Incorporation and degradation of lignoceric acid in cel

K. J. Friedman  
New Jersey Medical School

D. Glick  
New Jersey Medical School

Follow this and additional works at: https://newprairiepress.org/fgr

This work is licensed under a Creative Commons Attribution-Share Alike 4.0 License.

Recommended Citation
Incorporation and degradation of lignoceric acid in cel

Abstract
Incorporation and degradation of lignoceric acid in cel
"In situ" changer in enzyme activity during Neurospora conidial germination.

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

aGAD and GDH were from strain nda 2256 and SSADH, MDH and GOT were from strain noda 61-R-13.
bCells 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.
cConidia were dry harvested (Schmit and Brody, J. Bacteriol. 1975 124: 232). The permeabilized cells were taken after incubating for 3-5 hours in minimal glucose medium at 30°C.
dSamples were taken after incubation for 8-12 hours.
eGAD 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.
fGOT was assayed by measuring oxaloacetate production using MDH.

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 dinucleotidase 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.

Friedman, K. J. and D. Glick.

Incorporation and degradation of lignoceric acid in cel.

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 alter the fatty acid profile, we attempted to incorporate 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. cel cultures were grown on solidified medium at 31°C.
harvested ground mid-day. Freeze-dried hyphae were extracted for lipids using chloroform-methanol and a Folch washing procedure. The lipids were then separated into neutral and phospholipid via silicic acid column chromatography. Fatty acid methyl esters were made via a transesterification process and analyzed both quantitatively and qualitatively by gas chromatography. As shown in Table 1, lignoceric acid is a minor constituent of the phospholipid alkyl chains present in hyphoe grown in this manner.

### Table 1

<table>
<thead>
<tr>
<th>Fatty acid present</th>
<th>15:0</th>
<th>16:0</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>24:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>cel palmitic acid supplement</td>
<td>----</td>
<td>39.3</td>
<td>1.7</td>
<td>2.3</td>
<td>10.2</td>
<td>35.0</td>
<td>11.5</td>
<td>----</td>
</tr>
<tr>
<td>cel lignoceric acid supplement</td>
<td>2.24</td>
<td>15.3</td>
<td>.64</td>
<td>1.05</td>
<td>4.6</td>
<td>57.1</td>
<td>18.6</td>
<td>0.4</td>
</tr>
<tr>
<td>wild-type (no supplement)</td>
<td>----</td>
<td>19.4</td>
<td>0.1</td>
<td>.7</td>
<td>4.2</td>
<td>48.3</td>
<td>27.3</td>
<td>----</td>
</tr>
</tbody>
</table>

*Identified by retention time.

We have considered several possible explanations of these results. Since the cel mutant requires fatty acid supplementation for growth, we assume that the Tween-lignoceric acid satisfied this nutritional requirement. Since the Tween-lignoceric acid supplement contained 60% of the Tween conjugated to lignoceric acid, it is conceivable that the lignoceric acid was excluded from significant uptake or incorporation, and that the remaining 40% of nonreacted Tween 40 detergent supplied the growth requirements of the cel cultures. If this were the case, however, the fatty acid profile seen for cel growth with Tween-lignoceric acid supplementation would be similar to the profile seen for cel growth with Tween 40 (palmitic acid) supplementation. That this is not the case is shown in Table 1 which indicates that the fatty acid profile of cel grown on Tween-lignoceric acid more closely resembles the profile of wild-type Neurospora than that of cel grown on Tween 40.

Alternative explanations for the fatty acid profile observed for growth with lignoceric acid supplementation all involve some mechanism of fatty acid degradation. While we have no direct experimental evidence to support any particular degradative mechanism, the literature (reviewed by Weete 1974 Fungal Lipid Biochemistry) favors oxidation as the probable mechanism of lignoceric acid degradation in Neurospora. Such a system has been found in yeast and is active on fatty acid chain lengths between C12 and C26 (Fulco 1967 J. Biol. Chem. 242: 3608). β oxidation seems a less likely pathway for lignoceric acid degradation since it is believed (Weete 1974) that the enzymes responsible for β oxidation utilize fatty acid chain lengths between C4 and C12, or C8 and C18.

Although we have yet to achieve radical alteration of the fatty acid profile of Neurospora, our data indicate the existence of a metabolic mechanism which will degrade long chain fatty acids, when necessary, to maintain a wild-type-like fatty acid profile in the cel mutant. (Supported by Marshall Institute of General Medical Science Grant GM2017.) We are grateful to Dr. Josephine Readio for the custom synthesis of Tween-lignoceric acid. - - - Department of Physiology, New Jersey Medical School, 100 Bergen Street, Newark, NJ 07103.

Johnson, T. E. A search for position effects in Neurospora.

There are no reported instances of position effect variegation in Neurospora, and very few in the fungi. One reason for this might be the lack of cell autonomy, at least in Neurospora. Cell autonomy is required to observe such variegation. A locus which expresses such a cell autonomous effect is per-I on linkage group VR proximal to tv(6201).

I used this locus as a marker with which to observe possible position effects. The phenotypes of per-I are twofold. One set of alleles block the production of pigment in the perithecium and in the ascospores leaving them yellow; a second set of alleles block pigmentation formation in the perithecium but not in the neck of the perithecium or in the ascospores. A chromosome rearrangement with a breakpoint near the per-I locus might be expected to cause a position effect resulting in loss of pigment or mottling of the perithecium when the rearrangement strain is used as the female parent in a cross.

Appropriate strains were obtained from the stock center and screened for such effects. All strains were mating type A and involved single or multiple translocations with breakpoints in the right arm of linkage group V (221, 1445, 1548, 1879, 2006, 2025, 2031, 2034, 2062, 2093, 2098, 2185, 2427, 2447, 2629), or the left arm (1760, 2004, and 2411), or either arm (1483, 2021, 2179, 2334, 2397, 2416, 2423, 2633, 2680). None of these strains gave consistently light or motled perithecia as the protoperithecial parent in a cross to per-I. A number of alterations in normal perithecial morphology and/or development were observed in these crosses.

It is difficult to determine the significance of these findings without knowing the details of the cytological breakpoints involved. - - - Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309.