SHORT COMMUNICATION

L-Arabinose Metabolism in Rhizobia

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L-Arabinose is metabolized by different pathways in fast- and slow-growing rhizobia.

INTRODUCTION

L-Arabinose is a good carbon source for the growth of most rhizobia, and it has also been used in the growth medium which allows nitrogenase production in culture by certain strains (Pagan et al., 1975). The only information about the metabolism of L-arabinose in rhizobia was the demonstration in R. japonicum (Pedrosa & Zancan, 1974) of a pathway first described in Pseudomonad MSU (Dahms & Anderson, 1969):

\[ \text{L-Arabinose} \rightarrow \text{L-Arabinolactone} \rightarrow \text{L-Arabonate} \rightarrow \]
\[ \text{2-Keto-3-deoxy-L-arabonate} \rightarrow \text{Pyruvate + Glycolaldehyde} \]

in which reaction 1 is catalysed by L-arabinose dehydrogenase and reaction 2 by 2-keto-3-deoxy-L-arabonate aldolase.

In R. meliloti, however, we have recently shown (Duncan & Fraenkel, 1979) that L-arabinose is probably metabolized by the more common route, which has the same initial steps, but continues as follows:

\[ \text{2-Keto-3-deoxy-L-arabonate} \rightarrow \text{α-Ketoglutarate semialdehyde} \rightarrow \text{α-Ketoglutarate} \]

in which reaction 3 is catalysed by α-ketoglutarate semialdehyde dehydrogenase. Extracts of R. meliloti contained the enzymes which catalyse reactions 1 and 3, and a mutant blocked at the next step [α-ketoglutarate dehydrogenase (kgd)] was unable to grow on L-arabinose.

I have now surveyed other strains of rhizobia for the key enzymes of these pathways and find that the metabolism of L-arabinose is indeed different in fast- and slow-growing rhizobia.

METHODS

Bacterial strains. Rhizobium meliloti L5-30 was from the laboratory of Dr G. Martinez-Drets, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay; R. japonicum 61A76 was from the laboratory of Dr F. Ausubel, Biological Laboratories, Harvard University; R. leguminosarum DL-14 was from the laboratory of Dr R. Valentine, University of California, Davis, U.S.A.; R. trifolii T11, R. phaseoli K26 and Rhizobium sp. 32H1 (for cowpea) were from the laboratory of Dr E. R. Signer, Massachusetts Institute of Technology, Cambridge, Mass., U.S.A.

Growth of bacteria and preparation of extracts. Rhizobium meliloti L5-30 was grown to stationary phase in rich LBCG medium (Lennox, 1955) which contained (per litre) 10 g Bacto-tryptone, 5 g yeast extract, 5 g NaCl, 2·5 mm-CaCl₂, and 2 g glucose. Cultures were resuspended (to give an \( A_{600} \) value of 2) in minimal...
medium M63 (Cohen & Rickenberg, 1956) supplemented with carbon sources (0.4 %, w/v), arginine (25 μg ml⁻¹) and biotin (1 μg ml⁻¹). The other strains were grown in YP medium, i.e. 0.1 % (w/v) yeast extract in 7.5 mM-KH₂PO₄/KOH (pH 6.3), with 0.1 % (w/v) L-arabinose or 0.1 % (w/v) glucose. All strains were grown at 30 °C. For enzyme assays, bacteria were collected by centrifuging and resuspended (1 g wet weight per 3 ml) in 50 mM-K₂HPO₄/KOH buffer (pH 7.6) containing 2 mM-EDTA and 14 mM-2-mercaptoethanol.

Bacteria were disrupted ultrasonically for 1 min ml⁻¹ in an ice-bath with a Bronwill Biosonic III sonicator, allowing 2 min cooling after each minute of sonication. Extracts were centrifuged at 110000 g for 2 h at 4 °C in a Beckman L-5-50 ultracentrifuge.

**Enzyme assays.** NAD⁺-dependent L-arabinose dehydrogenase (EC 1.1.1.46) was assayed in 0.1 M KH₂PO₄/KOH buffer (pH 7.5) containing 1 μmol NAD⁺ ml⁻¹ and 0.7 μmol L-arabinose ml⁻¹. NAD⁺-linked α-ketoglutarate semialdehyde dehydrogenase (2,5-dioxovalerate: NAD⁺ oxidoreductase; EC 1.2.1.26) and glycolaldehyde dehydrogenase (EC 1.2.1.21) were measured under the same conditions using either α-ketoglutarate semialdehyde (0.1 μmol ml⁻¹) or glycolaldehyde (2 μmol ml⁻¹) as substrates. (α-Ketoglutarate semialdehyde was a generous gift from Dr Y. F. Chang, Dental School Department of Microbiology, University of Maryland.) Spectrophotometric assays were carried out at 25 °C with a Gilford model 240 spectrophotometer. For 2-keto-3-deoxy-L-arabonate aldolase (EC 4.1.2.18) assays (Pedrosa & Zancan, 1974), the reaction mixture (0.5 ml) contained 40 mM-HEPES buffer (pH 8.0), 10 μmol MgCl₂, 7.5 μmol sodium pyruvate and 10 μmol glycolaldehyde. The reaction was carried out for 30 min at 30 °C. Controls without substrates were performed and 2-keto-3-deoxy-L-arabonate formation was detected by the thiobarbituric acid/periodate method (Weissbach & Hurwitz, 1959). Specific activity is expressed as nmol min⁻¹ (mg protein)⁻¹. Enzyme activities were measured on the same day as the extracts were prepared. Aldolase activities were stable for up to 3 d in crude sonicates kept at 4 °C, while dehydrogenase activities fell as much as 70 % in 3 d.

Protein was assayed by the biuret method with bovine serum albumin as standard.

**RESULTS AND DISCUSSION**

L-Arabinose dehydrogenase activity was found in all six strains tested (Table 1). 2-Keto-3-deoxy-L-arabonate aldolase activity was not detected in three of the four fast-growing strains (R. trifolii, R. phaseoli and R. leguminosarum) but was found in the two slow-growers (R. japonicum and the cowpea strain). α-Ketoglutarate semialdehyde dehydrogenase activity was high in the fast-growing strains. The two slow-growing strains showed a low dehydrogenase activity with α-ketoglutarate semialdehyde and a similar level of activity with glycolaldehyde as substrate. Thus, this activity may be due to a non-specific dehydrogenase for the glycolaldehyde product of the L-arabinose pathway in these strains.

The results with R. meliloti confirm our previous data (Duncan & Fraenkel, 1979), except that low, rather than zero, aldolase activity was detected. We are uncertain as to its significance, considering the much higher level of α-ketoglutarate semialdehyde dehydrogenase, and the kdg mutant study implicating the α-ketoglutarate pathway in L-arabinose metabolism. [Since the aldolase reaction is assayed in the backward direction, it is possible that the apparent activity in R. meliloti is due to a different enzyme, such as that described for D-arabinose metabolism in Pseudomonas saccharophila (Palleroni & Doudoroff, 1957). Although the same reservation could apply to aldolase activity in the slow-growing strains, there is the additional evidence of pyruvate formation from L-arabinose (Pedrosa & Zancan, 1974).] Experiments with mixed extracts of R. trifolii and Rhizobium sp. 32H1 showed that the fast-grower did not contain an inhibitor for the aldolase activity of the slow-growing strain.

These results show that fast- and slow-growing rhizobia probably have different pathways of L-arabinose metabolism, and adds to the other known enzymic differences between the two groups (Martinez de Drets & Arias, 1972; Martinez de Drets et al., 1974).

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Table 1. Activities of enzymes in L-arabinose metabolism in rhizobia

All activities are expressed as nmol min⁻¹ (mg protein)⁻¹.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth type</th>
<th>Carbon source</th>
<th>L-Arabinose dehydrogenase</th>
<th>2-Keto-3-deoxy-L-arabonate aldolase</th>
<th>α-Ketoglutarate semi-aldehyde dehydrogenase</th>
<th>Glycol-aldehyde dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. meliloti</em> L5-30</td>
<td>Fast</td>
<td>L-Arabinose</td>
<td>14</td>
<td>15</td>
<td>304</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D-Glucose</td>
<td>11</td>
<td>23</td>
<td>185</td>
<td>13</td>
</tr>
<tr>
<td><em>R. trifolii</em> T1L</td>
<td>Fast</td>
<td>L-Arabinose</td>
<td>184</td>
<td>&lt; 0.5</td>
<td>375</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D-Glucose</td>
<td>21</td>
<td>&lt; 0.5</td>
<td>33</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td><em>R. phaseoli</em> K26</td>
<td>Fast</td>
<td>L-Arabinose</td>
<td>102</td>
<td>&lt; 0.5</td>
<td>70</td>
<td>10</td>
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<tr>
<td></td>
<td></td>
<td>D-Glucose</td>
<td>82</td>
<td>&lt; 0.5</td>
<td>61</td>
<td>4</td>
</tr>
<tr>
<td><em>R. leguminosarum</em> DL-14</td>
<td>Fast</td>
<td>L-Arabinose</td>
<td>42</td>
<td>&lt; 0.5</td>
<td>136</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D-Glucose</td>
<td>25</td>
<td>&lt; 0.5</td>
<td>113</td>
<td>&lt; 0.5</td>
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<tr>
<td><em>R. japonicum</em> 61A76</td>
<td>Slow</td>
<td>L-Arabinose</td>
<td>6</td>
<td>54</td>
<td>26</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D-Glucose</td>
<td>1</td>
<td>61</td>
<td>41</td>
<td>33</td>
</tr>
<tr>
<td><em>Rhizobium</em> sp. 32H1</td>
<td>Slow</td>
<td>L-Arabinose</td>
<td>34</td>
<td>42</td>
<td>44</td>
<td>25</td>
</tr>
<tr>
<td>(for cowpea)</td>
<td></td>
<td>D-Glucose</td>
<td>5</td>
<td>34</td>
<td>52</td>
<td>46</td>
</tr>
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</table>

REFERENCES


