 0.06 μmol/min·mg Chl, respectively, close to those values observed in the bundle sheath chloroplasts (Table I) indicating that both enzymes appear to be located in the chloroplasts of the bundle sheath strands.

DISCUSSION

The location of ATP sulfurylase in bundle sheath chloroplasts is in agreement with earlier findings (9). However, the activity of ATP sulfurylase expressed on a Chl basis was about five times higher than that previously reported for whole leaf and bundle sheath cell extracts from maize leaves (9). Since the values previously reported for ATP sulfurylase activity in both whole leaf and bundle sheath extracts of maize were about 20% of the average level of activity of the enzyme found in other C4 plants examined, the reported values may be an underestimation of the enzyme activity in maize leaf tissue.

Expressed on a Chl basis, the cysteine synthase activity in the mesophyll and bundle sheath chloroplasts are similar to those reported for pea and clover chloroplasts (15).

Significant levels of both cysteine synthase and cystathionine β-lyase were detected in both mesophyll and bundle sheath extracts. That the maize leaf cysteine synthase activity appears to be associated with chloroplasts in both mesophyll and bundle sheath cells is similar to the enzyme from spinach, white clover, and pea leaves (7, 15). When these results are combined with the finding that most, if not all, of the ATP sulfurylase activity is located in the bundle sheath cells, they indicate that sulfur must be transferred from bundle sheath to mesophyll cells following sulfate activation. The previous finding, that thiosulfonate reductase/sulfite reductase activity has been detected in both mesophyll and bundle sheath cell extracts (8), suggests that sulfate must be transferred from the bundle sheath to the mesophyll cells prior to reduction to sulfide. Some sulfite has been transferred from the bundle sheath chloroplasts, the mesophyll cells contain the enzymes to reduce sulfite to sulfide and to incorporate it into cysteine.

Although homocysteine may be synthesized by either transsulfuration or direct sulfhydration in plants, it appears that transsulfuration accounts for essentially all homocysteine biosynthesis in the plant kingdom (10). Therefore, the site of cystathionine β-lyase activity in plants can be used as an indicator of the site of homocysteine synthesis. With almost 85% of the cystathionine β-lyase activity located in the mesophyll chloroplasts, the major site of homocysteine synthesis appears to be the mesophyll chloroplasts. Since it was recently reported that methionine synthase (EC 2.1.1.13) is located in the cytoplasm of pea and barley leaves (17) no conclusions can be made regarding the location of methionine biosynthesis in maize.

The results presented in this paper together with previously published data (8, 9), indicate that SO₄²⁻ is activated in the bundle sheath chloroplasts of maize leaves and it is then transferred to the mesophyll cell chloroplasts in the form of APS or SO₄²⁻ (in a free or bound form—see Ref. 1).

The cellular compartmentation of sulfur assimilation in leaves of C₄ plants is analogous to that of carbon assimilation. Carbon assimilation is initiated in the mesophyll cells close to the source of gaseous carbon, CO₂, while sulfur assimilation is initiated in the bundle sheath cells close to the source of dissolved sulfur, SO₄²⁻.

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LITERATURE CITED