JOURNAL OF BACTERIOLOGY, Mar. 1979, p. 1119-1126
0021-9193/79/03-1119/08$02.00/0

Vol. 137, No. 3

Nitrogen Metabolite Repression of Nitrate Reductase in *Neurospora crassa*

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Received for publication 13 December 1978

The effect of different nitrogen compounds on the induction of reduced nicotinamide adenine dinucleotide phosphate-nitrate reductase was examined in *Neurospora crassa*. Whereas in the wild-type strain several amino acids and ammonia inhibit the formation of nitrate reductase, only glutamine, cysteine, and histidine are shown to inhibit the synthesis of nitrate reductase in a glutamine-requiring auxotroph. None of the amino acids inhibited nitrate reductase activity in vitro. The effects of cysteine and histidine are nonspecific, these amino acids being inhibitory to the growth of the organism. The effect of glutamine on the induction of nitrate reductase is not due to an inhibition of the uptake of the inducer nitrate. By the use of histidine-, pyrimidine-, and arginine-requiring auxotrophs, it was shown that glutamine appears to act per se and does not seem to be converted to another product in order to be effective in repression. The repression of nitrate reductase by ammonia appears, from the results described herein, to be indirect; ammonia has to be converted first to glutamine in order to be effective in repression.

Reduced NADPH-nitrate reductase from *Neurospora* catalyzes the first step in the assimilation of nitrate and is a cytochrome b_{557}-containing molybdoflavoprotein of about 230,000 molecular weight (7, 8).

Nitrate is required for the induced formation of nitrate reductase in *Neurospora* (12, 14, 18). The effect of nitrate is to stimulate transcription (19, 23) and to partially protect the enzyme from inactivation in vivo (20, 24).

Ammonia, or some metabolic product of ammonia, appears to repress the reductase in *Neurospora* at the transcriptional level (20, 23) by what seems to be a form of nitrogen metabolite repression, which has been observed in fungi (1, 16). Amino acids, purines, and pyrimidines, which are the major end products of inorganic nitrogen metabolism, are prime candidates for effectors in the regulation of nitrate reductase. Indeed, it has been shown that several amino acids repress nitrate reductase in *Neurospora* (3, 23). As metabolic interconversions of amino acids are possible in the wild-type strain, it was not feasible to distinguish the amino acid (or amino acids) responsible for the repression.

The purpose of the present investigation was to reinvestigate the effect of ammonia and some of the metabolic end products of ammonia (amino acids, adenine, uracil) on the induction of nitrate reductase in *Neurospora*. The approach used was the following: (i) to examine the effect of these metabolites on the net induced rate of appearance of nitrate reductase activity, as well as on the metabolic activities that add up to this net appearance of activity, i.e., effects on synthesis, on decay of the enzyme, on the uptake of the inducer nitrate, and on the activity of the reductase; and (ii) to carry out some of the studies mentioned above with amino acid- and pyrimidine-requiring auxotrophs and with strains which do not utilize ammonia, in order to identify effectors by preventing their metabolic conversion to other compounds.

(A preliminary report of this investigation was presented at the 21st Annual Meeting of the Canadian Federation of Biological Societies, London, Ontario, Canada, 20 to 23 June 1978.)

MATERIALS AND METHODS

***Neurospora* strains.** All stocks except His a came from the Fungal Stock Center, Humboldt State University Foundation, Arcata, Calif. Strain 3.1a (FGSC 988) was used as the wild-type strain. The mutant strains used were: *gln-a* (FGSC 1449), which requires glutamine for growth (E. Reich and S. Silagi, Proc. Int. Congr. Genet. 11th, Abstr. no. 1, p. 49-50, 1963) and has recently been shown to have an impaired glutamine synthetase (4); *arg-1A* (FGSC 325) and *arg-3A* (FGSC 1068), both of which require arginine; *pyr-3A* (FGSC 1964), which requires cytosine or uracil; His a (unknown origin), which requires histidine; and *am-1A* (FGSC 1184), which cannot use ammonia as the sole source of nitrogen, but can grow on amino acids like the wild-type strain (6).

Culture conditions. The basic medium without a nitrogen source has been described (22) and contained sucrose (20 g/liter) and macro- and micro-mineral elements. This medium was designated no nitrogen medium. One of the following nitrogen sources was included in the medium: ammonium tartrate, 4 g/liter, (ammonia medium); sodium nitrate, 20 mM (induction medium); glutamine, 2 g/liter, (glutamine medium); or sodium nitrite, 0.5 g/liter (nitrite medium). The wild type and strains *gln-a* and *am-1a* were grown in glutamine medium. Strains *arg-1A* and *arg-3A* were grown in glutamine medium supplemented with arginine (0.25 g/liter). Strain His a was grown in glutamine medium supplemented with histidine (0.4 g/liter). Strain *pyr-3A* was grown in ammonia medium supplemented with uracil (0.1 g/liter). The culture conditions were as described previously (29). When the induction of nitrate reductase was being studied, mycelial pads were grown from a conidial inoculum for 39 h at 27°C in standing culture with an appropriate supplement if required, washed, and subsequently transferred to induction medium (three pads per 30 ml of medium) supplemented or not with the test compound at the required concentration, and incubated with shaking for the desired time at 27°C. The mycelia were then collected, washed with distilled water, squeezed dry between paper towels, and either extracted immediately or frozen in liquid nitrogen and stored until used. When the decay of nitrate reductase was studied, the mycelia were grown for 39 h in stationary culture at 27°C, as described above, subsequently incubated in induction medium with shaking at 27°C for 3.5 h, washed, and finally transferred to induction medium supplemented with the desired test compound at the required concentration plus 6 mM sodium tungstate (decay medium) for 3.5 h at 27°C; after this the mycelial pads were harvested, washed with distilled water, squeezed dry with paper towels, and extracted immediately or frozen in liquid nitrogen and stored until used. When the uptake of nitrate was being studied, mycelia were grown from a conidial inoculum for 39 h at 27°C in standing culture in glutamine medium containing a trace element solution lacking molybdate. After growth, 30 mycelial pads at a time were washed with double-distilled water and transferred to 300 ml of induction medium lacking added molybdate from the trace element solution and containing 0.2 mM sodium metavanadate (17) plus the compound whose effect on uptake was to be tested; the culture was then shaken for 2 h at 27°C. The mycelia were subsequently washed thoroughly with double-distilled water, pressed dry between paper towels, weighed, and extracted with 0.1 M phosphate buffer, pH 7.0, containing 1 mM disodium EDTA (4 ml of buffer per g [wet weight] of mycelium). The brei was centrifuged at 15,000 × *g* for 15 min in a Sorvall centrifuge. A sample of the supernatant was placed in a boiling water bath for 30 min, and the precipitated protein was removed by centrifugation. Nitrate in the supernatant was determined enzymatically by its conversion to nitrite by partially purified nitrate reductase in a reaction mixture consisting of 0.8 ml of supernatant of boiled extract, 4 nmol of flavine adenine dinucleotide, 0.2 μmol of NADPH, and 5 U of partially purified nitrate reductase in a final volume of 1 ml. After incubation at 30°C for 15 min, the reaction was stopped with 1 ml of barium acetate (25% solution), and the nitrite formed was determined as described previously (14).

Extractions. The mycelia were extracted by grinding in an ice-cold mortar with an equal volume of silica in 0.1 M phosphate buffer, pH 7.0, containing 1 mM disodium EDTA (2 ml of buffer per 300 mg [blotted weight] of mycelium). The resulting brei was centrifuged at 12,000 × *g* for 15 min in a Sorvall refrigerated centrifuge. The supernatant was kept ice-cold until used.

Assay of nitrate reductase. NADPH-nitrate reductase was assayed as described previously (21), except that the reaction was stopped by the addition of 0.1 ml of 25% (wt/vol) barium acetate; the assay mixture was subsequently centrifuged at top speed in a bench top centrifuge for 5 min to remove precipitated protein and pyridine nucleotides. It was found that preincubation of crude extracts with all of the ingredients of the enzyme assay mixture except NADPH at 30°C for 30 min resulted in the maximum increase of nitrate reductase activity (unpublished data); for this reason extracts were routinely preincubated in this manner. The nitrate reductase assay reaction was started by the addition of NADPH after the above 30-min preincubation. One unit of nitrate reductase activity is defined as the production of 1 nmol of nitrite per min at 30°C. Specific activity is defined in terms of units of activity per milligram of protein.

Protein. Protein concentrations were determined by using the biuret reagent and crystalline bovine serum albumin as standard (9).

Materials. NADPH, flavine adenine dinucleotide, and bovine serum albumin were from Sigma Chemical Co. All other chemicals were of reagent grade and purchased locally.

RESULTS

The effect of amino acids, nucleosides, ammonia, and urea on the level of nitrate reductase in the wild type and in mutant strains *gln-a*, *arg-1A*, and *am-1a* was investigated. The results are summarized in Table 1. A number of amino acids and ammonia inhibited the induced expression of nitrate reductase to varying degrees in the wild-type strain. These results are in accord with the reported repression of the enzyme by ammonia (12) and by several amino acids (3, 23). The nucleosides and urea had no effect on the induced level of the enzyme.

None of the amino acids inhibited the activity of nitrate reductase in vitro, even at the final concentration of 20 mM used in our experiments (data not shown). Modulation of the activity of purified and extensively dialyzed nitrate reductase by some amino acids has been reported, but this effect is observed only in the absence of EDTA (11). Under our conditions, EDTA was used in the extraction buffer and was present in the assay mixture.

Arginine-requiring mutant *arg-1A* and mu-

TABLE 1. *Effect of different nitrogen compounds on the induced expression of nitrate reductase in the wild-type and mutant strains of Neurospora*[a]

Addition to induction medium	Nitrate reductase sp act (% of control)[b]			
	Wild type	*gln-a*	*arg-1A*	*am-1a*
None (control)	100	100	100	100
Ala	38	82	27	14
Gly	10	105	33	7
Ile	19	80	59	12
Leu	71	86	55	42
Val	24	88	36	24
Met	81	98	50	28
Cys	6	17	5	2
Ser	5	78	19	12
Thr	12	82	52	12
Pro	45	92	59	22
Asp	44	78	76	18
Glu	24	82	26	6
Asn	3	98	9	2
Gln	7	5	2	1
Arg	8	82	23	5
Lys	17	94	27	11
His	7	34	17	20
Tyr	75	83	55	42
Phe	61	97	58	41
Trp	31	104	22	40
Adenine	94	100	ND[c]	ND
Uracil	85	100	ND	ND
Ammonium tartrate	3	76	12	37
Urea	94	91	65	ND
Glu + ammonium tartrate	ND	44	ND	1

[a] Mycelia were grown in glutamine medium, transferred to induction medium plus or minus the test compound at a final concentration of 20 mM, and shaken for 3.5 h at 27°C as described in the text.

[b] The specific activities of nitrate reductase in control cell-free preparations of mycelia of wild type, *gln-a*, *arg-1A*, and *am-1a* were 47.2, 159, 47, and 145.9 U per mg of protein, respectively. These were each designated as 100%, and the specific activities of the preparations of mycelia induced in the presence of test compounds in the induction medium were expressed relative to these control values. The results given are representative of four different experiments.

[c] ND, Not determined.

tant *am-1a*, which does not utilize ammonia, showed a pattern of inhibition of the induced expression of nitrate reductase by different amino acids which was similar to that observed in the wild-type strain. In strains *am-1a*, which does not utilize ammonia and lacks NADP-dependent glutamate dehydrogenase (6), and *gln-a*, which does not utilize ammonia and has an impaired glutamine synthetase (4), ammonium did not repress the reductase to the same extent as glutamine. Glutamic acid plus ammonium repressed nitrate reductase effectively in NADP-dependent glutamic acid dehydrogenase-deficient strain *am-1a*, but not very effectively in glutamine synthetase-deficient strain *gln-a* (Table 1). The fact that there was any repression by glutamate and ammonia in this strain is probably due to its leakiness (4). This result suggests that ammonium has to be converted to glutamine before it can be effective in repression. Based on their studies using the double mutant *nit-1 am-2*, Dantzig et al. (3) reported that ammonium per se does not seem to mediate "ammonium repression" but that it must be metabolized in order to do so. Our results are obviously in agreement with this observation. The only other amino acids that were inhibitory in the glutamine auxotroph were histidine and cysteine. These two amino acids were found to inhibit the growth of the wild-type strain (Table 2); hence, the effect of these amino acids appears to be nonspecific.

The apparent repressive effect of amino acids could be due to an amino acid-dependent acceleration of the rate of decay of nitrate reductase in vivo. To examine this possibliity, the effect of different amino acids on the rate of decay of nitrate reductase was tested in the glutamine-requiring strain *gln-a*. The experiment consisted of inducing nitrate reductase in the mold, as described above, and subsequently exposing the mycelia to decay medium, which contained sodium tungstate plus the compound whose effect on the decay was to be investigated. The presence of tungstate in the culture medium prevents the synthesis of new active nitrate reductase; hence, nitrate reductase activity detected during and after incubation in the decay medium would

TABLE 2. *Effect of amino acids on growth of wild-type Neurospora*[a]

Amino acid concn in growth medium (mM)	Wt of mycelium (mg) in medium containing:[b]		
	His	Cys	Gln
0	43.65	43.65	43.65
0.5	25.60	35.70	41.10
1.0	24.00	24.25	64.50
2.5	25.50	3.00	31.00
5.0	20.85	1.20	71.65
10.0	18.40	No growth	68.10

[a] *Neurospora* was grown in ammonia medium supplemented with the indicated concentrations of histidine, cysteine, or glutamine, as described in the text. The resulting pads (two in each case) were washed, pressed between folds of filter paper, placed in aluminum foil, dried overnight at 200°F (ca. 92°C), and then weighed.

[b] The values given are the average weight of one mycelial pad and are typical of three repeats.

be due to the remaining active nitrate reductase that had been synthesized before the exposure of the mold to tungstate (21).

Subramanian and Sorger (26) showed that, in the presence of tungstate and nitrate, *Neurospora* synthesizes the complete nitrate reductase protein complex, but that only the component having NADPH-cytochrome *c* reductase activity is catalytically active. Incorporation of tungstate into the *Neurospora* enzyme complex under similar conditions has been reported (13). Immunological evidence shows that the decay of nitrate reductase observed when the inducer nitrate is removed from the culture medium is due to the disappearance of the nitrate reductase antigen, suggesting that the decay is due to the disappearance of the enzyme protein and not merely to the inactivation of the activity (21).

The rate of decay of nitrate reductase was very fast in a medium lacking any nitrogen source (Table 3) (29). From the results shown in Table 3, it is apparent that glutamine, which inhibited the induced appearance of nitrate reductase, did not accelerate the rate of decay of this enzyme in vivo; if anything, the presence of glutamine in the culture medium decreased the rate of decay of the reductase in vivo.

We observed in preliminary experiments that when low concentrations of glutamine were present in the induction medium, very little repression of nitrate reductase occurred. The reason for this observation was thought to be that glutamine was being utilized by the mold. To test this possibility, the time course of induction of nitrate reductase in the presence of 0, 2, and 20 mM glutamine was examined; the results are shown in Fig. 1. There was no induced expression of nitrate reductase activity initially, when glutamine was present in the medium. However, at the lower concentration of glutamine (2 mM), the specific activity of nitrate reductase began to rise after 2 h of incubation, indicating the beginning of relief of repression. Eventually, at the end of the fifth hour of incubation, nitrate reductase was induced to about 50% of its maximal level. Since the repression by glutamine persisted throughout the period of incubation at the higher concentration of glutamine (20 mM), the above result could be interpreted as being due to the depletion of glutamine by the mold. The effect of the concentration of glutamine on the induction of nitrate reductase was, therefore, tested by incubating the mycelia for 90 min in the induction medium containing the desired concentration of glutamine. Figure 2 shows that when the concentration of glutamine in the induction medium was as low as 2 mM, there was a resultant 85% repression of the induced expression of nitrate reductase.

TABLE 3. *Effect of different nitrogen compounds on the decay of nitrate reductase in vivo*

Addition to decay medium[a]	Nitrate reductase sp act (% of control)[b]
None	5.3
Ammonium tartrate	31.0
Sodium nitrate	31.0
Ala	11.0
Gly	14.0
Ile	5.6
Leu	6.8
Val	8.2
Met	6.6
Cys	5.4
Ser	12.4
Thr	5.7
Pro	8.9
Asp	13.7
Glu	17.2
Asn	12.3
Gln	22.4
Arg	23.3
Lys	11.6
His	7.7
Tyr	4.8
Phe	4.8
Trp	14.2
Adenine	13.4
Uracil	5.0

[a] Glutamine-requiring strain *gln-a* of *Neurospora* was grown in glutamine medium, transferred to induction medium for 3.5 h, and finally incubated in decay medium supplemented with the compound (20 mM) to be tested for 3.5 h, all as described in the text.

[b] The initial specific activity of nitrate reductase in extracts of mycelia in the decay medium was 159 U/mg of protein and was designated as 100%. Specific activities are expressed as the percentage of this control activity remaining in preparations of mycelia harvested and extracted at the end of the decay incubation. The results given are representative of three experiments.

Ammonia and numerous amino acids have been reported to inhibit the transport of nitrate into wild-type *Neurospora* mycelia (17). Arginine, tryptophan, valine, and threonine were reported to inhibit the transport of nitrate maximally, whereas glutamine was reported not to inhibit the uptake of nitrate at all (17). The effect of ammonia, arginine, and glutamine on the transport of nitrate in the glutamine-requiring mutant was investigated. The amount of nitrate present in mycelia incubated with glutamine and nitrate was found to be greater than that found in mycelia incubated with nitrate alone or with nitrate and either ammonium ions or arginine (Table 4). The specific activity of nitrate reductase, in contrast, was lowest in the above experiment in extracts of the mycelia that were incubated with glutamine and nitrate, in-

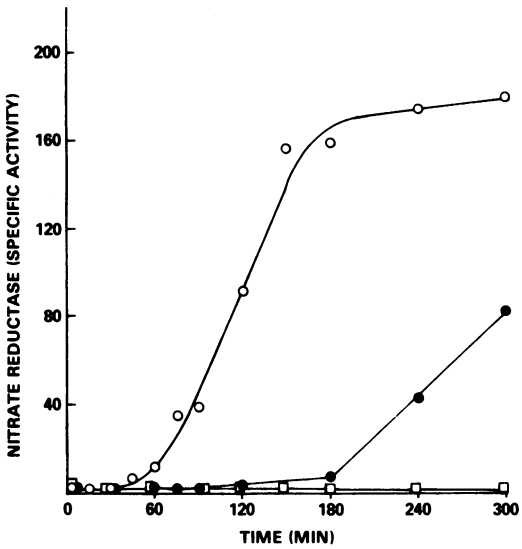

FIG. 1. *Time course of formation of nitrate reductase in the presence and absence of glutamine. Strain gln-a, a glutamine-requiring mutant of Neurospora, was grown in glutamine medium as described in the text. The mycelial pads were washed thoroughly and incubated in induction medium with shaking at 27°C in the absence* (○) *or in the presence of 2* (●) *or 20 mM* (□) *glutamine for the times indicated. The mycelia were then harvested and washed, and extracts were made and assayed as described in the text.*

dicating that glutamine was not preventing the uptake of nitrate.

The effect of glutamine on the formation of nitrate reductase was similar when the inducer was nitrite (Table 5). Nitrite is reportedly taken up by a system different from the nitrate uptake system in *Neurospora* and is reportedly not affected by the presence of ammonia or amino acids in the culture medium (18).

Nitrate reductase was synthesized by mycelia even when no nitrate was added to the culture medium (Fig. 3). The presence of arginine (which is reported to strongly inhibit the uptake of nitrate in the culture medium) at a concentration of 2 mM did not prevent the synthesis of nitrate reductase (Fig. 3); however, the level of nitrate reductase produced was lower than in its absence. When glutamine was present in the induction medium, on the other hand, the induced formation of nitrate reductase was completely inhibited (Fig. 3). The reason that nitrate reductase decayed so rapidly in nitrogen-deficient medium after 2 h in this experiment is that a rapid destruction mechanism is derepressed under these conditions (29).

It is apparent from the results displayed in Fig. 3 and 4 that extremely low concentrations of nitrate in the culture medium can effectively induce the formation of nitrate reductase. Schloemer and Garrett (17) reported a maximal 92% inhibition of the uptake of nitrate by amino acids. The remaining 8% is obviously more than sufficient to induce nitrate reductase in *Neuro-*

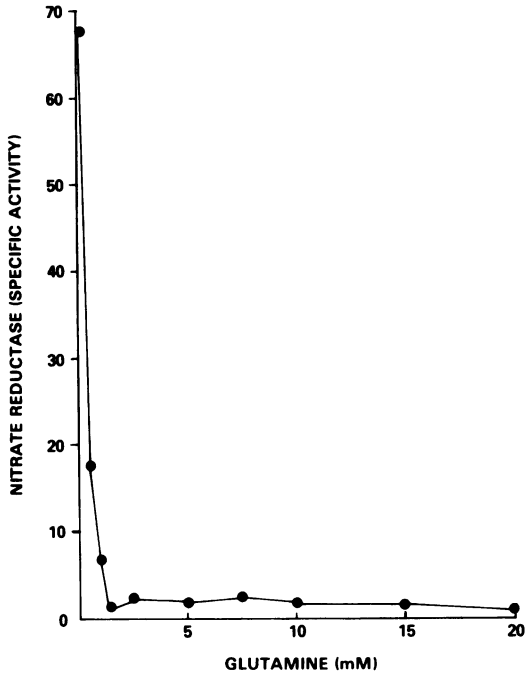

FIG. 2. *Effect of the concentration of glutamine in the culture medium on the induction of nitrate reductase. Strain gln-a, a glutamine-requiring auxotroph of Neurospora, was grown in glutamine medium, as described in the text. The mycelia were washed and subsequently incubated for 90 min with shaking at 27°C in induction medium containing the indicated concentrations of glutamine. The mycelia were then harvested, and extracts were prepared and assayed for nitrate reductase activity as described in the text.*

TABLE 4. *Effect of ammonium, arginine, and glutamine on the uptake of nitrate in the glutamine-requiring mutant*[a]

Addition to the vanadate-containing induction medium	Nitrate reductase activity (U/mg of protein)	Nitrate in the mycelial extract (nmol/g per 2 h)
None	11.6	4
Glutamine (2 mM)	1.0	41
Glutamine (20 mM)	0.5	45
Ammonium tartrate (20 mM)	6.8	11
Arginine (20 mM)	7.2	10

[a] The *gln-a* strain of *Neurospora* was grown in glutamine medium, induced for 2 h in induction medium containing no added molybdate and 0.2 mM sodium metavanadate plus the compound whose effect on uptake was to be tested.

TABLE 5. *Effect of glutamine on nitrate reductase activity induced with sodium nitrite in the glutamine-requiring mutant*[a]

Addition to *nitrite* induction medium	Nitrate reductase sp act (U/mg of protein)	Uptake of nitrite from the medium (μmol/g of mycelium per h)
None	121.7	141
Cycloheximide (2 μg/ml)	13.2	49
Glutamine (2 mM)	8	23
Glutamine (20 mM)	3.1	31

[a] The *gln-a* strain of *Neurospora* was grown in glutamine medium, transferred to nitrite medium containing the indicated test compound, and shaken at 27°C for 5 h.

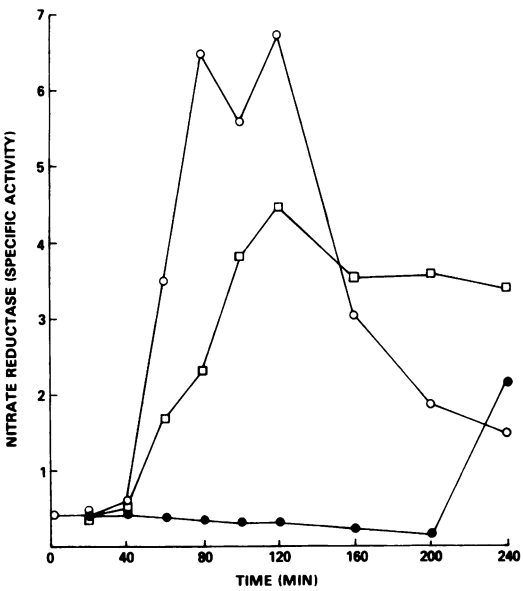

FIG. 3. *Effect of time of incubation, with and without arginine or glutamine, on the formation of nitrate reductase in mycelia incubated in no nitrogen medium. Strain gln-a, a glutamine-requiring mutant of Neurospora, was grown in glutamine medium as described in the text. The mycelial pads were then washed thoroughly with double-distilled water and incubated with shaking at 27°C in no nitrogen medium in the absence* (○) *or presence of either arginine* (□) *or glutamine* (●) *at a final concentration of 2 mM; after incubation for the indicated time intervals, the nitrate reductase activity in the mycelial extracts was assayed, as described in the text.*

spora bathed in a medium containing 20 mM nitrate.

To determine whether glutamine per se was active in repression, mutant strains lacking carbamylphosphate kinase (*pyr-3A*, *arg-3A*) and a strain unable to synthesize histidine (His a) were used. Glutamine repressed the formation of nitrate reductase (Table 6) in each of these strains, indicating that glutamine did not have to be converted to purines, pyrimidines, arginine, or histidine to be effective in repression. As tryptophan did not repress the enzyme (Table 1), glutamine was not repressing, obviously, by first being converted to tryptophan.

DISCUSSION

Our results suggest that glutamine is the main corepressor of nitrate reductase in *Neurospora*. The presence of glutamine in the culture medium greatly depressed the induction of nitrate reductase in the glutamine-requiring strain, whereas other metabolites, such as ammonium ions or asparagine, that behaved like glutamine in the wild-type strain (Table 1), were far less effective. This observation suggests that the compounds that inhibited the formation of nitrate reductase in the wild-type strain (Table 1) did so by being converted to glutamine. The mutation in the glutamine-requiring auxotroph *gln-a* has been shown to impair the conversion of glutamic acid to glutamine and to result in changes in both the activity and oligomeric structure of the enzyme glutamine synthetase (4). It is, therefore, reasonable to suggest that

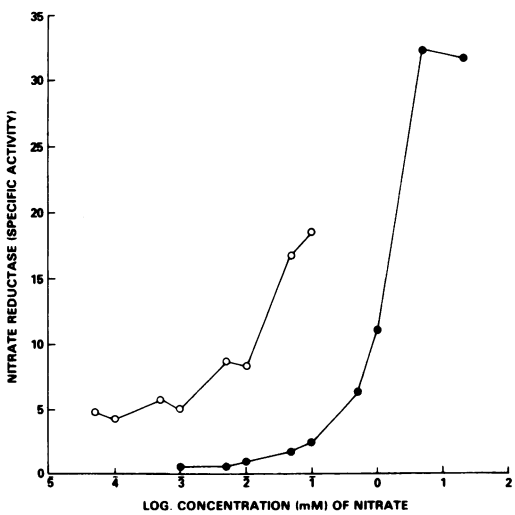

FIG. 4. *Effect of the concentration of nitrate on the formation of nitrate reductase in Neurospora. Strain gln-a, a glutamine-requiring mutant of Neurospora, was grown in glutamine medium, as described in the text. The mycelial pads were then washed and incubated with shaking at 27°C in induction medium, adjusted to contain different concentrations of nitrate, for 1.5* (○) *or 4 h* (●). *Mycelia were then harvested, and extracts were prepared and assayed for nitrate reductase as described in the text.*

TABLE 6. *Effect of glutamine on the induction of nitrate reductase in some mutant strains of N. crassa*[a]

Addition to induction medium	Nitrate reductase sp act (% of control)[b]			
	Wild-type strain	His a	*arg-3A*	*pyr-3A*
None (control)	100	100	100	100
Glutamine (2 mM)	46.9	12.9	1.5	3.6
Glutamine (20 mM)	0.9	2.95	1.4	0.7

[a] Histidine-requiring (His a), arginine-requiring (*arg-3A*), and pyrimidine-requiring (*pyr-3A*) auxotrophs were grown, transferred to induction medium containing glutamine at a final concentration of 2 or 20 mM, and shaken for 3.5 h at 27°C.

[b] The specific activities of nitrate reductase in control cell-free preparations from the wild type, His a, *pyr-3A*, and *arg-3A* strains exposed to induction medium containing no glutamine were 123, 139.8, 96.4, and 126.1 U/mg of protein, respectively. These were designated as 100%. Specific activities of other preparations were expressed as a percentage of these controls. The results given are representative of four separate experiments.

the compounds that inhibited the formation of nitrate reductase in the wild-type strain (Table 1) did so by being converted to glutamine. Our results are consistent with the observations of Dantzig et al. (3) and Subramanian et al. (23).

Glutamine prevented the induced appearance of nitrate reductase (Fig. 1) and did not enhance the rate of decay of the enzyme (Table 3). The effect of glutamine on the synthesis of nitrate reductase was not due to the inhibition of the uptake of the inducer nitrate. According to Schloemer and Garrett (17), certain amino acids repressed the nitrate transport system of wild-type *Neurospora*; tryptophan, arginine, valine, and threonine caused between 84 and 92% repression of the uptake system, whereas glutamine and alanine had no effect (17). Our observations on the *gln-a* mutant agree with these findings; the quantity of nitrate observed in extracts of mycelia exposed to nitrate plus glutamine was greater than that observed in mycelia exposed to nitrate alone or to nitrate plus arginine or to nitrate plus ammonia (Table 4). The intracellular nitrate which accumulates in the presence of glutamine is more than sufficient to induce a considerable level of nitrate reductase (Fig. 3 and 4), yet it does not induce the reductase. When nitrite was used as inducer, glutamine prevented the induced appearance of nitrate reductase (Table 5). The nitrite uptake system of *Neurospora* is reportedly not influenced by amino acids or ammonia (18). It appears, therefore, that the effect of glutamine cannot be due to inducer exclusion.

Glutamine did not inhibit the activity of nitrate reductase in vitro under our conditions. This observation is in agreement with the results of Garrett and Nason (8) and of Subramanian et al. (23). When the effect of different amino acids on purified nitrate reductase was tested in the complete absence of EDTA (11), glutamine, among other amino acids, stimulated the activity of nitrate reductase. Our results do not contradict these observations.

The effect of ammonium on nitrate reductase induction is probably the result of its conversion to glutamine. In strain *gln-a*, neither glutamic acid nor glutamic acid plus ammonium were as effective in repressing nitrate reductase as glutamine was (Table 1). On the other hand, in strain *am-1a*, which lacks NADP-dependent glutamic acid dehydrogenase, the reductase was not repressed by ammonium unless glutamic acid was also present (Table 1). The suggestion of Dantzig et al. (3) that ammonium repression of nitrate reductase is mediated by nitrogen metabolites other than ammonium is consistent with our results. In *Aspergillus nidulans*, three amidase enzymes and histidase are almost completely insensitive to ammonium but are strongly repressed by glutamine (10). In a strain of *Saccharomyces carlsbergensis* that lacks NADP-glutamate dehydrogenase, Van de Poll (28) found that allantoinase is repressed by glutamine and by glutamic acid plus ammonium, but not by ammonium alone. Arginase in *Neurospora* is repressed in *am-1a* either by glutamine or by ammonium plus glutamic acid but not by ammonium alone (27). These observations contrast with those of Dubois et al. (5) and Pateman et al. (15), suggesting a direct role of NADP-dependent glutamate dehydrogenase in nitrogen metabolite repression, and agree with those of Bossinger et al. (2), who dispute this contention.

The depressive effects of cysteine and of histidine on the induction of nitrate reductase appear to be nonspecific, as these amino acids also inhibited the growth of *Neurospora* on ammonia medium (Table 2) (23). Other amino acids had only slight effects on the induction of nitrate reductase in the glutamine-requiring mutant (Table 1), and it is difficult to distinguish whether these effects are specifically due to the amino acid tested or to its conversion to glutamine by degradation and a possible residual amount of glutamine synthetase in the mutant (4; unpublished data).

The question of whether glutamine itself was responsible for repression or whether it had to

be converted metabolically to some other product before it could be effective remains to be answered. Some of the most obvious metabolic products arising from glutamine, namely arginine, pyrimidines, histidine, and tryptophan (Table 1), did not repress nitrate reductase. Also, mutants auxotrophic for arginine and pyrimidines, lacking the carbamyl phosphate kinase of each of these pathways, and requiring histidine still had glutamine-repressible nitrate reductase, suggesting that glutamine or a very close metabolic product of glutamine was a corepressor.

ACKNOWLEDGMENTS

This work was supported by National Research Council of Canada grant A-3649.

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