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Effects of Ammonium, L-Glutamate, and L-Glutamine on Nitrogen Catabolism in *Aspergillus nidulans*

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During growth of *Aspergillus nidulans* in medium containing ammonium the specific activities of most enzymes involved in catabolism of nitrogen sources are low (ammonium repression). The *gdhA10* lesion, which results in loss of nicotinamide adenine dinucleotide phosphate-linked glutamate dehydrogenase activity, has been shown to lead to partial relief of ammonium repression of three amidase enzymes as well as histidase. The *areA102* lesion led to altered levels of these enzymes but did not greatly affect ammonium repression. The double mutant *areA102,gdhA10* was almost completely insensitive to ammonium repression of two of the amidase enzymes and histidase. This suggests that an interaction between the *areA* and *gdhA* genes in determining responses to ammonium occurs. Growth of mycelium in medium containing L-glutamate has been found to result in lowered levels of all four enzymes, and this occurs in strains insensitive to ammonium repression. Very strong repression in all strains occurred during growth in medium containing L-glutamine. Relief of these repressive effects of glutamate and glutamine was blocked by cycloheximide. Glutamate and glutamine had similar effects on the production of extracellular protease activity, and growth on glutamine led to low levels of urate oxidase. In contrast to the above enzymes, nitrate reductase was insensitive to the effects of glutamine and glutamate, even though this enzyme is very sensitive to ammonium repression. Although other possibilities exist, it is suggested that there may be mechanisms of general control of nitrogen-catabolic enzymes other than ammonium repression.

Enzymes involved in the catabolism of nitrogen sources have been commonly found to be present in low amounts during growth of microorganisms on ammonium salts (1, 6, 10, 12, 17, 20, 21). This phenomenon has been called by many names but will be referred to as ammonium repression in this paper, without making any specific inferences as to mechanism. Mutations leading to loss of nicotinamide adenine dinucleotide phosphate (NADP)-dependent glutamate dehydrogenase (GDH) (EC 1.4.1.4) have been found to result in loss of ammonium repression in yeast (10, 11, 24) and *Aspergillus nidulans* (3, 16). It has been proposed that the NADP-GDH enzyme molecule has a regulatory function in controlling enzyme synthesis (10, 20). However, van de Poll has reported that, in a strain of *Saccharomyces carlsbergensis* lacking NADP-GDH, allantoinase is repressed by glutamine and glutamate plus ammonium but not by ammonium alone (24). This has led to the suggestion that the NADP-GDH catalytic activity is necessary for ammonium repression. So far no mutants affecting the proposed regulatory function but not the catalytic activity of NADP-GDH have been described.

In *A. nidulans*, mutations in a gene, *areA* (called *amdT* in previous publications; see ref. 2), lead to pleiotropic effects on nitrogen source utilization (2, 14). A variety of phenotypic effects are observed in various *areA* mutants, including inability to grow on many nitrogen sources, the utilization of which are subject to ammonium repression. Others show altered sensitivities to ammonium repression of some of the enzymes of nitrogen catabolism. The *areA102* lesion results in increased growth on many nitrogen sources and leads to increased levels of some enzymes of nitrogen catabolism without relieving ammonium repression (2, 14, 15; unpublished data). Arst and Cove (2) have concluded that the product of the *areA* gene is required for the synthesis of nitrogen-catabolic enzymes and that ammonium repression involves an interference with the action of this positive regulatory factor. In addition, they suggest that carbon catabolite repression is mediated via a separate control system (2).

Results from this laboratory strongly support their conclusions.

This paper reports studies on ammonium repression of a number of nitrogen-catabolic enzymes in strains of *A. nidulans* with lesions in the *gdhA* gene (the proposed structural gene for NADP-GDH) and the *areA* gene. The effects of growth on L-glutamate and L-glutamine on enzyme levels have also been investigated. These studies raise the possibility that there may be control mechanisms, in addition to ammonium repression, affecting the levels of enzymes of nitrogen catabolism.

MATERIALS AND METHODS

Strains. Details of the strains used are described in Table 1. It should be noted that more limited experiments with other strains, of similar *gdhA* and *areA* genotypes, have given similar results to those reported here.

Media and growth conditions. The standard minimal medium used was that of Cove (8), where 1% (wt/vol) glucose is the sole carbon source and no nitrogen source is present. Nitrogen sources to the desired concentration were added to this medium. Glutamate was added as sodium L-glutamate and ammonium was added as ammonium tartrate. Mycelium was grown at 30 C in a Gallenkamp orbital incubator as described previously (13, 14). Harvested mycelium was stored frozen at −15 C and assayed within 5 days.

Preparation of crude extracts. All crude extracts were made in 100 mM orthophosphate buffer, pH 7.2, by methods described previously (13). Protein concentrations of crude extracts were determined by the method of Lowry et al. (18).

Plate tests for extracellular protease. These were carried out by the method of Cohen (6) using 1% dried milk powder.

Amidase enzyme assays. Formamidase and acetamidase (EC 3.5.1.4) activities were determined by methods described before (12, 13). Both of these enzymes are subject to strong repression by ammonium and derepress on transfer to medium lacking a nitrogen source (12). The acetamidase is inducible by amides (12, 13). The formamidase is not apparently inducible (as originally thought; 12, 13). Formamidase levels are no higher in medium containing formamide than in medium lacking a nitrogen source (unpublished data). A third amidase enzyme has recently been partially characterized (unpublished data). This enzyme hydrolyzes benzamide as well as other amides, but its precise substrate specificity has not been determined and so no Enzyme Commission number is available. Genetic and thermal inactivation experiments show that it is distinct from the other amidase enzymes (unpublished data). For the purposes of this paper this enzyme will be called the benzamidase. As shown here, this enzyme is subject to ammonium repression and derepresses on transfer to medium lacking a nitrogen source. The *areA102* lesion leads to much higher benzamidase activities than those present in wild-type (*areA*$^+$) strains (unpublished data). Benzamidase assays were performed using the method of assay for the other amidase enzymes, with 10 mM benzamide (recrystallized once from hot water) in 100 mM orthophosphate buffer (pH 7.2) as the substrate solution. All amidase-specific activities are expressed as milliunits per milligram of protein, where one unit is the amount of enzyme that catalyzes the release of 1 μmol of ammonium per min.

Histidase enzyme assays. A method (M. Polkinghorne and M. J. Hynes, unpublished data) based on measuring the formation of urocanate from histidine by histidase (EC 4.3.1.3) was used. The reaction was carried out at 30 C in a Unicam SP 1700 spectrophotometer using 1-cm^2 quartz cuvetts. The reaction mixture contained 2.8 ml of 100 mM orthophosphate buffer (pH 8.0) and 0.1 ml of crude extract. The reaction was started by the addition of 0.1 ml of L-histidine solution (3 mg per ml of water), and the increase in absorbance at 277 nm followed. Specific activities are expressed as milliunits per milligram of protein, where one unit is the amount of enzyme that catalyzes the conversion of 1 μmol of histidine to urocanate per min. It has been shown that histidase is subject to ammonium repression and does not require

TABLE 1. *Details of strains used in this study*

Strain	Genotype[a]	Origin	References
Wild type	*biA1*	Glasgow wild type	
gdhA10	*biA1; gdhA10*	H. Arst	3
areA102	*biA1; areA102; niiA4*	Formerly strain *amd*T102	2, 13-15
areA102,gdhA10	*yA1; areA102,gdhA10; riboB2*	Isolate from cross in this laboratory	
mauA2	*biA1; mauA2*	D. Cove	1, 4
mauA2; gdhA10	*biA1; gdhA10; mauA2*	Isolate from cross in this laboratory	
nirA$_1^c$	*yA1,adE20; nirA*$_1^c$, *riboB2*	J. Pateman	19

[a] *biA1*, Biotin auxotrophy; *niiA4*, lack of nitrite reductase; *yA1*, yellow conidia; *riboB2*, riboflavin auxotrophy; *mauA2*, lack of mono-amine oxidase; *adE20*, adenine auxotrophy. These genetic markers have no significance for the experiments reported here. The significance of the *gdhA10*, *areA102* and *nirA*$_1^c$ lesions is described in the text.

external induction by histidine (Polkinghorne and Hynes, unpublished data; this paper).

Urate oxidase enzyme assays. The assay method for urate oxidase (EC 1.7.3.3) was based on that of Scazzocchio and Darlington (23). The reaction mixture contained 2.8 ml of 100 mM orthophosphate buffer (pH 7.2), 0.1 ml of crude extract, and 0.1 ml of uric acid solution (0.336 mg per ml of water). The decline in absorbance at 292 nm at 30 C was followed using a Unicam SP 1700 spectrophotometer with 1-cm^2 quartz cuvettes. This enzyme has been shown to require induction by uric acid and to be subject to ammonium repression (23). Specific activities are expressed as milliunits per milligram of protein, where one unit is the amount of enzyme that catalyzes the oxidation of 1 μmol of uric acid per min.

Nitrate reductase enzyme assays. The method of Cove (8), with the modified control of Lewis and Fincham (17), was used to assay nitrate reductase (EC 1.6.6.3). This enzyme has an absolute requirement for induction by nitrate and is repressed by ammonium (8). Specific activities are expressed as milliunits per milligram of protein, where one unit is the amount of enzyme that catalyzes the reduction of 1 μmol of nitrate to nitrite per min.

Extracellular protease assays. Cohen (6, 7) has shown that *A. nidulans* produces protease enzyme activity in the external medium when mycelium is transferred to medium lacking a nitrogen source and that this activity does not appear when mycelium is growing in medium containing ammonium. In this study protease activity has been measured by following release of trichloroacetic acid-soluble colored compounds from azocasein (5). Mycelium was harvested by the normal method, and the external medium was collected and used for extracellular protease assays. The mycelium was dried and the dry weight was determined. One milliliter of azocasein solution (4 to 5 mg per ml of 100 mM orthophosphate buffer, pH 7.2) was added to plastic centrifuge tubes, and the reaction was initiated by adding 1 ml of the medium to be assayed. Incubation was carried out at 37 C in a shaking water bath. After a suitable time the reaction was stopped by addition of 5 ml of 5% (wt/vol) trichloroacetic acid. The insoluble material was spun down in a bench centrifuge, and the absorbance of the supernatant was read at 440 nm. The assay was linear within the times of incubation used. A control where the trichloroacetic acid was added before addition of the medium for assay was used in each case. Results were calculated as absorbance units per minute per milliliter of medium per milligram (dry weight) of mycelium and are expressed as relative arbitrary units in Table 6.

RESULTS

Preliminary observations on the effects of glutamate on histidase levels in the *areA102, gdhA10* strain (Polkinghorne and Hynes, unpublished data) led to a more detailed study of this problem. Since strains containing the *gdhA* lesion grow poorly in medium lacking glutamate, glutamine, or a source of glutamate (3), the initial experiments in this study involved growing mycelium in medium containing glutamate plus ammonium and then transferring to treatment media containing various nitrogen sources. It was found that, compared with wild-type and *areA102* strains, the *gdhA10* and *areA102,gdhA10* strains were at least partially insensitive to ammonium repression of formamidase, acetamidase, benzamidase, and histidase. In addition, it was found that mycelium transferred to medium containing glutamate (in both the presence and absence of ammonium) led to reduction in the levels of these enzymes compared with the levels in mycelium transferred to medium lacking a nitrogen source. Also, when mycelium was transferred to medium containing glutamine as the sole nitrogen source, the enzyme levels were very low in the wild-type and *areA102* strains and were at levels consistent with repression of enzyme synthesis and dilution during growth in the *gdhA10* and *areA102,gdhA10* strains. Figure 1 shows the results of a more detailed experiment on the *areA102* and *areA102,gdhA10* strains. It is seen that growth of *areA102* on glutamate plus ammonium led to very low levels of acetamidase and histidase, showing that this strain is sensitive to ammonium repression. These levels were maintained in mycelium transferred to medium containing glutamine. Transfer to medium containing glutamate alone led to enzyme levels approximately 50% of those in mycelium transferred to medium lacking a nitrogen source. In contrast to these results, the *areA102,gdhA10* strain grown on glutamate plus ammonium produced substantial levels of acetamidase and histidase, and this level was maintained on transfer to medium containing glutamate alone. Approximately 50% derepression occurred in medium lacking a nitrogen source. Transfer to medium with glutamine as the sole nitrogen source caused a decline in enzyme activity consistent with repression and dilution of enzyme.

These experiments led to the conclusion that the *gdhA10* lesion causes relief of ammonium repression but not relief of repression arising from growth on glutamate or glutamine. For convenience the effects of glutamate and glutamine will be referred to as glutamate repression and glutamine repression, respectively, in the rest of this communication (see Discussion).

It was then possible to carry out experiments in which mycelium was grown on medium with glutamine as the sole nitrogen source and then transferred to various treatment media. This avoided the complication of transferring myce-

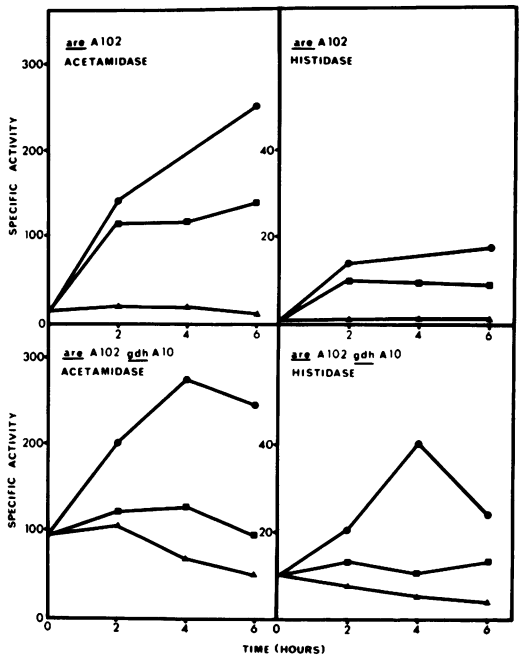

FIG. 1. *Effect of ammonium, glutamate, and glutamine on acetamidase and histidase activities in mycelium pregrown on glutamate plus ammonium. Mycelium was grown for 16 h in medium containing 10 mM glutamate plus 10 mM ammonium (as ammonium tartrate) for 16 h and then transferred to treatment media and incubated for various times before harvesting. Treatment media: (■), 10 mM glutamate; (▲), 10 mM glutamine; (●), no nitrogen source added. Specific activities in this and subsequent figures are in terms of milliunits per milligram of protein.*

lium containing substantial enzyme levels as occurred with mycelium of the *gdhA10* and *areA102,gdhA10* strains pregrown on glutamate plus ammonium medium.

The results shown in Table 2 indicate that the *gdhA10* mutant is partially relieved of ammonium repression of formamidase, acetamidase, and histidase but is sensitive to the effects of glutamate and glutamine when compared with the wild-type strain. The *areA102,gdhA10* strain is almost completely insensitive to ammonium repression of acetamidase, histidase, and benzamidase (Fig. 2), (enzyme levels in mycelium transferred to medium containing ammonium are similar to those in medium lacking a nitrogen source). Repression by glutamate and glutamine still occurs in the *areA102, gdhA10* strain (Fig. 2).

It should be noted here that the fluctuations in histidase-specific activity evident in Fig. 2B appear to constitute a genuine phenomenon,

TABLE 2. *Ammonium, glutamate, and glutamine repression of amidase enzymes and histidase in strains pregrown on glutamine medium*

Strain	Final growth conditions[a]	Enzyme sp act (mU/mg of protein)		
		Formamidase	Acetamidase	Histidase
Wild type	No nitrogen source	90	29	1.5
	10 mM NH_4^+	1	5	0
	10 mM glutamate	54	13	0.6
	10 mM glutamine	0	3	0.1
gdhA10	No nitrogen source	78	33	1.3
	10 mM NH_4^+	11	18	0.6
	10 mM glutamate	26	10	0.6
	10 mM glutamine	0	4	0.2

[a] Mycelium was grown for 16 h in 10 mM glutamine medium and then transferred to this final medium for 6 h before harvesting.

which is currently being studied (Polkinghorne and Hynes, unpublished data).

These experiments led to the conclusion that the *gdhA10* and *areA102* lesions interact to give a greater relief of ammonium repression of acetamidase and histidase than does the *gdhA10* lesion alone. Furthermore, both *gdhA10* and *areA102,gdhA10* are still sensitive to glutamate and glutamine repression. Cycloheximide was found to prevent the derepression of acetamidase, benzamidase, and histidase resulting from transfer of *areA102,gdhA10* mycelium from glutamate medium to ammonium medium (Table 3). Similarly, relief of glutamine repression was prevented by cycloheximide (Table 4). These results suggest that enzyme synthesis is repressed by glutamate and glutamine.

The *mauA1;gdhA10* strain was insensitive to ammonium repression of urate oxidase compared with the *mauA2* strain (Table 5). However, these strains showed similar sensitivity to glutamine repression.

It has also been found that the production of extracellular protease activity is sensitive to glutamate and glutamine repression. In contrast to an earlier report (3), it has been found that the *gdhA10* lesion leads to insensitivity to the effects of ammonium on extracellular protease. This has been observed in plate tests involving clearing of milk powder, as well as in enzyme assays. In plate tests the insensitivity of the *areA102,gdhA10* strain to ammonium was very obvious, but that of the *gdhA10* strain was more difficult to observe. This difficulty may account for the earlier failure to observe the effects of the *gdhA10* lesion on the milk clear-

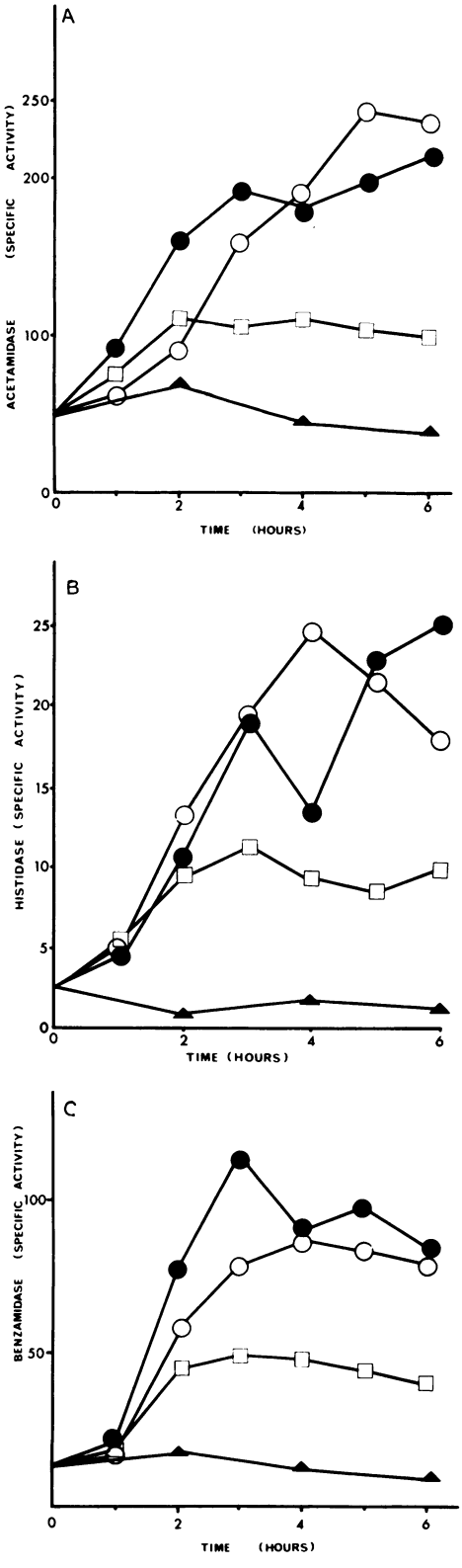

TABLE 3. *Effect of cycloheximide on relief of glutamate repression in the areA102,gdhA10 strain*

Final growth conditions[a]	Enzyme sp act (mU/mg of protein)		
	Acetamidase	Benzamidase	Histidase
10 mM NH_4^+	146	47	27.3
10 mM glutamate	74	17	9.3
10 mM NH_4^+ + cycloheximide	77	30	5.2
10 mM glutamate + cycloheximide	65	22	3.5

[a] Mycelium was grown for 16 h in 10 mM glutamate medium and then transferred to this medium for 4 h before harvesting. Cycloheximide was present at a concentration of 15 μg/ml.

TABLE 4. *Effect of cycloheximide on relief of glutamine repression in the areA102,gdhA10 strain*

Final growth conditions[a]	Enzyme sp act (mU/mg of protein)			
	Formamidase	Acetamidase	Benzamidase	Histidase
10 mM NH_4^+	21	212	52	20.7
10 mM NH_4^+ + heximide	0	30	0	0.6
10 mM glutamine	1	37	5	0.8
10 mM glutamine + cycloheximide	1	28	3	0.3

[a] Mycelium was grown for 16 h on 10 mM glutamine medium and then transferred to this medium for 4 h before harvesting. Cycloheximide was present at a concentration of 15 μg/ml.

ing. In protease enzyme assays, it has been found that growth of the *areA102,gdhA10* strain in ammonium medium leads to higher levels of activity than observed in medium lacking a nitrogen source (Table 6; unpublished data). This has not been observed for any other ammonium-repressible system. In plate tests glutamate was found to lead to some reduction in milk clearing, as has been observed previously (7). In addition, glutamine was observed to strongly reduce milk clearing. Slight insensitivity to the effects of glutamine was observed in

FIG. 2. *Ammonium, glutamate, and glutamine repression of acetamidase (A), histidase (B), and benzamidase (C) in strain areA102,gdhA10. Mycelium was pregrown in 10 mM glutamine medium and then transferred to the various treatment media and harvested at various times. Treatment media: (▲), 10 mM glutamine; (□), 10 mM glutamate; (○), 10 mM ammonium (as ammonium tartrate), (●), no nitrogen source.*

the *areA102,gdhA10* strain. The data presented in Table 6 support these results. The *gdhA10* and *areA102,gdhA10* strains were found to be sensitive to glutamate and glutamine repression of extracellular protease activity, although the *areA102,gdhA10* strain had slightly higher activity than *areA102* in glutamine medium. It should be noted that the strains containing the *areA102* lesion had higher protease activities than the wild-type strains (*areA*$^+$), in agreement with the results of plate tests (14).

An acidic amino-acid uptake system has been shown to be subject to ammonium and glutamine repression (22), and lesions in the *gdhA* gene have been shown to lead to insensitivity to ammonium repression (16, 20). In an experiment measuring [^{14}C]glutamate uptake by the *areA102,gdhA10* strain, it was found that uptake was completely insensitive to ammonium repression, but growth of mycelium in glutamine medium led to approximately 70% reduction in glutamate uptake capacity compared with uptake capacity of mycelium incubated in medium lacking a nitrogen source (unpublished data). Therefore, the acidic amino-acid uptake system appears to be sensitive to glutamine repression.

In contrast to all the systems mentioned above, there were clear indications that nitrate reductase was much less sensitive to glutamine repression than to ammonium repression. As found previously (3) the presence of the *gdhA10* lesion led to insensitivity of nitrate reductase to ammonium repression (Table 7). The presence of glutamine in the medium did not repress nitrate reductase greatly. The degree of repression can probably be accounted for by ammonium released from glutamine during growth. This suggestion is supported by the lower activity of the glutamine-grown culture of the *mauA2* strain compared with the *mauA2, gdhA10* strain. It is suggested that the effects of ammonium derived from glutamine on the other systems investigated is largely obscured by strong repression by glutamine. Some glutaminase activity has been observed in *A. nidulans* (unpublished data).

The findings of Cove (9) raise a possible objection to the conclusion that nitrate reductase is not very sensitive to glutamine repres-

TABLE 5. *Glutamine repression of urate oxidase*

Strain	Final growth conditions[a]	Urate oxidase (mU/mg of protein)
mauA2	Uric acid	96
	Uric acid + 10 mM NH_4^+	13
	Uric acid + 10 mM glutamine	24
mauA2; gdhA10	Uric acid	172
	Uric acid + 10 mM NH_4^+	95
	Uric acid + 10 mM glutamine	25

[a] Mycelium was grown on 10 mM glutamine medium for 16 h and then transferred to this medium for 4 h before harvesting. Uric acid was present in the medium at a concentration of 250 μg/ml.

TABLE 6. *Ammonium, glutamate, and glutamine repression of extracellular protease activity*

Strain	Final growth conditions[a]	Relative protease activity[b]
Wild type	No nitrogen source	45
	10 mM NH_4^+	3
	10 mM glutamine	7
	10 mM glutamate	16
gdhA10	No nitrogen source	25
	10 mM NH_4^+	41
	10 mM glutamine	7
	10 mM glutamate	10
areA102	No nitrogen source	100
	10 mM NH_4^+	2
	10 mM glutamine	2
	10 mM glutamate	75
areA102,gdhA10	No nitrogen source	122
	10 mM NH_4^+	222
	10 mM glutamine	21
	10 mM glutamate	63

[a] Mycelium was grown for 16 h on 10 mM glutamine medium and then transferred to this medium for 4 h before harvesting mycelium and collecting medium for extracellular protease assays.

[b] Arbitrary units (see Materials and Methods).

TABLE 7. *Ammonium and glutamine repression of nitrate reductase*

Strain	Final growth conditions[a]	Nitrate reductase (mU/mg of protein)
mauA2	10 mM NO_3^-	88
	10 mM NO_3^- + 10 mM NH_4^+	1
	10 mM NO_3^- + 10 mM glutamine	47
mauA2; gdhA10	10 mM NO_3^-	125
	10 mM NO_3^- + 10 mM NH_4^+	91
	10 mM NO_3^- + 10 mM glutamine	100

[a] Mycelium was grown for 16 h on 10 mM glutamine medium and then transferred to this medium for 4 h before harvesting. NO_3^- was added as sodium nitrate.

sion. He found that nitrate interferes with the utilization of many other nitrogen sources. Therefore, it is possible that the presence of nitrate as inducer results in reduced glutamine uptake and metabolism and so relieves glutamine repression. Therefore, the effects of growth in glutamine on nitrate reductase activities in the strain *nirA*$_1^c$, which produces nitrate reductase in the absence of inducer (19), was investigated. This strain is very sensitive to ammonium repression but not to glutamine repression (Table 8). Again, the effects of ammonium derived from glutamine might account for the 50% repression (comparison with the urea-grown culture) occurring during growth on glutamine. These experiments also show that growth on glutamate does not lead to lower activity than growth on urea, suggesting that nitrate reductase may not be sensitive to glutamate repression. This conclusion is not surprising in view of the inhibition of glutamate utilization by nitrate (9). It can be concluded that nitrate reductase is much less sensitive to glutamine repression than to ammonium repression.

DISCUSSION

An important finding of this work is that the *areA102,gdhA10* strain is less sensitive than the *gdhA10* strain to ammonium repression of a number of enzymes. The *areA102* lesion leads to higher levels of some but not all enzymes of nitrogen catabolism, but these enzymes are still subject to strong ammonium repression in the absence of the *gdhA10* lesion (this paper; unpublished data). These results therefore provide support for the proposed involvement of the *areA* gene in ammonium repression (2). There are at least two possible explanations for the observed interaction between *areA102* and *gdhA10*. The first suggestion is that a regulatory complex is formed between the products of these two genes and that this acts to stimulate enzyme synthesis in the absence of ammonium. In the presence of ammonium the action of this complex would be reduced, due to interaction between ammonium ion and the NADP-GDH part of the complex. This proposal combines the suggestions of Pateman et al. (20) and Arst and Cove (2). The second possibility is that the *gdhA10* lesion results in altered pool levels of ammonium (or a metabolite of ammonium), which then results in insensitivity to ammonium repression. If the *areA102* gene product was slightly altered in its interaction with ammonium ion (or a metabolite of ammonium), then this might result in a greater relief of ammonium repression in the double mutant. The proposed regulatory function for NADP-GDH can only be resolved by studies on more mutants, including those with defined effects on enzyme structure (e.g., deletion and nonsense mutants), as well as by looking for alterations in the regulatory function of NADP-GDH independent of catalytic alterations.

TABLE 8. *Effect of growth on glutamine, glutamate, and ammonium on nitrate reductase activity in the nirA*$_1^c$ *constitutive strain*

Growth conditions[a]	Nitrate reductase (mU/mg of protein)
5 mM urea	55
10 mM glutamate	55
10 mM glutamine	26
10 mM NH_4^+	1

[a] Mycelium was grown for 16 h in this medium and then harvested.

Growth of mycelium in glutamate medium leads to approximately 50% repression of some enzymes of nitrogen catabolism, when compared with mycelium incubated in medium lacking a nitrogen source. Since this occurs in strains insensitive to ammonium repression, it is unlikely that glutamate repression occurs via production of ammonium. Furthermore, there is no evidence for glutamate repression of nitrate reductase (8; Table 8) which is very sensitive to ammonium repression. Strong glutamine repression of many systems of nitrogen catabolism has been found. This occurs in mutants insensitive to ammonium repression, and nitrate reductase is much less sensitive to glutamine repression than to ammonium repression.

These data raise the question of whether the effects of ammonium, glutamate, and glutamine are all manifestations of the same regulatory mechanism or are due to different mechanisms. One possible way in which there could be a common regulatory mechanism is for the *gdhA10* lesion to affect the entry of ammonium into a pool of ammonium (or a metabolite of ammonium) which is the effector responsible for repression. The *gdhA10* lesion would not affect the levels of this pool of effector during growth on glutamate or glutamine medium. The effects of the *gdhA10* lesion on processing of ammonium could be attributed to loss of catalytic activity of NADP-GDH (as suggested by van de Poll [24]) or to an unknown role of NADP-GDH in the distribution of ammonium in various pools. In this connection it is known that mutants affecting ammonium pool levels can be altered in sensitivity to ammonium repression (4, 20). A common mechanism for repression by these compounds, however, is difficult to reconcile with strong ammonium repression of nitrate reductase (which is relieved by the *gdhA10*

lesion) but insensitivity to repression by glutamate or glutamine.

Therefore, it is tentatively suggested that regulation by glutamate and glutamine may be by different mechanism(s) from ammonium repression. Whether repression during growth on glutamate and glutamine is due to a single mechanism (e.g., repression by glutamine or a metabolite) cannot be determined at present. Glutamine is a strong nitrogen source for *A. nidulans* and therefore might be utilized in preference to many other nitrogen sources. In addition, glutamine and glutamate are the major end products of nitrogen catabolism. Therefore, mechanisms of regulation by these compounds are not unlikely.

Further studies on glutamine and glutamate metabolism are necessary to clarify the issues raised here. In addition, investigation of glutamine and glutamate repression in various *areA* mutants are necessary since the *areA* gene plays a fundamental role in nitrogen-catabolic regulation (2). Such experiments are complicated by the effects of gene *areA* on the utilization of glutamate and glutamine as nitrogen sources (2, 14; unpublished data). Pateman et al. (20) have proposed that it is the external concentration of ammonium which determines ammonium repression. At present there is no data on this point from this laboratory, except that in a preliminary experiment growth of mycelium in glutamine did not result in high levels of ammonium in the external medium. Experiments will be reported elsewhere (M. J. Hynes, Mol. Gen. Genet., in press) showing that methylammonium and cesium ion result in repression of some enzymes of nitrogen catabolism in strains insensitive to ammonium repression. These results further emphasize the complicated nature of regulation of nitrogen catabolism.

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ADDENDUM IN PROOF

Recent work has shown that *areA* lesions can alter ammonium repression of the acetamidase without affecting glutamate or glutamine repression of this enzyme. This supports the conclusion that repression by these compounds may be distinct from ammonium repression.

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