Mutations which Affect Amino Acid Transport in *Aspergillus nidulans*

By J. R. KINGHORN and J. A. PATEMAN

*Department of Genetics, University of Glasgow, Glasgow G11 5JS*

(Received 9 July 1974; revised 22 August 1974)

**SUMMARY**

Mutants deficient in amino acid transport (*aau*), and unable to utilize L-glutamate as a sole carbon and nitrogen source, have been isolated. There are four unlinked genes involved: *aauA*, *aauB*, *aauC* and *aauD*. The transport levels of certain amino acids, and the growth characteristics on certain nitrogen and carbon sources and toxic amino acid analogues, indicate that: *aauAI* has defective transport of acidic amino acids; *aauBI* has defective transport of acidic and neutral amino acids; *aauCI* and *aauDI* have defective transport of acid, neutral and aromatic amino acids. *aauAI* and *aauBI* are recessive for all three characteristics in the heterozygous diploid; *aauCI* and *aauDI* are dominant.

**INTRODUCTION**

In the fungi, a number of specific transport systems for amino acids and related substances have been described. These include ammonium (Pateman, Kinghorn, Dunn & Forbes, 1973; Pateman, Dunn, Kinghorn & Forbes, 1974) and urea (Dunn & Pateman, 1972) in *Aspergillus nidulans*, arginine, lysine, methionine and histidine in *Saccharomyces cerevisiae* (Grenson, 1966; Grenson, Mousset, Wiame & Béchet, 1966; Gits & Grenson, 1967; Crabeel & Grenson, 1970), and ammonium, methionine, cysteine, proline, lysine and arginine, as well as distinct acidic and basic systems, in *Penicillium chrysogenum* (Benko, Wood & Segel, 1967; Hackette, Skye, Burton & Segel, 1970; Skye & Segel, 1970; Hunter & Segel, 1971). Moreover, the isolation of mutants defective in the transport of specific amino acids is strong evidence for the existence of specific uptake systems. However, general amino acid transport systems have also been described in the fungi. The presence of a ‘general’ amino acid system has been reported for *Botrytis fabae* (Jones, 1963), *Arthrobotrys conoides* (Gupta & Pramer, 1970), *S. cerevisiae* (Grenson, Hou & Crabeel, 1970; Grenson & Hou, 1972), *Neurospora crassa* (Pall, 1969), and *P. chrysogenum* (Benko, Wood & Segel, 1969). Mutants have been isolated which have simultaneously lost the ability to transport a number of amino acids, e.g. *gap* and *apf* in yeast (Surdin et al. 1965; Grenson et al. 1970; Grenson & Hennaut, 1971), un-I, nap and mtr in *N. crassa* (Kappy & Metzenberg, 1965; Stadler, 1966; Lester, 1966; Jacobson & Metzenberg, 1968) and *fpaD* in *A. nidulans* (Sinha, 1969).

We describe the isolation and properties of new classes of mutants mapping at four loci in *A. nidulans* which are defective in the transport of a number of amino acids. A preliminary report of this work was given at a meeting of the Genetical Society of Great Britain (Kinghorn & Pateman, 1972).

**METHODS**

*Chemicals.* Analytical grade chemicals were used whenever possible: 14C-labelled L-amino acids, [14C]methylammonium and thio[14C]urea were obtained from The Radiochemical Centre, Amersham, Buckinghamshire.
Amino acid transport in A. nidulans

Strains. A biotin- and putrescine-requiring strain, biI puAa (Glasgow No. 0171), was used as the prototroph with respect to nitrogen metabolism.

Media. Medium used for genetic analysis was essentially that described by Pontecorvo et al. (1953). Nitrogen-less minimal medium (−N medium) described by Cove (1966), and carbon- and nitrogen-less minimal medium (−CN medium) were used. Most nitrogen and carbon sources used in media were kept as sterile stock solutions at 1 M. Others such as urea, L-asparagine and L-glutamine, which are unstable with ammonium as one of the products, were made up immediately before use.

Growth tests of mutants were carried out in Petri dishes containing 20 ml −N or −CN medium (solid) plus the nitrogen or carbon source under test. These were carefully point-inoculated with fresh conidia and incubated at 37 °C for approximately 30 to 36 h. Growth, in terms of colony size and density, was recorded as ++, + or −.

Selection of transport mutants. The putrescine technique of Herman & Clutterbuck (1966) was used to isolate mutants unable to utilize L-glutamate as a sole carbon and nitrogen source. N-methyl-N'-nitro-N-nitrosoguanidine treated conidia (Adelberg, Mandel & Chen, 1965) of biI puAa were point-inoculated on to −CN medium plus limiting putrescine (9.7 × 10⁻⁷ M) plus 100 mM L-glutamate. After 3 to 4 days' incubation at 37 °C, sectors showing spidery growth were isolated and purified.

Genetic analysis. The genetic techniques were those described by Pontecorvo et al. (1953) and McCully & Forbes (1965), with the exception that acridine yellow (0.06 mg/ml) was used as the haploidization agent instead of p-fluorophenylalanine.

Growth of mycelium for uptake assays. The uptake of 14C-labelled amino acids was determined during exponential growth phase in an attempt to study transport in young cells under as standard and reproducible conditions as possible. Mycelium was grown in culture shaken at 200 rev./min (rotary motion describing a 1 in circle) in a controlled environment incubator shaker (Pycrotherm; New Brunswick Scientific Co., New Brunswick, New Jersey, U.S.A.) for approximately 18 to 20 h at 25 °C, and harvested by filtering on nylon cloth (Cove, 1966). When mycelium was given some special treatment, it was transferred after harvesting to the preheated treatment medium for the appropriate time and then reharvested. The mycelium was pressed dry with paper towels and weighed. Yields were usually 5 to 8 g/l medium. Cells were used immediately for uptake assays.

Amino acid transport assay. The method is basically that described in previous communications (Kinghorn & Pateman, 1973, 1974a). The growth and treatment media are described in the text. The final concentration (µM) and specific activity (mCi/mmol, in parentheses) of each amino acid used were as follows: L-alanine, 100 (169); L-arginine, 100 (42); L-glutamate, 100 (231); L-glutamine, 200 (42); L-phenylalanine, 200 (4); L-serine, 100 (162); methylammonium, 500 (54); thiourea, 200 (18.3). The final approximate specific activities of each amino acid were as follows (µCi): L-alanine, 6.0; L-arginine, 1.5; L-glutamate, 0.5; L-glutamine, 3.5; L-phenylalanine, 2.0; L-serine, 9; methylammonium, 2.5; thiourea, 2.5. The amino acids were uniformly labelled.

RESULTS

Isolation of mutants

A large number of mutants unable to grow on L-glutamate as a sole carbon and nitrogen source were isolated (Kinghorn & Pateman, 1973, 1974b). Six of these mutants possessed low levels of L-glutamate transport.
J. R. KINGHORN AND J. A. PATEMAN

(a) $aauc$

Linkage group II  
\[ \text{cnxE} \quad aauc \quad ygA \]

\[
\begin{align*}
30.5 \pm 4.5 & \quad 23.7 \pm 4.1 \\
50 & 
\end{align*}
\]

(b) $aaub$

Linkage group VII  
\[ \text{wetA} \quad aaub \quad malA \]

\[
\begin{align*}
5.0 \pm 2.3 & \quad 23.9 \pm 4.5 \\
22.8 \pm 4.9 & 
\end{align*}
\]

Fig. 1. Linkage relationships of $aaub$ and $aauc$ to established markers, given as the mean percentage recombination ± S.E.M. An explanation of symbols and a complete linkage map are given by Clutterbuck (1974). The data in (b) do not exclude the possibility that $aaub$ is located to the left of $wetA$.

**Genetic analysis**

Mitotic and meiotic genetic analysis, as discussed below, showed that four gene loci were involved. These loci were designated $aaua$ (amino acid uptake), $aaub$, $aauc$ and $aaud$.

**$aaua$ locus.** Haploidization of the diploid between $bir$; $puA2$; $aaua1$ and master strain $D$ by the method of McCully & Forbes (1965) yielded segregants which showed free assortment between $aaua1$ and all chromosome markers except $nicB$. Analysis of a single mixed perithecium from the cross: $bir$; $puA2$; $aaua1$ by $yA2$; $palD$ $nicB$ $malA$ $wetA$ failed to locate $aaua1$, since $aaua1$ showed approximately 50% recombination with $palD$, $nicB$, $malA$ and $wetA$ (available markers in linkage group VII) amongst 161 progeny tested. Therefore the position of $aaua1$ in linkage group VII is not known.

**$aaub$ locus.** Haploidization analysis showed that a further two mutants were located in linkage group VII. The mutations were allelic, or closely linked, since no wild-type recombinant progeny were isolated in a total of 500 progeny scored. The locus was designated $aaub$ and the mutations $aaub1$ and $aaub2$. Crosses between $bir$; $puA2$; $aaub1$ and $yA1$; $pyroA4$; $aaua1$ gave approximately 50% recombination for the two genes (280 progeny tested). Crosses between $bir$; $puA2$; $aaub1$ or $bir$; $puA2$; $aaub2$ and $yA2$; $palD$ $nicB$ $malA$ $wetA$ located the $aaub$ locus near $wetA$ (Fig. 1).

**$aauc$ locus.** Haploidization analysis of the diploids between $bir$; $puA2$; $aauc1$ or $bir$; $puA2$; $aauc2$ and the tester strain $D$ was carried out with complete medium plus acridine yellow (0.06 mg/ml). Acridine yellow was used instead of $p$-fluorophenylalanine since the $aauc1$ and $aauc2$ mutations are dominant and resistant to $p$-fluorophenylalanine. Both mutant characteristics assorted freely with all chromosome markers except $acrA$, and consequently were assigned to linkage group II. The results of crosses between $bir$, $puA2$, $aauc1$ and $yA2$, $pyroA4$, $aauc2$ showed they were allelic or closely linked; no wild-type recombinants were isolated in 450 progeny. The results of crosses between $bir$, $puA2$, $aauc1$ or $bir$, $puA2$, $aauc2$ and $acrA$ $wA$ $thiA$ $abA$ $cnxE$ $ygA$ located the $aauc$ locus in the $cnxE$-$ygA$ interval in linkage group II (Fig. 1).

**$aaud$ locus.** As before, acridine yellow was used as the haploidization agent. $aaud1$ assorted freely with all chromosome markers except $riboB$ and was therefore assigned to
Amino acid transport in A. nidulans

The following amino acids and inorganic nitrogen compounds were used to determine the transport characteristics of aau mutants: L-glutamate (acidic), L-phenylalanine (aromatic), L-alanine and L-serine (neutral), L-arginine and L-glutamine (basic), thiourea and methylammonium (inorganic). From the data in Fig. 2, it can be seen that all classes of uptake mutants had low uptake of L-glutamate (< 10% of the wild type). The heterozygous diploids aauAr/aauA+ and aauBr/aauB+ appeared to have wild-type transport while aauCr/aauC+ and aauDr/aauD+ showed the mutant level, indicating that aauAr and aauBr mutants are recessive to their wild-type alleles while aauCr and aauDr are dominant (Fig. 3). The mutants aauBr, aauCr and aauDr, but not aauAr, had impaired transport of the neutral amino acids L-serine (Fig. 4) and L-alanine (Fig. 5), while only aauCr and aauDr had impaired uptake of the aromatic representative L-phenylalanine (Fig. 6). All genotypes had normal uptake of the basic amino acids L-arginine and L-glutamine, and also normal uptake of thiourea and methylammonium.

In summary, aauAr shows low uptake of only acidic amino acids and is recessive. aauBr shows low uptake of acidic and neutral amino acids and is recessive. aauCr and aauDr have low uptake of acidic, neutral and aromatic amino acids, and in contrast to aauAr and aauBr, are dominant.

Growth responses of aau mutants

Table 1 shows the growth characteristics of the four classes of uptake mutants on a range of amino acids provided as the sole nitrogen source. The aauAr mutant grows as wild type on inorganic nitrogen sources (e.g. nitrate, ammonium or urea), neutral amino acids (e.g. L-serine, L-alanine or L-valine), aromatic amino acids (e.g. L-phenylalanine, L-tryptophan or L-tyrosine) and basic amino acids such as L-arginine, L-ornithine, L-asparagine or L-glutamine, but grows poorly on the acidic amino acids, L-glutamate and L-aspartate. The heterozygous
Fig. 3. L-Glutamate transport by heterozygous diploids of wild-type and aau mutants. The cells were grown on -N medium + 10 mM-urea for 18 h.

Wild type; □, aau1; △, aau2; ○, aau3; ●, aau4; ▲, aau5.

Fig. 4. Transport of L-serine by wild-type and aau mutants. The cells were grown on -N medium + 10 mM-urea for 18 h. ■, Wild type; △, aau1; ○, aau2; ●, aau3; ▲, aau4.

Fig. 5. Transport of L-alanine by wild-type and aau mutants. The cells were grown on -N medium + 10 mM-urea for 18 h. ■, Wild type; △, aau1; ○, aau2; ●, aau3; ▲, aau4.

Fig. 6. Transport of L-phenylalanine by wild-type and aau mutants. The cells were grown on -N medium + 10 mM-urea for 18 h. ■, Wild type; △, aau1; ○, aau2; ●, aau3; ▲, aau4.
### Table 1. Growth responses of aau mutants on certain nitrogen sources

<table>
<thead>
<tr>
<th>Nitrogen source*</th>
<th>Wild type</th>
<th>aauAI</th>
<th>aauA&lt;sup&gt;+&lt;/sup&gt;</th>
<th>aauBI</th>
<th>aauB&lt;sup&gt;+&lt;/sup&gt;</th>
<th>aauCI</th>
<th>aauC&lt;sup&gt;+&lt;/sup&gt;</th>
<th>aauDI</th>
<th>aauD&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Ammonium</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Nitrate</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>L-Aspartate</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>L-Glutamate</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>L-Serine</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>L-Valine</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>L-Asparagine</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>L-Ornithine</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

+ +, Wild-type growth (this varies with the nitrogen source); +, about 50% of wild-type growth.
* Added at 10 mM final concentration to solid −N medium.

### Table 2. Growth responses of aau mutants on certain carbon sources

<table>
<thead>
<tr>
<th>Carbon source*</th>
<th>Wild type</th>
<th>aauAI</th>
<th>aauA&lt;sup&gt;+&lt;/sup&gt;</th>
<th>aauBI</th>
<th>aauB&lt;sup&gt;+&lt;/sup&gt;</th>
<th>aauCI</th>
<th>aauC&lt;sup&gt;+&lt;/sup&gt;</th>
<th>aauDI</th>
<th>aauD&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose 1 %</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Maltose 1 %</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Sedoheptulose 1 %</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Acetate 1 %</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Glycerol 1 %</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>L-Glutamate 100 mM</td>
<td>++</td>
<td>−</td>
<td>++</td>
<td>−</td>
<td>++</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L-Aspartate 100 mM</td>
<td>++</td>
<td>−</td>
<td>++</td>
<td>−</td>
<td>++</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L-Alanine 100 mM</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

+ +, Wild-type growth (this varies with the carbon source); −, poor growth.
* Basal medium = solid −N medium + 10 mM-ammonium.

diploid aauAI/aauA<sup>+</sup> grows as wild type on L-glutamate and L-aspartate, indicating that the aauAI mutation is recessive. Mutation in the aauB gene results in poor growth on acidic and neutral amino acids and is recessive for growth characteristics. The aauCI and aauD<sup>+</sup> mutants are rather similar to each other in that they both grow poorly on acidic, neutral and aromatic, but as wild type on basic amino acids. They are dominant in the heterozygous diploid.

All classes of mutants appear to have wild-type growth on certain carbon sources tested (Table 2). However, the utilization of L-glutamate, L-aspartate and L-alanine as sole carbon sources by the mutants was relatively poorer than their utilization of these amino acids as sole nitrogen sources. This is not surprising since larger quantities of the amino acid would be necessary to provide a sufficient energy source. The mutants aauAI and aauBI appear recessive for growth on L-glutamate as a carbon and nitrogen source, while aauCI and aauD<sup>+</sup> are dominant (Fig. 7).
Fig. 7. Growth of a homozygous wild-type diploid and diploids heterozygous for aau mutants with L-glutamate as sole carbon and nitrogen source. — CN medium (solid) plus 100 mM-L-glutamate for 48 h. +, Wild-type allele.

Resistance of aau mutants to certain toxic analogues of amino acids and inorganic nitrogen

An attempt was made to correlate the transport deficiencies of the various aau mutants with resistance to certain toxic analogues. A number of amino acid analogues reported to be inhibitory to bacteria and yeast did not inhibit wild-type *A. nidulans*. These included analogues of L-glutamate (D-glutamate), L-aspartate (D-aspartate), L-arginine (canavanine), L-proline (azetidine-2-carboxylic acid), L-lysine (thiosine), L-methionine (ethionine) and glutamine (glutamyl-hydrazine). However, three analogues were found to inhibit the growth of *A. nidulans*: aspartate hydroxamate, glutamate hydroxamate and D-serine. Auxanographic tests of a wild-type strain showed that the inhibitions of growth by the three analogues were reversed by L-asparagine, L-glutamine and L-serine, respectively. In addition, the aau mutants were tested for resistance to *p*-fluorophenylalanine, 3-amino tyrosine HCl (Sinha, 1969), methylammonium (Arst & Cove, 1969) and thiourea (Dunn & Pateman, 1972), which are toxic analogues of L-phenylalanine, L-tyrosine, ammonium and urea, respectively. The mutants *meaA8*, *uruA1*, *ser-9*, *fpaD43*, *ahyA1* and *gyyA1*, each known to be resistant to at least one of the analogues, were tested as controls and to determine if aau mutants shared any resistance characteristics with various known resistant mutants (Table 3). aauA1 is sensitive to all seven toxic analogues; aauB1 is resistant to D-serine only; aauC1 and aauD1 are resistant to *p*-fluorophenylalanine, D-serine and 3-amino tyrosine. aauB1 is recessive, while aauC1 and aauD1 are dominant for resistance. *uruA1*, *meaA8*, *ser-9*, *ahyA1* and
### Table 3. Resistance of aau mutants to toxic analogues of certain nitrogen sources

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Toxic analogue of metabolite</th>
<th>Control strains</th>
<th>aau strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N medium (solid) + 10 mM</td>
<td>Wild type</td>
<td>meaA8</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>p-Fluorophenylalanine (0.025%)</td>
<td>Nitrate</td>
<td>-</td>
</tr>
<tr>
<td>L-Serine</td>
<td>D-Serine (5 mM)</td>
<td>Nitrate</td>
<td>-</td>
</tr>
<tr>
<td>L-Asparagine</td>
<td>L-Aspartate hydroxamate (0.32 mM)</td>
<td>Nitrate</td>
<td>-</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>L-Glutamate hydroxamate (5 mM)</td>
<td>Nitrate</td>
<td>-</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>3-Amino tyrosine HCl (10 mM)</td>
<td>Nitrate</td>
<td>-</td>
</tr>
<tr>
<td>Ammonium</td>
<td>Methylammonium (200 mM)</td>
<td>Arginine</td>
<td>-</td>
</tr>
<tr>
<td>Urea</td>
<td>Thiourea (5 mM)</td>
<td>Nitrate</td>
<td>-</td>
</tr>
</tbody>
</table>

**Control strains:**
- **uruA1**: Resistant to thiourea only (Dunn & Pateman, 1972)
- **meaA8**: Resistant to methylammonium only (Arst & Cove, 1969)
- **fpaD43**: Resistant to p-fluorophenylalanine and 3-amino tyrosine HCl only (Sinha, 1969)
- **ser-9**: Resistant to D-serine only (Kinghorn, unpublished work)
- **ahyA1**: Resistant to aspartate hydroxamate only (Kinghorn, unpublished work)
- **ghyA1**: Resistant to glutamate hydroxamate only (Kinghorn, unpublished work)

+ , Appreciable growth (relatively normal colony size); - , extremely poor growth.
*aauA* mutants have no common resistance characteristics. *fpaD43* shares some resistance characteristics with *aauC1* and *aauD1*, i.e. dominance and resistance to *p*-fluorophenylalanine and 3-amino tyrosine (Sinha, 1969). However, *fpaD43* differs in that it is not resistant to D-serine.

**DISCUSSION**

It is clear that mutation in at least four loci, *aauA*, *aauB*, *aauC* and *aauD*, in *A. nidulans* results in impaired transport of certain amino acids. These are in addition to *fpaD* described by Sinha (1969). There are three main lines of evidence to support this: the transport levels of amino acids in the mutants; the phenotypes of the mutants on certain nitrogen or carbon sources; and their cross-resistance patterns to toxic analogues of certain nitrogen metabolites. Moreover, since the growth rates of the *aau* mutants are similar to that of the wild type when grown on minimal medium, it is concluded that the physiological defect is limited to the transport function.

The existence of *aauAr*, in which only L-glutamate and L-aspartate transport is impaired, suggests the presence of a specific system or component for L-glutamate and L-aspartate transport rather similar to that in *N. crassa* (Pall, 1969) and *P. chrysogenum* (Hunter & Segel, 1971).

Mutation in the *aauB* gene reduces the uptake of acidic and neutral amino acids and is rather similar to the *nap* and *un-t* mutations in *N. crassa*. Several explanations can be advanced to explain such mutations. The most likely is that *aauB1* results in an altered functional component (e.g. an energy-generating system) which is shared by both the acidic and neutral amino-acid transport systems. The fact that the *aauB1* mutation is recessive tends to support this.

Mutation at the *aauC* and *aauD* loci results in altered transport levels of acidic, neutral and aromatic amino acids. In contrast to *aauA1* and *aauB1*, but similar to *fpaD43*, both *aauC1* and *aauD1* are dominant in the heterozygous diploid.

Sinha (1969) discussed the possibility that *fpaD* plays a regulatory role in amino acid transport. However, the isolation of similar dominant mutations at another two loci rather discounts this hypothesis. It seems unlikely that all three loci are involved in regulation. An alternative explanation is that the loci code for structural proteins which are intimately associated with and shared by the acidic, neutral and aromatic amino acid transport systems. The proteins determined by these three loci (and perhaps others presently unknown) are essential for normal amino acid transport function. An extension of this hypothesis provides a plausible explanation for the dominance or semi-dominance of these mutations. The heterozygous diploid contains normal and mutant alleles which specify both normal and abnormal transport proteins. An actual transport site might contain all normal, all abnormal, or some combination of molecules of the transport protein. If all transport sites contained sufficient abnormal components to reduce transport, the mutation would appear dominant.

The support received from the Science Research Council (grant No. B/RG/48502) is gratefully acknowledged. We thank Mr James Heggie for excellent photographic assistance.
Amino acid transport in A. nidulans

REFERENCES


