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Interactions Between Amino Acid Transport Systems in *Neurospora crassa*

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Mutants of *Neurospora crassa*, selected as resistant to L-canavanine and L-thialysine, are partially deficient in the uptake of basic amino acids. Neutral amino acids completely inhibit uptake of basic amino acids, and this inhibition is dependent on the activity of a neutral amino acid permease. In contradistinction, mutants resistant to 4-methyl-DL-tryptophan are partially deficient in the uptake of neutral amino acids. Basic amino acids completely inhibit neutral amino acid uptake, and this inhibition is dependent on the activity of a basic amino acid permease. It is proposed that these specific transport systems compete with a general amino acid permease for some common element. The general permease is also regulated by ammonia, the amino acid pool, or both. The activity of the general permease can be eliminated phenotypically by a high concentration of glycerol or a high temperature. It is also shown that L-citrulline is transported by the neutral amino acid permease and by the general amino acid permease.

The detailed kinetic analysis of amino acid transport performed by Pall (15-17) has indicated the presence of several amino acid transport systems in *Neurospora crassa*. A general amino acid permease transports D-, L-, basic, neutral, and acidic amino acids. There are also three permeases each of which transports a generic group of L-amino acids: neutral, basic, or acidic. *Saccharomyces cerevisiae* has a general amino acid permease which translocates D-, L-, basic, and neutral amino acids in the absence of ammonia (8), permeases which transport basic L-amino acids (5), and permeases which are specific for individual amino acids, i.e., for L-lysine (6), L-methionine (4), and L-histidine (2). *Penicillium chrysogenum* has group permeases for basic, acidic, and neutral L-amino acids and specific permeases for L-arginine, L-lysine, L-proline, L-methionine, L-cystine, L-cysteine, and ammonia (9).

During the study of strains of *N. crassa* selected for resistance to the basic amino acid analogues, L-canavanine and L-thialysine, we found that these mutants were partially deficient in the transport of basic amino acids. In addition, the residual transport of basic amino acids was inhibited by neutral amino acids, and this inhibition was dependent on the activity of the neutral amino acid permease (15, 19).

This communication describes the genetics and physiology of L-canavanine- and L-thialysine-resistant mutants, the interactions between the basic, neutral, and general amino acid transport systems, and some new phenotypic characteristics of the general amino acid permease. It extends previous studies of L-citrulline transport in the fungus *N. crassa* (10, 20).

MATERIALS AND METHODS

Chemicals and growth conditions. *N. crassa* was grown in the minimal medium N of Vogel (21) supplemented with 1.5% sucrose. In certain experiments, an equimolar amount of KNO_3 was substituted for NH_4NO_3. Crosses were made on 1.7% corn meal agar (Difco), and the cultures were incubated in the dark at 25 C. Plating of ascospores and selection of mutants was done on Vogel medium N supplemented with glucose and fructose (0.05% each) in place of sucrose, plus 1% sorbose. Spot tests for nutritional requirements and resistance to amino acid analogues were made on solidified Vogel medium N containing 0.8% sorbose and 0.4% sucrose. Except when otherwise indicated, plates were incubated at 29 C. Conidia were harvested from slants of medium N supplemented with 1.5% sucrose which were incubated for 3 days in the dark at 29 C and 2 days with illumination at 25 C. Growth in liquid medium was in 250-ml Florence flasks containing 100 ml or in side-arm tubes (18 by 150 mm) containing 10 ml of Vogel's minimal medium plus sucrose (1.5%). The

vessels were inoculated with filtered conidia and aerated vigorously with hydrated air at 25 C.

L-Thialysine was obtained from the British Drug House, and 4-methyl-DL-tryptophan was from Mann Research Laboratories. Other nutrients were obtained from Calbiochem. They were sterilized by filtration. The radioactive amino acids, [^{14}C]L-arginine-guanido, [^{14}C]L-citrulline-ureido, and [3-^{14}C] L-tryptophan (side-chain label) were obtained from New England Nuclear Corp. Since it was found that the preparation of L-citrulline contained [^{14}C]urea and that the preparation of L-arginine liberated $^{14}CO_2$ in acid medium, these amino acids were further purified on Dowex 50W column (1.0 by 5 cm) 200 to 400 mesh, 8% cross-linked. After washing the column with 35 ml of 2 N HCl, the amino acids were eluted with 35 ml of 4 N HCl.

Stocks. All *N. crassa* stocks came from the collection of R. H. Davis (Department of Botany, University of Michigan) or from the Fungal Genetics Stock Center at Dartmouth College, Hanover, N.H. The basic stocks were the wild strain 74 (A) and 73 (a); the 4-methyl-DL-tryptophan-resistant mutant (Mtr); an arginine, citrulline, or ornithine auxotroph (Arg-5); a proline auxotroph (Arg-8); a lysine auxotroph (Lys-3); two tryptophan auxotrophs (Tryp-1, Tryp-2); and the pyrimidine auxotrophs Pyr-1 and Pyr-2.

Wild-type strains that are naturally sensitive and naturally resistant to L-canavanine have been examined for transport of basic amino acids (1). No difference has been found. The natural resistance or sensitivity to this antimetabolite is determined by the presence or absence of an enzyme which converts L-canavanine to L-homoserine and hydroxyguanidine (J. B. Logan, Ph.D. thesis, California Inst. of Technology, 1969). Except for the proline auxotroph Arg-8, all the other strains mentioned above were naturally resistant to L-canavanine (Can^r). From the cross Arg-8 × 74 (A), a wild-type strain was obtained which was sensitive to L-canavanine (Can^s). All the stocks were L-thialysine-sensitive.

Mutant selection and genetic analysis. A heavy suspension of spores of a wild strain (Can^s) was plated on a medium containing 50 μg of L-canavanine or 5 μg of L-thialysine per ml. Parent spores failed to germinate; rare colonies which appeared after the first day of incubation were isolated and transferred to slants supplemented with the antimetabolites. One of the mutants isolated was crossed with Pyr-2. Random spore analysis yielded 76% parental progeny, 10% double mutants, and 14% wild-type progeny. This preliminary genetic analysis indicated that the new mutation was in linkage group IVR.

Radioactive amino acid transport experiments. In a typical experiment of amino acid transport, a 10-ml sample of germinated conidia grown for 9 hr in Florence flasks was transferred to a 25-ml Erlenmeyer side-arm flask and incubated at 25 C with aeration for about 5 min. The rates of transport found under these conditions were similar to the rates obtained with cells 30 min after they have been filtered and resuspended in fresh medium. Radioactive amino acid, suitably diluted with carrier, was added. Immediately after the addition of the radioactive amino acid, samples (1 ml) with 50 to 80 μg of protein were removed at 20-sec intervals. The samples were filtered immediately through membrane filters (Millipore type HA 0.45-μm pore size) and washed with 20 ml of cold (4 C) minimal medium. Transport experiments run in the presence of glycerol were performed after 15 min of preincubation with 1.5 M glycerol before addition of the radioactive amino acid. Samples were counted in a gas flow for a time adequate to give at least 1,000 counts/min per sample; triplicates agreed within 5 to 10%.

Cell-free extracts were prepared by treating the germinated conidia with cold 5% trichloroacetic acid. The extracts were chromatographed on Whatman no. 3 MM paper in a solvent composed of *n*-butanol–acetic acid–water (4:1:5, v/v). In all cases, the major radioactive component was determined chromatographically as the one being assayed.

Protein determinations. Germinated conidia were collected on membrane filters. The filters were immersed in 5% trichloroacetic acid and then the residues were resuspended in 1 N NaOH. Protein in the supernatant fluids of these suspensions was determined by the method of Lowry et al. (13).

RESULTS

Characterization of mutants selected as resistant to basic and neutral amino acid analogues. Table 1 presents the growth response of different strains of *N. crassa* in the presence of certain amino acid analogues. The strains that were naturally resistant to L-canavanine, an analogue of L-arginine, were sensitive to the analogue of L-lysine, L-thialysine, i.e.; they were *can^r;bm-1$^+$;mtr$^+$;* (*74 A*). Mutants selected for resistance to one of these basic amino acid analogues were also resistant to the other, i.e., they were *can^s;bm-1;mtr$^+$*, or

TABLE 1. *Effect of amino acid analogues on the growth of different strains of Neurospora crassa*

Strain	Growth[a] on			
	Minimal medium	L-Canavanine-SO_4 (25–100 μg/ml)	L-Thialysine-hydrochloride (25–100 μg/ml)	DL-4-Methyl tryptophan (200 μg/ml)
Can^r;Bm-1$^+$;Mtr$^+$ (74-A)	+	+	−	−
Can^s;Bm-1$^+$;Mtr$^+$	+	−	−	−
Can^s;Bm-1;Mtr$^+$	+	+	+	−
Can^s;Thl;Mtr$^+$	+	+	+	−
Can^r;Bm-1$^+$;Mtr	+	+	−	+
Can^r;Bm-1;Mtr	+	+	+	+

[a] Growth on spot plates incubated at 29 C for 36 hr.

can^s;thl;mtr$^+$. When a strain with the single mutation bm-1 (selected as resistant to L-canavanine) was crossed with a strain with the single mutation *thl* (resistant to L-thialysine), all 500 progeny analyzed were found to be resistant to L-thialysine, suggesting that these mutations are closely linked or, very probably, allelic. Resistance to DL-4-methyl tryptophan (19) (*mtr*), an analogue of L-tryptophan, was phenotypically expressed independently of the resistance to the basic amino acid analogues.

The basic amino acid auxotrophs Arg-5 and Lys-3, grow optimally on L-ornithine or on L-arginine and on L-lysine, respectively. Table 2 demonstrates that when they combined with the mutation *bm-1*, the double mutants grow suboptimally on L-ornithine and L-lysine, respectively.

Furthermore, neutral amino acids such as L-citrulline or L-tryptophan completely inhibited the growth of these mutants on basic amino acids.

L-Tryptophan and L-citrulline, two neutral amino acids, inhibited only partially the growth of Arg-5 and Lys-3, but Arg-5;Bm-1 and Lys-3;Bm-1 were completely inhibited by these amino acids. These results are in agreement with previous work which shows that in strains without the *bm-1* mutation only a basic amino acid, i.e., L-lysine, completely inhibits the growth of a different basic amino acid auxotroph (3, 18).

The growth of neutral amino acid and pyrimidine auxotrophs carrying the *bm-1* mutation (Tryp-2;Bm-1 and Pyr-1;Bm-1), was not inhibited either by basic or neutral amino acids, respectively.

Growth of the neutral amino acid auxotrophs Tryp-1 on tryptophan, and of Arg-5 on citrulline, was completely inhibited by other neutral amino acids (Table 3). Basic amino acids such as L-lysine had little effect on the growth of these single mutants, and the effect, if any, was stimulation.

From these data it can be concluded that, in general, (i) the mutation *bm-1* prevents the growth of basic amino acid auxotrophs on their required nutrient if neutral amino acids are also present, and (ii) the mutation *mtr* prevents the growth of a neutral amino acid auxotroph on its required nutrient if a basic amino acid is also present.

The effect of these mutations is specific, since excellent growth was observed with strains carrying the double mutation *arg-5;bm-1* on L-citrulline plus L-lysine and carrying *arg-5;mtr* on L-arginine plus L-tryptophan.

To explain these data, we propose that the *bm-1* mutation is similar to the basic amino acid transport (Bat) mutant previously reported (20). The *bm-1* and the *mtr* mutations confer resistance to basic and neutral amino acid analogues, respectively, because these loci are responsible for partially inactivating the

TABLE 2. *Effect of basic and neutral amino acids on the growth of basic amino acid auxotrophs with and without the bm-1 mutation*

Strain	Growth[a]					
	Arg (50)	Orn (50)	Arg (50) Lys (1,000)	Orn (50) Lys (1,000)	Arg (50) Trp (1,000)	Orn (50) Trp (1,000)
Arg-5	50.4	50.4	0.15	0	30.4	28.0
Arg-5;Bm-1	47.4	28.4	1.45	2.45	0.95	3.8
	Lys (50)	Lys (50) Arg (1,000)	Lys (50) Orn (1,000)	Lys (50) Cit (1,000)	Lys (50) Trp (1,000)	
Lys-3	17.2	0.4	1.60	4.4	7.2	
Lys-3;Bm-1	2.7	0.3	0	0	0.3	

[a] Values shown are ratios of micrograms of protein per milliliter in medium with supplements divided by those in minimal medium. Growth was for 10 hr in liquid culture tubes at 25 C. Arg, L-arginine-hydrochloride; Orn, L-ornithine-hydrochloride; Lys, L-lysine-hydrochloride; Trp, L-tryptophan; Cit, L-citrulline. Inocula in micrograms of protein per milliliter: Arg-5 = 5.6, Arg-5;Bm-1 = 8.5, Lys-3 = 3.6, Lys-3;Bm-1 = 3.72. Figures in parentheses indicate micrograms of amino acid per milliliter of medium.

TABLE 3. *Effect of neutral and basic amino acids on the growth of neutral amino acid auxotrophs with and without the mtr mutation*

Strain	Growth[a] increase over minimal medium (μg of protein/ml)					
	Trp (10)	Trp (10) Gly (2000)	Trp (10) Cit (3,000)	Trp (50)	Trp (50) Lys (1,000)	Trp (50) Arg (1,000)
Trp-1	41.1	2.0	1.27	55.38	59.88	65.18
	Cit (50)	Cit (50) Gly (1,000)	Cit (50) Trp (1,000)	Cit (50) Lys (1,000)		
Arg-5	66.11	3.07	0	104.4		
Arg-5;Mtr	41.2	0	0	0		

[a] Growth was for 10 hr in liquid culture tubes at 25 C. Trp, L-tryptophan; Gly, glycine. Inocula in micrograms of protein per milliliter: Trp-1, 6.8; Arg-5, 5.74; Arg-5; Mtr, 6.9. Other conditions as in Table 2.

transport systems specific for basic and neutral amino acids. Thus, strains carrying *bm-1* are resistant to L-canavanine merely because this mutation affects the transport of this basic amino acid analogue. Stadler (19) has presented physiological and genetic data indicating that various *mtr* alleles decrease the rate of uptake of neutral amino acids by various degrees. Thwaites (20) and Pall (16) reported similar observations with regard to the transport of basic amino acids by the Bat mutant. To explain the inhibition of uptake of basic amino acids by neutral amino acids associated with *bm-1* and the effect of basic amino acids on the uptake of neutral amino acids associated with *mtr*, it might be assumed that these transport systems compete for some common element. This competition becomes more apparent when one of these transport systems is partially inactive. However, if these mutations completely inactivate the aforementioned transport systems, the existence of a general amino acid permease (*gap*) able to translocate basic and neutral amino acids must be invoked (15). Either alternative explains the growth of the double mutants carrying *arg-5;bm-1* on L-arginine or on L-ornithine, of mutants carrying *lys-3;bm-1* on L-lysine, of mutants carrying *arg-5;mtr* on L-citrulline, and the growth inhibition patterns by the amino acid mixtures described above.

To differentiate between these two hypotheses, the triple mutant carrying *arg-5;bm-1;mtr* was constructed. Since strain Arg-5 is auxotrophic for neutral or basic amino acids, it was possible to test, by using the triple mutant, the effect of neutral amino acids on the growth of basic amino acid auxotrophs and vice versa.

If a generalized amino acid permease exists, one would predict that the *bm-1* mutation should not affect Mtr mutants in their response to basic amino acids. Also, the *mtr* mutation should not affect Bm-1 mutants in their response to neutral amino acids. The reason for this is that neutral and basic amino acids would only compete for *gap* since, in the Bm-1 mutants, the basic amino acid permease is blocked and in the Mtr mutants the neutral amino acid permease is not functional.

Experiments were made to determine the effect of L-tryptophan and L-lysine on the growth response of the various Arg-5 mutants. The mutants Arg-5, Arg-5;Bm-1, and Arg-5;Bm-1;Mtr were grown on L-arginine, L-ornithine, and L-citrulline in the presence of varying concentrations of L-tryptophan or L-lysine. The data of a typical experiment are presented in Fig. 1. It is evident that the triple mutant carrying *arg-5;bm-1;mtr* shows resistance to growth inhibition by the neutral amino acid L-tryptophan and by the basic amino acid L-lysine, intermediate between that exhibited by either one of the double mutants Arg-5;Bm-1 and Arg-5;Mtr, and the single mutant Arg-5.

Since the inhibition pattern in the triple mutant was intermediate between the single and the double mutant, we postulate that the residual transport is due to the general amino acid transport system (15). In addition, we conclude that this system is competing for something that is common with the transport systems specific for neutral and basic amino acids. The presence of this general amino acid transport system predicts that its phenotypic inactivation would prevent the growth of the double mutant Arg-5;Bm-1 on arginine. It also predicts that the mutant Arg-5;Mtr should not grow on citrulline. It was found that spores of the strains Arg-5;Bm-1 and Arg-5;Mtr did not grow on L-arginine (nor L-ornithine) or L-citrulline, respectively, in the presence of ammonia when the temperature was raised from 29 to 42 C. Even at 29 C, glycerol (1.5 M) or previous amino acid starvation of the spore inoculum were growth-inhibitory (Table 4). In the absence of ammonia, the strains grew under the first two conditions but not under the last. These data are consistent with the existence of a general amino acid permease regulated by the amino acid pool, or ammonia, or both.

Transport of ^{14}C amino acids. To ascertain whether the growth data presented above could be explained by amino acid uptake, a series of measurements of amino acid transport were made. Data presented in Fig. 2a show that, in the wild-type strain 74(A), L-arginine uptake was inhibited only 35% by concentrations of L-tryptophan as high as 2.43 mM, a 50-fold excess over the L-arginine concentration used. We interpret the inhibition as being due to competition for *gap;* the remaining L-arginine uptake can be explained by transport via the basic amino acid permease. In the strain with the *bm-1* mutation, however, not only was the rate of L-arginine uptake reduced by 28%, but also 0.243 mM of L-tryptophan (only a fivefold excess) inhibited this uptake by more than 90% (Fig. 2b). This dramatic inhibition by L-tryptophan in the Bm-1 mutant is readily understood if the basic amino acid permease is completely blocked in this mutant, and that, furthermore, L-tryptophan competes with L-arginine for the general permease. The double mutant Bm-1;Mtr

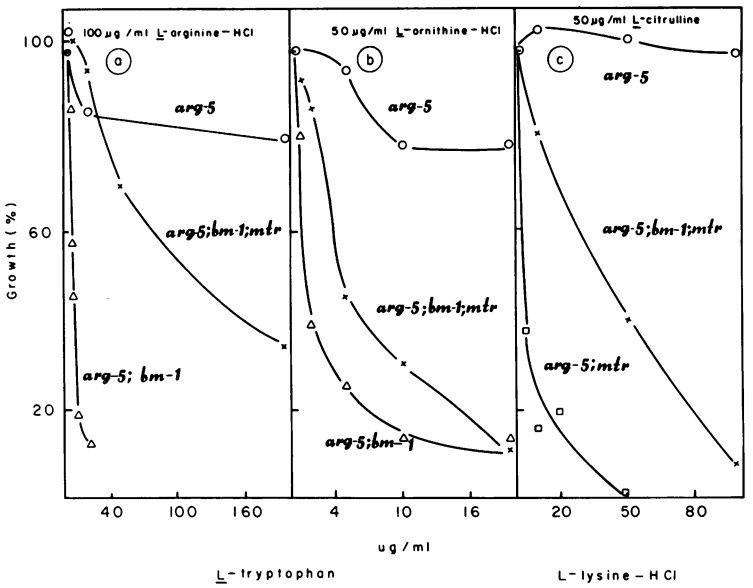

FIG. 1. *Effect of different* L-*tryptophan and* L-*lysine concentrations on the 10-hr liquid medium growth (in micrograms of protein) of mutants of N. crassa in the presence of* L-*arginine-hydrochloride (a),* L-*ornithine-hydrochloride (b), and* L-*citrulline (c).*

TABLE 4. *Effect of temperature, osmolarity, and amino acid starvation on the growth of basic and neutral amino acid auxotrophs with the bm-1 or the mtr mutation, or both*

Conditions	Amino acid	Arg-5		Arg-5; Bm-1		Arg-5; Mtr		Arg-5; Bm-1; Mtr	
		N[a]	W[b]	N	W	N	W	N	W
Control[c]	Arginine	+	+	+	+	+	+	+	+
	Ornithine	+	+	+	+	+	+	+	+
	Citrulline	+	+	+	+	+	+	+	+
Incubation at 42 C	Arginine	+	+	g[e]	+	+	+	g	+
	Ornithine	+	+	−	+	+	+	−	+
	Citrulline	+	+	+	+	g	+	−	+
Glycerol 1.5 M	Arginine	+	+	g	+	+	+	g	+
	Ornithine	+	+	g	+	+	+	g	+
	Citrulline	+	+	+	+	g	+	g	+
Inoculum: amino acid starved[d]	Arginine	+	+	−	−	+	+	−	−
	Ornithine	+	+	−	−	+	+	−	−
	Citrulline	+	+	+	+	−	−	−	−

[a] NH_4NO_3 as nitrogen source.
[b] KNO_3 as nitrogen source.
[c] All plates, except where indicated, were incubated at 29 C in the absence of glycerol and inoculated with spores obtained from 5-day-old slants.
[d] A spore suspension previously shaken for 24 hr in minimal medium at 22 C was used as inoculum.
[e] Spores only germinated; other symbols as in preceding tables.

transports L-arginine at the same rate as the single mutant Bm-1. Furthermore, 0.243 mM L-tryptophan inhibits this transport by only 31% (Fig. 2c). Thus, L-tryptophan competes with L-arginine not only for *gap* but, when being transported by the neutral amino acid permease, it must have competed as well for something in common with *gap*. This competition occurs under conditions in which the energy source in the medium is not limiting; only the presence of the *mtr* mutation reduced the inhibition of L-arginine transport by L-tryptophan. These data are in agreement with the results of the growth experiments presented in Fig. 1. Similar results were obtained when the rate of L-tryptophan uptake was measured in strains 74(A), Mtr, and Bm-1;Mtr in the presence or absence of L-arginine in the medium (Fig. 3). Moreover, in the wild strain 74(A), glycine and L-citrulline were more potent inhibitors of the uptake of L-tryptophan than was L-arginine. However, in the Mtr strain, L-arginine was almost as effective an inhibitor as either glycine of L-citrulline (Fig. 3a and b).

Recently it has been reported (20) that L-citrulline is transported mainly by *gap*. More data about the transport of neutral amino acids was obtained when the uptake of L-citrulline by strain 74(A) was measured. The rate

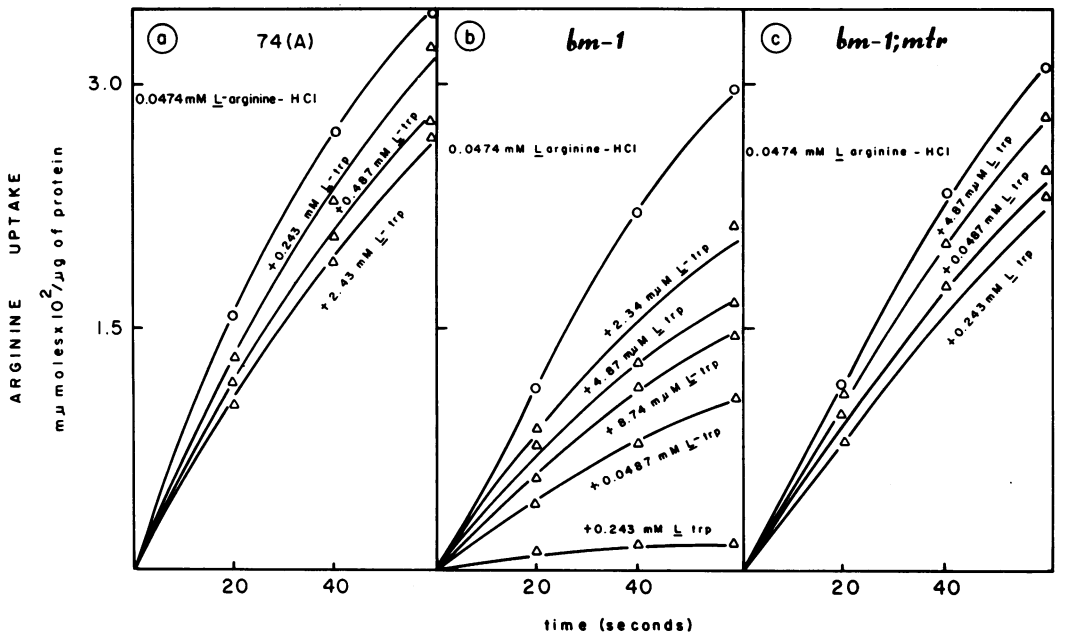

FIG. 2. *Effect of different* L-*tryptophan concentrations on the uptake of* L-*arginine-hydrochloride in strains 74 (A) (a), Bm-1 (b), and Bm-1; Mtr (c). Concentrations: 1.0 nmole* $\times 10^2$ *per μg of protein = 100 nmoles per μg of protein.*

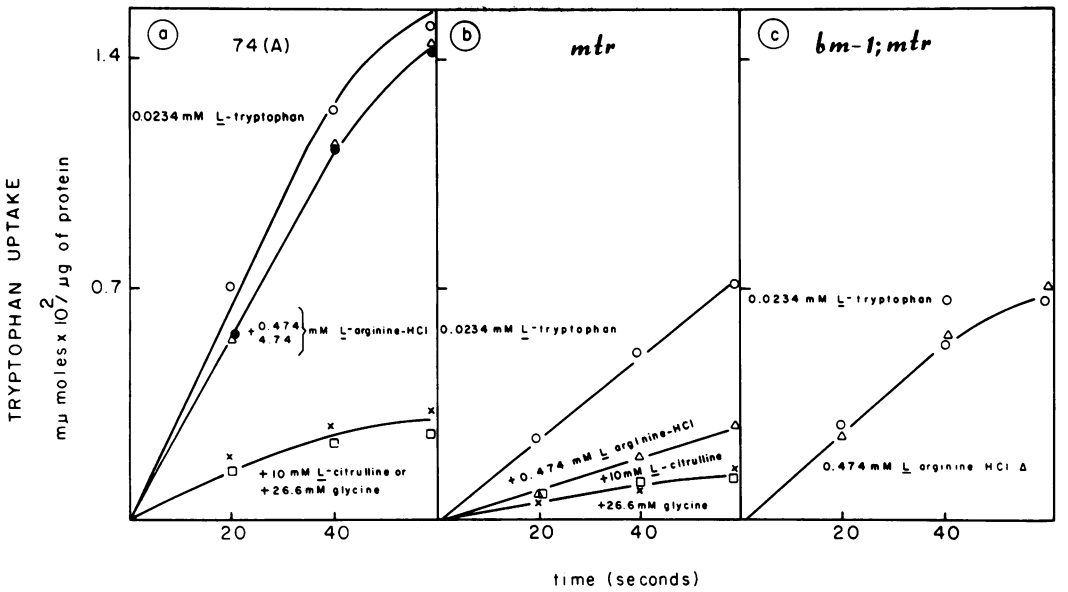

FIG. 3. *Effect on the* L-*tryptophan uptake of* L-*arginine-hydrochloride,* L-*citrulline, and glycine in strains 74(A) (a), Mtr (b), and* L-*arginine-hydrochloride in strain Bm-1;Mtr (c).*

of uptake of this amino acid was inhibited 60% by L-lysine and 85% by glycine (Fig. 4a). However, strains with the *mtr* mutation not only transported L-citrulline at a much reduced rate (55% of wild type), but L-lysine and glycine similarly inhibited this transport by 88% (Fig. 4b). These data indicate that glycine, L-citrulline, and L-tryptophan are transported by both

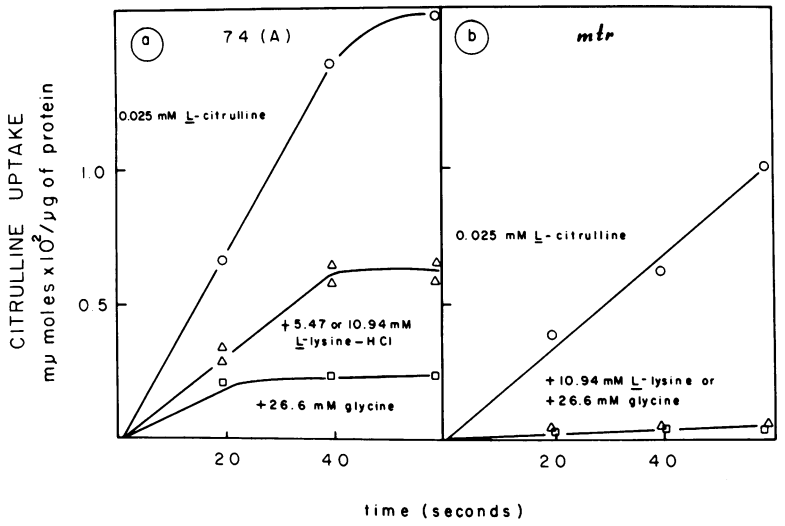

FIG. 4. *Effect of glycine and different* L*-lysine-hydrochloride concentrations on the uptake of* L*-citrulline in strains 74(A) (a) and Mtr (b).*

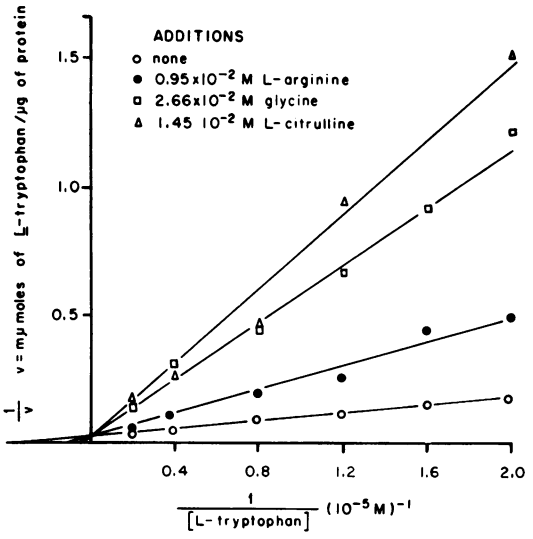

FIG. 5. *Competitive inhibition of* L*-tryptophan transport by* L*-arginine, glycine, and* L*-citrulline. The reciprocal of the initial velocity of* L*-tryptophan transport is plotted versus the reciprocal external* L*-tryptophan concentrations. The strain used was 74(A). 1/v = nanomoles of* L*-tryptophan transported in 30 sec per microgram of protein.*

the neutral and the general amino acid permeases. Furthermore, basic amino acids, such as L-lysine, appear to compete with neutral amino acids such as L-citrulline and L-tryptophan only for *gap*. This interpretation is supported by data showing that glycine, L-citrulline, and L-arginine competitively inhibited the transport of L-tryptophan in the wild strain 74(A) (Fig. 5). The two former amino acids are more effective inhibitors than the latter.

More direct evidence confirming the presence of a general amino acid permease in *N. crassa* was obtained when the rates of uptake of L-tryptophan and L-arginine were measured in the presence of 1.5 M glycerol. The growth data presented in Table 4 suggest that, in the presence of glycerol, a general amino acid permease was inhibited or inactivated. Under the conditions of high osmolarity produced by 1.5 M glycerol, there was a decrease in the rate of uptake of L-tryptophan. No further decrease was observed in the presence of an excess of L-arginine (Fig. 6a). Similar results were observed when the rate of uptake of L-arginine was measured in the presence of glycerol with or without an excess of L-tryptophan (Fig. 6b). However, in the mutant strain Bm-1, glycerol prevented almost completely the residual uptake of L-arginine (Fig. 6c). Similar results were obtained when the transport of L-citrulline and L-tryptophan were measured in the presence of glycerol, except that the decrease in the residual rate of uptake of these amino acids in the Mtr mutant was less spectacular. Lower concentrations of glycerol (1.0 and 0.5 M) had proportionally lower effects on the rates of L-arginine and L-tryptophan transport by mutants Bm-1 and Mtr, respectively.

These results confirm the presence of a general amino acid permease in *N. crassa* (15) and establish its inhibition or inactivation by a high glycerol concentration.

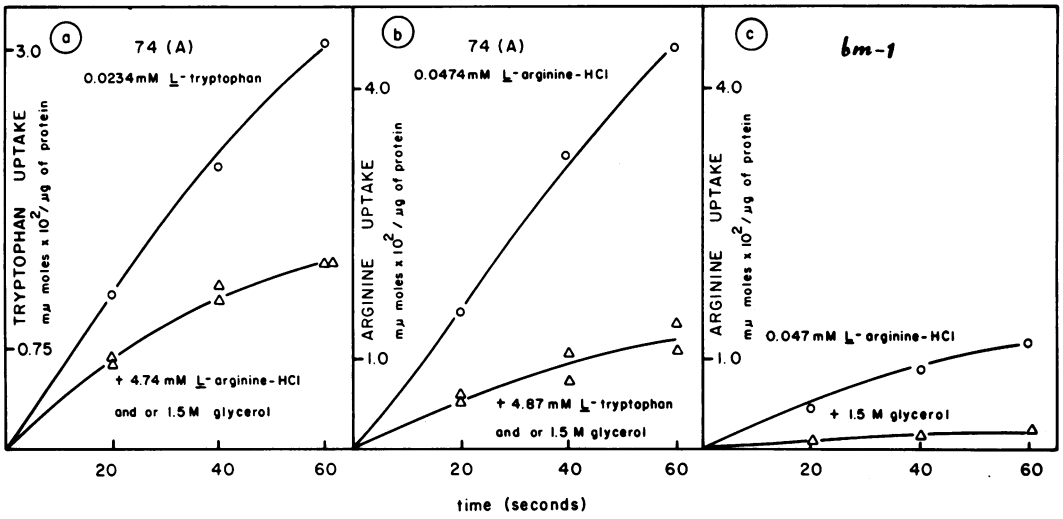

FIG. 6. *Effect of* L-*arginine-hydrochloride or glycerol, or both, on the uptake of* L-*tryptophan in strain 74(A) (a). Effect of* L-*tryptophan or glycerol, or both, on the uptake of* L-*arginine-hydrochloride in strains 74(A) (b) and Bm-1 (c).*

DISCUSSION

Since mutant strains selected as resistant to the basic amino acid analogues, L-canavanine and L-thialysine, lack the transport system specific for basic amino acids, we may conclude that these analogues are transported mainly by this specific system. Recent reports have afforded kinetic (16) and genetic evidence (20) demonstrating the existence of a basic amino acid permease in *Neurospora*.

This study also establishes that L-citrulline, as another neutral amino acid, is transported not only by *gap* (20) but by the neutral permease (15, 19).

The fact that ammonia regulates the activity of the general permease indicates that this transport system may be similar to the general permease that operates in *S. cerevisiae* (8). The general amino acid permease can be an amino-nitrogen permease in the sense that in the absence of ammonia any amino acid, with the possible exception of L-proline (S. Sánchez, and J. Mora, *unpublished data*), competes with others for entrance by this system. When ammonia is present, the activity of this permease is severely reduced and amino acids enter the cell mainly by the more specific amino acid transport systems. This paper presents evidence that the residual activity of *gap* in the presence of ammonia can be eliminated by raising the temperature to 42 C or by adding glycerol to 1.5 M.

Because L-arginine, through the action of arginase (14), is converted to L-ornithine and the latter is an arginine precursor, competition between L-arginine and L-ornithine for a basic amino acid permease in *Neurospora* does not pose a metabolic problem. However, the presence of an excess of any of these amino acids can prevent the utilization of L-lysine or L-arginine from the medium. *S. cerevisiae* has partially solved this problem by having an additional amino acid transport system specific for L-lysine (6). *P. chrysogenum* has completely solved this problem by the operation of a lysine-insensitive arginine transport and an arginine-insensitive lysine transport (9).

To explain the interaction between the neutral and the basic amino acid permeases and *gap*, it is proposed that they compete for some common element, such as an energy-coupling mechanism, e.g., the D-lactic acid dehydrogenase of bacteria. This enzyme has been shown to supply the energy for amino acid transport (11). A similar explanation has been advanced by Grenson (7) to acount for a mutant of *Saccharomyces* that has reduced activities of *gap* and of specific amino acid permeases. Other alternatives as a common binding protein for amino acids with specific translocation factors for each permease seem less likely since Wiley (22) observed a decreased capacity for binding tryptophan in shock fluids from the Mtr mutant.

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