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Regulation of Glutamate Dehydrogenases in *nit-2* and *am* Mutants of *Neurospora crassa*

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The regulation of the glutamate dehydrogenases was investigated in wild-type *Neurospora crassa* and two classes of mutants altered in the assimilation of inorganic nitrogen, as either nitrate or ammonium. In the wild-type strain, a high nutrient carbon concentration increased the activity of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-glutamate dehydrogenase and decreased the activity of reduced nicotinamide adenine dinucleotide (NADH)-glutamate dehydrogenase. A high nutrient nitrogen concentration had the opposite effect, increasing NADH-glutamate dehydrogenase and decreasing NADPH-glutamate dehydrogenase. The *nit-2* mutants, defective in many nitrogen-utilizing enzymes and transport systems, exhibited low enzyme activities after growth on a high sucrose concentration: NADPH-glutamate dehydrogenase activity was reduced 4-fold on NH_4Cl medium, and NADH-glutamate dehydrogenase, 20-fold on urea medium. Unlike the other affected enzymes of *nit-2*, which are present only in basal levels, the NADH-glutamate dehydrogenase activity was found to be moderately enhanced when cells were grown on a low carbon concentration. This finding suggests that the control of this enzyme in *nit-2* is hypersensitive to catabolite repression. The *am* mutants, which lack NADPH-glutamate dehydrogenase activity, possessed basal levels of NADH-glutamate dehydrogenase activity after growth on urea or L-aspartic acid media, like the wild-type strain, and possessed moderate levels (although three- to fourfold lower than the wild-type strain) on L-asparagine medium or L-aspartic acid medium containing NH_4Cl. These regulatory patterns are identical to those of the *nit-2* mutants. Thus, the two classes of mutants exhibit a common defect in NADH-glutamate dehydrogenase regulation. Double mutants of *nit-2* and *am* had lower NADH-glutamate dehydrogenase activities than either parent. A carbon metabolite is proposed to be the repressor of NADH-glutamate dehydrogenase in *N. crassa*.

Many eucaryotic microorganisms possess two distinct glutamate dehydrogenases for the interconversion of glutamate with ammonium and α-ketoglutarate, a key step in nitrogen metabolism (14). Evaluation of the factors important in the regulation of the glutamate dehydrogenases has proved difficult since the two enzymes are regulated concurrently but oppositely by both carbon and nitrogen nutrients in the medium; i.e., when one activity is high, the other is low (19, 30–33). This paper examines the regulation of the glutamate dehydrogenases in *Neurospora crassa*.

The nitrate assimilatory pathway converts inorganic nitrogen as nitrate to L-glutamate as follows:

$$NO_3^- \xrightarrow{1} NO_2^- \xrightarrow{2} NH_4^+ \xrightarrow{3} \text{L-glutamate}$$

$$NH_4^+ + \alpha\text{-ketoglutarate} \rightarrow \text{L-glutamate}$$

The first two steps are catalyzed, respectively, by nitrate reductase (12, 13, 25, 26) and nitrite reductase (5, 23), and the third, by the glutamate dehydrogenases. NADPH-glutamate dehydrogenase is believed to convert NH_4^+ and α-ketoglutarate to L-glutamate, whereas NADH-glutamate dehydrogenase degrades L-glutamate (30). Each enzyme has been purified to homogeneity (3, 39).

Most mutants of the pathway have been isolated by three selection procedures: lack of growth on nitrate as a sole nitrogen source has yielded the mutants *nit-1*, *nit-2*, and *nit-3* (36); lack of growth on ammonium, the *am* mutants

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(10, 11); and lack of growth on uric acid, the *amr* mutant (allelic with *nit-2*) (29). All of the *nit* mutants exhibit normal nitrate uptake (34) but are unable to assimilate nitrate because of a deficiency in NADPH-nitrate reductase activity (as well as one or more of the other three wild-type activities of nitrate reductase [7, 24]). The two mutants *nit-1* and *nit-3*, mapping respectively on chromosomes I and IV (36), synthesize an aberrant nitrate reductase enzyme due to the lack of the molybdenum-cofactor in the former and the presence of an aberrant apoprotein in the latter (1, 24). The product of the *nit-2* gene is unknown; the phenotype of *nit-2* is more complex and is discussed below. The *am* locus maps on chromosome V and codes for NADPH-glutamate dehydrogenase (10). Extracts of some *am* mutants possess a cross-reacting material immunologically related to the wild-type NADPH-glutamate dehydrogenase (32), and two *am* mutants have been shown to produce aberrant NADPH-glutamate dehydrogenase with an altered amino acid sequence (4, 41). The structural genes for nitrite reductase and NADH-glutamate dehydrogenase are not known in *N. crassa*, although a mutant lacking nitrite reductase activity has been reported (5).

Unlike the other *nit* mutants (described above), the *nit-2* mutants (including *amr* [unpublished data]) lack all four wild-type nitrate reductase activities as well as other enzymatic activities listed below (7, 24). They grow well on NH_4Cl medium but show no growth on nitrate or nitrite medium (16, 36) and poor growth on a variety of nitrogen sources including hypoxanthine, xanthine, uric acid, and various amino acids (16, 29); thus, they are similar to the *areA* mutant of *Aspergillus nidulans* (2). The *amr* mutant (allelic with *nit-2* on chromosome I) has pleiotropic changes involving extracellular protease (17) and enzymes of purine catabolism (29); consequently, *nit-2* has been proposed to be a master regulator gene for nitrogen metabolism (29).

The purpose of this paper is to reexamine the effects of carbon and nitrogen nutrients on the regulation of the glutamate dehydrogenases, as well as to further characterize the effect of the *nit-2* mutation.

(A portion of this work has been previously reported [A. H. Tifford and A. Nason, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, H73, p. 108].)

MATERIALS AND METHODS

Strains. The following strains were obtained from the Fungal Genetics Stock Center, Arcata, Calif.: wild type (STA4), *nit-1* (allele *34547*), *nit-2* (alleles *K31, nr37, I7*), *nit-3* (allele *14789*), am_1 (allele *32213*), and am_2 (allele *47305*). To obtain the double mutants, *nit-2* (allele *nr37*) was crossed with am_1 and am_2 (8, 40). The resulting progeny that lacked both NADPH-nitrate reductase and NADPH-glutamate dehydrogenase activities were assumed to be the double mutants: *nit-2* am_1 or *nit-2* am_2. One such strain from each cross was saved for further use.

Growth and induction of cultures. To obtain cells for enzyme assays, approximately 10^6 conidia were inoculated into 100 ml of Fries minimal medium in a 500-ml Erlenmeyer flask (7), grown at 25°C for 46 to 48 h, and harvested during exponential phase (7, 24). The sole nitrogen source is indicated in each experiment; freshly prepared urea solutions were membrane-filtered (Millipore Corp.) and aseptically added to give the indicated final concentration.

Extraction. For the glutamate dehydrogenase assays and the nitrate reductase assay, extracts were prepared as previously cited (7). For the nitrite reductase assay, extracts were prepared by the procedure of Lafferty and Garrett (21) except that 10 μM flavine adenine dinucleotide was included in the homogenizing buffer and dithionite was omitted.

Assays. NADPH-nitrate reductase and NADPH-glutamate dehydrogenase activities were assayed as described (7). The assay for NADH-glutamate dehydrogenase activity differed from that for NADPH-glutamate dehydrogenase: NADH replaced NADPH in the assay and the pH of the 0.2 M Tris-hydrochloride buffer was 8.5 (7). Nitrite reductase was assayed as indicated by Lafferty and Garrett (21). Protein was determined by the method of Lowry et al. (22). The activity of the glutamate dehydrogenases is given as the change in absorbance at 340 nm of 1.0 optical density unit per min per mg of protein.

RESULTS

NADPH-glutamate dehydrogenase activities of wild type and *nit* mutants. Since the activities of the first two enzymes of the nitrate assimilatory pathway, nitrate reductase and nitrite reductase, appear to be absent in the *nit-2* mutants, the activity of the biosynthetic NADPH-glutamate dehydrogenase might also be expected to be low (35). Table 1 compares the activity of this enzyme in NH_4Cl-grown and urea-grown cells of wild-type *Neurospora* and in three classes of *nit* mutants. When grown on NH_4Cl as a sole nitrogen source, the wild-type strain contained high enzyme activity. The *nit-1* and *nit-3* mutants possessed somewhat reduced activities (by 38% and 29%, respectively); but all three isolates of *nit-2* were conspicuously defective, with the enzyme activity reduced by 75%. After growth on urea medium, all strains possessed basal levels of NADPH-glutamate dehydrogenase activity.

These low activities could result by at least three mechanisms: (i) an inhibitor that reduces the activity might be present in *nit-2* extracts; (ii) the *nit-2* strains might possess a second mutation affecting the activity, or (iii) NADPH-

TABLE 1. *Specific activity of NADPH-glutamate dehydrogenase in the wild type and the nitrate-nonutilizing mutants*[a]

Strain	Sp act	
	NH_4Cl	Urea
Wild type (STA4)	4.5	0.6[b]
nit-1	2.6	1.0
nit-3	3.2	0.6
nit-2(K31)	1.0	0.9
nit-2(nr37)	0.9	0.8
nit-2(I7)	1.0	0.8

[a] Mycelia were grown on 800 ml of medium containing 2.0% sucrose and either 25 mM NH_4Cl or 150 mM urea as the sole nitrogen source.

[b] This value was not determined in this set and represents an average of six experiments.

TABLE 2. *Specific activity of NADH-glutamate dehydrogenase in the wild type and the nitrate-nonutilizing mutants*[a]

Strain	Sp act	
	NH_4Cl	Urea
Wild type (STA4)	<0.6	7.8
nit-1	<0.6	6.8
nit-3	<0.6	3.5
nit-2(K31)	<0.6	0.6
nit-2(nr37)	<0.6	0.6
nit-2(I7)	<0.6	0.6

[a] Mycelia were grown on 800 ml of medium containing 2.0% sucrose and either 25 mM NH_4Cl or 150 mM urea as the sole nitrogen source.

TABLE 3. *Specific activity of NADH-glutamate dehydrogenase in the wild type and in nit-2 after growth on medium containing two concentrations of sucrose*[a]

Urea concn (mM)	Sp act after growth on:			
	2% Sucrose		0.5% Sucrose	
	Wild type	*nit-2*	Wild type	*nit-2*
25	0.8	<0.6	8.2	1.5
50	2.1	<0.6	7.8	2.1
75	4.4	<0.6	14.1	4.7
100	3.7	<0.6	18.6	4.7
125	5.5	<0.6	16.7	3.5

[a] Mycelia were grown on 100 ml of medium containing the indicated urea concentration and either 2 or 0.5% sucrose. Results are representative of three trials.

glutamate dehydrogenase may be regulated directly by *nit-2*.

Inhibitor. When extracts of the wild type and *nit-2* (allele *nr37*) were mixed, the resultant NADPH-glutamate dehydrogenase activity was additive (data not shown). Thus, the low activity in *nit-2* is not due to the presence of an inhibitor.

Backcross. A backcross was done to determine whether the *nit-2* mutant (allele *nr37*) carried an independent mutation affecting NADPH-glutamate dehydrogenase, possibly a mutation in *am*, the structural gene for the enzyme. The cross resulted in one-to-one segregation: 25 of the 44 progeny resembled *nit-2* (lacking nitrate reductase and nitrite reductase and possessing low NADPH-glutamate dehydrogenase activity) and 21 were wild type. Since *nit-2* and *am* map on separate chromosomes (I and V, respectively [10, 36]), they would be expected to segregate independently. Strain *nit-2* (allele *nr37*) consequently does not carry a second mutation in *am* or any other unlinked gene, although the possibility of second mutation near *nit-2* on chromosome I cannot be ruled out.

NADH-glutamate dehydrogenase activity in wild type and *nit* mutants. Since the effect of the *nit-2* mutation appears to be pleiotropic, the activity of the catabolic NADH-glutamate dehydrogenase might also be altered in the *nit-2* mutants; consequently, the activity was surveyed in the wild type and the *nit* mutants. Similar basal levels of activity were present in all strains after growth on NH_4Cl medium containing 2% sucrose (Table 2). Marked differences, however, were observed after growth on urea medium. NADH-glutamate dehydrogenase activity has been demonstrated to be 10-fold higher after growth on urea medium than after growth on NH_4Cl medium (37). The NADH-glutamate dehydrogenase activity of wild type, *nit-1*, and *nit-3* was increased 6- to 10-fold, whereas the activity of the *nit-2* mutants showed no change (Table 2). Again, the presence of an inhibitor in the *nit-2* extracts was ruled out by mixing experiments.

The results thus far demonstrate that the *nit-2* mutants are defective in the ability to induce NADPH-glutamate dehydrogenase activity on NH_4-containing medium and NADH-glutamate dehydrogenase activity on urea-containing medium (Tables 1 and 2). Since both nitrogen and carbon nutrients are known to be important in glutamate dehydrogenase regulation, a further set of experiments was designed to vary each nutrient independently in the growth medium and to compare their effects on the activities of the wild-type and *nit-2* strains.

Effect of nitrogen source. Increasing the nitrogen concentration in the growth medium decreases the activity of NADPH-glutamate dehydrogenase and increases the activity of NADH-glutamate dehydrogenase in wild-type *N. crassa* and *A. nidulans* (27, 31). The effect of increasing the urea concentration from 25 to 125

mM in the growth medium was determined on the activities of both strains. As in the wild type, NADPH-glutamate dehydrogenase activity of *nit-2* decreased as the urea concentration was increased, although lower specific activities were observed (data not shown). By contrast, the NADH-glutamate dehydrogenase activity of the wild-type strain (grown on 2% sucrose medium) increased by nearly eightfold while the activity of the *nit-2* mutant remained constant (Table 3, left side).

Effect of carbon source. The effect of carbon on the regulation of the glutamate dehydrogenases has been demonstrated to be opposite to that of nitrogen (19, 31). While a high nutrient nitrogen concentration increases NADH-glutamate dehydrogenase activity and decreases NADPH-glutamate dehydrogenase activity (Table 3; 31), a high nutrient carbon concentration increases NADPH-glutamate dehydrogenase activity and decreases NADH-glutamate dehydrogenase activity (19). This phenomenon is confirmed in Fig. 1, which illustrates the effect of growth at increasing sucrose concentrations on the activities of glutamate dehydrogenases. In the wild-type strain, NADPH-glutamate dehydrogenase activity increased and NADH-glutamate dehydrogenase activity decreased as the sucrose concentration was raised. By contrast, both enzyme activities exhibited much smaller changes in *nit-2*. Similar results were obtained when either fructose or glucose replaced sucrose in the 79 mM NH_4Cl growth medium (data not shown).

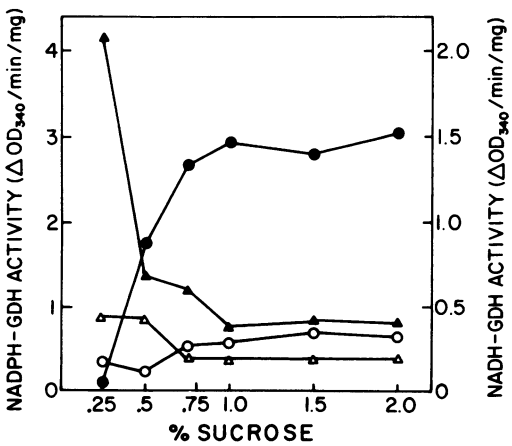

FIG. 1. *Effect of sucrose concentration on specific activities of NADPH-glutamate dehydrogenase (NADPH-GDH) and NADH-glutamate dehydrogenase (NADH-GDH). The sole nitrogen source was 79 mM NH_4Cl. Symbols: ●, NADPH-GDH of wild type; ○, NADPH-GDH of nit-2 (allele nr37); ▲, NADH-GDH of wild type; △, NADH-GDH of nit-2 (allele nr37).*

Since *nit-2* exhibited a slight increase in NADH-glutamate dehydrogenase activity when the sucrose concentration was reduced from 0.75 to 0.5% (shown in Fig. 1, in the presence of 79 mM NH_4Cl), it was important to reexamine the induction of this enzyme by increasing the urea concentration in a medium containing a low sucrose concentration. The results are presented in Table 3. As the nitrogen content of the growth medium was increased, the NADH-glutamate dehydrogenase activity of both strains increased. At a given urea concentration, the activity was higher after growth on a low sucrose concentration than on a high sucrose concentration (Table 3). For example, the wild-type strain possessed roughly threefold higher activity after growth on 75 to 125 mM urea medium containing 0.5% sucrose than on medium containing the same urea concentration and 2.0% sucrose. The effect of a low sucrose concentration on NADH-glutamate dehydrogenase activity of the *nit-2* mutant was most striking. The activity of the *nit-2* mutant increased with increasing urea concentrations on medium containing 0.5% sucrose and did not increase on 2.0% sucrose medium (Table 3). Consequently, the *nit-2* mutant possessed nearly eightfold higher activity after growth on low sucrose medium than on high sucrose medium (again, containing 75 to 125 mM urea). This eightfold increase is in sharp contrast to the threefold increase observed for the wild type under the same conditions. Even so, it is important to note that the specific activity of the *nit-2* mutant (grown on 75 to 125 mM urea medium and 0.5% sucrose) was nearly fourfold less than that of the wild-type strain (Table 3). Similar patterns, but lower specific activities, were observed when mycelia were grown on 0.5% sucrose, NH_4Cl-containing medium (data not shown). The observed patterns of increased and decreased activity cannot be accounted for by the addition of sucrose to the assay mixture since the glutamate dehydrogenase activities of both strains were not significantly affected.

NADH-glutamate dehydrogenase activity of the *am* mutants and *nit-2 am* mutants. Previously, the *am* mutants have been demonstrated to possess low levels of NADH-glutamate dehydrogenase activity when grown on urea medium containing 2.0% sucrose, and this is shown in Table 4. (Unlike an earlier report, am_2 was found also to possess low NADPH-glutamate dehydrogenase activity [32].) The low activities of the *am* mutants are similar to that described above for the *nit-2* mutants (Tables 2 and 4). This similarity could arise from their common defect (i.e., low or no NADPH-glutamate dehydrogenase activity, respectively, in *nit-2* and *am*), and, consequently,

TABLE 4. *Specific activity of NADH-glutamate dehydrogenase in the wild type and in the am mutants*[a]

Strain	Sp act after growth on urea medium		
	50 mM	100 mM	150 mM
Wild type (STA4)	3.7	6.0	6.9
am$_1$	<0.6	<0.6	<0.6
am$_2$	<0.6	<0.6	<0.6

[a] Mycelia were grown on 2.0% sucrose medium containing the indicated concentration of urea as the sole nitrogen source.

metabolites on either side of the enzymatic block may be important in the control of the glutamate dehydrogenases. For this reason, wild type, *nit-2*, *am*$_1$, *am*$_2$, and two double mutants, *nit-2 am*$_1$ and *nit-2 am*$_2$, were grown on several different media and the activities of NADH-glutamate dehydrogenase were compared. Table 5 illustrates the growth properties of these strains on media containing different nitrogen sources. Unlike the wild type and *nit-2*, the *am* mutants grew poorly on urea-containing medium and the double mutants did not grow at all on urea medium. All strains, however, grew well on the other media tested and used for the subsequent study. As shown in Table 6, basal levels of NADH-glutamate dehydrogenase activity were present in all strains after growth on L-aspartic acid medium. After growth on the other two nitrogen-rich media, the NADH-glutamate dehydrogenase activity was increased by six- to eightfold in the wild type, three- to fourfold in the parental strains (*nit-2*, *am*$_1$, and *am*$_2$), and one- to twofold in the double mutants (*nit-2 am*$_1$ and *nit-2 am*$_2$). Thus, the *nit-2* and *am* mutants showed lower activities than the wild type and the effects of the *nit-2* and *am* mutations were synergistic.

DISCUSSION

The *nit-2* mutants have been reported to possess basal levels of many nitrogen-utilizing enzymes, including nitrate reductase (36), nitrite reductase, extracellular protease, and enzymes of purine catabolism, xanthine dehydrogenase, uricase, alloantoinase, and allanoicase (29), although urease (29) and glutamine synthetase (A. H. Dantzig and A. Nason, unpublished data) are not affected. In addition, the *nit-2* mutants do not derepress for the uptake of several amino acids in the absence of ammonium, unlike the wild type (T. Facklan and G. A. Marzluf, Genetics **80**:s29, 1975), and they exhibit poor purine base uptake (probably resulting in the lack of induction of the enzymes of purine catabolism, listed above; 28, 38). Marzluf and co-workers have proposed that the *nit-2* gene product is responsible for the control of many nitrogen-utilizing enzymes (and transport systems) (29, 38).

The present report demonstrates that the *nit-*

TABLE 6. *Specific activity of NADH-glutamate dehydrogenase in the wild type and mutants*

Strain	Sp act after growth on:		
	Asp[a]	Asn[a]	Asp + NH_4Cl[b]
Wild type (STA4)	1.1	6.5	10.3
nit-2(nr37)	0.6	4.4	4.7
am$_1$	0.8	4.1	4.9
am$_2$	1.3	4.7	5.0
nit-2 am$_1$	0.6	2.2	1.2
nit-2 am$_2$	0.7	2.8	2.2

[a] Grown on medium containing 50 mM of aspartic acid (Asp) or asparagine (Asn) as the sole nitrogen source and 0.5% sucrose.

[b] The nitrogen source in the growth medium was 50 mM L-aspartic acid and 50 mM NH_4Cl. The medium also contained 0.5% sucrose.

TABLE 5. *Growth of the wild type and various mutants on several different nitrogen-containing media*

Strain	Growth medium[a]					
	CA[b]	Urea[c]	Asp[c]	Asn[c]	Asp + NH_4Cl[d]	Asp + urea[d]
Wild type (STA4)	+[e]	+	+	+	+	+
nit-2(nr37)	+	+	+	+	+	+
am$_1$	+	±	+	+	+	+
am$_2$	+	±	+	+	+	+
nit-2 am$_1$	+	0	+	+	+	+
nit-2 am$_2$	+	0	+	+	+	+

[a] Growth medium contained 0.5% sucrose.

[b] CA represents 2% Casamino Acids-containing medium.

[c] Urea, aspartic acid (Asp), or asparagine (Asn) was present at 50 mM in the growth medium as the sole source of nitrogen.

[d] Both compounds were present in the growth medium, each at 50 mM.

[e] Symbols: +, good growth; ±, poor growth (which is less than 25% of wild type's growth, based on wet weights); 0, no visible growth after 3 to 4 days.

2 mutants possess basal levels of two other nitrogen-utilizing enzymes, NADPH- and NADH-glutamate dehydrogenases, after growth on medium containing 2% sucrose. Unlike the other affected enzymes (listed above), the NADH-glutamate dehydrogenase activity of the *nit-2* mutant was found to be moderately enhanced under the appropriate growth conditions. By increasing the urea concentration in a medium containing a low sucrose concentration (0.5%, instead of the usual 2% concentration), the NADH-glutamate dehydrogenase activity of the *nit-2* mutant was increased nearly eightfold (Table 3). Even so, the NADH-glutamate dehydrogenase activity of *nit-2* was never found to be as high as that of the wild type when grown on a high urea concentration. This eightfold increase in activity of *nit-2* is in sharp contrast to the threefold increase in activity observed for the wild type under the same conditions, suggesting that the control of NADH-glutamate dehydrogenase of the *nit-2* mutant is more sensitive to catabolite repression than that of the wild type. This hypersensitivity, however, may represent a secondary effect of the mutation and may be due in part to the accumulation of carbon metabolites (as discussed below). The increase in the activity of this enzyme suggests that the phenotype of *nit-2* is more complex than previously believed and that increases in the activity of other affected enzymes may be observed under appropriate growth conditions.

A second finding is that the *am* mutants exhibit an altered ability to increase NADH-glutamate dehydrogenase activity, quite similar to that observed for the *nit-2* mutant (Tables 2, 3, 4, 6; reference 31). There are at least three mechanisms that could account for the low NADH-glutamate dehydrogenase activities of the *am* mutants. (i) The high intracellular concentrations of ammonium ions that accumulate at the enzymatic block might repress NADH-glutamate dehydrogenase. (ii) High levels of α-ketoglutarate (or another carbon metabolite) might accumulate and repress NADH-glutamate dehydrogenase synthesis. (iii) The *am* mutants may be unable to synthesize a nitrogen-containing metabolite necessary for induction of NADH-glutamate dehydrogenase.

Since NADH-glutamate dehydrogenase activity is enhanced by increasing the nitrogen concentration in the medium, it is unlikely that ammonium is acting directly as a repressor. In addition, the *am* mutants have been shown to escape ammonium repression of nitrate reductase and nitrite reductase (6, 7), and similar mutants of *Saccharomyces* and *Aspergillus* have been shown to escape ammonium repression of many nitrogen-utilizing enzymes (9, 15, 18, 20). The apparent lack of repression is consistent with the notion that the mediator of ammonium repression is a nitrogen metabolite other than ammonium, contrary to mechanism (i), above.

Induction by a nitrogen metabolite after the enzymatic block (mechanism iii) also seems unlikely since the wild type and the *am* mutants would then be expected to have moderate levels of NADH-glutamate dehydrogenase activity on medium containing L-aspartic acid as the sole nitrogen source. This is contrary to the basal levels of activity found in all strains after growth on L-aspartic acid medium (Table 6).

The present studies appear to support catabolite repression (mechanism ii, above). Wild type, *nit-2*, am_1, and am_2 were found to have moderate to high levels of NADH-glutamate dehydrogenase activity after growth on L-asparagine or L-aspartic acid plus ammonium (Table 6, reference 31). The observed moderate "induction" could be due to the lack of repression caused by the removal of the repressor (a carbon metabolite), perhaps via transamination.

Catabolite repression is also supported by the finding that the effects of the *nit-2* and the *am* mutations are synergistic (Table 6). The double mutants should accumulate high intracellular pools of α-ketoglutarate (and/or other carbon metabolites) as a result of their inability to convert α-ketoglutarate plus ammonium to L-glutamate; since they are hypersensitive to catabolite repression of NADH-glutamate dehydrogenase (caused by the *nit-2* mutation), the double mutants should exhibit increased repression of NADH-glutamate dehydrogenase activity when compared to the parental strains. This is consistent with the basal activities observed for the double mutants: *nit-2* am_1 and *nit-2* am_2 in Table 6. Other studies done by Kinghorn and Pateman have suggested that a carbon metabolite may be the repressor of NADH-glutamate dehydrogenase in *A. nidulans* (20).

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