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Regulation by Glutamine of the Synthesis of the Acidic Amino Acid Transport System of *Aspergillus nidulans*

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Intracellular concentrations of ammonia and amino acids, and the specific activity of the acidic amino acid transport system responsible for transport of low concentrations of glutamate in *Aspergillus nidulans* have been measured during germination on glutamate and ammonia and during various subsequent incubations of the germinated conidia. It is concluded that intracellular glutamine (and not ammonia) is involved in repression of synthesis of the transport system for low concentrations of glutamate and that intracellular ammonia (but not glutamine) is able to inhibit the preformed permease. This conclusion supports our suggestion that glutamine synthetase may well have a more significant role in the regulation of some aspects of nitrogen metabolism than does glutamate dehydrogenase as was previously proposed (Pateman *et al.*, 1973).

INTRODUCTION

Germinated conidia of *Aspergillus nidulans* possess a high affinity, acidic amino acid transport system (permease) which actively transports L-glutamate (K_m 180 μM), L-aspartate (K_m 100 μM) and L-cysteate (K_m 190 μM) (Robinson *et al.*, 1973*a*) and the same transport system has also been shown to occur in the mature mycelium (Pateman *et al.*, 1974). After an extensive investigation of the kinetics of synthesis of this transport system, Robinson *et al.* (1973*b*) proposed that its synthesis is repressed by ammonia or a metabolite of ammonia; that it is one of a number of metabolic systems whose synthesis is ammonia-repressible was also suggested by indirect analysis using mutants thought to be insensitive to ammonia repression (Pateman *et al.*, 1974). Robinson *et al.* (1973*b*) emphasized that the correlation between high intracellular concentrations of ammonia and low transport rates should be treated with caution because of the analytical methods used. In the work described below, this caution is seen to have been justified; we have found that glutamine and not ammonia is the metabolite whose large variations in intracellular concentration affect synthesis of the glutamate transport system.

These experiments were done at the same time as similar experiments on the regulation of the ammonia transport system of *A. nidulans* and the concentrations of ammonia and amino acids measured during the experiments described below have all been published in the account of that investigation (Cook & Anthony, 1978*a*, *b*).

METHODS

The wild-type strain (*biA1*) of *Aspergillus nidulans* was used. Growth media and methods for preparing conidial suspensions have been described previously (Robinson *et al.*, 1973*a*), as have the methods for germination of conidia (Cook & Anthony, 1978*a*). Transport of L-[U-^{14}C]glutamate was measured as described by Robinson *et al.* (1973*a*) and specific activities are expressed as units (g dry wt)$^{-1}$; 1 unit is defined as being able to transport 1 μmol glutamate min^{-1} at 30 °C at pH 6·5. Using the method of Ressler & Koga (1971) it was shown that the amount of transported glutamate oxidized to CO_2 during the assay period was

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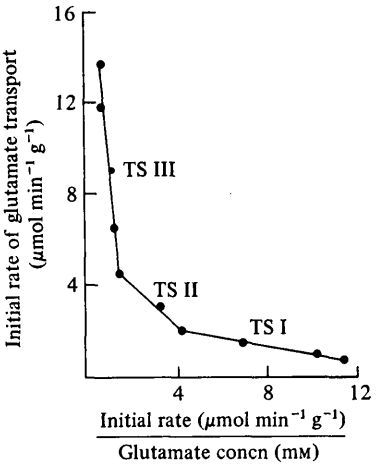

Fig. 1. Kinetics of L-glutamate transport by germinated conidia of *A. nidulans*. Conidia were germinated for 10 h at 30 °C in a glutamate (25 mM) medium and the rates of transport of various concentrations of L-[U-^{14}C]glutamate were measured. The K_m values from this plot of the data (Hofstee, 1952) are obtained from the slope of the curves.

insignificant and thus did not affect the measured rate of glutamate transport. Anaerobic assay conditions decreased the transport rate by more than 90 % indicating that the energy supply for this system requires aerobic metabolism. The methods and results of amino acid extractions and measurements have been described previously (Cook & Anthony, 1978*a*, *b*).

RESULTS

Demonstration of three glutamate transport systems

Our previous work on the regulation of glutamate transport in germinated conidia of *Aspergillus nidulans* concentrated on the system having a high affinity for acidic amino acids (Robinson *et al.*, 1973*a*, *b*), but it was later shown that *Aspergillus clavatus* has at least two systems capable of transport of glutamate (Robinson *et al.*, 1974). To facilitate comparison of conclusions described in the present work on the high affinity system with data that might be obtained by measuring glutamate transport at high concentrations it was essential briefly to characterize systems that might transport glutamate at high concentrations. The results (Fig. 1) show that in glutamate-germinated conidia there were three systems able to transport glutamate: the high affinity transport system I (TS I, K_m 180 μM) described previously (Robinson *et al.*, 1973*a*), transport system II (TS II, K_m 1 mM) and transport system III (TS III, K_m 11·5 mM). An indication of the specificities of the three systems was obtained by measuring glutamate transport in the presence of potentially competing amino acids at three different concentrations of glutamate (0·1, 1·5 and 15 mM). From the results (Table 1) we conclude that whereas the glutamate transport measured in the present work (using 0·1 mM-glutamate) is by way of the specific acidic amino acid permease (TS I) described previously, the use of higher concentrations might lead to confusion because transport would be by way of more than one transport system.

Correlation between high glutamine pools and low glutamate transport activity during germination

The specific activity of the acidic amino acid permease (TS I) reached a maximum at germ-tube emergence (8 to 10 h at 30 °C) during germination with either glutamate or ammonia as the sole nitrogen source, although the specific activity on ammonia was always

Table 1. *Inhibition of glutamate transport by various amino acids*

Conidia were germinated for about 11 h at 30 °C with glutamate (25 mM) as the sole nitrogen source. After washing, germinated conidia were stirred with potential inhibitor for about 90 s before addition of L-[U-^{14}C]glutamate at various concentrations. The initial rate of glutamate transport was measured during the first 3 min of the assay. Results are expressed as percentage inhibition (determined by comparing the initial rates in the presence and absence of inhibitor).

L-Glutamate concn (mM)	Transport system(s) operating at each glutamate concn	Inhibitor (concn, mM)	Inhibition (%)
0·1	TS I (K_m, 0·18 mM)	Glycine (1)	0
		Glycine (15)	0
		L-Arginine (1)	0
		L-Phenylalanine (1)	15
1·5	TS I (K_m, 0·18 mM)+Ts II (K_m, 1 mM)	Glycine (15)	35
		L-Arginine (15)	40
		L-Phenylalanine (15)	80
15·0	TS I (K_m, 0·18 mM)+TS II (K_m, 1 mM) +TS III (K_m, 11·5 mM)	Glycine (150)	70
		L-Arginine (150)	70
		L-Phenylalanine (150)	80

about 10 % of that measured during germination on glutamate (see Robinson *et al.*, 1973*b*, for a full analysis of the results for glutamate-germination).

The specific activities [units (g dry wt)$^{-1}$] during germination with glutamate (25 mM) for 0, 4, 8, 10, 12, 16, 20 and 24 h after initiation of germination were 0·005, 0·57, 1·34, 1·93, 1·67, 0·27, 0·19 and 0·17, respectively; the specific activities at the same times during germination with ammonia (25 mM) instead of glutamate were 0·005, 0·075, 0·20, 0·08, 0·05, 0·05, 0·05 and 0·05 units (g dry wt)$^{-1}$. Analysis of the ammonia and amino acid pool concentrations from conidia harvested at these times showed that the relatively low specific activity of the transport system (TS I) on ammonia correlated well with the very high concentrations of glutamine present compared with those measured during germination on glutamate; there was no other amino acid whose concentration correlated in such a marked manner (see Tables 1 and 2 in Cook & Anthony, 1978*b*, for the amino acid analyses for these experiments). These results implicate glutamine rather than ammonia in the regulation of TS I during germination of *A. nidulans*. It should be emphasized that this differs from the conclusion of Robinson *et al.* (1973*b*) who suggested that ammonia was the regulatory metabolite; but these authors noted that their single measurement of pool concentrations could not accurately distinguish between the ammonia initially present and that produced by hydrolysis during analysis. The method used in the present work is able to make this distinction (Cook & Anthony, 1978*b*).

Repression of synthesis of TS I by glutamine

The low activity of TS I in ammonia-germinated conidia must result either from repression of synthesis of the transport system or from inhibition of the preformed system by intracellular nitrogen compounds (e g. glutamine). To distinguish between these possibilities, ammonia-germinated conidia were incubated for 3 h either with ammonia (25 mM) or in nitrogen-free medium (with or without 10 μg cycloheximide ml^{-1}) and the glutamate transport activity and intracellular ammonia and amino acid concentrations were measured. The effect of cycloheximide on protein synthesis in this experiment and the pool concentrations of all amino acids are presented in full in Fig. 2 and Table 4 in Cook & Anthony (1978*b*). It was shown that nitrogen starvation resulted in a near-linear fivefold increase in the specific activity of TS I (from 0·28 to 1·4 units g^{-1} in 3 h) which did not occur in the presence of ammonia or cycloheximide. During the first hour of nitrogen starvation the intracellular concentration of glutamine was reduced from a high level (23 mM) to zero and the pool concentrations of asparagine, alanine, ammonia and glutamate were also reduced. After

the first hour the concentrations of most amino acids increased slightly; this probably was the result of protein hydrolysis in response to nitrogen starvation (Meyers & Knight, 1953). In the presence of ammonia there was little change in the concentrations of amino acids or in the specific activity of the transport system. Cycloheximide (10 μg ml^{-1}) prevented the increase in transport activity but had no effect on the dramatic drop in glutamine concentration to zero.

There was some variation in all amino acid concentrations in these experiments but the only clear correlation always observed was that between high glutamine (but not ammonia) concentrations and low activities of the transport system. These results suggest that during nitrogen starvation of ammonia-germinated conidia the increase in activity of TS I was probably due to derepression of its synthesis resulting from a decrease in the intracellular concentration of glutamine. That there was no increase in transport activity when protein synthesis was inhibited, even though the glutamine concentration still dropped to zero, shows that deinhibition of preformed TS I is not the regulatory mechanism by which nitrogen starvation led to increased transport activity.

Inhibition of preformed glutamate transport system (TS I) by ammonia

When ammonia-germinated conidia were starved of nitrogen for 2 h and then incubated with ammonia for a further hour the glutamate transport activity was reduced by only 40 % although an enormous increase in the concentration of glutamine (0 to 40 mM) occurred (Table 4 in Cook & Anthony, 1978*b*). This clearly suggests that ammonia does not lead to lowered activity by conversion to glutamine. The rapid decrease in activity has been previously observed in glutamine-germinated conidia by Robinson *et al.* (1973*b*) who concluded that ammonia was able to inhibit the preformed transport system; they also concluded that ammonia inhibits from within the organism because it had no effect when added simultaneously with glutamate in the transport assay. Glutamine, by contrast, appears to inhibit glutamate transport externally; incubation of glutamate-germinated conidia with 10 mM-glutamine immediately decreased the glutamate transport rate to 60 % and this level then remained constant during a 30 min incubation. The same extent of inhibition was also observed when conidia were preincubated with glutamine for 30 min before addition of radioactive glutamate. The inhibition by glutamine was not relieved by washing glutamine-incubated conidia before measuring glutamate transport. That ammonia inhibits only after its transport into the conidia was confirmed by the following experiment. Glutamate-germinated conidia were incubated with ammonia (10 or 25 mM) for 2, 5, 10, 20 or 30 min after which times they were washed and their glutamate transport activity was measured. It was found that ammonia had little effect during the first 5 min incubation but by 20 min there was considerable reduction in transport activity (55 % and 70 % reduction by 10 and 25 mM-ammonia, respectively). After 20 min there was a slight increase in transport activity indicating that inhibition may be relieved by metabolism of ammonia. This interpretation was confirmed by showing that incubation with methylamine (25 mM) (a slowly metabolized analogue of ammonia) produced a similar inhibition of transport but with no subsequent relief of inhibition.

DISCUSSION

Robinson *et al.* (1973*b*) concluded that ammonia or a metabolite of ammonia is involved in regulation of the acidic amino acid transport system of *A. nidulans*. The results presented above indicate that glutamine and not ammonia is the likely repressor metabolite (co-repressor) of synthesis of this transport system, but that ammonia is able to inhibit the preformed permease to some extent.

The conclusion that glutamine regulates synthesis of the transport system for low concentrations of glutamate is analogous with our conclusions with respect to regulation by glutamine of synthesis of the ammonia transport system of *A. nidulans*, but the regulation of

the two systems differs in one important respect. Whereas the preformed glutamate transport system is inhibited by intracellular ammonia, the ammonia transport system is inhibited by intracellular glutamine and asparagine (Cook & Anthony, 1978*b*). The results in the present paper support our previous conclusion (Cook & Anthony, 1978*b*) 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).'

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REFERENCES

Cook, R. J. & Anthony, C. (1978*a*). The ammonia and methylamine active transport system of *Aspergillus nidulans*. *Journal of General Microbiology* **109**, 265–274.

Cook, R. J. & Anthony, C. (1978*b*). Regulation by glutamine of ammonia transport in *Aspergillus nidulans*. *Journal of General Microbiology* **109**, 275–286.

Hofstee, B. H. J. (1952). On the evaluation of the constants Vm and Km in enzyme reactions. *Science* **116**, 329–331.

Meyers, E. & Knight, S. G. (1953). Studies on the intracellular amino acids of *Penicillium roqueforti*. *Mycologia* **53**, 115–122.

Pateman, J. A., Kinghorn, J. R., Dunn, E. & Forbes, E. (1973). Ammonium regulation in *Aspergillus nidulans*. *Journal of Bacteriology* **114**, 943–950.

Pateman, J. A., Kinghorn, J. R. & Dunn, E. (1974). Regulatory aspects of L-glutamate transport in *Aspergillus nidulans*. *Journal of Bacteriology* **119**, 534–542.

Ressler, C. & Koga, T. (1971). α-Cyanoamino acids and related nitriles as inhibitors of glutamate decarboxylase. *Biochimica et biophysica acta* **242**, 473–483.

Robinson, J. H., Anthony, C. & Drabble, W. T. (1973*a*). The acidic amino acid permease of *Aspergillus nidulans*. *Journal of General Microbiology* **79**, 53–63.

Robinson, J. H., Anthony, C. & Drabble, W. T. (1973*b*). Regulation of the acidic amino acid permease of *Aspergillus nidulans*. *Journal of General Microbiology* **79**, 65–80.

Robinson, J. H., Anthony, C. & Drabble, W. T. (1974). The utilization of nitrogen sources by *Aspergillus clavatus*. *Journal of General Microbiology* **85**, 23–28.