ALCOHOL DEHYDROGENASE AND ITS RELATION TO RESPIRATORY PATHWAYS IN LUPINE ROOT NODULES

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Changes in the isoenzymatic patterns of alcohol dehydrogenase (EC 1.1.1.1) accompanying ageing of the lupine root nodules were observed. Ethanol and other products of anaerobic metabolic pathways (lactate and malate) are better respiratory substrates for bacteroids and symbiosomes (peribacteroid units, PBUs) than glucose and pyruvate.

It is postulated that fermentative processes in lupine root nodule provide energy and substrates for bacteroids.

Alcohol dehydrogenase (ADH, EC 1.1.1.1) and lactate dehydrogenase (LDH, EC 1.1.1.27) are induced in lupin nodules [1, 2] which implies their physiological function. Mazurowa [3] found that both these enzymes occur in several molecular forms. Induction of these enzymes is probably associated with oxygen stress caused by leghemoglobin in the nodules [4] since it maintains concentration of free oxygen at the level of about 10 nM [5], i.e. too low for functioning of the respiratory chain of plant host mitochondria. However, bacteroids -microaerophiles can respire because of their terminal oxidases of high affinity to O₂.

In this paper we evidence participation of fermentative enzymes in the respiratory processes of nodules and the ability of Rhizobium bacteroids to metabolize products of anaerobic pathways.

MATERIALS AND METHODS

Experiments were performed with yellow lupine (Lupinus luteus L. cv. Ventus) obtained from the Wiatrowo Plant Breeding Station. Lupine seedlings were field grown on sandy soil of pH 6.0. Nodules of the plants at the stage of flower bud formation and fluorescence were analyzed. Bacteroids were isolated by the density gradient centrifugation according to Nishimura et al. [6] and symbiosomes according to Price et al. [7]. Stimulation of O₂ uptake on addition of a given compound was taken as the criterion of its utilization by
bacteroids. In the case of symbiosomes, in $^{14}\text{CO}_2$ evolved from U-$^{14}$C-labelled substrates was quantified. Oxygen uptake was measured according to Umbreit et al. [8]. $^{14}\text{CO}_2$ was trapped in the toluene-based scintillation mixture containing 3% (v/v) Protosol. Radioactivity was measured in Beckman scintillation counter.

Alcohol dehydrogenase (EC 1.1.1.1) was determined spectrophotometrically at 340 nm and 25°C according to Boyle & Yeung [9]. Non-denaturing electrophoresis was performed in 7.5% polyacrylamide gel slabs after Maurer [10]; positions of ADH were located according to Czosnowski [11].

RESULTS AND DISCUSSION

Total ADH activity increased up to 60th day of plant growth and then decreased gradually with nodule ageing (Fig. 1). The activity in nodules exceeded considerably that in roots.

A single highly active ADH form was present in root nodules isolated from 30 day-old plants (Fig. 2), the number of ADH forms in the nodules of two month-old plants increased to five, the initial form of the highest electrophoretic mobility being the most active. The activity of the form of the

![Graph](image-url)

Fig. 1. Alcohol dehydrogenase activity during lupine development; n. d., activity not detectable
Fig. 2. Developmental changes of the isoenzyme patterns of alcohol dehydrogenase in lupin nodules

lowest electrophoretic mobility was enhanced in older nodules of three month-old plants. ADH activity was not found on electrophoresis of root extracts.

The bacteroids and symbiosomes separated in the Percoll density gradient oxidized efficiently exogenous ethanol and lactate (Table 1). Malate proved the

**Table 1**

*Respiration of Rhizobium bacteroids and symbiosomes isolated from lupine root nodules*

The bacteroids and symbiosomes were separated in the Percoll density gradient. The results of mean of values of three independent experiments are given

<table>
<thead>
<tr>
<th>SUBSTRATES 50 mM or 185 kBq</th>
<th>BACTERIOIDS Oxygen consumption (µl O₂/hour/0.2 cm³ fraction)</th>
<th>SYMBIOSOMES ¹⁴CO₂ evolution (c.p.m./min/0.1 cm³ fraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>52</td>
<td>—</td>
</tr>
<tr>
<td>Glucose</td>
<td>50</td>
<td>100.412</td>
</tr>
<tr>
<td>Ethanol</td>
<td>218</td>
<td>885.924</td>
</tr>
<tr>
<td>Lactate</td>
<td>125</td>
<td>511.369</td>
</tr>
<tr>
<td>Malate</td>
<td>320</td>
<td>1.304.495</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>210</td>
<td>611.248</td>
</tr>
<tr>
<td>Succinate</td>
<td>242</td>
<td>1.231.672</td>
</tr>
<tr>
<td>Oxoglutarate</td>
<td>193</td>
<td>982.321</td>
</tr>
<tr>
<td>L- Asparagine</td>
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<td>307.683</td>
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<tr>
<td>L- Aspartate</td>
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<tr>
<td>L- Glutamine</td>
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<td>371.010</td>
</tr>
<tr>
<td>L- Glutamate</td>
<td>185</td>
<td>945.231</td>
</tr>
<tr>
<td>L- Alanine</td>
<td>102</td>
<td>384.997</td>
</tr>
</tbody>
</table>
best respiratory substrate and glucose did not affect respiration of bacteroids. Some differences in catabolic breakdown of the substrates tested were observed in bacteroids and symbiosomes. Aspartate, glutamate, and their amides, asparagine and glutamine were catabolized by bacteroids at a similar rate. On the other hand, symbiosomes utilized amino acids twice as intensively as their amides. Ethanol was assimilated and metabolized by symbiosomes at a lower rate than succinate and maltate, i.e. metabolites transported by specific carriers through peribacteroid membranes of symbiosomes. However, the intensity of ethanol metabolism was higher than that of glucose and pyruvate, commonly regarded as good substrates for bacteroids.

The results obtained enable to propose the following Scheme of metabolic relationship between the plant and symbiosome.

![Scheme 1](image)

Scheme 1. The proposed localization of respiratory processes in the lupine nodules. The oxygen stress caused by leghemoglobin induced anaerobic glucose breakdown. The products of anaerobic pathways are not toxic for plant cells of the nodule since they are utilized by bacteroids. Enzymes: 1, glycolysis; 2, ADH, alcohol dehydrogenase; 3, LDH, lactate dehydrogenase; 4, PEP-C, phosphoenolpyruvate carboxylase; 5, MDH, malate dehydrogenase; 6, dicarboxylate carrier; 7, the bacteroid specific respiratory chain; EtOH, ethanol; LA, lactic acid; PA, pyruvic acid; OAA, oxaloacetic acid; TCAC, tricarboxylic acid cycle; PBM, peribacteroid membrane.
REFERENCES