Enzyme profiles during synchronous development

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Abstract
Enzyme profiles during synchronous development

This research note is available in Fungal Genetics Reports: https://newprairiepress.org/fgr/vol19/iss1/2
Harding, R. W. and R. P. Wagner. Immunological studies with pyruvate dehydrogenase complex isolated from N. crassa.

300-350 A an $ value of 85, and to resemble in many respects the particle isolated from mammals. The complex obtained from E. coli and mammals has been separated into three constitutive enzymes: pyruvate dehydrogenase, dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase (Reed and Willm). In the present study, purified PDC obtained from N. crassa was injected into a rabbit four times over a period of five weeks. The immune serum thus obtained was found to form a single line with purified PDC on an Ouchterlony plate. When purified PDC was incubated for 10 min. at 37°C with the immune serum, the overall activity for the conversion of pyruvate to acetyl-CoA, as measured by NAD reduction, was completely lost. There is still a 22% inhibition of this activity even when the immune serum is diluted by a factor of 40.

The dihydrolipoyl dehydrogenase component of Neurospora PDC was assayed as described by Reed and Willm. Some modification in their assay for the dihydrolipoyl transacetylase activity was necessary, and no success was obtained by using their assay for measurement of the pyruvate dehydrogenase component. The immune serum at its highest concentration does not inhibit to any great extent either dihydrolipoyl dehydrogenase or dihydrolipoyl transacetylase. If the Neurospora PDC has a similar structure to PDC isolated from other sources, then the dihydrolipoyl transacetylase probably is the core of the molecule and is surrounded by the other two enzyme components. It would thus be predicted that the transacetylase activity would not be inhibited by the immune serum. Perhaps potential sites of inhibition on the dihydrolipoyl dehydrogenase are also blocked by another component of the complex. Presumably the overall activity of the particle is inhibited because the antibody inhibits the activity of the proposed third component, namely, pyruvate dehydrogenase. However, until a satisfactory assay can be devised for this enzyme, this hypothesis cannot be tested. * * * Department of Zoology, University of Texas, Austin, Texas 78712 (Present address of RWH: Radiation Biology Laboratory, Smithsonian Institution, Rockville Maryland 20852).

Totten, R. E. and H. B. Howe, Jr. Enzyme profiler during synchronous development of conidiophore and conidio in N. crassa.


Such pads were incubated in petri dishes containing 10 ml of 1% Bacto-Agar a + 35°C under fluorescent illumination for 0, 1, 4, or 7 hr, washed with deionized water in a Buchner funnel, pressed dry, and then frozen. Each frozen pod was ground intermittently with a Virtis 45 homogenizer for 15 min with 5 g of glass beads in 25 ml of 0.05 M phosphate buffer (pH 7) and then homogenate was centrifuged at 15,000 x g for 30 min. The precipitate was resuspended in 5 ml of phosphate buffer and sonicated intermittently for 2 min, the supernatant was centrifuged at 15,000 x g for 30 min. and the supernatant was combined with the first supernatant. All procedures were carried out in the cold. Seven-hour pads had developed conidiophores and conidia; 4-hr pads, conidiophores, but not conidia; and 1-hr pads, neither of these structures. Dry weights (mg) and total soluble protein (mg) of the pads at 0, 1, 4, and 7 hr, respectively, were: 1,080 and 133.1; 876 and 127.8; 818 and 129.6; 862 and 128.7.

The following enzymes have been investigated previously during the development of conidio: pyruvate dehydrogenase and glucose-6-phosphate dehydrogenase (Weiss and Turian 1966 J. Gen. Microbiol. 44: 407), invertase and trehalase (Hanks and Sussman 1969 Am. J. Botany 56: 1152), isocitrate lyase (Turian et al. 1962 Pathol. Microbiol 25: 737; Flavell 1968). The results are shown in the Table 1.

Although it was known that enzymatic activities in vitro may have little relationship to enzymatic activities in vivo, it was nevertheless anticipated that consistent trends might be found owing to our use of synchronous cultures and short incubation times. Even under these conditions, however, enzyme profiles seemed to have little functional significance, as previously found by others (e.g., Hess and Brand 1965 in Control of energy metabolism, Chance et al. (Eds.), Academic Press, New York.
Table 1. Total units and specific activities of 12 enzymes, extracted after four incubation times and % change in specific activities.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Total Units</th>
<th>Specific Activity</th>
<th>% change in specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incubation time (hours)</td>
<td>Incubation time (hours)</td>
<td>o-1</td>
</tr>
<tr>
<td>Aconitase</td>
<td>4.85</td>
<td>3.11</td>
<td>3.32</td>
</tr>
<tr>
<td>Fumarase</td>
<td>1.30</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>G-6-P dehydr.</td>
<td>58.9</td>
<td>49.2</td>
<td>49.2</td>
</tr>
<tr>
<td>Malate dehydr.</td>
<td>0.51</td>
<td>0.47</td>
<td>0.65</td>
</tr>
<tr>
<td>NAD-GDH</td>
<td>3.62</td>
<td>3.47</td>
<td>2.68</td>
</tr>
<tr>
<td>NADP-GDH</td>
<td>107</td>
<td>113</td>
<td>113</td>
</tr>
<tr>
<td>NADH-oxidase</td>
<td>2.03</td>
<td>1.98</td>
<td>1.91</td>
</tr>
<tr>
<td>Trehalase</td>
<td>4.11</td>
<td>3.69</td>
<td>3.27</td>
</tr>
<tr>
<td>Urease</td>
<td>22.4</td>
<td>21.8</td>
<td>21.3</td>
</tr>
</tbody>
</table>

- Expressed in millimicromoles of product/min/mg protein, except for fumarase, which is expressed as OD change/10 kc x 10^6.
- All enzymes were assayed from the same extract for each incubation time.

Ho C.C. Mutations blocking development of the protoperithecium in Neurospora.

The development of the protoperithecium or the female sexual organ in Neurospora, though essential for sexual development, is nevertheless dispensable for completion of the life cycle, due to alternative vegetative reproduction by conidia and vegetative hyphae. Mutants defective in the formation of protoperithecium are therefore valuable non-lethal developmental mutants in efforts to discover those genes that are responsible for the initiation of a developmental pathway. For genetic studies, these female-sterile mutants can be used or male parents to fertilize the protoperithecium from strains of opposite mating type.

Three classes of mutations blocking the different stages of the development of protoperithecium have now been obtained. All of them are spontaneous mutations. In most cases (except ty-1 and ty-2) only mutants with normal vegetative morphology and good growth rate were chosen, so that it can be certain that the mutation specifically affects the development of protoperithecium. It is already known that several morphological mutants, such as the modifiers of the colonial temperature-sensitive mutant (Terenzi and Reissig 1967 Genetics 56:321), have defective protoperithecium and are female sterile.

The modifications of the first group (f-1, f-2 and others) specifically prevent the formation of protoperithecium and have no effect on vegetative morphology or nutritional requirements. The f-1 mutation was mapped on the right arm of linkage group II between org-5 and tyr-3 (Tan and Ho 1970 Molec. Gen. Genet. 107:158). Another protoperithecium-less mutant also maps on linkage group II, but its precise location is not known. The location of f-2 is uncertain.

The second class of mutants (ty-1, ty-2) was first discovered by Westergaard, and the regulation of their tyrosinase synthesis was studied intensively (Horowitz et al. 1960 J. Mol. Biol.2: 96). They form a few small protoperithecia, which are generally defective in function. Rarely, a few of their protoperithecium can be mated to form perithecia. The mutant ty-1 has on abnormal vegetative morphology called "velvet", in that the aerial hyphae are short and bear few conidiophore. Velvet is inseparable from female sterility. Most ascospores of ty-1 are also probably lethal, as indicated by a large deficiency of ty-1 in the progeny of all crosses as determined by random ascospore analysis. The aerial hyphae of ty-2 are also shorter than those of the wild type. The mutant ty-1 was tentatively mapped by Walker (1963 Neurospora News1.3:15) near tyrosine-1 on the right arm of linkage group III. The present work confirms his result. The gene is located to the right of albino-2 on the right arm of linkage group 1. It is not allelic to the T locus (Horowitz and Ring 1956 Proc. Natl Acad. Sci. U.S.42:498), the structural gene of tyrosinase, which is proximal to al-2.