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Regulation of Lysine Transport by Feedback Inhibition in *Saccharomyces cerevisiae*

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A steady-state level of about 240 nmol/mg (dry wt) occurs during lysine transport in *Saccharomyces cerevisiae*. No subsequent efflux of the accumulated amino acid was detected. Two transport systems mediate lysine transport, a high-affinity, lysine-specific system and an arginine-lysine system for which lysine exhibits a lower affinity. Preloading with lysine, arginine, glutamic acid, or aspartic acid inhibited lysine transport activity; preloading with glutamine, glycine, methionine, phenylalanine, or valine had little effect; however, preloading with histidine stimulated lysine transport activity. These preloading effects correlated with fluctuations in the intracellular lysine and/or arginine pool: lysine transport activity was inhibited when increases in the lysine and/or arginine pool occurred and was stimulated when decreases in the lysine and/or arginine pool occurred. After addition of lysine to a growing culture, lysine transport activity was inhibited more than threefold in one-third of the doubling time of the culture. These results indicate that the lysine-specific and arginine-lysine transport systems are regulated by feedback inhibition that may be mediated by intracellular lysine and arginine.

Regulatory mechanisms of amino acid transport systems have been examined in several different species of fungi. In *Neurospora crassa* the neutral and basic amino acid transport systems are subject to feedback inhibition (26), whereas the methionine transport system is subject to induction by sulfur starvation as well as feedback inhibition (25). The acidic and general systems also appear to be regulated since they exhibit highest activity during carbon and/or nitrogen starvation (23, 24). Similarly, the cystine (34), acidic (15), and general (1, 16) amino acid transport systems of *Penicillium chrysogenum* are subject to feedback inhibition. In addition, a proline transport system is induced by carbon or nitrogen starvation (15), and a methionine system is induced during sulfur starvation (1). Amino acid transport is also subject to feedback inhibition in *Streptomyces hydrogenans* (30, 31), and the arginine-lysine transport system is induced during growth on the nitrogen source arginine or lysine (14). The acidic amino acid transport system of *Aspergillus nidulans* is apparently regulated by carbon catabolite repression (17, 27) as well as by nitrogen catabolite repression and/or inhibition (27, 28, 32). Proline transport in *Saccharomyces chevalieri* is subject to derepression by nitrogen starvation (20, 33).

In addition to specific transport systems for arginine (13), methionine (9), lysine (10), and histidine (5), *S. cerevisiae* possesses a general amino acid transport system for basic and neutral amino acids (12). The general system is inactive in the presence of ammonium ion (11, 12). As in *A. nidulans*, this ammonium ion effect is apparently part of the comprehensive regulation of various enzymes involved in nitrogen metabolism (6, 7, 8). The two histidine transport systems are regulated by feedback inhibition (5). Since methionine transport decays more rapidly during cycloheximide treatment than during methionine starvation, the methionine transport system may also be subject to feedback inhibition (21).

This study was undertaken to examine the regulation of lysine transport in *S. cerevisiae*. Evidence is presented showing that lysine transport is mediated by two transport systems and that these systems are regulated by feedback inhibition.

MATERIALS AND METHODS

Organisms, media, and growth conditions. *S. cerevisiae* ATCC 9896 was used in this study. Cells were grown in a medium containing per liter: 16 g of glucose, 0.16 g of DL-aspartic acid, 2.4 g of $(NH_4)_2SO_4$, 1.6 g of KH_2PO_4, 0.20 g of $CaCl_2$, 0.20 g of $MgSO_4 \cdot 7H_2O$, 0.80 mg of H_3BO_3, 0.80 mg of $ZnSO_4 \cdot 7H_2O$, 0.80 mg of $MnCl_2 \cdot 4H_2O$, 0.40 mg of $FeCl_3 \cdot 6H_2O$, 80 μg of $CuSO_4 \cdot 5H_2O$, 80 μg of KI, 2.4 mg of inositol, 0.80 mg of β-alanine, 80 μg of nicotinic acid, 16 μg of pyridoxine hydrochloride, 16 μg

of thiamine hydrochloride, 16 μg of potassium p-aminobenzoate, 0.25 μg of biotin, and sufficient 6 N NaOH to bring the pH to 4.0 (medium S). The medium was altered by omission of aspartic acid and/or supplementation with other amino acids where specified. Cultures were grown at 30 C with reciprocal shaking, and turbidity was monitored with a Klett-Summerson colorimeter (filter no. 42). *Saccharomyces* strain AB9 *(lys5)* (2) was kindly provided by J. K. Bhattacharjee, Miami University, Oxford, Ohio. Cells were grown in medium S supplemented with 0.2 mM lysine. *Enterobacter aerogenes* LC1 (18), an arginine auxotroph, was obtained from Michael Carsiotis, University of Cincinnati. Cells were grown in Vogel medium E (36) supplemented with 0.4% glucose and 0.02 mM arginine.

Chemicals. L-[U-^{14}C]lysine monohydrochloride (10 mCi/mmol) and L-[U-^{14}C]arginine monohydrochloride (10 mCi/mmol) were purchased from Amersham/Searle. Unlabeled amino acids were obtained from Calbiochem. Optically active amino acids were in the L form unless otherwise specified.

Transport assays. Midexponential-phase cultures of *S. cerevisiae* ATCC 9896 (about 125 Klett units) were harvested on nitrocellulose filters (0.65 μm, Millipore), washed twice with water at room temperature, and suspended in a solution of 1.0% glucose, 18 mM ammonium sulfate, and 50 mM potassium phosphate at pH 4 (solution A). During specificity studies and kinetic analyses, cell suspensions were stored in an ice-water bath for up to 75 min before use. Such treatment had no effect on lysine transport activity.

Uptake was initiated by rapid addition of a portion of cell suspension preincubated at 30 C for 5 min to an equal volume of 30 C solution A supplemented with radioactive amino acid and other ingredients where indicated. The initial cell concentration was about 0.8 mg (dry wt) per ml. Incubation at 30 C and reciprocal shaking were continued throughout the assay. At various time intervals, 1-ml samples were removed, filtered on 25-mm nitrocellulose filters (0.65 μm, Millipore), and immediately washed twice, each time with about 10 ml of water at room temperature. Filters were counted in a Packard Tri-Carb liquid scintillation spectrometer in vials containing 15 ml of Bray's solution (4). Subtracted control values of radioactivity were determined by omitting cells from the assay. The quantity of cells in the sample was estimated by employing a previously determined standard curve relating turbidity and dry weight. Uptake is expressed as nanomoles per milligram (dry weight).

For time course uptake experiments, cells were incubated in a final volume of 36 ml initially containing 2 mM radioactive substrate. When the time course was followed simultaneously by radioactive and microbiological assay, the final volume was increased to 128 ml. Lysine and arginine transport remain linear for at least 5 min. In efflux studies, the cells were filtered after 120 min of incubation and suspended in an equal volume of solution A supplemented with ingredients where indicated. Incubation and sampling were then continued for an additional 60 min.

For initial velocity measurements, cells were incubated in a final volume of 6 ml, and samples were withdrawn every 30 s for 2 min. The initial extracellular concentration of radioactive substrate was 20 μM except in kinetic analyses. From the resulting linear uptake curve, the slope of the line of best fit was calculated to obtain the initial velocity expressed as nanomoles per milligram (dry weight) per minute.

For preloading studies, harvested cells were suspended to about 0.8 mg (dry wt) per ml in solution A supplemented with 2 mM of the designated amino acid and incubated at 30 C with reciprocal shaking. A sample was removed after 60 min, filtered, washed with water, resuspended in 30 C solution A, and immediately assayed for the initial velocity of amino acid uptake as described above. Control values were obtained by assaying cells resuspended in unsupplemented solution A and similarly incubated.

Time course for inhibition. Lysine transport activity of an early exponential-phase culture of *S. cerevisiae* ATCC 9896 growing in unsupplemented medium S was monitored by removing a portion of the culture. These cells were filtered, washed, suspended in 30 C solution A, and immediately assayed for the initial velocity of lysine uptake. The culture was then filtered (0.45-μm Nalgene filter unit) and the cells were immediately suspended in an equal volume of fresh medium S supplemented with 1 mM lysine. Incubation of the culture and assay of lysine transport activity at intervals was continued as before.

Preparation of samples for microbiological assay. A portion of a suspension of *S. cerevisiae* ATCC 9896 was filtered (0.65 μm, Millipore) and washed with water at room temperature. These cells were suspended in 2 ml of water, incubated in a boiling-water bath for 10 min, and then cooled. Cell debris was removed by filtration (0.45-μm Millex filter unit, Millipore) and the filtrate was retained for assay.

Microbiological assay of lysine. Assay medium consisted of medium S supplemented with 1.0 mM glutamic acid and 0.5 mM each alanine, arginine, asparagine, aspartic acid, citrulline, cysteine, glutamine, glycine, histidine, DL-isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. Assay medium inoculated with *Saccharomyces* strain AB9 was added to samples and to suitable concentrations of lysine. After incubation at 30 C under stationary conditions, turbidities were measured with a Klett-Summerson colorimeter (filter no. 42). The quantity of lysine in each sample was determined by employing a standard curve relating turbidity and lysine concentration.

Microbiological assay of arginine. Assay medium consisting of Vogel medium E (36) supplemented with 0.4% glucose was added to samples and to suitable concentrations of arginine. After inoculation with *E. aerogenes* LC1 and incubation at 37 C with reciprocal shaking, turbidities were measured with a Klett-Summerson colorimeter (filter no. 42). The quantity of arginine in each sample was determined by employing a standard curve relating turbidity and arginine concentration.

RESULTS

Accumulation of lysine to a steady-state level. The time course of lysine uptake is depicted in Fig. 1. Within 120 min, [^{14}C]lysine was accumulated to a steady-state level of about 240 nmol/mg (dry wt), representing 10% of the available substrate. Results obtained by microbiological assay correlate well with the radioactive assay data, indicating that the determination of lysine uptake by use of [^{14}C]lysine is a valid method for the assay of lysine transport. The slight discrepancy between these assay methods is probably attributable to the initial intracellular lysine pool. Even in the presence of 20 mM unlabeled lysine, no efflux of accumulated lysine occurred when the radioactive substrate was removed (Fig. 2).

Specificity of lysine transport. A large variety of amino acids were tested for their effect on lysine transport. Only arginine had a substantial effect: when the ratio of arginine to lysine was 100:1, arginine caused a 62% inhibition of lysine transport activity (Table 1). Further information on the specificity of the transport system(s) involved in lysine transport was obtained from kinetic analyses.

Kinetics of lysine transport. The discontinuous Lineweaver-Burk plot shown in Fig. 3 suggests that a single transport system is not responsible for the transport of lysine. Assuming two saturable transport systems, kinetic constants were calculated by successive approximation (22): the higher-affinity component has a K_m of about 12 μM, and the lower-affinity component has a K_m of about 134 μM. Calculated initial velocities obtained with these estimated kinetic constants agree with the experimentally obtained values.

Arginine had no effect on the high-affinity

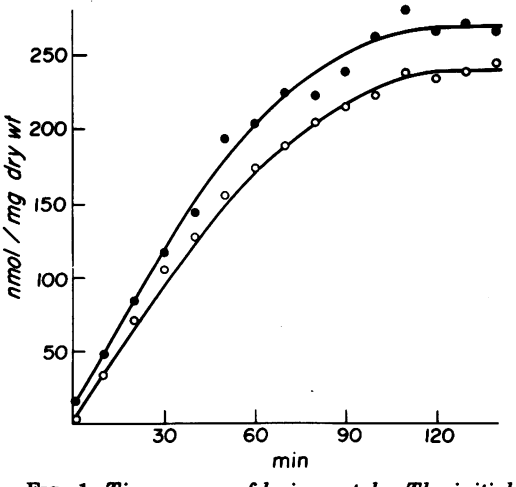

FIG. 1. *Time course of lysine uptake. The initial extracellular [^{14}C]lysine concentration was 2 mM. One set of samples was assayed microbiologically (●); the other set of samples was assayed for radioactivity (○).*

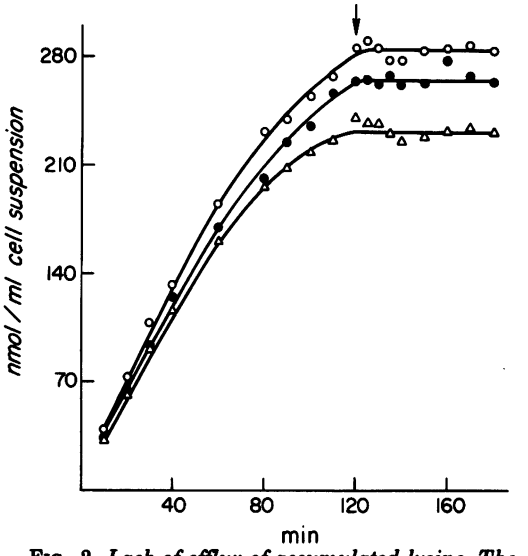

FIG. 2. *Lack of efflux of accumulated lysine. The initial extracellular [^{14}C]lysine concentration of each suspension was 2 mM. At the time indicated by the arrow, the [^{14}C]lysine solutions were replaced by a solution containing no lysine (●), 2 mM unlabeled lysine (△), or 20 mM unlabeled lysine (○).*

TABLE 1. *Lysine transport in the presence of various amino acids*

Amino acid added	% Initial velocity without added amino acid (added amino acid/lysine)		
	1	10	100
Alanine	106	108	103
Arginine	96	66	38
Asparagine	99	103	102
Aspartic acid	97	93	81
Citrulline	96	94	87
Cysteine	99	99	72
Glutamic acid	94	97	89
Glutamine	99	99	104
Glycine	102	110	86
Histidine	95	97	90
Isoleucine	96	94	86
Leucine	102	94	92
Methionine	98	92	84
Ornithine	101	109	72
Phenylalanine	96	104	90
Proline	116	104	112
Serine	97	96	98
Threonine	94	97	93
Tryptophan	97	98	85
Tyrosine	100	105	114
Valine	101	101	85

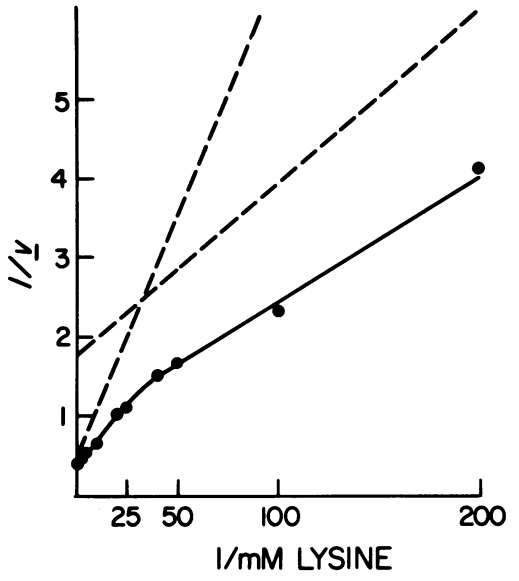

FIG. 3. *Lineweaver-Burk plot of lysine uptake by S. cerevisiae ATCC 9896. Initial velocities are expressed as nanomoles per milligram (dry weight) per minute. The dotted lines represent the individual components of lysine uptake derived by successive approximation.*

component of lysine transport: initial velocities obtained with low substrate concentrations in the presence of 2 mM arginine are equivalent to initial velocities calculated for the high-affinity component (Fig. 4). However, arginine was a strong competitive inhibitor of the low-affinity component of lysine uptake with an estimated K_i of 65 μM (Fig. 5). Based on these findings and the results illustrated in Table 1, we concluded provisionally that the high-affinity component represented a lysine-specific transport system, and the low-affinity component represented an arginine-lysine transport system.

Kinetics of arginine transport. A Lineweaver-Burk plot of arginine transport is linear over the range 5 to 200 μM, indicating a single, saturable transport process (Fig. 6). Arginine exhibits a K_m of 38 μM for this system, which approximates the arginine K_i for the low-affinity component of lysine transport. Furthermore, lysine is a competitive inhibitor of arginine transport (Fig. 7). These data support the contention that lysine is transported by both an arginine-lysine transport system and a higher-affinity, lysine-specific system.

Since the estimated kinetic constants of lysine for the lysine-specific and arginine-lysine transport systems are not widely separated, both systems contribute substantially to lysine transport activity. For example, at a substrate concentration of 20 μM, the lysine-specific system would contribute 55% and the arginine-lysine system would contribute 45% of the expected lysine transport activity. The impact of the arginine-lysine system even at low lysine concentrations is illustrated in Fig. 3 by the substantial difference between the experimentally obtained initial velocities and the derived values for the lysine-specific system.

Effect of growth in the presence of various amino acids on lysine transport. As an initial attempt to examine the regulation of lysine transport, we determined the effect of growth in

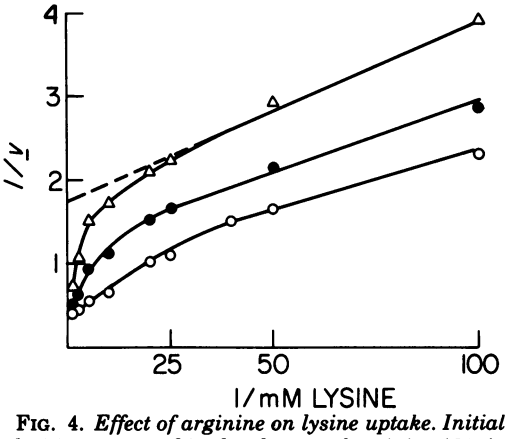

FIG. 4. *Effect of arginine on lysine uptake. Initial velocities measured in the absence of arginine (○), in the presence of 0.2 mM arginine (●), and in the presence of 2 mM arginine (△) are expressed as nanomoles per milligram (dry weight) per minute. The dotted line represents the high-affinity component of lysine uptake.*

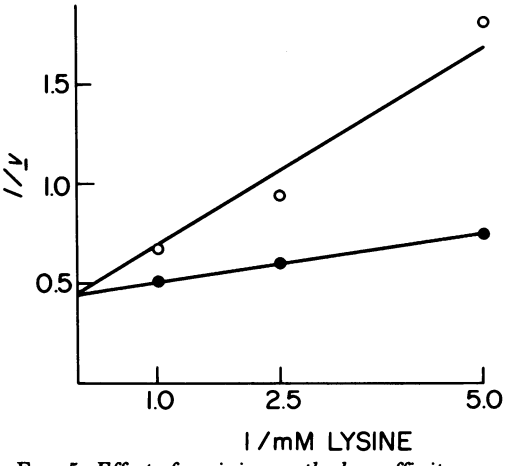

FIG. 5. *Effect of arginine on the low-affinity component of lysine uptake. Initial velocities measured in the absence of arginine (●) and in the presence of 0.2 mM arginine (○) are expressed as nanomoles per milligram (dry weight) per minute. Data are corrected for transport due to the high-affinity component of lysine uptake.*

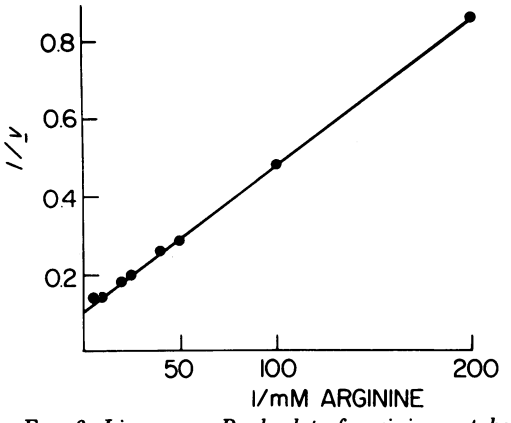

FIG. 6. *Lineweaver-Burk plot of arginine uptake by S. cerevisiae ATCC 9896. Initial velocities are expressed as nanomoles per milligram (dry weight) per minute.*

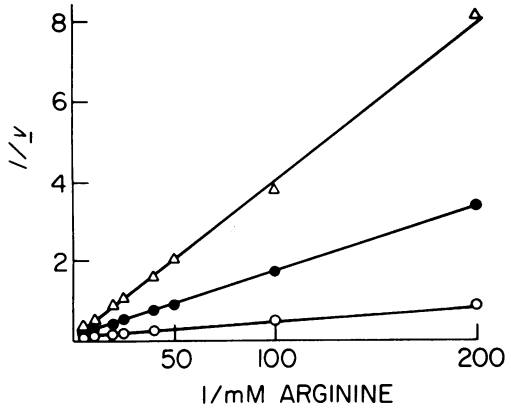

FIG. 7. *Effect of lysine on arginine uptake. Initial velocities measured in the absence of lysine (○), in the presence of 2 mM lysine (●), and in the presence of 4 mM lysine (△) are expressed as nanomoles per milligram (dry weight) per minute.*

the presence of various amino acids on subsequent lysine transport activity. The activity of cells grown in medium supplemented with 1 mM lysine was only 24% of the activity of cells grown in unsupplemented medium (Table 2). Growth in the presence of glutamic acid or arginine also caused a decrease in subsequent lysine transport activity, but the decrease was not as marked as that caused by growth in medium supplemented with lysine. Preloading studies were conducted to further explore the regulation of lysine transport.

Effect of preloading with various amino acids. Preloading with lysine inhibited lysine transport activity, whereas preloading with glutamine, glycine, methionine, phenylalanine, or valine caused little change (Table 3). On the other hand, preloading with histidine stimulated lysine transport. Although the effects were not as marked as that produced by lysine, preloading with arginine, aspartic acid, or glutamic acid also caused an inhibition of lysine transport activity. Based on the above results indicating the involvement of both a lysine-specific system and an arginine-lysine system in lysine transport, we hypothesized that these preloading effects were mediated by changes in the intracellular lysine and/or arginine pools that in turn would affect the activity of the lysine and arginine-lysine transport systems. The lysine and arginine pools of these cells were measured to test this hypothesis.

Preloading with arginine caused an expected large increase in the arginine pool (Table 4). Preloading with aspartic acid or glutamic acid

TABLE 2. *Lysine transport activity of cells grown in the presence of various amino acids*

Amino acid added[a]	Lysine transport activity[b]
None	0.885
Arginine	0.695
Aspartic acid	0.884
Glutamic acid	0.518
Glutamine	0.996
Glycine	0.778
Histidine	0.880
Lysine	0.213
Methionine	0.973
Valine	0.906

[a] Cells were grown in medium S minus aspartic acid supplemented with 1 mM of the indicated amino acid.

[b] Initial velocities expressed as nanomoles per milligram (dry weight) per minute.

TABLE 3. *Effect of preloading with various amino acids on lysine transport*

Amino acid used for preloading[a]	% Control transport activity[b]
Arginine	72
Aspartic acid	62
Glutamic acid	51
Glutamine	97
Glycine	110
Histidine	143
Lysine	34
Methionine	96
Phenylalanine	114
Valine	105

[a] Cells were incubated in solution A supplemented with 2 mM of the indicated amino acid for 60 min.

[b] Initial velocities of lysine uptake expressed as precentage of activity with no preloading.

caused increases in the arginine and/or lysine pool, whereas preloading with histidine resulted in decreases in both the arginine and lysine pools. These data correlate well with the effects of preloading on lysine transport activity and substantiate the hypothesis that regulation of lysine transport is mediated by lysine and arginine.

Since preloading with arginine or glutamic acid (treatments that increase the arginine pool) presumably led to inhibition of the arginine-lysine transport system, we directly tested the effect of arginine or glutamic acid preloading on arginine transport. Preloading with arginine caused an 87% decrease in arginine transport activity, whereas preloading with glutamic acid caused a 32% decrease in arginine transport activity (Table 5).

Time course of inhibition of lysine transport. The inhibition of lysine transport activity caused by lysine was studied in greater detail by monitoring the time course of inhibition in a growing culture. Addition of lysine was followed by a rapid decrease in lysine transport activity as shown in Fig. 8: transport activity decreased more than threefold in one-third of the doubling time of the culture. Thus, the decrease in lysine transport activity was too rapid to be accounted for by repression and subsequent dilution of the transport systems.

TABLE 4. *Arginine and lysine pools of cells preloaded with various amino acids*

Amino acid used for preloading[a]	Incubation time (min)	Lysine pool (nmol/mg [dry wt])	Arginine pool (nmol/mg [dry wt])
None	0	12	112
	60	12	110
Arginine	60	5	294
Aspartic acid	60	28	113
Glutamic acid	60	24	174
Histidine	60	4	79

[a] Cells were incubated in solution A supplemented with 2 mM of the indicated amino acid.

TABLE 5. *Effect of preloading with arginine or glutamic acid on arginine transport*

Amino acid used for preloading[a]	Incubation time (min)	Arginine transport activity[b]
Arginine	0	4.27
	60	0.57
Glutamic acid	0	4.40
	60	2.98

[a] Cells were incubated in solution A supplemented with 2 mM of the indicated amino acid.

[b] Initial velocities expressed as nanomoles per milligram (dry weight) per minute.

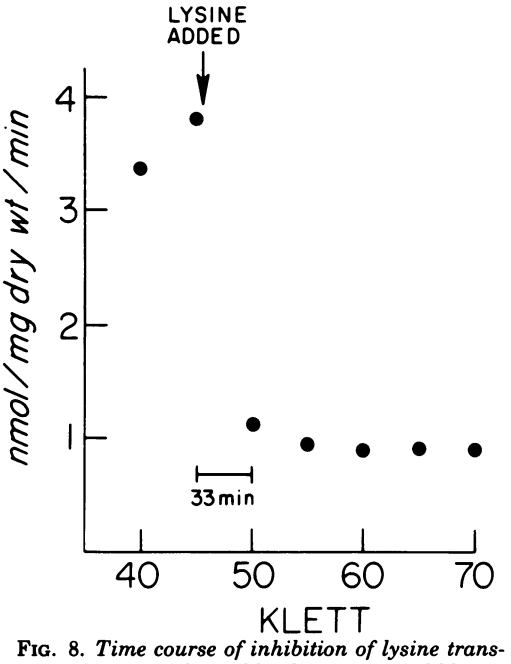

FIG. 8. *Time course of inhibition of lysine transport. At the time indicated by the arrow, 1 mM lysine was added to the medium of a growing culture. The generation time of the culture was 100 min.*

DISCUSSION

Although efflux of a component of the cellular amino acid pool concomitant with influx of amino acid has been observed in *S. hydrogenans* (14, 31), such exchange does not occur in *N. crassa* (26), *P. chrysogenum* (1, 16, 34), or *S. cerevisiae* (5, 19). Compartmentation of the bulk of some amino acids within vesicles in *N. crassa* (35, 37) probably prevents their efflux across the cytoplasmic membrane. Similarly, most of the amino acid pool of *S. cerevisiae* is located within vacuoles that are especially rich in basic amino acids (38). Thus, the sequestering of accumulated lysine within a vacuole probably explains the lack of efflux observed in the present studies. However, the necessity for regulation of lysine transport at the level of the cytoplasmic membrane persists.

In yeasts the initial translocation of some amino acids across the cytoplasmic membrane is followed by their translocation across a vacuolar membrane. For example, Boller et al. have characterized a highly specific arginine transport system in vacuoles isolated from *S. cerevisiae* (3). These findings suggest that accurate kinetic constants for amino acid transport systems located in the cytoplasmic membrane can-

not be obtained with intact yeast cells. However, the specificity of arginine transport in intact cells of *S. cerevisiae* noted by us and by others (13) coincides with the specificity observed in spheroplasts (3). The use of intact cells, therefore, remains a valid technique for differentiating amino acid transport systems located in the cytoplasmic membrane.

Two transport systems are responsible for the transport of lysine: a lysine-specific system for which lysine has a relatively high affinity and an arginine-lysine system for which lysine has a lower affinity. Two results support this conclusion. First, arginine (the only amino acid that inhibited lysine transport) had no effect on the high-affinity component of lysine transport, but competitively inhibited the low-affinity component; the arginine K_i for the low-affinity component of lysine transport approximates the K_m for arginine transport. Second, lysine competitively inhibited arginine transport.

Utilizing genetic as well as biochemical analyses, Grenson et al. also identified a lysine-specific system and an arginine system competitively inhibited by lysine using another strain of *S. cerevisiae* (10, 13). Subsequent studies revealed that lysine was also a substrate of a general amino acid transport system whose substrates include basic and neutral but probably not acidic amino acids (12). This system is inactive in the presence of ammonium ion (11, 12). *S. cerevisiae* ATCC 9896 evidently contains this system since lysine transport increased when ammonium sulfate was omitted from transport mixtures (data not shown). This increased lysine transport activity was sensitive to inhibition by valine but not by glutamic acid. Since ammonium sulfate was included throughout the present studies the effect of the general amino acid transport system could be ignored.

The following data support the conclusion that the lysine-specific and arginine-lysine transport systems of *S. cerevisiae* are regulated. (i) Preloading with lysine or other amino acids that cause increases in the intracellular lysine and/or arginine pools caused inhibition of lysine transport. (ii) The amount of inhibition caused by such preloading correlated with the relative amount of increase in the lysine and/or arginine pool. (iii) Preloading with histidine caused an increase in lysine transport and a concomitant decrease in both the arginine and lysine pools. (iv) Preloading with amino acids that cause increases in the arginine pool resulted in inhibition of arginine transport. Since the decrease in lysine transport activity after addition of lysine to a growing culture was too rapid to be accounted for by repression and subsequent dilution of the transport systems, feedback inhibition is apparently involved. However, simultaneous regulation of lysine transport by feedback inhibition and repression is indeed a possibility.

The excellent correlation of transport activity with lysine and arginine pool size provides evidence that the amino acids themselves may be the effector molecules mediating the feedback inhibition of their respective transport systems. Pall has obtained evidence that the basic (26), neutral (26), and methionine (25) transport systems of *N. crassa* are regulated by feedback inhibition that is apparently mediated by direct interaction of the substrates with their transport systems.

Both the arginine and lysine pools decreased during histidine preloading, which presumably caused an increase in the histidine pool. Similarly, a decrease in the lysine pool was observed concomitantly with a large increase in the arginine pool during arginine preloading. The basis of these reciprocal interactions among basic amino acids is unclear. During their studies of arginine metabolism in *S. cerevisiae*, Ramos et al. observed that the arginine pool of cells grown in the presence of ornithine or lysine was much lower than that of cells grown in the absence of these basic amino acids (29).

In addition to inhibiting the activity of the lysine-specific system, lysine apparently feedback-inhibits the arginine-lysine system as well. Two results support this conclusion. First, a steady-state level of accumulated lysine was reached with a substrate concentration of 2 mM. At this high lysine concentration, the arginine-lysine transport system would have been responsible for most of the lysine transport activity. Since this steady state occurred in the absence of efflux, all influx was terminated: the arginine-lysine system as well as the lysine-specific system was inhibited. Second, the amount of inhibition in lysine transport activity caused by preloading with lysine (66%) was in excess of that expected solely by an inhibition of the lysine-specific system (55%). Such feedback inhibition of the arginine-lysine system by intracellular lysine is consistent with the conclusion that feedback inhibition of the lysine-specific and arginine-lysine transport systems is mediated by their respective substrates.

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