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The regulation of NAD L-glutamate dehydrogenase in *Aspergillus nidulans*

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SUMMARY

Wild-type cells of *Aspergillus nidulans* have undetectable NAD L-glutamate dehydrogenase activity when utilizing glucose and high levels of NAD L-glutamate dehydrogenase when utilizing certain amino acids as sole carbon sources.

A mutant, designated *gdhC1*, has appreciable NAD-GDH activity when utilizing glucose as a carbon source. The *gdhC1* mutation is semi-dominant and is located in linkage group III.

1. INTRODUCTION

Many catabolic enzymes in fungi and bacteria are repressed by glucose or a metabolite derived from glucose. The phenomenon is known as 'the glucose effect', 'glucose repression' or 'catabolite repression' (Magasanik, 1961). Although relatively well documented for bacteria, e.g. galactosidase (Cohn & Monod, 1953), histidase (Neidhardt & Magasanik, 1956, 1957), inositol dehydrogenase (Magasanik, 1961) and certain systems responsible for amino acid oxidation (Jacoby, 1964), there are comparatively few instances of catabolite repression in eucaryotes. Perhaps the best studied system is the glyoxylate pathway in *Neurospora crassa* (Flavell & Woodward, 1971). The glyoxylate shunt enzymes isocitrate lyase and malate synthase are repressed when grown with glucose but derepressed with acetate as the sole carbon source.

In *Aspergillus nidulans* Hynes & Pateman (1970) found that some compounds such as acetamide are capable of being utilized as a carbon and nitrogen source. The enzyme responsible for the utilization of acetamide, acetamidase is repressed by glucose or metabolic products derived from glucose as well as being repressed by ammonium. Moreover, the studies of Cohen (1972, 1973) have revealed that extracellular protease is repressed by either carbon or ammonium or sulphur.

We report that L-glutamate NAD oxidoreductase E.C. 1.4.1.2 (NAD-GDH) is subject to catabolite repression, but unlike acetamidase and extracellular protease, NAD-GDH appears to be free from ammonium control. Some of the properties of a mutant, designated *gdhC1*, which appears to be abnormal with respect to carbon control, are described.

2. MATERIALS AND METHODS

Materials, supplements, growth of mycelium, L-glutamate dehydrogenase assays, protein estimation and genetic analysis were as described in a previous communication (Kinghorn & Pateman, 1973). Nitrogen less minimal medium (–N medium) (Cove, 1966) and carbon and nitrogen less minimal medium (–CN medium) were used. *xprD1* is an

ammonium derepressed mutant obtained by selecting for ammonium derepression of extracellular protease production (Cohen, 1972), kindly supplied by B. L. Cohen.

(i) *Selection of* gdhC1

Clumps of NTG-treated conidia (Adelberg, Mandel & Chen, 1965) of *biI puA2* were inoculated on to solid –N medium plus biotin, putrescine and 10 mM L-glutamate as the sole nitrogen source. A sector showing better growth than the wild-type was isolated, purified and the resultant strain designated *gdhC1*.

3. RESULTS

(i) *NAD-GDH activity in wild-type cells grown with various nitrogen sources*

Wild-type cells of *Aspergillus nidulans* grown with glucose and any one of ammonium, nitrate, L-glutamate, L-aspartate, L-alanine or casamino acids as the sole nitrogen source, possessed very low NAD-GDH activity. A similar result was obtained when wild-type cells were deprived of nitrogen for periods of up to 6 h.

(ii) *NAD-GDH activity in wild-type cells grown with various carbon sources*

The results presented in Table 1 show NAD-GDH levels in wild-type cells grown with various carbon sources. There was low activity in cells grown with 1 % glucose, but appreciable levels with 1 % acetate. L-glutamate, L-aspartate and L-alanine are relatively poor carbon sources especially when used in shake flask culture. Consequently, 0·1 % glucose was added to supplement these amino acids as carbon sources and high levels of NAD-GDH were found with all three amino acids as the main carbon and nitrogen source. High levels of activity were also found in cells grown with amino acids as the main carbon source and 10 mM ammonium added. The highest NAD-GDH activity was found in cells grown on 1·5 % casamino acids as the sole carbon and nitrogen source. This level was not significantly changed by the presence of 10 mM ammonium.

Table 1. *NAD-GDH activity in wild-type cells grown with various carbon sources*

Growth conditions	NAD-GDH activity (nmoles/min/mg protein)
–CN medium +	
1 % glucose + 10 mM ammonium	< 100
1 % acetate + 10 mM ammonium	850
0·1 % glucose + 10 mM L-glutamate	1855
0·1 % glucose + 10 mM L-aspartate	1620
0·1 % glucose + 10 mM L-alanine	2050
0·1 % glucose + 10 mM L-glutamate + 10 mM ammonium	1955
0·1 % glucose + 10 mM L-aspartate + 10 mM ammonium	1760
0·1 % glucose + 10 mM L-alanine + 10 mM ammonium	1830
1·5 % casamino acids	2740
1·5 % casamino acids + 10 mM ammonium	2675

(iii) *NAD-GDH levels in cells held in the presence of various carbon sources*

When wild-type cells, after growth on –N medium with 10 mM ammonium, were carbon-starved they developed low levels of NAD-GDH activity which were maximal after 3 h (Table 2). The level of activity decreased again if the carbon-starvation was continued to 6 h. If, instead, the cells were transferred to 100 mM L-glutamate or L-aspartate or L-alanine or 1·5 % casamino acids, they developed high activity.

(iv) *Genetic characterization of* gdhC1

Haploidization of the diploid between *biI puA2 gdhC1* and master strain *F* (McCully & Forbes, 1965) yielded segregants which showed free assortment between *gdhC1* and all markers except *galA1* which is in linkage group III. [An explanation of symbols and a complete linkage map of *Aspergillus nidulans* is given by Clutterbuck & Cove (1973).] The locus *gdhC* is therefore in linkage group III. The *gdhC1* mutant shows 50% recombination with *xprD1*, which is also in the same linkage group.

Table 2. *NAD-GDH activity in wild-type cells held in various carbon sources*

Growth conditions	Treatment	NAD-GDH (nmoles/min/mg protein)
–N medium +	–CN medium +	
10 mM ammonium	10 mM ammonium, 1·5 h	430
10 mM ammonium	10 mM ammonium, 3·0 h	510
10 mM ammonium	10 mM ammonium, 6·0 h	< 100
10 mM ammonium	100 mM L-glutamate, 1·5 h	560
10 mM ammonium	100 mM L-glutamate, 3·0 h	850
10 mM ammonium	100 mM L-glutamate, 6·0 h	1630
10 mM ammonium	100 mM L-aspartate, 1·5 h	370
10 mM ammonium	100 mM L-aspartate, 3·0 h	1150
10 mM ammonium	100 mM L-aspartate, 6·0 h	2020
10 mM ammonium	100 mM L-alanine, 1·5 h	660
10 mM ammonium	100 mM L-alanine, 3·0 h	1600
10 mM ammonium	100 mM L-alanine, 6·0 h	2425
10 mM ammonium	1·5% casamino acids, 1·5 h	290
10 mM ammonium	1·5% casamino acids, 3·0 h	1050
10 mM ammonium	1·5% casamino acids, 6·0 h	1930

Table 3. *NAD-GDH activity in* gdhC1

Initial growth treatment	Treatment	NAD-GDH (nmoles/min/mg protein) Wild-type	*gdhC1*	*gdhC1*/+
–N medium +				
10 mM ammonium	—	< 100	645	420
10 mM nitrate	—	< 100	810	530
10 mM L-glutamate	—	< 100	710	415
10 mM L-aspartate	—	< 100	595	390
10 mM L-alanine	—	< 100	830	555
0·15% casamino acids	—	< 100	610	315
–N medium +	–CN medium, 6 h			
10 mM ammonium	100 mM L-glutamate	1610	1645	1725
10 mM ammonium	100 mM L-aspartate	2010	1950	1950
10 mM ammonium	100 mM L-alanine	2215	2200	2305
10 mM ammonium	1·5% casamino acids	2035	1975	2350

(v) *NAD-GDH and NADP-GDH activity in* gdhC1

Table 3 shows that the *gdhC1* mutant has appreciable levels of NAD-GDH activity in the presence of glucose, while the wild-type has activity below the limit of detection. Wild-type and *gdhC1* cells held in the presence of L-glutamate, L-aspartate, L-alanine or casamino acids as the sole carbon and nitrogen source have similar levels of NAD-GDH

activity. The *gdhC1* mutation is semidominant in the heterozygous diploid with respect to NAD-GDH activity.

There is no apparent effect of the *gdhC1* mutation on the level of NADP-GDH activity. The levels of NADP-GDH in *gdhC1* are similar to those of the wild-type when the cells are grown on any one of nitrate, ammonium, L-glutamate, L-aspartate or L-alanine as sole nitrogen sources.

(vi) *Growth characteristics of* gdhC1

The results of growth tests (Table 4) carried out on solid media show that *gdhC1* grows better than the wild-type on 10 mM L-glutamate (Plate 1) or L-aspartate or L-alanine, but similar to the wild-type on 10 mM L-arginine or L-asparagine or ammonium or nitrate as the sole nitrogen source. Moreover, it shows normal growth on L-glutamate, L-aspartate or L-alanine as sole carbon and nitrogen source. The heterozygous diploid *gdhC1*/+ shows mutant growth on certain nitrogen sources including L-glutamate, indicating the semi-dominance or dominance of the *gdhC1* mutation.

Table 4. *Growth responses of* gdhC1

	Strains		
Growth conditions	Wild-type	*gdhC1*	*gdhC1*/+
N medium (solid) +			
10 mM ammonium	+	+	+
10 mM nitrate	+	+	+
10 mM L-glutamate	+	+ +	+ +
10 mM L-aspartate	+	+ +	+ +
10 mM L-alanine	+	+ +	+ +
10 mM L-arginine	+	+	+
10 mM L-glutamine	+	+	+
–CN medium (solid) +			
100 mM L-glutamate	+	+	+
100 mM L-aspartate	+	+	+
100 mM L-alanine	+	+	+

\+ = wild-type growth.
\+ + = better than wild-type growth (see Plate 1).

4. DISCUSSION

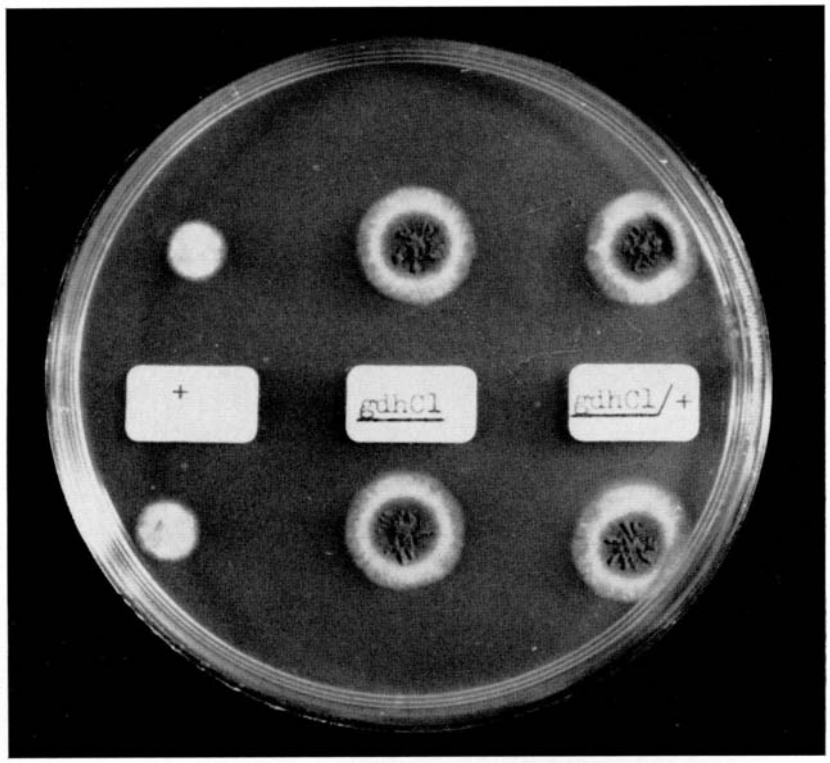
Our results suggest that NAD-GDH activity in *A. nidulans* is at least partly regulated by glucose repression. The main facts which support this argument are:

(1) Carbon starvation after growth on glucose results in appreciable NAD-GDH activity. This activity is maximal after 3 h.

(2) Maximal NAD-GDH activity is found in cells in which the carbon status is low, but probably sufficient for protein synthesis. This is the case when either acetate or L-glutamate or L-aspartate or casamino acids is the main or only carbon source.

Since NAD-GDH activity is found in cells after a short period of carbon starvation and in cells grown on acetate, the system is probably regulated by glucose repression alone or by repression and induction, induction being subordinate to repression. This seems similar to the situation in *N. crassa* (Strickland, 1971) but different to that in *Saccharomyces cerevisiae*, where ammonium represses NAD-GDH activity (Hierholzer & Holzer, 1963).

The *gdhC1* mutant has appreciable NAD-GDH activity when grown in the presence of glucose. This activity appears to be unaffected by the type of nitrogen source. The



Growth responses of *gdhC1* with L-glutamate as the sole nitrogen source. −N medium + 10 mM L-glutamate.

mutation at the *gdhC* locus allows better growth than the wild-type on L-glutamate or L-aspartate or L-alanine as nitrogen sources. This is probably due to the bonus of derepressed NAD-GDH activity which makes ammonium more easily available. The wild-type under these conditions does not make NAD-GDH and can only produce repressed levels of NADP-GDH activity (Pateman, 1969). Moreover, the normal function of NADP-GDH is to synthesize L-glutamic acid and it is relatively inefficient in the deamination reaction. This argument is supported by the fact that *gdhC1* grows as wild-type on nitrogen sources which provide easily accessible ammonium, e.g. ammonium, L-arginine and L-glutamine. The properties of this mutant could be explained if the *gdhC* codes for a regulatory product which on interaction with the carbon metabolite effector represses enzyme synthesis. The *gdhC1* mutation is semi-dominant in the heterozygous diploid. By analogy with the L-arabinose system in *E. coli* (Englesberg, Sheppard, Squires & Meronk, 1969), nitrate reductase (Pateman & Cove, 1967) and xanthine dehydrogenase (Scazzocchio & Darlington, 1968) in *Aspergillus nidulans*, such semi-dominance suggests that glucose repression of NAD-GDH may be a positive control system.

Further studies are being carried out to determine if the *gdhC1* mutant has altered glucose repression of other systems, e.g. amylase, glyoxylate enzymes, etc. Initial studies show that the *gdhC1* has wild-type carbon repression of extracellular protease (B. L. Cohen, personal communication).

Recent studies by Hynes (1972) have shown that mutation at the *amdT* locus results in loss of carbon regulation of acetamidase. The *gdhC* locus is not linked to *amdT* (also located in linkage group III), since *gdhC1* shows approximately 50 % recombination with *xprD1*, which is allelic with *amdT* (Arst & Cove, 1973), nor is the *gdhC* locus linked with *gdhA* (J. R. Kinghorn, unpublished work), mutation at which results in the abolition of NADP-GDH activity (Kinghorn & Pateman, 1973).

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