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Isocitrate lyore-2 from N. crassq.

Growth of N. crassa on a medium supplemented with acetate brings about a derepression of isocitrate lyase (IL) (Turian 1960 Bull. Soc. Bot. Suisse 70: 451). Two forms of the enzyme (IL-I ond IL-Z) hove been separated by ion-exchange chromatography of cell-free homogenates obtained from derepressed cultures (Sjogren and Romano 1967 J. Bacteriol. 93: 1638).

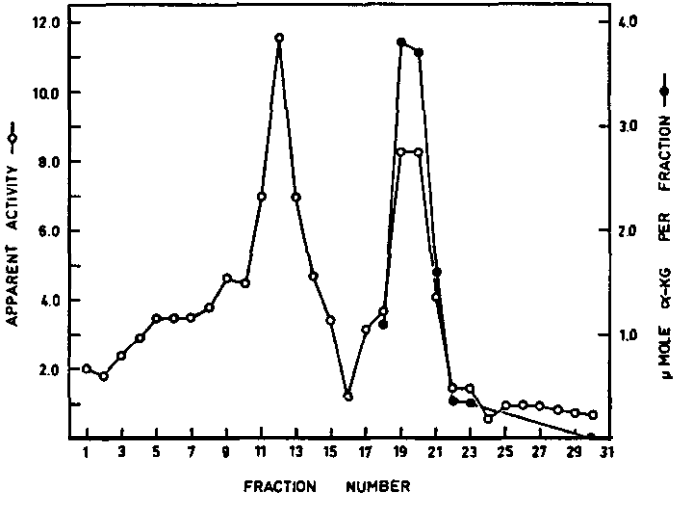

Figure 1. Elution profile of DEAE-cellulose column loaded with non-purified homogenate from on acetate-derepressed culture. Left ordinate: rote of formotion of phenylhydrazone measured at 324 nm and expressed as μmoles of glyoxylate-phenylhydrazone/hour/fraction. Right ordinate: amount of o-KG/fraction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

In on attempt to study the kinetic properties of IL-2, acetate-derepressed cultures of N. crassa strain Lindegren (+)A (FGSC# 853) were homoaenized in a medium containing IO mM Tris-HCl (pH 7.8), 1 mM EDTA, 2.5 mM $MgCl_2$ and 1 mM mercaptoethanol (TEM buffer). The homogenate was clarified by centrifugation at 500 x g (IO min) and 48,000 x g (45 min). The supernatant of the last centrifugotion 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 (Io-300 mM in Tris). Method B: IL was precipitated from the homogenate by $(NH_4)_2SO_4$ (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 rote of appearance of the glyoxylate-phenylhydrazone at 324 nm (Kobr et al. 1969 Biochem. Biophys. Rer. 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 ond Woodword 1971 J. Bacteriol. 105: 200). However, the activity profile obtained by Method B exhibits one peak only, corresponding to IL-I. Whatever the conditions of preliminary purification, only IL-I con 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-ketoglutaric acid (a-KG). This keto-acid is also present in crude extracts from acetate derepressed cultures. o-KG was identified by: (1) TLC on silica gel G in either: n-butanol/acetic acid/H20 (4:1:1, 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 NH_4^+ and commercial glutamic dehydrogenase. From Figure 1 it appears that the distribution of α-KG closely parallels the second peok of IL activity (IL-2). Furthermore, exogenous o-KG added to a homogenate prepared from a repressed (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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