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Mutant of *Escherichia coli* K-12 Defective in the Transport of Basic Amino Acids

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Escherichia coli K-12 possesses two active transport systems for arginine, two for ornithine, and two for lysine. In each case there is a low- and a high-affinity transport system. They have been characterized kinetically and by response to competitive inhibition by arginine, lysine, ornithine and other structurally related amino acids. Competitors inhibit the high-affinity systems of the three amino acids, whereas the low-affinity systems are not inhibited. On the basis of kinetic evidence and competition studies, it is concluded that there is a common high-affinity transport system for arginine, ornithine, and lysine, and three low-affinity specific ones. Repression studies have shown that arginine and ornithine repress each other's specific transport systems in addition to the repression of their own specific systems, whereas lysine represses its own specific transport system. The common transport system was found to be repressible only by lysine. A mutant was studied in which the uptake of arginine, ornithine, and lysine is reduced. The mutation was found to affect both the common and the specific transport systems.

Previously a mutant of *Escherichia coli* K-12 was described in which the uptake of label from ^{14}C-arginine, ^{14}C-ornithine, and ^{14}C-lysine was reduced (12). It was concluded that this mutant had a defective transport system for the three basic amino acids. However, other interpretations are possible for the observed results, such as a block in the formation of the two polyamines, putrescine and cadaverine, or overproduction of endogenous arginine and lysine. In the present paper and in the accompanying paper by Rosen (19), these possibilities are examined and experiments are described which rule them out. Our studies reveal a complex system for the transport of the three basic amino acids, similar to systems described for other amino acids (16), with common elements for the three structurally related amino acids and components specific for each amino acid. The mutation responsible for the reduced uptake of arginine, lysine, and ornithine was found to affect both the common and the specific components.

The mutation has been mapped and assigned to a locus named *argP* at min 56 on the *E. coli* linkage map (13).

MATERIALS AND METHODS

Bacterial strains and media. JC182 is a double male strain of *E. coli* K-12 (4) and requires thiamine and adenosine for growth. It is sensitive to inhibition by canavanine. JC182-5 is a canavanine-resistant mutant isolated from it after treatment with *N*-methyl-*N'*-nitro-*N'*-nitroso-guanidine (1).

The minimal medium used was medium A (5) with 30 mM glucose as the carbon source. Medium HALF (8) is an arginine-free, synthetic, enriched medium modified from the AF medium of Novick and Maas (15) by omitting histidine and lysine.

Chemicals. L-[3-^{3}H]arginine, L[*U*-^{14}*C*]arginine, L-[4,5-^{3}*H*]lysine, L-[*U*-^{14}*C*]lysine, and L-[*U*-^{14}*C*]ornithine were purchased from New England Nuclear Corp. L-[5-^{3}*H*]ornithine was purchased from Schwartz/Mann. Aminooxyacetic acid hemi-hydrochloride (AOA) was from Sigma Chemical Corp. All amino acids were of the L-form unless otherwise specified.

Transport assays. For all the experiments described except those involving initial rate of transport, bacteria taken from the middle of the exponential growth phase were incubated for 30 min at 37 C with 200 μg of chloramphenicol (CAP) per ml in minimal medium or in HALF medium containing 8 mM AOA. The cells were then centrifuged at room temperature and concentrated into a small volume of minimal medium with 1 mM glucose, CAP, and AOA, in the same amounts as above. This suspension was diluted to a final concentration of 1 mg (wet wt)/ml into minimal medium with 1 mM glucose, CAP, AOA, and 0.02 μCi of radioactive amino acid per ml and adjusted with cold carrier amino acid to the desired concentration. Bacterial concentration was determined by optical density (OD) measurements with a Lumetron colorimeter at 490 nm. After incubation for varying times, fractions of the mixture were filtered

on membrane filters (0.45-μm pore size and 25-mm diameter; Millipore Corp.). The disks were washed twice with 4-ml portions of minimal medium kept at room temperature, dried, and counted in a Nuclear-Chicago Mark I liquid scintillation counter in vials containing a solution of 4 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)] benzene per liter of toluene.

For experiments involving initial rate of transport, this procedure was modified. Cells harvested by centrifugation from the middle of the exponential growth phase were washed twice by centrifugation with medium A at room temperature and a third time with medium A containing 8 mM AOA. The cells were then incubated for 5 min at room temperature in medium A containing 80 μg of CAP per ml, 8 mM AOA, and 20 mM glucose. The cells were used for transport assays within 20 min after the 5-min incubation period. To initiate a transport assay, a portion of the cell suspension, usually 0.1 ml, was added to a reaction mixture containing (final concentration) 80 μg of CAP per ml, 20 mM glucose, 8 mM AOA, and labeled amino acid. After 15 and 30 s, 0.5-ml portions were filtered onto membrane filters (Millipore Corp.) and washed with a solution containing 10 ml of 0.01 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.3), 0.15 M NaCl, and 5×10^{-4} M $MgCl_2$ (3). The concentration of cells was adjusted so that less than 10% of the labeled amino acid was taken up during the first 15 s.

The values of the 15-s samples were used for the calculation of initial transport rates after subtraction of the values found for zero-time samples (approximately 10% of the radioactivity taken up during the first 15 s).

Enzyme measurements. The preparation of cell extracts and the assays for agmatine ureohydrolase and the inducible and constitutive arginine decarboxylases have been described previously (9).

RESULTS

Normal conversion of arginine into putrescine in strain JC182-5. In previous experiments with wild-type cells in the presence of CAP and ^{14}C-arginine, it was noted that the level of radioactivity continued to increase for 40 to 60 min before a plateau was reached (12). This was different from uptake studies with other amino acids where, under similar conditions, a plateau is reached within a few minutes (11); it suggested that we were measuring not only equilibration with an internal pool of arginine but conversion of arginine to other products. In *E. coli* the only pathway of arginine conversion, besides protein synthesis, is decarboxylation to form agmatine, which is then converted to putrescine and spermidine. It was shown by Wilson and Holden (21) that the decarboxylation of arginine can be blocked by AOA. They found that, in the absence of AOA, within a few minutes most of the label from external ^{14}C-arginine was in putrescine and agmatine. In the presence of about 4 mM AOA, most of the label remained in arginine and the total uptake of label was greatly reduced. Their work was done with *E. coli* W, and we obtained similar results with *E. coli* K-12. We have shown, furthermore, that with a mutant blocked in arginine decarboxylase the uptake of ^{14}C-arginine is the same with and without AOA and that it is like the uptake of wild-type cells in the presence of AOA (14).

Since our *argP* mutant JC182-5 showed reduced uptake of ^{14}C-arginine in the absence of AOA as compared to wild-type cells, and since the plateau level of label in wild-type cells is much higher in the absence of AOA than in its presence, it seemed conceivable that the block in the mutant was in the conversion of arginine to polyamines. We therefore compared the uptake of ^{14}C-arginine of wild-type and mutant cells with and without AOA (Fig. 1 and 2). It can be seen that the difference between the two strains persists in the presence of AOA. We also measured the levels of the enzymes involved in the conversion of arginine to putrescine. There are two arginine decarboxylases, one constitutive and one inducible; the latter is formed in the presence of arginine under acid conditions (pH 5–6). It can be seen (Table 1) that the levels of these enzymes are not affected by the *argP* mutation. Thus, the reduced uptake of ^{14}C-arginine in the mutant cannot be ascribed to a block in the conversion of arginine to putrescine.

Reduced ^{14}C-arginine uptake in strain JC182-5 is not due to dilution of labeled arginine by excreted ^{12}C-arginine. In the accompanying paper (19), Rosen describes canavanine-resistant mutants which have reduced uptake for basic amino acids and also excrete arginine into the culture medium. This raises the possibility that the reduction in ^{14}C-arginine uptake may only be an apparent one and may be due to dilution of ^{14}C-arginine by endogenously formed ^{12}C-arginine. He could rule out this possibility by constructing a double mutant which, in addition to the mutation in uptake, had a block in arginine biosynthesis. This double mutant still had reduced uptake when compared with the corresponding single arginine auxotrophs. We have also constructed, by mating with an arginine auxotroph, such a double mutant from JC182-5 and have compared it with a corresponding arginine auxotroph obtained by mating with JC182. We also found that the difference in the uptake of ^{14}C-arginine persisted between the *argP* and *argP*$^+$ arginine auxotrophs. Furthermore, we found that the growth rate of the *argP arg* strain with arginine was about half that of the *argP*$^+$

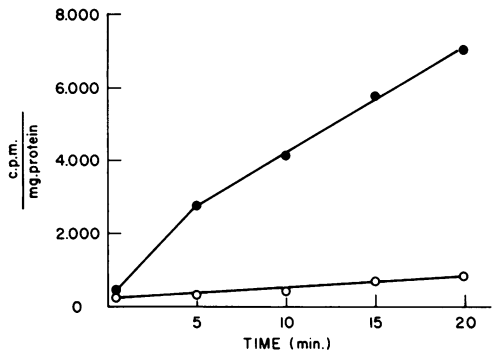

FIG. 1. *Time-dependence uptake of ^{14}C-arginine at 10 μM external concentration in wild-type E. coli and JC-182-5. Uptake was measured as outlined in Materials and Methods. Arginine transport is expressed as radioactivity taken up per milligram of bacterial protein. Symbols: ●, ^{14}C-arginine uptake in wild-type E. coli; ○, ^{14}C-arginine uptake in JC-182-5.*

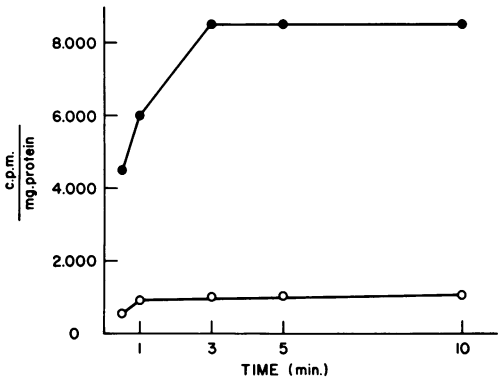

FIG. 2. *Time-dependence uptake of ^{14}C-arginine at 10 μM initial external concentration in wild-type strain and JC-182-5. Uptake was measured in the same conditions as in Fig. 1, except that the cells were incubated with AOA for 30 min before transport was measured and AOA was also present in the "uptake medium." Symbols: ●, ^{14}C-arginine uptake in wild-type strain; ○, ^{14}C-arginine uptake in JC-182-5.*

arg strain (Peyru and Maas, unpublished results).

Regulation of transport. The fact that the *argP* mutation affects the transport of arginine, ornithine, and lysine suggested the existence of a single transport system for the three basic amino acids. The first indication that the situation may be more complex came from studies on the regulation of formation of this transport system. It was noted that wild-type bacteria grown in an enriched, but arginine- and lysine-free medium (HALF), were more active for arginine transport than were cells grown with arginine (Fig. 3). Similar results were obtained with minimal medium A with or without arginine. To obtain this effect, it was necessary that the bacteria were actually growing in the presence of arginine. Exposure of cells to arginine in the absence of growth, followed by washing out the cold arginine, did not result in a decrease of the transport activity. The effect of arginine may, therefore, be ascribed to repression. After pregrowth without arginine, the maximal level of repression is reached within one generation during subsequent growth in the presence of arginine.

In further studies it was noted that pregrowth with arginine or ornithine diminished markedly the transport activity for these amino acids, whereas lysine transport was only slightly affected (Table 2). On the other hand, prior growth with lysine reduced lysing transport activity markedly, while transport of arginine and ornithine were hardly affected. In contrast to the effect of the *argP* mutation, repression is specific for lysine on the one hand and arginine and ornithine on the other hand.

To determine whether repression control of

TABLE 1. *Levels of enzymes involved in the conversion of arginine into putrescine*[a]

Enzyme	Product measured	Enzyme sp act (μmol/min/mg of protein)	
		Parent JC-182	Mutant JC-182-5
Agmatine ureohydrolase	Urea	0.0050	0.0055
Arginine decarboxylase (constitutive)	CO_2	0.0051	0.0055
Arginine decarboxylase (inducible)	CO_2	0.29	0.29

[a] Techniques used for enzyme assays have been described previously (9).

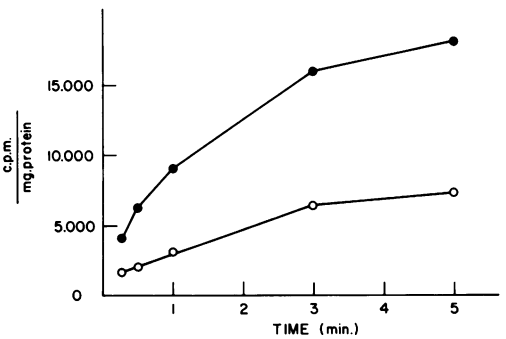

FIG. 3. *Arginine uptake in wild-type strain grown in the presence or absence of arginine. Cultures were grown in HALF medium without arginine (●) or in the presence of 200 μg of arginine (○) per ml. Uptake assays were performed as described in Materials and Methods.*

transport was exerted via the same system as that for arginine biosynthesis, we tested strain JC182-9 (*argR*) which is genetically derepressed for the enzymes of arginine synthesis. We found the same degree of repression for arginine transport as in the parent strain JC182 (*argR*$^+$).

It should be noted that the reduction of transport activity due to the *argP* mutation is much greater than the reduction due to repression. The latter is about 60% for arginine and ornithine (Table 2), whereas the former is about 90% (Fig. 1).

Kinetics of arginine, lysine, and ornithine transport. Further evidence for several transport systems came from kinetic studies. The data for the variations in initial rate of arginine uptake with external arginine (Fig. 4) could not be described by a straight line in a plot of S/V versus S. They were best fitted by a curvilinear plot whose constants were calculated the procedure developed by Reid et al. (17). Similarly,

TABLE 2. *Repression of amino acid transport in cells pregrown with arginine, ornithine, or lysine*[a]

Addition to HALF medium	Control (%) on substrate:		
	^{14}C-Arginine	^{14}C-Ornithine	^{14}C-Lysine
L-Arginine	45	14	90
L-Ornithine	58	8	80
L-Lysine	88	100	35

[a] Cells were grown in HALF medium (control) and in HALF medium supplemented with 1 mg of arginine, ornithine, or lysine per ml. Uptake was performed as outlined in Materials and Methods.

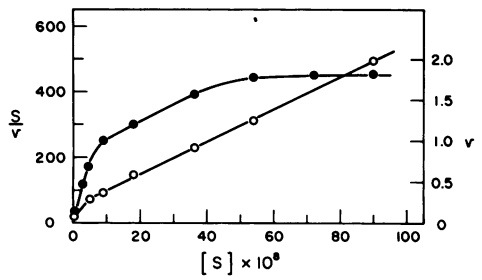

FIG. 4. *Kinetics of L-arginine uptake in wild-type E. coli K-12. Cells were grown in minimal medium, and uptake was performed as outlined in Materials and Methods, by using L-[^{3}H]arginine at different concentrations between 0.9 × 10^{-9} and 10^{-4} M. The opened circles represent the experimental initial rates; the line was drawn by using the theoretical values calculated from the equation for two (6) simultaneous reactions on the same substrate. Symbols: O, S/V = molarity per nanomole per milligram of protein per minute; ●, initial rate of uptake (nanomoles per milligram of protein per minute) (V).*

initial rates of lysine and ornithine uptake gave evidence for two kinetically distinguishable transport systems. With the assumption that only two active transport systems were functioning and no contribution of diffusion, the values for the two kinetically distinct systems for the transport of arginine, lysine, and ornithine (Fig. 4 to 6) were calculated. They are presented in Table 3.

The contribution of each system to transport at a given substrate concentration can be calculated from the equation (6) $v_i = (sV_{max_1}/s + K_{max_1}) + (sV_{max_2}/s + K_{max_2})$, where v_i is the measured initial rate of transport and s is the initial concentration of substrate. Consequently, it is possible to choose extracellular substrate concentrations for one or another system—depending upon the K_m values—and to determine the contribution of each system at such concentration. Thus, at 0.01 μM external concentration of arginine and 0.1 μM of lysine and ornithine, the concentrative ability of the high-affinity system is minor for arginine but substantial for lysine and ornithine. Only 20% of arginine is transported through the high-affinity system at 0.01 μM initial concentration, but as much as 60% of ornithine and 95% of lysine are transported by this system when an external concentration of 0.1 μM of these amino acids is used.

The high-affinity systems, like the low-affinity systems, bring about concentration of their substrates against a gradient. For example, arginine and ornithine, both of which have low V_{max_1} values, are concentrated more than 100-fold. At 10^{-8} M external arginine, the internal concentration after equilibrium is approximately 7×10^{-6} M, disregarding endogenously formed arginine and assuming 0.73 μliters of water per mg of wet weight (22); at 10^{-7} M external ornithine, the internal concentration is 3×10^{-5} M.

Specificity of the basic amino acid transport systems. Since kinetic studies revealed two transport systems for each of the three structurally related amino acids, it was of interest to see to what extent these systems were shared among the amino acids. Inhibition studies of initial rates of transport of arginine, lysine, and ornithine, each at low and high concentrations, in the presence of a 100-fold excess of presumptive competitors are presented in Tables 4 and 5. The results suggest the presence of a single high-affinity system of low specificity [the LAO system of Rosen (18)] for the three amino acids and also for N-α-acetylornithine and L-canavanine, and of three low-affinity systems, each specific for one of the

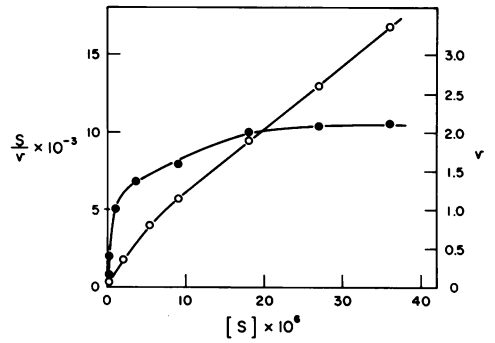

FIG. 5. *Same as Fig. 3, with* L-[^3H]*lysine. Symbols:* O, *S/V = molarity per nanomole per milligram of protein per minute;* ●, *initial rate of uptake (nanomoles per milligram of protein per min) (V).*

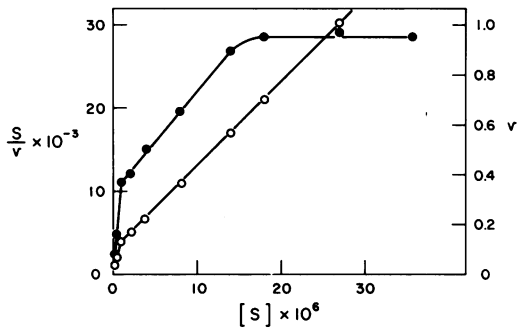

FIG. 6. *Same as Fig. 3 with* L-[^3H]*ornithine. Symbols:* O, *S/V = molarity per nanomole per milligram of protein per minute;* ●, *initial rate of uptake (nanomoles per milligram of protein per min) (V).*

TABLE 3. *K_m values for uptake of* L-*arginine,* L-*lysine and* L-*ornithine*[a]

Substrate	High-affinity transport system		Low-affinity transport system	
	K_m (M)	V_{max}	K_m (M)	V_{max}
L-Arginine	5.0×10^{-9}	0.01	1.25×10^{-7}	1.8
L-Lysine	1.0×10^{-7}	1.5	5.0×10^{-6}	2.5
L-Ornithine	1.0×10^{-7}	0.1	3.0×10^{-6}	1.0

[a] The constants were calculated from Fig. 4 to 6 by the method of Reid et al. (17). V_{max}, nanomoles per milligram of protein per minute. Initial rates of uptake were measured as described in Materials and Methods.

amino acids. The weak inhibition observed in the uptake of arginine at 0.01 μM external concentration (Table 4) is because only 20% of arginine is transported by the high-affinity system at this concentration. The low-affinity system for ornithine shows some inhibition by the presumptive competitors. However, with ornithine at a concentration of 10 μM, raising the concentrations of lysine and arginine above the 100-fold excess does not increase the inhibition of ornithine uptake, suggesting that in addition to the specific system yet another nonspecific ornithine transport system may be present.

The K_i values of lysine and ornithine for arginine transport were determined by varying

TABLE 4. *Inhibition of the high-affinity basic amino acid transport systems*[a]

Addition	Control (%) on substrate:		
	H^3-Arginine	^{3}H-Lysine	^{3}H-Ornithine
L-Arginine		60	30
L-Lysine	75		35
L-Ornithine	71	56	
L-Histidine	67	60	40
L-Acetylglutamate	87	93	85
α-Acetyl-L-ornithine	63	56	50
L-Canavanine	67	24	20

[a] The concentrations of labeled substrates were: ^{3}H-arginine, 0.01 μM; ^{3}H-lysine and ^{3}H-ornithine, 0.1 μM. Each unlabeled competitor was used at 100-fold molar excess over the labeled substrate. Canavanine concentration was 1.0 mM (a K_m of 0.4 mM has been found [B. Rosen, personal communication]). The amounts of ^{3}H-arginine, ^{3}H-lysine, and ^{3}H-ornithine taken up in the absence of competitor were 0.24, 1.20 and 0.20 nmol per mg of protein per min, respectively. Initial rates of uptake were measured as described in Materials and Methods.

TABLE 5. *Specificity of the low-affinity basic amino acid transport systems*[a]

Addition	Control (%) on substrate:		
	^{3}H-Arginine	^{3}H-Lysine	^{3}H-Ornithine
L-Arginine		103	33
L-Lysine	78		43
L-Ornithine	80	100	
L-Histidine	88	105	52
L-Acetylglutamate	103	100	74
α-Acetyl-L-ornithine	84	100	42
L-Canavanine	80	104	34

[a] The concentrations of labeled substrates were: ^{3}H-arginine, 1.0 μM; ^{3}H-lysine and ^{3}H-ornithine, 10 μM. Each unlabeled competitor was used at 100-fold molar excess over the labeled substrate. Canavanine concentration was 1.0 mM. The amounts of ^{3}H-arginine, ^{3}H-lysine, and ^{3}H-ornithine taken up in the absence of competitor were 1.60, 2.60, and 1.20 nmol per mg of protein per min, respectively. Initial rates of uptake were measured as described in Materials and Methods.

the concentrations of these two amino acids in the presence of a constant initial concentration of arginine 10^{-8} M (7). The K_i for lysine was 2.5×10^{-7} M and that for ornithine was 3.0×10^{-7} M. These values are close to their respective K_m values for the high-affinity systems, providing further evidence that both amino acids are transported via the LAO system. From the competition studies it can be concluded that the transport of arginine, lysine, and ornithine is mediated by at least four physiologically distinct systems, one common to the three (LAO system) and three specific ones (one for each amino acid).

Since the existence of several systems was first indicated by studies on repression (Table 2), we tested the effect of pregrowth with each of the amino acids on the high- and low-affinity systems. The three low-affinity systems showed results similar to those described in Table 2, with arginine or ornithine repressing transport of arginine and ornithine and with lysine being specific for its own transport. The LAO system can be tested best by measuring lysine transport because at the lowest possible arginine concentration (10^{-8} M) which permits reliable measurements only 20% of the arginine is transported by this system. Similarly, measurements of ornithine transport are difficult because of further metabolic conversions of ornithine. With 10^{-7} M concentration of lysine, 95% is transported by the LAO system. Under these conditions only lysine represses the formation of the LAO system (Fig. 7). Thus, there are two ways of regulating the formation of these transport systems, one common to arginine and ornithine and affecting the low-affinity systems for these two amino acids, and one for lysine and affecting the low-affinity lysine system and the LAO system.

Energy requirements for arginine, lysine, and ornithine uptake. The uptake systems for arginine, lysine, and ornithine appear to be active transport systems. The initial rates of uptake of both high- and low-affinity systems are stimulated by the addition of glucose or succinate and reduced by the addition of a metabolic inhibitor such as 2,4-dinitrophenol (Table 6).

Transport of arginine, lysine, and ornithine in strain JC182-5. Since *argP* mutant JC182-5 shows reduced uptake of arginine, lysine, and ornithine, it was of interest to investigate its transport of these amino acids through the high- and low-affinity systems. We therefore compared the uptake of labeled substrates of wild-type and mutant cells at two different external concentrations (Table 7).

There is a significant reduction in the mutant in transport of the three amino acids through all systems. This reduction is less drastic with ornithine transport. This may be related to the fact that the conversion of ornithine into citrulline is not blocked and, therefore, no steady state is reached. At low initial external concen-

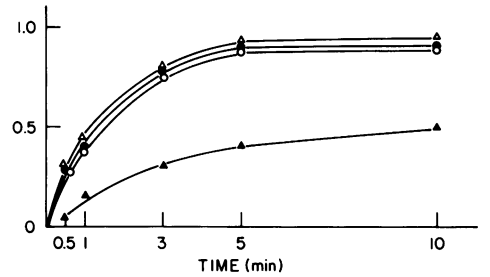

FIG. 7. *Time dependence of uptake of ^{3}H-lysine at 0.1 μM initial external concentration in wild-type E. coli. Cells were pregrown in HALF medium (○), in HALF medium supplemented with 1.000 μg of ornithine per ml (●), in HALF medium supplemented with 1.000 μg of arginine per ml (△), or in HALF medium supplemented with 1.000 μg of lysine per ml (▲). Uptake was measured as outlined in Materials and Methods.*

TABLE 6. *Dependence of basic amino acid transport on metabolic energy*[a]

Substrate and addition	Uptake (nmol/min/mg)	
	High-affinity system	Low-affinity system
Arginine		
None	0.01	0.05
Glucose	0.08	0.65
Succinate	0.24	1.02
DNP	0.00	0.00
Lysine		
None	0.24	2.00
Glucose	0.51	2.84
Succinate	0.98	2.99
DNP	0.00	0.00
Ornithine		
None	0.03	0.05
Glucose	0.09	0.59
Succinate	0.30	1.52
DNP	0.00	0.00

[a] The initial concentrations of the substrates were: (for the high-affinity system) arginine, 0.01 μM; lysine and ornithine, 0.1 μM; and (for the low affinity system) arginine, 1.0 μM, lysine and ornithine, 10 μM. Glucose and succinate were used at 0.2%; the concentration of 2,4-dinitrophenol (DNP) was 2 mM. Cells were grown in minimal medium (Materials and Methods) supplemented with 0.2% glucose. Initial rates of uptake were performed as described in Materials and Methods.

tration of arginine (0.01 μM), there is no measurable uptake in the mutant after 1 min, and similarly the initial uptake for lysine is markedly reduced at low initial concentration (0.1 μM). With incubation times longer than 1 min, some uptake of arginine, lysine, and ornithine can be detected in the mutant, and it is presumably carried out mainly through the low-affinity systems with greatly reduced maximal velocities. Due to these low initial values in JC-182-5, it was impossible to obtain accurate kinetic constants.

Transport of histidine in JC-182-5. Since histidine is also a basic amino acid and since it inhibits the high-affinity system for arginine, lysine, and ornithine (Tables 4 and 5), it seemed conceivable that histidine is a substrate for this system. We therefore compared the histidine uptake of wild-type and mutant cells at 10 and 0.01 μM initial external concentrations. No significant differences were detected between wild type and JC-182-5. Thus, several possibilities remain to explain the histidine transport in wild type and JC-182-5. Either histidine is an inhibitor of the high-affinity system for arginine, lysine and ornithine, but not a substrate for it, or the reduction in histidine transport in JC-182-5 is not detected because of the high residual level of histidine transport due to the other histidine-transporting systems.

TABLE 7. *Amino acid transport in wild type and in JC-182-5*[a]

Substrate	Initial external concn (μM)	V	
		Wild type	JC-128-5
^{3}H-Arginine	0.01	0.30	0.00
	1.00	1.30	0.10
^{3}H-Lysine	0.10	1.60	0.20
	10.0	2.40	0.28
^{3}H-Ornithine	0.10	0.15	0.05
	10.0	1.00	0.25

[a] Initial rate of uptake was measured as outlined in Materials and Methods. *V*, nanomoles per milligram of protein per minute.

DISCUSSION

During recent years it has become apparent that the transport of a single amino acid is usually mediated by several systems of varying degrees of specificity (16). For example, histidine is transported in *Salmonella typhimurium* through at least five permeases with different patterns of specificity and affinity (2). This is in contrast to the biosynthesis of amino acids for which there is usually only one pathway for each. Several pathways involved in the transport of a single amino acid may have common elements, such as the P protein which is necessary for the functioning of two histidine permeases. The results described here show similar complexities for the transport of basic amino acids as have been observed for other amino acids. The transport of arginine, ornithine, and lysine is mediated by at least four physiologically distinct systems, one common to the three (LAO system) and three specific ones (one for each amino acid). The evidence for separate systems is based on nonlinear S/V versus S plots obtained in kinetic studies and on competitive inhibitions at low, but not at high, substrate concentrations. The pattern of repression is also complex, the formation of the low-affinity systems for arginine and ornithine being repressible by either arginine or ornithine and the formation of the low-affinity lysine system and the LAO system being repressible by lysine.

It should be noted that Rosen (18) found no transport of arginine via the LAO system. His S/V versus S plot is a straight line. This discrepancy between his results and ours has not been elucidated. It may be due to strain differences.

Since the experiments reported here have been carried out with whole cells, we cannot say anything about the number of physical entities involved in these transport systems. We have carried out experiments on binding proteins like those described in the accompanying paper by Rosen (19) and have obtained similar results (Celis and Maas, unpublished). We also found three arginine-binding proteins, two specific for arginine and one (LAO) common to arginine, ornithine, and lysine.

Mutation in the *argP* gene reduces transport by all four systems. Rosen (19) has postulated that the mutation results in an uncoupling of carrier proteins from a postulated common concentrative apparatus. He has found no differences in the binding proteins between strains JC-182 and JC-182-5. In our preliminary experiments we found no difference for the arginine-specific binding proteins, but we did find a consistent difference for the LAO protein. The LAO protein from JC-182-5 had a five- to tenfold greater dissociation constant than the LAO protein from JC-182. Nevertheless, we feel that this is not conclusive evidence that the LAO-binding protein is altered in JC-182-5, and we are now trying to isolate *argP* mutants which have a greater reduction in transport activity than does JC-182-5. Since *argP* is closely linked to *serA*, we can use the localized mutagenesis method of Hong and Ames (10) to isolate *argP* mutants.

We have found that histidine inhibits the

transport of arginine, ornithine, and lysine by the LAO system. However, the *argP* mutation does not visibly affect histidine transport. It may be that the reduction is not detected, as mentioned above. Alternately, it may be that there is a component of the LAO system which is shared by a histidine transport system, or that histidine is an inhibitor but not a substrate for the LAO system. In *S. typhimurium*, Rosen and Vasington (20) have reported that histidine transport can be inhibited by arginine and lysine and that arginine interferes with the binding of histidine to the histidine-binding protein. In the same organism, Ames and Lever (2) have found that histidine transport can be inhibited by arginine, ornithine, and lysine and that these three amino acids also interfere with the binding of histidine to the J protein, which is known to be involved in histidine transport.

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