Cytidylytransferases in chol and wild type strains

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Abstract
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RESEARCH NOTES


The existence of the PE PMMAE CMP-PkMAE table phospholipid 2.a PE-PDH4AE CMP-PDH4AE CMP-PC 2.92 4.04 4.0, the CMP derivatives of the respective em.222:196). to lecithin biosynthesis, from CDPcholine and 1,2-diglyceride, was discovered in the same pathway was revealed by in vitro experiments with the wild type strain of N. crassa (Radominska-Pyrek et al., 1969 Acta Biochim. Polon. 16:357), as the synthesis of appropriate phosphobases was observed. The formation of cytidine biosynthesis, as the phospholipid fraction become significantly cytidine phosphates (unpublished results).

The present paper concerns the activities of cytidylyltransferases in the chol-1 and chol-2 strains of N. crassa as compared to those of the wild type strain. The metabolic blocks in the two cholineless mutants and the enzymic reactions studied are shown in Figure 1.

Figure 1: Two alternate pathways of lecithin (phosphatidylycholine) biosynthesis. The dashed arrows symbolize reactions which have not been studied yet in Neurospora. Abbreviations used: E = ethanolamine; MMAE = monomethylaminoethanol; DMAE = dimethylaminoethanol; C = choline. PE, PMMAE, PDMAE and PC are the phosphoric esters of the respective aminoethanols. CMP-PE, CMP-PMMMAE, CMP-PDMAE and CMP-PC are the CMP derivatives of the respective aminoalcohol phosphates.

The following strains were used: wild type 74-OR-23-IA, chol-1 (34484) and chol-2 (47904) A. Mycelia were grown at 30°C in liquid shake cultures in Vogel's minimal medium plus 1% sucrose. Where indicated (in Table 1), this was supplemented with choline chloride (0.2 mmole/liter medium) or dimethylaminoethanol (0.1 mmole/liter medium). The culture of the chol-2 strain in unsupplemented medium was grown for 3 days. All other cultures were grown for 2 days. The methods of determination of the activity of cytidylyltransferases used in the present report were described previously (Radominska-Pyrek et al., 1969 ibid.)

Table 1 shows the specific activities of cytidylyltransferases residing in the 105,000 x g supernatants of the wild type, chol-1 and chol-2 strains cultivated in different media.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>wild type</th>
<th>chol-1</th>
<th>chol-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>7.70</td>
<td>7.65</td>
<td>5.96</td>
</tr>
<tr>
<td>PMMAE</td>
<td>2.48</td>
<td>2.28</td>
<td>1.80</td>
</tr>
<tr>
<td>PDMAE</td>
<td>1.56</td>
<td>1.52</td>
<td>0.97</td>
</tr>
<tr>
<td>PC</td>
<td>2.84</td>
<td>4.04</td>
<td>3.20</td>
</tr>
</tbody>
</table>

Each entry in this table stands for nanomoles of nucleotide formed per mg protein/15 min.

Table 1 shows about 50% of PE- and PMMAE-cytidylyltransferase activities and only 6% of PDMAE-activities when compared with the wild type. The chol-2 strain grows in the supplemented medium has PE- and PMMAE-cytidylyltransferase activities only slightly lowered, whereas the PC-cytidylyltransferase activity, similarly as in the chol-1 strain, is slightly increased.

The results suggest that the limitation in amount of endogenous choline in the chol-2 strain of N. crassa, lowered markedly the activities of all cytidylyltransferases and, in particular, those after the block in the methylation pathway. The addition of exogenous choline for cultivation of this strain restored to a large extent the PE- and PMMAE-cytidylyltransferase activities and increased the activity of PC-cytidylyltransferase. Similar stimulation of PC-cytidylyltransferase activity was observed for the chol-1 strain.
The present results indicate that the system studied is not repressed by the choline or DMAE, but on the contrary, that these compounds could be the inducers of K- and PMMAE-cytidylyltransferase.

Louie, S., A. Chan and G. Soika. Serine-induced formation of oeriol hyphae and conidia by a Neurospora mutant.

Ser-2 (isolate 65004) is a very "leaky" serine brodytoph. It grows rapidly on minimal medium but does not form abundant oeriol hyphoe or pigmented conidio unless supplemented with L-serine. It also responds to glycine, but no other amino acid, or intermediate in the serine biosynthetic pathway, can substitute for serine. The addition of swine to cultures growing on solid media causes the mutant to form aerial hyphoe and pigmented conidio at approximately the same rate as do wild type strains on minimal media. This property was examined by comparing growth rates of ser-2 and a wild type strain (STA4), employing a variety of growth parameters.

Figure 1 shows the results of an experiment designed to compare the rate of hyphal elongation on solid Vogel's minimal medium (2% sucrose as carbon source). This method ignores penetration of hyphae into the agar and oeriol hyphae formation (Zolokar 1959 Am. J. Botany 46:555). Under these conditions STA4 and ser-2 show identical growth rates in the absence of serine.

When these strains are grown in Vogel's minimal liquid medium with vigorous agitation, dry weight increases logarithmically for at least 24 hours (Luck 1963 J. Cell Biol. 16:483). Formation of oeriol hyphoe and conidio is minimized in submerged culture, yet Figure 2 (which is representative of many such experiments) indicates that ser-2 grows more slowly than does STA4 under these conditions.

Growth in stationary liquid culture is essentially unrestricted and 3-dimensional (Marshall and Alexander 1960 J. Bacteriol. 80:412) and can best be expressed as the cube root of the increase in dry weight (Emerson 1950 J. Bacteriol. 60:221). After approximately two days of incubation, wild type organisms begin to form oeriol hyphoe above the mycelial mat. The appearance of these structures is delayed at least one week in ser-2. The defect can be completely overcome by addition of 0.1 ml serine to the growth medium. From Figure 3 it can be seen that ser-2 and STA4 on minimal and serine-supplemented media hove--lo, growth rates for approximately 2 days, while the mycelial mat is being formed across the surface of the liquid. The failure of ser-2 cultures on minimal medium to form aerial hyphoe results in

Figure 1. Hyphal elongation in solid medium. Growth tube culture at 30°C in constant darkness on Vogel's minimal medium. (STA4 open circles; ser-2 darkened circles).

Figure 2. Logarithmic growth in minimal liquid medium. Cultures grown at 30°C in constant darkness. 30 ml. Vogel's minimal medium in 125 ml. Erlenmeyer flasks agitated at 150 rpm.