

Effect of Ammonium Ions on the Induction of Nitrite Reductase in *Neurospora crassa*

H. C. P. CHANG AND G. J. SORGER*

Department of Biology, McMaster University, Hamilton, Ontario, Canada

Received for publication 26 January 1976

Results with strain *am-la*, a glutamate dehydrogenaseless mutant, showed that ammonium ions must first be metabolized in order to repress nitrite reductase in *Neurospora*.

Nitrite reductase catalyzes the second step in the assimilation of nitrate, i.e., the reduction of nitrite to ammonia. The enzyme is thought to be induced by its substrate nitrite and also by nitrate (1, 4-7, 9, 13). Nitrite reductase is believed to be repressed by ammonia (3, 5, 7-9, 13). The purpose of this investigation was to determine whether ammonium ions inhibit the synthesis of nitrite reductase in *Neurospora* and whether they can do so directly or only after being metabolized.

Neurospora strain 74 A was used as the wild-type strain. The basic medium (10) contained one of the following nitrogen sources: ammonium tartrate, 4 g/liter (ammonium medium); Casamino Acids, 5 g/liter (CA medium); or sodium nitrate, as stated in the experiments (nitrate medium). Enzyme preparation was as described previously (1). Nitrite reductase was assayed as described by Chang et al. (1). One unit of nitrite reductase activity is defined as the reduction of 1 nmol of nitrite per min, and 1 unit of ammonia-producing activity is defined as the production of 1 nmol of ammonia per min. Both activities measure the assimilatory nitrite reductase, but the nitrite-reducing activity also measures an interfering activity. Nicotinamide adenine dinucleotide phosphate nitrate reductase was assayed as described by Subramanian et al. (11). One unit of activity is defined as the production of 1 nmol of nitrite per min. Protein concentration was determined by the biuret method (2), with bovine serum albumin as standard.

When ammonium ions were present in the nitrate induction medium, the nitrite-reducing, ammonia-producing activity of the wild-type mycelia was partially repressed (Table 1). The extent of repression depended on the concentration of ammonium ions in the culture medium. Similar repression was observed when the mycelia were pregrown in CA medium.

Ammonium ions do not inhibit nitrite reduc-

TABLE 1. Effect of the concentration of ammonium ions in the nitrate induction media on the accumulation of nitrite reductase in wild-type mycelia^a

Nitrogen source and concn in induction medium	Sp act (U/mg of protein)	
	Nitrite reduction	Ammonia production
NO ₃ ⁻ (20 mM)	17.9	15.1 (100)
NO ₃ ⁻ (20 mM) + NH ₄ ⁺ (2 mM)	16.2	13.8 (91)
NO ₃ ⁻ (20 mM) + NH ₄ ⁺ (4 mM)	14.0	11.5 (76)
NO ₃ ⁻ (20 mM) + NH ₄ ⁺ (8 mM)	13.8	10.1 (67)
NO ₃ ⁻ (20 mM) + NH ₄ ⁺ (15 mM)	11.7	9.5 (63)
NO ₃ ⁻ (20 mM) + NH ₄ ⁺ (30 mM)	10.5	8.2 (54)

^a Wild-type mycelial pads pregrown into late log phase on ammonia medium were induced for 10 h on basic medium containing different concentrations of sodium nitrate and ammonium tartrate as shown. The mycelia were harvested and extracted, and the cell-free preparations were assayed immediately for nitrite reductase activity. The same experiment was repeated five times, and the results shown are representative of the five experiments. Figures in parentheses are percentages of control activity.

TABLE 2. Effect of the concentration of ammonium ions in the induction medium on the accumulation of nitrite reductase in *am-la* mycelia^a

Nitrogen source and concn in induction medium	Sp act (U/mg of protein)	
	NO ₂ ⁻ reduction	NH ₄ ⁺ production
NO ₃ ⁻ (20 mM)	23.2	19.2 (100)
NO ₃ ⁻ (20 mM) + NH ₄ ⁺ (2 mM)	28.6	23.0 (120)
NO ₃ ⁻ (20 mM) + NH ₄ ⁺ (4 mM)	27.1	21.1 (110)
NO ₃ ⁻ (20 mM) + NH ₄ ⁺ (8 mM)	25.8	19.9 (104)
NO ₃ ⁻ (20 mM) + NH ₄ ⁺ (15 mM)	25.2	20.1 (105)
NO ₃ ⁻ (20 mM) + NH ₄ ⁺ (30 mM)	24.3	18.5 (96)

^a Mycelial pads pregrown in CA medium until late log phase were induced for 10 h on basic medium containing different concentrations of sodium nitrate and ammonium tartrate as shown. The mycelia were harvested, extracted, and assayed for nitrite reductase activity. The results are representative of four repeated experiments. Figures in parentheses are percentages of control activity.

tase in vitro. The presence of 3 mM ammonium chloride in the assay mixture had no effect on extract-dependent reduction of nitrite (K. A.

TABLE 3. Nitrate content of extracts of wild-type and *am-la* mycelia that had been exposed to nitrate medium containing different concentrations of ammonium ions^a

Nitrogen sources and concn in the induction medium	Nitrate content of mycelial extract (nmol/mg of protein)	
	Wild type	<i>am-la</i>
NO ₃ ⁻ (10 mM)	2.0 (100)	3.6 (100)
NO ₃ ⁻ (10 mM) + NH ₄ ⁺ (5 mM)	1.7 (85)	2.2 (61)
NO ₃ ⁻ (10 mM) + NH ₄ ⁺ (10 mM)	1.3 (67)	2.3 (64)
NO ₃ ⁻ (10 mM) + NH ₄ ⁺ (20 mM)	2.7 (135)	2.0 (56)
NO ₃ ⁻ (10 mM) + NH ₄ ⁺ (40 mM)	4.4 (220)	4.5 (125)

^a Mycelial pads pregrown in CA medium (1) into late log phase were induced for 3.5 h on nitrate medium (1) containing 10 mM nitrate plus different concentrations of ammonium tartrate. The mycelia were harvested and extracted (1). Samples of extracts were autoclaved and centrifuged. The nitrate content of the clear supernatant fluids was measured by reducing nitrate to nitrite with nicotinamide adenine dinucleotide phosphate nitrate reductase and determining the resultant nitrite colorimetrically (12). The results are the average of two sets of determinations (which were within 15% of each other).

TABLE 4. Content of nitrate reductase and of ammonia of extracts from mycelia exposed to media containing nitrate (10 mM) and different concentrations of ammonium tartrate^a

Nitrogen sources and concn in the induction medium	Ammonia content of extract (nmol/mg of protein)		NADPH nitrate reductase sp act (units/mg protein)	
	Wild type	<i>am-la</i>	Wild type	<i>am-la</i>
NO ₃ ⁻ (10 mM)	23.6	42	21.2	17.1
NO ₃ ⁻ (10 mM) + NH ₄ ⁺ (5 mM)	19.4	32.6	6.75	9.85
NO ₃ ⁻ (10 mM) + NH ₄ ⁺ (10 mM)	20.5	41	6.12	8.0
NO ₃ ⁻ (10 mM) + NH ₄ ⁺ (20 mM)	20.8	34.4	3.45	9.4
NO ₃ ⁻ (10 mM) + NH ₄ ⁺ (40 mM)	46.2	46.4	2.0	9.2
NO ₃ ⁻ (10 mM) + NH ₄ ⁺ (60 mM)	48.1	53.6	ND ^b	ND

^a Mycelial pads pregrown in CA medium were induced for 3.5 h on basic media containing 10 mM NaNO₃ plus different concentrations of ammonium tartrate. The mycelia were harvested, extracted, and assayed for nicotinamide adenine dinucleotide (NADPH) nitrate reductase activity. One-milliliter aliquots of extracts were used to determine the ammonia content by the Conway microdiffusion method and by using Nessler's reagent (1). The results on ammonia content are representative of two sets of determinations. NADPH nitrate reductase activity shown is the average of duplicate measurements and is representative of three experiments.

^b ND, Not done.

TABLE 5. Effect of glutamic acid in the induction medium on the accumulation of nitrite reductase in wild-type and *am-la* mycelia^a

Nitrogen sources and concn in the induction medium	Sp act (U/mg of protein)			
	Wild type		<i>am-la</i>	
	Nitrite reduction	Ammonia production	Nitrite reduction	Ammonia production
NO ₃ ⁻ (10 mM)	18.6	15.3	26.5	19.6
NO ₃ ⁻ (10 mM) + glu (5 mM)	14.5	9.7	15.5	11.5
+ glu (10 mM)	13.9	8.5	14.8	10.0
+ glu (20 mM)	11.4	7.0	13.7	9.4
+ glu (40 mM)	11.2	6.3	11.5	8.2

^a Wild-type and *am-la* mycelial pads pregrown in ammonia medium and CA medium, respectively, until late log phase were induced for 9 h on basic media containing 10 mM nitrate and different concentrations of glutamic acid (glu) neutralized with sodium bicarbonate. The mycelia were harvested, extracted, and assayed for nitrite reductase activity. The results are representative of three experiments.

Cook and G. J. Mulkins, M.S. theses, McMaster Univ., Hamilton, Ontario, Canada, 1968 and 1970, respectively). No extractable inhibitor of nitrite reductase was produced when mycelia were exposed to ammonium ions. This was examined by mixing an extract of mycelia in which the nitrite reductase had been repressed by exposure to ammonium ions with an extract of fully induced mycelia. The nitrite reductase activity of the mixture was the sum of the activities of the mixture components. It thus appears that ammonium ions or a metabolic product of ammonium ion represses the accumulation of nitrite reductase in *Neurospora* mycelia. A similar conclusion, from different evidence, has been drawn from the situation in *Chlorella* (8).

When mycelia of strain *am-la*, which lacks glutamate dehydrogenase (4; our unpublished observations), were exposed to nitrate medium containing a concentration of ammonia ions that caused repression of nitrite reductase in

the wild-type strain, no effect on nitrite reductase formation was observed (Table 2). The lack of effect was apparently not due to differences in the effect of ammonium ions on the intracellular concentration of nitrate (Table 3) or ammonia (Table 4) between the wild-type and *am-la* strains.

The simplest explanation for the lack of repression of nitrite reductase by ammonium ions in *am-la* is that unmetabolized ammonium ions by themselves are not sufficient for repression. Two plausible alternatives can be entertained at this point: (i) ammonium ions must first be metabolized by a pathway using active glutamate dehydrogenase in order to repress nitrite reductase; or (ii) ammonium ions plus some other component missing in strain *am-la* are needed for repression of nitrite reductase.

When wild-type and *am-la* mycelia were exposed to nitrate medium containing different concentrations of glutamate, a metabolic product of ammonia, nitrite reductase induction was repressed in both strains (Table 5). The effect of glutamate was very similar to that observed with wild-type mycelia exposed to nitrate medium containing ammonium ions (Table 1). This observation would favor possibility (i), that ammonium ions must be metabolized in order to repress nitrite reductase.

We thank the National Research Council of Canada for grant no. A3649, which financed this work.

LITERATURE CITED

1. Chang, H. C. P., G. J. Mulkins, J. C. Dyer, and G. J. Sorger. 1975. Enzymatic and non-enzymatic reduction of nitrite by extracts of *Neurospora crassa*. *J. Bacteriol.* 123:755-758.
2. Dawson, R. M. C., D. C. Elliott, W. H. Elliott, and K. M. Jones (ed.). 1959. Data for biochemical research, p. 284. Oxford University Press, New York.
3. Eppley, R. W., and J. N. Rogers. 1970. Inorganic nitrogen assimilation of *Ditylum brightwellii*, a marine plankton diatom. *J. Phycol.* 6:344-351.
4. Fincham, J. R. S. 1954. Effects of a gene mutation in *Neurospora crassa* relating to glutamate dehydrogenase formation. *J. Gen. Microbiol.* 11:236-246.
5. Garrett, R. H. 1972. The induction of nitrite reductase in *Neurospora crassa*. *Biochim. Biophys. Acta* 264:481-489.
6. Ingle, J., K. W. Joy, and R. H. Hageman. 1966. Regulation of activity of the enzymes involved in the assimilation of nitrate by higher plants. *Biochem. J.* 100:577-588.
7. Kemp, J. D., and D. E. Atkinson. 1966. Nitrite reductase of *Escherichia coli* specific for reduced nicotinamide adenine dinucleotide. *J. Bacteriol.* 92:628-623.
8. Losada, M., A. Peneque, P. J. Aparicio, J. M. Vega, J. Cárdenas, and J. Herrera. 1970. Inactivation and repression by ammonium of the nitrate reducing system in *Chlorella*. *Biochem. Biophys. Res. Commun.* 38:1009-1015.
9. Pateman, J. A., B. M. Rever, and D. J. Cove. 1967. Genetic and biochemical studies of nitrate reduction in *Aspergillus nidulans*. *Biochem. J.* 104:103-111.
10. Sorger, G. J., and N. H. Giles. 1965. Genetic control of nitrate reductase in *Neurospora crassa*. *Genetics* 52:777-788.
11. Subramanian, K. N., G. Padmanaban, and P. S. Sarma. 1968. The regulation of nitrate reductase and catalase by amino acids in *Neurospora crassa*. *Biochim. Biophys. Acta* 151:20-32.
12. Subramanian, K. N., and G. J. Sorger. 1972. Regulation of nitrate reductase in *Neurospora crassa*: stability in vivo. *J. Bacteriol.* 110:538-546.
13. Vega, J. M., M. G. Guerrero, E. Leadbetter, and M. Losada. 1973. Reduced NAD⁺-nitrite reductase from *Azotobacter chroococcum*. *Biochem. J.* 133:701-707.