MUTANTS OF *ASPERGILLUS NIDULANS* LACKING MALATE SYNTHASE

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1. Introduction

The enzymes isocitrate lyase and malate synthase are unique to the operation to the glyoxylate cycle, which functions anaplerotically during growth on acetate [1]. Although these enzymes have been studied in a number of fungi [2–4], little is known of their regulation in these eukaryotic organisms.

We have previously described [5] mutants of *Aspergillus nidulans* lacking isocitrate lyase activity, and have shown that this enzyme is necessary for growth upon acetate. It is the purpose of this paper to describe mutants of *A. nidulans* that lack malate synthase activity and to show that malate synthase is also necessary for growth upon acetate as sole source of carbon. The malate synthase-less mutants have been allocated to a gene designated *mas* which is located in linkage group I of *A. nidulans*, whereas the structural gene for isocitrate lyase maps in linkage group V [5]. Thus the two genes are unlinked, although the enzymes show coincident regulation.

2. Experimental procedures

Media and routine procedures for genetic analysis in *Aspergillus nidulans* were those described by Pontecorvo et al. [6]. Cultures for the determination of enzyme activities were grown in a defined liquid minimal medium [7]. Carbon sources and magnesium sulphate were sterilised and added separately. Cultures were started by inoculation with suspensions of washed conidia to give 10^6 conidia/ml of medium, and incubated at 37° with vigorous rotation of a gyrotary shaker.

Mycelium was harvested by filtration, washed with distilled water and resuspended in buffer, pH 7,5, containing 20 mM tris, 2 mM $MgCl_2$ and 1 mM EDTA. Cell-free extracts for enzyme assays were prepared, and their protein contents were measured, as previously described [5]. Malate synthase activity was assayed as the rate of release of coenzyme A.SH when extracts were incubated with acetyl-coenzyme A and glyoxylate [8].

3. Results and discussion

3.1. *Malate synthase activity in mycelium grown upon different carbon sources*

Low malate synthase activity was found in wild-type *A. nidulans* grown upon sucrose whereas this enzyme was present in high activity after growth on acetate (table 1). Malate synthase, like isocitrate lyase [5], is thus induced by growth on acetate. Growth of mycelium upon a mixture of acetate and glucose results in a malate synthase activity as high as that found on acetate alone, but mycelium grown on acetate and sucrose has about half this amount. A similar response was also observed for isocitrate lyase in mycelium grown upon these mixtures [5], and both sets of data are compatible with the observation that *A. nidulans* utilises acetate preferentially from a mixture of acetate and glucose but not from acetate with sucrose [9]. The activities of these two enzymes of the glyoxylate cycle in *A. nidulans* are thus coincident, indicating a common control mechanism.

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Table 1
Malate synthase activity after growth on different carbon sources.

Carbon source	Concn. (M)	Time of harvesting (hr after inoculation)	Specific activity*
Acetate	0.1	24	4.0
Sucrose	0.02	18	0.1
Acetate and Sucrose	0.1 0.02	18	1.4
Acetate and Glucose	0.1 0.02	18	3.7

* Malate synthase activity was measured as the rate of glyoxylate dependent release of CoA-SH from acetyl-coenzyme A, in the presence of the enzyme and glyoxylate [8]. Specific activities are expressed as μmoles of -SH formed/mg of protein/hr.

3.2. *Mutants lacking malate synthase activity*

Mutants unable to utilise acetate for growth were isolated by filtration enrichment and replica-plating following ultraviolet irradiation. Among 93 such mutants, which grew normally on sucrose but did not grow on acetate alone, 9 had no detectable malate synthase activity after growth on a mixture of acetate and sucrose. All of these mutants were non-complementary in heterokaryon tests, indicating loss of the same function; they all map very closely to the *bi-1* (biotin) locus in linkage group 1 and identify a single gene *(mas)* which we interpret as the structural gene for malate synthase. The *mas* gene is unlinked to the structural gene for isocitrate lyase (*icl*), which is located in linkage group V [5]. Both genes are distinct from the *fac A*, *fac B* and *fac C* loci described by Apirion [10], which confer resistance to fluoroacetate; such *fac* mutants also grow very poorly on acetate. However, *fac* mutants contain both malate synthase and isocitrate lyase, albeit in low activity; *mas* mutants and *icl* mutants retain the wild-type sensibility of fluoroacetate.

The *mas* mutants from normal levels of isocitrate lyase when grown upon acetate with sucrose, and similarly the *icl* mutants have normal malate synthase activity. This latter observation argues against a sequential induction of malate synthase by the glyoxylate formed by isocitrate lyase.

3.3. *Reversion of mas mutants and the role of malate synthase*

Spontaneous revertants, able to grow on acetate, were selected from 4 different *mas* mutants; of these, 26 were tested for malate synthase activity after growth on acetate. All were found to have regained the enzyme, though some had less activity than wild-type; this supports the view that *mas* specifies a structural gene for malate synthase.

Together with the results of studies on the rate of isocitrate lyase [5], the present observations on malate synthase confirm that both these component enzymes of the glyoxylate cycle are necessary for the growth of *A. nidulans* upon acetate. Two main differences are apparent between the genetic control of this anaplerotic sequence in *E. coli* and in *A. nidulans*. Whereas the bacterium forms two types of malate synthase [11], *A. nidulans* forms only one; whereas in *E. coli*, the structural genes for isocitrate lyase and one of these malate synthases are co-transducible [12] in *A. nidulans* there is no close genetic linkage.

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