Isocitrate lyore-2 from N. crassa

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Abstract
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DNA "uptake" at "0" time of incubation depends upon the concentration of DNA in the incubation medium. From a DNA solution of 10 µg/ml, 0.2-0.4 µg DNA/mg dry weight of mycelium is taken up at "0" time. This may increase 2-10 fold during 60 minutes of incubation. There are tenfold differences between the values of the absolute amount of DNA taken up at "0" time, depending upon various factors of cultivation and incubation. Therefore, the difference between radioactivity of samples removed after 0 and 60 minutes' incubation, respectively, is taken as the measure of the continuing rate of transport of DNA into the cell interior. DNA uptake is dependent upon the physiologic state of the culture. Mycelial fragments from 7-day-old agar slants or 72-hour-old submerged cultures take up DNA very poorly or not at all. The best uptake was seen with young (20-hour-old) mycelia; on B-10 fold increase of the "zero" minute value was obtained.

There is no continuing DNA uptake at 4°C; sodium azide (10⁻² M) also blocks DNA uptake. The uptake of high molecular weight DNA is much better than that of its DNAse-digested fragments. There is no continuing uptake if DNA digested with DNase (30 µg/ml in 5 x 10⁻³ M MgCl₂ for 30 min) is present at 27°C for one hour in the incubation mixture instead of native DNA. The uptake of high molecular weight DNA is not inhibited by the addition of 10 µg/ml non-radioactive digested DNA (30 µg/ml DNase, 30 min at 27°C); 75 µg/ml of this preparation is required to decrease intact DNA uptake by about 50%, whereas cold intact DNA molecules, even at such a low concentration as 10 µg/ml, decrease uptake to 50% of the control. Increasing the DNA concentration from 0.02 µg/ml to 1.0 µg/ml in the incubation mixture led to proportionately higher values of uptake. The pH of the incubation medium influences DNA uptake. There is no continuing uptake at pH 7.3 or above; on optimum was found between pH 7.5 and 6.7. EDTA does not influence uptake. There is no continuing uptake at 10⁻⁴ M but inhibits it at 10⁻³ M or 10⁻² M concentrations. Mg²⁺ and Co²⁺ ions have a slight enhancing effect upon DNA uptake.

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Figure 1. Elution profile of DEAE-cellulose column loaded with non-purified homogenate from on acetate-derepressed culture. Left ordinate: rate of formation of phenylhydrazone measured at 324 nm and expressed as µmoles of glyoxylate-phenylhydrazone/hour/fraction. Right ordinate: amount of o-KG/fraction measured by enzymatic analysis.

In an attempt to study the kinetic properties of IL-2, acetate-derepressed cultures of N. crassa strain Lindegren (+A) (FGSC # 853) were homogenized in a medium containing 10 mM Tris-HCl (pH 7.8), 1 mM EDTA, 2.5 mM MgCl₂ and 1 mM mercaptoethanol (TEM buffer). The homogenate was clarified by centrifugation at 500 x g (10 min) and 48,000 x g (45 min). The supernatant of the last centrifugation was processed by either of two methods. Method A: the clarified homogenate was loaded on a DEAE-cellulose column and eluted with a linear gradient of TEM buffer (0-300 mM in Tris). Method B: IL was precipitated from the homogenate by (NH₄)₂SO₄ (50-75% saturation), desalted in a Sephadex G-25 column, adsorbed on a DEAE-cellulose column, and eluted as described above. IL activity was measured by following the rate of appearance of the glyoxylate-phenylhydrazone at 324 nm (Kobr et al. 1969 Biochem. Biophys. Res. Commun. 37: 640).

The elution pattern obtained by Method A exhibits two peaks of apparent IL activity (Figure 1). This result duplicates the data of the literature (Sjogren and Romano; Flavell and Woodword 1971 J. Bacteriol. 105: 200). However, the activity profile obtained by Method B exhibits one peak only, corresponding to IL-1. Whatever the conditions of preliminary purification, only IL-1 can be eluted from the ion-exchange column.

A kinetic study of the fractions corresponding to IL-2 revealed that the formation of a phenylhydrazone in this portion of the gradient is independent of isocitrate, Mg⁺⁺, EDTA and/or SH. These fractions were found to contain almost exclusively α-keto-glutaric acid (a-KG). This α-keto acid is also present in crude extracts from acetate derepressed cultures. a-KG was identified by: (1) TLC on silica gel G in either: n-butanol/acetonic acid/H₂O (4:1:1, v:v:v), or: ether/formic acid (7:1, v:v); (2) UV spectra of phenylhydrazones or semicarbazones; (3) enzymatic analysis with 0.12 mM NADPH, excess NH₄⁺ and commercial glutamic dehydrogenase. From Figure 1 it appears that the distribution of a-KG closely parallels the second peak of IL activity (IL-2). Furthermore, exogenous o-KG added to homogenate of derepressed (sucrose-grown) culture, is eluted as a second peak from the DEAE-cellulose column.
There observations show that the increase of absorbancy detected in the middle port of the elution gradient (150 mM in Tris) does not result from an enzymatic catalysis, but rather from a chemical condensation with phenylhydrazine of the α-KG eluted from the column. Even though they do not rule out the possibility of the existence of IL-Z, our results indicate the need for re-evaluation of the occurrence of isoenzymes of IL and of the conclusions regarding their role in N. crassa.

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Sagarría, E. Esterase polymorphism in Neurospora.

<table>
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<th>Strain designation</th>
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The comparative study of esterases of ten wild strains of Neurospora has been carried out. The strains used were received from the FGSC and are listed in Table 1.

Mycelial extracts were submitted to acrylamide gel electrophoresis and esterases were revealed with 4 and 6-naphthyl acetate. In the study of the zymogram, it was possible to distinguish three groups of strains with different mobilities for the esterases. Considering all strains together, fourteen bands were observed for these enzymes. The sites of bands have been numbered from 1 to 14. The number 1 corresponds to the band of greatest mobility towards the anode, and the number 14 to the bond closest to the origin. (Figure 1)

Figure 1.

The strains Honduras a, Java a, Panama a, Panama A, Fiji N6-6 A, and Fiji N6-1a, present a rapid zone of great esterase activity formed by bands 6, 7 and 8; Panama A also possesses a weak band in position 14. Strains Liberia-4 A and North Africa A have three bands of heavy activity (9, 11 and 13), bond 6 is weak and bond 3 is very weak. Strain Costa Rica A contains bands 10, 12 and 14; bond 6 and band 5 are very weak. Strain Singapore-2 A has bands 11 and 13 of heavy activity, whereas bands 6 and 8 are much weaker, as are also bands 1 and 2. In all strains there is a zone of heavy esteratic activity formed by three bonds. This zone seems to be composed of two bands only in strain Singapore-2 A. All strains present activity in site 6.

This esterase polymorphism is present in both wild type strains of N. crassa and among Neurospora strains of uncertain species collected in nature. It is a strain Singapore-2 A that shows the most differentiation upon comparison with the rest of the strains analyzed. Crosser between the different strains lead us to conclude that four independent systems of isoenzymes exist. Each one is controlled by two alleles, except the system which manifests the bonds of greatest activity, which would be controlled by three.

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Tsai, J. H.J. and H. Tsai. Localization of Neurospora ornithine aminotransferase in mitochondria.