CARBON DIOXIDE FIXATION AND SALT UPTAKE IN PARTICULATE PREPARATIONS FROM BARLEY ROOTS

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Previous workers (4, 6) have shown that CO₂ fixation in excised roots is related to ion absorption since an absorption of excess cations results in an increase in both the CO₂ fixation and in the formation of labelled malic acid. The accumulation of cations and anions has been demonstrated in both animal (1, 5, 8) and plant (7) mitochondria, and in view of Robertson’s hypothesis that the mitochondria may be involved in electrolyte accumulation in the plant cell, it was of interest to determine whether the CO₂ fixation by particulate preparations from barley roots (3, 9) is influenced by the absorption of salts.

MATERIALS AND METHODS

The particles were isolated from 6-day-old barley seedlings and exposed to C¹⁴O₂ as previously described (3). Potassium was determined by means of a flame photometer. The organic acids were isolated by ether extraction followed by silica gel chromatography after the method of Bulen et al (2).

RESULTS AND DISCUSSION

Preliminary experiments were performed to investigate the potassium content of the barley root particles after exposure to potassium salt solutions; plants were grown in culture solutions containing 0.1 meq K/liter and 50 meq K/liter and the particulate preparations from these plants were analyzed for potassium. The results, given in table I, showed that the plants grown in the higher concentration of potassium yielded particles containing a higher level of potassium.

Particulate preparations were incubated with potassium phosphate for 45 minutes, in each case duplicate experiments were carried out with heat-inactivated particles as controls. The samples were then centrifuged for 15 minutes at 16,000 × G at −5°C, the supernatant was decanted and the residue pellet was quickly washed with 0.5 M sucrose before being transferred with distilled water to a dish in preparation for potassium analysis. The data in table I showed that particles prepared from plants grown in a culture solution containing a low concentration of potassium took up more potassium during exposure to potassium phosphate than those particles prepared from plants grown in culture solution containing 50 meq K/liter; moreover, in almost every case, the level of potassium was higher in the heat-inactivated particles than in the active particles. As the potassium content of both active and heat-inactivated particles was proportional to the concentration of the external medium, the results suggested that there was surface adsorption of the potassium ions, and the

Table I

Potassium Content of Barley Root Particles

<table>
<thead>
<tr>
<th>EXP. NO.</th>
<th>SOURCE OF PARTICLES</th>
<th>K (M × 10⁻⁴)</th>
<th>POTASSIUM µM/MG N</th>
<th>INITIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IN REACTION MEDIUM</td>
<td></td>
<td>ACTIVE</td>
<td>HEAT-INACTIVATED</td>
</tr>
<tr>
<td>1.</td>
<td>A</td>
<td>800</td>
<td>8</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>2.</td>
<td>A</td>
<td>1000</td>
<td>7</td>
<td>190</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>29</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>3.</td>
<td>A</td>
<td>500</td>
<td>26</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>500</td>
<td>14</td>
<td>110</td>
</tr>
<tr>
<td>4.</td>
<td>A</td>
<td>2000</td>
<td>14</td>
<td>22</td>
</tr>
</tbody>
</table>

Reaction medium: Exp. 1. Potassium phosphate, pH 5.6 as shown, 120 micromoles (µM) sucrose, 1 µM PEP (cyclohexylamine salt) in a total volume of 3 ml. Exp. 2, 3. Potassium phosphate, pH 5.6 as shown, 500 µM sucrose, 3 µM PEP (cyclohexylamine salt), 90 µM glucose in a total volume of 10 ml. Exp. 4. Potassium bromide as shown, 300 µM sucrose in a total volume of 15 ml. Particles washed 3 times with 0.5 M sucrose.

Incubation time: 45 minutes at room temperature.

A Roots grown in culture solution containing 0.1 meq K/l.
B Roots grown in culture solution containing 50 meq K/l.
A and B extracted with 0.05 M TRIS and 0.5 M sucrose.

Temporary address.
higher level of potassium in the heat-inactivated particles might be explained on the grounds of a larger surface area being available due to the breaking up of the particles in heating. To remove the adsorbed potassium, the particles were subjected to successive washing with 0.5 M sucrose. The results (table I, Exp. 4) showed that after washing, the active particles contained 3 times as much potassium as the heat-inactivated particles, indicating an active binding of the cation.

To study the effects of salts on CO₂ fixation in the particles, single salts, KBr, K₂SO₄ and CaBr₂, were added to the reaction medium during exposure to C¹⁴O₂. The results are given in table II. In the pyruvate system there was some correlation between the CO₂ fixation and the cations present in the reaction medium, since the addition of potassium bromide and potassium sulphate increased the level of CO₂ fixation, and the activity was highest in the presence of potassium sulphate. However, the stimulation of the CO₂ fixation was small compared to that found in excised roots; this was possibly due to the presence of excess cations in the particles through the use of phosphate buffer in the extraction, and also to the presence of other ions in the medium. In the case of excised roots the presence of excess cations resulted in up to 90% of the fixed CO₂ being incorporated into malate (4). With particular preparations we have failed to obtain any appreciable quantity of labelled malate formed by the pyruvate system, even in the presence of a wide range of cofactors. In some cases we have found a considerable amount of labelled succinate formed in the presence of potassium bromide.

In the PEP system the addition of salts resulted in a reduction in the level of CO₂ fixation even in the presence of added DPNH. The only exception occurred when the particles were extracted with TRIS instead of phosphate buffer; here the addition of potassium bromide and potassium sulphate increased the amount of CO₂ fixation, but the use of TRIS as an extraction medium results in a low level of CO₂ fixation (3). The very low level of fixation in the presence of calcium bromide was probably due to the injurious effects of the calcium ions and the competition between calcium and magnesium, the magnesium ions being essential for the PEP system (3). The decreased fixation in the presence of both potassium phosphate and potassium sulphate, compared to potassium bromide, indicated that there was no correlation between the CO₂ fixation by the PEP system and the particular salt present. This was further confirmed by the results for the activity of the ether soluble fraction extracted from the reaction media to which potassium bromide and potassium phosphate had been added in the presence of PEP and DPNH. Total C¹⁴O₂ fixation in these 2 systems expressed as a percentage of the total carbon-14 added was 11.14 and 7.52, and the ether extract activity 4.61 and 3.22 respectively. These results showed that the total CO₂ fixation and the activity in the ether fraction were lower in the presence of potassium phosphate, and that the fixation into the ether extract expressed as a percentage of the total carbon-14 fixed were 41.4 and 42.8 for the 2 systems, there being no increased formation of labelled malate in the presence of potassium phosphate.

**TABLE II**

**EFFECT OF SALTS ON C¹⁴O₂ FIXATION IN BARLEY ROOT PARTICLES**

<table>
<thead>
<tr>
<th>SYSTEM</th>
<th>PEP + DPNH**</th>
<th>PEP†</th>
<th>PYRUVATE‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TRIS</td>
<td>PO₄</td>
<td>PO₄</td>
</tr>
<tr>
<td>1. None</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2. KBr</td>
<td>126</td>
<td>93</td>
<td>74</td>
</tr>
<tr>
<td>3. K₂SO₄</td>
<td>110</td>
<td>84</td>
<td>63</td>
</tr>
<tr>
<td>4. KPO₄</td>
<td>88</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>5. CaBr₂</td>
<td>...</td>
<td>...</td>
<td>5</td>
</tr>
</tbody>
</table>

Reaction media:
* Activity as percentage of carbon-14 activity in the absence of added salt.
** PEP + DPNH: 1 ml particles containing 1.11 mg nitrogen, 1 μM PEP (tricyclohexylamine salt), 3 μM magnesium chloride, 1.2 μM manganese chloride, 9 μM AMP, 30 μM TRIS buffer at pH 7.4, 1.6 μM DPNH, 54 μM single salt.
† PEP: 1 ml particles containing 0.74 mg nitrogen, 1 μM PEP (tricyclohexylamine salt), 4.5 μM magnesium chloride, 0.6 μM manganese chloride, 9 μM AMP, 90 μM sodium phosphate buffer at pH 7.4, 200 μM single salt.
‡ Pyruvate: 1 ml particles containing 0.89 mg nitrogen, 60 μM sodium pyruvate, 1.5 μM magnesium chloride, 0.6 μM manganese chloride, 90 μM ATP, 90 μM sodium phosphate buffer at pH 5.6, 200 μM single salt incubated with 1 μM potassium bicarbonate-¹⁴C with 2.5 × 10⁶ counts/sec carbon-14 activity in a total volume of 3.5 ml.

Experimental conditions: Temperature 25°C; exposure period 1 hr.; reaction terminated by 0.5 ml 2N sulphuric acid; excess C¹⁴O₂ removed by bubbling with inert CO₂ for 15 minutes. Particles from roots grown in culture solution containing 0.1 meq. K/l.

**SUMMARY**

Our results showed that although potassium was taken up by the particulate preparations from barley roots, we failed to observe either an increase in the total CO₂ fixation, or an increase in the formation of labelled malate, under conditions which induce excess cation uptake in excised barley roots.

* The following abbreviations will be used: PEP, phosphoenolpyruvic acid; DPNH, reduced diphosphopyridine; TRIS, tris(hydroxymethyl)aminomethane; AMP, adenosine monophosphate; ATP, adenosine diphosphate-
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LITERATURE CITED


EFFECT OF TISSUE AGE ON HEXOSE METABOLISM. I. AN ENZYME STUDY WITH PEA ROOT1, 2

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Through the use of a method involving the measurement of the rate of glucose 6-C¹⁴ and glucose 1-C¹⁴ oxidation (C₆/C₁ ratio) by plant tissues, Gibbs and Beevers (5) found that while immature tissue respired glucose either exclusively or to a large extent via the classical EMP glycolytic pathway, the participation of the HMP pathway was increasingly pronounced as the tissue aged and differentiated. It is clear that the relative concentrations of the enzymes of the various pathways might determine the extent to which each participates in the metabolism of a given tissue. The purpose of this investigation was to determine whether the concentration of some enzymes of the two pathways varied with the developmental age of the plant tissue. Fructose 1,6-diphosphate aldolase, fructose 1,6-diphosphatase and 6-phosphogluconate dehydrogenase were selected as enzymes representing the two pathways. Also assayed were the enzymes catalyzing the anaerobic disappearance of ribose 5-P. The root of the pea plant was selected as the test tissue for the following reasons: 1) large numbers of this plant can be conveniently grown in hydroponic tanks; 2) root tissue in comparison to other plant organs showed a sharper decreasing C₆/C₁ ratio with successive tissue segments; and 3) previous publications from this laboratory (4, 6, 7) have demonstrated that these enzymes could be quickly and easily determined in cell-free extracts of this plant.

In this paper, attention is directed to the entire root. In a subsequent report, enzyme activity in parts of the root will be treated. A preliminary report of this investigation has appeared (2).

MATERIALS AND METHODS

GROWTH OF PLANTS: Pea seeds (Pisum sativum var. Alaska, Rogers Brothers Seed Company, Idaho Falls, Idaho) were immersed overnight in distilled water at 4°C. The following day which corresponds to day 1 in the table, they were washed for 15 minutes with a 3.5% calcium hypochlorite solution, followed