JOURNAL OF BACTERIOLOGY, Apr. 1972, p. 313-320

Vol. 110, No. 1
Printed in U.S.A.

Histidine Uptake in Strains of *Neurospora crassa* with Normal and Mutant Transport Systems

CLINT W. MAGILL, HUGH SWEENEY, AND V. W. WOODWARD

Department of Plant Sciences, Genetics Section, Texas A&M University, College Station, Texas 77843; Department of Agriculture and Fisheries, Leixlip, Co. Kildare, Ireland; and Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55100

Received for publication 22 December 1971

Kinetic parameters for three systems of active histidine uptake by germinated conidia of *Neurospora crassa* have been measured. Each system appears to follow typical Michaelis-Menten kinetics when studied separately from the other systems. Under the conditions studied, the general amino acid transport system was found to account for the major portion of histidine uptake from low concentrations. Three types of transport mutants with altered growth inhibition patterns were selected in a histidine auxotroph. Growth of one mutant, type *bas*^{a}, could be inhibited by the addition of methionine to a histidine-supplemented medium, and another type, *neu*^{a}, could be inhibited by the addition of arginine. These mutants were shown to be lacking active histidine uptake by the basic amino acid and neutral amino acid transport systems, respectively. Another type of double mutant (*his-3,neu*^{r}) could be inhibited only by the addition of very high concentrations of methionine in the presence of arginine and histidine, and the mutation appeared to have altered the specificity of the neutral amino acid permease.

Amino acid uptake in *Neurospora crassa* requires energy and occurs against a concentration gradient (i.e., uptake is active; references 4, 14, and 24). The presence of multiple amino acid-uptake systems in *Neurospora* has been well documented. A number of experimental observations demonstrate that separate transport systems exist for the classes of amino acids based on net charge (i.e., basic, neutral, and acidic). These observations include: (i) mutual antagonisms between members of the same family, such as the inability of arginine auxotrophs to grow when lysine is also present in the growth medium (6); (ii) mutual inhibition of uptake by amino acids within a family (1, 2, 11, 16, 21); and (iii) the demonstration that amino acid analogue-resistant mutants have altered ability to accumulate one or more classes of amino acids.

Among the mutations which lower the rate of uptake of neutral amino acids are the methyl tryptophan-resistant mutation (*mtr*) isolated by Stadler (16; see reference 10) and the *nap* mutation isolated by Jacobson and Metzenberg (8), which is resistant to ethionine and fluorophenylalanine. In contrast, the *mod-5* mutant described by St. Lawrence et al. (14) shows increased sensitivity to fluorophenylalanine and methyl tryptophan, correlated with an increased rate of uptake of aromatic amino acids.

Mutations which cause an alteration in basic amino acid transport specificity include *bat* (18), *hlp-1* (3), and strain *CR-10* which is resistant to the arginine analogue canavanine (13). In addition to the permeases with charge specificity, a general amino acid transport system with very broad substrate specificity has been described in *Neurospora* and found to be present under certain conditions (10).

It was our intent to examine the multiple permease model with respect to a particular amino acid and to determine the relative importance of each system in the transport of this amino acid. The amino acid histidine is uniquely suited for such a study, because it is a member of at least two families under physiological conditions. Since the secondary pK_a of histidine is 6, at high *p*H values it has the characteristics of a neutral amino acid, and, at

low *p*H values, it has a net positive charge.

The fact that histidine has alternate modes of uptake has allowed us to select mutants in the basic and neutral permease systems without the use of analogues. This paper defines the kinetic parameters of histidine transport in the various systems and describes the effects of three new transport mutations.

MATERIALS AND METHODS

Strains. The *N. crassa* strain *his-3; a* (K-57) obtained from D. G. Catcheside, or mutants derived from this strain, were used in all experiments.

Chemicals. Only L-isomers of amino acids were used throughout. Uniformly labeled L-[^{14}C] histidine was obtained from New England Nuclear Corporation. Unlabeled L-amino acids were obtained from Nutritional Biochemical Corporation.

Media. The basic minimal medium was Vogel's medium N (20) supplemented with 1.5% (w/v) sucrose. For conidial production, cultures were grown for 6 days at 30 C in 125-ml flasks containing 20 ml of minimal medium plus 0.1 mg of L-histidine per ml (0.64 mM) and were solidified with 2% agar.

Selection of mutants. Conidia from the *his-3;a* strain were ultraviolet-irradiated in sterile water. To select mutants of the basic amino acid transport system (*bas*^{a} mutants), the treated conidia were placed in a medium containing 0.64 mM L-histidine plus 6.7 mM L-methionine, and growing colonies were filtered off at 6-hr intervals for 48 hr (22). Colonies which then grew on the minimal medium plus histidine were tested for inhibition in the presence of methionine. A similar screening technique was used to select neutral transport (*neu*^{a}) mutants, with 5.7 mM arginine rather than methionine used to inhibit growth. Another type of *his-3* transport double mutant (*his-3,neu*^{r}) was selected for ability to grow on a histidine-supplemented medium also containing arginine and methionine at the previously given concentrations.

Uptake of ^{14}C-labeled histidine. Conidia were collected in a liquid medium supplemented with 0.13 mM L-histidine, and hemacytometer estimates of the concentration were made. After 5 hr of shaking at 30 C to initiate germination, an appropriately sized sample was centrifuged, and the conidia were suspended in minimal medium (*p*H 5.8) to give a concentration of 2×10^6 or 4×10^6 conidia per ml. The conidia were then equilibrated for 2 hr at 30 C with stirring, before uptake studies began. The mutant strains selected for kinetic assays all germinated and grew at the same rate as *his-3;a* in the histidine-supplemented preincubation medium, as determined by dry weight samples.

Experiments designed to measure the time course of uptake were started by adding L-[^{14}C] histidine at the desired concentration. Two-ml samples were collected at varying time intervals on membrane filters (type HAWP, 0.45 μm; Millipore Corp.), washed with 10 ml of ice cold Vogel's medium, and dried overnight before counting. Counts were made in a toluene base scintillation fluid containing Omnifluor (New England Nuclear Corp.).

Experiments to determine initial velocity and kinetic properties were made by preparing reaction tubes containing 2 ml of the liquid medium with double the desired concentration of histidine and inhibitors, which were allowed to equilibrate to 30 C. To initiate the reaction, a 2-ml suspension of conidia was added to give a final concentration of cells equal to 2×10^6/ml. Uptake was stopped, and the samples were rinsed, dried, and counted as before.

RESULTS

Selection of mutant strains. Mutants which have the properties shown in Table 1 were selected as described above. Several independent isolates of the *his-3,neu*^{r} type were found, and, in each case, it was found that growth could be inhibited by increasing the amount of methionine 16- to 80-fold, without changing the arginine concentration. It was observed that 5.4 mM lysine could be substituted for arginine in any of the growth media of Table 1, and the other neutral L-amino acids which were tested (3.8 mM leucine, 2.8 mM tyrosine, 2.4 mM tryptophan, 4.3 mM valine, and 4.8 mM serine) gave results identical to those recorded for methionine. In the case of the *his-3, neu*^{r} mutant, growth could not be inhibited by adding 3.4 mM L-glutamic acid, 2.8 mM L-cysteine, 4.3 mM L-proline, or 1.9 mM adenosine to the test medium containing histidine, arginine, and methionine. Growth of the *his-3,neu*^{r} strain could be prevented however by substituting 3 mM L-phenylalanine for methionine in the test medium.

Other tests with *his-3* and *his-3,neu*^{r} were made using 0.2 mM L-histidine. The addition of 2 mM arginine and methionine to germinated or ungerminated conidia of *his-3,neu*^{r} did not prevent growth, but 20 mM arginine and meth-

TABLE 1. *Growth patterns of wild-type and mutant strains on various media*[a]

Strain	Minimal media supplemented with[b]				
	None	His	His + Met	His + Arg	His + Met + Arg
Wild type	+	+	+	+	+
his-3	−	+	+	+	−
his-3,bas^a	−	+	−	+	−
his-3,neu^a	−	+	+	−	−
his-3,neu^r	−	+	+	+	+

[a] Histidine concentration was 0.64 mM, and arginine and methionine concentrations were 2.9 and 3.4 mM, respectively. Plus indicates growth; minus indicates no growth.

[b] His = histidine; Met = methionine; and Arg = arginine.

ionine did prevent growth. Growth did not occur in the *his-3* strain at either the 2-mM or 20-mM combinations of inhibitor.

Preliminary mapping data suggest that the *bas*^{a} gene is located in linkage group II, and *neu*^{r} in IVR, so perhaps it is allelic to *mtr*. The location of *neu*^{a} has not been determined.

Transport by the general amino acid permease. Uptake at low histidine concentrations was the same in the mutant strains as in *his-3*. This was true both in the time course of uptake and for a number of kinetic parameters which were determined. Initial studies of histidine uptake were made using micromolar concentrations of histidine. Both *his-3* and the transport mutants showed rapid accumulation of 2 μM L-histidine during time course studies. The initial rate of uptake remained linear for only a short time, as is shown for *his-3,bas*^{a} (Fig. 1).

To determine if one-min fixed-time assays could be used to calculate initial velocities from low histidine concentrations, the amount of histidine accumulated in 1 min from 2 μM L-histidine (specific activity = 2) was measured as a function of conidial concentration. It was found to be linear to at least 12×10^6 conidia per ml. This linear relationship was taken to mean that kinetic measurements made at 1 min utilizing 2 μM L-histidine and 2×10^6 conidia per ml are valid estimates of the initial velocity.

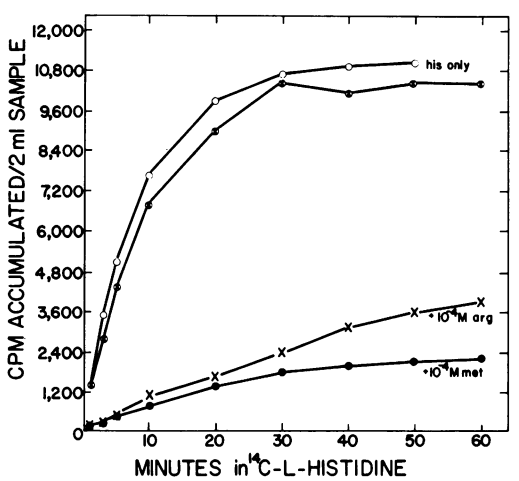

FIG. 1. *Time course of histidine uptake in germinated conidia from the his-3, bas*^{a} *transport mutant in the presence and absence of inhibitors. Conidia were grown in 2 μM L-histidine (2 μCi/μmole). Each point is an average from three repetitions.* (○) *and* (⊗) *histidine only; data from two independent experiments are shown to demonstrate repeatably* (×) *histidine + 0.1 mM L-arginine;* (●) *histidine + 0.1 mM L-methionine.*

A similar relationship was demonstrated for 0.5 mM histidine using 5- and 10-min fixed-time assays.

The rate of histidine uptake was measured over a wide range of substrate concentrations. When data from all concentrations were included in double reciprocal plots, a hyperbolic, rather than linear relationship, was obtained, indicative of the ability of more than one permease system to transport histidine.

Several lines of evidence indicate that uptake at low concentrations (<50 μM) is almost entirely the result of the action of the general amino acid transport system described by Pall, and that this system is not affected by the *neu*^{a}, *neu*^{r}, or *bas*^{a} mutations. Either arginine or methionine was able to inhibit more than 80% of the histidine (2 μM) uptake in all strains (Fig. 2). Lineweaver-Burk plots made over varying concentrations below 50 μM gave typical Michaelis-Menten kinetics and showed that both arginine and methionine were competitive inhibitors (Fig. 3).

Kinetic parameters for the general permease were measured by two methods. To minimize the amount of histidine entering by other systems, inhibition constants (K_i) for methionine and arginine were obtained from Dixon-type plots (5) using 2 and 4 μM histidine. The inhibition constants, calculated by solving for the intersection of regression lines fitted to the data (Fig. 4), are given in Table 2. The apparent K_m for histidine may be determined from these plots by the equation: $K_m = (-K_i [S])/(i + K_i)$, where S is the substrate concentration and i is the intercept on the base line (5). Under the conditions used, i (negative) was very near in absolute value to K_i, with the result that estimates of K_m were often very different in repeated experiments even though the K_i values obtained were nearly identical. For this reason, the apparent affinity constants (K_m) for histidine in Table 2 were calculated from Lineweaver-Burk plots at low histidine concentrations. The estimated V_{max} was 3.6 ($S_{\bar{x}}$ = 1.5) nmoles per sample per min. Estimates of K_m and K_i for the general permease in the mutant strains all fell within the range of observations for *his-3* (Table 2).

Incorporation of accumulated histidine. Because histidine uptake by the general permease was found to be normal in the mutant strains, the possibility that arginine inhibition of the *his-3,neu*^{a} strain or methionine inhibition of the *his-3,bas*^{a} strain might reflect competition for incorporation of histidine into protein was investigated. This was done by growing germinated conidia in 2 μM L-[^{14}C]

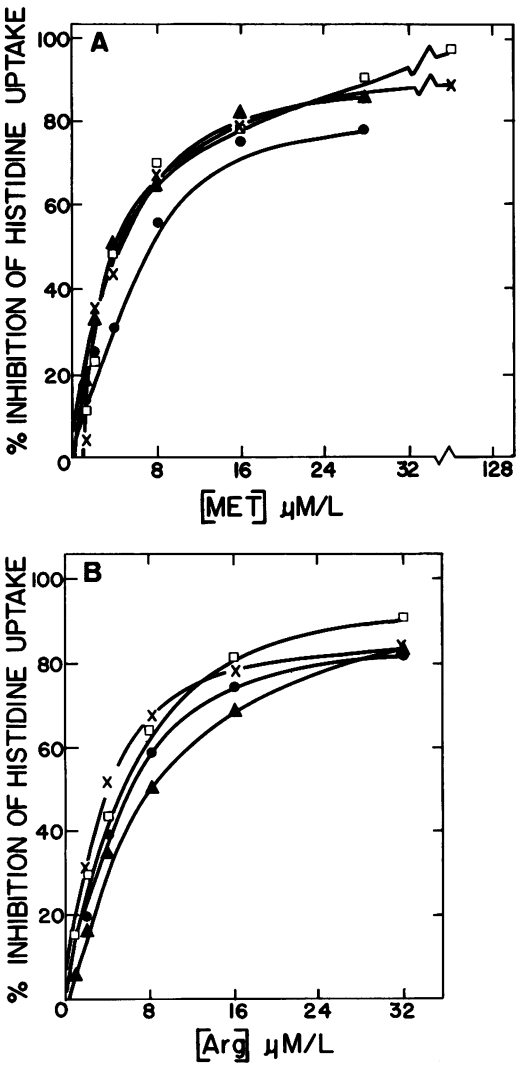

FIG. 2. *Relative inhibition of* L-*histidine uptake in germinated conidia of his-3 and transport mutants. The histidine concentration in all cases was 2 μM, with a specific activity of 2 μCi/μmole, and the incubation time was 1 min. The strains are represented as follows:* (×) *his-3;* (□) *his-3,neu^a;* (●) *his-3,bas^a;* (▲) *his-3,neu^r. In part A, methionine was added as inhibitor; in part B, arginine was added as inhibitor.*

histidine in the presence and absence of inhibitors. Although the inhibitors greatly lowered the initial rate of histidine uptake, the fraction of accumulated histidine which was incorporated into protein after various time intervals was not lowered by the presence of inhibitors in *his-3* or the transport mutants. The data for *his-3, bas^a* are given in Table 3. These data, as well as those for the other strains, show that the ability to utilize accumulated histidine was not altered by the *bas^a*, *neu^a*, or *neu^r* mutations.

Histidine transport by the basic and neutral permeases. As the histidine concentration was increased, the proportion of uptake attributable to the basic and neutral systems rose appreciably. To measure histidine uptake via the basic amino acid uptake system, the general and neutral systems were blocked by the addition of 0.02 M L-methionine. The amount of histidine accumulated per unit of time, in the presence of excess methionine which exceeded the amount taken up when both inhibitors were present, was attributed to the basic amino acid transport system (Fig. 5A). A double reciprocal plot of this informa-

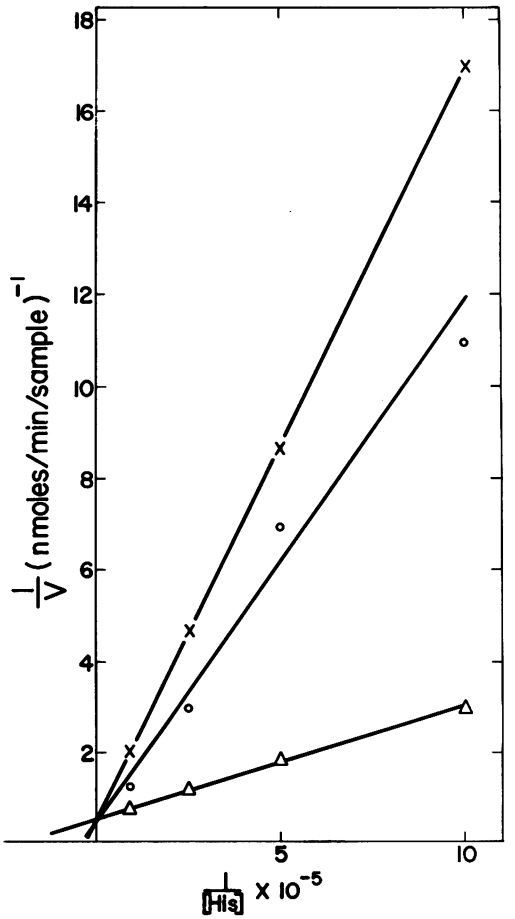

FIG. 3. *Lineweaver-Burke plots of histidine uptake from concentrations less than 50 μM.* (△) *No inhibitors;* (○) *16 μM* L-*methionine,* (×) *16 μM* L-*arginine. V is measured as nanomoles absorbed per minute per sample. Conidia* (2×10^6/*ml*) *were from the his-3;a strain.*

tion for the *his-3* strain gave an apparent K_m of 3.5 mM, and the V_{max} was 30.5 nmoles per sample per min. Likewise, by blocking the basic and general transport systems with 0.02 M arginine, it was possible to measure uptake by the neutral system. The apparent K_m of the neutral amino acid permease of *his-3* was 0.65 mM, and the V_{max} was 12.4 nmoles per sample per min.

Total histidine uptake was somewhat lower in both the *his-3,bas*^{a} and the *his-3,neu*^{a} double-mutant strains as compared to the *his-3* strain with normal transport. In the *his-3,bas*^{a} mutant, histidine uptake was reduced to the background rate by the presence of methionine alone (Fig. 5B). Arginine alone inhibits only that part of the total active uptake of the *his-3,bas*^{a} strain attributable to the general permease. The uptake of histidine by the *his-3,neu*^{a} mutant may be essentially eliminated by arginine, whereas uptake in the presence of methionine is similar to that found in the *his-3* conidia under conditions where only the basic permease is expected to function (Fig. 5C). The apparent K_m (histidine) for the neutral transport system in the *his-3,bas*^{a} strain was 0.8 mM, and the apparent K_m (histidine) for the basic transport system in *his-3,neu*^{a} was 1.3 mM. Both of these measurements agree within the error of measurement for the values obtained with *his-3*.

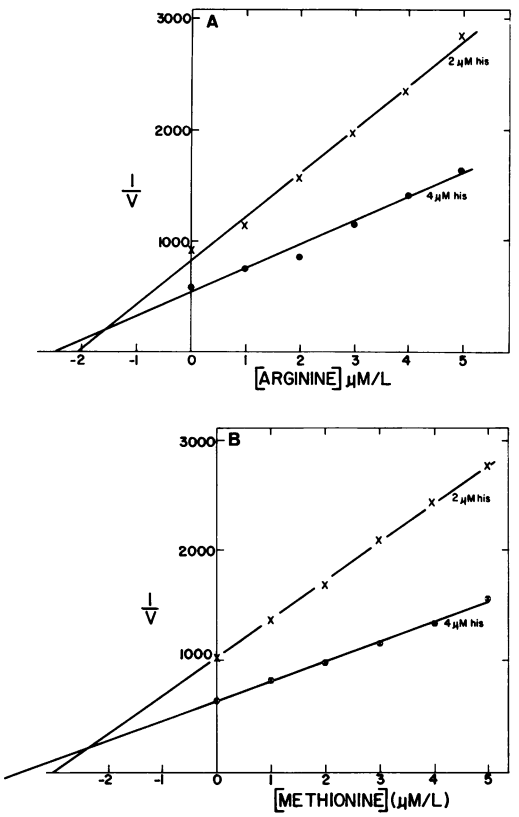

FIG. 4. *Dixon-type plots of the reciprocal velocity of* L*-histidine uptake in the presence of* L*-arginine or* L*-methionine. Velocity (V) is measured as μmoles of* L*-[*^{14}*C]histidine (2 μCi/μmole) accumulated in 2 min by a 4-ml sample containing 2 × 10*^{6} *germinated conidia per ml. All lines shown are fitted by regression analysis. Part A: his-3,neu*^{a}*, arginine as an inhibitor; part B: his-3,neu*^{a}*, methionine as an inhibitor.*

TABLE 2. *Apparent affinity constants (μM) of histidine, methionine, and arginine for the general amino acid permease of Neurospora strains*

Strain	K_m (histidine)[a]	K_i (arginine)[b]	K_i (methionine)[b]
his-3	5.3	3.2	3.0
his-3,neu^a	5.8	2.2	3.1
his-3,bas^a	6.2	4.7	6.0
his-3,neu^r	4.3	5.5	3.6

[a] The K_m for histidine for strain *his-3* is an average of six independent estimates from Lineweaver-Burk plots. All others are estimated from single plots.

[b] The Dixon-plot estimates of K_i for *bas*^{a} and *neu*^{r} are averaged from three experiments, and those for *his-3* and *neu*^{a} are averaged from two.

TABLE 3. *Relative incorporation of 2 μM* L*-[*^{14}*C] histidine into trichloroacetic acid-insoluble counts at various times*

Min in L-[^{14}C] histidine	% Counts per min (insoluble) in trichloroacetic acid[a] when grown in inhibitor % counts per min (insoluble) in trichloroacetic acid when grown without inhibitor		
	20 μM arginine	20 μM methionine	20 μM arginine + 20 μM methionine
15	1.0	1.0	1.3
30	0.9	1.0	0.89
60	1.2	1.5	1.1
120	0.73	1.3	0.72
240	1.1	1.0	1.1

[a] As compared to the total counts/min accumulated in identical samples. Trichloroacetic acid samples were precipitated for 20 min in ice cold 5% trichloroacetic acid.

Stadler found that mutants lacking a phenylalanine transport system could be suppressed by a second mutation which allowed phenylalanine to be transported via the basic transport system. No evidence for a new mechanism for histidine uptake was found in the *his-3,neu*^{r} mutant. Tests for growth inhibition

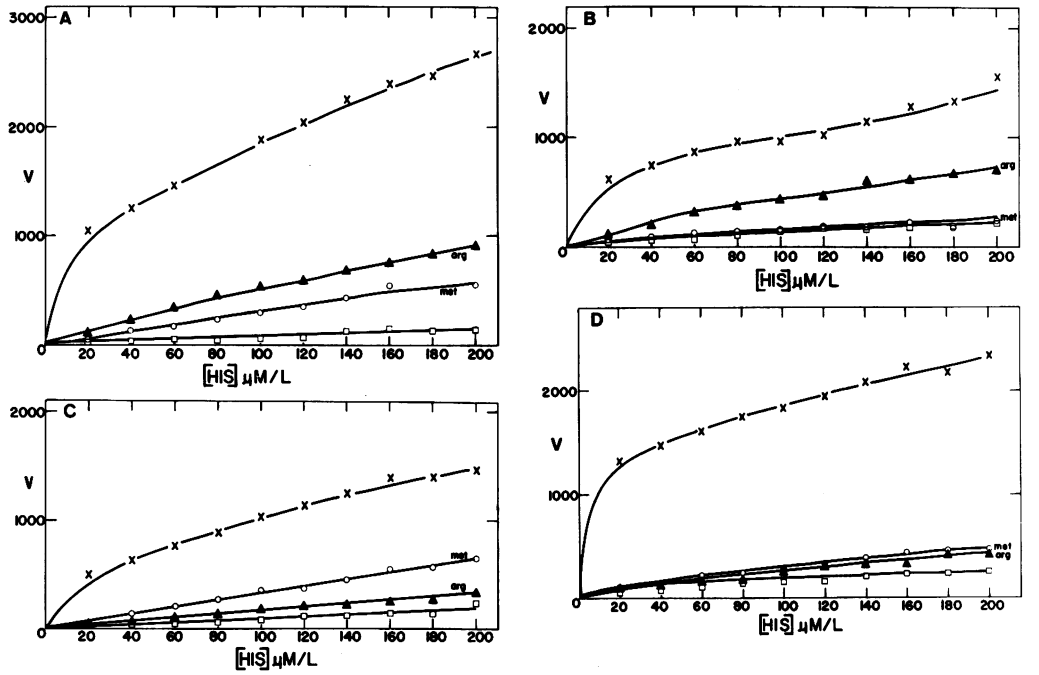

FIG. 5. *Initial velocity of histidine uptake by germinated conidia of his-3 and three transport mutants in the presence and absence of inhibitors. Velocity (V) is measured as counts/min accumulated in 5 min by 8 × 10^6 conidia in a 4-ml suspension.* (×) *histidine only;* (▲) *histidine + 0.02 M arginine;* (○) *histidine + 0.02 M methionine;* (□) *histidine + 0.02 M arginine + 0.02 M methionine. A, his-3; B, his-3, bas[a]; C, his-3, neu[a]; D, his-3, neu[r].*

by adding excess amounts of compounds known to have uptake systems, including acidic amino acids (11), nucleosides (15), and proline (24), gave no evidence that these systems might now accept histidine.

In the *his-3, neu[r]* strain, histidine uptake was lowered to nearly the same extent by 20 mM arginine or methionine as by a combination of the two inhibitors (Fig. 5D). At 2 mM concentrations, methionine exhibited a much lower degree of inhibition of uptake than arginine (Fig. 6). At both the 2- and 20-mM concentrations, arginine appeared to be a slightly more effective inhibitor of histidine uptake in *his-3, neu[r]* than in *his-3* (Fig. 5A, 5D, and 6). These data suggest that arginine is still effective in inhibiting uptake by the general and basic permeases but that 2 mM methionine does not effectively inhibit the neutral permease. The low rate of histidine uptake in the presence of arginine indicates that the neutral permease in the *his-3, neu[r]* strain may also have a slightly lowered capacity for histidine transport. Due to the incomplete inhibition of histidine uptake by a combination of methionine and arginine in the *his-3, neu[r]* mutant, it was not possible to make estimates of the K_m for histidine of the neutral and basic permeases with the available data.

DISCUSSION

When Haas et al. (7) first observed that histidine auxotrophs could not grow with both excess neutral and basic amino acids and when Mathieson and Catcheside (9) showed growth inhibition to be related to the ability to assimilate histidine, a two-site model for histidine uptake evolved. Discovery of mutants which could be blocked by arginine or methionine alone (23) gave apparent support for this two-site model. More recently, Pall (10) described a general amino acid uptake system present under certain conditions which has affinity for nearly all α-amino acids. This system was found to play a large role in histidine uptake in germinated conidia. Thus the total rate of active histidine uptake by germinated conidia is a sum of contributions from the general, basic, and neutral amino acid entry systems. Each of these transport mechanisms appears to follow Michaelis-Menten kinetics, giving a total rate of uptake of V (nanomoles per 8 ×

10^6 conidia per minute) = (3.6 [His]/[His] + 5.6 μM) (general) plus 30 [His]/([His] + 3.5 mM) (basic) plus (12.4 [His])/([His] + 0.65 mM) (neutral).

As can be verified by differentiation, no maxima or minima exist for positive values of histidine concentration [His] other than ∞, and there is no point of inflection. Thus, the additive model for velocity versus substrate concentration plots in which each component obeys Michaelis-Menten kinetics produces a smooth hyperbolic curve with no intermediary plateaus (17). The substrate saturation curves for *his-3* and the transport mutants were consistent with this model.

Both the observations on growth inhibition and the complete inhibition of histidine uptake in the presence of methionine indicate that the *bas*^{a} mutation eliminates the permease which normally functions for basic amino acids. Thus in the *his-3,bas*^{a} strain, only the general and neutral transport systems are available for histidine transport, and both of these systems can be inhibited by methionine or other neutral amino acids. Similarly the *neu*^{a} mutation is lacking a functional neutral permease, so that growth and histidine uptake can be prevented by competitive inhibition of the general and basic transport systems with lysine or arginine.

The effect of the *neu*^{r} mutation is not so obvious. Perhaps the simplest explanation of the data is that *neu*^{r} alters the specificity of the neutral amino acid permease. An affinity for histidine and possibly phenylalanine is retained, but other neutral amino acids are not as effective competitive inhibitors in the mutant as in wild type. Though less probable, it is also possible that the mutation could have increased the affinity for histidine, which would have the same effect. Another possibility for increasing histidine uptake by the neutral system despite the presence of inhibitors is a control type mutation causing overproduction of the neutral permease. In either of the latter cases, uptake in the presence of arginine would be expected to increase, rather than decrease as was observed.

The high level of activity of the general permease in germinated conidia was not predicted. Pall (10) reported little or no activity in young cultures, but found high activity of the general permease in older nitrogen-starved mycelium. He proposed that this permease serves a scavenger function for nitrogen. The conidia in our experiments were rapidly growing after 5 hr of germination. They were,

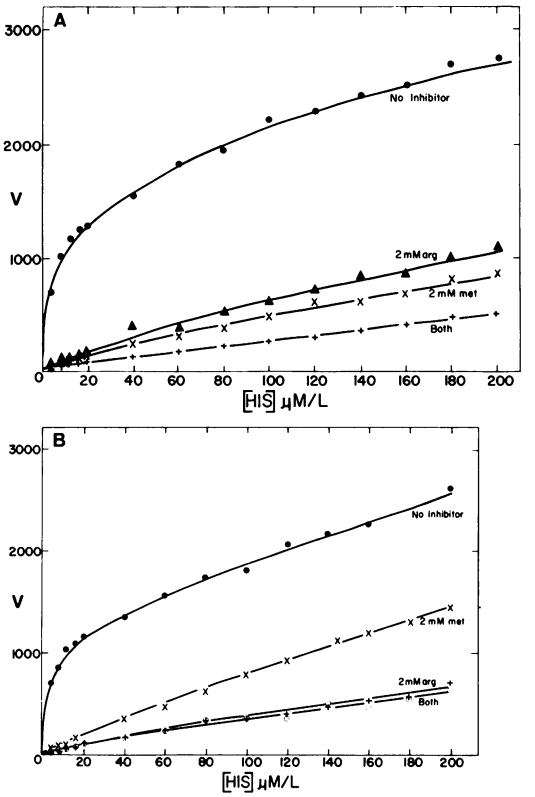

FIG. 6. *Initial velocity of histidine uptake by his-3- and neu*^{r}*-germinated conidia. Velocity is measured as counts/min accumulated in 5 min by 8 × 10*^{6} *conidia in a 4-ml suspension. Inhibitors present at 2 mM concentrations are indicated on the appropriate lines. A, his-3; B, his-3,neu*^{r}.

however, starved for histidine (nitrogen present) for 2 hr before uptake studies began, so perhaps lack of an essential amino acid simulates starvation conditions. However, Tisdale and Debusk (19) have recently reported a rapid increase in amino acid transport, especially of the general transport system prior to visible germination. It would thus seem that the general transport system is present in germinated conidia.

The large contribution to uptake by the general uptake system as compared to the basic and neutral systems may account for the lack of histidine transport noted by Thwaites and Pendyala (18) while measuring a system specific for basic amino acids.

The presence of the general permease in germinated conidia also raises an interesting question: how do neutral and basic transport mutants survive in the presence of analogues?

There is of course the possibility that the general system alone could not transport sufficient analogues to give lethal effects. It is also possible that molecules transported by different systems are used differentially (e.g., amino acids transported by the general system may be deaminated to provide nitrogen rather than used directly in protein synthesis). Our results indicated, however, that the accumulated histidine was efficiently incorporated into protein. Another possibility is that the presence of analogues suppress the formation of the general permease. Wiley and Matchett (21) observed that preloading with tryptophan decreased the rate of tryptophan uptake and Pall and Kelly (12) found that the inhibition extended to other members of the same family of amino acids. Tisdale and DeBusk (19) found that incubation in the presence of amino acids lowered the rate of uptake. The ideal way to study the control and properties of the general transport system is in a strain lacking the other permeases. To date, our efforts to construct such a strain through recombination have not been successful. This may be a result of low viability, because Tisdale and DeBusk (19) have reported finding such a strain. In our crosses, the transport double mutant has the genotype *his-3*,*bas*^{a},*neu*^{a}, which may be an indication the general permease does not function in ascopores to fulfill the histidine requirement.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant 1 F2-GM-39, 299-01 from the National Institute of General Medical Sciences and by Texas A&M Research Council Grant 15428.

LITERATURE CITED

1. Baurle, R. H., and H. R. Garner. 1964. The assimilation of arginine and lysine in canavanine resistant and sensitive strains of *Neurospora crassa*. Biochim. Biophys. Acta **93**:316-322.
2. Brockman, H. E., A. G. DeBusk, and R. P. Wagner. 1959. Mutant-associated antagonisms in *Neurospora crassa*. Arch. Biochem. Biophys. **84**:455-461.
3. Choke, H. K. 1969. Mutants of *Neurospora* permeable to histidinol. Genetics **62**:725-733.
4. DeBusk, B. G., and A. G. DeBusk. 1965. Molecular transport in *Neurospora crassa*. I. Biochemical properties of a phenylalanine permease. Biochim. Biophys. Acta **104**:139-150.
5. Dixon, M., and E. C. Webb. 1964. Enzymes, 2nd ed. Academic Press Inc., New York.
6. Doermann, A. H. 1944. A lysineless mutant of *Neurospora* and its inhibition by arginine. Arch. Biochem. **5**:373-384.
7. Haas, F., M. B. Mitchell, B. N. Ames, and H. K. Mitchell. 1952. A series of histidineless mutants of *Neurospora crassa* whose growth is inhibited by a combination of neutral and basic amino acids. Genetics **37**:217-226.
8. Jacobson, E. S., and R. L. Metzenberg. 1968. A new gene which affects uptake of neutral and acidic amino acids in *Neurospora crassa*. Biochim. Biophys. Acta **156**:140-147.
9. Mathieson, M. J., and D. G. Catcheside. 1955. Inhibition of histidine uptake in *Neurospora crassa*. J. Gen. Microbiol. **13**:72-83.
10. Pall, M. L. 1969. Amino acid transport in *Neurospora crassa*. I. Properties of two amino acid transport systems. Biochim. Biophys. Acta **173**:113-127.
11. Pall, M. L. 1970. Amino acid transport in *Neurospora crassa*. III. Acidic amino acid transport. Biochim. Biophys. Acta **211**:513-520.
12. Pall, M. L., and K. A. Kelley. 1971. Specificity of transinhibition of amino acid transport in *Neurospora*. Biochem. Biophys. Res. Commun. **42**:940-947.
13. Roess, W. B., and A. G. DeBusk. 1968. Properties of a basic amino acid permease in *Neurospora crassa*. J. Gen. Microbiol. **52**:421-432.
14. St. Lawrence, P., B. D. Maling, L. Altberger, and M. Rachmeler. 1964. Mutational alteration of permeability of *Neurospora*: effects on growth and the uptake of certain amino acids and related compounds. Genetics **50**:1383-1402.
15. Schlitz, J. R., and K. D. Terry. 1970. Nucleoside uptake during the germination of *Neurospora crassa* conidia. Biochim. Biophys. Acta **209**:278-288.
16. Stadler, D. R. 1966. Genetic control of the uptake of amino acids in *Neurospora*. Genetics **54**:677-685.
17. Teipel, J., and D. E. Koshland, Jr. 1969. The significance of intermediary plateau regions in enzyme saturation curves. Biochemistry **8**:4656-4663.
18. Thwaites, W. M., and L. Pendyala. 1969. Regulation of amino acid assimilation in a strain of *Neurospora crassa* lacking basic amino acid transport. Biochim. Biophys. Acta **192**:455-461.
19. Tisdale, J. H., and A. G. DeBusk. 1970. Developmental regulation of amino acid transport in *Neurospora crassa*. J. Bacteriol. **104**:689-697.
20. Vogel, J. 1956. A convenient growth medium for *Neurospora* (Medium N). Microbial Genet. Bull. **13**:42-43.
21. Wiley, W. R., and W. H. Matchett. 1966. Tryptophan transport in *Neurospora crassa*. I. Specificity and kinetics. J. Bacteriol. **92**:1698-1705.
22. Woodward, V. W., J. R. DeZeeuu, and A. M. Srb. 1954. The separation and isolation of particular biochemical mutants of *Neurospora* by differential germination of conidia, followed by filtration and selective plating. Proc. Nat. Acad. Sci. U.S.A. **40**:192-200.
23. Woodward, C. K., C. P. Read, and V. W. Woodward. 1967. Neurospora mutants defective in their transport of amino acids. Genetics **56**:598.
24. Zalokar, M. 1961. Kinetics of amino acid uptake and protein synthesis in Neurospora. Biochim. Biophys. Acta **46**:423-432.