Regulation by Glutamine of Ammonia Transport in *Aspergillus nidulans*

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Conidia of *Aspergillus nidulans* had a very low activity of a high affinity ammonia transport system. During germination the activity increased, reaching a maximum soon after the onset of exponential growth. The activity in germinated conidia varied with the nitrogen source in the germination medium. Analyses of intracellular ammonia and amino acids revealed that a low level of ammonia transport activity was associated with a high intracellular concentration of glutamine and *vice versa*. The intracellular concentration of glutamine is probably involved in repression of synthesis of the ammonia transport system. It is suggested that glutamine and asparagine (rather than ammonia itself) regulate the pre-formed ammonia transport system by inhibition from within the intracellular pool. It is concluded that glutamine and glutamine synthetase may be more important in the regulation of some aspects of nitrogen metabolism rather than ammonia and glutamate dehydrogenase as has been previously suggested.

INTRODUCTION

Cook & Anthony (1978) have shown that there is a single active transport system for ammonia and its analogue methylamine in germinated conidia of *Aspergillus nidulans*, the activity being measured with [^{14}C]methylamine. Intracellular ammonia or a metabolite of ammonia has been implicated in the regulation, by inhibition and repression, of the active transport system for acidic amino acids in germinated conidia of this organism (Robinson *et al.*, 1973*b*) and a similar mode of regulation has been suggested for the regulation of the transport of ammonia itself (Anthony & Cook, 1973). This suggestion is supported by the work of Pateman *et al.* (1973) who have proposed a complex heuristic model for regulation in *A. nidulans* based on measurements of ammonia pool sizes and of various enzymes and transport systems involved in nitrogen metabolism in a variety of mutant strains. Important proposals were that intracellular ammonia and membrane-bound glutamate dehydrogenase are involved in the regulation of ammonia transport. The present paper tests this aspect of their model.

Variation in the specific activity of a transport system may arise either by a change in the activity of pre-formed system or by a change in the rate of its synthesis. It is difficult to distinguish directly between these two modes of regulation because transport systems cannot be assayed in cell-free extracts.

In the present paper this difficulty has been overcome by two types of experiment, both involving the measurement of intracellular pool concentrations of ammonia and of amino acids. The first type involved measurement of transport activity and pool concentrations during nitrogen starvation of germinated conidia having an initial low transport activity. The second type involved measurement of changes, during incubation with a

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variety of nitrogen compounds, in these activities and concentrations in germinated conidia having an initial high ammonia transport activity. The results of these experiments suggest that regulation of the transport of low concentrations of ammonia may be effected by the intracellular concentration of glutamine rather than by ammonia as such. It appears that regulation is achieved by both repression of synthesis of the transport system and by inhibition of activity of the pre-formed system.

METHODS

Chemicals. All chemicals were reagent grade and almost all were obtained from BDH. The exceptions were: L-[4,5-³H]leucine (38 Ci mmol⁻¹), [¹⁴C]methylamine hydrochloride (56 mCi mmol⁻¹) and [5-³H]-uridine from The Radiochemical Centre, Amersham; cycloheximide (actidione) from Sigma. Other chemicals were obtained from the sources given in the preceding paper (Cook & Anthony, 1978).

Organism and growth media. The wild-type strain (*biA1*) of *Aspergillus nidulans* was used. The growth media and methods used in preparing conidial suspensions have been described previously (Robinson *et al.*, 1973*a*), as have the methods for germination of conidia in aerated liquid cultures (Cook & Anthony, 1978).

Extraction and measurement of intracellular ammonia and amino acids. Conidia and germinated conidia were extracted with trichloroacetic acid (5%, w/v) twice for 10 min at 2 °C. After centrifugation to remove cell debris, the supernatant was further extracted with two 50 ml volumes of diethyl ether to remove trichloroacetic acid. The extracts were stored at -20 °C and assayed for ammonia and amino acids using a JEOL 6L amino acid analyser. Equal volumes of trichloroacetic acid extract were injected into the basic and acid-neutral columns of the analyser. L-Norleucine (50 nmol ml⁻¹) was added to the extract as an internal standard. To calculate the concentration of amino acids in the intracellular pool it was assumed that the intracellular volume of germinated conidia is three times their dry weight (Pall, 1970; Robinson *et al.*, 1973*b*). Glutamine and asparagine were not hydrolysed during analysis and the concentrations of ammonia were the same when assayed by the automatic analyser and by the glutamate dehydrogenase method (Cook & Anthony, 1978), indicating that there was no substantial hydrolysis of any amino acid to ammonia during analysis. Storage of conidial extracts for up to 2 months at -20 °C led to no loss of amino acids.

It should be emphasized that many of the conclusions in this paper assume that all amino acids and ammonia are extracted with more or less equal efficiency. This cannot be readily tested but no evidence to the contrary was ever obtained. It is also feasible that separate 'regulatory pools' of ammonia and amino acids occur. If the concentrations in these pools do not reflect the overall concentrations as measured in the present work then it is difficult to evaluate the evidence with respect to the nature of metabolites regulating the ammonia transport system.

Incorporation of L-[³H]leucine into protein and of [³H]uridine into RNA. The method used to determine the incorporation of L-[4,5-³H]leucine into protein by germinated conidia was that described by Robinson *et al.* (1973*b*). For measurement of RNA synthesis, conidia were germinated, harvested and resuspended in various media as indicated in Results. After 10 min incubation at 30 °C, [5-³H]uridine [6.6 nM; 2 µCi (6.6 pmol)⁻¹] was added. The conidia were further incubated and harvested at the appropriate time. Weighed samples of conidia were extracted by the method of Tisdale & DeBusk (1972). The conidia were extracted in trichloroacetic acid (10%, w/v) for 30 min at room temperature. The extracts were centrifuged and the supernatants discarded. The pellets were resuspended in 5 ml distilled water and filtered under vacuum through a Millipore filter (type HAWP, 0.45 µm pores, 25 mm diam.), washed twice with 5 ml volumes of cold (2 °C) distilled water, sucked dry and added to 10 ml butyl-PBD scintillation fluid (Cook & Anthony, 1978) for determination of radioactivity.

Measurement of transport of [¹⁴C]methylamine. Methods were as previously described (Robinson *et al.*, 1973*a*; Cook & Anthony, 1978). The initial rate of uptake was expressed as µmol substrate transported (g dry wt)⁻¹ min⁻¹. The specific activity of the transport system was expressed as units (g dry wt)⁻¹, one unit being defined as a rate of transport of 1 µmol substrate min⁻¹ at 30 °C at pH 6.5.

Fig. 1. Development of the ammonia transport system during germination. Conidia (approx. 10^7 ml^{-1}) were germinated with either glutamate (25 mM; ●) or ammonia (25 mM; ○) at 30°C on a gyrotary shaker (240 rev. min^{-1}). For convenience, separate flasks were used for each sample. In all experiments the ammonia transport system was assessed by measuring the transport of [^{14}C]-methylamine (20 μM) as described in Methods. The morphology of germinating conidia was observed under a phase-contrast microscope at intervals during germination. For the first 2 h the diameter of the conidia was $3.1\ \mu\text{m}$. After this time the diameter increased until at 8 h more than 90% of the germinating conidia had a diameter of $5\ \mu\text{m}$. At 9 h conidia were seen to darken and by 10 h more than 90% had visible germ tubes.

RESULTS

Activity of the ammonia transport system

Activity during germination. The specific activity of the ammonia transport system was very low in conidia [0.005 units ($\text{g dry wt})^{-1}$] but increased 400-fold after germination in a glutamate medium for 10 h, and subsequently decreased to 2.5% of the maximum level. Maximum activity was associated with germ tube emergence and the onset of exponential growth (Fig. 1). By contrast, activity remained low in conidia germinated with ammonia as the nitrogen source; the specific activity increased 18-fold during germination but reached only 2.5% of the maximum measured in glutamate-germinated conidia.

The total concentration of the ammonia/amino acid pool was greater at the time of germ tube emergence in ammonia-germinated conidia than in those germinated with glutamate (Tables 1 and 2). During germination, the total pool concentration was greatest at approximately 8 to 10 h; this was probably associated with the elevated protein synthesis occurring during germ tube production. During germination with ammonia (Table 2), the glutamine, asparagine and ammonia pool concentrations were very high compared with the concentrations in glutamate-germinated conidia, the variation in size of the glutamine pool being most marked. This suggested that one of these compounds (or a combination of them) might be important in the regulation of the ammonia transport system.

Activity in conidia germinated with various nitrogen sources. When ammonia, urea or L-glutamine (either singly or in mixtures) were present in the germination medium there was a very low level of transport activity (Table 3). These results are similar to those for glutamate transport (Robinson *et al.*, 1973b) and indicate that ammonia and/or glutamine play a role in the regulation of ammonia transport. When asparagine was the sole nitrogen source there was a 10-fold higher activity than in conidia germinated with glutamine or ammonia, indicating that asparagine is not as effective as glutamine and ammonia in this regulation.

Table 1. *Intracellular concentrations of ammonia and amino acids in conidia during germination with glutamate*

Conidia (approx. 10^7 ml $^{-1}$) were germinated with glutamate (25 mM) at 30 °C on a gyrotary shaker (240 rev. min $^{-1}$) for the periods indicated. For convenience, separate flasks were used for each sample. The transport of [14 C]methylamine (20 μ M) was measured as described in Methods and recorded as specific activity [units (g dry wt) $^{-1}$]. The intracellular concentrations (mM) of ammonia and amino acids were determined as described in Methods (—, not detected).

Compound in pool	0 h	4 h	8 h	10 h	12 h	16 h	20 h	24 h
Glutamine	2.54	—	—	0.60	0.96	0.83	0.31	0.31
Asparagine	1.93	1.25	1.51	1.40	1.10	0.78	1.30	1.08
Ammonia	1.47	1.69	2.69	2.39	3.43	3.96	3.43	7.26
Glutamate	4.53	30.81	47.07	44.78	28.84	33.60	18.84	20.23
Alanine	4.55	3.57	11.23	7.41	11.17	33.95	47.26	34.58
Ornithine	0.12	0.18	0.45	0.17	0.41	1.85	2.23	—
Lysine	3.86	4.14	5.75	6.01	4.39	6.59	7.27	10.70
Histidine	—	0.27	0.33	0.19	0.45	0.24	0.39	0.33
Arginine	0.91	0.49	1.33	0.51	0.59	1.03	1.39	1.19
Aspartate	0.24	3.28	4.08	3.08	2.95	1.84	1.76	1.06
Threonine	0.28	0.96	1.69	1.62	1.62	2.05	2.11	1.94
Serine	0.45	2.67	3.46	3.13	2.33	2.20	1.50	2.00
Glycine	0.64	0.54	1.08	0.88	0.79	1.52	1.79	1.59
Valine	0.62	1.48	0.93	1.65	0.85	2.31	2.05	4.04
Methionine	0.84	0.86	1.12	0.96	0.34	1.44	2.71	2.43
Isoleucine	0.25	0.30	0.28	0.29	0.20	0.40	0.76	0.52
Leucine	0.21	0.78	0.52	0.58	0.51	0.43	0.81	0.53
Tyrosine	0.12	—	—	0.11	—	0.17	0.23	0.23
Phenylalanine	0.08	—	—	—	—	0.19	0.11	0.10
Total pool	23.64	52.64	83.52	75.76	60.93	95.38	96.25	90.12
Specific activity of ammonia transport system	0.005	0.58	1.30	2.06	0.85	0.16	0.07	0.05

Regulation of the ammonia transport system by repression of its synthesis

Effect of nitrogen starvation on ammonia transport activity of ammonia-germinated conidia.

If the low activity of the system with ammonia-germinated conidia is a result of repression of synthesis or inhibition of a pre-formed transport system by intracellular nitrogen compounds, then a period of nitrogen starvation should lead to increased transport activity. To investigate this possibility, ammonia-germinated conidia were incubated for 3 h either with ammonia (25 mM) or in nitrogen-free medium (with or without cycloheximide, 10 μ g ml $^{-1}$) and the ammonia/amino acid pool concentrations and ammonia transport activity were measured. Protein and RNA synthesis were also monitored during this experiment (see below). Incubation with ammonia for a further 3 h had little effect on the composition of the amino acid pool or on ammonia transport activity (Fig. 2*a*; Table 4). By contrast, nitrogen starvation for a similar period led to a 20-fold increase in the specific activity of the ammonia transport system, a high proportion of this increase occurring during the second hour of incubation. Intracellular glutamine completely disappeared during the first hour of the incubation during which time there were also substantial reductions in the intracellular concentrations of asparagine, alanine, ammonia and glutamate. During the latter part of the incubation there was an increase in the pool sizes of most amino acids and ammonia; this was possibly the result of protein hydrolysis arising from the nitrogen starvation (see Meyers & Knight, 1973).

Cycloheximide, an inhibitor of protein synthesis, prevented the increase in transport activity but did not prevent the concentration of glutamine from falling to zero during the first hour of the incubation (Table 4).

These results suggest that, during nitrogen starvation of ammonia-germinated conidia,

Table 2. *Intracellular concentrations of ammonia and amino acids in conidia during germination with ammonia*

Conidia (approx. 10^7 ml $^{-1}$) were germinated with ammonia (25 mM) at 30 °C on a gyrotary shaker (240 rev. min $^{-1}$) for the periods indicated. For convenience, separate flasks were used for each sample. The transport of [14 C]methylamine (20 μ M) was measured as described in Methods and recorded as specific activity [units (g dry wt) $^{-1}$]. The intracellular concentrations (mM) of ammonia and amino acids were determined as described in Methods (—, not detected).

Compound in pool	0 h	4 h	8 h	10 h	12 h	16 h	20 h	24 h
Glutamine	2.54	16.22	24.94	29.95	27.26	19.43	18.65	20.19
Asparagine	1.93	2.27	4.33	6.50	5.67	3.76	4.36	5.33
Ammonia	1.47	8.75	4.15	6.30	6.22	6.55	5.58	4.48
Glutamate	4.53	19.24	40.40	31.48	30.04	16.63	14.25	15.38
Alanine	4.55	10.51	11.77	35.60	38.17	24.13	26.01	27.51
Ornithine	0.12	0.61	0.88	3.45	5.93	7.91	6.75	6.46
Lysine	3.86	4.75	7.35	7.29	6.79	5.22	6.61	8.42
Histidine	—	0.55	0.85	0.58	0.50	0.57	0.86	0.70
Arginine	0.91	5.09	7.22	3.92	3.52	3.72	4.04	3.30
Aspartate	0.24	1.27	3.41	3.11	1.29	1.75	1.55	1.96
Threonine	0.28	0.48	1.63	1.71	2.21	1.37	1.71	1.85
Serine	0.45	1.70	3.64	2.17	1.80	1.27	1.53	1.74
Glycine	0.64	0.51	1.36	1.14	1.33	1.04	1.43	1.42
Valine	0.62	2.49	1.55	3.09	3.22	1.26	1.83	2.08
Methionine	0.84	0.61	0.66	2.20	0.55	0.50	0.95	1.47
Isoleucine	0.25	0.26	0.34	0.52	0.65	0.60	0.60	0.74
Leucine	0.21	0.28	0.54	1.26	1.31	1.05	0.73	0.89
Tyrosine	0.12	0.20	0.17	—	0.32	0.31	0.29	0.13
Phenylalanine	0.08	0.48	0.51	—	0.10	—	0.11	0.56
Total pool	23.64	76.27	115.65	140.27	136.88	97.07	97.84	104.61
Specific activity of ammonia transport system	0.005	0.005	0.090	0.012	0.015	0.015	0.015	0.020

Table 3. *Specific activities of the ammonia transport system in conidia germinated with various nitrogen sources*

Conidia (10^7 ml $^{-1}$) were germinated at 30 °C in liquid media (see Methods) with the nitrogen sources indicated. The transport of [14 C]methylamine (20 μ M) was measured as described in Methods and the values are expressed as a percentage of the specific activity measured in conidia germinated with glutamate [0.96 units (g dry wt) $^{-1}$]. The growth rate of the conidia depended on the nitrogen source in the germination medium so, for valid comparisons with different nitrogen compounds, the specific activity of the transport system was measured in conidia at the time of germ tube emergence.

Nitrogen source	Time of germ tube emergence (h)	Relative transport rate (%)
L-Phenylalanine	11	210
L-Arginine	10	120
L-Alanine	12	110
L-Glutamate	12	100
Nitrate	12	90
Glycine	12	87
L-Asparagine	10	20
L-Glutamate + ammonia	10	6
Urea	11	4
L-Glutamine	10.5	2
Ammonia	10	2

Fig. 2. Effect of nitrogen starvation on the specific activity of the ammonia transport system and on protein and RNA synthesis. Conidia were germinated for 10 h at 30 °C with ammonia (25 mM) as the sole nitrogen source, and then harvested and resuspended (2 mg dry wt ml⁻¹) in various growth media. Some incubation media contained cycloheximide to inhibit protein synthesis; some (b) contained L-[4,5-³H]leucine (0.1 mM; 0.2 μ Ci μ mol⁻¹) to measure protein synthesis; and some (c) contained [5-³H]uridine [6.6 nM; 2 μ Ci (6.6 pmol)⁻¹] to measure RNA synthesis. The conidia were incubated for 1, 2 or 3 h; they were then harvested and the ammonia transport system (a), protein (b) and RNA (c) synthesized during these times was measured as described in Methods. ●, Nitrogen-free medium; ○, ammonia (25 mM); ▲, nitrogen-free medium containing cycloheximide (10 μ g ml⁻¹); △, ammonia (25 mM) added to nitrogen-free medium after 2 h incubation.

the increase in activity was probably due to derepression of synthesis of the transport system resulting from a decrease in the intracellular concentration of glutamine. That there was no increase in transport activity when the intracellular concentration of glutamine decreased in the presence of cycloheximide shows that de-inhibition of a pre-formed transport system is not the regulatory mechanism by which nitrogen starvation of ammonia-germinated conidia led to greatly increased ammonia transport activity. This, however, does not preclude the possibility that, once formed, the transport system is regulated by inhibition from within the cells (see below).

Incorporation of L-[4,5-³H]leucine into protein and [5-³H]uridine into RNA by nitrogen-starved conidia. Conidia incubated with ammonia synthesized protein at a high rate (Fig. 2b), as would be expected of nitrogen-sufficient, exponentially growing conidia. The rate of protein synthesis in nitrogen-starved conidia was 70% lower, on average, than in nitrogen-sufficient conidia. Cycloheximide almost totally inhibited protein synthesis. The results

Table 4. Intracellular concentrations of ammonia and amino acids in ammonia-germinated conidia incubated in various media

Conidia were germinated for 10 h at 30 °C with ammonia (25 mM) as the sole nitrogen source. The germinated conidia were harvested, re-suspended [2 g wet wt (100 ml)⁻¹] in various media (as indicated) and incubated at 30 °C for 1, 2 or 3 h. They were then harvested and the transport of [¹⁵C]methylamine (20 µM) was measured as described in Methods; the results are expressed as units (g dry wt)⁻¹. The intracellular concentrations (mM) of ammonia and amino acids were determined as described in Methods (—, not detected).

	No incu- bation	25 mM-Ammonia			Nitrogen-free medium			Nitrogen-free medium + 10 µg cycloheximide ml ⁻¹			Nitrogen-free medium + 25 mM-ammonia at 2 h	
		1 h	2 h	3 h	1 h	2 h	3 h	1 h	2 h	3 h	1 h	3 h
Glutamine	22.48	16.71	23.93	25.47	—	—	—	—	—	—	—	40.24
Asparagine	3.64	3.54	5.16	4.09	0.24	0.48	0.59	0.83	0.67	0.21	1.45	1.45
Ammonia	6.50	3.85	4.55	5.55	2.48	6.85	12.26	3.63	3.19	3.20	25.92	25.92
Glutamate	42.77	39.44	45.24	44.57	8.63	13.03	19.08	24.53	19.57	12.77	51.90	51.90
Alanine	12.33	2.89	3.69	5.89	0.84	1.32	2.04	2.72	2.12	2.13	10.32	10.32
Ornithine	0.74	0.30	0.68	0.91	0.19	0.05	0.03	0.31	0.09	0.13	1.22	1.22
Lysine	0.81	0.99	0.90	1.14	0.60	0.07	0.09	1.25	1.44	1.47	1.11	1.11
Histidine	0.93	0.58	0.76	0.71	0.11	0.07	0.13	0.71	0.74	0.74	0.93	0.93
Arginine	5.18	5.74	7.45	8.57	1.45	0.12	0.09	5.41	5.34	4.26	4.76	4.76
Aspartate	1.59	2.49	1.42	0.96	0.35	0.52	0.81	0.72	1.10	0.84	4.62	4.62
Threonine	3.67	2.44	2.77	2.92	1.29	1.54	1.61	3.66	3.50	3.07	2.72	2.72
Serine	3.60	3.60	4.76	4.43	1.75	2.27	3.29	2.89	2.10	1.99	4.05	4.05
Glycine	1.78	1.04	1.58	1.61	0.45	0.86	1.27	0.94	1.16	1.25	1.35	1.35
Valine	2.92	1.94	2.45	2.51	1.05	1.33	1.49	4.71	2.26	2.42	2.51	2.51
Methionine	0.99	0.69	0.73	0.78	0.38	0.22	0.87	1.65	2.07	0.46	0.64	0.64
Isoleucine	1.42	1.15	1.33	1.07	0.67	0.76	0.77	1.41	0.96	1.04	0.88	0.88
Leucine	0.51	0.34	0.28	0.32	0.32	0.45	0.36	0.71	0.56	0.78	0.38	0.38
Tyrosine	0.11	0.08	0.05	0.09	0.11	0.11	0.06	0.30	0.31	0.18	0.08	0.08
Phenylalanine	0.06	0.05	0.05	0.04	0.09	0.08	0.04	0.31	0.30	0.22	0.06	0.06
Total pool	112.03	87.86	107.78	111.72	21.00	30.31	44.88	56.69	47.48	37.98	155.14	155.14
Specific activity of ammonia transport system	0.127	0.127	0.170	0.105	0.550	2.140	2.460	0.080	0.090	0.110	0.050	0.050

Fig. 3. Effect of incubation of glutamate-germinated conidia with glutamine or asparagine on the specific activity of the ammonia transport system and on the glutamine and asparagine concentrations within the intracellular pool. Conidia (approx. 10^7 ml⁻¹) were germinated for approximately 10 h at 30 °C in a liquid medium containing glutamate (25 mM) as the sole nitrogen source. Harvested conidia were incubated in uptake assay medium plus (a) glutamine (0.2 mM) or (b) asparagine (0.2 mM) for 2, 5, 10 or 20 min. They were then harvested and washed, and their ammonia transport activity (measured with [¹⁴C]methylamine) and ammonia and amino acid pool concentrations were determined. ●, Transport activity; ▲, glutamine concentration; ■, asparagine concentration; □, glutamine plus asparagine concentration.

in Fig. 2(c) show that during the first hour of incubation there was little difference in the rate of RNA synthesis between nitrogen-starved and nitrogen-sufficient conidia. In the subsequent two hours of nitrogen sufficiency there was a steady rate of RNA synthesis. A similar result was recorded for nitrogen-starved conidia in the presence of cycloheximide. During the second hour of nitrogen starvation in the absence of cycloheximide there was a rapid increase in the synthesis of RNA which coincided with the increase in the specific activity of the ammonia transport system.

These observations are consistent with the conclusion that the increase in transport activity during nitrogen starvation of ammonia-germinated conidia results from derepression of synthesis of the ammonia transport system.

Regulation of the ammonia transport system by inhibition

Effect of ammonia on nitrogen-starved conidia. When ammonia-germinated conidia were starved of nitrogen for 2 h and then incubated with ammonia for 1 h, ammonia transport activity was reduced by 98% and the total concentration of ammonia and amino acids increased fivefold (Table 4). The elevated pool concentrations were mainly due to large increases in glutamine (100-fold) and in alanine, glutamate and ammonia (three- to fourfold); all other amino acids increased to some extent with the exception of leucine, phenylalanine and tyrosine. These large increases were probably the result of rapid metabolism of ammonia to provide amino acids for the growth of the previously nitrogen-starved conidia. The rapid and large decrease in transport activity correlated with the very large increase in glutamine concentration strongly suggests that the ammonia transport system, once formed, is regulated by inhibition by intracellular glutamine. This conclusion was confirmed by the following experiment in which a high initial rate of ammonia transport was ensured by germinating the conidia on glutamate.

Table 5. Intracellular concentrations of ammonia and amino acids in germinated conidia incubated with glutamine

Conidia (approx. 10^7 ml⁻¹) were germinated for approximately 10 h at 30 °C with glutamate (25 mM) as the sole nitrogen source. Harvested conidia were incubated in uptake assay medium plus glutamine (0.2 mM) for 2, 5, 10 or 20 min. They were then washed, and their ability to transport [¹⁴C]methylamine (20 µM) was measured as described in Methods and expressed as units (g dry wt)⁻¹. The intracellular concentrations (mM) of ammonia and amino acids were determined as described in Methods. No lysine was detected in these analyses.

Compound in pool	No incubation	2 min incubation	5 min incubation	10 min incubation	20 min incubation
Glutamine	4.57	7.61	7.91	10.33	9.46
Asparagine	1.25	2.14	1.19	1.98	1.59
Ammonia	10.09	5.29	6.56	9.84	6.87
Glutamate	11.58	9.19	8.18	10.70	9.70
Alanine	2.16	0.57	0.66	0.75	0.73
Ornithine	0.07	0.07	0.15	0.18	0.19
Lysine	—	—	—	—	—
Histidine	0.14	0.33	0.16	0.32	0.43
Arginine	0.40	0.75	0.92	1.59	1.83
Aspartate	2.49	0.02	1.97	3.04	2.53
Threonine	1.10	0.51	0.83	0.84	0.68
Serine	1.33	0.95	1.30	2.11	1.76
Glycine	0.64	0.24	0.45	0.60	0.44
Valine	0.97	0.73	0.46	0.49	0.39
Methionine	0.84	0.02	0.75	0.61	0.64
Isoleucine	0.49	0.25	0.31	0.34	0.27
Leucine	0.19	0.12	0.16	0.22	0.14
Tyrosine	0.10	0.09	0.12	0.13	0.04
Phenylalanine	0.06	0.02	0.03	0.06	0.02
Total pool	38.07	28.90	32.11	44.13	37.71
Specific activity of ammonia transport system	1.32	0.80	0.60	0.48	0.65

Effect of glutamine and asparagine on the activity of the pre-formed ammonia transport system. Incubation of glutamate-germinated conidia with glutamine (0.2 mM) for up to 20 min led to a progressive decrease in activity which correlated inversely with an increase in the glutamine concentration in the cells (Fig. 3*a*; Table 5). Doubling the pool concentration of glutamine halved the activity of the ammonia transport system; no other amino acid or ammonia showed any such correlation. The rapidity of the decrease in activity was not consistent with dilution of components of the transport system or with protein breakdown. As glutamine was the only compound increasing in concentration during the short incubation, it is concluded that glutamine inhibits the pre-formed system in germinated conidia.

Incubation with asparagine (0.2 mM) gave similar results to those obtained with glutamine (Fig. 3*b*; Table 6). During the first 10 min, there was a 75% decrease in the transport activity associated with an increase in both asparagine and glutamine pool concentrations. In the next 10 min, the glutamine concentration decreased and there was an equivalent increase in asparagine concentration. That the transport activity remained inhibited during this period suggests that asparagine also inhibits the transport system. The inverse correlation between transport activity and the combined glutamine plus asparagine concentrations (Fig. 4) supports the suggestion that the ammonia transport system is inhibited by both glutamine and asparagine.

Fig. 4. Correlation between glutamine plus asparagine concentration and ammonia transport activity. The data are taken from Fig. 3. ●, Glutamate-germinated conidia (zero time); ▲, conidia incubated with glutamine; ■, conidia incubated with asparagine.

Table 6. *Intracellular concentrations of ammonia and amino acids in germinated conidia incubated with asparagine*

Conidia (approx. 10^7 ml⁻¹) were germinated for approximately 10 h at 30 °C with glutamate (25 mM) as the sole nitrogen source. Harvested conidia were incubated in uptake assay medium plus asparagine (0.2 mM) for 2, 5, 10 or 20 min. They were then washed, and their ability to transport [¹⁴C]methylamine (20 μ M) was measured as described in Methods and expressed as units (g dry wt)⁻¹. The intracellular concentrations (mM) of ammonia and amino acids were determined as described in Methods. No lysine was detected in these analyses.

Compound in pool	No incubation	2 min incubation	5 min incubation	10 min incubation	20 min incubation
Glutamine	4.57	5.27	9.02	10.77	6.24
Asparagine	1.25	2.11	2.11	2.77	7.10
Ammonia	10.09	4.66	6.23	5.38	6.17
Glutamate	11.58	9.13	9.24	10.16	9.93
Alanine	2.16	0.84	0.64	0.72	0.59
Ornithine	0.07	0.05	0.08	0.10	0.10
Lysine	—	—	—	—	—
Histidine	0.14	0.16	0.36	0.37	0.51
Arginine	0.40	0.92	1.54	1.82	2.45
Aspartate	2.49	1.90	2.83	1.73	0.66
Threonine	1.10	0.94	0.53	0.84	0.74
Serine	1.33	1.28	1.09	1.62	0.79
Glycine	0.64	0.53	0.24	0.47	0.23
Valine	0.97	0.78	1.23	0.53	0.39
Methionine	0.84	0.71	0.02	0.02	0.02
Isoleucine	0.49	0.37	0.36	0.27	0.37
Leucine	0.19	0.22	0.18	0.16	0.20
Tyrosine	0.10	0.13	0.02	0.02	0.11
Phenylalanine	0.06	0.06	0.02	0.02	0.02
Total pool	38.07	30.06	35.71	37.77	37.62
Specific activity of ammonia transport system	1.32	1.05	0.60	0.35	0.32

DISCUSSION

The results in the present paper indicate that the system for active transport of low concentrations of ammonia was absent in conidia of *A. nidulans* and that its synthesis was repressed during germination by high intracellular concentrations of glutamine. Nitrogen starvation of ammonia-germinated conidia resulted in a large reduction in the glutamine pool concentration with concomitant derepression of the transport system which was also shown to be subject to inhibition by intracellular glutamine and asparagine. (See note of caution, in Methods, with respect to interpretation of concentrations of extracted amino acids and ammonia.)

When conidia were germinated with asparagine as the sole nitrogen source, the transport activity was 10-fold higher than in those germinated with glutamine or ammonia, indicating that asparagine may not be as effective as glutamine in repressing the synthesis of the ammonia transport system. The activity of the pre-formed system was, however, more sensitive to inhibition by intracellular asparagine than it was to glutamine.

The function of the high affinity ammonia transport system is probably not the 'bulk' transport of ammonia (Cook & Anthony, 1978). Nitrogen starvation of germinated conidia resulted in a rapid increase in activity after a lag period of approximately 1 h indicating that synthesis of the transport system is specifically derepressed in response to nitrogen starvation. These observations suggest that the system functions as a scavenger for ammonia during nitrogen starvation. A similar function has been ascribed to the ammonia transport system of *Penicillium chrysogenum* (Hackett *et al.*, 1970). If the system is an ammonia scavenger, active during nitrogen starvation, then its derepressed synthesis during germination on alanine, arginine, glutamate, glycine, phenylalanine or nitrate must be explained. These nitrogen sources were sufficient for germination and subsequent growth of conidial germ tubes and all, with the exception of nitrate, contributed to the carbon metabolism of the organism. Carbon catabolite repression by glucose of glutamate transport is known to occur (Hynes, 1973) and it is possible that glucose also controls the assimilation of other amino acids. Glucose (1%) was always present as the carbon source during germination in the experiments described in the present work and it is suggested that the flow of α -amino nitrogen from amino acids may be so stringently controlled by glucose that a state of nitrogen deprivation may have occurred which allowed the derepression of the ammonia transport system.

Previous workers on 'ammonium' regulation in *A. nidulans* have concluded that ammonia itself is the regulatory molecule, rather than glutamine or asparagine as appears to be indicated by the data in the present paper. This difference in conclusions reflects the different experimental approaches used; our observations do not markedly disagree with previous observations where the experiments have been similar. The most thoroughly described model for regulation has been that of Pateman *et al.* (1973). These workers proposed a unifying hypothesis to account for 'paradoxical and complex relationships between ammonium transport and the ammonium regulation of various transport and enzyme systems' in a wide variety of mutants. The demonstration in the present paper that intracellular glutamine and asparagine may be the regulators of ammonia transport suggests that the model may be incorrect in its proposal that the 'intracellular ammonium concentration affects the level of ammonium uptake'.

It is possible that the levels of glutamine and asparagine (rather than ammonia) function in the regulation of nitrogen metabolism by reflecting the flow or amount of nitrogen for biosynthesis. This conclusion directs attention to the possibility that glutamine synthetase may well have a more significant role in the regulation of some aspects of nitrogen metabolism than does glutamate dehydrogenase as previously proposed in the model of Pateman *et al.* (1973). Similar studies to those described here using mutants lacking glutamine

synthetase and glutaminase might be effective in confirming this proposed role for glutamine and glutamine synthetase in the regulation of ammonia transport.

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REFERENCES

- ANTHONY, C. & COOK, R. J. (1973). Regulation of methylamine transport during germination of *Aspergillus nidulans*. *Journal of General Microbiology* **77**, vii.
- COOK, R. J. & ANTHONY, C. (1978). The ammonia and methylamine active transport system of *Aspergillus nidulans*. *Journal of General Microbiology* **109**, 265–274.
- HACKETTE, S. L., SKYE, G. E., BURTON, C. & SEGAL, I. H. (1970). Characterization of an ammonium transport system in filamentous fungi with methylammonium-¹⁴C as the substrate. *Journal of Biological Chemistry* **245**, 4241–4250.
- HYNES, M. J. (1973). Pleiotropic mutants affecting the control of nitrogen metabolism in *Aspergillus nidulans*. *Molecular and General Genetics* **125**, 99–107.
- MEYERS, E. & KNIGHT, S. G. (1953). Studies on the intracellular amino acids of *Penicillium roqueforti*. *Mycologia* **53**, 115–122.
- PALL, M. L. (1970). Amino acid transport in *Neurospora crassa*. II. Properties of a basic amino acid transport system. *Biochimica et biophysica acta* **203**, 473–483.
- PATEMAN, J. A., KINGHORN, J. R., DUNN, E. & FORBES, E. (1973). Ammonium regulation in *Aspergillus nidulans*. *Journal of Bacteriology* **114**, 943–950.
- ROBINSON, J. H., ANTHONY, C. & DRABBLE, W. T. (1973a). The acidic amino acid permease of *Aspergillus nidulans*. *Journal of General Microbiology* **79**, 53–63.
- ROBINSON, J. H., ANTHONY, C. & DRABBLE, W. T. (1973b). Regulation of the acidic amino acid permease of *Aspergillus nidulans*. *Journal of General Microbiology* **79**, 65–80.
- TISDALE, J. H. & DEBUSK, A. G. (1972). Permeability problems encountered when treating conidia of *Neurospora crassa* with RNA synthesis inhibitors. *Biochemical and Biophysical Research Communications* **48**, 812–822.