JOURNAL OF BACTERIOLOGY, June 1977, p. 1234-1243
Vol. 130, No. 3

Printed in U.S.A.

Properties of an *Escherichia coli* K-12 Mutant Defective in the Transport of Arginine and Ornithine

T. F. R. CELIS

Department of Microbiology, New York University School of Medicine, New York, New York 10016

Received for publication 29 December 1976

A canavanine-resistant mutant strain, defective in the transport of arginine and ornithine, was isolated and characterized. Experiments presented show that both the kinetics of influx and the steady state of accumulation of arginine and ornithine are affected by the mutation, whereas the activity of other related transport systems remains unchanged. On the basis of competitive studies, it is concluded that L-canavanine can inhibit efficiently the arginine-specific uptake system. D-Arginine appears to be a moderate inhibitor. None of the basic amino acid-binding proteins of the mutant strain showed detectable alterations in terms of quantity, physical properties, or affinity constants. Studies on the relationship between the number of transport carriers and the steady state of accumulation of arginine suggested the presence of a reduced number of membrane carriers in the mutant strain. It is proposed that the mutation affects a regulatory gene concerned with controlling the amount of membrane carriers produced, which are components of the arginine- and ornithine-specific uptake systems. The mutation maps at min 62 on the recalibrated linkage map of *Escherichia coli* K-12, in a locus closely linked or identical to *argP*.

The transport of the basic amino acids arginine, lysine, and ornithine is mediated by at least four physiologically distinct systems in *Escherichia coli* K-12. There are one common, high-affinity transport system for arginine, lysine, and ornithine (LAO system) and three low-affinity specific systems, one for each amino acid (8, 33). Evidence for separate systems is based on biphasic kinetics of entry of all three basic amino acids and on competitive inhibitions at low, but not at high, substrate concentrations.

The four transport systems are repressible and the pattern of repression is also complex. The formation of the specific systems for arginine and ornithine is repressible either by arginine or ornithine, and the formation of the specific lysine system and the LAO system, by lysine (8).

After treatment of cells with a mild osmotic shock, three binding proteins can be isolated from the shock fluid (33). Two of these periplasmic proteins bind specifically to arginine and one binds to arginine, lysine, and ornithine (LAO binding protein). The relationship between these binding proteins and the four transport systems has not yet been defined. Two transport-deficient mutant strains, JC-182-5 (8, 23) and CanR-22 (34), have been previously isolated and studied. JC-182-5 is a defective strain in the transport of the three basic amino acids with the mutation affecting the four transport systems. In the case of CanR-22, the mutation is also pleiotropic in that it affects the transport of arginine, lysine, and ornithine through a reduced activity of the common (LAO) system and the arginine-specific system. Both mutants were isolated as canavanine-resistant strains and map at min 62 on the recalibrated linkage map of *Escherichia coli* K-12 (5), close to the *serA* locus.

Reported here are studies carried out on another mutant strain defective in the uptake of arginine and ornithine, with normal levels of lysine transport. The mutation responsible for the reduced transport of the two amino acids was found to affect the arginine- and the ornithine-specific systems. It maps close to the *argP* locus on the linkage map of *E. coli* and confers on the mutant strain a canavanine-resistant condition.

MATERIALS AND METHODS

Bacterial strains and media. All strains used in these studies were derived from *E. coli* K-12 and are listed in Table 1. Prototrophic revertants of the serine marker in the parent and mutant strains were isolated by transduction with a P1 phage grown on a wild-type *E. coli* K-12 strain. Prototrophic serine revertants were used to avoid the complication of dealing with L-serine-sensitive mutants (10), which appear spontaneously when serine auxotrophic strains are grown on minimal medium supple-

TABLE 1. *List of E. coli strains*

Strain	Genotype[a]	Canavanine[b]	Source or comment
AB856	*thi serA*	s	B. Bachmann via N. Kelker
RC-02	*thi serA*	r	Canavanine-resistant mutant of AB856
RC-04	*thi*	s	*serA*$^+$ revertant of AB856
RC-06	*thi*	r	*serA*$^+$ revertant of RC-02
MA176	*thr leu serA lysA thi* Sm^r	s	W. Maas
MA177	*pro trp argE serA speB thi*	s	W. Maas

[a] For gene symbols see Bachmann et al. (5).
[b] Abbreviations: s, sensitive; r, resistant.

mented with L-serine. For transport experiments or for experiments in which binding proteins were isolated, the cells were grown in minimal medium A described by Davis and Mingioli (11) with 20 mM D-glucose as the carbon source. Medium AF is an arginine-free synthetic enriched medium described by Novick and Maas (28). Neopeptone broth was prepared from a digest of beef heart to which was added neopeptone (Difco) (10 g/liter) and NaCl (5 g/liter). Solid medium was prepared by the addition of agar to a final concentration of 2%. For the testing of canavanine sensitivity L-canavanine was added at 100 μg/ml. Putrescine was used at 10 μg/ml. Cells were grown with gyratory shaking at 37°C to mid-exponential phase, except for large-scale cultures used for the isolation of binding proteins which were grown to stationary phase. Growth was measured by determining the optical density (OD) with a Lumetron colorimeter.

Chemicals. L-[3-^{3}H]arginine, L-[U-^{14}C]arginine, L-[4,5-^{3}H]lysine, L-[3-^{3}H]ornithine, L-[3-^{3}H]histidine, L-[U-^{14}C]glutamine, and L-[U-^{14}C]proline were purchased from New England Nuclear Corp. Aminooxyacetic acid hemi-hydrochloride (AOA), L-canavanine, and chloramphenicol were from Sigma Chemical Co. All amino acids were of L-form unless otherwise specified. Chemical contamination with arginine of amino acids and analogues used in competition experiments was monitored by using a Beckman amino acid analyzer (model 120C).

Isolation of canavanine-resistant mutants. Strain AB856 was mutagenized with *N*-methyl-*N'*-nitro-*N'*-nitrosoguanidine (1). After washing, 0.2 ml of the mutagenized culture was inoculated into neopeptone broth and allowed to grow for 15 h. The culture was then washed twice with medium A and canavanine-resistant strains were selected on AF agar plates containing canavanine. Colonies that grew after 16 h of incubation at 37°C were purified by restreaking on the same medium and then grown in liquid cultures for the preparation of transport studies.

Assay for transport activity. Cells harvested by centrifugation from the middle of the exponential phase were washed twice by centrifugation with medium A 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 chloramphenicol (CAP), 8 mM AOA, and 20 mM glucose. AOA was included in the assay as an inhibitor of amino acid decarboxylation (8, 37). The cells were used for transport studies 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. Portions (0.5 ml) of the mixture were filtered onto membrane filters (25 mm, 0.45-μm pore size, Millipore Corp.) and washed with a solution containing 10 ml of 0.01 M tris(hydroxymethyl)-aminomethane (Tris)-hydrochloride (pH 7.3), 0.15 M NaCl, and 5×10^{-4} M $MgCl_2$ (4), at room temperature. The filters were dried and counted in a Nuclear Chicago Mark I liquid scintillation counter in vials containing 7 ml of LSC complete [5.0 g of 2,5-diphenyloxazole and 0.2 g of 1,4-bis-(5-phenyloxazolyl)benzene, per liter of toluene, York Town Research]. The concentration of cells was adjusted so that less than 10% of the labeled substrate was taken up during the first 15 s of incubation time. The values of 15 s and 30 s were used to calculate initial rates of influx. When only initial rates of uptake were measured, CAP and AOA were not included in the reaction mixture. The amount of labeled substrate bound on the surface of cells in addition to the amount of substrate trapped on the filter and the intercellular space was determined by adding cells to the filters followed by the addition of a volume of radioactive reaction mixture (equal to the volume poured onto the filters after incubation with cells) and immediately thereafter the addition of 10 ml of washing buffer.

Exit of arginine. To 1 ml of the above-described cell suspension 9 ml of L-[^{3}H]arginine was added and incubated at room temperature until steady state was reached. A 0.5-ml volume was withdrawn to determine the steady state of accumulation of radioactive arginine. Immediately thereafter unlabeled arginine in 20 μl of concentrated solution was added at time zero, and 0.5-ml samples were withdrawn and filtered. Time was recorded when each sample was filtered.

Osmotic shock. Osmotic shock was performed by the procedure of Neu and Heppel modified for the isolation of binding proteins (35). In stage I of the shock procedure the concentration of ethylenediaminetetraacetic acid was 1 mM and in stage II cold deionized water was used in place of 0.5 mM $MgCl_2$.

DEAE-cellulose chromatography. Fractionation of the crude shock fluid on diethylaminoethyl (DEAE)-cellulose was performed at 4°C, following the procedure described by Rosen (33). The crude shock fluid was concentrated in an Amicon model

401 ultrafiltration cell equipped with a PM-10 filter. The buffer to equilibrate the Whatman DEAE-cellulose DE-52 (microgranular, 1 meq/g) was 5 mM Tris-hydrochloride, pH 7.3. After the crude shock fluid was applied, the column was washed with 2 column volumes of 5 mM Tris-hydrochloride (pH 7.3), followed by a linear gradient in which the mixing chamber contained 5 mM Tris-hydrochloride buffer (pH 7.3) and the reservoir contained 0.1 M NaCl in the same buffer.

Assay for binding activity. Binding activities were measured by the technique of equilibrium dialysis in Plexiglas cells described by Furlong et al. (14) with 0.1 ml of protein solution in one side and 0.1 ml of radioactive substrate in the other side. For measuring the dissociation constants of the binding protein-amino acid complexes the labeled substrates were prepared in 0.01 M potassium phosphate buffer (pH 7.0) containing 0.05 M NaCl (33). To detect binding activity on fractions eluted from the DEAE-cellulose column the labeled substrates were prepared in 5 mM Tris-hydrochloride buffer (pH 7.3), containing 0.05 M NaCl. The binding reaction was allowed to proceed to equilibrium by rotating the binding chambers for 16 h at 4°C. Volumes (25 μl) were then removed from each side and counted in 7.5 ml of Aquasol.

Enzyme assays. Ornithine transcarbamylase (OTC) activity was determined by a variation of Jones et al. (18) in suspension of toluenized cells (25). One unit of OTC activity represents the amount of enzyme which catalyzes the production of 1 μmol of citrulline in 1 h at 37°C under the standard assay conditions.

Arginyl-transfer ribonucleic acid (tRNA) synthetase activity was determined by measuring the incorporation of L-[^{3}H]arginine into tRNA (General Biochemicals). Extracts were obtained after harvesting the cells by centrifugation and washing them in a solution containing 0.01 M Tris-hydrochloride (pH 7.3), 0.01 M $MgCl_2$, and 0.006 M mercaptoethanol (TMM buffer) (16). The washed cells were suspended in TMM buffer and disrupted by sonic extraction in a Branson Sonifier (model 125) at setting 5 for 1 min. After centrifugation, the supernatant extract was dialyzed for 16 h at 4°C against TMM buffer. The assay system contained, in a total volume of 0.1 ml: 10 μmol of Tris-hydrochloride buffer (pH 7.5), 0.1 μmol of adenosine 5'-triphosphate, 1.0 μmol of $MgCl_2$, 5.0 nmol of L-[^{3}H]arginine (100 μCi/μmol), 1.0 mg of *E. coli* tRNA, and cell-free extract containing between 5 and 10 μg of protein. After incubation at 37°C for 30 s, 1 min, and 10 min, 50 μl was pipetted onto 3 MM Whatman filter paper disks (diameter, 24 mm). The disks were plunged immediately into ice-cold 5% trichloroacetic acid containing 1% nonradioactive arginine. After 15 min, these were treated twice with fresh solution of the same composition, each time for 15 min, three times with 5% trichloroacetic for 15 min, and two times with ethanol-ether (1:1) for 15 min. Finally they were washed with ether at room temperature, dried, and placed into glass vials containing 10 ml of LSC complete and counted. One unit of enzyme activity represents the amount of enzyme which catalyzes the incorporation of 1 nmol of arginine into tRNA in 1 min at 37°C.

Biosynthetic arginine decarboxylase, biodegradative (inducible) arginine decarboxylase, and agmatine ureahydrolase were measured by the methods of Hirshfield et al. (17).

Protein determinations. Protein determination was carried out by the method of Lowry et al. (22) with crystalline bovine serum albumin as standard.

Transductions. Transductions employing the P1-like bacteriophage 363 were carried out as described by Glansdorff (15).

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis used the discontinuous buffer system and apparatus described by Studier (36). Details of the procedure are described in the accompanying paper (7).

RESULTS

Comparative uptake of amino acids in parent and mutant strains. Figure 1 shows that arginine and ornithine uptake by the mutant are markedly lower than in the parent strain. At the initial external concentrations used in these experiments—1 μM for arginine and 10 μM for ornithine—most of the substrate is taken up by the specific transport system of each amino acid. The concentration ability of the general system (LAO system) can be tested best by measuring lysine transport at 0.1 μM and that of the specific lysine system can be

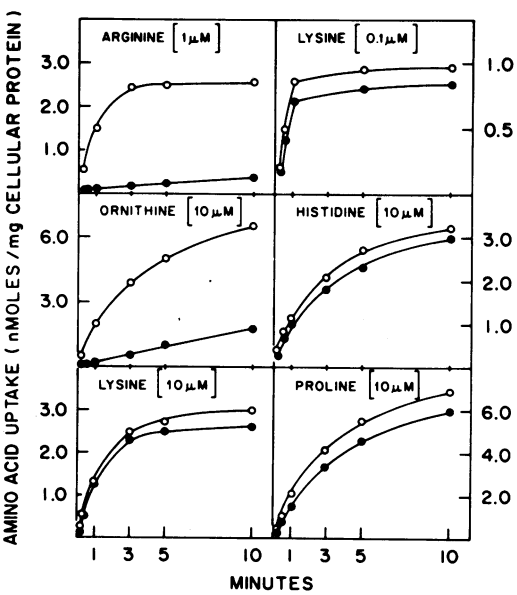

FIG. 1. *Time dependence uptake of labeled amino acids in wild-type E. coli and mutant (RC-06). Uptake was measured as outlined in the text. The initial external concentration is indicated for each substrate. Symbols:* ○, *uptake in wild-type E. coli;* ●, *uptake in RC-06.*

tested best by using lysine at 10 μM (8). The levels of lysine transport in the mutant are normal at both concentrations. The transport activity of histidine, a compound related to the transport systems of basic amino acids in *E. coli* (8), as well as unrelated substrates such as proline and glutamine, were also investigated in both strains. In all cases, similar levels of transport were found in the mutant and wild-type strains. The maximum concentration ratio (ratio of intracellular concentration to extracellular concentration) of arginine was found to be reduced from 410 (parent strain) to 85 in the mutant, and from 200 to 25 in the case of ornithine. A value of 2.7 μl of cell water per mg of dry weight was used for the calculations of internal concentrations (40).

Kinetics of arginine, ornithine, and lysine transport in parent and mutant strains. Since the time dependence uptake experiments suggested an unaffected lysine transport activity in the mutant, it was of interest to confirm the difference in uptake ability of lysine and the other two amino acids (arginine and ornithine) in this strain. Therefore, the kinetics of transport of lysine, arginine, and ornithine in wild-type and mutant strains were investigated.

The initial rates of arginine, ornithine, and lysine influx into RC-06 mutant and isogenic RC-04 at different substrate concentrations were compared. The data were analyzed by applying methods described by Reid et al. (31) for obtaining two K_m and two V_{max} values from a single biphasic plot. The S/V versus S plot of data typical of lysine entry is presented in Fig. 2. The two lysine transport systems are present in the mutant with K_m and V_{max} values close to that of wild-type strain. In contrast, the S/V versus S plot (Fig. 3) of data for the variation in initial rates of arginine influx in the mutant can be best described by a straight line, giving a single set of constants (Table 2). While the apparent K_m value found in the mutant for arginine entry is comparable to the K_m value of the specific arginine transport system in the parent strain, the maximal rate constant is considerably lower. The two ornithine systems

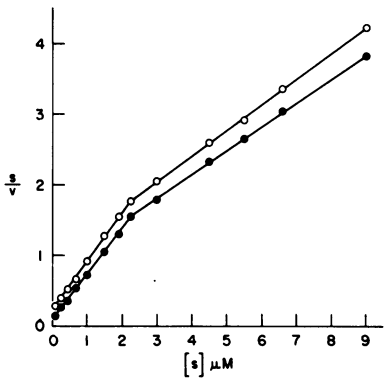

FIG. 2. *Kinetics of L-lysine in parental and mutant strains. Cells were grown in minimal medium and initial rates of uptake were measured as outlined in the text using L-[^{3}H]lysine at different concentrations between 10^{-8} and 10^{-4} M. The circles represent the experimental initial rates; the line was drawn by using the theoretical values calculated from the equation for two (12) simultaneous reactions on the same substrate. Symbols: ●, wild-type strain; ○, mutant strain; S/V, molarity per nanomole per milligram of cellular protein per minute.*

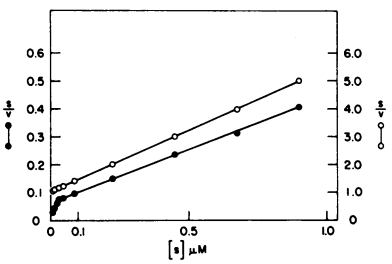

FIG. 3. *Same as Fig. 2, but with L-[^{3}H]arginine. Symbols: ●, parent strain; ○, mutant strain; S/V, molarity per nanomole per milligram of cellular protein.*

TABLE 2. *Kinetic values for uptake of lysine, arginine, and ornithine on isogenic strains RC-04 (parent) and RC-06 (mutant)*[a]

Substrate	Strain RC-04 (parent)		Strain RC-06 (mutant)	
Lysine	$K_1 = 2.25 \times 10^{-6}$ M	$V_1 = 2.8$	$K_1 = 3.0 \times 10^{-6}$ M	$V_1 = 2.7$
	$K_2 = 1.0 \times 10^{-7}$ M	$V_2 = 1.5$	$K_2 = 2.5 \times 10^{-7}$ M	$V_2 = 1.6$
Arginine	$K_1 = 1.25 \times 10^{-7}$ M	$V_1 = 2.5$	$K_1 = 2.0 \times 10^{-7}$ M	$V_1 = 0.23$
	$K_2 = 1.0 \times 10^{-8}$ M	$V_2 = 0.1$		
Ornithine	$K_1 = 3.0 \times 10^{-6}$ M	$V_1 = 2.0$	$K_1 = 1.25 \times 10^{-6}$ M	$V_1 = 0.3$
	$K_2 = 1.0 \times 10^{-7}$ M	$V_2 = 0.4$	$K_2 = 2.0 \times 10^{-7}$ M	$V_2 = 0.16$

[a] The constants were calculated from S/V versus S plots as described in the text. V_1 and V_2 were measured in nanomoles per milligram of cellular protein per minute. Initial rates of uptake were measured as described in the text.

were found to be functioning in the mutant, the specific one with a reduced efficiency for ornithine accumulation (Fig. 4).

Canavanine inhibition of arginine-specific transport system. As previously shown, canavanine inhibits the entry of arginine, lysine, and ornithine through the general (LAO) and ornithine-specific transport systems in *E. coli* (8). However, when arginine influx was measured at 1 μM external concentration (for testing the arginine-specific permease), only 20% of transport activity was inhibited with 100 molar-fold excess of canavanine (8). Since the mutation on strain RC-06 seems to affect the arginine-specific system, in addition to the ornithine permease, it was necessary to explain why a transport mutant, selected as a canavanine-resistant strain, may have a defective arginine-specific transport system. The inhibition of arginine influx by canavanine was studied in the parent strain by measuring arginine uptake at the minimal initial external concentration (0.25 μM) which is still able to saturate the specific system, in the absence and the presence of increasing concentrations of canavanine. When canavanine was included in the assay at a concentration 1,000-fold higher than that of arginine, a substantial reduction (65%) in arginine entry was detected (Table 3). This suggests that canavanine also inhibits the arginine-specific system and is presumably a substrate of such system.

Metabolism of arginine in strain RC-06. In addition to transport-defective mutants, three classes of canavanine-resistant strains have been reported in *E. coli* (24). In nonrepressible (*argR*) strains, the resistance to canavanine is due to an increase in the internal level of arginine as a result of derepression of the arginine biosynthetic enzymes (19). A second type of mutant, *argS*, has a defective arginyl-transfer RNA synthetase, and the partial block in the incorporation of arginine into tRNA leads also to an increase in the arginine pool (16). In a third class of mutants, Can^R, no alteration in metabolism or transport was detected (24). In a related connection, it is possible that a block in the conversion of arginine into putrescine would also lead to a larger pool of endogenous arginine. It was, therefore, desirable to rule out the possible influence of changes in the pool of arginine on the transport of amino acids in these studies, and the metabolism of arginine was explored in the mutant and parent strains. As shown in Table 4, the repression by arginine of the arginine-forming enzymes, as measured by the rate of formation of OTC, is not impaired in the mutant, and extracts prepared from cells of each of the strains have similar levels of arginine tRNA synthetase activity. The levels of enzymes involved in the conversion of arginine into putrescine are presented in Table 5.

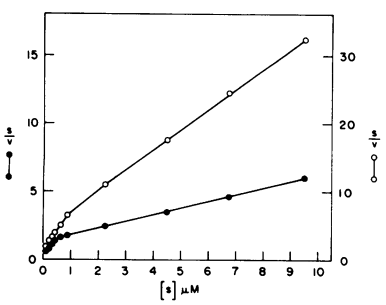

FIG. 4. *Same as Fig. 2, with* L-[^{3}H]*ornithine. Symbols:* ●, *parent strain;* ○, *mutant strain; S/V, molarity per nanomole per milligram of cellular protein.*

TABLE 3. *Inhibition of the arginine-specific transport system*[a]

Addition (μM)	[^{3}H]arginine uptake (nmol/min per mg)	% of control
	1.80	100
L-Lysine, 25	1.55	86
L-Lysine, 250	1.46	81
L-Ornithine, 25	1.51	84
L-Ornithine, 250	1.49	83
L-Histidine, 25	1.65	92
L-Histidine, 250	1.60	89
L-Canavanine, 25	1.29	72
L-Canavanine, 250	0.63	35
L-Homoserine, 25	1.62	87
L-Homoserine, 250	1.64	91
D-Arginine, 25	1.27	71
D-Arginine, 250	1.20	67

[a] The cells, from wild-type *E. coli*, were grown on minimal medium A (11). Initial rates of uptake were measured as outlined in the text. L-[^{3}H]arginine was used at 0.25 μM initial external concentration.

TABLE 4. *Levels of OTC and arginyl-tRNA synthetase in parent and mutant strains*

Enzyme	Enzyme sp act (U/mg of protein)[a]	
	Parent (RC-04)	Mutant (RC-06)
OTC	1.84	2.2
Arginyl-tRNA synthetase	14.24	10.80

[a] OTC units are micromoles of citrulline produced in 1 h at 37°C; arginyl-tRNA synthetase units are nanomoles of arginine incorporated into tRNA in 1 min at 37°C. Cells were grown on AF medium (28), supplemented with 100 μg of arginine per ml. Enzyme assays are described in the text.

TABLE 5. *Activities of enzymes involved in the conversion of arginine into putrescine*[a]

Enzyme	Product measured	Enzyme sp act (μmol/min per mg of protein)	
		Parent (RC-04)	Mutant (RC-06)
Arginine biosynthetic decarboxylase	CO_2	0.0090	0.0080
Arginine biodegradative Decarboxylase (inducible)	CO_2	0.173	0.188
Agmatine ureahydrolase	Urea	0.072	0.066

[a] Procedures used for enzyme assays were those of Hirshfield et al. (17).

Here again it can be seen that the activities of these enzymes are not affected by the mutation in strain RC-06. Thus, the reduction of transport activity in strain RC-06 cannot be attributed to changes in the internal pool of arginine by a nonrepressible arginine biosynthetic pathway or a block in the conversion of the amino acid into putrescine or arginyl-tRNA.

Efflux of arginine and determination of a diffusion coefficient involved in arginine transport. It has been suggested, as a possible explanation for the reduced uptake of a labeled substrate by a mutant strain, that these cells may be leakier than those of the wild type. In this situation the mutant may not be capable of retaining the pool of the tested substrate, and the observed reduction in the accumulation of such compound could rather be due to dilution of the labeled substrate by endogenously formed compound.

To determine whether exit of arginine by the mutant could account for the defective uptake of arginine, cells from wild-type and mutant strains were allowed to a accumulate radioactive arginine after which the rates of efflux were measured. Most of the intracellular arginine could be eliminated, by a cold chase, in wild-type strain, and loss of half the accumulated arginine occurred in approximately 1 min (Fig. 5). Efflux of the amino acid from the mutant strains was markedly lower; the rate of arginine exit was equivalent to only 20% of that observed in cells from the wild-type strain. Even though a transmembranal efflux effect, due to the large excess of unlabeled arginine used in these experiments, may have some influence on the true exit (32), it is clear that the reduction of uptake by the mutant cannot be ascribed to an increased efflux of arginine. Rather, the experiment suggests an impaired mechanism of exit of the amino acid in the mutant strain.

The relative contribution of diffusion on arginine transport was determined experimentally from the kinetics of entry and equilibration (9) by measuring arginine uptake on the mutant strain in the presence of canavanine (1 mM) as a competitive inhibitor. Cells were grown on minimal medium supplemented with labeled arginine to reduce the remaining transport activity to a basal level by repression. A value of D, equivalent to 0.05 μmol/min per ml of cell water for a gradient of 1 mM, was obtained from these kinetics. Under the conditions used in these experiments, comparable values of D were obtained with wild-type cells.

Binding proteins in strain RC-06. Binding activities between labeled arginine, ornithine, and lysine on crude shock fluid released from parent and mutant strains are presented in Table 6. As shown, the same amounts of binding activities were detected by equilibrium dialysis when preparations from the two strains were studied. In addition, binding proteins were fractionated by DEAE chromatography of shock fluids. Their affinities with arginine, lysine, and ornithine were investigated. Fraction of the crude shock fluid produced two peaks with arginine-specific binding activity, in agreement with the work of Rosen (33) and Wilson and Holden (38). The first protein (arginine-specific binding protein I) was detected in the 5 mM Tris-hydrochloride (pH 7.3) wash

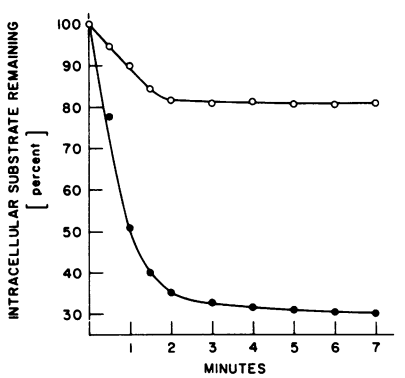

FIG. 5. *Efflux of L-[^{3}H]arginine from parent and mutant strains. The cells were loaded with labeled arginine at 1 μM initial external concentration at room temperature under the conditions described in the text. The cells from the wild-type strain were incubated for 15 min and those from the mutant strain were incubated for 30 min. The amount of substrate accumulated was estimated by filtering a portion. At zero time, unlabeled arginine (20 μl) was added to give a final concentration of 1 mM. Cells were filtered at the indicated times. At zero time the cells had accumulated arginine to the following extents, expressed as nanomoles per milligram of cellular protein: RC-04, 2.20; RC-06, 0.31. Symbols: ●, strain RC-04 (parent), ○, strain RC-06 (mutant).*

TABLE 6. *Binding activity in shock fluid*[a]

Strain	Total units[b]			Sp act[c]		
	Arg	Lys	Orn	Arg	Lys	Orn
RC-04 (parent)	117	60	59	0.65	0.34	0.33
RC-06 (mutant)	122	65	65	0.59	0.32	0.35

[a] The activity for each substrate was determined at 10 μM initial concentration by equilibrium dialysis as outlined in the text.
[b] Nanomoles of amino acid bound to the shock fluid obtained from a 10-liter culture.
[c] Nanomoles of amino acid bound per milligram of shock fluid protein.

buffer fraction, and the second (arginine-specific binding protein II) was eluted with 0.03 M NaCl. The LAO binding protein (33) was released from the column with 0.02 M NaCl. When the dissociation constants of binder-amino acid complexes were determined by equilibrium dialysis, for each of the three amino acids, similar values were obtained from the Scatchard plots of data with preparations of the mutant and parent strain. The arginine-specific binding protein I from both strains has a K_d of approximately 1 μM; the arginine-specific binding protein II extracted from the parent strain gives a K_d value of 0.1 μM; and the same molecule from the mutant has a K_d of 0.2 μM. The comparative affinity values for each of the three amino acids with the LAO binding proteins also were found to be of the same order of magnitude.

From studies carried out on histidine transport-deficient mutants in *Salmonella typhimurium* (21), it has been shown that mutations affecting the histidine-J binding protein can produce a molecule with altered electrophoretic mobility in the presence of sodium dodecyl sulfate, with the protein still having a normal affinity for histidine. The conclusion suggested from these findings is that an active site in the binding protein, other than its histidine-binding site and necessary for interaction with other transport component, may be affected by the mutation. To test this possibility, the shock fluids and the binding proteins isolated after DEAE chromatography from parent and mutant strains were subjected to electrophoresis on sodium dodecyl sulfate acrylamide on slab gels. The analysis of the electrophoretic patterns did not reveal any significant difference between preparation of mutant and parent strains. The three binding proteins from the mutant and wild-type strains were found to be released in similar amounts and each of them from both strains exhibited protein bands with identical mobilities. The conclusion drawn from these studies is that the periplasmic binding proteins are not affected by the mutation on strain RC-06.

Mapping of the mutation on strain RC-06. An early indication of genetic linkage between the *serA* locus and the mutation conferring canavanine resistance on strain RC-06 was revealed (Fig. 6) by studying protrotrophic revertants of the serine A marker obtained by transduction with a P1 phage made on a wild-type *E. coli* K-12 strain as donor and strain RC-02 as recipient. Ninety percent of the *serA*$^+$ transductants were canavanine sensitive and 10% remained canavanine resistant. Transport studies of arginine, ornithine, and lysine on 12 of these (*serA*$^+$, canavanine resistant) transductants showed that all had the transport characteristics of the original isolated mutant (RC-02).

The mutation was further localized by P1-transduction experiments, crossing strain RC-06 (*serA*$^+$, *lysA*$^+$, canavanine resistant) as donor and strain MA176 (*serA*, *lysA*, canavanine sensitive) as recipient. Among 451 *lysA*$^+$ colo-

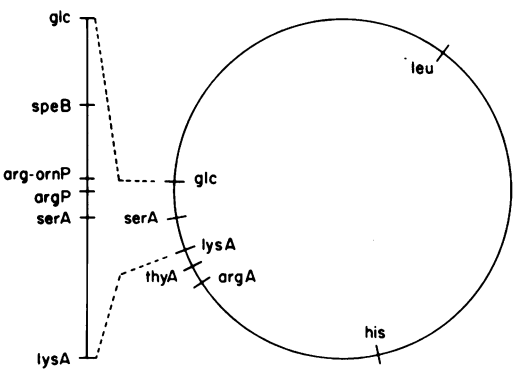

FIG. 6. *Simplified map of the E. coli chromosome showing the positions of relevant genes used in this study. Genetic symbols are those of Bachman et al. (5), except for arg-ornP, in the expanded serA region, which indicates the position of the mutated locus in the arginine and ornithine transport-defective mutant strain.*

nies tested, 11 were *serA*$^+$ and 3 of these were canavanine resistant with low arginine and ornithine uptake. These results suggested that the mutation is affecting a locus located on the *argP* side of the *serA* gene. This was confirmed by another transduction experiment, using RC-06 (*serA*$^+$, *speB*$^+$, canavanine resistant) as donor and strain MA177 (*serA*, *speB*, canavanine sensitive) as recipient. The co-transduction frequencies with *serA*$^+$ in this experiment were 90% for canavanine resistance and 45% for *speB*$^+$. All *speB*$^+$ were canavanine resistant. Investigation of transport activity on 20 canavanine-resistant transductants showed that all had reduced levels of arginine and ornithine uptake. The conclusion is that the mutation maps in a locus closely linked or identical to *argP*.

DISCUSSION

From recent studies on the transport of arginine, ornithine, and lysine in bacteria, some useful conclusions are beginning to emerge. The presence of one common transport system for the three amino acids has been observed in *E. coli* (8, 23), *S. typhimurium* (29), and *Pseudomonas putida* (13). In addition, specific systems for each of the three amino acids have been found in most of the organisms studied (8, 13, 20, 29, 33, 37).

The ability of the arginine-specific transport system to recognize arginine but to reject ornithine and lysine has been well defined in *S. typhimurium* (29). Compounds such as L-homoserine and *trans*-hydroxyproline were found to be good inhibitors, although no substrates, of the system in *S. typhimurium*, and evidence was obtained for the structural requirements of the transport receptor site of this permease. It has been suggested that the secondary nitrogen of arginine may act as a hydrogen donor in the recognition process (29). When L-homoserine was tested as a competitive inhibitor of arginine uptake in *E. coli*, no inhibition was detected and a minimal reactivity was observed, not only toward the simple diamino acids but also with most of the analogues studied (Table 3 and reference 8). Only L-canavanine can compete visibly with arginine uptake and D-arginine appears to be a moderate inhibitor. With the concentration of arginine used in these experiments, the main route of entry is the specific transport system, the contribution of the general system being less than 5%. Therefore, the observed inhibition by canavanine of arginine uptake is consistent with the transport properties of the mutant strain RC-06, isolated as a canavanine-resistant strain, which has a defective arginine-specific permease and an intact general transport system. Studies on a related mutant (described in the accompanying paper [7]) isolated as a strain able to utilize D-arginine as a source of L-arginine, which shows an increased sensitivity to inhibition of growth by canavanine and high levels of arginine-specific transport system activity, suggest that both compounds, canavanine and D-arginine, might enter the cell as substrates of the arginine-specific permease in *E. coli* K-12 (7).

The finding of a single kinetics for arginine transport in the mutant, with a K_m value comparable to that of the arginine-specific system from wild-type cells (Fig. 3, Table 2), could be an indication that the high-affinity system (general transport system) for arginine uptake is not operating in the mutant. Alternatively, it may simply represent the addition of two sets of values that now are linear in the S/V versus S plot of data, within experimental error. Since the general transport system appears to be functional (Fig. 2), and the contribution given by this system to the uptake of arginine is low, even at low substrate concentrations (8), the most plausible explanation for the single K_m is that both specific and general systems are operating in the mutant. It would appear that the substantial reduction in the apparent V_{max} value of the arginine-specific transport system renders the "break" in the S/V versus S plot of data undetectable. A similar situation has been found in a leucine transport mutant where a single K_m value, intermediate between the affinity constants of the two leucine transport systems present in the wild-type strain, was obtained (3).

Evidence has been previously obtained that indicates the existence of a common regulatory mechanism for the synthesis of the arginine and ornithine transport systems (8). Either arginine or ornithine represses simultaneously the formation of both systems. This evidence, in conjunction with the observation that only arginine and ornithine are affected in their active transport in the mutant, without any detectable change in the periplasmic binding proteins, led us to consider the proposal that the mutation affects the synthesis of membrane carriers which are components of the arginine and ornithine transport systems.

Experiments presented here show that both the rates of uptake and steady state of accumulation of arginine and ornithine are affected by the mutation on strain RC-06. At the steady state of accumulation, the rate of entry equals the rate of exit, a situation that can be formally described by the steady-state equation (26, 31) as follows:

$$V_{max}\ (\text{influx})\frac{So}{K_m\ (\text{influx}) + So} + D\ So = V_{max}\ (\text{efflux})\frac{Si}{K_m\ (\text{efflux}) + Si} + D\ Si$$

The rate of entry of substrate is given by the terms to the left of the equals sign and the rate of exit is given by the terms to the right. V_{max} and K_m are the Michaelis-Menten terms, and D is a coefficient describing a "diffusion" component. At the initial external concentrations of substrate used, the contribution by diffusion is usually minimal. The concentration gradient (Si/So) at the steady state will be determined, in the absence of a diffusion component, by a higher affinity (K_m) of the membrane carriers for entry than for exit, or by a higher number of carriers (V_{max}) involved in influx than in efflux. By the same analysis a reduction in the ratio Si/So (as it is seen in the mutant strain) would depend on changes in the "affinity ratio" K_m efflux/K_m influx, on fewer functional membrane carriers implicated in entry than in exit, or on a combination of both circumstances. Analysis of the steady-state equation shows that the gradient (Si/So) is independent of the number of membrane carriers (V_{max}), which implies that the same steady-state gradient would be attained in cells with different numbers of membrane carriers. Cells containing more carriers than those of the normal organism would reach the steady state of accumulation in a shorter time. In cells with less carriers, the time necessary to attain the steady state would increase. However, it has been shown experimentally, in the lactose transport system (26), that the gradient (Si/So) at the steady state is entirely dependent on the number of membrane carriers. In uninduced cells, with 3% of the normal number of carriers, the steady-state gradient of thiomethylgalactoside accumulation is equivalent to only 15% of that found in fully induced cells. It has been suggested that a contribution by the diffusion component may account for this discrepancy. In particular, when the number of carriers is low, diffusion at the steady state would be responsible for a substantial amount of substrate that leaves the cell (26). The apparent K_m values for arginine and ornithine entry in the mutant and wild-type strains, described here, are of the same order of magnitude. In contrast, a significant reduction in the maximal influx (V_{max}) values for entry of both amino acids in the mutant was revealed by the kinetic analysis. The relative contribution of diffusion on the exit of arginine from cells of both strains, calculated from the steady-state equation (D equals 0.05 μmol/min per ml of cell water for a gradient of 1 mM), revealed that diffusion accounts for approximately 5% of arginine efflux in the wild type, whereas more than 30% of the substrate leaves the cell via a diffusion pathway in the mutant strain. These results are taken to mean that the difference between the wild-type and mutant strains in their capacity to accumulate arginine might be due, by analogy with the lactose transport system, to the presence of fewer membrane carriers for arginine transport in the mutant. They strongly suggest that the mutation may have affected the synthesis of membrane carriers which are components of the arginine- and ornithine-specific transport systems. The locus in which these mutations have occurred presumably is a regulatory gene concerned with controlling the amount of membrane carriers produced. The finding of a related mutant (described in the accompanying paper [7]) with derepressed levels of arginine- and ornithine-specific transport systems activity lends further support to this suggestion.

Transport mutants affected in a regulatory mechanism with either defective or derepressed levels of transport have been found in other systems such as leucine (30), glutamine (39), cystine (6), and glutamate (27) in *E. coli*, and histidine in *S. typhimurium* (2). The effect of a reduced number of transport elements has also been simulated by the osmotic shock procedure, where the K_m value is unaffected and the apparent V_{max} is reduced (34).

The term "regulation" (of synthesis of transport carriers) is used here with a definitional connotation, i.e., to correlate changes observed in transport activity with the absence or presence of substrates on the growth media. The molecular basis of this phenomenon at present is not apparent since no information is available on the repression complex, its mechanism of action, or site of action.

The mutant strain RC-06 has been useful in confirming previous findings on the complexities of the transport of basic amino acids in *E. coli* (8). The existence of a shared mechanism of regulation on the formation of components of the arginine- and ornithine-specific transport systems, suggested from repression studies, is now confirmed genetically with the isolation and characterization of the mutant strain described in this report.

ACKNOWLEDGMENTS

I thank Roseanne Prince for her valuable assistance and Ellena Kappa for typing the manuscript.

This work was supported by Public Health Service grant 5 RO1 AI 12062 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Adelberg, E. A., M. Mandel, and G. C. Chen.** 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitro-nitrosoguanidine in *Escherichia coli* K-12. Biochem. Biophys. Res. Commun. 18:788-795.
2. **Ames, G. F. L., and J. Lever.** 1970. Components of histidine transport: histidine-binding proteins and *hisP* protein. Proc. Natl. Acad. Sci. U.S.A. 66:1096-1103.
3. **Anderson, J. J., S. T. Quay, and D. L. Oxender.** 1976. Mapping of two loci affecting the regulation of branched-chain amino acid transport in *Escherichia coli* K-12. J. Bacteriol. 126:80-90.
4. **Anraku, Y.** 1968. Transport of sugars and amino acids in bacteria. J. Biol. Chem. 243:3128-3135.
5. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. 40:116-167.
6. **Berger, E. A., and L. Heppel.** 1972. A binding protein involved in the transport of cystine and diaminopimelic acid in *Escherichia coli* K-12. J. Biol. Chem. 247:7684-7694.
7. **Celis, T. F. R.** 1977. Independent regulation of transport and biosynthesis of arginine in *Escherichia coli* K-12. J. Bacteriol. 130:1244-1252.
8. **Celis, T. F. R., H. J. Rosenfeld, and W. K. Maas.** 1973. Mutant of *Escherichia coli* K-12 defective in the transport of basic amino acids. J. Bacteriol. 116:619-626.
9. **Christensen, H. N.** 1975. Kinetics of transport, p. 107-164. *In* H. N. Christensen (ed.), Biological transport. W. A. Benjamin, Inc., New York.
10. **Cosloy, S. D., and E. McFall.** 1970. L-Serine-sensitive mutants of *Escherichia coli* K-12. J. Bacteriol. 103:840-841.
11. **Davis, B. D., and E. S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B12. J. Bacteriol. 60:17-28.
12. **Dixon, M., and E. C. Webb.** 1964. Enzymes, p. 329. Academic Press Inc., New York.
13. **Fan, C. L., D. L. Miller, and V. W. Rodwell.** 1972. Metabolism of basic amino acids in *Pseudomonas putida*. Transport of lysine, ornithine and arginine. J. Biol. Chem. 247:2283-2288.
14. **Furlong, C. E., R. G. Morris, M. Kandrach, and B. P. Rosen.** 1972. A multichamber equilibrium dialysis apparatus. Anal. Biochem. 47:514-526.
15. **Glansdorff, N.** 1965. Topography of cotransducible arginine mutations in *Escherichia coli* K-12. Genetics 51:167-179.
16. **Hirshfield, I. N., R. DeDeken, P. C. Horn, D. A. Hopwood, and W. Maas.** 1968. Studies on the mechanism of repression of arginine biosynthesis in *Escherichia coli*. J. Mol. Biol. 35:83-93.
17. **Hirshfield, I. N., H. J. Rosenfeld, Z. Leifer, and W. K. Maas.** 1970. Isolation and characterization of a mutant of *Escherichia coli* blocked in the synthesis of putrescine. J. Bacteriol. 101:725-730.
18. **Jones, M. E., L. Spector, and F. Lipman.** 1955. Carbamyl phosphate, the carbamyl donor in enzymatic citrulline synthesis. J. Am. Chem. Soc. 77:819-820.
19. **Kadner, R. J., and W. K. Maas.** 1971. Regulatory gene mutations affecting arginine biosynthesis in *Escherichia coli*. Mol. Gen. Genet. 111:1-14.
20. **Kustu, S. G., and G. F. Ames.** 1973. The *hisP* protein, a known histidine transport component in *Salmonella typhimurium*, is also an arginine transport component. J. Bacteriol. 116:107-113.
21. **Kustu, S. G., and G. F. Ames.** 1974. The histidine-binding protein J, a histidine transport component, has two different functional sites. J. Biol. Chem. 249:6976-6983.
22. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.
23. **Maas, W. K.** 1965. Genetic defects affecting an arginine permease and repression of arginine synthesis in *Escherichia coli*. Fed. Proc. 24:1239-1242.
24. **Maas, W. K.** 1972. Mapping of genes involved in the synthesis of spermidine in *Escherichia coli*. Mol. Gen. Genet. 119:1-19.
25. **Maas, W. K., R. Maas, J. Wiame, and N. Glansdorff.** 1964. Studies on the mechanism of repression of arginine biosynthesis in *Escherichia coli*. J. Mol. Biol. 8:359-364.
26. **Maloney, P. C., and T. H. Wilson.** 1973. Quantitative aspects of active transport by the lactose transport system of *Escherichia coli*. Biochim. Biophys. Acta 330:190-205.
27. **Marcus, M., and Y. Halpern.** 1969. Genetic analysis of the glutamate permease in *Escherichia coli* K-12. J. Bacteriol. 97:1118-1128.
28. **Novick, R. P., and W. K. Maas.** 1961. Control by endogenously synthesized arginine of the formation of ornithine transcarbamylase in *Escherichia coli*. J. Bacteriol. 81:236-240.
29. **Quay, S., and H. N. Christensen.** 1974. Basis of transport discrimination of arginine from other basic amino acids in *Salmonella typhimurium*. J. Biol. Chem. 249:7011-7017.
30. **Rahmanian, M., D. R. Claus, and D. L. Oxender.** 1973. Multiplicity of leucine transport systems in *Escherichia coli* K-12. J. Bacteriol. 116:1258-1266.
31. **Reid, K. G., N. M. Utech, and J. T. Holden.** 1970. Multiple transport components for dicarboxylic amino acids in *Streptococcus faecalis*. J. Biol. Chem. 245:5261-5272.
32. **Robbie, J. P., and T. H. Wilson.** 1969. Transmembrane effects of β-galactosides on thiomethyl-β-galactoside transport in *Escherichia coli*. Biochim. Biophys. Acta 173:234-244.
33. **Rosen, B. P.** 1971. Basic amino acid transport in *Escherichia coli*. J. Biol. Chem. 246:3653-3662.
34. **Rosen, B. P.** 1973. Basic amino acid transport in *Escherichia coli*: properties of canavanine-resistant mutants. J. Bacteriol. 116:627-635.
35. **Rosen, B. P., and L. A. Heppel.** 1973. Present status of binding proteins that are released from Gram-negative bacteria by osmotic shock, p. 209-234. *In* L. Leive (ed.), Bacterial membranes and walls. Marcel Dekker Inc., New York.
36. **Studier, F. W.** 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. J. Mol. Biol. 79:237-248.
37. **Wilson, O. H., and J. T. Holden.** 1968. Arginine transport and metabolism in osmotically shocked and unshocked cells of *Escherichia coli* W. J. Biol. Chem. 244:2737-2742.
38. **Wilson, O. H., and J. T. Holden.** 1968. Stimulation of arginine transport in osmotically shocked *Escherichia coli* W cells by purified arginine-binding protein fractions. J. Biol. Chem. 244:2743-2749.
39. **Weiner, J. H., and L. Heppel.** 1971. A binding protein for glutamine and its relation to active transport in *Escherichia coli*. J. Biol. Chem. 246:6933-6941.
40. **Winkler, H. H., and T. H. Wilson.** 1966. The role of energy coupling in the transport of β-galactosides by *Escherichia coli*. J. Biol. Chem. 241:2200-2211.