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Physiological and Regulatory Properties of the General Amino Acid Transport System of *Neurospora crassa*

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The fundamental properties of the general amino acid transport system of *Neurospora crassa* were investigated in the conidial stage of the life cycle. The transport activity was found to be under genetic control, and an isogenic set of mutants deficient for the neutral, basic, or general amino acid transport systems and combinations thereof was constructed and used for analyzing the properties specific to the general permease. Amino acid transport by this system was found to be a carrier-mediated active process with broad specificity for the neutral and basic amino acids. Kinetic analysis revealed that a common binding site functioned to transport both neutral and basic amino acids and that the permease had a high affinity for its substrates. The kinetic parameters K_m, V_{max}, and K_i were defined for several substrates. Two modes of regulation were detected: substrate inhibition and ammonium repression. Activity of the general system was enhanced by the removal of ammonium ions from the incubation medium with a concomitant decline in either neutral or basic permease activity, suggesting that a common component exists between the neutral and the general systems and between the basic and the general systems.

For several years, amino acid transport has served as a useful probe for examining the movement of small molecules across the outer membrane of eucaryotic cells. Considerable attention has been focused on the transport of amino acids in the fungi because, unlike most eucaryotes, they offer the advantage of both a genetic and a physiological dissection of the transport process and its regulation. The common strategy used by the fungi appears to be one of a limited number of permeases which transport families of structurally related amino acids.

In the ascomycete *Neurospora crassa*, three constitutive amino acid transport systems have been detected: the neutral (N) system, which transports the family of neutral aliphatic and aromatic amino acids, the basic (B) system, which is specific for the family of cationic amino acids, and a general (G) system, which has affinity for all classes of amino acids (4, 13, 14, 17, 26, 27).

Because of its broad specificity, the G system is particularly representative of the transport strategy of eucaryotic cells. Several investigators have reported evidence for the existence of G-system activity throughout the life cycle. Pall (13) first detected the system in carbon-starved mycelia and has described some of its kinetic properties in this late developmental stage. Young mycelia also possess a G system which is quite active and have activity that is influenced by the nitrogen source in the incubation medium (20). Wolfinbarger and DeBusk (26) have detected G-system activity in the conidial stage which precedes the young mycelial stage of early development. As in the other developmental stages, the conidial G system transports a variety of amino acids.

Initially the activity of the G system was studied by competition analysis. Because the N and B systems transport only neutral or basic amino acids, respectively, transport of a substrate by one of these systems is subject to competition only by a member of the family of amino acids for which the system has affinity. In contrast, since the G system transports all classes of amino acids, either class of amino acids readily interferes with the uptake of any amino acid by this system. This unique property allowed for the initial characterization of G-system substrate specificity. With the isolation of mutants defective in either the N or B permeases, it became possible to monitor G system activity apart from N and B activity and to eventually isolate a mutant defective in the transport of amino acids through the G system.

An isogenic set of these transport mutants was constructed in order to use a genetic and kinetic approach to extend further the earlier observations concerning the nature of the G system activity. By genetically eliminating the complications of having a second functional system for a family of amino acids, we have used these mutants to delineate the fundamental genetic,

physiological, and regulatory properties of the general system during the earliest stages of development.

MATERIALS AND METHODS

Strains. To obtain an isogenic set of amino acid transport-defective mutants, the triple mutant *pm nbg* was backcrossed four times to the St. Lawrence wild type, 74a. The *pm nbg* mutant (FGSC 2605, 2606) and the wild type (FGSC 988) are available from the Fungal Genetics Stock Center, Arcata, Calif. Crosses were performed on the synthetic cross medium described by Westergaard and Mitchell (25). The transport mutants were recovered in all possible combinations by ordered tetrad dissection, and genotypes were verified by amino acid transport analysis.

The various mutant combinations were used to study the activity of a single transport system in isolation. Since neutral amino acids are transported via the N and G systems, a *pm n* mutant defective for the N system was used to study G-system activity, and a *pm g* mutant was used to monitor N-system activity. Similarly, since basic amino acids enter through the B and the G systems, a B-system-defective strain (*pm b*) was used to study G-system basic amino acid transport and a G-system-defective strain (*pm g*) was used to study B-system activity. The *pm n* and *pm b* mutants were used for all G-system studies except those assays involving histidine. Since histidine enters through the N, B, and G permeases, the N system must be studied in a *pm bg* mutant, the B system in a *pm ng* mutant, and the G permease in a *pm nb* strain. A *pm nb* was required for G-system inhibition kinetic analyses involving histidine and was, therefore, used for all G-system inhibition assays rather than using a *pm n* mutant to study neutral amino acids and a *pm b* mutant to study basic acids in reference to the G system.

Growth of cultures. Strains were preserved on silica gel according to the method of Brockman and deSerres (3). Primary cultures were obtained by transferring gel crystals to fresh minimal medium containing Vogel medium N (H. J. Vogel, Microb. Genet. Bull. 13:42–43, 1956) plus 2% sucrose and 1.5% agar. Cultures were grown in constant light at 35°C for 3 days followed by 3 days at 25°C, after which time they were stored at 4°C and used to inoculate secondary cultures for the ensuing 4 weeks. The secondary cultures were grown under the same regimen. This constant light/temperature shift regimen yields cultures with substantial conidiation. The use of the primary culture for subsequent inoculation of secondary cultures attempts to ensure genetic stability by minimizing the occurrence of spontaneous mutations which may arise when cultures are transferred serially. We have obtained excellent reproducibility of growth and transport assays with cultures handled according to this regimen.

Transport assays. Secondary cultures were grown for 6 days in 125-ml Erlenmeyer flasks containing 25 ml of medium N plus 2% sucrose and 1.5% agar under the standard temperature/light regimen described above. Cell suspensions were prepared by harvesting the conidia aseptically into ice-cold sterile glass-distilled water. Mycelial fragments were removed by passing the cell suspension through a filter of cheesecloth and glass wool. The dry weights of the cell suspensions were obtained by pipetting portions onto tared nitrocellulose filters (Millipore Corp., type AA, 0.8 μm, 25-mm diameter) and drying to constant weight. Cell suspensions were then stored in an ice bath at 4°C and used for 1 day thereafter.

For conidial transport assays, cells were incubated at 25°C in 1× Vogel medium N (0.1 mg/ml, final concentration) plus radiolabeled substrate (uniformly labeled) at a final concentration of 0.005 μCi/0.01 μmol per ml. In most cases, an equimolar concentration of NH_4Cl or KNO_3 was substituted for the NH_4NO_3 of Vogel medium N to achieve a nitrogen-sufficient or a nitrogen-deficient medium, respectively. For competition analysis, the competitor was present at a final concentration of 2 mM. The metabolic inhibitors sodium azide and 2,4-dinitrophenol were present at a concentration of 0.5 mM. Assays were initiated by the addition of cells to the incubation flasks which had been equilibrated to 25°C in a reciprocal-shake constant-temperature water bath. The cultures were shaken gently throughout the assay to provide a homogeneous suspension. Portions (5 ml) were withdrawn at regular intervals, filtered onto nitrocellulose filters, washed three times with ice-cold water, glued to aluminum planchets, dried, and counted in a Beckman gas flow proportional counter.

For postconidial assays, cells were incubated at a final concentration of 0.1 mg/ml for 3 h at 25°C in 1 × Vogel medium N (containing NH_4Cl or KNO_3) plus 1% D-glucose. This regimen allowed the cells to develop beyond the conidial stage but before visible germination and mass increase. Transport assays were initiated by the addition of radiolabeled amino acid (0.005 μCi/0.01 μmol per ml, final concentration) containing competitor (2 mM, final concentration) or an equivalent amount of water in the case of the control flasks. The radioisotopes were uniformly labeled. Portions were sampled and counted as above.

Initial rate determinations. Initial rate measurements were used to determine transport velocities for kinetic analyses, pH and temperature optima experiments, and concentration gradient determinations. During the first 8 min of transport, the transport rate was linear, which allowed for initial rate determinations. Under the extremes of either pH or temperature, however, the linear period of transport was greater than 8 min and appropriate incubation times were used. The regimen described for conidial transport assays was used.

All saturation and inhibition kinetic analyses were performed at 35°C, the temperature at which maximum amino acid transport rates were obtained. All substrates were chromatographed on Whatman No. 1 filter paper (0.16-mm thickness, medium flow rate), using three different solvents to ensure homogeneity. To determine the degree of metabolic conversion of transported substrates during the initial rate period, cells were preloaded with 50 μM radioactive substrate under standard conidial incubation conditions and extracted with boiling water for 30 min. The extracts were filtered, concentrated, and chromatographed in 88% phenol. No secondary metabolic products were detected.

The kinetic constants K_m and V_{max} were determined from Hofstee transformations (6, 9) of velocities measured for substrate ranges at least 10-fold above and 10-fold below the K_m. The kinetic constant K_i was derived according to the method of Dixon (5), using two different substrate concentrations. Because of the complications of substrate inhibition, the total concentration of substrate plus inhibitor never exceeded 50 μM for K_i determinations.

The pH studies used the standard procedure for initial rate determinations. A pH range of 2 to 11 was achieved by adding monobasic potassium phosphate (pH range, 2 to 7) or dibasic potassium phosphate (pH range, 7 to 11) to the NH_4Cl Vogel medium N from which the calcium and magnesium salts were omitted to prevent the formation of precipitates. The pH was adjusted with 6 N HCl or saturated KOH.

To determine the extent of the concentration gradient established (internal concentration/external concentration), cells were preloaded for 8 min as above. A known external concentration of 10 μM was maintained. The internal concentration was obtained by measuring the nanomoles of free amino acid (calculated from a known specific activity of the intracellular trichloroacetic acid-soluble amino acid pool) and an intracellular water content of 5.3×10^{-4} ml per mg (dry weight) of cells. This method is set forth by Wolfinbarger and DeBusk (26) for the purpose of calculating internal concentrations of transported amino acids.

RESULTS

Genetic analysis. Two components appear to be involved in the transport of neutral amino acids in wild-type *N. crassa*: one component, the N system, whose activity is subject to competition solely by another member of the family of neutral amino acids, and a second component, the G system, whose activity is subject to competition by either a neutral or a basic amino acid, a phenomenon described as "cross-family competition." Basic amino acid transport also appears to involve two components, a B system, which is subject to competition only by another basic amino acid, and a G system, which is subject to competition by either a basic or a neutral amino acid. Therefore, the G system is suspected of being a generalized transport system with broad specificity for amino acid substrates.

The isolation of a mutant deficient for this generalized transport activity allows us to prove that the G system is a single system with broad specificity and that three distinct constitutive amino acid permeases exist in conidia, two which are involved with the transport of neutral amino acids, the N and the G, and two which are involved with transport of basic amino acids, the B and the G, for a total of three distinct systems.

Elimination of a functional *pm g* gene product eliminated the cross-family competition component of the wild type with respect to both neutral (phenylalanine) and basic (lysine) amino acids (Fig. 1). This substantiates the concept of a single generalized system capable of transporting structurally distinct classes of amino acids. The *pm g* mutation is specific for the G system, since no alteration of N or B permease activity was detected in the conidial stage.

When the *pm g* mutation was combined with a mutation at the *pm n* locus which controls the activity of the N system (10, 22), no transport of any of the neutral amino acids phenylalanine, tryptophan, valine, leucine, citrulline, and glutamine was observed, confirming that neutral amino acid transport is mediated via the N and the G systems. Similarly, when the *pm g* mutation was combined with a mutation at the *pm b* locus, which controls the activity of the B system (17, 23), all transport of the basic amino acids arginine, lysine, and ornithine was eliminated. Therefore, basic amino acid transport is likewise mediated via two systems, the B and the G. The triple mutant, *pm nbg*, lacked transport activity for all of these amino acids, suggesting that three distinct constitutive amino acid transport systems exist in *N. crassa* conidia. Figure 2 provides an overview of conidial amino acid transport.

Amino acid transport analysis of 22 ordered tetrads of the cross *pm nbg* × 74a (wild type) demonstrated that the *pm g* mutation segregates as a single Mendelian gene. Linkage of *pm g* to the centromere was detected, but no linkage to the *mtr* (*pm n*) locus (LG IVR), to the *bat* (*pm b*) locus, also on LG IVR (S. Ogilvie-Villa, personal communication), or to the *m.t.* locus (LG IL) was detected. Since *pm n* and *m.t.* are linked to their respective centromeres, it is concluded that the *pm g* locus is probably not on linkage group IL or IVR. The location of the locus is as yet undetermined because there is no readily assayable phenotype.

Physiological characterization of the G system. Since it was shown that the neutral amino acids phenylalanine, tryptophan, valine, and leucine were transported only by the G system in a *pm n* mutant which lacks a functional N system, transport of phenylalanine in this mutant was chosen as representative of the properties of the G system with respect to neutral amino acids for the purpose of physiological characterization. Similarly, the basic amino acids lysine, arginine, and ornithine were transported only through the G system in a *pm b* mutant which lacks the B system. Lysine transport in a *pm b* mutant was chosen as representative of G-system activity with respect to basic amino acids. The double mutant *pm nb* was used to study histidine transport through the G system since histidine exists as both a neutral and

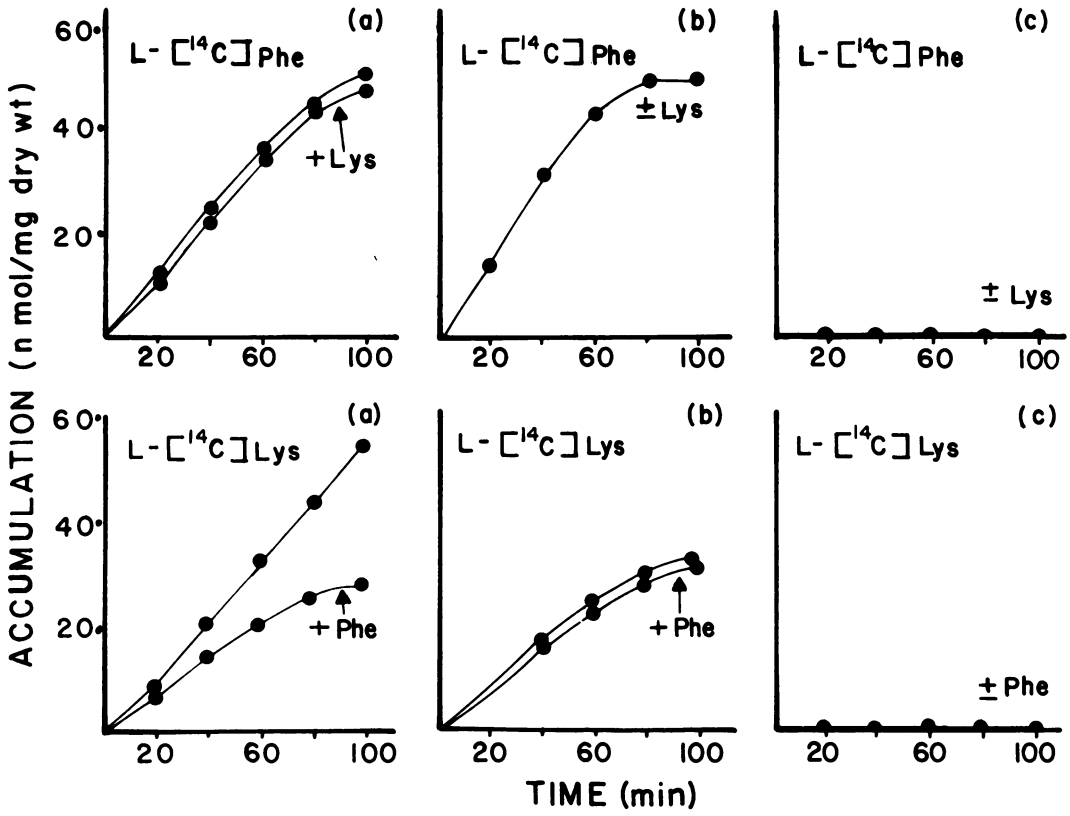

FIG. 1. *Effects of the pm g mutation on conidial stage transport of phenylalanine and lysine. (a) Wild-type strain 74a; (b) strain pm g defective for G-system activity; (c) strain pm nbg defective for N-, B-, and G-system activities. Nitrogen source was KNO_3; substrate concentration was 10 μM; competitor concentration was 2 mM.*

a basic species at the pH of the transport studies (pH 5.8) and thus would be transported by all three systems if they were functional.

Several properties of the G system were similar to those properties commonly associated with enzymatic processes. A pH optimum of 5 to 6 and a temperature optimum of 35 to 40°C were observed for both phenylalanine and lysine transport. Transport of these two amino acids exhibited Michaelis-Menten saturation kinetics (Fig. 3), was eliminated by the metabolic inhibitors sodium azide and 2,4-dinitrophenol (Fig. 4), and occurred against a concentration gradient, suggesting that transport by the G system is a carrier-mediated active transport process. The concentration gradient was rapidly established, the internal/external ratio being 9:1 for phenylalanine and 112:1 for lysine after 8 min of transport.

Kinetic analysis of the G system. Examination of the affinity and velocity parameters by means of saturation kinetics demonstrated that transport of substrates by the G system was a high-affinity-low-velocity process (Table 1). This was in contrast to the profiles generated for the N and B systems (Table 2). The N system exhibited a lower affinity for phenylalanine than did the G system, but the velocity of phenylalanine transport via the N system was much greater. The B system exhibited a high affinity for basic amino acids similar to the affinity values of the G system, but the velocity of the B-system transport of these amino acids exceeded that of the G permease.

Inhibition kinetic analysis revealed all inhibition to be competitive in nature, with the K_i values closely correlating with the K_m values, suggesting that only a single binding site exists for transport of both neutral and basic amino acids via the G system (Table 3). Inhibition analysis also revealed that the L-stereoisomer of an amino acid is preferred over the D-stereoisomer of both neutral and basic substrates (Table 3) and that an α-amino group is an essential structural component for transport of an amino acid by the G system. The imino acid proline

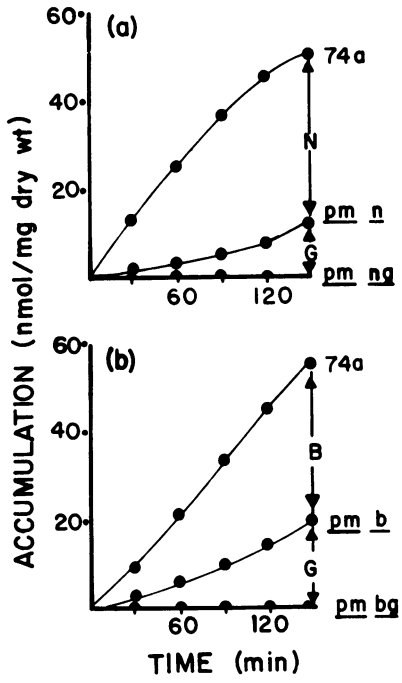

FIG. 2. *Summary of amino acid transport in N. crassa conidia. 74a is the wild type. The following strains are defective for one or more amino acid transport systems: pm n (N-system defective); pm ng (N-system defective and G-system defective); pm b (B-system defective); pm bg (B-system defective and G-system defective). (a) Transport of neutral amino acids; (b) transport of basic amino acids.*

failed to inhibit G-system transport of phenylalanine, suggesting that the system is specific for alpha-L-amino acids.

Histidine accumulation. Studies by Pall (14) and by Magill et al. (12) have suggested that histidine is not a favored substrate for the B system in mycelia. Magill found the G system to be the major contributor to total histidine accumulation. Histidine transport was examined in the conidial stage in reference to each of the three transport systems. Because it exists as both a neutral and a basic species, histidine can potentially be transported by the N, the B, and the G permeases. The systems can be dissected physiologically by the use of competitors to eliminate two of the three systems or genetically by eliminating two systems by mutation. Either approach serves to isolate a single system for investigation of its properties with respect to histidine transport (Fig. 5). No transport was observed in the triple mutant *pm nbg*, demonstrating that histidine entry is confined to one or more of the previously suspected transport systems. Clearly the G system was the most active of the three systems in the conidial stage. The kinetic parameters for histidine transport through the G system are given in Tables 1 and 3.

Regulation of the G system. At least three factors appear to modulate G-system activity: the substrate concentration, the developmental stage of the organism, and the presence of ammonium ions in the medium.

Inhibition of amino acid accumulation at high substrate concentrations was observed for phenylalanine, tryptophan, valine, histidine, lysine, and arginine. Substantial reduction in velocity was evident at 100 μM, as can be seen with the representative amino acids in Fig. 6. Neither

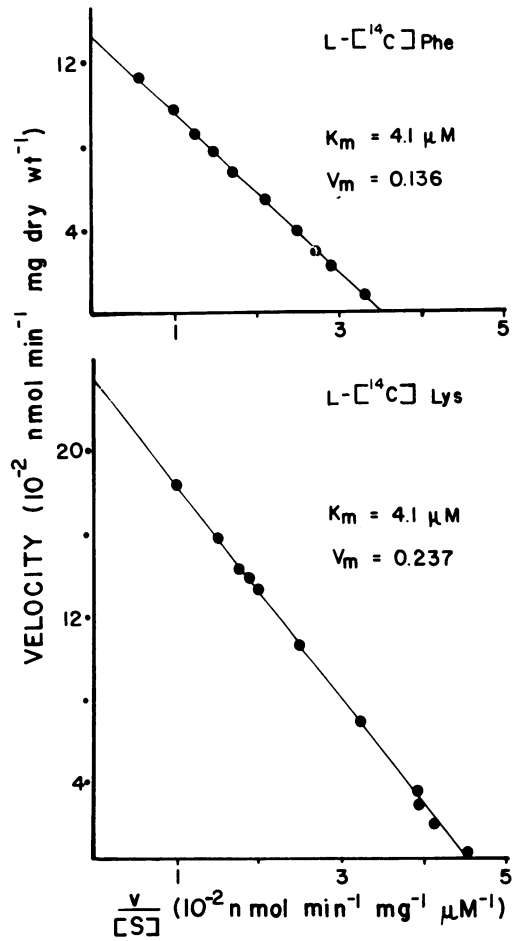

FIG. 3. *Hofstee transformation of initial velocities of phenylalanine and lysine transport by the G system. The pm n strain is defective for N-system activity and was used to assay phenylalanine transport by the G system. The pm b strain is defective for B system activity and was used to assay lysine transport by the G system. V_{max} is expressed as nanomoles per minute per milligram. Nitrogen source was NH_4Cl.*

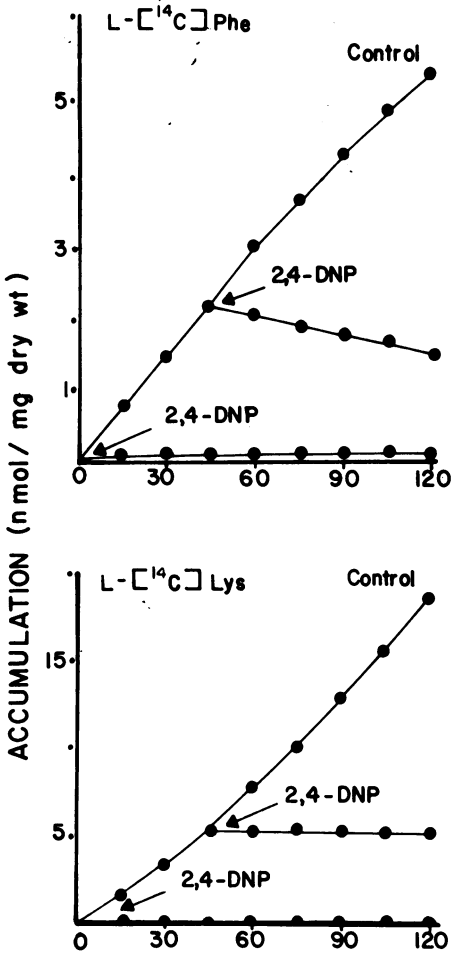

FIG. 4. *G-system transport of phenylalanine and lysine in the presence of the metabolic inhibitor 2,4-dinitrophenol (0.5 mM). Phenylalanine transport was monitored in the pm n strain, and lysine transport was monitored in the pm b strain as explained in Fig. 3. Nitrogen source was NH_4Cl; substrate concentration was 10 μM.*

leucine nor ornithine, however, exhibited a decline in velocity with increasing substrate concentration.

Both the developmental stage and the ammonium content of the incubation and transport media influenced the activity of the G system. In the conidial stage, no difference was observed between the transport of phenylalanine or lysine in the presence of ammonium ions (NH_4Cl) or in their absence (KNO_3). If, however, cells were allowed to develop past the conidial stage but before visible germination (postconidial stage), two points were noted: (i) the N-, B-, and G-system activities increased over the conidial activity, regardless of nitrogen source used, and (ii) whereas no difference in transport activity of any of the systems was noted in the conidial stage in the presence and absence of ammonium ions, there was a considerable difference in the postconidial stage. These points are illustrated (Fig. 7) by wild-type transport of lysine as a function of developmental stage and of nitrogen content of the incubation and transport media. The total transport remained the same regardless of the nitrogen source, but the activity of the G system increased with a concomitant decrease in the B-system activity. Similarly, with phenylalanine as the substrate, the G-system activity increased with a concomitant decrease in the N-permease activity (data not shown).

Whereas it is not clear whether the nitrogen effect is developmental or whether the transport systems are affected directly, the effect of glucose appears to be developmental. The presence of glucose during the 3-h incubation allows cells to develop from the conidial to the postconidial stage with a concomitant increase in activity of all three transport systems. Cells incubated for 3 h in the absence of glucose continue to exhibit the transport pattern characteristic of the conidial stage and, therefore, do not appear to enter the postconidial stage with its enhanced trans-

TABLE 1. *Kinetics of amino acid transport via the general permease*

Amino acid substrate	Strain	K_m[a] (μM)	V_{max}[a] (nmol min^{-1} mg^{-1})	No. of replicates
Neutral				
L-Phenylalanine	*pm n*	4.1 ± 0.3	0.136 ± 0.007	3
L-Tryptophan	*pm n*	0.9 ± 0.1	0.017 ± 0.001	1
L-Leucine	*pm n*	4.4 ± 0.2	0.126 ± 0.004	2
L-Valine	*pm n*	8.6 ± 0.9	0.108 ± 0.008	2
Neutral/basic				
L-Histidine	*pm nb*	4.8 ± 0.3	0.103 ± 0.004	1
Basic				
L-Lysine	*pm b*	4.1 ± 0.3	0.237 ± 0.010	3
L-Arginine	*pm b*	1.6 ± 0.1	0.151 ± 0.002	1
L-Ornithine	*pm b*	6.2 ± 0.2	0.216 ± 0.004	1

[a] Error figures are the standard error of the mean except for single determinations, where the standard error of the Hofstee plot is given.

TABLE 2. *Kinetics of amino acid transport via the neutral and basic permeases*

Permease	Substrate	Strain[a]	K_m[b] (μM)	V_{max}[b] (nmol min^{-1} mg^{-1})
Neutral	L-Phenylalanine	74a + Lys	56.7 ± 4.0	4.64 ± 0.26
		pm g	48.4 ± 3.2	3.90 ± 0.19
Basic	L-Lysine	74a + Phe	3.3 ± 0.1	0.594 ± 0.012
		pm g	3.5 ± 0.2	0.609 ± 0.012
	L-Arginine	74a + Phe	1.5 ± 0.1	0.542 ± 0.009
		pm g	1.6 ± 0.1	0.427 ± 0.009

[a] N-system activity was monitored by elimination of the G activity by competition with 2 mM L-lysine or by mutation (*pm g*). B-system activity was monitored by elimination of the G system by competition with 2 mM L-phenylalanine or by mutation (*pm g*).

[b] Error figures are the standard error of the Hofstee plot. Values are for single determinations.

TABLE 3. *Kinetic properties of inhibitors of the general amino acid transport system*[a]

Inhibitor	K_m[b] (μM)	K_i[b] (μM) of given substrate		
		L-Phenylalanine	L-Lysine	L-Valine
L-Phenylalanine	4.1	4.8	4.4	4.9
D-Phenylalanine		62.6		
L-Tryptophan	0.9	1.9	2.4	
L-Leucine	4.4	4.7	6.0	
D-Leucine		180.1		
L-Isoleucine		14.5	16.5	15.4
L-Valine	8.6	8.3	11.1	9.6
L-Citrulline		14.0		
L-Histidine	4.8	4.7	5.4	
L-Arginine	1.6	3.7	2.4	2.4
L-Lysine	4.1	4.1	3.8	8.3
D-Lysine			250.3	
L-Ornithine	6.2		5.1	

[a] All inhibition observed was competitive in nature.

[b] The *pm n* mutant was used for K_m determinations for neutral amino acids, the *pm b* mutant for basic amino acid K_m determinations, and the *pm nb* mutant for the K_m determination for histidine and for all K_i measurements.

port activities (J. H. Tisdale, unpublished data). Glucose, then, appears to affect the transport systems indirectly through its effects on development.

DISCUSSION

The physiological and regulatory properties of the general amino acid transport system of *N. crassa* have been described. The system is characterized by high-affinity-low-velocity carrier-mediated active transport with broad specificity for amino acids. G-system activity is under genetic control since the transport ability is lost upon mutation at the *pm g* locus. Kinetic analysis demonstrated that the system has a high affinity for all L-amino acids tested but a very low velocity of transport. The system does exhibit affinity for the D-stereoisomers, but the L-isomers are the preferred alpha-amino acid substrates. Inhibition kinetic analysis suggested that the various substrates compete for a single common binding site before entry into the cell. Two modes of regulation have been described: the loss of ability to accumulate most amino acids at substrate concentrations of 100 μM or greater, and the ability of ammonium ions to decrease the magnitude of transport activity.

The broad specificity of the G system is in keeping with the original observations of Pall (13), Wolfinbarger and DeBusk (26), and Magill et al. (11, 12), who collectively showed the general permease to be capable of transporting neutral, basic, and acidic amino acids. The inability of the imino acid proline to inhibit phenylalanine transport via the G system is consistent with the conclusions of Magill et al. (11) that a free alpha-amino group is required for G-system transport of an amino acid. The kinetic parameters observed here for conidial stage cells correlate well with the values reported by Pall for older mycelia, suggesting that high affinity and low velocity accurately characterize the activity of the G permease.

Pall (13) also reported that the system had affinity for D-amino acids since D-phenylalanine was transported. The K_i for D-phenylalanine was, however, 10- to 15-fold higher than that for L-phenylalanine. The present study substantiates Pall's observation with D-phenylalanine, but the K_i values for D-leucine and D-lysine suggest 36- and 50-fold reductions in affinities, respectively, in comparison with the L-amino acid counterparts. These observations suggest that the L-stereoisomer is the preferred substrate for the G system. D-Amino acids have also been reported to be substrates for the general permease of the fungus *Saccharomyces cerevisiae* (19). The low affinity of the *Neurospora* permease for D-amino acids raises the question of whether transport into the cell via this route is physiologically significant. Experiments are in progress to monitor D-amino acid utilization by an amino acid auxotroph as a function of the transport system by which the amino acid enters the cell.

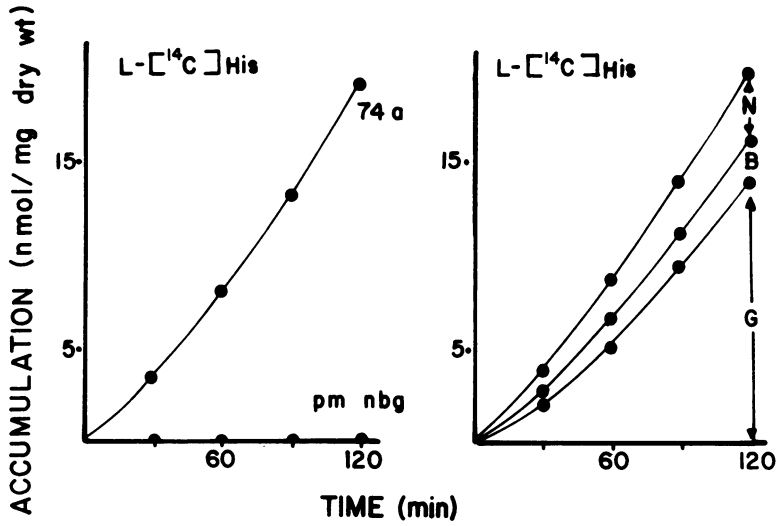

FIG. 5. *Transport of histidine by the N, B, and G systems. N-system activity was determined with pm bg, B-system activity with pm ng, and G-system activity with pm nb. Nitrogen source was NH_4Cl. Histidine concentration was 10 μM.*

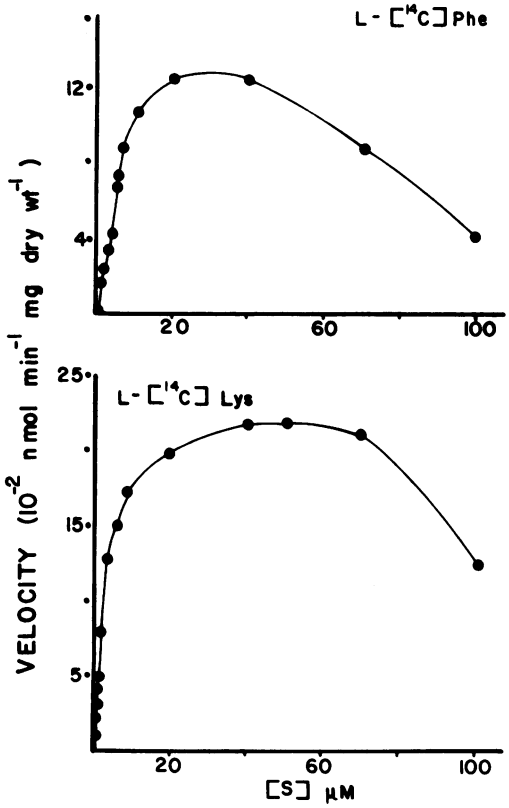

FIG. 6. *Saturation kinetics of phenylalanine and lysine transport by the G system. Phenylalanine transport was monitored in the pm n strain, and lysine transport was monitored in the pm b strain as explained in Fig. 3. Nitrogen source was NH_4Cl.*

The properties of the G system contrast with those of the N and B permeases. The G system has a high affinity for all L-amino acids thus far examined, yet this system has a very low velocity. Additionally, the G system differs from the other systems in that it exhibits substrate inhibition. These properties result in a limitation in the total amount of amino acid which can be transported, suggesting that the G permease is not a major contributor to the normal flux of amino acids into the cell. These observations raise questions concerning the physiological significance of the G system to the cell. It is conceivable that amino acids are channeled into different metabolic fates intracellularly depending upon the transport system of entry. Perhaps the N and B systems function as the primary source of exogenous amino acids for use in protein synthesis and the G system serves a different, as yet unknown, function. Pall (13) has suggested the permease serves as a scavenging system for amino nitrogen. Sanchez et al. (20) have also suggested that the G system serves as an amino-nitrogen permease. The data presented here which reveal the system to be maximally operative under conditions of low substrate concentrations and nitrogen starvation are not inconsistent with the possibility that the G permease may serve as a scavenging system.

It was noted that two amino acids, leucine and ornithine, did not cause substrate inhibition of the G permease. One explanation for this phenomenon is that an additional system other than the N and G systems exists for the transport of leucine at high substrate concentrations and an

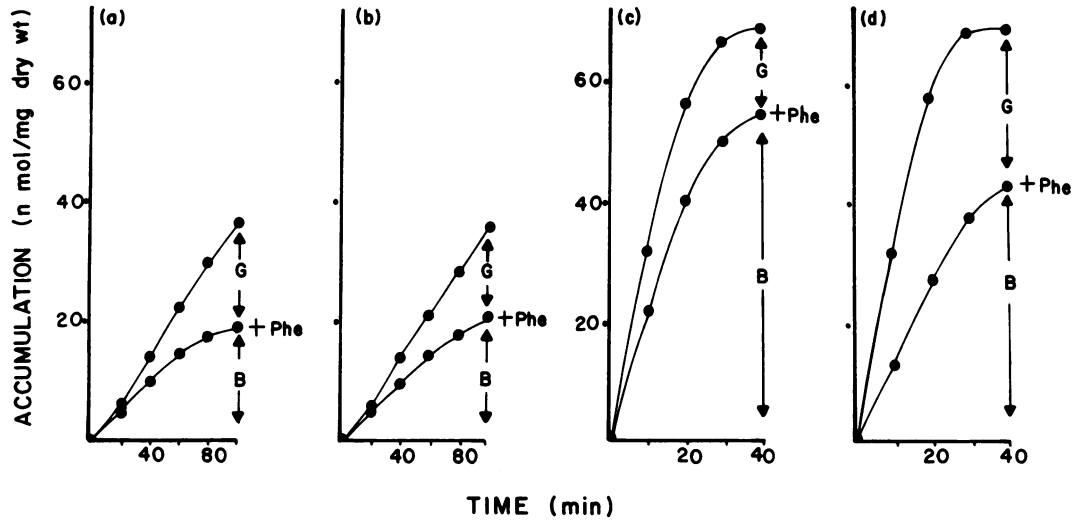

FIG. 7. *Transport of lysine by the wild type (74a) as a function of developmental stage and nitrogen deficiency. (a) Conidial stage; nitrogen source, NH_4Cl. (b) Conidial stage; nitrogen source, KNO_3. (c) Postconidial stage; nitrogen source, NH_4Cl. (d) Postconidial stage; nitrogen source, KNO_3.*

additional system other than the B and G systems exists for the transport of ornithine. Current data argue against this possibility since there was no leucine transport in a *pm ng* mutant and no ornithine transport in a *pm bg* strain. The observed activity, therefore, in the *pm n* mutant (leucine) and the *pm b* mutant (ornithine) is presumed due to the G permease. It is not known at this point what is unique about the G-system interaction with respect to these two amino acids, but they are currently being investigated as probes for the substrate inhibition phenomenon.

Enhanced G-system activity in *Neurospora* in response to germination and to nitrogen starvation is characteristic of the general amino acid permeases described for other fungi: *Aspergillus oryzae* (21), *Penicillium chrysogenum* (1, 2), and *S. cerevisiae* (7, 8, 18). In contrast to the situation in which the general permease is functional only under nitrogen starvation conditions as has been observed with *Penicillium* and *Saccharomyces*, the *Neurospora* permease is constitutive. In the conidial stage no differences were observed in G-system activity in the presence or absence of ammonium ions. It is only upon development that a difference became evident. The early developmental period is characterized by an increase in amino acid transport, most notably the G system (15, 24). Superimposed on this developmental increase in G activity is the influence of the nitrogen source in the incubation medium. The depression of G activity by the presence of ammonium ions has been reported previously by Sanchez et al. (20), Rao et al. (16), and Facklam and Marzluf (T. Facklam and G. A. Marzluf, Genetics **80**:s29). The influence of the nitrogen source does not appear to be confined solely to the G system, however. The studies presented here demonstrate that, whereas the total transport in a wild-type cell remains the same regardless of the nitrogen source, the increase in G activity in the absence of ammonium ions occurs at the expense of N- and B-system activity. These data suggest that one or more common elements exist among the transport systems. Sanchez et al. (20) suspected the existence of a common element and proposed an energy-coupling mechanism as a likely candidate. Since our data show that loss of the basic transport system in a wild type does not alter the transport of neutral amino acids and vice versa, we feel that whatever the nature of the common element, it is most likely family specific. That is, there is not an element common to all three systems but one which is confined to the two basic permeases, the B and the G, and the one which is confined to the two neutral permeases, the N and the G. Studies designed to examine the nitrogen regulation of all three transport systems and the possible nature of the family-specific common element(s) are currently in progress.

The G permease, therefore, exhibits kinetic and regulatory properties sufficiently unique in comparison with the other two constitutive permeases to suggest it may serve a function distinct from the other permeases. Additionally, all three systems are influenced by the nitrogen sufficiency of their environment in such a way as to

suggest the existence of common elements. The findings reported here provide a foundation for determining the molecular architecture of the transport systems and their physiological significance to the cell.

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