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Phosphorylation of the Periplasmic Binding Protein in Two Transport Systems for Arginine Incorporation in *Escherichia coli* K-12 Is Unrelated to the Function of the Transport System

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In *Escherichia coli* K-12, the accumulation of arginine is mediated by two distinct periplasmic binding protein-dependent transport systems, one common to arginine and ornithine (AO system) and one for lysine, arginine, and ornithine (LAO system). Each of these systems includes a specific periplasmic binding protein, the AO-binding protein for the AO system and the LAO-binding protein for the LAO system. The two systems include a common inner membrane transport protein which is able to hydrolyze ATP and also phosphorylate the two periplasmic binding proteins. Previously, a mutant resistant to the toxic effects of canavanine, with low levels of transport activities and reduced levels of phosphorylation of the two periplasmic binding proteins, was isolated and characterized (R. T. F. Celis, J. Biol. Chem. 265:1787–1793, 1990). The gene encoding the transport ATPase enzyme (*argK*) has been cloned and sequenced. The gene possesses an open reading frame with the capacity to encode 268 amino acids (mass of 29.370 Da). The amino acid sequence of the protein includes two short sequence motifs which constitute a well-defined nucleotide-binding fold (Walker sequences A and B) present in the ATP-binding subunits of many transporters. We report here the isolation of canavanine-sensitive derivatives of the previously characterized mutant. We describe the properties of these suppressor mutations in which the transport of arginine, ornithine, and lysine has been restored. In these mutants, the phosphorylation of the AO- and LAO-binding proteins remains at a low level. This information indicates that whereas hydrolysis of ATP by the transport ATPase is an obligatory requirement for the accumulation of these amino acids in *E. coli* K-12, the phosphorylation of the periplasmic binding protein is not related to the function of the transport system.

The translocation of hydrophilic molecules across bacterial membranes is mediated by specific transport systems that are present in the membrane and are composed of one or more proteins called transport carriers or porters. These systems allow the cell to incorporate nutrients against large concentration gradients at the expense of metabolic energy.

The transport of arginine in *Escherichia coli* K-12 is mediated by two transport systems, one common to arginine and ornithine (AO system) (11) and one common to lysine, arginine, and ornithine (LAO system) (15, 23). Each system includes a specific periplasmic protein and therefore, is sensitive to the effects of cold osmotic shock (15, 23). In addition, a third periplasmic transport system for arginine in *E. coli* has been reported recently (29).

Periplasmic transport systems are present in gram-negative bacteria, and the energy required for the intracellular accumulation of the substrate is provided by a donor of activated phosphoryl groups (6).

Membrane proteins of periplasmic binding protein-dependent transport systems couple ATP binding or ATP hydrolysis to the translocation of a wide variety of solutes, including amino acids, sugars, peptides, polysaccharides, and inorganic ions in prokaryotic organisms (8).

Bacterial periplasmic permeases are complex transport systems. A common view holds that a soluble periplasmic binding protein acting as a receptor, recognizes the incoming substrate, and defines the selectivity of the system. The complex of substrate and protein then interacts with an inner membrane-associated complex that usually is made of three or four protein subunits (8). The molecular mechanism by which transport systems that include an ATP-binding protein carry out their functions is not clear. Similarly, the mechanism through which the free energy of ATP binding or its hydrolysis is coupled to changes leading to the translocation of the substrate is poorly understood.

We previously reported the isolation and characterization of a membrane enzyme of *E. coli* K-12 with an intrinsic ATPase activity related to the incorporation of arginine, ornithine, and lysine into the cell (13). The finding and characterization of a mutant (14) with defective enzymatic activity and significantly reduced levels of substrate incorporation through the AO and LAO transport systems suggested a role for the ATPase activity of the enzyme in the operation of the two transport systems (14).

Here we report the amino acid sequence of the ATPase protein, deduced from the nucleotide sequence of its gene, and the purification of the enzyme. We also report the isolation and characterization of suppressor mutations of the original mutated locus (*argK*) and examine several of their properties, including canavanine phenotypes, transport activities, effects on the ATPase and kinase activities of the ArgK protein, and DNA sequences.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. All strains used in this study were derived from *E. coli* K-12 and are listed in Table 1. Strain DH5αF′ was the host for pTZ18R derivatives and was used for plasmid DNA preparations. DH5αF′

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TABLE 1. Strains and plasmids used

Strain or plasmid	Genotype and/or characteristics	Reference or source
Strains		
RC04	*thi-1* Can^s	14
RC101	*thi-1* Can^r	14
RC120	*thi-1* Ap^r Can^s	14
DH5αF′	*supE44* Δ*lacU169* (ϕ80 *lacZ*ΔM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Gibco-BRL
GI724	λ$^-$ *lacI*^{q} *lacPL8 ampC*::P*trp mcrA mcrB* INV(*rrnD-rnE*)	Invitrogen
AH724	Isogenic of GI724, harbors pRC67	
AH48	*thi-1* Can^s	This study
AH56	*thi-1* Can^s	This study
AH126	*thi-1* Can^s	This study
AH202	*thi-1* Can^s	This study
AH212	*thi-1* Can^s	This study
AH265	*thi-1* Can^s	This study
AH296	*thi-1* Can^s	This study
AH318	*thi-1* Can^s	This study
AH206	*thi-1* Can^s	This study
AH88	*thi-1* Can^s	This study
AH126	*thi-1* Can^s	This study
AH618	*thi-1* Can^s	This study
AH332	*thi-1* Can^s	This study
AH420	*thi-1* Can^s	This study
Plasmids		
pTZ18R	Ap^r Ori *lacZ*^{1} S1IG	14
pRC96	Ap^r Ori f1IG *argK*$^+$	pTZ18R carrying a 9.8-kbp fragment containing *argK*
pRC152	Ap^r Ori f1IG *argK*$^+$	pTZ18R carrying a 2.2-kbp fragment containing *argK*
pLex	Ap^r, expression vector with p_L promoter	Invitrogen
pRC67	Ap^r, pLEX derivative harboring *argK*$^+$	This study

was also the recipient for M13 phage derivatives mp19 and was used to isolate single-stranded DNA. *E. coli* C600 served as the host for λ phages (EMBL4 derivatives of the Kohara collection [20]). The pLEX expression system (Invitrogen) was used for expression of *argK* in *E. coli*. Strain GI724 was used as the host for plasmid pRC67 (Table 1). The minimal medium used was medium A (9) with 20 mM glucose as the carbon source. Medium AF is an arginine-free synthetic enriched medium (10). For testing canavanine sensitivity, L-canavanine was added to medium AF at 100 μg/ml. Induction medium for growing strain AH724 (Table 1) includes 0.2% Casamino Acids, 1.0 mM $MgCl_2$, 20 mM glucose, 6% Na_2HPO_4, 5% NaCl, and 1% NH_4Cl (Invitrogen). The procedures for growing cells have been described elsewhere (9).

Chemicals. [γ-^{32}P]ATP, L-[3-^{3}H]arginine, L-[3^3-H]ornithine, L-[4,5-^{3}H]lysine, [α-^{35}S]dATP, and [α-^{33}P]dATP were purchased from Du Pont-New England Nuclear. Amino-oxyacetic acid hemihydrochloride, L-canavanine, chloramphenicol, ampicillin, and isopropyl-β-D-thiogalactopyranoside were purchased from Sigma. Restriction endonucleases, T4 DNA ligase, exonuclease III, and DNA polymerase I large (Klenow) fragment were purchased from New England Biolabs. RNase, X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and S1 nuclease were purchased from Boehringer Mannheim. 2′-Deoxynucleoside-5′-triphosphates were purchased from Pharmacia Biotech.

Assay for transport activity. Amino acid uptake was measured as described previously (11).

Purification of periplasmic transport proteins. The AO and LAO periplasmic binding proteins (AO and LAO proteins) were purified as previously described (13).

Assay of enzyme activities. The ATPase and kinase activities of the ArgK protein were determined as described previously (13).

Cloning and sequencing. The 9.5-kbp *Eco*RI DNA fragment from plasmid pRC96 (14) was isolated and used to subclone several restriction fragments into the vector pTZ18R. Restoration of transport activity in strain RC101 was investigated by testing ampicillin-resistant transformants for canavanine phenotype and arginine uptake. A restored function of the *argK* locus was detected with plasmid pRC152 carrying a 2.2-kbp *Bam*HI fragment (Fig. 1). The 2.2-kbp DNA fragment was cloned into M13 mp19 bacteriophage in both orientations. To make a nested set of unidirectional deletions, double-stranded replicative-form DNA from the mp19 derivatives was digested with restriction enzymes *Sma*I and *Sac*I. After phenol extraction and ethanol precipitation, the DNA was digested with exonuclease III and S1 nuclease. The ends were repaired and ligated. A series of clones with deletions in increments of 200 to 300 bp was chosen for sequencing. Overlapping sequence was obtained for the 2,140-bp region on both strands. DNA sequencing was performed by the dideoxynucleotide termination method (26), using Sequenase (United States Biochemical). Nested deletions for DNA sequencing were constructed as described in reference 3. Plasmid DNA and M13 bacteriophage were purified and transformation experiments were carried out as described by Sambrook et al. (25).

The nucleotide sequence of the *argK* mutations was determined by direct sequencing of amplified DNA. Isolation and amplification of DNA from the *argK* gene was performed with two synthetic 22-nucleotide (nt) primers complementary to the 3′ boundaries of the gene. A GeneAmp PCR reagent kit (Perkin-Elmer) was used according to the directions included in the kit. The DNA was purified by NuSieve GTG low-melting-temperature agarose; after phenol extraction and ethanol precipitation, it was used as the template in the sequence reactions. We used five additional 20-nt-long primers equally spaced across the *argK* gene. The nucleotide sequence was determined with an AmpliCycle sequencing kit (Perkin-Elmer). For computer analysis of the DNA and protein sequences, the University of Wisconsin Genetics Computer Group software package was used.

Purification of the ATP-binding protein. The *argK* gene was isolated and amplified as described above, using a GeneAmp PCR reagent kit (Perkin-Elmer). Purified DNA was cloned in frame with the lambda *c*II initiation triplet of the expression vector pLEX (Invitrogen) in order to utilize the p_L promoter and ribosome binding site of the pLEX expression system. Expression of the target gene was monitored by analysis of the recombinant protein by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Strain AH7124 was grown in induction medium (see above) at 30°C to an optical density at 600 nm of 0.5. Expression of *argK* was then induced by adding tryptophan to a final concentration of 100 μg/ml, and the culture was incubated for 3 h. The induced cells were harvested by centrifugation and washed twice with a cold buffer of Tris-HCl (pH 7.3) containing 1.0 mM EDTA and 1.0 mM dithiothreitol (DTT). Membrane vesicles were prepared by two passages through a French press cell at 10,000 lb/in^2. Unbroken cells were removed by a centrifugation at 3,000 × *g* for 10 min, and the supernatant was centrifuged at 150,000 × *g* for 60 min. The pellet was washed twice with 0.01 M Tris-HCl (pH 7.3) containing 1.0 mM EDTA and 1.0 mM DTT. For solubilization, 300 mg of membrane proteins was incubated for 30 min in an ice bath at a concentration of 3.0 mg/ml in a solution of 0.05 M potassium phosphate (pH 7.5) containing 1.1% (wt/vol) octylglucoside, 3.0% (wt/vol) *E. coli* phospholipids (Avanti Polar Lipids, Inc.), 1.0 mM EDTA, and 20% (vol/vol) glycerol (2).

Unextracted material was pelleted at 150,000 × *g* for 1 h at 4°C. The clarified supernatant was dialyzed against 0.01 M Tris-HCl buffer (pH 7.3) containing 20% glycerol and 1.0 mM DTT and then loaded on a DEAE-Sephacel column (2 by 41 cm) equilibrated with the same buffer. The protein was eluted with a 0 to 2.0 M NaCl linear gradient (800 ml). Fractions with ATPase activity (13) were pooled, concentrated by ultrafiltration, and chromatographed in a Mono Q HR 10/10 column attached to a Pharmacia FPLC system, using a 0.05 to 0.15 M NaCl

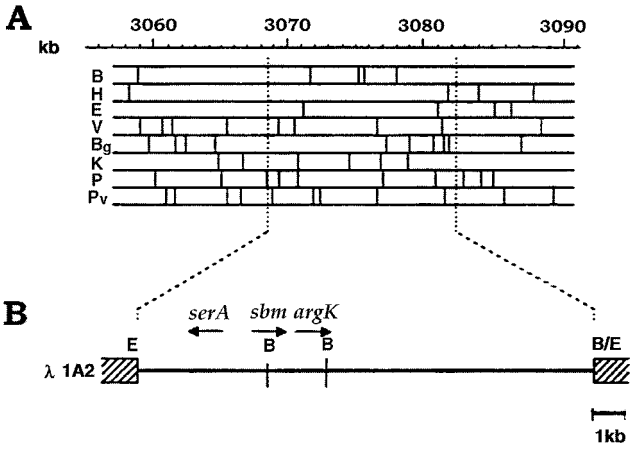

FIG. 1. Physical map of the 65.8-min chromosomal area and locations of λ phage clone 1A2 and the *argK* gene. (A) Chromosomal fragments produced by the eight restriction enzymes used to map the chromosome (20). Kilobase coordinates are shown at the top. (B) Physical map of the λ phage clone 1A2 (miniset 471) from the Kohara collection (20) and location of the sequenced *Bam*HI fragment encoding the *argK* gene. The *argK* gene is located downstream of the *sbm* gene (24) and is transcribed in the same direction. B, *Bam*HI; H, *Hin*dIII; E, *Eco*RI; V, *Eco*RV; Bg, *Bgl*I; K, *Kpm*I; P, *Pst*I; Pv; *Pvu*I.

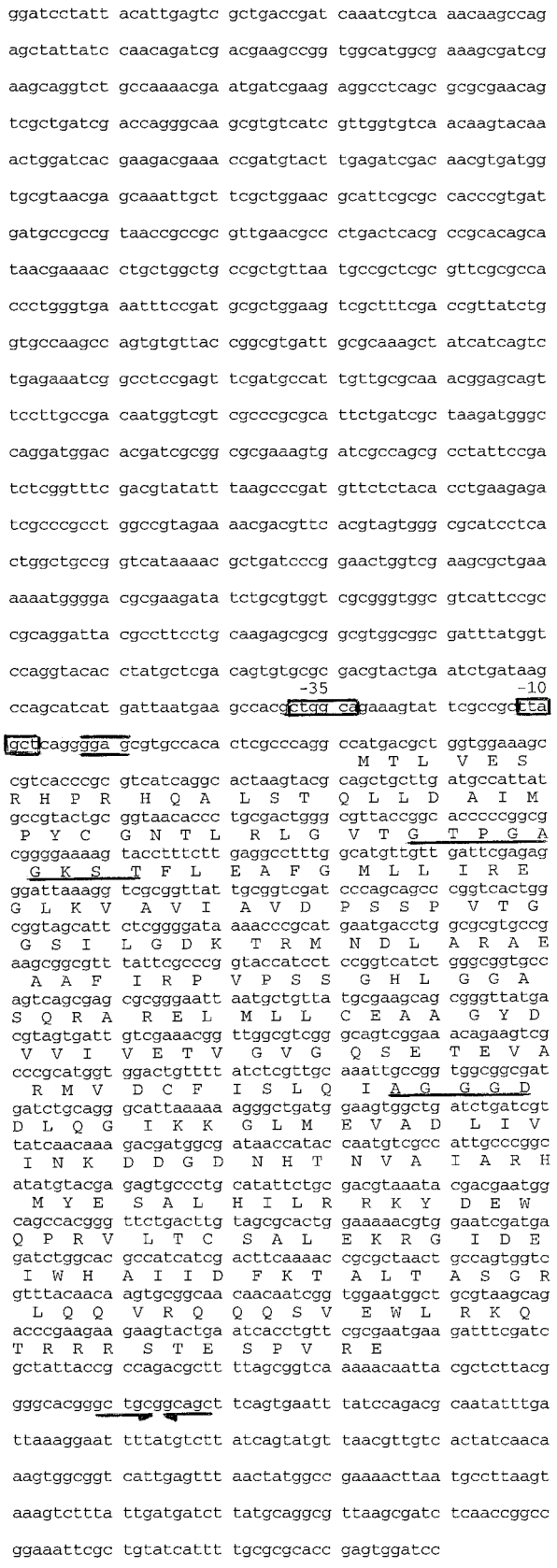

FIG. 2. Nucleotide sequence of the 2,140-bp *Bam*HI fragment encoding the *argK* gene and its deduced amino acid sequence. The conserved sequences for nucleotide binding (Walker sequences A and B) are underlined. Indicated at nt 1008 to 1011 is the putative Shine-Dalgarno sequence. Sequences conforming to the consensus for *E. coli* promoters (−35 and −10) are boxed. An inverted repeat with the potential to form a hairpin structure for transcriptional termination is shown by the two arrows between nt 1909 and 1919.

gradient in 0.01 M Tris-HCl buffer (pH 7.3) supplemented with 10% glycerol and 0.05 M NaCl. In the final step of purification, fractions with ATPase activity were pooled and dialyzed against 0.01 M Tris-HCl buffer (pH 7.3) supplemented with 10% glycerol and 0.05 M NaCl. A dye-ligand chromatography column of red agarose (reactive red 120; Sigma) equilibrated with dialysis buffer was loaded with the dialyzed preparation. The column was washed with several bed volumes of dialysis buffer containing 1.0 M NaCl. The enzyme was then eluted with the same buffer supplemented with 5.0 mM ATP. Eluted fractions were pooled after measuring ATPase activity and analysis by SDS-PAGE.

Isolation of *argK* suppressor mutations. Suppressor mutations were isolated by a standard genetic procedure, with specific modifications (22). Strain RC101 was grown at 37°C for 16 h in medium A supplemented with thiamine (1.0 μg/ml) (15). One hundred microliters of cell suspension was inoculated and grown in medium AF supplemented with thiamine and 100 μg of L-canavanine per ml. Upon reaching a concentration of approximately 10^7/ml, ampicillin (20 μg/ml) was added, and then incubation with shaking continued for 90 min. The number of cells grown in the presence of ampicillin was reduced to approximately 10^3/ml. The culture was then centrifuged, washed twice with medium A, and grown in medium A containing thiamine. After a second cycle of ampicillin treatment in the presence of canavanine, the cells were washed and aliquots were plated in AF-thiamine medium. Several hundreds of single colonies were tested by replica plating in two plates, AF-thiamine and AF-thiamine-canavanine. Canavanine-sensitive clones (12) were purified by restreaking in the same plates and then grown in liquid medium A in preparation for transport studies. Initial rates of uptake (11) of arginine and ornithine were then measured. Strains that showed restored values of incorporation of the two amino acids were selected for further studies.

Protein determinations. Protein determinations were carried out by the Bradford procedure (Bio-Rad).

Nucleotide sequence accession number. The sequence reported in this paper has been deposited in the GenBank database (accession no. U65074).

RESULTS

Cloning and location of the *argK* gene. We have previously shown that the effect of mutation of the arginine transport ATPase gene (*argK*) in *E. coli* K-12 strain RC101 can be corrected by a 9.5-kbp DNA fragment ligated into the *Eco*RI site of pTZR18, which was then designated pRC96 (14). The 9.5-kbp *Eco*RI DNA segment was originally isolated from λ phage clone 1A2 from the Kohara library (20) (Fig. 1). The *Eco*RI DNA fragment from plasmid pRC96 was isolated and used to generate different subclones in pTZR18 (data not shown). This led to the identification of a 2.2-kbp *Bam*HI fragment which was positive in restoring the function of *argK* (Fig. 1). The *argK* gene was then located near kb 3076, corresponding to 65.8 min on the *E. coli* chromosomal map (Fig. 1). DNA sequence analysis confirmed its location near the *sbm* gene (24). The *sbm* gene, which is transcribed clockwise on the genomic map, encodes a protein which is highly homologous to other methylmalonyl-coenzyme A mutase proteins (24).

Sequence analysis of the *argK* gene. The sequence of the 2,140-bp *Bam*HI fragment (Fig. 2) revealed an open reading frame (nt 1033 to 1836) capable of encoding a 268-amino-acid polypeptide of 29,370 Da, consistent with the size of 29 kDa determined for the ArgK protein by SDS-PAGE (Fig. 3). Sequences conforming to the consensus for *E. coli* promoters (−35 and −10) as well as a putative Shine-Dalgarno sequence are present upstream of the start codon for the *argK* gene. The translation initiation site was determined by a primer extension experiment (data not shown).

Purification of the ArgK protein. The final step of purification produced a homogeneous preparation with a size consistent with the molecular weight calculated from the deduced amino acid composition of the protein (Fig. 3). The predicted isoelectric point (pH 7.51) is near neutrality. A Kyte-Doolittle

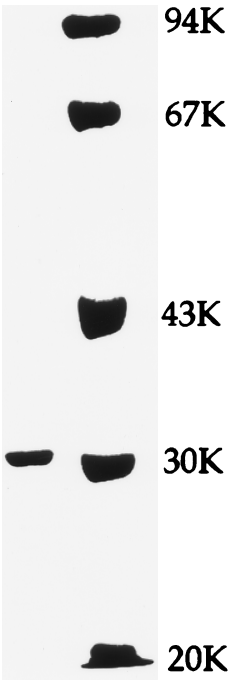

FIG. 3. SDS-PAGE of a purified preparation of the ATPase enzyme. Electrophoresis was performed as described previously (14), using 12.6% polyacrylamide gels. Molecular weight standards were phosphorylase *b* (M_r = 94,000), bovine serum albumin (M_r = 67,000), ovalbumin (M_r = 43,000), carbonic anhydrase (M_r = 30,000), and soybean trypsin inhibitor (M_r = 20,100).

hydrophobic profile, calculated with a moving window of 9 residues (21), predicted a relatively hydrophilic protein, without the long hydrophobic stretches found in hydrophobic membrane proteins (data not shown). The enzyme was eluted from a Superose 12HR 10/30 column, equilibrated with five protein markers of different molecular weights, at the same position as carbonic anhydrase (M_r = 29,000), indicating that the purified protein is a monomer.

Analysis of mutants. The characterization of mutant RC101 revealed that the mutation in *argK* produced a strong inhibition of arginine, ornithine, and lysine uptake concomitantly with a reduced specific transport ATPase activity and an inability of the enzyme to phosphorylate the AO and LAO proteins (14). The nucleotide sequence analysis of the *argK* gene revealed that strain RC101 carries two mutations in the *argK* locus. The amino acid serine at position 43 in the protein sequence had been replaced by arginine, and threonine 129 had been changed to alanine. Serine 43 is located in the glycine-rich loop of the protein, which is part of the ATP-binding domain of the molecule (28). This glycine-rich loop is thought to be involved in critical interactions with the β and γ phosphates of ATP (27). The replacement of threonine 129 is located in a portion of the protein which is not involved in energy utilization (8). Presumably, threonine 129 is related to the transfer of phosphate to the periplasmic proteins.

Twenty-six canavanine-sensitive derivatives were generated and analyzed. Fourteen of these strains (described in Table 2) carry independent intragenic suppressor mutations. A partially purified preparation of the enzyme, after DEAE-Sephacel chromatography, was used to measure the enzymatic activities of the protein. Nucleotide analysis of the back mutations in the *argK* gene of revertant strains revealed that the suppressed phenotype resulted from restoration of the ATPase activity of the enzyme by reversion to the original serine triplet, by changes in the same serine codon that could be tolerated by the enzyme, or by second-site mutations that could compensate the original serine-to-arginine mutation still present in the protein (Table 2). The change of threonine 129 to alanine remained unchanged in all mutations analyzed, and none of the second-site mutations could compensate the primary defect at position 129. Except for a partial (25%) restoration of phosphorylation of the binding protein in strain AH618, the presence of alanine at position 129 seems to abolish the ability of the enzyme to transfer phosphate to the periplasmic transport protein in cells with restored transport activity. It can be concluded that the role of the ArgK protein as a kinase enzyme is not related to the function of the transport system.

DISCUSSION

We have cloned and sequenced an *E. coli* K-12 gene encoding a membrane ATPase protein (ArgK) required for the active incorporation of arginine, ornithine, and lysine into cells. The amino acid sequence of the protein, as deduced from the nucleotide sequence, indicates that the protein is an ATP-binding subunit of the AO and LAO transport systems. Comparison of the amino acid sequence of the protein with sequences of ATP-binding proteins that belong to the family of ABC transport systems showed that the ArgK protein does not have significant sequence identity, over the entire domain, with the ATP-binding subunit of ABC transporters. This absence of homology in sequence libraries might reflect constraints imposed by the requirements in an unusual ATPase that is also a kinase enzyme. It can be concluded that although the AO and LAO systems of *E. coli* are periplasmic binding protein-dependent transport systems, they do not belong to the family of ABC transporters.

It was shown that a cloned copy of the wild-type gene (*argK*$^+$) was positive in restoring the activity of an *argK* mutation in cells with low uptake of arginine, ornithine, and lysine. The *argK* mutation affects the AO and LAO transport systems (14). The wild-type *argK*$^+$ gene is able to restore both transport systems. These systems, therefore, contain two different periplasmic proteins and presumably deliver their substrates to a common set of inner membrane components. A similar situation has been found with the LIV-1 and LS systems of *E. coli* and the His and LAO systems of *Salmonella typhimurium* (1).

Data from analysis of the mutants presented in Table 2 are consistent with a proposed role for the sequences for nucleotide binding in many nucleotide-binding proteins (28). These data are also consistent with information obtained from analyses of mutations affecting residues that are part of the ATP-binding subunits of the histidine transport system of *S. typhimurium* (27), the yeast SteA protein (8), and the multidrug resistance P-glycoprotein (P-gp) (4). The low levels of phosphate incorporated into the periplasmic transport proteins found in all canavanine-sensitive revertants that display normal values for transport provided conclusive evidence that phosphorylation of the periplasmic protein is not related to the function of the permease.

Studies with P-gp have suggested that phosphorylation of this transport protein by protein kinase C (5, 16) may modulate the rate of drug transport by this system. It has been shown, however, that phosphorylation of P-gp does not affect its own intrinsic transport activity (18). Since P-gp can function also as a modulator of cell-swelling-activated chloride channels in eukaryotic organisms (17), it has been proposed that the protein kinase C-mediated phosphorylation of P-gp may play a role in the efficiency with which the protein regulates heterologous channels rather than its own transport activity (18). Similarly, phosphorylation of components of the cystic fibrosis trans-

TABLE 2. Properties of suppression mutations in the *argK* locus[a]

Strain	Canavanine sensitivity[b]	% Uptake		ATPase (%)	% Phosphorylation		Changes	
		Arginine	Ornithine		AO protein	LAO protein	Base	Amino acid
RC04	S	100.0	100.0	100.0	100.0	100.0		
RC101	R	16.0	21.0	20.1	1.2	4.5	AGT → AGG	S → R at 43
							ACG → GCG	T → A at 129
AH48	S	96.0	101.0	92.0	1.9	2.1	AGG → AGC	R → S at 43
							ACG → GCG	T → A at 129
AH56	S	84.3	80.6	78.0	2.3	3.4	AAA → GAA	K → E at 163
							ACG → GCG	T → A at 129
AH124	S	91.2	92.7	83.4	0.9	2.0	AGG → TGG	R → W at 43
							ACG → GCG	T → A at 129
AH202	S	89.2	73.5	101.0	2.2	1.9	AGG → GGG	R → G at 43
							ACG → GCG	T → A at 129
AH212	S	76.4	81.4	89.6	5.2	2.2	AGG → ACG	R → T at 43
							ACG → GCG	T → A at 129
AH265	S	74.5	80.8	83.2	4.8	1.9	AGG → ATG	R → M at 43
							ACG → GCG	T → A at 129
AH294	S	86.3	78.6	80.6	4.2	2.5	AAA → GAA	K → E at 42
							ACG → GCG	T → A at 129
AH318	S	92.1	86.3	87.5	3.7	4.0	GCG → GAG	A → E at 40
							ACG → GCG	T → A at 129
AH206	S	65.0	68.0	71.0	6.1	4.9	CAT → TAT	H → Y at 197
							ACG → GCG	T → A at 129
AH88	S	74.2	67.0	80.0	4.6	5.6	TTA → TTT	L → F at 241
							ACG → GCG	T → A at 129
AH126	S	97.1	96.0	98.6	4.5	6.7	AGG → AGT	R → S at 43
							ACG → GCG	T → A at 129
AH618	S	96.0	89.0	98.0	25.0	21.3	CAT → GAT	H → D at 182
							ACG → GCG	T → A at 129
AH332	S	80.4	79.6	79.8	6.3	5.6	AAG → ATG	K → M at 59
							ACG → GCG	T → A at 129
AH420	S	63.5	67.0	69.2	4.8	8.6	GTT → GAT	V → D at 173
							ACG → GCG	T → A at 129

[a] The sequence analysis of each mutation refers to the transport properties of strains harboring that mutation. Effects of mutations on the ATPase and kinase activities of the enzymes isolated from the mutants are indicated. Values for wild-type strain RC04 and defective strain RC101 are included. Transport and enzyme activities were measured as indicated in Materials and Methods as follows: One hundred percent arginine uptake equals 2.0 nmol/min/mg of protein, incorporated through the AO system with an initial external concentration of 1.0 μM radioactive arginine (15). Ornithine was measured at 0.1 μM initial external concentration to test the LAO transport system. The wild-type strain showed an uptake value of 0.2 nmol/min/mg of protein (15). ATPase activity in wild-type strain RC04 equals 25.8 nmol/min/mg of protein as 100% of P_i released (13). Phosphorylation of the AO protein in wild-type *E. coli* has a value of 32.4 nmol/min/mg of protein as 100% of P_i incorporated (13). Phosphorylation of the LAO protein equals 26.3 nmol/min/mg of protein as 100% of P_i incorporated (13).

[b] S, sensitive; R, resistant.

membrane conductance regulator CFTR seems to regulate the expression of heterologous membrane-associated proteins (19). We are now exploring the possibility of a second and distinct function of the phosphorylated periplasmic binding protein by the ArgK enzyme of *E. coli* K-12.

The finding of a required ATPase activity for the functioning of the AO and LAO transport systems strongly suggests that these systems may use ATP as the source of energy.

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