The Effects of the Carbon Source on Glutamate Dehydrogenase Activities in *Aspergillus nidulans*

By M. J. HYNES

*Department of Genetics, La Trobe University,
Bundoora, Victoria 3083, Australia*

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SUMMARY

The NADP-specific glutamate dehydrogenase (NADP-GDH) activity of *Aspergillus nidulans* was rapidly lost from cultures starved for a carbon source. This loss of NADP-GDH was blocked by protein synthesis inhibitors. Glutamate repressed NADP-GDH but did not cause rapid loss of activity. Since NADP-GDH is involved in the participation of ammonium in the regulation of nitrogen metabolism, the loss of NADP-GDH activity accompanying carbon starvation may be important in the interaction between carbon and nitrogen metabolism. Increased NAD specific glutamate dehydrogenase activity (NAD-GDH) was observed when mycelium was transferred to medium lacking glucose. The increase in NAD-GDH activity was greatest when glutamate was present. Protein synthesis inhibitors did not prevent this increase in activity.

Two mutants, *amdT102* and *amdT19*, which are altered in regulation of nitrogen metabolism, are similar to the wild-type strain with regard to regulation of NADP-GDH and NAD-GDH.

INTRODUCTION

Mutants in a gene, *amdT*, in *Aspergillus nidulans* pleiotropically affect the utilization of many nitrogen sources (Hynes, 1973*a*). The utilization of L-glutamate is affected in *amdT* mutants. One mutant, *amdT19*, is unable to utilize glutamate as a sole nitrogen source in the presence of glucose, but grows normally on glutamate as the sole source of carbon and nitrogen. Another mutant, *amdT102*, grows slightly more strongly than wild-type strains on glutamate as the sole nitrogen source in the presence of glucose and is normal on glutamate as the sole carbon and nitrogen source. This has led to a study of the effects of the carbon source on glutamate metabolism. This paper describes the effects of the carbon source on glutamate dehydrogenase activities in the wild type as well as *amdT* mutants. Studies on glutamate uptake will be reported elsewhere (Hynes, 1973*b*).

METHODS

Strains, media and genetic techniques. These have been described in previous papers (Hynes, 1970, 1972).

Growth of mycelium. Mycelium was grown as described previously (Hynes, 1972). In some experiments 20 to 30 ml of mycelium was sampled at various times and harvested by the normal method.

Preparation of cell-free extracts. Crude cell-free extracts were made in 10^{-1} M-tris (hydroxy methyl) amino methane (tris)-hydrochloride buffer (pH 8.5) by the method of Hynes (1972).

Enzyme assays. Nicotinamide adenine dinucleotide phosphate (NADP)-specific glutamate dehydrogenase (NADP-GDH) was assayed by following the glutamate-dependent reduction of NADP⁺ at 340 nm. The method of Pateman (1969) was followed except that double the concentration of glutamate was used.

Nicotinamide adenine dinucleotide (NAD)-specific glutamate dehydrogenase (NAD-GDH) was assayed by following the 2-oxoglutarate-dependent oxidation of reduced NAD at 340 nm. The method was based on that of Kapoor & Grover (1970) and H. Arst (personal communication). The reaction mixture contained: 1.0 ml 10⁻¹ M-tris-hydrochloride buffer (pH 8.5), 1.8 ml water, 6.6 mg (NH₄)₂SO₄, 0.5 mg reduced NAD, and 0.1 ml crude extract. The assay was begun by adding 0.1 ml of 2-oxoglutarate (14.6 mg/ml). The assays were carried out at 25 to 28 °C in a Beckman DB spectrophotometer. All NAD-GDH assays of mycelium were performed within 48 h of harvesting as there were indications that the enzyme was unstable under the conditions employed for storing harvested mycelium (−12 °C).

RESULTS

It has been found that the presence or absence of glucose in the growth medium has profound effects on both NADP-GDH and NAD-GDH activities.

NADP-GDH specific activity was found to be maintained in medium containing glucose. Pateman (1969) has presented evidence for repression of NADP-GDH by glutamate in *Aspergillus nidulans*. It was found that incubation in glucose–25 mM-glutamate medium did not lead to rapid loss of enzyme activity, but rather to an activity consistent with cessation of enzyme synthesis and dilution of preformed enzyme during subsequent growth.

NADP-GDH activity was rapidly lost in medium lacking glucose (Fig. 1, Table 1). This was most rapid when glutamate was present. The protein synthesis inhibitors, cycloheximide and acriflavin, prevented this rapid loss of activity, suggesting the possibility that an inactivating protein (possibly a protease) is active in the absence of glucose. J. R. Kinghorn (personal communication) has also observed this rapid loss of NADP-GDH. Preliminary results indicate that glutamine synthetase activity is rapidly lost with carbon starvation. Another enzyme of nitrogen metabolism, nitrate reductase, also rapidly decreases with carbon starvation, but this loss of activity is only slightly inhibited by cycloheximide (Hynes, 1973c). In other fungi and higher eukaryote systems enzyme inactivation has been observed (Ferguson, Boll & Holzer, 1967; Ferguson & Sims, 1971; Katsunuma, Schött, Elsässer & Holzer, 1972; Lewis & Fincham, 1970; Subramanian & Sorger, 1972; Schimke & Doyle, 1970).

A basal NAD-GDH specific activity was found under all growth conditions. This activity increased approximately five- to eightfold during growth on glutamate as the sole carbon and nitrogen source. A smaller increase (three- to fourfold) in activity occurred in medium lacking a carbon source with ammonium present. Only a very slight increase occurred in medium lacking both carbon and nitrogen sources and no increase in NAD-GDH occurred in the presence of glucose. Surprisingly it was found that cycloheximide and acriflavin did not inhibit the increase in NAD-GDH specific activity as a result of the absence of glucose (Fig. 1, Table 1). In fact, acriflavin may have caused some increase in activity. Acriflavin and cycloheximide were certainly effective under these conditions because they simultaneously inhibited the loss of NADP-GDH activity. These results suggest that protein synthesis is not required for the increase in NAD-GDH activity. There is an apparent anomalous result in the lack of effect of the inhibitors on the increase in NAD-GDH activity in medium in which glutamate is the sole carbon and nitrogen source. It might have

Fig. 1. NAD-GDH and NADP-GDH specific activities of the wild-type strain. Wild-type mycelium was grown for 18 h in glucose- 0.5×10^{-2} M-urea medium and then transferred to various media. Samples for enzyme assay were taken at intervals. ●, No carbon or nitrogen source; ○, 10^{-2} M-glutamate; ▲, 10^{-2} M-glutamate + cycloheximide ($10 \mu\text{g/ml}$) – all added to minimal salts solution. NAD-GDH specific activity expressed as nmol reduced NAD oxidized/min/mg protein. NADP-GDH specific activity expressed as nmol NADP^+ reduced/min/mg protein.

Table 1. Glutamate dehydrogenase activities of wild-type strain (*amdT*⁺)

Growth conditions*	Specific activities	
	NAD-GDH†	NADP-GDH‡
Glucose + 10^{-2} M- NH_4^+	26	311
No carbon source + 10^{-2} M- NH_4^+	92	78
No carbon source + 10^{-2} M- NH_4^+ + cycloheximide ($15 \mu\text{g/ml}$)	65	267
No carbon source + 10^{-2} M- NH_4^+ + acriflavin ($15 \mu\text{g/ml}$)	93	154
Glucose + 10^{-2} M-glutamate	26	250
Glucose + 10^{-2} M-glutamate + cycloheximide ($15 \mu\text{g/ml}$)	24	307
Glucose + 10^{-2} M-glutamate + acriflavin ($15 \mu\text{g/ml}$)	76	312
10^{-2} M-glutamate	217	28
10^{-2} M-glutamate + cycloheximide ($15 \mu\text{g/ml}$)	131	406
10^{-2} M-glutamate + acriflavin ($15 \mu\text{g/ml}$)	171	447
Glucose + no nitrogen source	23	343
No carbon or nitrogen source	47	42
No carbon or nitrogen source + cycloheximide ($15 \mu\text{g/ml}$)	46	455
No carbon or nitrogen source + acriflavin ($15 \mu\text{g/ml}$)	77	398

* Mycelium was grown for 16 h on glucose- 10^{-2} M- NH_4^+ medium and then transferred to this medium for 4 h.

† Expressed as nmol of reduced NAD oxidized/min/mg protein.

‡ Expressed as nmol of NADP^+ reduced/min/mg protein.

been expected that glutamate uptake capacity would not develop under these conditions, and NAD-GDH activity would be similar to that on medium lacking both carbon and nitrogen sources. A tentative explanation of this result is that the inhibitors, by blocking protein synthesis, lead to intracellular accumulation of amino acids, including glutamate.

Table 2. *Comparison of glutamate dehydrogenase activities of amdT mutants*

Growth conditions*	Specific activities†					
	<i>amdT</i> ⁺		<i>amdT</i> 19		<i>amdT</i> 102	
	NAD-GDH	NADP-GDH	NAD-GDH	NADP-GDH	NAD-GDH	NADP-GDH
Glucose + 10^{-2} M-NH ₄ ⁺	34	315	46	372	44	360
No carbon source + 10^{-2} M-NH ₄ ⁺	111	55	94	52	110	42
Glucose + 10^{-2} M-glutamate	23	216	34	322	23	185
10^{-2} M-Glutamate	150	27	181	24	161	21

* Mycelium was grown for 16 h on glucose- 10^{-2} M-NH₄⁺ medium and then transferred to this medium for 4 h.

† Units of specific activity as for Table 1.

The glutamate dehydrogenase activities of *amdT*⁺, *amdT*19 and *amdT*102 strains are compared in Table 2. There were no apparent differences between the strains in NAD-GDH activities. Similarly, the NADP-GDH activities of the *amdT* mutants were similar to wild type. The only evident difference occurred in strains grown on glucose-10⁻² M-glutamate. NADP-GDH activity of the *amdT*19 strain was higher than that of the *amdT*⁺ and *amdT*102 strains in this medium. This can be explained by greatly reduced glutamate uptake capacity in the presence of glucose in *amdT*19 (Hynes, 1973*b*) and the consequent inability to grow on glutamate in the presence of glucose. Therefore little repression of NADP-GDH by glutamate and dilution of enzyme by growth was possible in the *amdT*19 mutant.

DISCUSSION

The isolation of mutants of *Aspergillus nidulans* lacking each of the glutamate dehydrogenases has provided clear evidence for the NADP-GDH being responsible for amination of 2-oxoglutarate (i.e. a biosynthetic role), and NAD-GDH being responsible for the deamination of glutamate (i.e. a catabolic role) (Kinghorn & Pateman, 1973; Arst & MacDonald, 1973). Compatible with these functions are the repression of NADP-GDH by glutamate and the increased activity of NAD-GDH in medium containing glutamate as the sole carbon source.

In mutants deficient in NADP-GDH activity in both yeast and *Aspergillus nidulans* a number of enzymes and uptake systems have been found to have reduced sensitivity to the effects of ammonium (Dubois, Grenson & Wiame, 1973; Arst & MacDonald, 1973; Pateman, Kinghorn, Dunn & Forbes, 1973). It has therefore been suggested that NADP-GDH is necessary for ammonium repression of these enzymes and uptake systems. Starving *A. nidulans* for a carbon source leads to relief of ammonium repression of the acetamidase enzyme (Hynes, 1970) and glutamate uptake (Hynes, 1973*b*). The utilization of acetamide and glutamate as sole carbon sources is not inhibited by ammonium. The rapid loss of NADP-GDH activity upon transfer to medium lacking glucose provides an important factor in proposing explanations of these results. When glucose is absent, NADP-GDH is rapidly lost (degraded) and this leads to relief of the effects of ammonium. However, Arst & Cove (1973) have evidence, supported by results from this laboratory, for additional regulatory mechanisms which are active when glucose is absent.

The *amdT*19 and *amdT*102 mutants seem to be normal in their regulation of glutamate dehydrogenase activities. Therefore the pleiotropic effects of these *amdT* lesions on nitrogen source utilization do not appear to result from alterations in amination or deamination of 2-oxoglutarate. This is particularly significant in view of the recent finding that the *amdT* locus is involved in ammonium repression (Arst & Cove, 1973). Recent results indicate that glutamine synthetase activities are not significantly altered in the *amdT* mutants.

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