"In situ" changes in enzyme activity during Neurospora conidial germination

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Abstract
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"In situ" changer in enzyme activity during Neurospora conidial germination.

Two enzymes of the γ-aminobutyric acid (GABA) bypass of the citric acid cycle, glutamic acid decarboxylase (GAD) and succinate semialdehyde dehydrogenase (SSADH) have been detected in conidia. Neither of these enzymes have been assayed previously in Neurospora. GAD and SSADH comprise part of a new pathway that may be responsible for metabolizing glutamic acid during conidial germination (Schmit and Brody 1975 J. Bacteriol. 124: 232). GAD appears to be stored at high levels in dormant conidia (Table 1). The specific activity of this enzyme decreases during germination and early log-phase growth. SSADH appears to be a constitutive enzyme. The activities of NADP glutamate dehydrogenase, malate dehydrogenase and glutamate oxaloacetate transaminase increase as conidia germinate and enter log-phase growth.

All of these enzymes were assayed "in situ" using cells permeabilized by the procedures of Basabe et al. (1979 Anal. Biochem. 92: 356). Strains containing the noda mutation deficient in nicotinamide adenine dinucleotide activity (Nelson et al. 1975 J. Bacteriol. 122: 695) were used to eliminate the problems of NAD and NADP destruction that occurs with conidia from wild type strains. By combining the cell permeabilization techniques and use of the noda mutant strains, we have simplified the procedures for assaying enzymes during conidial germination. We are in the process of using these techniques to measure "in situ" changes in enzyme activities throughout the asexual cycle of Neurospora.

**RESEARCH NOTES**

**TABLE 1.**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific Activity (nmoles/min/mg dry weight)sup b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conidia(c)</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>32.0</td>
</tr>
<tr>
<td>Decarboxylase (GAD)(f)</td>
<td>32.0</td>
</tr>
<tr>
<td>Succinic Semialdehyde</td>
<td>1.8</td>
</tr>
<tr>
<td>Dehydrogenase (SSADH)</td>
<td>2.0</td>
</tr>
<tr>
<td>Glutamate Oxaloacetate Transaminase (GOT)</td>
<td>410.0</td>
</tr>
<tr>
<td>Malate Dehydrogenase (MDH)</td>
<td>28.0</td>
</tr>
</tbody>
</table>

GAD and GDH were from strain noda 2256 and SSADH, MDH and GOT were from strain noda 61-R-13.

Cells were permeabilized with the toluene-ethanol procedure of Basabe et al. (And. Biochem. 1979) 92: 356). The permeabilized cells were washed with buffer three times to remove all tracer of ethanol.

Conidia were dry harvested (Schmit and Brody, J. Bacteriol. 1975 124: 232).

Samples were taken after incubating for 3-5 hours in minimal glucose medium at 30° C.

Samples were taken after incubation for 8-12 hours.

GAD was arrayed by measuring γ-aminobutyric acid production by "GABAase" (Sigma). All other dehydrogenases were arrayed at 20° C with optimal substrate concentration by measuring changes in NAD(P) (H) concentrations. GOT was assayed by measuring oxaloacetate production using MDH.

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Friedman, K. J. and D. Glick.

Incorporation and degradation of lignoceric acid in cel.

alter the fatty acid profile, we attempted to incorporate

Several analyses (Friedman 1977 J. Membr. Biol. 32: 33; Kushwaha and Kates 1976 Lipids 1: 778) of the fatty acid composition of Neurospora indicate that a small number of fatty acids serve as the alkyl moieties of Neurospora phospholipids. Using the cel mutant (FGSC 165), and fatty-acid supplemented media, it has been possible to change the proportions of the fatty acids present in the phospholipid profile and to incorporate a branched-chain fatty acid (phytanic acid) normally not present in Neurospora (Brody and Allen 1972 J. Supramolec. Struct. 1: 125). In efforts to radically lignoceric acid (C24) into the phospholipids of the cel mutant.

Our results (Table 1) suggest that lignoceric acid is degraded mainly into C18 and, to a lesser extent, into C16 chain lengths prior to incorporation into Neurospora phospholipids.

Our experimental protocol was similar to that previously employed (Friedman 1977). Lignoceric acid was added to Vogel's minimal medium (containing 20 gms. sucrose/liter) as the Tween detergent (240 mg/liter). Tween-lignoceric acid was synthesized by transesterifying Tween 40 with the methyl ester of lignoceric acid. 1 cel cultures were grown on solidified medium at 31° Cond