The use of cycloheximide as an inhibitor of protein synthesis in Neurospora

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The use of cycloheximide as an inhibitor of protein synthesis in Neurospora

Abstract
Cycloheximide as inhibitor of protein synthesis

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C. Notes: 1. Lyophilized material can be used only if the culture has been lyophilized immediately after harvesting. Frozen and thawed cultures which were extracted, or lyophilized and then extracted, gave variable results, possibly due to glycogen breakdown. Also aerobic cultures grown to the point of carbon-source exhaustion had lower levels of glucose-6-P. Therefore, only actively growing cultures can be used for the assay.

2. Extraction by cold 1 M HClO₄ (and subsequent neutralization with cold KOH) gave comparable results to the ethanol extractions, as did extraction with cold 10% TCA. In both cases the glucose-6-P could be assayed properly only after inhibitors were removed by chromatography.

3. Extracts are not concentrated to dryness since phosphorylated compounds occasionally adhere to glass surfaces.

4. Almost complete clarification can be obtained by 100,000 × g for 90 minutes.

5. Glucose-6-P in extracts tends to trail somewhat, particularly on paper which has not been treated with EDTA.

6a. Core must be taken to avoid small bits of paper in the eluate as they subsequently interfere with the assay.

7. Other sources occasionally contained significant amounts of 6-phosphogluconic acid dehydrogenase (thereby doubling all values obtained), as well as traces of isomerases.

8. It is not known in what form the glucose-6-P is isolated, so a molecular weight of 340 (i.e., Na₂glucose-6-P·2H₂O) was assumed.

Brody, al temperature-sensitive mutant strains for isolation of additional mutants at a given site.

For purposes of correlation with the results of studies on N. crassa strain col-2 (Y5331), it was desirable to isolate additional strains altered at col-2 locus and to examine the properties of their glucose-6-P dehydrogenases. However, the isolation of a particular colonial strain would involve numerous mutant hunts and extensive mapping and/or biochemical screening since the colonial phenotype can be due to mutation at many different genes (40 at least). The selection and isolation of temperature-sensitive revertants circumvented these problems. The rationale for this approach is as follows: revertants from a presumed point mutation in a structural gene may be due to mutations at the original site, elsewhere in the same gene, or at another locus. Quite often compensatory mutations at another site in the gene, the so-called second-rite revertants, lead to the production of temperature-sensitive proteins. Therefore, some of the temperature-sensitive revertants may be second-site revertants. Approximately 40 wild-type revertants were isolated at 25°C and 4 of these were found to be colonial at 35°C. One of these temperature-sensitive strains was the desired strain; i.e., it had a temperature-sensitive glucose-6-P dehydrogenase.

Another temperature-sensitive revertant, which proved to be a temperature-sensitive suppressor strain, was helpful in that it was used as a source of conidia for the inositol-less mutant isolation technique. Selection of mutants in most colonial strains is difficult due to the lock of conidiation. However, this difficulty is bypassed by harvesting conidia from this suppressor strain grown at 25°C and then performing the mutant selection at 35°C. Selection of certain mutants in colonial strains may be advantageous, since the altered metabolism of the colonial strains might not allow the growth of certain "leaky" mutants during inositol deprivation. * * * Rockefeller University, New York, New York. 10021.

Pall, M. L. The use of cycloheximide as an inhibitor of protein synthesis in Neurospora.

The antibiotic cycloheximide (Actidione) has been reported to be an inhibitor of protein synthesis in some fungi, higher animals, and higher plants. It is shown here to be an effective inhibitor of protein synthesis in Neurospora crassa.

Wild-type strain 69-1113a was grown for 2 days at 25°C in 20 ml of Vogel's Medium N + 2% sucrose. Cycloheximide was added and the flasks were shaken on a reciprocal shaker for one hour. They were then given a 12-minute pulse of 0.5 µCi ¹⁴C L-lysine. The mycelial pads were fractionated according to the procedure of Roberts, et al. (1955 Carnegie Inst. Washington Publ. 607: 207).

<table>
<thead>
<tr>
<th>Concentration of cycloheximide (µg/ml Vogel's medium)</th>
<th>Percent of counts token up incorporated into protein</th>
<th>Percent inhibition of protein synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>63.3 %</td>
<td>93.0 %</td>
</tr>
<tr>
<td>1</td>
<td>4.44 %</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.11 %</td>
<td>98.2 %</td>
</tr>
</tbody>
</table>

Cycloheximide has also been used to study the inducible enzyme tyrosinase. When ethionine is added during the log period, no activity develops. When it is added during the synthetic period, the activity remains at the level reached at the time of addition of the cycloheximide. These results are consistent with the idea that the tyrosinase is a novo protein synthesis and the cycloheximide inhibits any further synthesis of the enzyme. * * * Division of Biology, California Institute of Technology, Pasadena, California. 91109.