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Glyoxylate cycle mutants in Neurospora

Abstract

Glyoxylate cycle mutants in Neurospora

Flavell, R. B. Glyoxylate cycle mutants in Neurospora.

Acetate is metabolised in microorganisms via the glyoxylate cycle. The steps of the glyoxylate cycle which are not part of the citric

acid cycle are those involving the conversion of isocitrate to glyoxylate ond succinate and the synthesis of malate from glyoxylate and acetyl-CoA. These two steps require the enzymes isocitrate lyase and malate synthetase, respectively. The presence of isocitrate lyase in Neurospora grown on acetate has previously been reported by Turian (1962 NN#1:6). Before acetate is metabolized it is firstly converted to its metabolically active form, acetyl-CoA, by the enzyme acetic thiokinase. All enzymes of the glyoxylate cycle are repressed when Neurospora is grown on sucrose.

Neurospora, grown up at 30°C an a shaker in Vogel's minimal medium with 1.5% sucrose as sole carbon source, harvested, washed and then shaken at 30°C in Vogel's minimal medium with 50 mM acetate as sole carbon source, showed maximum specific activities of isocitrate lyase, malate synthetase, and acetic thiokinose approximately 7 hours after transfer to acetate medium. Isocitrate lyase and malate synthetase showed a 20-fold induction and acetic thiokinase a IO-fold induction.

Mutants have been isolated (using N-methyl-N'-nitro-N-nihoroguanidine **as** a mutagen followed by filtration enrichment) which cannot grow on acetate but which can grow on sucrose. These mutants fall into 6 distinct complement&ion groups where all mutants of each group complement all members of other groups. No within-group complementation has been observed.

The mutants were grown up on sucrose medium, transferred to acetate medium for 7 hours as outlined above and assayed for the presence of the glyoxylate cycle enzymes.

Members of one complementotion group appear to lack completely isocitrate lyase activity or have only low levels of it and gre being considered as mutants of the structural gene for this enzyme. Two other groups can be tentatively regarded as consisting of structural gene mutants for malate synthetase and acetic thiokinase, respectively.

Mutants in a fourth complementation group possess all the glyoxylate cycle enzymes, inducible by acetate as in the wild type. The metabolic deficiency which results in these mutants being unable to grow on acetate seems obscure at present.

In the mutants in a fifth complementotion group the enzymes of the glyoxylate cycle ore not induced by transfer to acetate; all mutants possess the low levels of enzyme activities typical of the repressed state on sucrose. These mutations ore recessive in heterocaryonr. Investigations to see if there is an acetate permease deficiency here are being carried out.

The sixth complementation group contains mutants in which isocitrate lyase is not induced by acetate but malate synthetase and acetic thiokinase are. After transfer to acetate, isocitrate lyase specific activities remain at the low sucrose-grown levels. Presumably these mutants lack a gene-determined, cytoplasmic product necessary for the induction of isocitrate lyase. The mutant gene is recessive to its wild type allele in heterocaryons.

The presumed structural gene for isocitrate lyore has been mapped between pab-2 and asp on linkage group 'JR.

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