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AMINO ACID TRANSPORT IN *NEUROSPORA CRASSA*

III. ACIDIC AMINO ACID TRANSPORT

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SUMMARY

Kinetic studies of uptake demonstrate the existence of an active transport system for D- and L-acidic amino acids in *Neurospora crassa*. This system, designated amino acid Transport System IV, has K_m's for L-cysteic, L-aspartic and L-glutamic acids of 7, 13 and 16 μM, respectively. It has higher affinity for D-aspartic acid than for L-aspartic acid but lower affinity for D-glutamic than for L-glutamic acid. Transport System IV has low activity in growing mycelial pads but has higher activity in carbon-, nitrogen- or sulfur-starved pads.

The characterization of Transport System IV appears to complete the outlining of the major amino acid transport systems in Neurospora. A comparison is made among the substrate affinities and regulation of several amino transport systems in Neurospora and in two other fungi.

INTRODUCTION

Three amino acid transport systems have been described in *Neurospora crassa*, one for L-neutral amino acids, another for D- or L-basic, neutral or acidic amino acids and a third for L-basic amino acids[1,2]. It has been suggested that a fourth transport system, possibly specific for acidic amino acids, must also be active[1]. This report concerns the properties of this last system, designated amino acid Transport System IV. Its affinity for various amino acids and activity under various physiological conditions are investigated.

MATERIALS AND METHODS

Most experiments were performed with mycelial pads of wild type strain of Neurospora, ST74A, grown in 1/2 × Vogel's medium N with 0.5 % sucrose[1]. In this medium, pads are primarily limited in their growth by carbon starvation. Growing pads or more strictly carbon-, nitrogen- or sulfur-starved mycelial pads were grown as described previously[2]. Uptake was performed as described previously[2].

Labeled L-cysteic acid was synthesized by the method of CLARKE[3] from L-[^{35}S]cystine and added unlabeled cystine. L-[^{14}C]Aspartic acid, L-[^{14}C]glutamic

acid and L-[^{35}S]cystine were obtained from Schwarz BioResearch. Amino acids and their derivatives were obtained from Nutritional Biochemicals.

RESULTS

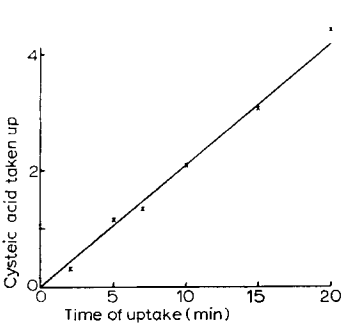
Kinetic studies

The uptake of 0.1 mM L-cysteic acid proceeds at a constant rate for at least 20 min (Fig. 1). At 20 min, the ^{35}S label from the cysteic acid is about 20 times more concentrated in the cell water than in the medium. Incubation with NaN_3 (10 mM) or dinitrophenol (1 mM) for 5 min inhibits uptake over 95 %. These results support the conclusion that the cysteic acid uptake involves active transport.

High concentrations of basic or neutral amino acids inhibit the transport of cysteic acid only to a small extent (Table I). Acidic amino acids, however, give almost complete inhibition. Both D- and L-acidic amino acids are excellent inhibitors. These findings suggest that cysteic acid may be transported by an acidic amino acid transport system. This system, confirmed by the kinetic studies performed below, is designated amino acid Transport System IV.

As shown in Fig. 2, the uptake of L-cysteic acid follows Michaelis–Menten kinetics with a Michaelis constant (K_m) of 7 μM and a maximum velocity of transport of 0.22 nmole/mg dry wt. per min. As is also shown in Fig. 2, L-glutamic acid and D-aspartic acid are competitive inhibitors of the uptake of L-cysteic acid with inhibitor constants (K_i's) of 16 and 5.4 μM, respectively. Similar studies indicate that L-aspartic acid and D-glutamic acid are also competitive inhibitors of cysteic acid uptake. Affinity constants for the five amino acids studied are shown in Table II.

When the uptake of L-aspartic acid or L-glutamic acid is measured, it is found that the uptake is inhibited by each of the other acidic amino acids*. For example, the uptake of L-aspartic acid follows Michaelis–Menten kinetics with a K_m of 12 μM



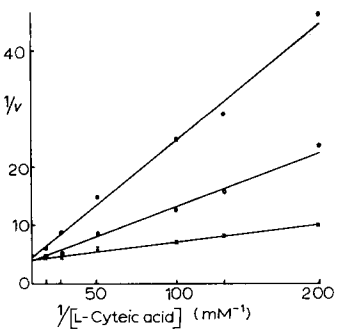


Fig. 1. Time-course of cysteic acid uptake. The uptake of 0.1 mM L-cysteic acid into mycelial pads of ST 74A was measured for various periods of time. Uptake is expressed as nmoles of L-cysteic acid taken up per mg dry wt. of mycelium.

Fig. 2. Cysteic acid uptake: inhibition by L-glutamic and D-aspartic acids. The 5-min uptake of various concentrations of L-cysteic acid was measured in the presence or absence of L-glutamic or D-aspartic acids. v is expressed as nmoles of L-cysteic acid taken up per mg dry wt. of mycelium per min. ●—●, 30 μM D-aspartic acid; ○—○, 40 μM L-glutamic acid; ×—×, no inhibitor.

* Studies on aspartic and glutamic acid uptake in Neurospora by DUSENBERY[13] led to the studies reported here.

TABLE I

CYSTEIC ACID UPTAKE IN PRESENCE OF VARIOUS AMINO ACIDS

3-day-old mycelial pads grown in 1/2 × Vogel's medium N with 0.5% sucrose were used for 5 min uptake. The uptake of 10 μM L-cysteic acid was measured in the presence of 1 mM various amino acids.

Amino acid used as inhibitor	*Inhibition of cysteic acid uptake* (%)
L-Arginine	23
L-Lysine	26
β-Alanine	25
Glycine	10
D-Phenylalanine	36
D-Aspartic acid	99
L-Aspartic acid	97
D-Glutamic acid	84
L-Glutamic acid	96

TABLE II

AFFINITY CONSTANTS FOR TRANSPORT SYSTEM IV

The affinity constants listed are in agreement with those found in studies of L-cysteic, L-aspartic and L-glutamic acids.

Amino acid	K_m *or* K_i (μM)
L-Cysteic acid	7
L-Aspartic acid	13
D-Aspartic acid	5.4
L-Glutamic acid	16
D-Glutamic acid	90

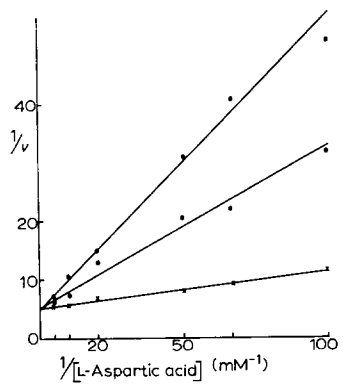


Fig. 3. L-Aspartic acid uptake: inhibition by D-aspartic and L-cysteic acids. The 5-min uptake of various concentrations of L-aspartic acid was measured in the presence of 1 mM L-arginine. D-Aspartic acid (30 μM) or L-cysteic acid (20 μM) were used as inhibitors of cysteic acid uptake. v is expressed as nmoles of L-aspartic acid taken up per mg dry wt. of mycelium per min. ●—●, 30 μM D-aspartic acid; ○—○, 20 μM L-cysteic acid; ×—×, no inhibitor.

and maximum velocity of transport of 0.20 nmole/mg dry wt. per min (Fig. 3). D-Aspartic acid and L-cysteic acid are competitive inhibitors with K_i's of 5.6 and 6 μM, respectively. The affinity constants are in excellent agreement with those found by observing L-cysteic acid uptake. Similar agreement is found for affinity constants determined from measurements of L-glutamic acid uptake. These agreements and the competitive nature of the inhibitions provide strong evidence that a single transport system, System IV, is responsible for the acidic amino acid uptake being studied.

Other possible inhibitors

In order to test the range of compounds with affinity for System IV, a variety of compounds were tested as potential inhibitors of cysteic acid uptake (Table III). A variety of basic and neutral amino acids show little inhibition. Aspartic and glutamic acids and their analogues are effective inhibitors. The lower inhibition exhibited by the analogues shows that their structural differences from the normal amino acids tend to decrease affinity for Transport System IV. The esters of aspartic and glutamic acids inhibit cysteic acid transport more effectively than do other neutral amino acids. It is possible that an extracellular esterase may cleave the ester

TABLE III

INHIBITION OF CYSTEIC ACID UPTAKE BY VARIOUS COMPOUNDS

3-day-old mycelial pads grown in 1/2 × Vogel's medium N with 0.5 % sucrose were used for 5 min uptake. The uptake of 10 μM L-cysteic acid was measured in the presence of 1 mM various compounds.

Compound used as possible inhibitor	*Inhibition of cysteic acid uptake* (%)
L-Arginine	20.5
L-Lysine	14.5
β-Alanine	10
L-Cysteine	15.5
Glycine	10
DL-Methionine sulfoxide	17
D-Phenylalanine	23
L-Serine	14
L-Aspartic acid	95
D-Aspartic acid	99
DL-β-Methylaspartic acid	94
DL-Erythrohydroxyaspartic acid	69
L-Aspartic acid β-methyl ester	67
Malic acid	5
Oxaloacetic	3
Succinic acid	0
N-Methyl-DL-aspartic acid	2
L-Glutamic acid	95
D-Glutamic acid	85
DL-Alloglutamic acid	72
DL-α-Methylglutamic acid	52
L-Glutamic acid γ-methyl ester	57

linkages, leaving the free acidic amino acids to inhibit. The possibility of metabolism makes it difficult to interpret the inhibition by the esters.

Malic, oxaloacetic and succinic acids which can be derived from aspartic acid through removal of the α-amino group have no affinity for System IV. Similarly *N*-methylaspartic acid has no affinity. These findings suggest that a free α-amino group is necessary for binding to Transport System IV. It should also be noted that β-alanine, the decarboxylation product of aspartic acid shows little affinity for Transport System IV.

System IV activity under various conditions

In order to test the activity of amino acid Transport System IV under different physiological conditions, the uptake of 10 mM L-aspartic acid was measured into different mycelial pads. The uptake was measured in the presence of arginine to avoid possible aspartic acid uptake by System II (ref. 1). Several amino acids were tested as possible inhibitors of aspartic acid uptake (Table IV). In each case, the uptake was not greatly inhibited by the neutral or basic amino acids used but was effectively inhibited by the acidic amino acids. This is the pattern of inhibition expected where aspartic acid is taken up by System IV. Since it is very unlikely that another transport system

TABLE IV

ASPARTIC ACID UPTAKE INTO VARIOUS CULTURES: INHIBITION BY SEVERAL AMINO ACIDS

10 μM L-aspartic acid was taken up for 4 min in the presence of 1 mM L-arginine.

Unlabeled amino acid added (1 mM)	*Inhibition of uptake of 10 μM L-aspartic acid by unlabeled amino acids (%)*		
	Carbon-starved pads	*Sulfur-starved pads*	*Nitrogen-starved pads*
D-Aspartic acid	97	97	99
L-Cysteic acid	98	99	98
L-Glutamic acid	97	97	97
L-Lysine	15	22	11
D-Phenylalanine	6	5	4
L-Serine	6	6	4

TABLE V

SYSTEM IV ACTIVITY UNDER VARIOUS CONDITIONS

10 μM L-aspartic acid was taken up for 4 min in the presence of 1 mM L-arginine. *v* is expressed in nmoles taken up per mg dry wt. per min. As for other determinations in this report, *v* was determined from counts in the trichloroacetic acid-soluble pool. Because counts measured in protein (NaOH-soluble fraction) or released as CO_2 were small, in each case being less than 20% of the total, the listed transport velocities provide an accurate measure of relative transport activities.

Type of culture	*Uptake of 10 μM L-aspartic acid (v)*
Growing mycelial pads	0.0074
Carbon-starved pads	0.053
Sulfur-starved pads	0.233
Nitrogen-starved pads	0.330

would show this same pattern, the aspartic acid uptake can be concluded to be due to the activity of amino acid Transport System IV.

The K_m values for L-aspartic acid uptake (in the presence of arginine) into carbon-, nitrogen- or sulfur-starved cultures were also measured. In each case, values obtained were within 40% of the K_m determined above for System IV (13 μM), providing further support for the conclusion that the uptake under these conditions is due to the activity of amino acid Transport System IV.

The velocity of aspartic acid uptake under various physiological conditions is shown in Table V. Little uptake occurs in growing mycelial pads. Carbon-, nitrogen- or sulfur-starved pads have substantial activity. Thus System IV has high activity under starvation conditions but low activity under conditions of rapid growth. It may serve to supply carbon, nitrogen or sulfur when these elements are in limited supply in the mycelium.

DISCUSSION AND CONCLUSIONS

Amino acid Transport System IV is an acidic transport system, taking up aspartic, glutamic and cysteic acids. It has substantial affinity for a number of analogues of aspartic and glutamic acids. It has higher affinity for D-aspartic acid than for L-aspartic acid but has lower affinity for D-glutamic acid than for L-glutamic acid. Thus the relative stereospecificity varies with the amino acid involved.

Transport System IV is regulated by the physiological state of the culture. It has low activity in growing mycelial pads but has high activity in carbon-, nitrogen- or sulfur-starved pads. It may serve to sustain growth under conditions of deprivation by transporting aspartic, glutamic and cysteic acids*.

The characterization of Transport System IV appears to complete the outlining of the major amino acid transport systems in *N. crassa*. Most of the amino acid transport in Neurospora is performed by the four transport systems described in Table VI**. Each of these systems is capable of transporting a variety of substrates. In this way, amino acid transport in Neurospora is similar to that found in mammalian cells but differs from that found in bacteria where narrow substrate specificity appears to be the rule. The four systems differ from each other in their pattern of regulation. System I (neutral amino acids) and System III (basic amino acids) have high activity under conditions of rapid growth but low activity under conditions of carbon-, nitrogen- or sulfur-starvation (refs. 1, 2 and unpublished work). In contrast, System IV, characterized here, and possibly also System II have high activity under starvation conditions but lower activity under conditions of rapid growth. Prolonged starvation lowers the activity of all the systems.

It is possible to compare amino acid transport in Neurospora with transport in Penicillium and yeast where several systems appear to be active. Under conditions of rapid growth and nitrogen sufficiency, yeast[5–7] and probably also Penicillium[8] have a number of transport systems with narrow specificity which are active. Thus the

* Cysteic acid has been found in natural protein, formed from the oxidation of cystine residues[4]. It is not unreasonable to suppose that cysteic acid may be a physiologically important source of sulfur.

** Transport systems for proline and for methionine have been characterized by the author (unpublished results) but these are of narrow specificity and are not considered in this discussion. It is possible, of course, that additional systems may be active under conditions not yet studied.

TABLE VI

MAJOR AMINO ACID TRANSPORT SYSTEMS IN NEUROSPORA

The amino acids in this table are only a partial listing of the amino acids having affinity for the different transport systems. In most cases, amino acids with similar properties to those listed will also have affinity. Affinity constants (K_m or K_i) are all expressed in μM.

	System I: L-*Neutral amino acids*	*System II*: D- *or* L-*basic, neutral and acidic amino acids*	*System III*: L-*Basic amino acids*	*System IV*: D- *or* L-*acidic amino acids*
Amino acids transported and affinity constants (μM)	L-Tryptophan (60) L-Leucine (110) L-Phenylalanine (50)	L-Arginine (0.2) L-Phenylalanine (2) D-Phenylalanine (25) Glycine (7) L-Aspartic acid (1200)	L-Arginine (2.4) L-Lycine (4.8)	L-Cysteic acid (7) L-Aspartic acid (13) L-Glutamic acid (16)
Other amino acids showing affinity	L-Valine L-Alanine Glycine L-Histidine L-Serine	L-Lysine L-Leucine α-Aminoisobutyric acid β-Alanine L-Histidine	L-Ornithine L-Canavanine L-Histidine (low affinity)	D-Aspartic acid D-Glutamic acid

functions performed by Systems I and III in Neurospora appear to be performed by a larger number of narrower transport systems in those other fungi. Under conditions of nitrogen starvation, however, Penicillium[8,9] and probably also yeast[7,10] have an active transport system with very broad specificity. These latter systems appear to be quite similar to System II in Neurospora. Yeast also has a proline transport system which demonstrates regulation similar to that found for Systems II and IV in Neurospora[11,12].

The data available make precise comparisons difficult. Nevertheless, the results in each of these fungi suggests that certain transport systems have their highest activity under conditions of rapid growth, perhaps serving mainly to provide amino acids for net protein synthesis. Still other systems have their highest activity under starvation conditions, perhaps serving mainly as sources of nitrogen, sulfur or reduced carbon. Further comparative studies may not only serve to help discern general patterns of regulation but may also help in phylogenetic studies of fungi.

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