ALTERATIONS IN THE CONTROL OF GLUTAMATE UPTAKE IN MUTANTS OF ASPERGILLUS NIDULANS

M. J. Hynes

Department of Genetics, La Trobe University, Bundoora, Victoria 3083, Australia.

Received August 6, 1973

SUMMARY

A mutation, amdT19, which leads to inability to grow on glutamate as the sole nitrogen source but does not affect growth on glutamate as the sole source of carbon and nitrogen, is shown to result in increased repression of glutamate uptake by glucose. An allelic mutation, amdT102, results in insensitivity to glucose repression. Glutamate uptake is still sensitive to NH_4^+ repression in the presence of glucose in these strains. Starvation for a carbon source leads to relief of NH_4^+ repression.

Mutants in a gene, amdT, in Aspergillus nidulans have been found to pleiotropically affect the utilization of many nitrogen sources (1, 2). One allele, amdT19, results in lack of growth on glutamate as a sole nitrogen source in the presence of glucose and other carbon sources, but does not affect the utilization of glutamate as the sole source of carbon. Another allele, amdT102, results in slightly stronger growth than wildtype, on glutamate as the sole nitrogen source in the presence of glucose. The properties of amdT mutants have suggested that this gene plays an important role in regulating the interaction between carbon and nitrogen metabolism (1, 2). Therefore, since glutamate is the major point at which assimilation of inorganic nitrogen into intermediary metabolism occurs, a study of glutamate utilization by amdT mutants has been undertaken. In this communication it is reported that glutamate uptake is subject to

Copyright © 1973 by Academic Press, Inc.
All rights of reproduction in any form reserved.

increased repression by glucose in the amdT19 strain, while this effect of glucose is relieved in strain amdT102.

MATERIALS AND METHODS

Strains, media and growth of mycelium have been described in previous papers (1, 3).

Glutamate uptake measurements. Portions of mycelium were transferred to 250 ml. Ehrlenmeyer flasks containing 25 ml of the standard salts solution, 1 mM glutamate and 0.5 μCi of uniformly ^{14}C-labelled L-glutamate (285 mCi per m mole - obtained from The Radiochemical Centre, Amersham, England). The mycelium was incubated at 37° in a Gallenkamp orbital incubator. Five ml samples (containing 5-20 mg dry weight of mycelium) were taken at intervals for about 20 minutes and mycelium was rapidly collected by suction filtration on filter paper (Whatman No. 1) and washed with excess cold water. The filters were dried overnight at 60°, weighed and then counted in 5 ml of BBOT (4 gms per litre of toluene) scintillation fluid in a Packard Tri-carb scintillation counter (model 3375).

RESULTS

Table 1 shows that the altered growth of amdT mutants on glutamate can be accounted for by the effects of growth on glucose on glutamate uptake capacity. The amdT19 strain had very low glutamate uptake capacity when grown in glucose medium, but was able to take up glutamate at rates equivalen to the amdT$^+$ and amdT102 strains when deprived of a carbon source or when grown in medium in which glutamate was the sole source of carbon and nitrogen. The amdT102 strain had higher uptake capacity than the wildtype strain when grown in glucose medium. Cycloheximide (10 μg per ml) was found to block the increase in glutamate uptake capacity resulting from

TABLE 1 : Glutamate uptake by amdT mutants.

Growth conditions		Glutamate uptake[c]		
Carbon source	Nitrogen source	amdT$^+$	amdT102	amdT19
Glucose	20 mM NH_4^+ [a]	0.19	0.47	0.17
None	20 mM NH_4^+ [a]	1.67	1.89	1.52
Glucose	10 mM Glutamate[b]	0.78	2.02	0.22
None	10 mM Glutamate[b]	3.62	2.46	1.60
Glucose	None[b]	1.86	2.62	0.29
None	None[b]	1.82	2.37	1.78

a Mycelium was grown for 16 hours on glucose - 20 mM NH_4^+ medium and then transferred to this medium for 4 hr.

b Mycelium was grown for 19-20 hours on glucose - 5 mM urea medium and then transferred to this medium for 4-5 hr.

c Expressed as nanomoles glutamate taken up per minute per milligram dry weight of mycelium.

starvation of strain amdT19 for a carbon source, suggesting that the synthesis of an essential component of the glutamate uptake system may be subject to control by the amdT gene.

Growth in medium containing NH_4^+ leads to low capacity for glutamate uptake (4, 5). This was observed for all strains in these experiments (Table 1). However, carbon starvation or growth on glutamate as a sole carbon source, led to relief of this effect of NH_4^+ in all strains. Methylammonium is not a nitrogen source in the mauA2 (formerly mau2) mutant of A. nidulans and apparently acts as an analogue of NH_4^+ (6). Growth of the mauA2 mutant on glutamate as sole nitrogen source with glucose as the carbon source is inhibited by methylammonium. However it was found that

methylammonium (10 mM) did not affect growth of the mauA2 mutant on glutamate as the sole carbon and nitrogen source. In addition it was found that NH_4^+ (20 mM) does not prevent growth of any of the three strains on glutamate as the sole carbon source. These results are explicable in terms of the observed effects of NH_4^+ on glutamate uptake. NH_4^+ results in reduced glutamate uptake capacity when glucose is present as the carbon source, but does not affect glutamate uptake when glutamate is the sole carbon source. It has previously been reported that NH_4^+ does not repress an acetamidase enzyme when acetamide is present as the sole carbon source, but does repress this enzyme when glucose is present (3).

DISCUSSION

This paper presents evidence for glutamate uptake in A. nidulans being subject to control by NH_4^+ and glucose. The amdT gene is involved in glucose repression of glutamate uptake capacity. The amdT102 lesion leads to insensitivity to glucose repression, while the amdT19 lesion leads to increased sensitivity to glucose repression of glutamate uptake. The amdT mutants do not appear to be altered in their response to NH_4^+ repression of glutamate uptake.

The amdT lesions are highly pleiotropic in their effects on nitrogen source utilization (2). Growth on many other amino acids as sole nitrogen sources is altered in the mutants (2). The effects of glucose on the uptake of some of these amino acids is currently being studied. In other fungi it has been shown that starvation for a carbon source can lead to derepression of amino acid uptake systems (7, 8).

The effects of the amdT lesions on acetamide utilization have been shown to be very similar to their effects on glutamate uptake. Synthesis of

the acetamidase enzyme is not subject to glucose repression in amdT102 and is subject to increased glucose repression in amdT19 (1). Therefore the results presented here provide further evidence for the fundamental role of the amdT gene in control of the interaction between carbon and nitrogen metabolism.

This work was supported by the Australian Research Grants Committee.

REFERENCES

1. Hynes, M.J. : J.Bacteriol. 111, 717 (1972).
2. Hynes, M.J. : Molec.Gen.Genetics in press (1973).
3. Hynes, M.J. : J.Bacteriol. 103, 482 (1970).
4. Robinson, J.H., Anthony, C. and Drabble, W.T. : Biochem.J. 124, 75p (1971).
5. Kinghorn, J.R. and Pateman, J.A. : Heredity 29, 128 (1972), and in press (1973).
6. Arst, H.N. and Cove, D.J. : J.Bacteriol. 98, 1284 (1969).
7. Pall, M.L. : Biochim.Biophys.Acta 211, 513 (1970).
8. Oxender, D.L. : Ann.Rev.Biochem. 41, 777 (1972).